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Clinical Pharmacology of Lamotrigine

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Klinisk farmakokinetikk av lamotrigin

Sammendrag

Lamotrigin (LTG) er det mest brukte antiepileptikum i Norge, som også brukes i ut-strakt grad ved manisk-depressive lidelser og andre psykiatriske sykdommer. Dette arbeidet undersøker bruken av LTG samt faktorer som påvirker metabolismen og ut-skillelsen av LTG, med fokus på kvinner og kvinnelige hormoner. Både retrospektive database-analyser og prospektive kliniske studier ble brukt.

Det langt største antall serumkonsentrasjonsmålinger på LTG utføres på prøver fra kvinner. De fleste som bruker LTG får midlet for en psykiatrisk lidelse, mange av disse utenfor godkjent indikasjon. Kvinner har høyere serumkonsentrasjoner enn menn ved samme dose. Barn, ungdom og eldre har høyere serumkonsentrasjoner enn 20-65 år gamle voksne. Utover kjente interaksjoner med klassiske enzyminduktorer og –hem-mere ble det oppdaget at etinyløstradiol i p-piller reduserer LTG-serumkonsentrasjo-nen med over 50 %, noe som ofte medfører tap av anfallskontroll og behov for dose-økning av LTG. De fleste psykofarmaka kan trygt kombineres med LTG. De fysiologiske hormonsvingningene gjennom en normal menstruasjonssyklus synes ikke å påvirke LTG i en klinisk relevant grad. Svangerskap derimot fører til et raskt og stort fall av LTG-serumkonsentrasjonen allerede tidlig i første trimester. Dette skyldes sannsynligvis den fysiologisk økte blodgjennomstrømning i nyrene. Senere i graviditeten tilkommer raskere metabolisme av LTG gjennom østrogen-indusert økt aktivitet av leverenzymet UGT1A4.

Funnene i dette arbeidet understreker nytten av serumkonsentrasjonsmålinger av LTG, spesielt hos barn og eldre, men også hos pasienter som samtidig bruker andre lege-midler inkludert kombinerte p-piller, og spesielt hos gravide kvinner.

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Acknowledgements

List of original papers

This thesis is based on the following papers:

Paper I
Arne Reimers:
Trends and changes in the clinical use of lamotrigine. Pharmacoepidemiology and Drug Safety 2009;18(2):132-9.

Paper II
Arne Reimers, Eirik Skogvoll, Janne Kutschera Sund, Olav Spigset:
Drug interactions between lamotrigine and psychoactive drugs: Evidence from a therapeutic drug monitoring service. Journal of Clinical Psychopharmacology 2005;25(4):342-348

Paper III
Arne Reimers, Grethe Helde, Eylert Brodtkorb :
Ethinyl estradiol, not progestogens, reduces lamotrigine serum concentrations. Epilepsia 2005;46(9):1414-7

Paper IV
Arne Reimers, Eylert Brodtkorb, Grethe Helde, Olav Spigset:
Lamotrigine serum concentrations throughout the menstrual cycle – a study of two subjects. Journal of Clinical Neuropharmacology 2006;29(3):160-2

Paper V
Arne Reimers, Grethe Helde, Geir Bråthen, Eylert Brodtkorb:
Lamotrigine and its N2-glucuronide during pregnancy: The significance of renal clearance and estradiol. Epilepsy Research 2011;94(3):198-205

Paper VI
Arne Reimers, Eirik Skogvoll, Janne Kutschera Sund, Olav Spigset:
Lamotrigine in children and adolescents: The impact of age on its serum concentrations and on the extent of drug interactions. European Journal of Clinical Pharmacology 2007;63(7):627-629

Summary in English

Background and objectives

Lamotrigine (LTG) is a first-line drug for the treatment of epilepsy in adults and children. Besides, it is widely used as a mood stabilizer for the treatment of affective disorders. Due to its favorable safety profile and its comparatively low potential for drug interactions, many neurologists and psychiatrists prefer LTG to other drugs, especially for the treatment of women of childbearing age.

After oral administration, LTG is rapidly and almost completely absorbed. Metabolism takes place by conjugation with glucuronic acid, catalyzed by UDP-glucuronosyltransferase 1A4 (UGT1A4). The resulting LTG-N2-glucuronide is then excreted via the kidneys, and up to 90 % of an oral dose of LTG appears as the N2-glucuronide in the urine. There is, however, considerable inter-individual variation in the pharmacokinetics of LTG. Several factors may account for this. Apart from pharmacogenetic polymorphisms, these factors may include concomitant diseases, drug interactions, age-dependent changes in metabolic capacity, as well as hormonal influences.

The aim of the present work was to identify and describe such factors and their impact on the clinical pharmacokinetics of LTG, with special focus on the use of LTG in women.

Materials and methods

Both retrospective analyses and prospective clinical studies were used in this work. The database of the Department of Clinical Pharmacology provided pharmacoepidemiological information, as well as information on drug interactions and the relevance of age and gender (papers I, II and VI).

Prospective clinical studies at the Department of Neurology and Clinical Neurophysiology investigated the impact of female sexual hormones on the pharmacokinetics of LTG (papers III, IV and V). These studies focused on endogenous estrogen as well as ethinyl estradiol and progestins used for hormonal contraception. The largest of these studies (paper V) examined the pharmacokinetic changes induced by pregnancy and their underlying mechanisms.

The analysis of LTG and LTG-N2-glucuronide in serum and urine was performed by liquid chromatography-mass spectrometry methods developed at the Department of Clinical Pharmacology.

Results

- The vast majority of LTG serum concentration analyses are performed in samples from women. Most patients used LTG for a broad variety of psychiatric disorders, many of them off-label. These trends appear to continue and accelerate (paper I).

- The median serum concentration-to-dose ratio (CDR) of LTG is about 0.06 which means that, e.g., a daily dose of 200 mg LTG will give an average serum concentration of 12 µmol/l. Women have a slightly higher CDR than men. Children, adolescents and the elderly show higher CDRs than adults aged 20 – 65 years (papers II and VI).

- With regard to drug interactions, the findings of previous studies could not only be confirmed, but also further quantified. Classical enzyme inducers such as carbamazepine, oxcarbazepine, or phenytoin reduce LTG serum concentrations, while co-administration of valproate leads to considerably higher serum levels. Most psychotropic drugs can be safely combined with LTG (paper VI).

- Ethinyl estradiol-containing combined oral contraceptives reduce LTG serum concentrations by up to 60 %, whereas progestin-only contraceptives do not. Evidence was found that it is the ethinyl estradiol component in combined oral contraceptives which reduces LTG levels, and not the progestin-component (paper III).

- The fluctuations of estradiol and progesterone during a normal menstrual cycle do not seem to affect LTG kinetics to a clinically relevant degree (paper IV).

- Pregnancy may reduce LTG serum levels by over 50 %, with a rapid, marked decline already in the first trimester. This first-trimester decline is probably due to increased renal blood flow, whereas the following, additional decrease is caused by estradiol-dependent induction of LTG-glucuronidation (paper V).

Conclusions

The preponderance of LTG use in women and in psychiatric conditions is of interest both from a clinical and from a public health view. As these trends appear to accelerate, they may become even more important in the future.

UGT1A4 activity is low in young children, but increases by age. This phenomenon affects the metabolism of LTG and is subject to large interindividual variability. Thus, the

treatment of children and adolescents with LTG demands close clinical and laboratory monitoring of these patients.

Oral contraceptives containing ethinyl estradiol will often necessitate an increase of the LTG dose to maintain effective seizure control. Progestin-only contraceptives can be safely combined with LTG treatment without such adjustments.

Pregnancy increases the metabolism and excretion of LTG considerably, a phenomenon that needs to be kept in mind by clinicians who treat women wishing to become pregnant. Special attention should be paid to the marked fall of LTG serum concentrations already in the first trimester.

Renal blood flow emerged as a significant factor with respect to the accelerated elimination of LTG and its main metabolite in pregnancy. It may also become significant in individuals in whom renal blood flow is altered due to other causes, e.g. in renal disease or in elderly patients.

Sammendrag på norsk

Bakgrunn og malsetting

Lamotrigin (LTG) er et førstehåndspreparat mot epilepsi både for voksne og barn. Det brukes dessuten som stemningsstabilisator ved behandling av affektive lidelser. Den gunstige sikkerhetsprofilen og det forholdsvis lave interaksjonspotensialet har medført at mange nevrologer og psykiatere foretrekker LTG fremfor andre legemidler, særlig hos kvinner i fertil alder.

LTG metaboliseres ved konjugering med glukuronsyre, katalysert av UDP-glukuronosyl-transferase 1A4 (UGT1A4). Metabolitten, LTG-N2-glukuronid, skilles ut gjennom nyrene. Inntil 90 % av en oral dose finnes igjen i urinen som N2-glukuronid. Farmakokinetikken til LTG viser imidlertid betydelig interindividuell variasjon. Dette har ulike årsaker, f.eks. farmakogenetiske polymorfismer, sykdom, interaksjoner med andre legemidler, aldersavhengige forskjeller i metabolsk kapasitet eller hormonell påvirkning.

Målet med dette arbeidet var å identifisere og beskrive disse faktorene og hvordan de påvirker farmakokinetikken til LTG, særlig med fokus på spesielle forhold hos kvinner.

Materiale og metode

Både retrospektive og prospektive studier ble brukt i dette arbeidet. Databasen ved Avdeling for klinisk farmakologi leverte farmako-epidemiologisk informasjon, data om legemiddelinteraksjoner, samt informasjon om betydningen av alder og kjønn (publikasjonene I, II og VI).

Prospektive kliniske studier ble gjennomført ved Avdeling for nevrologi og klinisk nevrofysiologi for å undersøke effekten av kvinnelige seksualhormoner på farmakokinetikken til LTG (publikasjonene II, IV og V). Studiene II og IV fokuserte på endogene hormoner såvel som etinyløstradiol og progestiner, som begge brukes i hormonell kontrasepsjon. Publikasjon V undersøkte hvilke farmakokinetiske forandringer et svangerskap induserer og hvilke mekanismer som ligger bak.

Analysene av LTG og LTG-N2-glukuronid i serum og urin ble gjennomført med væskekromatografisk-massespektrometriske metoder (LC-MS) utarbeidet ved Avdeling for klinisk farmakologi.

Resultater

- Det overveiende flertall av serumkonsentrasjonsmålinger på LTG utføres på prøver fra kvinner. De fleste pasienter får LTG for ulike psykiatriske lidelser, mange av dem utenfor godkjent indikasjon. Disse trendene ser ut til å vedvare (publikasjon I).

- Den mediane serumkonsentrasjons/dose-ratio (CDR) til LTG er 0,06. Det betyr at eksempelvis en døgndose på 200 mg LTG vil gi en median serumkonsentrasjon på 12 µmol/l. Kvinner har en noe høyere CDR enn men. Barn, ungdom og eldre har høyere CDR enn 20-65 år gamle voksne (publikasjonene II og VI).

- Funn fra tidligere studier angående legemiddelinteraksjoner ble bekreftet og nærmere kvantifisert. Klassiske enzyminduktorer som karbamazepin, oxkarbazepin og fenytoin reduserer LTG-serumkonsentrasjonen, mens bruk av valproat gir betydelig høyere serumkonsentrasjoner. De fleste psykofarmaka kan trygt kombineres med LTG (publikasjon VI).

- Kombinerte orale prevensjonsmidler (p-piller) reduserer LTG-serumkonsentrasjonen med opptil 60 %. Prevensjonspreparater som kun inneholder et progestin påvirker ikke LTG. Vi viste dermed at det er etinyløstradiol i kombinerte "p-piller" som reduserer LTG-serumkonsentrasjonen og ikke progestiner (publikasjon III).

- Svingningene i østradiol- og progesteron-serumkonsentrasjoner gjennom en normal menstruasjonssyklus ser ikke ut til å påvirke farmakokinetikken til LTG i klinisk rele-vant grad (publikasjon IV).

- Under svangerskap kan LTG-serumkonsentrasjonen synke med over 50 %. Det opptrer et raskt og kraftig fall allerede i første trimester. Denne tidlige nedgangen skyldes mest sannsynlig økt blodgjennomstrømning i nyrene, mens den påfølgende nedgangen sannsynligvis skyldes østradiolavhengig økt glukuronidering av LTG (publikasjon V).

Konklusjoner

Både fra samfunnsmedisinsk og klinisk perspektiv er det av interesse at det er flest kvinner som bruker LTG, og at de fleste bruker det for psykiatriske lidelser. Da denne trenden er økende, vil dette bli mer uttalt i fremtiden.

UGT1A4-aktiviteten er lav hos barn, men øker med alderen. Dette har betydning for metabolismen til LTG. Den interindividuelle variasjonen er stor. Barn og unge som bruker LTG bør derfor følges tett, både klinisk og med serumkonsentrasjonsmålinger.

Eldre mennesker utvikler ofte høyere CDR; redusert renal blodgjennomstrømning kan være årsaken.

P-piller som inneholder etinyløstradiol vil ofte medføre behov for doseøkning av LTG for at anfallskontrollen skal opprettholdes. Preparater som kun inneholder et progestin påvirker ikke LTG-konsentrasjonen.

Under graviditet øker metabolismen og utskillelsen av LTG betydelig. God kjennskap til dette er viktig i behandlingen av kvinner med graviditetsønske. Det raske og store fallet i LTG-serumkonsentrasjonen i første trimester bør vies særlig oppmerksomhet. Økt renal blodgjennomstrømning synes å være årsaken til dette.

Abbreviations and definitions

AED	antiepileptic drug
BL	baseline
CDR	concentration/dose-ratio
CL	clearance
COC	combined oral contraceptive
CYP	cytochrome P450
DDD	defined daily dose
EE	ethinyl estradiol
LTG	lamotrigine
LTG-GLUC	lamotrigine-N2-glucuronide, the main metabolite of LTG
PG	progestin
t1/2	serum half-life
TDM	therapeutic drug monitoring
UGT	uridinediphosphate (UDP) glucuronosyltransferase

LTG-concentrations are sometimes given in molar units. For conversion to mass units, the molar concentration has to be divided by 3.9. E.g., a lamotrigine concentration of 15 µmol/l equals 15/3.9 = 3.8 mg/l. Vice versa, mass units have to be multiplied by 3.9 to give molar units.

1. Introduction

1.1. The history of lamotrigine

Lamotrigine (6-(2,3-dichlorophenyl)-1,2,4-triazine-3,5-diamine; LTG) was introduced in 1992 as one of the first so-called second-generation antiepileptic drugs (AED). It has no structural similarity with any other AED (Fig 1). LTG was originally developed as a folate antagonist [1] as it had been observed that some older AEDs were associated with low folate serum concentrations. This in turn led to the assumption that folate antagonism by itself might represent an antiepileptic mechanism (a hypothesis which is now abandoned) [2]. LTG's antiepileptic effect has later been shown to be based on blocking of sodium channels and anti-glutamatergic effects. Moreover, its antifolate properties appear to be too weak to be clinically relevant [3].
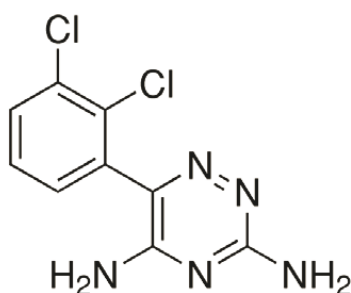

Figure 1: Chemical structure of lamotrigine.

After first having been used as add-on treatment together with older-generation AEDs, LTG is now licensed worldwide for monotherapy of partial and generalised epilepsy in adults, and for add-on treatment in children.

During the clinical development as an AED, patients reported considerable improvements in mood and general well-being. [4]. At that time, carbamazepine and valproate were the only AEDs which were established as treatment for mood disorders (apart from lithium, antidepressants and antispychotics). However, their use is compromised by numerous adverse effects and their teratogenic potential. Being a potent enzyme inducer and -inhibitor, respectively, their use is further complicated by pharmacokinetic drug interactions. LTG, which has a more favourable safety and drug interaction

profile, was therefore tried as an alternative treatment in mood disorders. It was found to be efficient in the prophylaxis of depressive episodes in bipolar II disorder, but not in the treatment of mania, acute depressive episodes, or unipolar depression. Today, LTG is licensed worldwide for the prevention of depressive episodes in patients with bipolar disorder.

The favorable safety profile of LTG was a crucial factor for its success. Soon after the launch of LTG for partial epilepsy, it was found that it was effective in generalized epilepsy as well. VPA had for decades been the first-line AED for generalized epilepsies, but the focus on safety issues of VPA increased, particularly with respect to teratogenicity and endocrine side effects. Hence, LTG soon became a first choice alternative for the treatment of women, not only in neurology but also in psychiatry.

LTG is the most used AED in Norway, both in terms of Defined Daily Doses (DDD) and number of users. According to the Norwegian Prescription Registry, 22 977 408 DDD of LTG were prescribed to 22 348 individual users in 2009 [5]. The DDD of LTG has been set to 300 mg by the WHO.
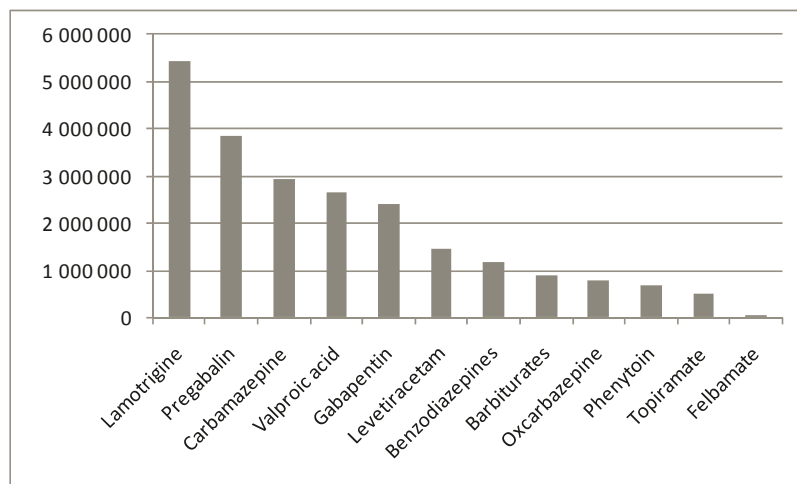

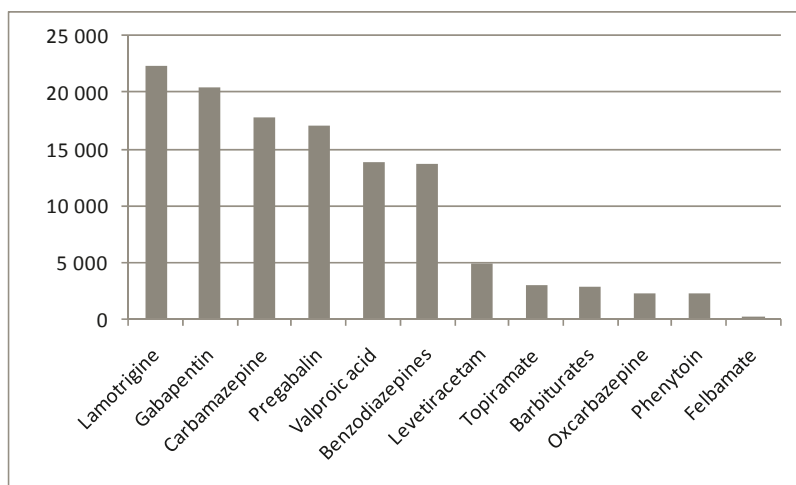*Figure 2: Prescribed defined daily doses (DDD) of AEDs in Norway in 2009*

Figure 3: Number of individual users of antiepileptic drugs in Norway in 2009

1.2. Pharmacokinetics

The common daily dose of LTG is 200 - 400 mg, ranging from 100 to 800 mg [6]. After oral dosing, absorption of LTG is almost complete and unaffected by food [7]. LTG is mainly biotransformed by uridinediphosphate (UDP-)-glucuronosyltransferase 1A4 (UGT1A4) to a pharmacologically inactive metabolite, lamotrigine-N2-glucuronide (LTG-GLUC; figure 4). Seventy to 90 % of an orally administered dose appear as LTG-GLUC in urine, and approx. 10% as unchanged LTG. Two percent may appear in the faeces [8, 9]. Glucuronidation in the N5-position has been postulated [9], but the putative LTG-N5-glucuronide has never been demonstrated. Other metabolites have been found in animals, but occur only in very small amounts in humans [9-11].
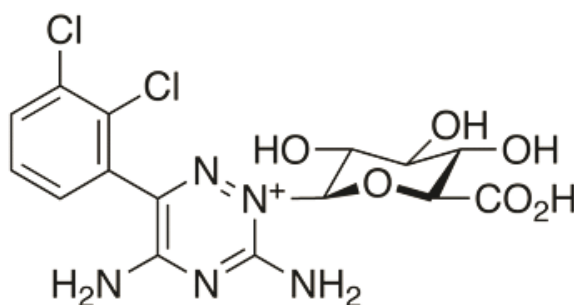

Figure 4: Lamotrigine-N2-glucuronide, the main metabolite of lamotrigine

Basic pharmacokinetic data of LTG are shown in table 1 [12-17]. LTG shows first-order, linear pharmakokinetics, which means that the maximum, trough, and average serum concentrations are proportional to the dose.

The relationship between serum concentration and the clinical effect of LTG has not been firmly established, but based upon the results of several clinical studies, a reference range of 3-14 mg/L (or 10-50 µmol/L) has been accepted by most neurologists [18]. A reference range for the use of LTG in mood disorders has not been established and remains a matter of discussion. However, a standard daily dose of 200 mg is recommended by the manufacturer for this indication. Based on the bioavailability and the V_D stated in table 1, this dose would give serum concentrations of 1.7 - 2.2 mg/L (or 7-9 µmol/L) in a person weighing 75 kg.

Table 1. Basic pharmacokinetic data of lamotrigine in healthy adults (averaged from multiple studies).

Oral bioavailability	98 %
t_{max}	2-3 hours
$t_{1/2}$	23-37 hours
CL	2.14 +/- 0.81 L/h
V_D	1.2-1.5 L/kg

t_{max}: time to maximum serum concentration after oral intake; $t_{1/2}$: serum half-life; CL: plasma clearance; V_D: apparent volume of distribution.

Although LTG pharmacokinetics are quite predictable, there is a certain degree of interindividual variation, as with all drugs. Age, sex, pregnancy, co-medication and, putatively, genetic polymorphisms of UGT enzymes may all have an impact on LTG kinetics. These issues are among the main subjects of this thesis and will be discussed in detail.

1.3. Pharmacokinetic drug interactions

No drug interactions of LTG regarding absorption, distribution or elimination have been established so far. Pharmacokinetic drug interactions with LTG appear to be restricted to metabolism, as the clearance of LTG by UGT1A4 can be increased or decreased by other drugs. Enzyme inducers, e.g. phenytoin, phenobarbital or carbamazepine, as well as combined oral contraceptives (COC) may reduce the half-life and serum concentrations of LTG by more than 50 %. By contrast, valproic acid increases LTG's half-life and serum concentrations by about 100 % [19-23]. Especially the interaction of LTG with COC has received much attention in recent years. This interaction was first discovered 10 years after the introduction of LTG and was an unexpected finding [23]. Enzyme-inducing AEDs had for decades been known to impair the anticontraceptive effect of COCs. LTG was the first AED to have its efficacy reduced by a COC. It has later been found that COCs also may reduce the serum concentrations of valproate [24, 25]. Drug interactions with LTG, represent one of the main subjects of this thesis.

1.4. UDP-glucuronosyl transferases (UGTs)

UDP-glucuronosyltransferases (UGTs) are a mammalian superfamily of phase II metabolizing enzymes, of which UGT1A and UGT2B are the most important subfamilies. To date, 25 UGT1A and UGT2B isozymes have been identified in humans [26]. UGTs catalyze the binding of glucuronic acid to endo- and exogenous compounds, e.g. hormones or drugs. This is an important process that increases their solubility in water and aids in their urinary excretion. UGTs have been found in a variety of organs and tissues, including liver, lungs, intestinal mucosa, brain, uterus and placenta, in both animals and humans [26-30].

UGT activity is regulated by various pre- and posttranslational mechanisms, e.g. hormones, liver-enriched transcription factors, ligand-activated transcription factors, and the aryl hydrocarbon receptor [31, 32]. These findings relate mostly to experiments with hepatic UGT. Very little is known on the extent and intrinsic regulation of human placental UGT activity. External factors like enzyme inducing drugs, ethinyl estradiol or cigarette smoke may increase UGT-activity. Some UGT1A4-substrates, e.g. LTG, may even induce their own metabolism to a certain degree [15, 19, 33-35].

1.5. Pharmacoepidemiology

Data from prescription registries often form the basis for pharmacoepidemiological studies. Their value may be enhanced by coupling with other databases which give information on indications and dosing [36-38]. Information on serum concentrations may further increase the usefulness of pharmacoepidemiological investigations.

Serum concentration measurement of LTG was established soon after its introduction as an AED [19]. Collected systematically over long time, data from such measurements may represent a platform for pharmacoepidemiological research and may reveal trends and changes in the pattern of clinical drug use. Apart from giving information about dosages, indications and co-medications, such research may also address the clinical relevance of recommended reference ranges, and to what degree treatment guidelines reflect clinical reality (and vice versa). Previous naturalistic studies on pharmacoepidemiological aspects of LTG-treatment investigated mainly its clinical effectiveness and safety issues [39-43]. Little is known about changes in its pattern of use over time [44].

1.6. Gender issues

1.6.1. Contraceptives

By contrast to VPA, LTG has so far not been associated with endocrine or metabolic disturbances like, e.g., polycystic ovary syndrome or diabetes, and it has therefore been regarded as a preferable choice in women [45, 46]. However, LTGs interaction potential with sexual hormones still remains largely unexplored. Women of child-bearing age often use hormonal contraception , and it is now well-established that COCs reduce LTG serum concentrations by more than 50 % [23, 47, 48], which may require dose adjustment of LTG. In turn, LTG may reduce serum levels of levonorgestrel, although not to a clinically relevant degree [48]. COCs contain a combination of a synthetic estrogen derivative, usually ethinyl estradiol (EE), and a progestin (a synthetic progesterone derivative, often levonorgestrel). There are also several progestin-only contraceptives on the market. Moreover, in addition to oral compounds, hormone-containing parenteral, transdermal, intravaginal, and intrauterine products are available. Therefore, it was desirable to find out whether the interaction between LTG and hormonal contraception was due to the EE or to the progestin. Moreover, it was useful to know whether this interaction is restricted to oral

application of the hormones or whether it also is present with topical/parenteral methods.

1.6.2. Menstrual cycle

With respect to the interaction of LTG with COC, it has been found that LTG serum levels increase quickly and by over 100 % during the pill-free week [48]. Thus, one might hypothesise that the effect of changing estrogen-levels on the metabolism of LTG may both develop and disappear within a few days. The question arises whether the physiological fluctuations of female sex hormones during a normal menstrual cycle as well may lead to short-term fluctuations of the LTG serum levels. If this was the case, it might have clinical consequences for the treatment of women with epilepsy. It might also have implications for choosing the day of the cycle for measuring the LTG serum concentrations. The study described in paper IV was designed to answer this question.

1.6.3. Pregnancy

Epilepsy is the most common neurological problem which requires pharmacological treatment in pregnant women [49]. Indeed, a considerable number of female patients take LTG during pregnancy [50]. The balance between seizure control and potential teratogenic risks, and the pharmacokinetic changes of AEDs during pregnancy and puerperium often represent significant challenges to the clinician [49, 51, 52].

Several studies have shown that LTG clearance may increase by 65 - 230 % during gestation. Accordingly, serum concentrations may decrease by more than 60 %, often requiring dose adjustments to maintain seizure control. [33, 53-60]. These pharmacokinetic changes are subject to marked interindividual variability [57]. They are thus largely unpredictable, and close therapeutic drug monitoring is recommended [61]. Serum concentrations return to pre-pregnancy values within 2-3 weeks postpartum [54, 55, 59], a phenomenon which also requires close clinical follow-up of the patients. Increased glucuronidation of LTG in the N2-position has been proposed as the mechanism behind its increased clearance during pregnancy [33]. However, pregnancy induces a variety of significant physiological changes which may affect LTG pharmacokinetics and lead to a fall in its serum concentrations [62, 63]. Further knowledge of the mechanisms behind pregnancy-induced changes in the

pharmacokinetics of LTG was desirable. The study presented in paper V was performed to address this issue.

2   Aims

The general objective of this thesis was to extend the knowledge on the clinical pharmacokinetics and use of lamotrigine, with a particular focus on women. The specific aims were:

- to collect and analyse epidemiological data and to document current status, trends and historical changes in the clinical use of LTG

- to document and evaluate possible drug interactions with other drugs

- to study and describe age- and gender-related aspects of LTG pharmacokinetics

- to investigate practical aspects related to the increasing use of LTG in women, especially the impact of hormonal contraception and pregnancy on LTG pharmacokinetics

- to describe and investigate the nature of the underlying physiological mechanisms which lead to changes in LTG pharmacokinetics in pregnancy

3 Methods

3.1 Data collection

Table 2 gives a methodological overview of the papers included in this thesis.

Table 2. Methodological overview

	Study design	Study subject	Pro-/retro-spective	n
Paper I	Database analysis	routine samples	retrospective	12 107
Paper II	Database analysis	routine samples	retrospective	1 733
Paper III	Clinical study	women with epilepsy	prospective	45
Paper IV	Case study	women with epilepsy	prospective	2
Paper V	Clinical study	pregnant women with epilepsy	prospective	21
Paper VI	Database analysis	routine samples from patients aged 2-19 years	retrospective	744

For papers I, II and VI, the database of the Department of Clinical Pharmacology at St. Olavs University Hospital was used. This database consists of data from routine samples sent to the department for measurement of the serum concentration of drugs. The request form asks for exact times of intake of the last dose and blood sampling, daily dose, number of daily intakes, diagnosis, and co-medication. Age and gender was derived from each individual's population registry number found on the request form. Samples without information on dose and/or time interval between last dose and sampling were excluded from evaluation. Moreover, only samples taken 10-24 hours

after intake were included. Samples with a serum concentration below the lower limit of quantification of the analytical method were excluded from all studies.

Paper III was a three-armed, open, prospective study. LTG serum concentrations of 45 consecutively enrolled women, either using no hormonal contraception, an EE-containing COC, or a progestin-only compound, were measured. Some women participated in more than one group and thus served as their own controls in a cross-over fashion. Blood samples were drawn drug-fasting in the morning and at steady-state conditions, but not standardized in relation to the menstrual cycle. Time intervals between blood samplings in patients who changed group were at least one month to allow wash-out, and their LTG doses remained unchanged.

For paper IV, repeated blood samples were taken from two female outpatients, and the serum concentrations of LTG, estradiol and progesterone were determined. All blood samples were taken by the same nurse under identical conditions. The time intervals from intake of the last dose to blood sampling were always between 10-14 hours. The time intervals between consecutive samples did not exceed three days. This study design required an extremely high degree of co-operation from the participants, which may explain that only two patients could be recruited. In both patients, compliance was found to be excellent.

Paper V was an open, prospective clinical study. A total of 21 pregnant outpatients treated for epilepsy with LTG, were consecutively included. Exclusion criteria were: liver- or kidney disease and co-medication with carbamazepine, oxcarbazepine, phenytoin, phenobarbital, primidone, valproate, topiramate, rifampicin, fluoxetine or lithium [64]. Patients with a history of low treatment adherence or substance abuse were also excluded. Patients were asked to enroll as soon as the attending neurologist had been informed about their pregnancy. Thus, all patients were in gestational week 5 or later at the time of the first visit. Baseline samples were collected at least four weeks postpartum since it had been shown that LTG pharmacokinetics return to pre-pregnancy values within two to three weeks after delivery [54, 55, 59, 65].

Morning trough blood samples were collected at the first visit, and then once every month throughout pregnancy. Body weight was recorded at each visit.
On two occasions, once in the third trimester and once at least four weeks after delivery, blood samples were taken at 0800 hours (drug fasting, immediately before the morning dose), and then 2, 4, 8 and 12 hours after the morning dose. The 12-hour period was chosen because all patients were on a twice-daily-regimen of LTG. The patients´ urine was collected in the same 12-hour period.

3.2 Sample analysis

Blood samples were centrifuged at 350 G for 10 minutes and the serum supernatant was carefully transferred to sample vials. The total volume of the collected urine was recorded and a 20 ml urine sample was taken for analysis of LTG and LTG-GLUC. Serum and urine samples were stored at -18 °C until analysis.

Before analysis, urine samples were diluted 1:100 because of their very high LTG-GLUC concentrations exceeding the assay's measuring range. Apart from this, serum and urine samples were treated identically. To 100 μL sample volume, 25 μL minoxidil (internal standard) and 75 μL 1 % formic acid were added. Serum and urine sample preparation was then performed on OMIX™ Tomtec mixed mode phase SPE tips (Varian, Walnut Creek, CA) by means of a Tomtec Quadra 96 model 320 automatic liquid handler (Tomtec, Hamden, CT) equipped with 1.2 ml Varian 96-well plates. The OMIX tips were conditioned successively by methanol and 0.1 % formic acid. After sample aspiration, the OMIX tips were washed with 1 % methanol and elution was performed with 50 μL methanol:ammonia (95:5).

The eluent was transferred to a deep well plate and injected on an Agilent MSD 1100 LC-MS system (Agilent, Palo Alto, CA). The LC-MS system consisted of a G1379A degasser, a G1311A quaternary pump, a G1313A auto sampler, a G1316A column oven and a G1946D mass spectrometer. Separation was performed on a Supelguard Discovery 18 (20 x 4 mm) column with a mobile phase consisting of methanol:formic acid:ammonium acetate (3:6:91) at a flow of 1000 ml/min. LTG was monitored after positive APCI ionization at m/z 256.3 (target ion) and 258.3 (qualifier ion), LTG-GLUC at m/z 432.3 (target ion) and 434.3 (qualifier ion), and the internal standard minoxidil at m/z 210.1 (target ion) and 164.1 (qualifier ion).

The calibrated ranges in both serum and urine were 0.5 – 10 μg/mL (LTG) and 1-20 μg/mL (LTG-GLUC). Three quality control samples of LTG and LTG-GLUC, covering the range from 0.5 - 20 μg/mL, were analysed with every sample batch. Between-day analytical variation of quality controls in serum was better than 8.4 % at 0.5 μg/mL and 10.4 % at 10 μg/mL for LTG, and 10.3 % at 1 μg/mL and 17.5 % at 20 μg/mL for LTG-GLUC. Analytical variation in urine was better than 4.1 % at 1 μg/mL and 2.6 % at 10 μg/mL for LTG, and 7.4 % at 2 μg/mL and 10.5 % at 20 μg/mL for LTG-GLUC.

Serum estradiol analysis was performed on a Roche Modular E 170. The calibrated measuring range of this method was 5.0-4300 pg/mL (CV ranging from 2.2 to 12 %). Progesterone, erythrocyte volume fraction (EVF), serum sodium, serum creatinine and

serum bilirubine were analyzed by the respective routine methods of the Department of Medical Biochemistry at St. Olavs University Hospital.

3.3 Data analysis

Calculations were performed by Kinetica 5.0, Microsoft Excel 2007, SPSS 15, and the statistical software R. Where necessary, dose-corrected LTG and LTG-GLUC serum concentrations were calculated by dividing the serum concentration (mg/L) by the daily LTG dose (mg/day) in order to compensate for possible dose adjustments. In paper V, some pharmacokinetic calculations were performed after dose-normalisation.

For the pharmacokinetic calculations in paper V, a one-compartment model with first-order absorption and elimination was chosen [7, 14]. Five serum concentration/time points per participant were available for calculation of baseline and month 8 pharmacokinetic parameters, respectively. To enable comparison between dose-dependent pharmacokinetic parameters at baseline and at month 8 despite dose adjustments, dosages and the corresponding values were normalized to 400 mg/day. Renal clearance (CL_R) was calculated using the following formula:

$$CL_R = \frac{C_U * Q}{C_{ss,av}}$$

where C_U = concentration in collected urine (in mg/L); Q = total urine volume excreted during the 12-hour collection period (in L/h); $C_{ss,av}$ = average serum concentration at steady state during one dosing interval (in mg/L).

Because of different times of enrolment and possibility to participate, study subjects contributed in various degrees to paper V. Mean values were therefore calculated from pooled data of a varying number of contributing individuals (minimum n = 5, maximum n = 20).

Results in all papers are generally presented as mean ± standard deviation except where otherwise stated. According to the various study designs, different statistical tests were used for comparison between groups (t-test, z-test, Wilcoxon rank sum test). A p-value ≤0.05 was considered statistically significant in all studies.

3.4 Ethics

All studies were approved by the Regional Ethics Committee in Mid-Norway. With respect to the prospective studies (papers III, IV and V), all participants were informed both orally and in written form, and gave their written consent before enrolment.

4 Results

4.1 Overview

According to papers I and II, the median CDR is about 0.06, which means that, e.g. a daily dose of 200 mg LTG will give a serum concentration of 12 µmol/l. Male patients will have only slightly lower serum concentrations than women. Children and the elderly have higher serum concentrations per mg LTG given than adults aged 20 - 65.

The majority of today's LTG-users are women, and most prescriptions of LTG are for psychiatric disorders (papers I, II and VI).

With regard to drug interactions, the findings of previous studies could not only be confirmed, but also further quantified. Classical enzyme inducers such as carbamazepine, oxcarbazepine, or phenytoin reduce LTG serum concentrations, while co-administration of valproate leads to higher serum levels. EE-containing COCs reduce LTG serum concentrations, while progestin-only contraceptives do not (papers II and III). Moreover, evidence is provided that it is indeed the EE in COCs which reduces LTG levels, and not the progestin-component (paper III).

The fluctuations of estradiol and progesterone during a normal menstrual cycle do not seem to affect LTG kinetics to a clinically relevant degree (paper IV). By contrast, pregnancy may reduce LTG serum levels by over 50 % with a rapid decline in the first trimester. This is mainly due to increased renal blood flow, followed by estradiol-induced glucuronidation (paper V).

4.2 Summary of paper I

Trends and changes in the clinical use of lamotrigine.
Arne Reimers. Pharmacoepidemiology and Drug Safety 2009;18(2):132-9.

Purpose: To investigate long-term trends and changes in the pattern of use of LTG.

Methods: Retrospective survey of a routine therapeutic drug monitoring database.

Results: 12,107 samples analysed from October 1999 to May 2007 were surveyed retrospectively. During this period, the mean daily dose rose from 183 to 253 mg. The majority of samples are taken from female LTG users. The distribution of neurological

and psychiatric diagnoses differed between male and female patients. The mean patient age increased from 34 to 41 years. The proportion of samples from psychiatric patients became larger than that of neurological patients, and is still growing. A total of 131 different diagnoses were stated, most of them psychiatric. The mean serum concentration was 14.8 µmol/L, and remained quite stable during the whole observation period. Neurological patients had higher mean serum concentrations than psychiatric patients. Thirty percent of the neurological and 41 % of the psychiatric patients had serum concentrations below the reference range. 68 % of the patients used additional medication. Among the 10 most frequent co-medications were five psychotropic drugs, two anticonvulsants, and two sedatives.

Conclusions: Significant trends and changes in the pattern of use of LTG have taken place during the observation period. These findings may be useful for discussions where detailed pharmacoepidemiological information is needed.

4.3 Summary of paper II

Drug interactions between lamotrigine and psychoactive drugs: Evidence from a therapeutic drug monitoring service.

Arne Reimers, Eirik Skogvoll, Janne Kutschera Sund , Olav Spigset. Journal of Clinical Psychopharmacology 2005;25(4):342-348

Purpose: To present a systematic study on the interaction potential of LTG, with focus on psychoactive drugs.

Methods: A review of routine serum concentration measurements of LTG performed in our laboratory yielded a total of 1733 serum samples from 829 patients (530 women, 299 men) suitable for statistical analysis.

Results: Main results for the whole study population were (median; interquartile range in parentheses): dose, 200 (100–300) mg; serum concentration, 2.97 (1.82–4.74) mg/mL; LTG-CDR, 14.8 (9.9–24.6) (ng/mL)/(mg/d). A linear mixed model, allowing multiple observations from the same patient, was used to identify and quantitate the effect of factors influencing the LTG-CDR. In addition to age and gender, a total of 35 different co-medications (25 drugs used in psychiatry as well as 10 other drugs) were evaluated. With women younger than 70 years as the reference group, factors found to lower the LTG-CDR significantly were: male gender, and co-medication with carbamazepine, ethinyl estradiol, fluoxetine, lithium, phenytoin, phenobarbital, or topi-

ramate. Factors associated with a significantly higher LTG-CDR were: age $\geq$ 70 years, and co-treatment with valproate. No antidepressants other than fluoxetine and none of the antipsychotic drugs included were associated with an altered LTG-CDR.

Conclusions: Concerning pharmacokinetic drug interactions, we conclude that LTG can be safely combined with most psychotropic drugs.

4.4   Summary of paper III

Ethinyl estradiol, not progestogens, reduces lamotrigine serum concentrations.
Arne Reimers, Grethe Helde, Eylert Brodtkorb. Epilepsia 2005;46(9):1414-7

Purpose: To study the interaction between LTG and hormonal contraception.

Methods: LTG serum concentrations of female patients using either no hormonal contraception (n= 18), an EE-containing (n = 11) or a PG-only-containing compound (n = 16) were analysed. Patients were recruited prospectively, and blood samples were drawn drug-fasting and at steady-state conditions. Comedication with enzyme inducers, valproate, topiramate, or sertraline was not allowed.

Some patients changed group and thus served as their own controls. Samples were analysed by a GC/MS method. The Mann-Whitney U-test was used for statistical comparison of the groups.

Results: The LTG-CDR, expressed as (mg/L)/(mg/d) was significantly lower in women using EE than in the control group (mean ± SD, 0.010 ± 0.004 vs. 0.017 ± 0.006 , p = 0.003). The CDR in women using PG was 0.02 ± 0.007 which was not statistically different from controls. Also, there was no difference in CDR between women using either oral, topical or parenteral PG. Five women switched from the control to the EE group and experienced a considerable reduction in CDR. A rise of the CDR towards control level was seen in the two women who changed from EE to PG.

Conclusions: It is the EE component of oral contraceptives which interacts with LTG. The PG-only compounds did not alter LTG serum concentrations in this study. These findings should be considered when treating and counselling women with epilepsy in childbearing age.

4.5   Summary of paper IV

Lamotrigine serum concentrations throughout the menstrual cycle – a study of two subjects.

Arne Reimers, Eylert Brodtkorb, Grethe Helde, Olav Spigset. Journal of Clinical Neuropharmacology 2006; 29(3):160-2

Purpose: To measure the serum concentrations of LTG throughout a complete menstrual cycle.

Methods: Serum concentrations of LTG, estradiol, and progesterone throughout a menstrual cycle were measured in two young women not using hormonal contraception.

Results and Conclusions: The physiological hormonal fluctuations during an ovulatory cycle were not associated with clinically relevant changes in LTG serum concentrations.

4.6   Summary of paper V

Lamotrigine and its N2-glucuronide during pregnancy: the significance of renal clearance and estradiol.
Arne Reimers, Grethe Helde, Geir Bråthen, Eylert Brodtkorb. Epilepsy Research. Submitted November 01, 2010.

Purpose: To investigate the physiological mechanisms behind the pronounced decline of LTG serum concentrations during pregnancy.

Methods: Serum and urine concentrations of LTG and its main metabolite, LTG-GLUC, were measured monthly in 21 pregnancies of 19 women using LTG. Simultaneously, a panel of biochemical variables was monitored to evaluate liver and kidney function and possible hemodilution effects. Pharmacokinetic parameters were calculated once at baseline and once in gestational month 8.

Results: Initially, LTG and LTG-GLUC serum concentrations fell simultaneously by 27 % and 38 %, respectively (gestational month 2). Subsequently, the ratio of the LTG-GLUC/LTG serum concentrations increased gradually, correlating strongly with rising serum estradiol concentrations. In gestational month 8, the ratio was 164 % higher than at baseline. At that time, LTG total clearance had increased by 118 %, and the

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amount of unchanged LTG in urine had dropped by 40 % while the amount of LTG-GLUC had increased by a corresponding 37 %.

Conclusions: The simultaneous decline of LTG and LTG-GLUC serum concentrations in early pregnancy suggests that in this phase, increased renal blood flow is the major cause. After gestational month 2, estradiol-induced glucuronidation of LTG becomes more important, leading to a further fall of LTG serum concentrations and a gradual rise of the LTG-GLUC/LTG-ratio through the remaining pregnancy. An expanded volume of distribution may also contribute to reduced LTG serum concentrations in pregnancy.

4.7   Summary of paper VI

Lamotrigine in children and adolescents: The impact of age on its serum concentrations and on the extent of drug interactions.

Arne Reimers, Eirik Skogvoll, Janne Kutschera Sund, Olav Spigset. European Journal of Clinical Pharmacology 2007;63(7):627-629

Purpose: To investigate the impact of age and co-treatment with other drugs on the serum concentrations of LTG in children and adolescents.

Methods: A review of routine serum concentration measurements of LTG performed in our laboratory yielded a total of 744 serum samples from 296 subjects (110 males, 186 females, age 2 to 19 years) suitable for statistical analysis. The primary outcome variable was the dose-corrected lamotrigine serum concentration, expressed as the CDR. A linear mixed model, allowing multiple observations from the same patient, was used to identify and quantify the effect of factors influencing the LTG-CDR.

Results: According to the model, the LTG-CDR decreases by 6 % per year of age. Valproate and levetiracetam were found to raise the LTG-CDR, whereas the following co-medications reduced it: carbamazepine, clobazam, fluoxetine, clonazepam, and ethinyl estradiol. The effect of carbamazepine decreased with increasing age. No gender difference was detected.

Conclusions: Age is an important factor with respect to the pharmacokinetics and the extent of drug interactions of LTG in children and adolescents. In this population, older individuals will need higher doses than younger ones in order to achieve the same serum concentrations.

5 Discussion

5.1 Pharmacoepidemiological aspects

Previous pharmacoepidemiological studies of LTG investigated mainly its clinical effectiveness and safety issues [39-44]. The study presented in paper I adds more specific, diagnosis-related information on the clinical use of LTG.

Since its introduction, LTG has undergone an interesting development. In the beginning an add-on drug for localisation-related epilepsy, it is now the most used AED in Norway [5]. It has not only become a first-line monotherapy treatment for both localisation-related and generalised epilepsies, it has also become a widely used drug in psychiatry, being prescribed for a plethora of diagnoses, most of them being off-label. It also appears that LTG is emerging as a first-line treatment for women during their reproductive years [66] and during pregnancy [67, 68]. Paper I suggests that prescriptions for psychiatric conditions exceed the number of prescriptions for neurological disorders in Norway, and that females appear to represent the majority of LTG users. In fact, in 2009, 13208 individual LTG users were females vs. 9140 males, according to the Norwegian prescription registry [5]. However, this may not necessarily be the case in other countries, where treatment guidelines, health care regulations and medical traditions may lead to different patterns of use of LTG [69].

Comparisons of pharmacoepidemiological studies from different countries should be done with caution if such studies are based on data from prescription databases where the mere number of prescriptions is counted, either as original prescriptions or as Defined Daily Doses (DDDs). AEDs are not only used for treating epilepsy. In Norway, LTG is now predominantly used for psychiatric disorders, as suggested by paper I. Other AEDs as well are widely used for non-epilepsy conditions like mood disorders, migraine or pain, where different daily doses may be used [36, 37, 70-72]. Thus, the number of prescribed DDDs alone does not indicate the disorder that the drug was prescribed for and gives weak, if not false, results when used for calculation of, e.g. disease prevalences [73]. In addition, the prevalence of psychiatric comorbidity in epileptic patients may be as high as 32 % [74]

Moreover, the DDD of a certain drug as defined by the WHO is "*the assumed average maintenance dose per day for a drug used for its main indication in adults*" [75]. From this definition, it becomes clear that the DDD does not necessarily reflect the real average dose used in everyday clinical practice, especially when a drug classified as an AED in reality is mainly used against non-epilepsy conditions, where other mean daily dosages may be common [72]. Indeed, both the mean and the median doses found in

paper I were considerably lower than the DDD of 300 mg as suggested by the WHO [76]. This finding confirms the results of a previous study suggesting that, compared to clinical practice, the DDD appears to be too high and should be reconsidered [77]. In addition, commonly used doses and, thus, serum concentrations, of LTG may differ considerably between countries, as discussed in paper I.

Papers I and II show that the gender difference was small during the first years after LTG was launched, but has increased ever since (figure 5).
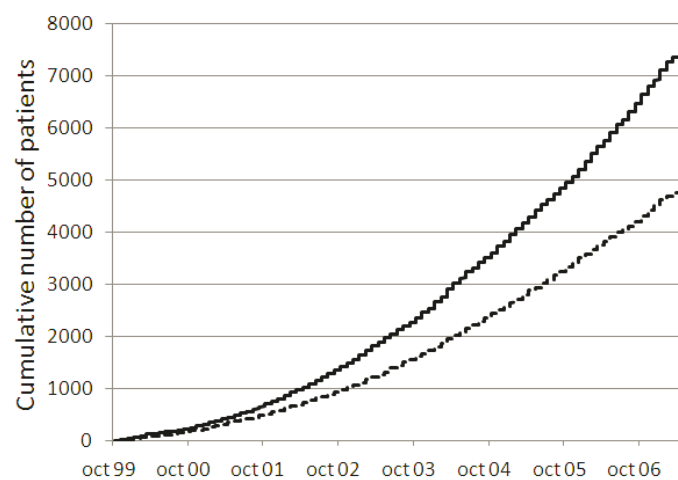
Figure 5: Cumulative number of female (solid line) and male (dotted line) patients during the observation period (October 1999 – May 2007).

Data from the Norwegian prescription database confirm this [5]. This development may be due to changing use of LTG which initially was restricted to epilepsy where the gender difference is indeed small, whereas affective disorders are more frequent in females. Additionally, it may also reflect that LTG is particularly recommended for fertile women, mainly because it does not impair hormonal contraception, its lack of endocrine side effects, and the notion that it is less teratogenic than other drugs, particularly, valproate [78-82]. On this background, one would expect a lower mean age of females compared to male LTG users. However, paper I did not confirm such a difference, and it can only be speculated on the reasons.

On average, females have seven percent higher LTG serum concentrations per mg given than males, but this difference is hardly of clinical relevance. Also, the mean and median daily doses of males and females are practically identical. Consequently, the CDR also is essentially similar. However, this applies to the entire study population. The large standard deviations indicate that there may be considerable differences between individuals.

As shown by paper I, the majority of LTG prescriptions is at present given for psychiatric conditions (figure 6).
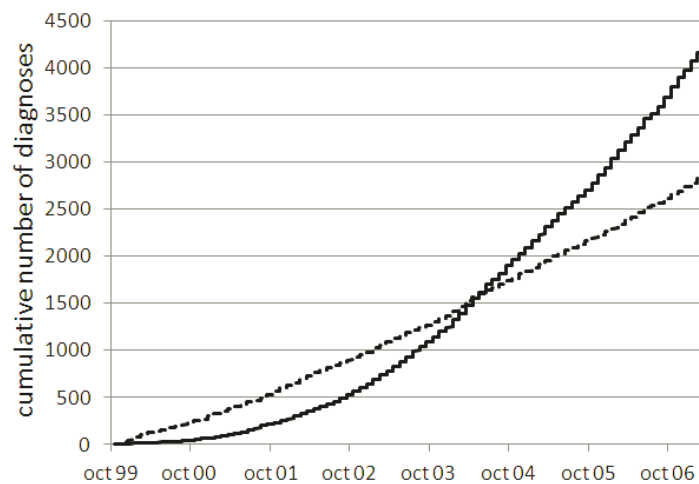
Figure 6: Cumulative number of psychiatric (solid line) and neurologic (dotted line) diagnoses during the observation period (October 1999 – May 2007).

LTG is licensed in many countries, including Norway, for the prevention of depressive episodes in patients with bipolar disorder [83]. However, about 45 different psychiatric diagnoses (ICD-10 first level codes) were stated on the laboratory request forms, which implies a considerable degree of off-label use of LTG (table 3).

Table 3: Diagnoses as provided on the laboratory request forms. Number of samples (n) in brackets. Second level codes/subdiagnoses (e.g., G40.1) not shown.

	Major (first level) ICD-10 code
Neurologic (2,877)	
epilepsy (2,843)	G40; G41
other neurologic (34)	G10; G25; G32; G35; G43; G50; G61
Psychiatric (4,289)	
bipolar (1,750)	F31
manic (14)	F30
other affective (935)	F32; F33; F34; F38; F39
schizoaffective (242)	F25
schizophrenic and delusional (186)	F20; F21; F22; F29
neurotic, stress-related and somatoform (169)	F40; F41; F42; F43; F44; F45; F48
behavioural syndromes (15)	F50; F51
personality disorders (140)	F60; F62; F63; F69
mentally retarded (4)	F70; F71; F79
impaired psychological development (37)	F84; F89;
dementia (9)	F00; F01; F02; F03
other psychiatric (86)	F06; F10; F12; F13; F15; F19; F90; F91; F94; F95
unspecified psychiatric (702)	F99
Other (43)	Q90; nursed infant of mother using LTG

In other words, LTG is widely being used for poorly documented or even undocumented conditions. The reasons remain to be elucidated, but this finding recalls the gabapentin story which received much attention some years ago. In brief, the AED Neurontin® (gabapentin) had been massively marketed for off-label indications, mainly bipolar disorder, despite two double-blind, randomised controlled trials demonstrating its lack of efficacy in this indication. At one point, over 78 % of all Neurontin® prescrip-

tions were for off-label uses [84]. The story ended in 2004 in a lawsuit where the manufacturer pleaded guilty and was fined 430 million dollars for illegal marketing practices [84-87].

Papers I and II demonstrate that roughly 70 % of LTG users take at least one drug in addition to LTG, although this figure does not distinguish between neurological and psychiatric patients. However, polypharmacy is very common in psychiatry. The number of additional drugs in these two studies ranged from 1-19. Interestingly, female patients used significantly more additional drugs than men, on average three (vs. two). Polypharmacy implies a risk for drug-drug interactions, and of course, the risk grows with increasing number of drugs prescribed to a single person. LTG is subject to various drug interactions because of its extensive metabolism [88].

5.2   Concentration-dose relationship

The serum concentration of LTG is directly proportional to its dose (linear, first-order kinetics) [7, 8, 16, 17]. In the individual patient, the new serum concentration after a dose change may thus be predicted on the basis of any serum concentration measured before the dose change. A practical tool for calculation is the CDR, which is derived by simply dividing the measured trough serum concentration by the actual daily dose. The CDR may also be used reversely, i.e., to determine the dose needed to achieve a desired serum concentration.

We found that the mean LTG-CDR of the whole study population was 0.06, which means that, on average, 1 mg LTG will give a serum concentration of 0.06 µmol/l. Accordingly, the median daily LTG-dose of about 230 mg (papers I and II) would give 13.8 µmol/l, which is near the lower bound of the generally accepted reference range of 10-50 µmol/l. However, these are average values for a whole population. Age, sex, co-medication and, presumably, pharmacogenetic polymorphisms all are factors which may affect the LTG-CDR significantly in the individual patient, as described in detail in papers I, II, III and VI.
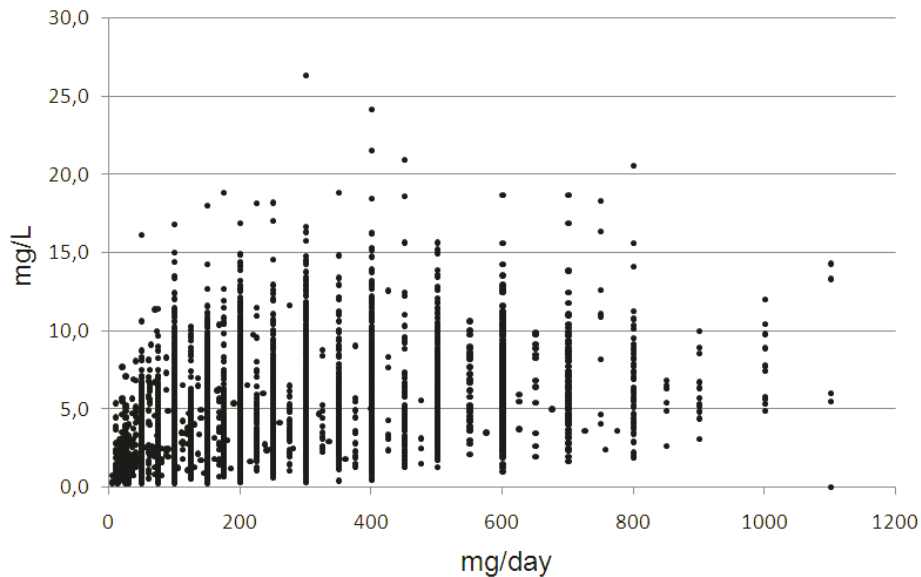
Figure 7: Scatterplot of serum concentration vs. daily dose of LTG (n = 11 855). Each dot represents one sample.

In fact, the 10- and 90-percentiles of the LTG-CDR were 0.03 and 0.16, respectively, indicating large interindividual variation, and underlining the usefulness of therapeutic drug monitoring (figure 7).

On the other hand, the intraindividual variation of the LTG-CDR is low (paper II). Thus, the CDR of the individual patient may be a simple and useful tool in the management of patients treated with LTG.

5.3 Drug interactions

Drug-drug interactions are a major problem in both primary care and hospital medicine [89-92]. They may lead to adverse drug reactions, cause or prolong hospital admissions, and they may even be fatal [93-96]. Since LTG is often prescribed together with other drugs (papers I, II and VI), physicians should be aware of its interaction potential.

LTG is a weak inducer of its own metabolism [15], but apart from single reports, there is no evidence that it affects the pharmacokinetics of other drugs. It has been observed, though, that the combination with carbamazepine may put patients at a higher risk for neurotoxic side effects, but this appears to be due to a pharmacodynamic interaction rather than a pharmacokinetic one [97-100].

However, the metabolism of LTG itself may be considerably affected by other drugs. Classical enzyme inducers like carbamazepine, phenytoin and phenobarbital decrease its serum concentrations by about 50 %, whereas valproate, a classical enzyme inhibitor, roughly doubles them. Treatment with LTG in patients using valproate requires particular attention to avoid potentially lethal hypersensitivity reactions like Stevens-Johnson syndrome or toxic epidermal necrolysis, as high serum concentrations of LTG may facilitate the formation of toxic or reactive metabolites [101-107]. These important interactions were discovered soon after the introduction of LTG [19] and have since been confirmed by multiple studies, including papers I and II. Interactions with enzyme inducers and -inhibitors can, theoretically, completely be compensated for by simply adjusting the LTG dose. In addition, serum level monitoring of LTG is easily accessible in Norway, and represents a practical tool to find the right dose. Despite of that, the mean LTG serum concentration in users with concomitant valproate is considerably higher than the population average, while it is considerably lower in patients using enzyme inducers (paper I). Several clinical studies have postulated a therapeutic synergism between LTG and valproate which is independent of pharmacokinetic interactions [107, 108]. However, due to methodological weaknesses in these studies, it cannot be ruled out that this putative synergism is in fact caused by increased LTG serum levels.

Less well established than the above discussed interactions is the interaction with topiramate, described in paper II, which may lead to decreased serum levels of LTG. While one previous study found similar results [109], two others did not [110, 111]. However, given in doses >200 mg/day, topiramate induces the metabolism of EE [112], and EE, like LTG, is a UGT-substrate [113]. Thus, clinicians should be aware of this possible drug interaction.

While these interactions were not really surprising, the interaction of LTG with COCs was unexpected. Epileptologists had for decades been aware of the fact that enzyme inducing AEDs weaken the effect of hormonal contraception [114-116]. By contrast, a drug interaction in the form of an impaired effect of the AED while the effect of the COC remains unaltered was new and surprising at the time of its discovery. This may explain why the LTG-COC interaction was not reported until 2001 [23], more than 10 years after the first launch of LTG in the UK [117]. On the other hand, EE, which

represents the estrogen component in most COCs, has long been known for its potential to affect other drugs through induction of UGT [118-120]. Today, it is well established that COCs may reduce LTG serum concentrations by >50 % [23, 47, 121-124]. The growing interest in the effect of COCs on the metabolism of LTG raised the question whether COCS also affect the serum concentrations of valproate, an AED which also is mainly metabolised by glucuronidation [125, 126]. Indeed, COCs have been found to increase the clearance of valproate, although to a much lesser, and presumably clinically irrelevant, degree than LTG [24, 25].

COCs consist of a synthetic estrogen (usually EE) and a progestin component. Thus, the decrease of LTG serum concentrations might theoretically be caused by a) EE alone, b) the progestin alone, or c) both of them. Paper III strongly suggests that it is indeed EE alone which decreases LTG serum concentration, and that progestins do not affect LTG kinetics, regardless of their mode of administration (figure 8). On the other hand, LTG may reduce the serum levels of levonorgestrel (a progestin commonly used in COCs), but only to a minor, clinically insignificant degree [122].
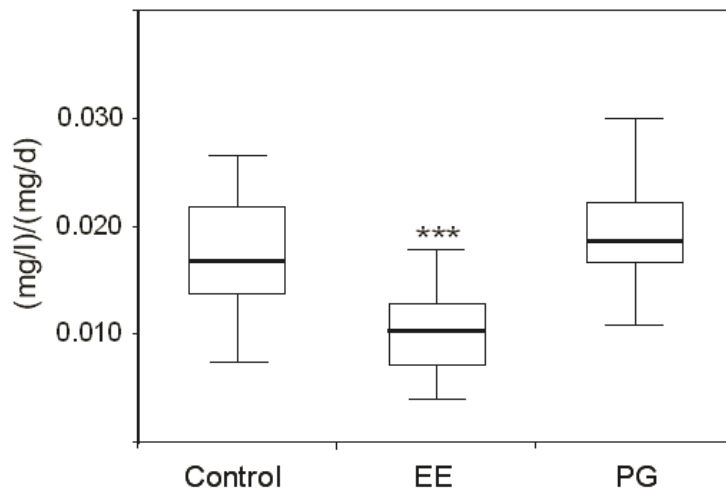
Figure 8: Boxplot showing the median (horizontal line), interquartile range (lower and upper edge of the box) and total range of the LTG-CDR of the 3 study groups. EE: ethinyl estradiol; PG: progestin. ***: p = 0.003 vs. control group.

Interestingly, it has also been found that in COC users, LTG serum levels increase by up to 116 % within the pill-free week [48, 121], which practically represents a complete

reversal of the enzyme-inducing effect of the COC. In fact, LTG levels increased by 27, 63 and 116 % on days 3, 5 and 7 days after cessation of the COC, respectively [48]. Likewise, the inducing effect of EE-containing COCs develops within one week [121]. Thus, the LTG-EE interaction is characterised by an unusually rapid induction and dein-duction of UGT1A4.

5.4 Hormones and the menstrual cycle

The discovery of the LTG-EE interaction raised the question if, and to what extent, en-dogenous female sex hormones, particularly estradiol, do interact with LTG. Endoge-nous estradiol is known as both a substrate and an inducer of UGT [31, 127]. Because of the unusually rapid induction and de-induction of LTG metabolism, it was of special interest to find out whether the physiologic fluctuations of estradiol during a normal menstrual cycle may have an impact on LTG pharmacokinetics. Paper IV showed that LTG serum concentrations are quite stable throughout the whole cycle, although one of the subjects in this paper showed a slight, clinically insignificant decrease in the lu-teal phase when estradiol and progesterone serum concentrations are high. A similar decrease has been described in a later study [24], but also here the change was consi-dered clinically insignificant. Moreover, yet another study did not find significant fluc-tuations of the LTG clearance during the menstrual cycle [128].

Different types of estrogen vary in the strength of their pharmacologic effects, and several forms of synthetic estrogens that are used in COCs may be more enzyme induc-ing than estradiol [129, 130]. This might possibly account for the apparently greater effect of COCs than the cyclic variation of estradiol during the natural menstrual cycle. If this hypothesis is correct, it implies that larger variations of estradiol serum levels than those present during a menstrual cycle may affect LTG serum concentrations. During pregnancy, estradiol levels rise several hundred times and, indeed, LTG levels may decrease by more than 60 %.

5.5 Pregnancy

The decrease of LTG serum concentrations during pregnancy was first described by a case report in 1997 [53] and later confirmed by several small studies [33, 54-59]. These studies showed that LTG clearance increases by 65 - 230 %, while its serum concentra-

tions may decrease by more than 60% in pregnant women. The results presented in paper V confirmed these previous findings, and provided additional evidence for increased glucuronidation of LTG during pregnancy, as we could demonstrate that the ratio of the mean LTG-GLUC/LTG serum concentrations increases throughout gestation (figure 9).
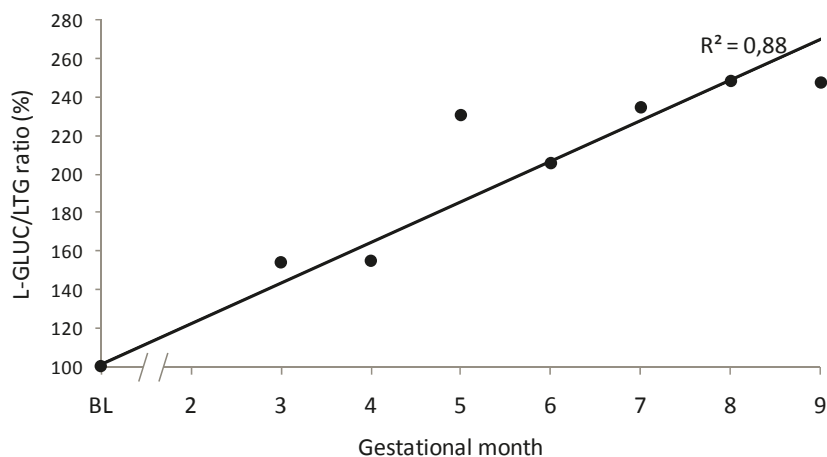
Figure 9: Relative change of the mean LTG-GLUC/LTG serum concentration ratio during pregnancy (baseline = 100%, n = 5-20). BL: baseline

The maximum increase, observed in gestational month 8, was is in good agreement with the results of a previous study [33]. Moreover, while the proportion of LTG excreted in urine as the unchanged parent drug decreased by 40 %, the amount found as the LTG-GLUC metabolite increased by almost the same amount (37 %; table 4 in paper V). These findings strongly suggest increased glucuronidation of LTG during pregnancy.

In addition, paper V also shows a strong correlation between rising estradiol serum concentrations and the increasing LTG-GLUC/LTG ratio (figure 10).
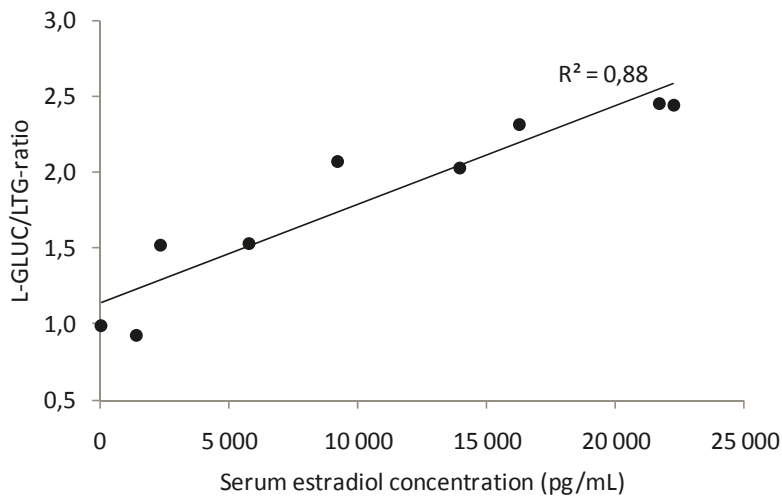
Figure 10: LTG-GLUC/LTG ratio at each gestational month as a function of estradiol serum levels (n = 7-13)

Of course, such a correlation may be due to coincidence. However, there are several findings which strongly suggest a causal relationship:

1. Ethinyl estradiol, a synthetic estrogen, induces UGT enzymes and may reduce LTG serum levels by over 50 % [34, 47, 119, 120].

2. Animal data show that UGT1A1 and UGT1A4 are in fact induced during pregnancy [131].

3. In-vitro experiments with human liver- and breast-cancer cells show that endogenous estradiol induces the up-regulation of UGT1A4 [127] and UGT2B15 [132].

4. Hormone replacement therapy with natural equine estrogens may reduce LTG serum levels by 25-30 % [133].

Thus, there appears to be sufficient reason to assume that the strong correlation between elevated estradiol levels during pregnancy and increased glucuronidation of LTG is due to causality rather than coincidence.

An important finding in paper V, which previously was unrecognised, is that LTG serum concentrations decrease markedly already in gestational month 2 and by 50% in month 3, and that this early decrease is mostly due to increased renal blood flow. It appears that induced glucuronidation becomes predominant first later in pregnancy,

as can be seen from the course of the LTG-GLUC serum concentrations. It is important that clinicians are aware of this early, first-trimester fall of LTG serum levels, as decreased serum concentrations of LTG have previously been found to represent a significant risk for seizure deterioration in pregnant patients [57, 60, 65].

5.6   UDP-glucuronosyltransferase (UGT)

Glucuronidation is the conjugation of glucuronic acid to an exogenous or endogenous substrate with a functional group, e.g. an amine, carboxylic acid, thiol, or hydroxyl group. This conjugative reaction is catalysed by UGTs. Although predominantly expressed in the liver, UGTs have been found in a large variety of tissues and organs [26-30]. Glucuronidation is an important metabolic pathway for many drugs, including AEDs like LTG and valproate which are mainly excreted as their glucuronides [125, 126]

Generally, UGTs are less well studied than the cytochrome P450 (CYP) system, as clearly illustrated by the number of publications. A PubMed search for "UGT" on November 25, 2010 gave 1304 hits, while "CYP" yielded 7762 hits [134]. However, a large number of medicinal drugs are primarily metabolised by UGTs, and there is growing interest and increasing knowledge concerning UGTs.

There is considerable interindividual variation in UGT activity. Age, gender, co-medication with enzyme inducers or -inhibitors, and genetic polymorphisms are factors that have been implicated as sources of this variability. However, the significance of these factors may vary for the individual UGT isozyme [135, 136]. A four to five-fold interindividual variability in the activity of UGT1A4 has been demonstrated in experiments with human liver cells [137]. Papers II and VI also revealed considerable interindividual variability in the kinetics of LTG.

LTG is mainly metabolised by UGT1A4, although a minor role of UGT2B7 is currently being disputed [137-139].

Two genetic polymorphisms associated with reduced UGT1A4 activity, with a prevalence of eight and nine percent, respectively, have so far been described [140]. The degree at which UGT1A4 activity is reduced differs between the two mutations and may range from about 30 % to 100 % (i.e., a complete loss of catalytic activity), depending on the substrate studied. However, the effect of these polymorphisms on LTG biotransformation has not been examined and, thus, their clinical relevance remains unclear.

Sex differences in UGT activity are comparatively small, if we put pregnancy aside [135]. Indeed, paper II found only minimal and clinically insignificant differences between male and female users of LTG. Other (albeit smaller) studies did not find any gender difference at all [13-15].

As shown by papers II, V and VI, the most important and clinically relevant factors affecting LTG kinetics are co-medication, pregnancy and age.

5.7   Age

The expression of UGTs prior to and immediately following birth is quite limited, which might explain the susceptibility of neonates and nursing infants to certain drug toxicities [141, 142]. In the case of LTG, serum concentrations in nursing infants have been reported to reach 9.2 to 22.7% of the mother's serum concentration [143]. Although LTG is generally regarded as safe with breastfeeding, severe apnoea in a fully breast-fed infant has recently been reported [144].

Previously, it has been proposed by population pharmacokinetic modeling that LTG pharmacokinetics in children may not be related to age but to body weight [145]. Unfortunately, information on body weight was generally not available in our material. However, in children, body weight is not an independent variable since it is strongly determined by age [146]. Moreover, age does not only determine whole body weight, but organ weight, enzymatic function and regional blood flows as well [142, 147-151]. Thus, it appears logical to focus on age as a determinant of drug disposition in children, and we found indeed that age has a highly significant effect on LTG kinetics in children.

Paper VI shows clearly that the great interindividual variation in LTG CDR in 2-year old children becomes less with increasing age (figure 11).
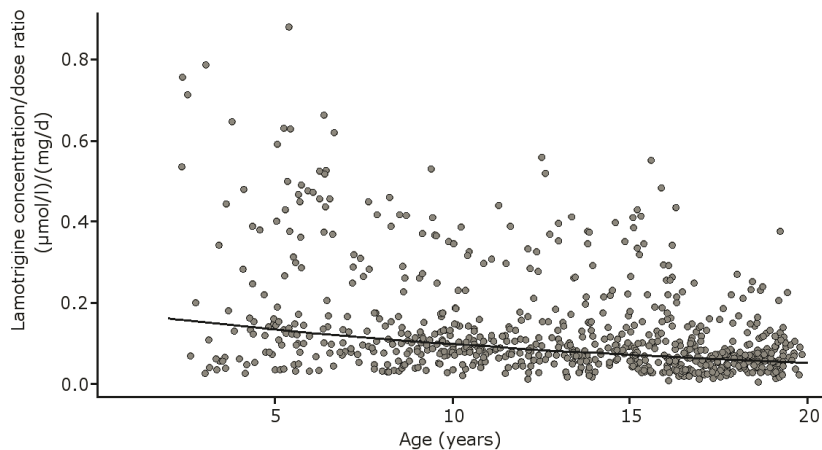
Figure 11: LTG-CDR, expressed as (µmol/L)/(mg/day), versus age (in years). Each dot represents one sample. The line shows the expected effect of age on the LTG-CDR.

It is most likely that this phenomenon is due to maturating enzyme activity since it has been suggested that maximum UGT1A4 activity is not reached before 19 years of age [152]. The interaction of age with the effects of carbamazepine on LTG CDR fits very well into this concept. We found that the impact of carbamazepine was most pronounced in younger children and decreased with increasing age. Carbamazepine is a classical enzyme inducer and enhances the glucuronidation of LTG [19, 21, 22, 153]. Thus, it appears logical that the enzyme-inducing effect of carbamazepine is greater at a younger age, when the baseline glucuronidation capacity is low. The great interindividual variability of LTG kinetics in young children clearly requires close clinical follow-up of these patients, and serum level measurements of LTG should be used as an aid in finding the right dose.

Interestingly, it has recently been shown that human liver microsomes from elderly subjects (>65 years) metabolise LTG as fast as microsomes from younger subjects [137]. This is apparently in contrast to the finding of paper II, that elderly patients have a higher LTG CDR than younger patients. However, serum concentrations of LTG do not depend on glucuronidation capacity alone. As indicated by paper V, renal blood flow is a very important factor, and renal blood flow is usually decreased in the elderly. Moreover, the elimination half-life of lamotrigine has been shown to be approximately doubled in uraemic patients, compared to healthy volunteers [154].

6 Future perspectives

The papers presented in this thesis confirm and extend relevant knowledge on the clinical pharmacokinetics of LTG. The methods used have their strengths, but also some limitations, as discussed in detail in the respective papers. Thus, particularly the new findings should be reproduced to confirm their validity.

Our results also raise new questions. For example, if part of the increased clearance of LTG during pregnancy is due to estradiol-induced glucuronidation, does this glucuronidation take place only in the liver? Since UGT is expressed in many extrahepatic tissues including the placenta, does placental UGT contribute? If yes, to what extent? It has been shown that LTG crosses the human placenta and is found in the newborn and umbilical cord blood in considerable amounts [53, 58, 59, 155]. *In vitro* and *ex vivo* studies suggest that human placenta at term does not metabolise LTG to a significant degree [155-157]. On the other hand, UGT enzymes of the 1A family have been found in human placenta [158]. Unfortunately, more specific data on the expression of the various UGT1A isozymes in placenta is not available at present. There is some evidence that not only UGT1A4, but also UGT2B7 may glucuronidate LTG to some (low) degree [138, 139]. UGT2B7 has indeed been found in human placenta [159], but studies on the significance of UGT2B7 for the metabolism of LTG have been contradictive [137]. More research is needed to clarify this issue.

Another question relates to the significance of pharmacogenetic polymorphisms of UGT1A4. There is great interindividual variation in the pharmacokinetics and metabolism of LTG and the impact of drug interactions or pregnancy varies considerably among individuals. As mentioned in the Discussion section, two UGT1A4 polymorphisms have been discovered so far, but their impact on LTG metabolism has not been examined. Moreover, further, yet undiscovered polymorphisms may exist. Since UGT1A4 does not only metabolise LTG but other, frequently used drugs as well, pharmacogenetic polymorphisms of UGT1A4 definitely deserve further study. A great number of genetic polymorphisms have also been discovered for other UGT isozymes. Their clinical significance is largely unknown and should be explored.

It was the emerging knowledge on the LTG-COC interaction and decreased LTG serum concentrations during pregnancy that opened a new perspective also with respect to other drugs that are primarily metabolised by UGT. In recent years, the possible interaction of the AED valproate with COCs has received some attention. Other drugs which are primary UGT substrates include the new AED retigabine and more established drugs like, e.g., morphine, acetaminophen, benzodiazepines like oxazepam, irinotecan,

tolbutamide or olanzapine, one of the most frequently used antipsychotics. The possible interactions of these and other UGT-metabolised drugs with COCs, and the possible impact of pregnancy on their pharmacokinetics represent important areas for future research.

7 Conclusions

The analysis of extensive material from a routine drug monitoring database and three prospective clinical studies yielded interesting and clinically relevant findings concerning the clinical pharmacokinetics of LTG.

Paper I gave insight into the historical development and the current pattern of use of LTG in Norway. From an epidemiological and from a public health viewpoint, it is important information that the majority of LTG users are females, and that most prescriptions are for a large variety of psychiatric conditions, many of them off-label. Moreover, these trends appear to increase further and will, thus, become even more important in the future.

Drug interactions with LTG are well-studied, and have been confirmed by papers II, III and VI. Moreover, paper III provided first-time evidence that it is the estrogen component in COCs (i.e., EE) which causes low LTG serum concentrations. By contrast, progestin-only contraceptives may safely be combined with LTG treatment.

Since up to 90 % of an LTG dose is metabolised by UGT1A4, this enzyme plays a major role concerning drug interactions with LTG. Apart from classical enzyme inducers like carbamazepine or phenytoin, or the classical enzyme inhibitor valproate, estrogens have turned out to affect LTG kinetics considerably. The synthetic estrogen derivative EE as well as endogenous estradiol obviously increase UGT1A4 activity. Paper IV demonstrates that the comparatively small physiological fluctuations of estradiol during a normal menstrual cycle do not affect LTG kinetics to a clinically relevant degree. By contrast, paper V shows a strong correlation between the several hundred-fold increase of estradiol levels during pregnancy and the increasing rate of glucuronidation of LTG.

On a general basis, UGT activity is relatively poor in young children, but increases with age. This phenomenon is subject to large interindividual variability, as shown by paper VI. Thus, the treatment of children with LTG or other drugs which are UGT substrates demands close monitoring of these patients. Although the interindividual variability of LTG kinetics decreases with age, it is still of considerable magnitude even in adults. Drug interactions, pregnancy and, possibly, genetic polymorphisms may explain most of these variations in this age group. Moreover, paper V demonstrates that renal blood flow also may become a significant factor. This is of importance not only during pregnancy, when increased renal blood flow may contribute to low LTG serum concentra-

tions. It may also become significant in individuals with reduced renal blood flow, e.g. in renal disease, or in elderly patients treated with LTG.

Increased renal blood flow is assumed to be the major cause of the rapid fall of LTG serum concentrations in early pregnancy (paper V). This is a previously unrecognised finding and should be carried in mind when treating patients on LTG who want to become pregnant.

Continued research is needed to further elucidate these issues.

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Appendix: Papers I - VI

Paper I

ORIGINAL REPORT

Trends and changes in the clinical use of lamotrigine[a]

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SUMMARY

Purpose   To investigate long-term trends and changes in the pattern of use of lamotrigine (LTG).
Methods   Retrospective survey of a large, routine therapeutic drug monitoring database.
Results   Twelve thousand one hundred and seven samples from 4123 subjects were analysed from October 1999 to May 2007. Within this period, the mean daily dose rose from 183 to 253 mg, whereas the median dose remained unchanged at 200 mg. Females became the majority of LTG users, and they had a higher proportion of psychiatric diagnoses than male patients. The mean patient age increased from 34 to 41 years. The proportion of samples from psychiatric patients became larger than that of neurologic patients. A total of 130 different diagnoses were stated, most of them psychiatric off-label. The mean serum concentration was 3.8 mg/L and it remained quite stable during the whole observation period. Neurologic patients had a higher mean serum concentration than psychiatric patients. 30% of the neurologic and 41% of the psychiatric patients had serum concentrations below the reference range. Sixty-eight per cent of the patients used additional drugs. Females used a higher number of additional medications than males. The 10 most frequent co-medications consisted of seven psychotropic drugs, two anticonvulsants, and thyroxine.
Conclusions   Significant changes in the pattern of use of LTG have taken place during the observation period and some significant trends could be identified. Copyright © 2008 John Wiley & Sons, Ltd.

KEY WORDS — lamotrigine; pharmaco-epidemiology; pattern of use; trends

INTRODUCTION

Lamotrigine (LTG) was introduced in the early 1990s and is today widely prescribed as an anticonvulsant to adults and children. In recent years, it has also been licensed for the treatment of bipolar disorder in many countries. Serum concentration measurement of LTG was established soon after its introduction.[1] Collected systematically over long time, data from such measurements may represent a platform for pharmaco-epidemiological research and may reveal trends and changes in the pattern of clinical drug use. Apart from giving information about dosages, indications and co-medications, such research may also address the clinical relevance of recommended reference ranges,

and to what degree treatment guidelines reflect clinical reality (or *vice versa*).

Previous naturalistic studies on pharmaco-epidemiological aspects of LTG-treatment investigated mainly its clinical effectiveness and safety issues.[2–6] Little is known about changes in its pattern of use over time.[7]

Our laboratory receives serum samples from many parts of Norway and has built up a database containing data from several thousand serum samples analysed for LTG over many years. In this article, we present a survey of this database. The main aim was to investigate trends or changes in the clinical use of LTG, and to identify differences between patient groups.

METHODS

All serum samples sent to our laboratory and analysed for LTG in the period from October 1999 to May 2007

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were reviewed. Data from samples analysed before October 1999 were not accessible due to technical reasons.

All raw information linked to the samples was transferred from the routine laboratory data system (NonStopLab ver. 5.5.3, Tietoenator, Oslo, Norway) to a spreadsheet program (Microsoft® Excel 2007, Microsoft Corp., Redmond, WA, USA). Patient names were replaced by an electronically generated ID number to secure privacy. Information on gender was derived from each patient's population registry number. After calculation of the patient's age at the time of sampling, the population registry number (which contains the date of birth) was also deleted. Serum concentrations, originally recorded in molar units, were converted to mass units using a conversion factor of 3.9. The LTG serum concentration-to-dose ratio (CDR) was calculated by dividing the serum concentration by the total daily dose, i.e. CDR = (mg/L)/(mg/day). Thus, the CDR represents the serum concentration per each mg LTG given. (Note that by transformation, this formula becomes $CDR = 1/L \times$ day, which is equivalent to 1/oral clearance.)

In the final database, each sample had its own dataset, consisting of the following variables: ID number, sampling date, age at time of sampling, gender, time between last drug intake and blood sampling, total daily dose, CDR, diagnosis (ICD-10 coded), all co-medication (generic names), and eventual other non-numeric information given on the request form in free text.

Data are presented as mean ± standard deviation (SD) or as median and range, as appropriate. The *t*-test or, where appropriate, the *z*-test, were used to calculate *p*. Given the large sample sizes, the level for statistical significance was set to $p \leq 0.01$. As a rule, testing for statistical significance was performed only in cases where numerical differences were considered clinically relevant.

Ethics

This survey has been performed in accordance with the Norwegian rules and laws concerning privacy protection.

RESULTS

The final database consisted of datasets of 12 107 samples analysed from October 1999 to May 2007. In many cases, the request forms had been filled in only partially, yielding many incomplete datasets. Accordingly, the number of samples used for each statistical analysis varied.
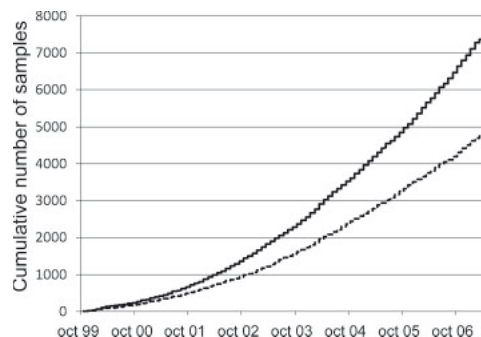
Figure 1. Cumulative number of samples from female (solid line) and male (dotted line) patients during the observation period (October 1999–May 2007)

The mean time between last drug intake and blood sampling was 13.9 ± 5.2 h, respectively (range: 1–38 h; $n = 10\,515$). Most (80%) of the samples had been taken within 10.0–24.0 h.

The mean number of samples analysed per month was 136 ± 70. However, the mean number of samples analysed per month in the first complete 6 months was 39 ± 9 (November 1999–April 2000), whereas it was 258 ± 29 for the last complete 6 months (September 2006–February 2007) ($p < 0.01$).

Gender and age

Of the 12 107 samples, 4745 were from males, and 7362 from females. The samples could be assigned to 1695 male and 2428 female individuals. Thus, each male and female patient contributed with an average number of three samples. Over time however, there was a clear trend towards a higher proportion of samples from female patients (Figure 1).

Mean age ± SD of the total study population was 39.3 ± 18.3 years (range: 0.2–94.5 years). There was no significant difference between males and females: the mean age of the male and female patients was 39.2 ± 18.7 years (range: 0.3–92.1 years), and 39.4 ± 18.0 years (range: 0.2–94.5 years), respectively.

The mean age of subjects with a neurologic diagnosis was 35 ± 20 years, while it was 41 ± 15 years for subjects with a psychiatric diagnosis ($p < 0.0001$). Among neurologic patients, 7.8% were younger than 10 years, and 25.5% were younger than 20 years, while among those with a psychiatric diagnosis, it was only 0.1 and 4.7%, respectively.

Over time, the mean patient age rose. While the mean age for the first 1000 samples was 34 ± 19 years (range: 1–89 years), it was 41 ± 18 years (range: 0–92 years) for the last 1000 samples. This difference was highly significant ($p < 0.01$).

Table 1. Diagnoses as provided on the request forms

	Major ICD-10 code
Neurologic (2877)	
Epilepsy (2843)	G40; G41
Other neurologic (34)	G10; G25; G32; G35; G43; G50; G61
Psychiatric (4289)	
Bipolar (1750)	F31
Manic (14)	F30
Other affective (935)	F32; F33; F34; F38; F39
Schizoaffective (242)	F25
Schizophrenic and delusional (186)	F20; F21; F22; F29
Neurotic, stress-related and somatoform (169)	F40; F41; F42; F43; F44; F45; F48
Behavioural syndromes (15)	F50; F51
Personality disorders (140)	F60; F62; F63; F69
Mentally retarded (4)	F70; F71; F79
Impaired psychological development (37)	F84; F89;
Dementia (9)	F00; F01; F02; F03
Other psychiatric (86)	F06; F10; F12; F13; F15; F19; F90; F91; F94; F95
Unspecified psychiatric (702)	F99
Other (43)	Q90; nursed infant of mother using LTG

Number of samples (n) in brackets. Subdiagnoses (e.g. G40.1) not shown

Diagnosis

The diagnosis was provided in 59.5% ($n = 7209$) of all samples. Table 1 gives an overview of the diagnoses stated on the request forms. A total of 130 different diagnoses were stated, most of them psychiatric off-label. In 97 cases, more than one diagnosis was stated. In 43 of them it was one or more psychiatric diagnoses in addition to a neurologic (epilepsy: 91%), the remaining cases were combinations of either 2 or 3 psychiatric diagnoses.

While females represented 53% of all neurologic patients, they represented 65% of all psychiatric patients. Also, the proportions of neurologic and psychiatric diagnoses differed between males and females. Of all male patients, 52.7% ($n = 1479$) had one or more psychiatric diagnoses and 47.3% ($n = 1327$) one or more neurologic, while the respective numbers for females were 64.3% psychiatric ($n = 2728$) and 35.7% neurologic ($n = 1515$). These differences were statistically significant ($p < 0.01$ for both diagnoses). The proportion of psychiatric indications rose over time (Figure 2).

Dose

The mean total daily dose of the entire study population ($n = 11\,855$) was 229 ± 146 mg (median: 200 mg/day; range: 5–1100 mg). As can be seen from the moving average line shown in Figure 3, the mean daily dose rose from 183 ± 136 mg (median, 150 mg/day; first 500 samples) to 253 ± 158 mg (median, 200 mg/day; last 500 samples). The difference between the mean values was statistically highly significant ($p < 0.01$). However, it should be noted that the median value
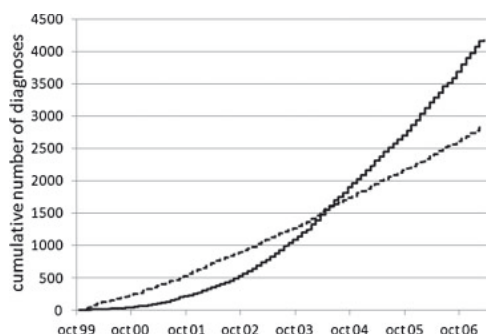
Figure 2. Cumulative number of psychiatric (solid line) and neurologic (dotted line) diagnoses during the observation period (October 1999–May 2007)
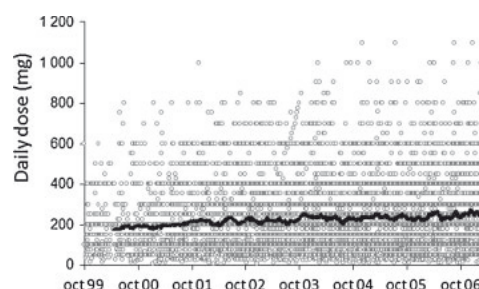
Figure 3. Scatterplot of stated daily doses of LTG *versus* time ($n = 11\,855$). Each circle represents one sample. The line indicates the moving average of 500 samples

remained unchanged throughout the observation period.

The mean daily doses per age group were as follows: 0–9 years, 112 mg/day ($n = 384$); 10–19 years, 188 mg/day ($n = 1250$); 20–29 years, 241 mg/day ($n = 2313$); 30–39 years, 252 mg/day ($n = 2495$); 40–49 years, 255 mg/day ($n = 2092$); 50–59 years, 238 mg/day ($n = 1704$); 60–69 years, 201 mg/day ($n = 706$); 70–79 years, 186 mg/day ($n = 530$); ≥ 80 years, 170 mg/day ($n = 314$).

Female patients used a numerically higher mean daily dose than male patients (232 ± 148 mg/day vs. 224 ± 142 mg/day), whereas the median dose for both genders was 200 mg/day.

Patients with a neurologic diagnosis ($n = 2788$) were prescribed a mean total daily LTG dose of 226 ± 162 mg/day (median: 200 mg; range: 5–1000 mg/day). Patients with a psychiatric diagnosis ($n = 4158$) were prescribed a mean dose of 232 ± 136 mg/day (median: 200 mg/day; range: 5–1100 mg/day). A more detailed distribution of dose versus diagnosis is given in Table 2.

Serum concentration

The mean serum concentration of all samples was 3.8 ± 2.6 mg/L (median: 3.2 mg/L; range: 0.3–41.4 mg/L). After exclusion of those cases where overdose or intoxication was stated ($n = 24$), the numbers were virtually the same: 3.8 ± 2.5 mg/L (median: 3.4; range: 0.3–32.2 mg/L). Mean serum concentrations for male and female patients were 3.7 ± 2.5 mg/L (median: 3.3 mg/L; range: 0.3–24.2 mg/L), and 3.8 ± 2.6 mg/L (median: 3.2 mg/L; range: 0.3–32.2 mg/L), respectively.

There was only an insignificant trend of the serum concentration to rise with time. The mean serum concentration of the first 500 samples was 3.7 ± 2.6 mg/L (median: 3.2 mg/L), while for the last 500 samples it was 3.9 ± 2.5 mg/L (median: 3.4 mg/L) ($p = 0.07$).

Figure 4 displays clearly that there is great variability in the LTG serum concentration at any given dose. The distribution of serum concentration versus dose, according to neurologic or psychiatric diagnosis, can be seen from Table 2.

The reference range for LTG in our laboratory is 10–50 μmol/l, which corresponds to 2.6–12.8 mg/L and thus is almost identical with that reported by Morris et al.[19] Of the samples linked to a psychiatric diagnosis, 40.7% were below this reference range, and 59% were lower than 4 mg/L. For samples linked to a neurologic diagnosis, the values were 29.7 and 56%, respectively.

Table 2. Distribution of daily dose and serum concentration according to diagnosis (as provided on the request form)

		Daily dose (mg/day)								
	All	5–100	101–200	201–300	301–400	401–500	501–600	601–700	701–800	>800
Neurologic diagnosis % (n)	100 (2789)	30.6 (853)	34.3 (957)	13.8 (385)	11.0 (306)	4.7 (130)	3.3 (93)	1.3 (35)	0.7 (19)	0.4 (11)
Serum conc. (mg/L)*	3.6 (0.3–24.2)	2.8 (0.3–1)	3.4 (0.3–13.9)	3.7 (0.5–16.7)	4.8 (1.0–24.2)	4.7 (1.3–15.6)	6.1 (1.5–18.7)	6.1 (1.9–18.7)	5.6 (2.0–11.2)	5.3 (4.4–7.4)
Psychiatric diagnosis % (n)	100 (4159)	21.5 (896)	40.4 (1680)	20.4 (849)	10.7 (444)	3.3 (139)	2.4 (98)	0.7 (28)	0.3 (12)	0.3 (13)
Serum conc. (mg/L)*	2.9 (0.3–17.0)	1.4 (0.3–7.9)	2.6 (0.3–14.8)	3.7 (0.3–17.0)	4.1 (0.4–14.7)	4.9 (1.3–15.6)	5.5 (1.0–14.3)	6.4 (1.6–10.2)	5.8 (2.9–8.3)	6.5 (2.6–14.3)

*Median (range).
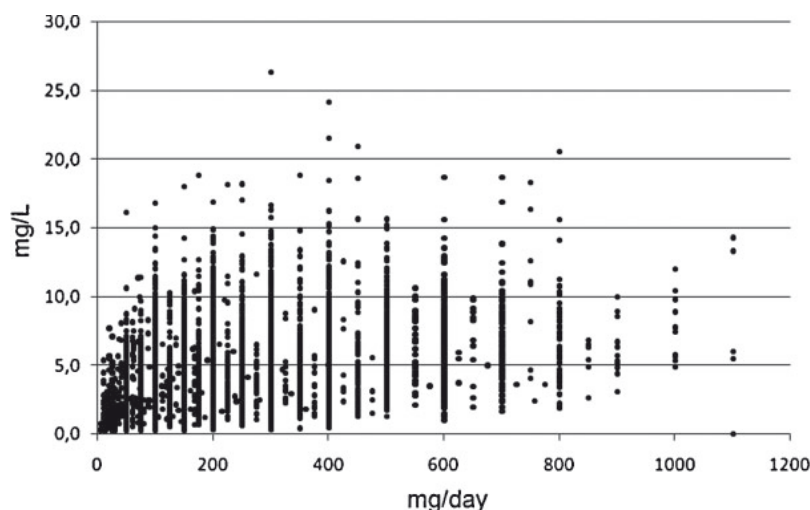
Figure 4. Scatterplot of serum concentration *versus* daily dose ($n = 11\,855$). Each dot represents one sample

Concentration-to-dose ratio (CDR)

The mean CDR of all samples with known dose ($n = 11\,847$) was 0.022 ± 0.023 (mg/L)/(mg/day) while the median was only 0.015 (range: 0.001–0.536). Indeed, the frequency distribution of the CDR was considerably right-skewed, as can be seen from Figure 5. After exclusion of all samples with any co-medication, the mean CDR was 0.019 ± 0.017 (mg/L)/(mg/day) (median 0.016; $n = 3674$). Without any co-medication, a daily dose of 200 mg LTG would thus be expected to give a serum concentration of $200 \times 0.019 = 3.8$ mg/L, while 300 mg would give 5.7 mg/L.

The mean CDR of all samples with valproate as co-medication was 0.05 ± 0.03 ($n = 1508$). The mean CDR of samples with other interacting co-medication (but without valproate) was: carbamazepine, 0.01 ± 0.01 ($n = 565$); oxcarbazepine, 0.013 ± 0.01 ($n = 85$); fenytoin, 0.008 ± 0.008 ($n = 76$), phenobarbital, 0.01 ± 0.005 ($n = 43$), topiramate, 0.015 ± 0.01 ($n = 186$); ethinyl estradiol, 0.01 ± 0.008 ($n = 300$), fluoxetine, 0.015 ± 0.008 ($n = 113$); lithium, 0.015 ± 0.008 ($n = 617$). All mean values were significantly different from both the total study mean CDR and the mean CDR of all samples where no co-medication was stated ($p < 0.01$ in all cases).

The median CDR according to age was as follows: 0–9 years, 0.03 ($n = 358$); 10–19 years, 0.02 ($n = 1248$); 20–29 years, 0.015 ($n = 2313$); 30–39 years, 0.013 ($n = 2493$), 40–49 years 0.013 ($n = 2092$); 50–59 years, 0.015 ($n = 1704$); 60–69 years, 0.015 ($n = 796$); 70–79 years, 0.02 ($n = 529$); ≥ 80 years, 0.02 ($n = 314$).

There was no clinically relevant difference in the mean and median CDR between male ($n = 4634$) and female ($n = 7213$) patients. Mean values were 0.023 ± 0.023 and 0.021 ± 0.023; median values were 0.015 and 0.016, respectively.

Over time, the CDR decreased. While the mean CDR of the very first 1000 samples was 0.027 ± 0.029 (median: 0.017; range: 0.001–0.232), it was 0.020 ± 0.019 (median: 0.014; range: 0.001–0.188) for the last 1000 samples. This difference was highly significant ($p < 0.01$)

Patients with a neurologic diagnosis ($n = 2788$) had a mean CDR of 0.027 ± 0.028 (median: 0.018; range: 0.002–0.387), while psychiatric patients ($n = 4158$)

Figure 5. Frequency distribution of the LTG serum concentration/dose-ratio ($n = 11\,855$)

had a mean CDR 0.017 ± 0.016 (median: 0.014; range: 0.001–0.536). The difference was highly significant ($p < 0.001$)

Co-medication

Co-medication was stated on 68.4% ($n = 8285$) of all request forms. A total of 274 different generic entities were stated (antibiotics and vitamin preparations were each counted as one generic type of co-medication). The mean and median number of co-medications per sample was 2 (range: 1–19). Samples from female patients had a mean number of 3 ± 2 co-medications (range: 1–16; $n = 5031$), which was significantly different from the number of 2 ± 2 (range: 1–19; $n = 3252$) co-medications for samples from male patients ($p < 0.01$).

The relative frequency of the number of additional drugs was: (1) 39.8% ($n = 3294$); (2) 22.9% ($n = 1899$); (3) 14.2% ($n = 1174$); (4) 10.0% ($n = 831$); (5) 6.0% ($n = 493$); (6) 3.8% ($n = 318$); (7) 1.9% ($n = 160$), (8) 0.7% ($n = 57$); (9) 0.3% ($n = 28$); (≥ 10) 0.4% ($n = 31$).

The 25 most frequently stated co-medications were valproate ($n = 1508$), citalopram/escitalopram ($n = 1035$), olanzapine ($n = 873$), venlafaxine ($n = 742$), zopiclone ($n = 734$), lithium ($n = 653$), carbamazepine ($n = 650$), thyroxine ($n = 639$), quetiapine ($n = 555$), oxazepam ($n = 530$), clonazepam ($n = 492$), alimemazine ($n = 453$), acetylsalicylic acid ($n = 431$), mirtazapine ($n = 407$), chlorprothixene ($n = 355$), levetiracetam ($n = 327$), oral contraceptives containing ethinyl estradiol ($n = 304$), mianserin ($n = 290$), sertraline ($n = 277$), clozapine ($n = 276$), diazepam ($n = 276$), risperidone ($n = 275$), vitamins ($n = 255$), topiramate ($n = 217$), metoprolol ($n = 215$).

Of the samples where valproate was stated as co-medication and a diagnosis was stated, 72.6% ($n = 670$) were assigned a neurologic diagnosis, 26.5% ($n = 245$) a psychiatric and 0.9% ($n = 8$) a neurologic plus a psychiatric one.

Patients using valproate ($n = 1422$) had a mean LTG serum concentration of 6.2 ± 3.2 mg/L (median: 5.6; range: 0.33–24.2), which was significantly higher than the total population mean of 3.8 ± 2.6 mg/L (median: 3.2 mg/L; range: 0.3–41.4 mg/L) ($p < 0.01$). Conversely, patients using carbamazepine ($n = 564$) had a mean serum concentration of only 2.9 ± 2.3 mg/L (median: 2.4; range: 0.26–23 mg/L) ($p < 0.01$ vs. total study population mean). The serum concentration of patients using both valproate and carbamazepine (4.0 ± 1.8 mg/L; $n = 86$) was not statistically different from the total population mean.

More detailed investigations on pharmacokinetic drug interactions were not performed since this issue has been subject to previous research.[8,9]

Overdose/intoxication

Twenty-four samples from 24 patients aged 17–78 years (8 males, 17 females) were marked as known or suspected overdose/intoxication. Of these, eight samples had serum concentrations above 12.8 mg/L (50 µmol/L), ranging from 13.9 to 41.3 mg/L. Clinical data were mostly unclear and incomplete. Taken dosages were stated in some cases of known intoxication, ranging from '3 daily doses' to '30–40 tablets of 100 mg'. The patient's diagnosis (the underlying disease) was stated in only three cases (epilepsy: two; depression: one).

DISCUSSION

Due to the nature of the underlying database, this survey has both strengths and limitations. The strengths are the large number of samples, the long observation period and the naturalistic setting which allows a unique insight into everyday clinical practice. A limitation is that the diagnosis and the clinical indication for the analysis were not stated in all cases. It should also be remembered that all data were collected in one single country, and that results may be strongly influenced by national treatment routines and health care premises which may differ from those of other countries. Nevertheless, Scandinavia is a suitable part of the world to perform such research since therapeutic drug monitoring for decades has been considered a valuable tool in the treatment of various diseases. This survey confirms that monitoring of serum concentrations of LTG is common practice in Norway, as reflected by the number of samples analysed monthly.

Gender and age

Females seem to represent the majority of today's LTG users. This gender difference was small during the first years but has increased ever since, which may reflect that the initial use of LTG was restricted to epilepsy where the gender difference is indeed small, whereas affective disorders are more common in females.[10] It may also reflect that LTG soon after its launch was particularly recommended for fertile women, mainly due to its lack of induction of the metabolism of hormonal contraception, its lack of endocrine side

effects and the notion that it was less teratogenic than other drugs. [11–13] Having this in mind, it is interesting that there was no difference in the mean age between males and females.

There was no clinically significant difference in the mean and median dose and serum concentration between males and females. Indeed, the values were practically identical. Consequently, the CDR was also essentially similar. However, this applies to the entire study population. The large SDs indicate that there may be considerable differences between individuals.

In accordance with previous findings, there was a U-shaped effect of age on the CDR, with higher serum concentrations per mg in children and in the elderly. [14,15] Interestingly, the average serum concentration itself did not seem to change very much with age, while the prescribed doses (and consequently, the CDR) did.

Diagnosis

More samples are now taken from psychiatric patients than from those with a neurologic diagnosis. Psychiatrists may consider serum concentration measurements more important than neurologists because of a greater need to follow up patient compliance. The finding that the median age of patients with a psychiatric diagnosis was higher than that of patients with a neurologic one was not surprising. This simply reflects that bipolar and schizoaffective disorders are less common in the very young, compared to epilepsy.

Dose

The mean prescribed dose rose during the observation period. However, the median dose remained unchanged at 200 mg/day. Both the mean and the median doses found in this survey are considerably lower than the defined daily dose (DDD) as suggested by the WHO, which is 300 mg. [16] This finding confirms the results of a previous study suggesting that, compared to clinical practice, the DDD appears to be too high and should be reviewed. [17]

While the mean prescribed doses were essentially identical among psychiatric and neurologic patients, there was a trend towards higher serum concentrations in neurologic patients. This may be explained by the fact that roughly three-fourth of patients using valproate (which inhibits LTG metabolism) were neurologic patients. Accordingly, the mean CDR was higher among patients with a neurologic diagnosis.

Serum concentrations, CDR and co-medication

Generally, the results with respect to drug interactions of LTG were as expected and will not be discussed in detail since this issue has been subject to previous investigations. [8,9] It should however be noted that patients on valproate had higher serum concentrations than the total population mean, while patients using enzyme inducers had considerably lower concentrations. This suggests that the effects of enzyme-inhibiting and -inducing co-medications are not completely compensated by, e.g. dose adjustments.

Figure 4 and Table 2 show clearly that there is no obvious correlation between dose and serum concentration. Whether this might be explained by different degrees of non-compliance, different time between last intake and blood-sampling, interindividual differences in metabolic capacity, drug–drug interactions or other factors: in any case, these results indicate that one and the same LTG dose can give very different serum concentrations in different patients, and that measurement of LTG serum concentrations thus may be useful.

Another interesting finding is the distribution of measured serum concentrations in relation to established reference ranges. Of the three largest laboratories in Norway, two (including our own) use a reference range of 2.6–12.8 mg/L (10–50 μmol/L), and one uses 2.6–15.4 mg/L (10–60 μmol/L) for epilepsy (for bipolar disorder, no reference range has been established). The present survey shows that the average serum concentration lies around 3.8 mg/L, which is close to the lower limit of these reference ranges. This could mean that an average serum concentration of 3.8 mg/L represents an optimum risk/benefit balance for the most patients. It may also reflect the common clinical aim of treating the patient with as low a dose as possible.

The very first suggested reference range for LTG was 1–4 mg/L. [18] Other reference ranges have been suggested later, with lower limits of 1–3 mg/L, and upper limits of 10–20 mg/L. [19–23] The fact that 41% of the psychiatric and 30% of the neurologic patients in the present survey had serum concentrations below 2.5 mg/L may suggest that the currently used lower limit is too high. It might also be speculated whether the majority of these patients had problems with regular drug intake, and if suspected non-compliance was the reason for analysis. However, when considering the average daily dose and the CDR of LTG, it becomes more likely that these low serum concentrations are due to low prescribed doses rather than low compliance. Interestingly, a similar survey as the present one, recently performed in Australia, found

that 75% of all serum concentrations were above 7 mg/L.[23] This indicates that drug treatment routines may differ considerably between countries, a finding that should be taken into consideration when discussing reference ranges or treatment guidelines on an international level.

In conclusion, this survey revealed significant trends and changes in the pattern of use of LTG during recent years.

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Paper II

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Paper III

Ethinyl Estradiol, Not Progestogens, Reduces Lamotrigine Serum Concentrations

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Summary: *Purpose:* To study the interaction between lamotrigine (LTG) and hormonal contraception.

Methods: LTG serum concentrations of female patients using either no hormonal contraception (n = 18), an ethinyl estradiol (EE)-containing (n = 11), or a progestogen (PG)-only–containing compound (n = 16) were analyzed. Patients were recruited prospectively, and blood samples were drawn during drug fasting and at steady-state conditions. Comedication with enzyme inducers, valproate, topiramate, or sertraline was not allowed. Some patients changed groups and thus served as their own controls. Samples were analyzed by a gas chromatography/mass spectroscopy method. The Mann–Whitney *U* test was used for statistical comparison of the groups.

Results: The LTG serum concentration-to-dose ratio (CDR), expressed as (mg/L)/(mg/d) was significantly lower in women using EE than in the control group (mean $\pm$ SD, 0.010 ± 0.004 vs. 0.017 ± 0.006; p = 0.003). The CDR in women using PG was 0.02 ± 0.007, which was not statistically different from controls. No difference was found in CDR between women using either oral, topical, or parenteral PG. Five women switched from the control to the EE group and experienced a considerable reduction in CDR. An increase of the CDR toward control level was seen in the two women who changed from EE to PG.

Conclusions: It is the EE component of oral contraceptives that interacts with LTG. The PG-only compounds did not alter LTG serum concentrations in this study. These findings should be considered when counselling women with epilepsy in the childbearing ages. **Key Words:** Lamotrigine—Ethinyl estradiol—Progestogens—Serum concentration—Drug interaction.

Lamotrigine (LTG) is a broad-spectrum antiepileptic drug (AED) that is increasingly used worldwide for both epileptic and psychiatric disorders. Currently, much attention is directed toward possible endocrine side effects of AEDs in women (1). LTG has so far not been reported to be associated with such disturbances and has therefore been regarded as a preferable choice in this patient group (1,2). However, its interaction potential with sexual hormones has still not been extensively studied. Women of childbearing age often use hormonal contraception, and it has recently been reported that combined oral contraceptives reduce LTG serum concentrations by ≤64% (3,4), which may require dose adjustment of LTG. Most oral contraceptives contain a combination of an estrogen derivative, ethinyl estradiol (EE), and a progestogen (PG). In addition to oral compounds, hormone-containing parenteral, intravaginal, and intrauterine products are on the market. We wanted to know whether the interaction between LTG and hormonal contraception is due to the EE or the PG component. Moreover, we wanted to investigate whether this interaction is restricted to oral application of the hormones or whether it also can be seen with topical/parenteral methods.

METHODS

This was a three-armed, open, prospective study. LTG serum concentrations of consecutively enrolled female patients of childbearing age using no hormonal contraception, an EE-containing compound, or a PG-only-containing compound were analyzed.

Some patients participated in more than one group and thus served as their own controls.

Concomitant treatment with drugs known or suspected to reduce (enzyme inducers, topiramate) or increase (valproate, sertraline) LTG serum concentrations was not allowed (1,5–7). Patients with liver or kidney disease, known compliance problems, or a history of drug abuse were not included. Blood samples were drawn during drug fasting in the morning and at steady-state conditions, but not standardized in relation to the menstrual cycle. Time intervals between blood samplings in patients who changed

group were ≥1 month, and their LTG doses remained unchanged.

All samples were analyzed with a liquid chromatography/mass spectrometry (LC/MS) method. LTG was extracted from 0.1 ml serum with 0.5 ml dichloromethane/isopropanol (9:1) after addition of internal standard solution (50 μl of 10 μg/ml minoxidil in methanol) and alkalinization with 0.1 ml 0.2M sodium bicarbonate. After mixing and centrifugation, the organic extract was transferred to vials and injected on an Agilent MSD 1100 LC/MS system (Agilent, Palo Alto, CA, U.S.A.). The LC/MS system consisted of a G1379A degasser, a G1311A quaternary pump, a G1313A autosampler, a G1316A column oven, and a G1946A mass spectrometer. Separation was performed on a SB-LC18 Zorbax (30 × 4.6 mm, 3 μm) column with a mobile phase consisting of methanol/ammonium acetate, 45:55. LTG was monitored after positive electrospray ionization at m/z 256.0, and the internal standard minoxidil, at m/z 210.2. The calibrated range was from 0.13 to 25.6 μg/ml. Six quality-control samples covering the range from 0.5 to 11.5 μg/ml were analyzed with every batch of unknown samples. Between-day relative standard deviation calculated from quality-control samples was >9% at 0.5 μg/ml and 5% at 9 μg/ml. The limit of quantification of the method was <0.06 μg/ml.

To correct for varying daily doses, the concentration-to-dose ratio (CDR) was calculated by dividing serum concentration by total daily dose. By accepting an α-error of 0.05 and a β-error of 0.10, the minimal number of participants necessary to detect a difference greater than the usual fluctuation (defined by us as >33% deviation from the mean LTG CDR value of the control group) was calculated to be seven in the test groups and 13 in the control group. The nonparametric Mann–Whitney U test was applied for comparison of the EE and the PG groups vs. control, respectively. The study was approved by the Regional Committee for Ethics in Medical Research. All patients gave written informed consent.

RESULTS

Forty-five women, age 17 to 44 years, were included in the study, 18 without hormonal contraception, 11 using combined contraceptives containing EE, and 16 using PG-only compounds. All comedications with LTG, including the administration mode of the contraceptive method, are shown in Table 1. No significant differences were found in mean age or body mass index (BMI) or mean daily LTG dose between groups, although the doses were somewhat lower in the EE group (225 ± 193 mg) than in the control group and the PG group (323 ± 182 mg and 291 ± 110 mg, respectively).

EE users had clearly lower serum concentrations at identical doses, compared with controls and PG users. The mean serum concentrations were 5.6 ± 3.1 mg/L (control group), 2.0 ± 1.3 mg/L (EE group), and 5.4 ± 2.1 mg/L (PG group). The difference between the EE group and controls was highly significant (p < 0.001).

The ranges, medians, and percentiles of the dose-corrected serum concentrations, expressed as serum concentration-to-dose ratio (CDR), are shown in Fig. 1. The difference between the EE group and the control group was highly significant (p = 0.003), whereas no statistical difference was found between the controls and the PG group. The patients in the latter group had been using PG for a median period of 8 weeks (range, 4 to 208 weeks) before blood sampling.

Figure 2 shows the change in CDR of patients taking part in more than one group. Switches occurred in either direction along the X-axis in individual patients, and the time interval between measurements was ≥4 weeks. LTG serum concentrations of patients who switched from EE

TABLE 1. *Comedications with lamotrigine*

| Group | No. | Contraceptive method | | Other drugs (no.) |
		Administration	Compounds and doses	
Contr.	12	–	–	–
	6	–	–	LEV (4); ZNS (1); GBP (1)
EE	3	Oral	EE, 35 μg; cyproterone acetate, 2 mg	LEV (1)
	3	Oral	EE, 30/40/30 μg; levonorgestrel, 50/75/125 μg (triphasic)	–
	2	Oral	EE, 30 μg; drospirenone, 3 mg	–
	1	Oral	EE, 30 μg; desogestrel, 150 μg	–
	1	Oral	EE, 30 μg; levonorgestrel, 150 μg	–
	1	Vaginal ring	EE, 15 μg + etonogestrel, 120 μg/24 h	–
PG	3	Oral	Desogestrel, 75 μg	–
	1	Oral	Noretisteron, 0.35 mg	–
	7	Subdermal	Etonogestrel, 68 mg	LEV (1), ZNS (1)
	1	Subdermal	Levonorgestrel, 36 mg	–
	1	IM	Depot-medroxyprogesterone, 150 mg every 12 wk	–
	3	Intrauterine	levonorgestrel, 20 μg/24 h	LEV (1)

Contr, controls; EE, ethinyl estradiol; PG, progestogens; LEV, levetiracetam; ZNS, zonisamide; GBP, gabapentin.
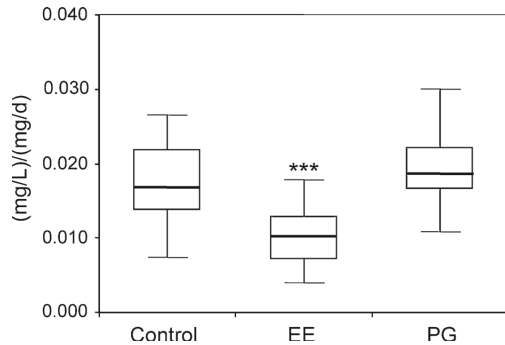
FIG. 1. Box plot showing the median (*horizontal line*), interquartile range (*lower and upper edge of the box*), and total range of the serum concentration-to-dose ratio of the three study groups. EE, ethinyl estradiol; PG, progestogen. ***p = 0.003 (EE group compared with controls).

to PG increased to control value levels. Patient A first switched from control to the EE group, and then from the EE to the PG group. Accordingly, her CDR decreased from 0.026 to 0.011, and then returned to 0.022. One patient of the control group (patient D) was later treated with a topical EE–containing vaginal ring. Her CDR decreased from 0.023 to 0.012, whereas her LTG dose remained unchanged.

DISCUSSION

This study suggests that PG-only contraceptive compounds do not reduce LTG serum concentrations. In accordance with previous findings (3,4), our results
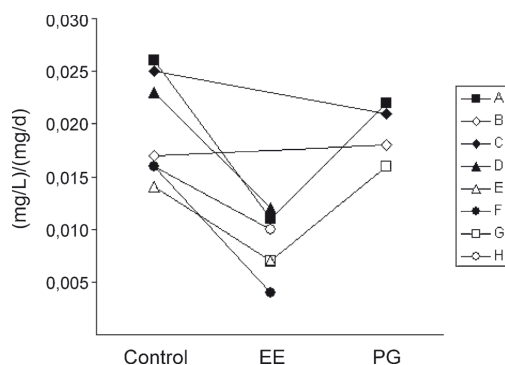
FIG. 2. Lamotrigine serum concentration-to-dose ratios in individual patients **(A–H)** who changed groups. Contraceptive methods were as follows. **A:** Ethinyl estradiol (EE) + levonorgestrel, depot-medroxyprogesterone IM. **B, C:** Etonogestrel subdermal implant. **D:** Etonogestrel vaginal ring. **E, F:** EE + cyproteronacetate. **G:** EE + levonorgestrel, etonogestrel subdermal implant. **H:** EE + cyproteronacetate.

show that the use of combined oral contraceptives is associated with considerably reduced LTG serum levels. Interestingly, reduced LTG concentrations also were demonstrated in one patient using an EE-containing vaginal ring (see Fig. 2). Although the mean EE serum concentrations achieved with this device generally are somewhat lower than those with conventional oral contraceptives, the average minimal EE concentrations are quite similar (8).

Although no reports indicate significant fluctuations of the LTG serum concentrations throughout the physiologic menstrual cycle, it has recently been shown that LTG concentrations may increase considerably toward the end of the pill-free week in women taking combined oral contraception (9). In the previous studies by Sabers et al. (3,4), and in the present study, blood sampling was not standardized in relation to the menstrual cycle. Nevertheless, the effect of EE on the LTG serum concentrations could be clearly demonstrated. The more patients who were in the pill-free period, the more would our results underestimate the true effect of EE on the LTG serum concentration. With respect to PG, it should be noted that the PG compounds used in our study were administered on a continuous long-term basis without regular drug-free intervals.

LTG is metabolized mainly via glucuronidation by uridine-diphosphate glucuronosyltransferase (UGT) 1A4 at the N2 position and then eliminated via the kidneys (10). EE, which itself is glucuronidated by UGT1A1, is a known inducer of UGTs and may thereby increase the clearance of other glucuronidated drugs (11). Hence it is reasonable to assume that the reduction of LTG serum concentration also is due to increased glucuronidation, leading to accelerated renal elimination of LTG. This remains to be proven by studies of the LTG metabolite pattern. The observed reduction in LTG-CDR by >50% is of comparable magnitude to the effect of carbamazepine comedication (12) and may thus require dose adjustment in many patients.

PG-only compounds did not influence LTG serum concentrations, regardless of the administration mode, which either was oral, subdermal, intramuscular, or intrauterine in our study. Moreover, we could not detect any differences related to the various application forms, but the number of patients was limited. Marked differences are found in the pharmacokinetic and pharmacodynamic properties of the various PGs on the market. Only limited information is available on their dose–response relation in humans, and only progestational effects have been studied extensively. Nevertheless, all PG-only compounds in this study, except the intrauterine device used by three participants, were administered systemically, and all of them have demonstrated contraceptive efficacy at the doses used, indicating biologically active serum concentrations (13). Moreover, no differences in LTG-CDR were related to the administration mode of the PG compounds in our study.

Removal of the three patients using an intrauterine drug-delivery system caused only marginal statistical changes. Hence all PG users were considered a uniform group in the final statistical analysis.

We also could also not see any association between the CDR and the duration of PG treatment, which ranged from 1 to 6 months (median, 8 weeks), a period that should have been sufficiently long to allow any potential effect on glucuronidating liver enzymes to develop. In contrast to our results, it was recently reported that the oral PG compound, desogestrel, increased LTG serum concentrations in seven of 10 patients (14). However, the effect varied considerably (0–96%), and similar effects of PG on other drugs have, to our knowledge, not previously been reported in the literature.

Recently, LTG was shown to reduce the area under the concentration–time curve (AUC) of the PG component of some combined oral contraceptives, as in levonorgestrel, by 19% (9). The clinical relevance of this interaction remains to be elucidated. Given the comparatively more brittle mechanism of PG-only pills exclusively targeting the cervical mucus and the endometrium, the risk for contraceptive failures should be considered in women who also use LTG. However, the novel 75-μg desogestrel-only pill differs from other PG-only pills in having a more robust mechanism, providing consistent ovulation inhibition with a performance appearing to be very similar to that of combined oral contraceptives (15).

In conclusion, our study adds further information on the interaction potential of LTG that should be considered when counselling women with epilepsy of childbearing age.

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Paper IV

Paper V

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Lamotrigine and its N2-glucuronide during pregnancy: The significance of renal clearance and estradiol

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Summary
Purpose: To investigate the physiological mechanisms behind the pronounced decline of lamotrigine (LTG) serum concentrations during pregnancy.
Methods: Serum and urine concentrations of LTG and its main metabolite, LTG-N2-glucuronide (LTG-GLUC), were measured monthly in 21 pregnancies of 19 women using LTG. Simultaneously, a panel of biochemical variables was monitored to evaluate liver and kidney function and possible hemodilution effects. Pharmacokinetic parameters were calculated once at baseline and once in gestational month 8.
Results: Initially, LTG and LTG-GLUC serum concentrations fell simultaneously by 27% and 38%, respectively (gestational month 2). Subsequently, the ratio of the LTG-GLUC/LTG serum concentrations increased gradually, correlating strongly with rising serum estradiol concentrations. In gestational month 8, the ratio was 164% higher than at baseline. At that time, LTG total clearance had increased by 118%, and the amount of unchanged LTG in urine had dropped by 40% while the amount of LTG-GLUC had increased by a corresponding 37%.
Conclusions: The simultaneous decline of LTG and LTG-GLUC serum concentrations in early pregnancy suggests that in this phase, increased renal blood flow is the major cause. After gestational month 2, estradiol-induced glucuronidation of LTG becomes more important, leading to a further fall of LTG serum concentrations and a gradual rise of the LTG-GLUC/LTG-ratio through the remaining pregnancy. An expanded volume of distribution may also contribute to reduced LTG serum concentrations in pregnancy.
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Introduction

Epilepsy is the most common neurological problem which requires pharmacological treatment in pregnant women (Brodtkorb and Reimers, 2008). The balance between seizure control and potential teratogenic risks, and the pharmacokinetic changes of antiepileptic drugs during pregnancy and puerperium, often represent challenges to the clinician (Pennell, 2006; Brodtkorb and Reimers, 2008; Sabers and Tomson, 2009). Lamotrigine (LTG) is a widely used antiepileptic drug, indicated for various forms of epilepsy as well as for mood disorders (Viguera et al., 2002; Yonkers et al., 2004). Females constitute the majority of patients taking LTG, with a large proportion in childbearing age (Reimers, 2009). Consequently, a considerable number of female patients take LTG during pregnancy (Sabers et al., 2004).

Several studies have shown that LTG clearance may increase by 65—230% during gestation (Tomson et al., 1997; Ohman et al., 2000; Tran et al., 2002; de Haan et al., 2004; Pennell et al., 2004; Petrenaite et al., 2005). Accordingly, serum concentrations may decrease by more than 60%, often requiring dose adjustments. These pharmacokinetic changes are subject to marked interindividual variability (Petrenaite et al., 2005). They are thus largely unpredictable, and close therapeutic drug monitoring is recommended (Sabers and Petrenaite, 2009). Serum concentrations return to pre-pregnancy values within 2—3 weeks postpartum (Ohman et al., 2000; Tran et al., 2002; de Haan et al., 2004).

After oral dosing, LTG is completely absorbed (Cohen et al., 1987) and mainly metabolised by uridinediphosphate-glucuronosyltransferase 1A4 (UGT1A4) in the N2-position. Up to 90% of an orally administered dose appear as the pharmacologically inactive N2-glucuronide (LTG-GLUC) in urine, and 10% as unchanged LTG. Two percent appear in the faeces (Doig and Clare, 1991; Dickens and Chen, 2002). An N5-glucuronide has been postulated (Doig and Clare, 1991), but never demonstrated. Other metabolites occur only in very small amounts in humans (Doig and Clare, 1991; Wootton et al., 1997; Beck et al., 2006). Increased glucuronidation of LTG in the N2-position has therefore been proposed as the mechanism behind its increased clearance during pregnancy (Ohman et al., 2008).

However, pregnancy induces a variety of physiological changes which may affect LTG pharmacokinetics and lead to a fall in its serum concentrations (Anderson, 2005; Pavek et al., 2009). The aim of the present study was therefore to further investigate possible mechanisms behind the pregnancy-induced changes in the pharmacokinetics of LTG.

Methods

Study design

Twenty-one LTG-using pregnant outpatients with epilepsy were prospectively and consecutively included in this study. Exclusion criteria were: liver- or kidney-disease, or co-medication with carbamazepine, oxcarbazepine, phenytoin, phenobarbital, primidone, valproate, topiramate, rifampicin, fluoxetine or lithium (Reimers et al., 2005b). Patients with a history of compliance problems or substance abuse were also excluded. Patients were asked to enrol as soon as the attending neurologist had been informed about their pregnancy. Thus, most participants were in gestational week 8 or later at the time of the first visit. Baseline samples were collected at least four weeks postpartum as it has been shown that LTG pharmacokinetics return to pre-pregnancy values within two to three weeks after delivery (Ohman et al., 2000; Tran et al., 2002).

Morning trough blood samples (10—16 h after the last dose) for analysis of LTG, LTG-GLUC and for various biochemical parameters (see next paragraph) were obtained at the first visit, then monthly throughout pregnancy, and at baseline. Body weight was recorded at each visit.

On two occasions, once in the third trimester (gestational month 8) and once at least four weeks after delivery (= baseline), blood samples were taken at 0800 (immediately before the morning dose), and then 2, 4, 8 and 12 h later. The 12-h period was chosen as all patients were on a twice-daily-regimen of LTG. Urine was collected during the same 12-h period.

Sample analysis

Blood samples were centrifuged at $350 \times g$ for 10 min and the serum supernatant was carefully transferred to sample vials. The total volume of the collected urine was recorded and a 20 mL sample was taken for analysis of LTG and LTG-GLUC. Serum and urine samples were stored at $-18\,^\circ$C until analysis.

Before analysis, urine samples were diluted 1:100 because of their very high LTG-GLUC concentrations exceeding the assay's measuring range. Serum and urine samples were then treated identically. To 100 μL sample volume, 25 μL minoxidil (internal standard) and 75 μL 1% formic acid were added. Serum and urine sample preparation was then performed on OMIX^{TM} Tomtec mixed mode phase SPE tips (Varian, Walnut Creek, CA) by means of a Tomtec Quadra 96 model 320 automatic liquid handler (Tomtec, Hamden, CT) equipped with 1.2 mL Varian 96-well plates. The OMIX tips were conditioned successively by methanol and 0.1% formic acid. After sample aspiration, the OMIX tips were washed with 1% methanol and elution was performed with 50 μL methanol:ammonia (95:5).

The eluent was transferred to a deep well plate and injected on an Agilent MSD 1100 LC—MS system (Agilent, Palo Alto, CA). The LC—MS system consisted of a G1379A degasser, a G1311A quaternary pump, a G1313A auto sampler, a G1316A column oven and a G1946D mass spectrometer. Separation was performed on a Supelguard Discovery 18 (20 mm × 4 mm) column with a mobile phase consisting of methanol:formic acid:ammonium acetate (3:6:91) at a flow of 1000 mL/min. LTG was monitored after positive APCI ionization at m/z 256.3 (target ion) and 258.3 (qualifier ion), LTG-GLUC at m/z 432.3 (target ion) and 434.3 (qualifier ion), and the internal standard minoxidil at m/z 210.1 (target ion) and 164.1 (qualifier ion).

The calibrated ranges in both serum and urine were 0.5—10 μg/mL (LTG) and 1—20 μg/mL (LTG-GLUC). Three quality control samples of LTG and LTG-GLUC, covering the range from 0.5 to 20 μg/mL, were analysed with every sample batch. Between-day analytical variation of quality controls in serum was better than 8.4% at 0.5 μg/mL and 10.4% at 10 μg/mL for LTG, and 10.3% at 1 μg/mL and 17.5% at 20 μg/mL for LTG-GLUC. Analytical variation in urine was better than 4.1% at 1 μg/mL and 2.6% at 10 μg/mL for LTG, and 7.4% at 2 μg/mL and 10.5% at 20 μg/mL for LTG-GLUC.

Serum estradiol analysis was performed on a Roche Modular E 170. The calibrated measuring range of this method was 5.0—4300 pg/mL (CV ranging from 2.2 to 12%).

In order to monitor general liver- and kidney function, as well as to discover potential hemodilution effects, further blood samples were taken monthly and analysed for erythrocyte volume fraction (EVF), serum sodium, serum creatinine and serum bilirubine.

Calculations and data analysis

All calculations were performed by Kinetica 5.0 and Microsoft Excel 2007. Where necessary, dose-corrected LTG and LTG-GLUC serum concentrations were calculated by dividing the serum concentration (mg/L) by the daily LTG dose (mg/day) in order to compensate for possible dose adjustments.

For pharmacokinetic calculations, a one-compartment model with first-order absorption and elimination was chosen, and the trapezoidal rule was used. Five serum concentration/time points per participant were available for calculation of both baseline and month 8 pharmacokinetic parameters. To enable comparison between dose-dependent pharmacokinetic parameters at baseline and at month 8 despite dose adjustments, dosages were normalized to 400 mg/day.

Renal clearance (CL_R) was calculated using the following formula: C_U (mg/L) $\times$ urine flow (L/h)/$C_{ss,\,av}$ (mg/L) where C_U = concentration in collected urine; urine flow = total urine volume excreted during the 12-h collection period; $C_{ss,av}$ = average serum concentration at steady state during one dosing interval.

Because of different times of enrolment and variable ability to participate, all study subjects could not contribute to all analyses. Mean values were therefore calculated from pooled data of a varying number of contributing individuals (minimum $n = 5$, maximum $n = 20$) and are presented with $\pm$standard deviation except where otherwise stated. The t-test for unpaired samples was used for comparison between groups. A p-value ≤ 0.05 was considered statistically significant.

This study was approved by the regional ethics committee and all participants gave their informed written consent.

Results

A total of 21 pregnancies in 19 subjects were included. Two pregnancies were spontaneously aborted (one in week 11 and one in week 12), and one was terminated by caesarean section in week 30. The remaining 18 pregnancies were uneventful. Demographic data, treatment and pregnancy characteristics are summarised in Table 1.

In 10 of the 21 pregnancies, the dose was adjusted according to clinical judgement. This led to a rise of the mean daily LTG dose of the study population from 255 ± 144 mg/d at conception to 342 ± 180 mg/d at child-

Table 1 Demographic, treatment and pregnancy characteristics.

Number of pregnancies, n	21
Number of participants	19
Age at inclusion (years), mean (range)	26.8 (17—39)
Co-medication, n	
Levetiracetam	2
Zonisamide and clobazam	1
Chlorprothixene + mirtazapine	1
Daily LTG dose (mg), mean (range)	
At concepation	255 (100—800)
At childbirth	344 (200—800)
Body weight (kg), mean (range)	
At inclusion	72.9 (54—98)
At childbirth	82.4 (66.8—106.2)
Gestational age (weeks), mean (range)	
At inclusion	11 (5—23)
At childbirth	38 (30—42)

birth, an increase by 34%. Body weight rose by a mean of 13% (range, 5.3—27.7%).

Fig. 1 shows an initial decline of the LTG and LTG-GLUC serum concentrations in early pregnancy. Subsequently, while LTG concentrations continued to fall and stay low, LTG-GLUC concentrations began to rise accordingly. The relative change over time in the ratio between the absolute serum concentrations of the LTG-GLUC metabolite and the LTG mother substance is displayed in Fig. 2 (baseline = 100%). A maximum increase by 148% was found (gestational month 8). This increase correlated strongly ($R^2 = 0.88$) with the increase in the mean estradiol serum concentration, which rose from 41 pg/mL at baseline to 22,235 pg/mL in month 8 (Fig. 3).

Changes in the basic pharmacokinetic parameters of LTG at baseline and at gestational month 8 are presented in Table 2. Compared to baseline, c_{min}, c_{max}, and AUC were significantly reduced at month 8, while total LTG clearance, volume of distribution and $t_{1/2}$ all were increased.

Solid line with squares: lamotrigine (n = 5-20); dashed line with circles: lamotrigine-N2-glucuronide (n = 5-18); BL: baseline
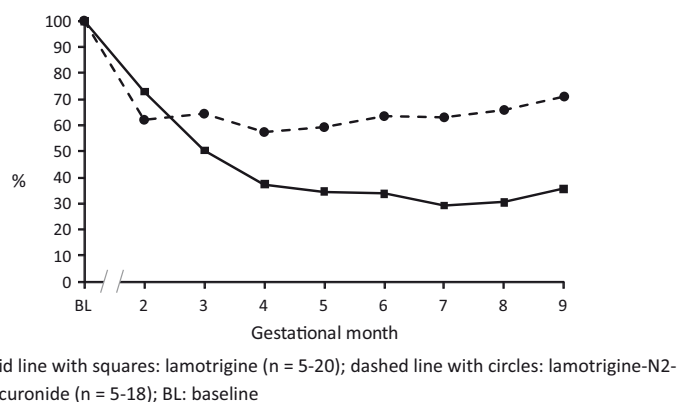
Figure 1 Relative change of mean dose corrected LTG and LTG-GLUC serum concentrations, respectively, during pregnancy. Solid line with squares: lamotrigine ($n = 5—20$); dashed line with circles: lamotrigine-N2-glucuronide ($n = 5—18$); BL: baseline.

BL: baseline; LTG-GLUC: lamotrigine-N2-glucuronide; LTG: lamotrigine
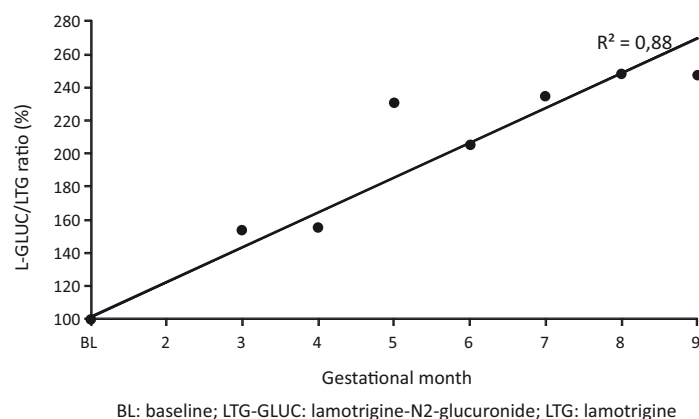
Figure 2   Relative change of the mean LTG-GLUC/LTG serum concentration ratio during pregnancy (baseline = 100%, $n = 5-20$). BL: baseline; LTG-GLUC: lamotrigine-N2-glucuronide; LTG: lamotrigine.

Urine data are shown in Table 3. At month 8, the renal clearances (CL_R) of LTG and LTG-GLUC were 77% and 63% higher than at baseline.

Table 4 shows the results of the biochemical laboratory analyses. As expected, serum sodium remained fairly stable. Bilirubin concentrations showed a small, non-significant decrease, and returned to baseline level by gestational month 9. EVF and serum creatinine concentrations declined modestly, but significantly. However, all biochemical values stayed within their proposed reference ranges for pregnant women (Jamjute et al., 2009; Klajnbard et al., 2010).

Discussion

Pharmacokinetics

This prospective, naturalistic study provides a detailed time profile of the changing LTG serum concentrations from the 2nd gestational month throughout pregnancy. A rapid decline was seen already in the first trimester. Decreased serum concentrations of LTG have previously been found to represent a significant risk for seizure deterioration in preg-

Figure 3   LTG-GLUC/LTG ratio at each gestational month as a function of estradiol serum levels ($n = 7-13$).

nant patients (Petrenaite et al., 2005; Pennell et al., 2008). Our results indicate that this risk is present already from the early phase of pregnancy. This has previously not been shown in such detail as most previous studies presented pooled data trimester-wise.

Baseline pharmacokinetic parameters of LTG in our study were consistent with previously published data on LTG pharmacokinetics in non-pregnant populations (Cohen et al., 1987; Garnett, 1997; Chan et al., 2001; Milovanovic and Jankovic, 2009). Also, the observed changes in the third trimester were within the range of previous reports (Tran et al., 2002; de Haan et al., 2004; Pennell et al., 2008; Fotopoulou et al., 2009).

Interestingly, while the dose-corrected LTG serum concentration fell by as much as 70%, serum half-life ($t_{1/2}$) increased. This unexpected finding may be explained by the following relation between serum half-life ($t_{1/2}$), apparent volume of distribution (V_d), total clearance (CL), and the fact that V_d increased slightly more than CL:

$$t_{1/2} = \frac{0.693 \times V_d}{CL}$$

However, because of the interdependence of $t_{1/2}$, CL, and V_d, these calculated parameters should be interpreted with caution. Also, all women in this study were on a twice daily dosing regimen. Samples for calculation of pharmacokinetic parameters were taken during the dosing interval of 12 h which is considerably shorter than the serum half-life of LTG, making our results for $t_{1/2}$ somewhat uncertain. However, the fact that our calculated values for V_d and $t_{1/2}$ at baseline are in good agreement with previous studies in non-pregnant populations suggests that our data are reasonably valid (Cohen et al., 1987; Garnett, 1997; Chan et al., 2001; Milovanovic and Jankovic, 2009).

Renal blood flow

During pregnancy, renal blood flow and glomerular filtration rate increase by 50—80%, starting shortly after conception
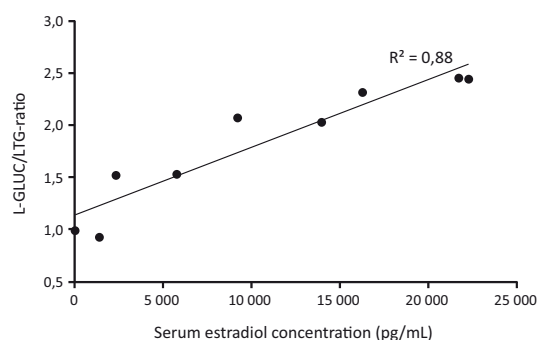
Table 2 Biochemical laboratory parameters at baseline and throughout pregnancy (mean ± SD).

	Baseline	Gestational month								
		2	3	4	5	6	7	8	9
Sodium (mEq/L) ($n=7$–14)	140 ± 1.6	138 ± 1.7	138 ± 1.9	137 ± 1.5	138 ± 1.4	139 ± 2.2	137 ± 1.6	138 ± 1.6	137 ± 1.9
Total bilirubin (mg/dL) ($n=5$–14)	0.40 ± 0.15	0.41 ± 0.18	0.33 ± 0.06	0.30 ± 0.05	0.29 ± 0.06	0.28 ± 0.06	0.29 ± 0.09	0.39 ± 0.07	0.45 ± 0.05
Creatinine (mg/dL) ($n=6$–12)	0.8 ± 0.1	0.7 ± 0.0[*]	0.6 ± 0.0[**]	0.6 ± 0.1[**]	0.5 ± 0.1[**]	0.6 ± 0.1[**]	0.6 ± 0.1[**]	0.6 ± 0.1[**]	0.7 ± 0.0[**]
Estradiol (pg/mL) ($n=6$–14)	41 ± 27	1382 ± 1463	2314 ± 1120	5804 ± 2776	9224 ± 3778	13958 ± 4491	16292 ± 6902	21672 ± 6946	22235 ± 6880
EVF (%) ($n=5$–13)	0.39 ± 0.02	0.39 ± 0.03	0.39 ± 0.02	0.38 ± 0.02	0.37 ± 0.02[*]	0.36 ± 0.02[*]	0.35 ± 0.02[*]	0.35 ± 0.02[*]	0.36 ± 0.02[*]

[*] $p \leq 0.05$ vs. baseline.
[**] $p \leq 0.001$ vs. baseline.
Estradiol not tested.

with persistence throughout the second trimester and with some reduction in late pregnancy. In addition, plasma volume increases as early as 6 weeks after conception, reaching its maximum by trimester 2 and leading to a hemodilution effect (Davison, 1987; Sturgiss et al., 1994; McAnulty et al., 2008). The observed increase in renal clearance (CL_R) of both LTG and LTG-GLUC corresponds well with the above mentioned physiological increase in renal blood flow and may thus account for about one half of the 118% increase of the total clearance (CL) of LTG, suggesting that the other half may be due to induced glucuronidation. Theoretically, impaired gastrointestinal absorption or reduced protein binding (due to lower albumin levels in pregnancy) may also lead to lower total LTG concentrations. However, it is unlikely that these mechanisms are clinically relevant, as previously discussed by others (Tran et al., 2002; Ohman et al., 2008). Moreover, t_{max} in our study was practically unaltered by pregnancy, indicating unchanged intestinal absorption.

Increases in renal blood flow and plasma volume may also explain why both LTG and LTG-GLUC concentrations dropped almost simultaneously in early pregnancy (Fig. 1). Indeed, the decline in serum creatinine by 18% already in month 2 was within the expected range (Kristensen et al., 2007; Klajnbard et al., 2010) and may explain most of the 27% decrease of the LTG serum concentration at that point of time.

Other biochemical parameters

While serum creatinine concentration mainly reflects renal blood flow, EVF is a classical marker for plasma volume. EVF fell gradually, but only by an observed maximum of 11% (achieved first in month 7), while LTG concentrations were reduced by 50% already in month 3. Thus, with respect to the decreased LTG serum concentrations, increased renal blood flow seems to be much more important than possible hemodilution effects. In addition, the fact that not only LTG, but also the LTG-GLUC concentration showed a rapid initial fall, supports the idea that in early pregnancy, increased renal blood flow is more important than induction of UGT activity.

As expected, serum sodium concentrations remained stable throughout pregnancy, whereas total bilirubine (a UGT substrate) declined moderately, although not statistically significant in our study. However, all biochemical laboratory parameters remained within their proposed reference ranges for pregnant women (Jamjute et al., 2009; Klajnbard et al., 2010).

Glucuronidation

After the first trimester, the mean LTG and LTG-GLUC serum concentrations showed only modest changes, possibly because of maximum enzyme induction. Nevertheless, the ratio of the mean LTG-GLUC/LTG serum concentrations continued to increase (Fig. 2). The maximum increase, observed in month 8 is in good agreement with the results of a previous study (Ohman et al., 2008). Moreover, while the proportion of LTG excreted in urine as the unchanged parent drug decreased by 40%, the amount found as the LTG-GLUC metabolite increased by almost the same amount

Table 3   Steady state LTG pharmacokinetics at baseline and at month 8 (mean ± SD).

	Baseline ($n = 6$)	Month 8 ($n = 7$)	Relative change (%)	p-Value
t_{max} (h)	2.0 ± 0.2	2.3 ± 0.7	+15	0.35
C_{max} (mg/L)[a]	14.5 ± 9.2	5.3 ± 4.3	−63	0.04
C_{min} (mg/L)[a]	8.5 ± 3.1	2.2 ± 1.6	−74	0.001
CL (L/h)	1.7 ± 0.7	3.7 ± 1.5	+118	0.004
AUC (mg/L[a]h)[a]	137.3 ± 66.4	43.4 ± 34.2	−68.4	0.009
V_d (L/kg)	1.0 ± 0.1	2.4 ± 0.6	+140	<0.001
$t_{1/2}$ (h)	25.8 ± 5.8	35.5 ± 11.1	+38	0.02

t_{max}: time from intake to maximum serum concentration; C_{max}: maximum serum concentration; C_{min}: minimum serum concentration; CL, total apparent clearance; AUC: area under the curve during one dosing interval; V: apparent volume of distribution; $t_{1/2}$: serum half-life.
[a] Dose-corrected values (standard daily dose of 400 mg) in order to compensate for dose adjustments.

Table 4   Urine data (12-h collected urine, i.e. during one dosing interval).

	Baseline ($n = 6$)	Month 8 ($n = 8$)	Relative change (%)	p-Value
CL_R (L/h)				
LTG	0.09 ± 0.06	0.16 ± 0.06	+77	0.04
LTG-GLUC	2.7 ± 1.3	4.4 ± 1.5	+63	0.03
% of LTG dose appearing in urine as				
LTG[a]	5.5 ± 2.2	2.8 ± 1.5	−40	0.04
LTG-GLUC[a]	65 ± 18	89 ± 23	+37	0.02

CL_R: renal clearance; LTG: lamotrigine; LTG-GLUC: lamotrigine-N2-glucuronide.
[a] Calculated on a mg basis.

(37%; Table 4). These findings strongly suggest increased glucuronidation of LTG during pregnancy.

Estradiol

There was a high correlation between the rising estradiol serum concentrations and the increasing LTG-GLUC/LTG ratio. Although other gestational hormones such as progesterone and HCG also rise during pregnancy, they were not monitored because we were not aware of any evidence for their possible interaction with LTG pharmacokinetics or UGT enzymes. By contrast, endogenous estrogen is known both as a substrate and an inducer of UGT (Chen et al., 2009; Bock, 2010). While the comparatively small physiological fluctuations of endogenous estradiol during a normal menstrual cycle apparently do not seem to affect LTG serum concentrations to a clinically significant degree (Reimers et al., 2006; Wegner et al., 2009), our data as well as results from other studies indicate that the huge increase in estradiol serum levels during pregnancy does (Fig. 3). Animal data showing induction of UGT1A1 and UGT1A4 during pregnancy also support the idea of estradiol-induced LTG-glucuronidation (Chen et al., 2005). Moreover, ethinyl estradiol is a strong inducer of UGT enzymes and may reduce LTG serum levels by over 50% (Shenfield, 1993; Sabers et al., 2003; Reimers et al., 2005a), and hormone replacement therapy with equine estrogens may reduce LTG serum levels by 25—30% (Harden et al., 2006). Taken together, there appears to be sufficient reason to assume that the correlation between increased estrogen levels and increased glucuronidation of LTG is due to causality rather than coincidence.

Study design

The naturalistic setting of this study had its strengths and weaknesses. Participants were volunteering outpatients, and only patients with a history of good compliance were included. Due to the varying opportunity and willingness of the pregnant women to attend to all scheduled visits, the number of data points (n) per parameter measured or calculated ranged from five to 20, which, however, is of the same magnitude as in previous studies on LTG in pregnancy (Tomson et al., 1997; Ohman et al., 2000; Tran et al., 2002; de Haan et al., 2004; Pennell et al., 2004; Petrenaite et al., 2005). Moreover, where comparison with earlier studies (in both pregnant and non-pregnant populations) was possible, our data were in good agreement, indicating their validity.

Conclusions

The rapid, initial fall of LTG and LTG-GLUC serum concentrations in early pregnancy appears to be mainly caused by increased renal blood flow. Later, estradiol-induced increased glucuronidation of LTG seems to predominate, leading to a further fall of LTG serum concentrations and a rise of the LTG-GLUC/LTG-ratio. An expanded volume of distribution may also contribute to lower LTG serum concentrations, although to a minor degree.

The temporal pattern of the decline of LTG serum concentrations should be acknowledged in the management of pregnant women, particularly the marked early first trimester fall. It should also receive attention in the pre-gestational counselling and education of these women.

Acknowledgements

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Paper VI

PHARMACOKINETICS AND DISPOSITION

Lamotrigine in children and adolescents: the impact of age on its serum concentrations and on the extent of drug interactions

**Arne Reimers · Eirik Skogvoll ·
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Abstract

Objective To investigate the impact of age and co-treatment with other drugs on the serum concentrations of lamotrigine in children and adolescents.

Methods A review of routine serum concentration measurements of lamotrigine performed in our laboratory yielded a total of 744 serum samples from 296 subjects (110 males, 186 females, age: 2–19 years) suitable for statistical analysis. The primary outcome variable was the dose-corrected lamotrigine serum concentration, expressed as the lamotrigine concentration/dose (C/D) ratio. A linear mixed model that allowed multiple observations from the same patient was used to identify and quantify the effect of factors influencing the lamotrigine C/D ratio.

Results According to the model, the lamotrigine C/D ratio decreases by 6% per year of age. Valproate and levetiracetam were found to raise the lamotrigine C/D ratio, whereas the following co-medications reduced it: carbamazepine, clobazam, fluoxetine, clonazepam and ethinyl estradiol. The effect of carbamazepine decreased with increasing age. No gender difference was detected.

Conclusions Age is an important factor with respect to the pharmacokinetics and the extent of drug interactions of lamotrigine in children and adolescents. In this population, older individuals will need higher doses than younger ones in order to achieve the same serum concentrations.

Keywords Age · Children · Drug interactions · Lamotrigine · Serum concentration

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Introduction

Lamotrigine is an anticonvulsant drug with efficacy in various kinds of epileptic disorders in adults and in children. It has become a first-choice drug for a variety of seizure types and epileptic syndromes in childhood [1] and is also licensed for the treatment of bipolar disorder [2]. Children tolerate lamotrigine well, although the incidence of rash is higher than in adults [3, 4].

In humans, lamotrigine is not biotransformed by the hepatic cytochrome P-450 (CYP) system. Instead, it is metabolised almost exclusively via glucuronidation by uridindiphosphate glucuronosyltransferase (UGT), mainly UGT1A4. Only 10% of a given dose of lamotrigine is excreted unchanged [5]. Although these properties prevent lamotrigine from drug interactions involving the CYP system, several drugs, including combined oral contraceptives, may

alter lamotrigine serum concentrations considerably, often necessitating dose adjustment [6–9].

Several studies on the pharmacokinetics of lamotrigine in pediatric populations have been published [10–14]. However, infants, children and adolescents do not form a homogenous group since it is well documented that drug metabolism is subject to developmental changes within childhood [15]. This applies particularly to the glucuronidation capacity, which may take from months to years postpartum to reach adult values [16, 17]. Recently, there has been growing interest for such developmental aspects of drug disposition [18]. While the pharmacological concept of clearance is central in this context, serum concentration and dose are more familiar terms for most clinicians [19]. The ratio formed by serum concentration divided by dose (C/D ratio) is essentially equivalent to 1/apparent clearance and thus equally suited to describe the relation between serum concentration and dose.

The aim of this study was to investigate the impact of age and drug interactions on the C/D ratio of lamotrigine, based on a large number of serum samples sent to our routine therapeutic drug monitoring service.

Materials and methods

Collection and analysis of samples

We reviewed all serum samples of patients aged 0–19 years which were analysed for lamotrigine in our laboratory over a period of 40 months. Our request form requires, among other information, statements of exact time for intake of the last dose and time of blood sampling, daily lamotrigine dose, number of daily intakes, diagnosis, co-medication and body weight. Age and gender were obtained from the population registry number on the request form. Unfortunately, body weight was not stated in the vast majority of samples. Samples without information on dose and/or time interval between last dose and sampling were excluded from the analysis. Moreover, samples taken less than 10 h or more than 24 h after intake as well as samples with a serum concentration below the limit of quantification of the analytical method were also excluded. Following these procedures, we obtained a total of 744 samples from 296 subjects aged 2.4 to 19.9 (median: 13.8) years that were suitable for statistical analysis.

All samples were analysed with a liquid chromatography-mass spectrometry (LC-MS) method as described previously [8]. In brief, the calibrated range of this method was 0.5–100 μmol/L. Six quality control samples covering the range from 2 to 45 μmol/L were analysed with every batch of unknown samples. The between-day coefficient of

variation calculated from quality control samples was better than 9% at 2 μmol/L and 5% at 35 μmol/L. The lower limit of quantification was 0.2 μmol/L.

Statistical analysis

Basic descriptive statistic analysis of the raw data was performed with Microsoft Excel 2000 (Microsoft Corp, Redmond, Wash.), and with SPSS ver. 12 for Windows (SPSS Chicago, Ill.). Data are presented as means with standard deviations (SD) or 95% confidence interval (95% CI), or as medians with inter-quartile ranges (IQR) and/or total ranges, as appropriate. The lamotrigine C/D ratio was calculated by dividing the serum concentration of lamotrigine (expressed as μmol/L) by the total daily dose (in mg), and thus expresses the serum concentration per milligram lamotrigine given.

In terms of the elimination phase, we assumed a simple exponential model [5]. The distribution of the lamotrigine C/D ratio was found to be heavily right-skewed, and to achieve near normality, the natural logarithm of lamotrigine C/D ratio [i.e. $\log_e$ (lamotrigine C/D ratio)] was employed as the outcome variable in the analysis.

Multiple samples were often available in the same patient. In order to utilise these data, we employed a linear mixed model that allows correlation between repeated observations [20]. This model assumes that each individual patient possesses a random intercept, i.e. an individual "offset", in addition to being affected by the fixed factors. Model parameters, including variance components, were estimated using the method of restricted maximum likelihood (REML) with the software programme R ver. 2.4.0 [21, 22].

Model estimation proceeded backwards, starting with all potential explanatory variables (gender, age, and co-medications) in the model. At each successive step, the least significant factor was removed, and the model was re-fitted until only statistically significant factors, defined as $p<0.05$, were present in the model. The generalised coefficient of determination, analogous to r^2 in multiple linear regression, which can take values between 0 and 1, was calculated from the residual variance under the null and full model, respectively [23]. According to the model, the expected lamotrigine C/D ratio can be calculated by the following equation:

$$lamotrigine\ C/D\ ratio = e^{\beta_0+\beta_1+\beta_2+...\beta_i}$$

where β_0 represents the overall intercept and β_1 to β_i represent the coefficients of additional fixed factors.

The following co-medication was included in the model: valproate ($n=164$), carbamazepine ($n=95$), topiramate ($n=30$), oxcarbazepine ($n=18$), nitrazepam ($n=14$), clobazam ($n=13$), clonazepam ($n=12$), combined oral contraceptives ($n=$

Table 1 Demographic data and lamotrigine doses in the various age groups

Total number of patients, n	296
Males, n (%)	110 (37)
Females, n (%)	186 (63)
Lamotrigine dose, mg/day (median, range)	
All samples (n=744)	125 (5–850)
Age: 0–4.9 years (n=41)	60 (10–250)
Age: 5–9.9 years (n=177)	75 (5–400)
Age: 10–14.9 years (n=204)	125 (20–750)
Age: 15–19.9 years (n=322)	200 (25–850)

12), diazepam (n=11), levetiracetam (n=9), fluoxetine (n=9) and phenobarbital/primidone (n=8).

Results

Basic demographic and clinical data are given in Table 1. The distributions of dose, serum concentration and lamotrigine C/D ratio were all right-skewed, and values are therefore given as median with the IQR range in parentheses (n= 744): dose, 125 (75–200) mg/day; serum concentration, 13.1 (7.8–19.4) μmol/L; lamotrigine C/D ratio, 0.087 (0.055–0.148) (μmol/L)/(mg/day).

Table 2 gives the values for β_0 (which represents the overall intercept) and for β_1 to β_i (representing the coefficients of additional fixed factors). No effect of gender was detected, but a significant age effect of −0.062 per year of age was estimated (note the logarithmic scale). The minus sign implies that the lamotrigine C/D ratio decreases with increasing age. We present here an example of how the β-coefficients are used: the intercept (β_0) has a value of −1.694. The expected lamotrigine C/D ratio in a 4-year-old child (regardless of gender, and without co-medication) is therefore 0.14 (μmol/L)/(mg/day), as given by

$$\text{lamotrigine C/D ratio} = e^{-1.694+4\times(-0.062)} = 0.14$$

since the value for β_1 is −0.062 (per year of age). In other words, each 1 mg lamotrigine given to a 4-year-old would be expected to raise the serum concentration by 0.14 μmol/L, and a daily dose of 100 mg would yield an expected concentration of 14 μmol/L. In contrast, a 12-year-old child would have a lamotrigine C/D ratio of $e^{-1.694+12\times(-0.062)} = 0.09$ (μmol/L)/(mg/day), and a daily dose of 100 mg would therefore yield a concentration of only 9 μmol/L. This is illustrated in Fig. 1.

If one or more of the other fixed factors were present, the expected C/D ratio would be altered accordingly (Table 2). If, for example, the above-mentioned 4-year-old child also was taking valproate, the expected lamotrigine C/D ratio would be $e^{-1.694+4\times(-0.062)+1.013} = 0.40$ (μmol/L)/(mg/day), with the profound consequence that a daily dose of 100 mg is expected to yield a serum concentration of 40 μmol/L.

Among the 13 drugs included in the initial model, we found not only the classical interacting agents valproate and carbamazepine to have a significant impact on lamotrigine C/D ratio, but also combined oral contraceptives, clobazam, clonazepam, levetiracetam, and fluoxetine (Table 2). Compared to valproate and carbamazepine, the effects of these drugs on the lamotrigine C/D ratio were smaller, and the relatively wide confidence intervals for these effects should
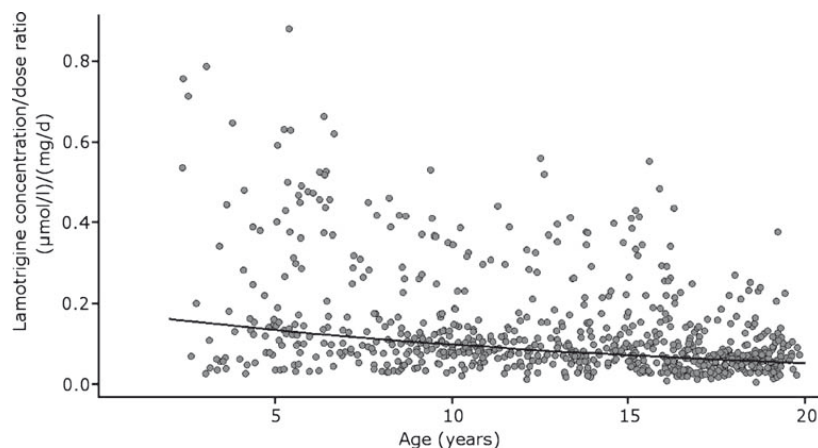
Fig. 1 Lamotrigine serum concentration/dose ratio, expressed as (μmol/L)/(mg/day), according to age (in years). Each *dot* represents one sample. One outlier is removed to enhance visual clarity. The *line* shows the expected effect of age

be noticed. Oxcarbazepine (18 samples) and phenobarbital/primidone (eight samples) did not have statistically significant effects in our model.

The effect of carbamazepine on the lamotrigine C/D ratio was strongly age-dependent, as can be seen from the significant age and carbamazepine interaction term. The positive sign means that the effect of carbamazepine decreases with increasing age. As a final example of the calculation, consider again the 4-year old child, who this time is taking carbamazepine in addition to lamotrigine. The expected lamotrigine C/D ratio now equals $e^{-1.694+4\times(-0.062)-1.138+4\times0.039} = 0.05 (\mu mol/L)/(mg/day)$, and the expected serum concentration at a daily dose of 100 mg would be 5 μmol/L. This means a reduction in C/D ratio by 65%, compared to lamotrigine monotherapy (C/D ratio: 0.14; expected serum concentration at 100 mg/day: 14 μmol/l). Accordingly, a 12-year-old with the same medication would have a C/D ratio of $e^{-1.694+12\times(-0.062)-1.138+12\times0.039} = 0.045$, which means a lower reduction of the lamotrigine C/D ratio by carbamazepine of only 50%, (compared to the C/D ratio in monotherapy: 0.09). No interaction between age and valproate was detected.

For practical reasons, it may be desirable to know the dose necessary to achieve an intended target serum concentration. Using the above equation, and since the C/D ratio equals the serum concentration divided by dose, the required dose D can be found by the equation:

$$D = \frac{C}{e^{\beta_0+\beta_1+\beta_2+...\beta_I}}$$

which is equivalent to:

$$D = C \times e^{-(\beta_0+\beta_1+\beta_2+...\beta_I)}$$

where D is the daily dose in milligrams, and C is the target serum concentration in μmol/L (note the sign change of the exponent after transformation). For example, to achieve a target concentration of 20 μmol/L, an 8-year old individual not taking any of the drugs listed in Table 2 will need a dose of $20 \times e^{-(-1.69+8\times(-0.062))} = 178$ mg/d. If this patient was also taking clonazepam, a dose increase towards 250 mg would be necessary to achieve the same serum concentration, according to the model.

On the log scale, the inter-individual (i.e. between patients) and intra-individual (i.e. within patients) variance components were estimated to be 0.41^2 and 0.35^2, respectively, yielding an intra-class correlation coefficient of 0.58, or 58%. This figure may be interpreted as the percentage of residual variation that can be attributed to individual patients [20].

The generalized coefficient of determination was found to be 0.35; thus, age and co-medication in Table 2 may be interpreted as explaining approximately 35% of the variation in the C/D ratio. Graphical residual analysis revealed no important deviations from the model assumptions of conditional normality or homoscedasticity (not shown).

Discussion

In accordance with established knowledge, we found that valproate was associated with an increased lamotrigine C/D

Table 2 Model parameter estimates, including intercept and explanatory factors, found to influence the lamotrigine serum concentration-to-dose ratio (lamotrigine C/D ratio)

Variable [samples (n); patients (n)][a]	log_e(lamotrigine C/D ratio)			Lamotrigine C/D ratio [(μmol/L) (mg/d)]		
	Value	95% CI	p	Expected value	95% CI	% change vs. intercept
Intercept[b]	−1.694	−1.869; −1.519	<0.0001	0.184	0.154; 0.219	n.a.
Age[c]	−0.062	−0.074; −0.050	<0.0001	0.173	0.171; 0.175	−6
Valproate (164; 75)	1.013	0.910; 1.117	<0.0001	0.506	0.423; 0.606	+175
Carbamazepine (95; 38)	−1.138	−1.454; −0.821	<0.0001	0.061	0.045; 0.083	−68
Clobazam (13; 7)	−0.278	−0.511; −0.044	0.0200	0.139	0.105; 0.185	−24
Levetiracetam (9; 6)	0.324	0.040; 0.609	0.0256	0.254	0.182; 0.355	+38
Fluoxetine (9; 4)	−0.522	−0.879; −0.166	0.0042	0.109	0.073; 0.163	−41
Clonazepam (12; 9)	−0.340	−0.608; −0.071	0.0134	0.131	0.094; 0.181	−29
Ethinyl estradiol (12; 8)	−0.416	−0.682; −0.150	0.0023	0.121	0.088; 0.168	−34
Age : carbamazepine[c]	0.039	0.015; 0.063	0.0017	0.191	0.187; 0.196	+4

The lamotrigine C/D ratio is the expected increase in serum concentration of lamotrigine (in μmol/L) per mg lamotrigine administered daily. The last column displays the percentage change in serum concentration when the respective factor is present. 95% CI=95% confidence interval; n.a.=not applicable

[a] n is the number of samples and patients, respectively

[b] Reference group; i.e. age set to 0 years, taking lamotrigine but none of the other listed medications

[c] The value of the estimate must be multiplied with the age in years

ratio and that carbamazepine and combined oral contraceptives were associated with a decreased lamotrigine C/D ratio. These effects were statistically highly significant. The extent of these interactions were very similar to what has been reported earlier [8, 9, 14, 24], which supports the validity of our present model.

In addition, fluoxetine was identified as a factor reducing the lamotrigine C/D ratio. This result appears to be unexpected, but a study in adult patients [8] found the same effect, and of a similar magnitude. Fluoxetine is generally known as a potent enzyme inhibitor [25]. However, the enzymes known to be inhibited by fluoxetine all belong to the CYP system. Lamotrigine is not metabolized by CYP enzymes, but by UGT. As such, it can not be ruled out that fluoxetine exerts a dual effect on drug-metabolizing enzyme systems such as, for example, ethinyl estradiol. Ethinyl estradiol inhibits the CYP enzymes but induces UGT enzymes [26, 27].

We also found that the benzodiazepines clobazam and clonazepam reduced the lamotrigine C/D ratio, although this effect was comparatively weak. Clonazepam has previously been reported to reduce the serum levels of phenytoin to a small degree [28, 29]. However, other studies have failed to reproduce these findings [30–32], and it should be noted that the confidence intervals of our results were rather wide (Table 2). One may therefore speculate whether some of these findings may have arisen by chance. The same applies for levetiracetam. Levetiracetam has not previously been reported to alter the metabolism of other drugs, and its pharmacokinetic properties provide no good reason for the slightly increased lamotrigine C/D ratio.

Surprisingly, the effects of the enzyme-inducing drugs phenobarbital/primidone and oxcarbazepine did not reach statistical significance ($p=0.89$; 95% CI: −0.394, 0.454 and $p=0.11$; 95% CI: −0.404, 0.039, respectively). However, these drugs were used by a few patients serving with multiple samples, and many of these patients also used valproate. It is well known that the enzyme−inhibiting effect of valproate generally overrides the effects of concomitantly used enzyme-inducers [9, 22, 31]. Thus, these findings are most likely less reliable than the other observations in the present study.

In general, it should be noted that the results of this study do not enable firm conclusions to be drawn on possible causal relationships in terms of drug interactions. Routine data from a TDM service unit has its inherent limitations, mainly from the uncertain reliability and incompleteness of the clinical information accompanying the blood samples. Nevertheless, it is reasonable to believe that the large number of observations to some degree counterbalance these weaknesses.

In the recent years, there has been a growing interest in the ontogenic aspects of pharmacokinetics, and age is, of course,

a crucial factor in this respect [18]. It has been proposed – based on population pharmacokinetic modeling – that lamotrigine pharmacokinetics in children may not be related to age but to body weight [33]. Unfortunately, information on body weight was generally not available in our material. In children, nevertheless, body weight is not an independent variable since it is mainly determined by age [34]. Moreover, age does not determine only body weight, but also organ weight, enzymatic function and regional blood flows [15–18, 35, 36]. Thus, it appears logical to focus on age as a determinant of drug disposition, and we found that age indeed has a highly significant effect on the lamotrigine C/D ratio, resulting in lower dose-normalized lamotrigine serum concentrations in older children.

On the basis of the data presented in Fig. 1, it seems that the variation in the lamotrigine C/D ratio decreases with increasing age. This may be due to a larger variation in the metabolic capacity among younger children compared to older ones as a result of differences in the degree of the maturation of their glucuronidating capacity [37]. The interaction of age with the effects of carbamazepine on the lamotrigine C/D ratio fits very well with this concept. We found that the impact of carbamazepine was most pronounced in younger children and decreased with increasing age. It has long been known that carbamazepine induces the glucuronidation of lamotrigine [9, 11, 14, 24]. Thus, it appears logical that the enzyme-inducing effect of carbamazepine is greater at a younger age, when the baseline glucuronidation capacity is low.

In summary, our results suggest that age influences the C/D ratio of lamotrigine in children and adolescents. This is supported by the finding that the impact of carbamazepine on lamotrigine serum concentration is also age-dependent. In accordance with previous studies, we found that valproate, carbamazepine and combined oral contraceptives had a significant impact on the lamotrigine serum levels in children. Clobazam, clonazepam, levetiracetam, and fluoxetine may also alter the serum levels of lamotrigine, but only to a small degree. Whether these latter findings are real or caused by method artifacts remains to be confirmed.

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