DRUG EPIDEMIOLOGY, INTERACTIONS AND PHARMACOGENETICS

A post-mortem database study

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ACADEMIC DISSERTATION

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Science seeks the truth. And it does not discriminate. For better or worse it finds things out. Science is humble. It knows what it knows and it knows what it doesn't know. It bases its conclusions and beliefs on hard evidence - evidence that is constantly updated and upgraded. It doesn't get offended when new facts come along. It embraces the body of knowledge.

Ricky Gervais

CONTENTS

ABBREVIATIONS

ADI	adverse drug interaction
ADR	adverse drug reaction
ADE	adverse drug event
CNS	central nervous system
CI	confidence interval
CYP	cytochrome P450
CYP2D6	cytochrome P450 enzyme 2D6
CYP2D6	gene encoding CYP2D6
DDD	defined daily dose
DDI	drug-drug interaction
EM	extensive metabolizer
FTI	fatal toxicity index
GC	gas chromatography
HEM	heterozygous extensive metabolizer
HPDI	highest posterior density interval
ICD-10	International Classification of Diseases, 10th revision
IM	intermediate metabolizer
INR	international normalised ratio
LC	liquid chromatography
LOD	limit of detection
LOQ	limit of quantification
MS	mass spectrometry
NSAID	non-steroidal anti-inflammatory drug
N-VEN	*N*-desmethylvenlafaxine
O-VEN	*O*-desmethylvenlafaxine
PCR	polymerase chain reaction
PM	poor metabolizer
SFINX	Swedish, Finnish, INteraction X-referencing
SNRI	serotonin and norepinephrine reuptake inhibitor
SSRI	selective serotonin reuptake inhibitor
TCA	tricyclic antidepressant
TOF	time-of-flight
UM	ultra-rapid metabolizer
VEN	venlafaxine
WHO	World Health Organization

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following articles, which are referred to in the text by Roman numerals **I**-**IV**:

I Launiainen T, Vuori E, Ojanperä I. Prevalence of adverse drug combinations in a large post-mortem toxicology database. *Int J Legal Med* 2009; 123:109-115.

II Launiainen T, Sajantila A, Rasanen I, Vuori E, Ojanperä I. Adverse interaction of warfarin and paracetamol: evidence from a post-mortem study. *Eur J Clin Pharmacol* 2010; 66:97-103.

III Launiainen T, Rasanen I, Vuori E, Ojanperä I. Fatal venlafaxine poisonings are associated with a high prevalence of drug interactions. *Int J Legal Med* 2011; 125:349-358.

IV Launiainen T, Broms U, Keskitalo-Vuokko K, Pitkäniemi J, Pelander A, Kaprio J, Ojanperä I. Nicotine, alcohol and drug findings in young adults in a population-based post-mortem database. *Nicotine Tob Res* 2011; Published online 21 April DOI 10.1093/ntr/ntr069.

Related electronic supplementary material is included in this thesis.

ABSTRACT

Multi-drug use increases the risk of drug-drug interactions, giving rise to an often predictable and preventable subgroup of adverse drug reactions. Ageing of the population and the growing number of available drugs emphasise the importance and awareness of drug interactions in both clinical and forensic work. At its best post-mortem forensic toxicology analysis produces high-quality data that can be used in studies on pharmacoepidemiology and substance abuse. In this thesis, the data stored in a comprehensive forensic toxicology database was combined with information from death certificates, and a commercial drug interaction database was used to identify interacting drugs. A new GC-MS method for routine screening of acidic and neutral drugs was developed and additional metabolite and genotype analyses were performed to complete the profile of selected cases. Previous inclusion of nicotine use markers in routine urine analysis allowed the estimation of nicotine use prevalence in young adults in the database and subsequent comparison of the nicotine and non-nicotine users groups.

The incidence of drug combinations possessing serious adverse drug interactions was generally low (0.71%), but it was notable for the two individually studied drugs, a common anticoagulant warfarin (33%) and a new-generation antidepressant venlafaxine (46%). Serotonin toxicity and adverse cardiovascular effects were the most prominent possible adverse outcomes. However, the specific role of the suspected adverse drug combinations was rarely recognized in the death certificates. The frequency of bleeding was found to be elevated when paracetamol and warfarin were used concomitantly. The contraindicated use of non-steroidal anti-inflammatory drugs along with warfarin therapy was rare. Pharmacogenetic factors did not play a major role in fatalities related to venlafaxine, but the presence of interacting drugs was more common in cases showing high venlafaxine concentrations. Venlafaxine was used in suicides more often than SSRIs or mirtazapine. Nicotine findings in deceased young adults were roughly three times more prevalent than the estimated smoking frequency in those still alive. A majority of substance abusers and users of psychopharmaceutical drugs had also used nicotine. No difference in the proportion of suicides was observed between nicotine users and non-nicotine users, but there were fewer natural deaths in the nicotine users group.

The possibility of drug interactions and pharmacogenetic issues should be taken into account in cause-of-death investigations, especially in unclear cases, suspected medical

malpractice, and cases where toxicological findings are scarce. Electronic databases make it easier to handle the steadily growing volume of drug interaction data. A sufficiently high autopsy frequency and broad-spectrum post-mortem toxicology were the prerequisites for gathering comprehensive data that can also be used in epidemiological studies to promote drug safety and public health.

INTRODUCTION

Sales and use of drugs are on the increase worldwide as populations are ageing in many developed countries and new drugs are constantly emerging. Simultaneous use of multiple drugs is daily life for many patients and elderly people. Multi-drug use predisposes to adverse drug interactions, a problem that could often have been avoided beforehand with appropriate prescribing. However, evaluation and control of an individual's prescription drug assortment can be challenging due to scattering of medical and prescription records, while self-reported inventories may also be incomplete. Even patients under hospital care may be treated with adverse drug combinations, and use of over-the-counter drugs further complicates assessment of the risk of interactions. Fortunately, the vast amount of research data about non-recommended drug combinations has now been assembled in compact form within interaction databases that can be easily browsed by health care professionals.

Prescription drugs with abuse potential, such as opioids and benzodiazepines, are being increasingly distributed and abused along with illicit drugs. Furthermore, abusers might not even be aware of the real contents of the preparation they are taking. Besides illicit substances, tobacco and alcohol dependence are known to be interconnected with mental health problems and increased mortality. Interviews, surveys and questionnaires might not give a realistic view of substance abuse problems due to poor participation and reliability.

Post-mortem pharmacoepidemiology can be used to promote drug safety and public health. It allows problems in prescribing practices, drug use and trends in substance abuse to be detected. Advances in analysis methods are leading to more comprehensive screenings with lower limits of detection and subsequently to an ever increasing number of toxicological findings. New drugs and multi-drug use pose challenges for the interpretation of the forensic toxicology results. The general purpose of this thesis is to explore the information stored in the post-mortem toxicology database, together with added new experimental analytical and pharmacogenetic data on specific substances (warfarin, venlafaxine). The findings are expected to provide a better understanding of drug use practices and to highlight the role of drug interactions in cause-of-death investigations.

REVIEW OF THE LITERATURE

1 Pharmacotherapy

There is no such thing as a potent and safe drug. Besides the expected outcome, a drug that has a desired effect on the body is also able to introduce adverse effects. The outcome of drug therapy is influenced not just by appropriate choice of drug and dosage, but also by numerous other factors like compliance, adherence, external factors (e.g. diet, other drugs, alcohol and smoking), diseases (e.g. liver, kidney and heart conditions) and genetic variations in response to drugs (pharmacogenetics). It has been estimated that only half of patients take their drugs as prescribed (Evans and Spelman 1983). Patients may not purchase or take their drugs, or they might discontinue their use. On the other hand, people use drugs that are not prescribed for them: in a survey conducted in the United States, every fourth responder admitted to borrowing or sharing their prescription medication (Petersen et al. 2008). Prescription drugs might also be used concomitantly with over-the-counter drugs or drugs from previous therapies without consulting a pharmacist or a doctor. Furthermore, the use of herbal or complementary products is considered 'natural' and safe and is seldom reported to the prescribing doctor or in the medical records (Eisenberg et al. 1993; Cohen et al. 2002). Prescription drugs are also widely abused (Zarocostas 2007) and they can be used to enhance the effect of illicit drugs (Vuori et al. 2003).

1.1 Adverse drug reactions and events

According to the World Health Organization (WHO), adverse drug reaction (ADR) is defined as an unintended response to a drug occurring at a conventional dose used for disease in prophylaxis, diagnosis, therapy or for modification of physiological functions. This definition excludes therapeutic failures, non-compliance and intentional or accidental poisonings (WHO, factsheet N°293). The term adverse drug event (ADE) can be defined as ADRs and/or events caused by medication errors (Bates et al. 1993). ADRs are commonly divided into type A and type B reactions (Pirmohamed et al. 1998). Type A reactions are related to the pharmacological effect of the drug, and are dose-related, predictable, common and less serious, while Type B reactions are unexpected, bizarre, non-dose-related, rare and more serious and are often due to hypersensitivity reactions. ADRs have been further categorised as dose-related accumulation reactions (C), delayed reactions (D), withdrawal reactions (E) and unexpected failure of therapy (F) (Edwards and Aronson 2000). The definitions enable national ADR data to be gathered for the ADR report database of the Uppsala Monitoring Centre (UMC) from member countries of WHO's International Drug Monitoring Programme.

ADRs are a notable cause of morbidity and mortality. Besides the harm experienced by individuals, ADRs result in significant costs to society. Recent systematic reviews have estimated that approximately 5% of hospital admissions are related to ADEs and elderly people in particular have a high risk of ADEs and hospitalisation as they often use several prescription drugs simultaneously and have multiple diseases (Beijer and de Blaey 2002; Kongkaew et al. 2008). The most commonly observed ADRs are gastrointestinal lesions and bleeding, central nervous system (CNS) bleeding and other bleeding, renal dysfunction and cardiovascular disorders (Pirmohamed et al. 2004; Wester et al. 2008). Although most ADRs are relatively mild, fatal ADRs do occur. A prospective Scandinavian study of patients of internal medicine resulted in a fatal ADE incidence of 9.5 per 1000 hospitalised patients (Ebbesen et al. 2001). A large meta-analysis conducted in the United States and a single Finnish hospital study found fatal ADR frequencies of 4.6% and 5%, respectively, in deceased hospitalised patients (Lazarou et al. 1998; Juntti-Patinen and Neuvonen 2002). In Swedish post-mortem studies approximately 3% of all deaths were assigned to fatal ADRs (Wester et al. 2008), and 1% of the deaths in a Swedish population were considered to have been caused by suspected fatal ADRs and fatal poisonings that could have been prevented (Jönsson et al. 2010). The drugs most commonly implicated in fatal ADRs are antithrombotic drugs, non-steroidal anti-inflammatory drugs (NSAIDs), antidepressants and cardiovascular drugs (Wester et al. 2008).

1.2 Drug-drug interactions

Multi-drug use is a well recognized and growing problem that poses a challenge in the treatment of the elderly and patients with long-term illnesses (Lampela et al. 2007; Hovstadius et al. 2010). It predisposes to adverse drug interactions (ADIs), which are a significant but often predictable and avoidable class of ADRs (Mozayani and Raymon 2003; Baxter 2005). A drug-drug interaction (DDI) can sometimes be beneficial, e.g. penicillin can be administered together with probenecid, which inhibits the renal excretion of the antibiotic and thus extends its half-life in plasma (Kampffmeyer et al. 1975). Most of the known DDIs are harmful and can lead not only to unwanted and even toxic reactions but also to loss of therapeutic effect. These latter interactions are harder to detect because the lack of efficacy can be explained by other factors such as poor compliance. In a study conducted among elderly outpatients, the risk of a subtherapeutic effect as a potential DDI was as common as the risk of noxious reactions (Björkman et al. 2002). Besides drugs, important interactions may occur with other pharmacologically active agents such as alcohol and nicotine (Koski et al. 2003; Koski et al. 2005a; Koski et

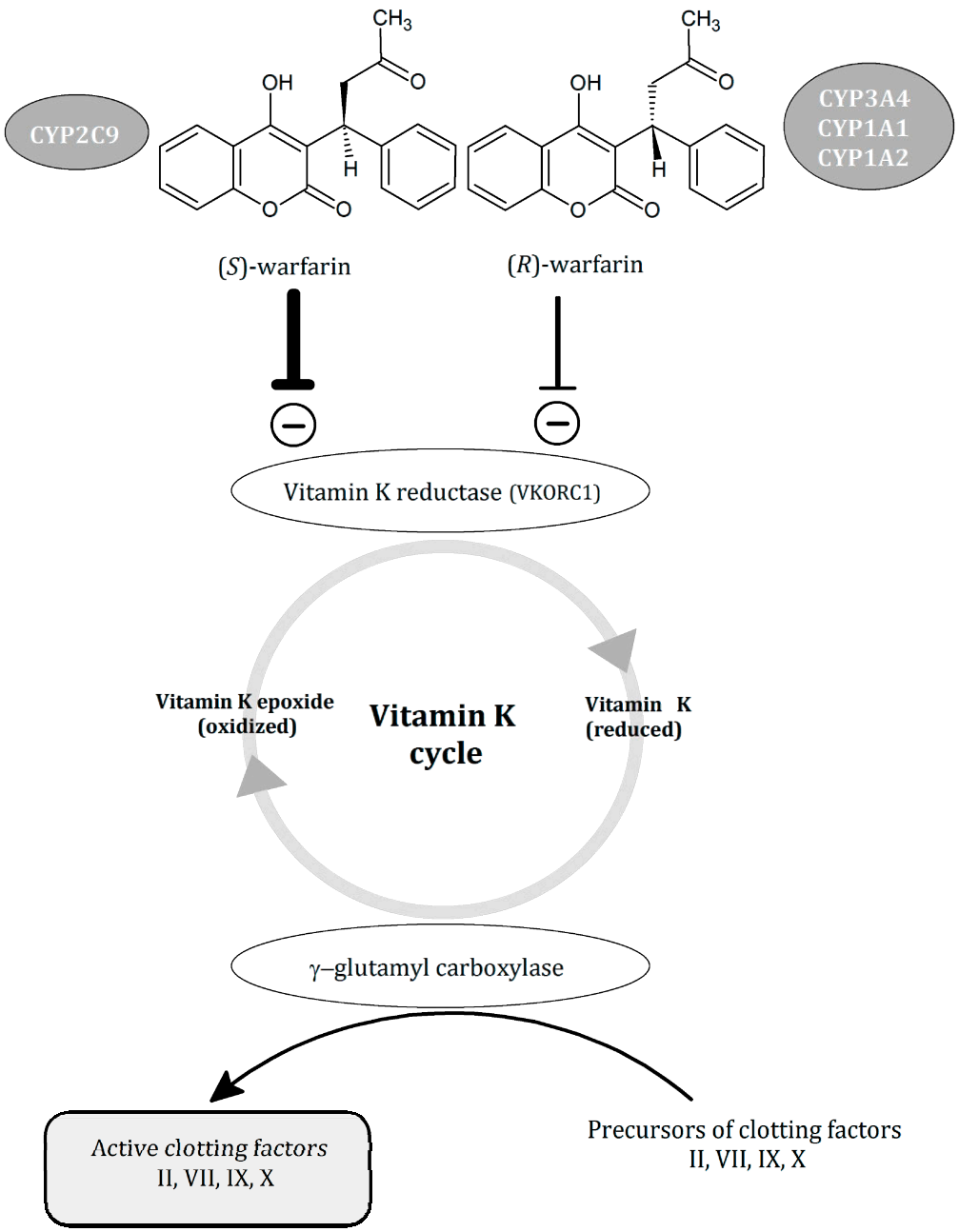

Figure 1. Metabolism and site of action of warfarin (modified from Schwartz and Stein 2006). Warfarin prevents the recycling of vitamin K by inhibiting vitamin K reductase. Vitamin K is a co-substrate needed for the γ-carboxylation-mediated activation of clotting factors, and inhibition by warfarin leads to anticoagulation. Warfarin is administered as a racemic mixture: the (*S*)-enantiomer is more potent than the (*R*)-enantiomer and they are metabolized by different enzymes.

al. 2005b; Kroon 2007). The prevalence of different ADIs has been studied in hospital and pharmacy records and in spontaneous reporting databases (Peng et al. 2003; Chen et al. 2005; Åstrand et al. 2006; Strandell et al. 2008). Studies done with post-mortem material are rather scarce and involve limited numbers of cases and/or drugs (Neuvonen et al. 1993; Preskorn and Baker 1997; Rogde et al. 1999; Preskorn 2002; Vuori et al. 2003). No systematic post-mortem surveys have so far been conducted.

ADIs can have either a pharmacokinetic or a pharmacodynamic mechanism, and some drug combinations possess both. A pharmacokinetic interaction can lead to concentration changes (increase or decrease) by altering the absorption, protein binding, distribution, metabolism or excretion of the drug. In a pharmacodynamic interaction the effect of the drug is changed by the presence of another drug. This can occur at receptor level or, more indirectly, involve interference with physiological mechanisms. Besides additive or synergistic effects and combined toxicity, antagonistic or opposing effects are also possible. Drug metabolism can be affected by inhibition or induction of the metabolizing enzymes. Inhibition can manifest itself fast as it blocks existing enzyme molecules. This can lead to elevated substrate concentration, or in the case of a pro-drug, to decreased production of an effective metabolite. The effect of induction of gene expression becomes evident (and disappears) more slowly as it requires production of new enzyme molecules. Induction can lead to a decrease in substrate concentration and subsequently to an insufficient therapeutic effect, but also to an increase in the concentration of effective metabolite from a pro-drug.

1.2.1 Interactions of warfarin

Warfarin (*Marevan*®) is a good example of a widely used drug possessing a large amount of possible clinically important ADIs by several mechanisms (Seymour and Routledge 1998; Holbrook et al. 2005). Warfarin is an oral anticoagulant that exerts its effect by lowering the amount of active vitamin K available for the activation of clotting factors II, VII, IX and X (Figure 1). It is used to control blood coagulation in numerous conditions such as chronic atrial fibrillation, prosthetic heart valves, venous thromboembolisms and coronary artery disease (Hirsh et al. 2001). Too little anticoagulation can lead to thrombosis and too much anticoagulation to bleeding. Bleeding tendency is also affected by risk factors such as diet, advanced age, liver failure, smoking and alcohol consumption (Delaney et al. 2007; Shalansky et al. 2007). The dose required to obtain suitable anticoagulation is highly patient-specific and requires monitoring of the international normalised ratio (INR) value, which is calculated from the time of clot formation *in vitro*. The therapeutic range of warfarin is narrow (1-3 mg/l for plasma) (Schulz and Schmoldt 2003) and the target INR value for most clinical indications is 2.0–3.0 (Hirsh et al. 2001).

Table 1. Examples of clinically significant drug-drug interactions of warfarin

Interaction type	Interacting drugs	Probable mechanism	Effect on anticoagulation
Kinetic	Cholestyramine Cholestipol	Impaired absorption, increased elimination	Reduction
	Carbamazepine Phenytoin Primidone Rifampicin	Induction of metabolism	Reduction
	Amiodarone Fluconazole Metronidazole	Inhibition of metabolism	Increase
Dynamic	NSAIDs Acetylsalicylic acid	Additive effect	Increase
	Vitamin K	Antagonism	Reduction

(Modified from Seymor and Routledge 1998)

Concurrent use of interacting drugs has been reported to result in a 3-4.5 times higher risk of bleeding (Gasse et al. 2005). Examples of interactions with commonly used drugs are listed in Table 1. The metabolism of warfarin can be inhibited and induced, and drugs like acetylsalicylic acid and NSAIDs can affect the haemostasis by impairing platelet function. Warfarin is also extensively (97%) bound to albumin (Osselton et al. 1980) and is thus susceptible to changes in the concentration of free drug in the plasma, e.g. in the case of competitive binding by another drug, but this mechanism is not considered to be of clinical significance (Hersh et al. 2007). Dietary vitamin K and complementary and alternative medicine products can also interfere with warfarin therapy (Greenblatt and von Moltke 2005; Shalansky et al. 2007).

NSAIDs and acetylsalicylic acid are not recommended to be used concomitantly with warfarin, but as over-the-counter drugs they are easily available and their concomitant use is hard to prevent or study without software-based electronic warning systems in pharmacies (Heikkilä et al. 2006). Few reports of possible adverse tramadol-warfarin interaction cases have been published (Scher et al. 1997; Sabbe et al. 1998; Hedenmalm et al. 2004; Dumo and Kielbasa 2006). Tramadol is a common analgesic available on prescription in Finland. It acts as a partial μ-opioid receptor agonist but also inhibits reuptake of noradrenalin and serotonin, and the latter property might impair platelet aggregation and increase the risk of bleeding (de Abajo et al. 1999; Dalton et al. 2003).

Paracetamol (acetaminophen) is the first-line drug for pain relief for warfarinized patients (Toes et al. 2005). The presumed safety of this drug combination has been challenged after numerous publications of ADRs and increases in INR values (Hylek et al. 1998; Whyte et al. 2000; Andrews 2002; Gebauer et al. 2003; Mahe et al. 2005; Ornetti et al. 2005; Mahe et al. 2006; Parra et al. 2007). The interaction mechanism remains unclear although several candidates have been proposed (Lehmann 2000; Whyte et al. 2000; Gebauer et al. 2003; Thijssen et al. 2004). Conversely, some studies have shown paracetamol to have no enhancing effect on anticoagulation by warfarin (Kwan et al. 1999; Gadisseur et al. 2003).

2 Pharmacogenetics

Genetic variations caused by inherited differences in nucleotide sequences in DNA contribute to interindividual differences in the response to drugs. Variation in the genes encoding the proteins involved in drug response, e.g. enzymes, transporters and receptors, can affect adsorption, distribution, metabolism and elimination by producing a functionally altered protein or a change in the amount of the protein. Genotypic variations in enzyme-coding genes result in differences in phenotypic enzyme activity. These phenotypic profiles can be categorized as poor metabolizer (PM), intermediate metabolizer (IM), extensive metabolizer (EM) and ultra-rapid metabolizer (UM) (Zanger et al. 2004) (See Table 2 for details). Another category termed heterozygous extensive metabolizer (HEM) has been introduced to describe EMs carrying one active and one inactive allele (Hermann et al. 2008).

2.1 CYP enzymes

Xenobiotic metabolism, which includes drug metabolism, protects the body against the potentially harmful effects of foreign compounds. The reactions used to accomplish this task by increasing the water solubility of the substrates can be divided into

Table 2. Classification of phenotypes based on genotypes and their predicted clinical outcomes

Phenotype	Genotype	Enzyme activity	Substrate	Therapeutic response
Poor metabolizer (PM)	or	None	Active parent drug Prodrug	Excessive Failure
Intermediate metabolizer (IM)	or	Reduced	Active parent drug Prodrug	Excessive Reduced
Extensive metabolizer (EM)	or or	Normal	Active parent drug Prodrug	Expected Expected
Ultarapid metabolizer (UM)	... or	Excessive	Active parent drug Prodrug	Failure Excessive

whole gene deletion; partially active allele; increased activity allele
inactive allele; active allele

(Modified from Zanger et al. 2004 and Pilgrim et al. 2010b)

phase I and phase II reactions. In phase I reactions (oxidation, reduction, hydrolysis) the functional groups of the substrate are modified, and in phase II reactions the compound is conjugated with highly polar endogenous agents such as glucuronic acid. The superfamily of cytochrome P450 (CYP) enzymes is the most important metabolic system of phase I metabolism (Danielson 2002). CYP enzymes are expressed mainly in the liver, but also in extra-hepatic tissues such as the mucosa of the small intestine, kidney, brain and lung (Krishna and Klotz 1994). So far, 57 human CYP isoenzymes have been detected and classified into 18 families (Nelson 2009). Besides being remarkably polymorphic genes, *CYPs* also show interethnic variation (Bradford 2002; Home Page of the Human Cytochrome P450 (*CYP*) Allele Nomenclature Committee 2008).

Table 3. Examples of substrates and inhibitors of CYP2D6

Substrates	**Inhibitors**
Analgesics, antitussives Codeine, dextromethophan, ethylmorphine, oxycodone, tramadol	
Antiarrhythmics Flecainide, mexiletine, procainamide, propafenone	*Antiarrhythmics* Flecainide, propafenone, quinidine
Antidepressants Amitriptyline, doxepin, fluoxetine, fluvoxamine, imipramine, maprotiline, mianserin, nortriptyline, paroxetine, venlafaxine	*Antidepressants* Amitriptyline, bupropion, doxepin, fluoxetine, nortriptyline, paroxetine, reboxetine, sertraline, trimipra
Antiemetics Ondansetron, tropisetron	*Antifungal* Tebinafine
Antiestrogen Tamoxifen	*Antimalarial* Chloroquine, primaquine
Antipsychotics Haloperidol, perphenazine, risperidone, thioridazine, perphenazine, thioridazine zuclopenthixol	*Antipsychotics* Haloperidol, levomepromazine,
ß-blockers Metoprolol, propranolol, timolol	**Inducers**
Stimulant MDMA *(Ecstasy)*	None described

(Rendic 2002; SFINX 2009)

2.1.1 CYP2D6

CYP2D6 is responsible for the metabolism of many commonly used drugs including opioid analgesics, antidepressants, neuroleptics, β-blockers and antiarrhythmics (Ingelman-Sundberg 2005) (Table 3). *CYP2D6* is highly polymorphic; more than 70 different alleles have been reported, and the variants include copy number variations, single nucleotide substitutions and frameshift, insertion and deletion mutations (Home Page of the Human Cytochrome P450 (*CYP*) Allele Nomenclature Committee 2008). The genetic variation of *CYP2D6* has considerable phenotypic effects (Kirchheiner et al. 2004). There are several known CYP2D6-inhibiting drugs, but unlike other drug-metabolizing CYPs, no inducers have been described for *CYP2D6* (Table 3). Based on population genotyping studies it has been estimated that 5-10% of the European population are PMs (Bradford 2002; Ingelman-Sundberg et al. 2005; Sistonen et al. 2009) and ~3% are UMs (Dahl et al. 1995; Sachse et al. 1997; Sistonen et al. 2007). However, there are indications that genotyping for duplicated alleles explains only a fraction (10-30%) of the UM phenotypes in Caucasians (Løvlie et al. 2001).

2.1.2 CYP3A4

CYP3A4 is the most abundant P450 isoenzyme in the liver and has the widest range of substrates, including prescription drugs and endogenous substrates such as steroid hormones (Shimada et al. 1994; Guengerich 1999; Anzenbacher and Anzerbacherova 2001). Thus numerous drug interactions are CYP3A4 related (Rendic 2002). Furthermore, the CYP3A4 activity of the intestinal wall can be down-regulated by diet constituents such as grapefruit juice, the interaction leading to an increase in the oral bioavailability of substrate drugs (Bailey et al. 1991; Lown et al. 1997). Initially *CYP3A4* was thought not to be influenced by polymorphisms, but recent studies have shown otherwise (Keshava et al. 2004). However, CYP3A4 expression has been observed to demonstrate great interindividual variation that has not been successfully explained by common *CYP34A* variants (Lamba et al. 2002; Lamba et al. 2010).

2.2 Clinical pharmacogenetics

Predicting phenotype from genotype provides a way of optimising drug dose for each patient and for identifying patients that have a specific risk of suffering adverse drug effects or therapeutic failure. The traditional classification of phenotypes and the expected therapeutic outcomes of drug therapy are summarized in Table 2. Translation of genotype data into phenotype data is still challenging for several reasons (Gaedigk et al. 2008). Usually only the PM phenotype represents a separate subgroup as substantial

overlap exists between other phenotype groups (Zanger et al. 2004). Drugs may also have complementary metabolic pathways preventing accumulation. Furthermore, the apparent phenotype can be altered by co-medication possessing pharmacokinetic interactions; inhibitors can lead to mimicking of the PM phenotype, and inductors can accelerate the metabolism towards that of UMs.

Clinically important polymorphisms are seen, e.g. with genes coding for CYP2D6, CYP2C9 and CYP2C19 (Johansson and Ingelman-Sundberg 2010). CYP2D6 catalyzes the activation reaction of tamoxifen, a pro-drug used in breast cancer therapy. An alternative therapy can be considered for a predicted PM or IM who would not benefit much from this drug therapy (Goetz et al. 2008). CYP2D6 and CYP2C19 have been shown to affect the pharmacokinetics of many commonly used antidepressants and antipsychotic drugs, and drug-specific guidelines are available for dose adjustments (Kirchheiner et al. 2004).

The genetic factors affecting the dose of warfarin required for optimal anticoagulation include variation at *CYP2C9* and the warfarin target receptor (VKOR) gene *VKORC1*, but it has been estimated that together they account for only 35% of the variance in the dose (Au and Rettie 2008). Although algorithms have been generated for estimating the suitable individual warfarin dosage (Gage et al. 2008), predictive genetic testing does not obviate the need for INR follow-ups and patient guidance.

Myopathy is a plasma concentration-dependent ADR of the widely used lipid-lowering drugs called statins. A common genetic variant of the *SLCO1B1* gene, which encodes a transporter mediating the hepatic intake of statins, can have a profound and statin-specific effect on the pharmacokinetics and, at worst, the increase in plasma concentration can lead to life-threatening rhabdomyolysis. In order to reduce this risk, genotype-specific recommended maximum doses are now available for each statin (Niemi 2010).

2.3 Venlafaxine metabolism and pharmacogenetics

Venlafaxine (*Efexor*®) (VEN) is a new generation antidepressant that inhibits the reuptake of serotonin, noradrenalin, and to a lesser degree dopamine (Holliday and Benfield 1995). It is metabolized mainly to its active metabolite *O*-desmethylvenlafaxine (O-VEN) by CYP2D6 (Figure 2). CYP3A4 (and possibly CYP2C19 and CYP2C9) catalyzes the demethylation of venlafaxine to its minor metabolite *N*-desmethylvenlafaxine (N-VEN) (Otton et al. 1996; Fogelman et al. 1999). Both metabolites are further demethylated to *N,O*-venlafaxine (Ereshefsky and Dugan 2000). Besides the parent drug, only O-VEN has been shown to have similar pharmacological activity to the parent drug. Recently O-VEN

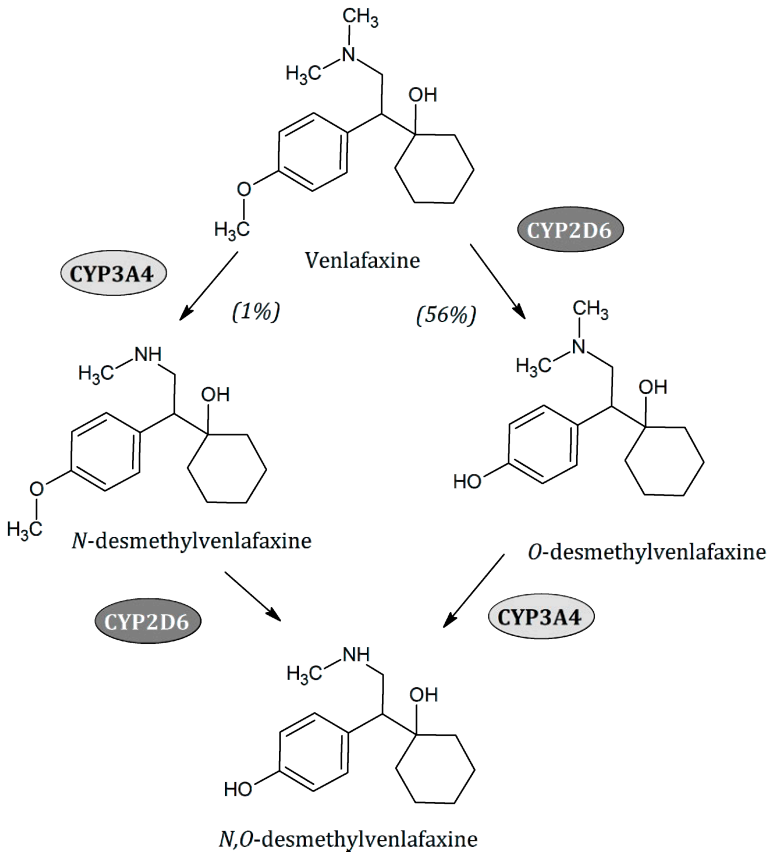

Figure 2. Outline of venlafaxine phase I metabolism (modified from Ereshefsky and Dugan 2000). The parent drug and metabolites are excreted predominantly in urine (free or conjugated) in the following proportions: 1-10% unchanged drug, 30% *O*-desmethylvenlafaxine, 6-19% *N,O*-desmethylvenlafaxine and 1% *N*-desmethylvenlafaxine. The half-life of venlafaxine in plasma is 4 h and of *O*-desmethylvenlafaxine 10 h (Moffat et al. 2004).

has been introduced onto the pharmaceutical market as a distinct formulation (*Pristiq*®, desvenlafaxine) (Deecher et al. 2006; Preskorn et al. 2009), but the clinical benefits of this metabolite drug have not convinced all researchers (Sopko et al. 2008). Venlafaxine is administered as a racemic mixture; the *S*(-)-enantiomer inhibits both noradrenalin and serotonin uptake, while the *R*(+)-enantiomer inhibits primarily serotonin uptake (Holliday and Benfield 1995). CYP2D6 has been shown to exhibit stereoselectivity towards the *R*(+)-enantiomer (Eap et al. 2003). A correlation between *CYP2D6* genotype and the metabolic ratio of VEN to O-VEN has been observed in several clinical studies (van der Weide et al. 2005; Whyte et al. 2006; Preskorn et al. 2009).

In a study of psychiatric patients whose medication was primarily dependent on CYP2D6 mediated metabolism, the number of ADRs was observed to be higher and the duration of hospitalization longer in patients exhibiting PM phenotype (Chou et al. 2000). More specifically, it has been proposed that PMs could be more susceptible to ADRs caused by VEN (Lessard et al. 2001; Langford et al. 2002; Shams et al. 2006). Reports have been published of cardiac toxicity (Howell et al. 2007; Megarbane et al. 2007; Bosse et al. 2008), hyponatremia (Egger et al. 2006; Roxanas et al. 2007), serotonin syndrome and seizures (Whyte et al. 2003; Kelly et al. 2004) and rhabdomyolysis (Wilson et al. 2007). ADRs have been found to lead to a higher risk of discontinuation of VEN drug therapy (Stahl et al. 2005; Gartlehner et al. 2008; Weinmann et al. 2008). UM status has also been connected with an increased incidence of therapeutic failure (Kawanishi et al. 2004). PMs are not affected by inhibitors of CYP2D6, but they might be more susceptible to inhibition or induction of the normally minor pathway via CYP3A4 (Ereshefsky and Dugan 2000).

2.4 Post-mortem pharmacogenetics

In 1999 it was shown that CYP genotyping was possible in post-mortem blood material (Druid et al. 1999), and since then pharmacogenetics has successfully been used in cause-of-death investigations. Poor drug metabolism can lead to accumulation of the drug or even fatal intoxications, as illustrated by the case of a nine-year-old boy who died of fluoxetine intoxication. The cause-of-death examination revealed a completely defective CYP2D6 genotype, resulting in compromised ability to metabolise fluoxetine, a CYP2D6 substrate (Sallee et al. 2000). In addition the child was prescribed an increasing dose of fluoxetine despite a long period of symptoms of toxicity. A defective CYP2D6 genotype has also been associated with a fatal doxepin poisoning (Koski et al. 2007a). In contrast to these cases, two studies of fatal drug intoxications reported underrepresentation of PMs (Holmgren et al. 2004; Zackrisson et al. 2004). In the case of a pro-drug, ultra-rapid metabolism can lead to accumulation of the active compound, and reports of accumulation of morphine after administration of codeine have been published (Gasche et al. 2004; Koren et al. 2006).

Post-mortem sample material has been used to show a correlation between genotype and phenotype, i.e. parent drug to metabolite ratios, even in the presence of other possible confounding factors such as multi-drug use (Levo et al. 2003; Koski et al. 2006). Post-mortem pharmacogenetics is not limited to drug metabolism, and some fatalities might be explained by mutations in the long QT syndrome genes that predispose to cardiac arrhythmia (Lunetta et al. 2003). Despite the available analysis methods and

the intriguing cases published so far, post-mortem pharmacogenetics is not yet widely utilized in cause-of-death investigations (Sajantila et al. 2010).

3 Epidemiology and public health

3.1 Pharmacovigilance and -epidemiology

Pharmacovigilance is defined as the science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other drug-related problem (WHO). It is a known fact that rare or delayed adverse effects seldom manifest themselves in the small population of healthy young volunteers who participate in the relatively short clinical testing periods of new drugs (Amery and ISPE 1999). Consequently, new drugs may be withdrawn from the market after accumulation of data on ADRs, as happened recently with rofecoxib (*Vioxx*®) (Bottone and Barry 2009). Pharmacoepidemiology may be defined as the non-experimental study of the use and effects of drugs in large numbers of people. Pharmacovigilance and pharmacoepidemiology will never succeed in preventing ADRs but they do provide an effective means of detecting them, at the same time educating both health care professionals and the public, which, it is hoped, will lead to better use of medication. Successful examples of improved drug safety include tightened prescription regulations for dextropropoxyphene (*Abalgin*®) after related fatalities (Smith et al. 1979) and replacement of barbiturates by the less hazardous benzodiazepines (Stead et al. 1981; Osselton et al. 1984).

The burden of ADRs and ADEs to society is hard to estimate. Spontaneous reporting databases suffer from underreporting of ADRs (Heeley et al. 2001; Jönsson et al. 2006; Narum et al. 2011) and incidence data is mostly available from hospitalized patients (Lazarou et al. 1998). Even in clinical settings ADRs are difficult to identify, and it has been reported that elderly patients might ignore ADRs, considering them to be an unavoidable part of normal ageing (Lampela et al. 2007). The incidence of ADIs is even harder to estimate, and cases where the outcome is therapeutic failure are particularly hard to recognise. The use of contraindicated drug combinations may sometimes be a justified and calculated procedure offset by dose adjustments or close monitoring. Furthermore, not everyone who uses a potentially adverse drug combination suffers an adverse reaction, and it is difficult to predict which patients will ultimately experience an adverse outcome (Seymour and Routledge 1998). Clinical studies have evaluated the nature and prevalence of ADIs by extracting overlappings in prescription databases (Peng et al. 2003; Chen et al. 2005; Åstrand et al. 2006; Heikkilä et al. 2006) or co-

reporting in ADR report databases (Strandell et al. 2008; Strandell and Wahlin 2011). Electronic drug interaction databases and software have been created to help health care professionals avoid erroneous prescriptions and to manage the constantly accumulating data on drug interactions (Grönroos et al. 1997; Indermitte et al. 2007; Strandell et al. 2008).

3.2 Substance abuse

Substance abuse is usually considered to involve alcohol and illicit drugs, but many prescription drugs are also abused (Zarocostas 2007). Prescription drugs are easily accessed through "doctor shopping" or over-prescribing or by diverting substitution treatments, and these drugs are considered to be safer than street drugs. The monitoring and prevention of this abuse is difficult (Mounteney and Haugland 2009), but there is a clear demand for studying this phenomenon as it has been estimated that abuse of prescription drugs could soon exceed that of illicit narcotics (Zarocostas 2007). Post-mortem toxicology analysis results combined with sufficient background data could offer a way to profile this abuse scene.

It can be presumed that individuals with substance abuse problems participate poorly in population-based surveys such as self-reporting questionnaires or interviews. Unconventional approaches can be applied to overcome the problem of poor participation: for example, illicit drug use can be detected and estimated in municipal wastewater (Zuccato et al. 2008). Biomarkers can be used to validate the response of participants who tend to underreport their use. For example, levels of cotinine, a major metabolite of nicotine, can be measured to differentiate between smokers and non-smokers (Vartiainen et al. 2002). Besides related long-term health issues, nicotine and alcohol dependence have been shown to aggregate in the same users and to be highly co-morbid (Istvan and Matarazzo 1984; Li et al. 2007). Furthermore, there is evidence that smoking, illicit drug use, mental disorders and increased risk of suicide are interconnected (Hughes 2008; Sihvola et al. 2008; Berlin et al. 2010). Drug-related harm has been evaluated by scoring the harm not only to the user but also to others (Nutt et al. 2010). In this multicriteria decision analysis, alcohol was considered to be the most harmful drug and tobacco was ranked sixth after heroin, crack cocaine, methamphetamine and cocaine in the United Kingdom (Nutt et al. 2010).

3.3 Post-mortem pharmacoepidemiology

Unlike in most clinical studies, post-mortem toxicology data is based on the actual measurements of medicines in blood or other biological specimens and is usually

accompanied by other laboratory results for abused substances. Post-mortem toxicology produces reliable and up-to-date information on drug poisoning statistics (Vuori et al. 2009) and substance abuse, such as new trends in drug abuse (Ojanperä et al. 2008) and the effects of alcohol sales on mortality (Poikolainen et al. 2002; Koski et al. 2007b). Multiple findings of legal drugs, illicit drugs and alcohol(s) are typical of post-mortem cases (Vuori et al. 2009). This makes interpretation of the results challenging, but also allows studies that could not be conducted in clinical settings, portraying the reality often faced in drug therapy. The relative toxicity of co-administering commonly used drugs like benzodiazepines or antidepressants with ethanol has been evaluated in post-mortem material (Koski et al. 2002; Koski et al. 2003; Koski et al. 2005a, Koski et al. 2005b). In addition, post-mortem toxicology produces data on therapeutic and fatal concentrations of different drugs in blood; compilations have been published from concentrations measured in post-mortem cases (Stead and Moffat 1983; Druid and Holmgren 1997; TIAFT 2004; Reis et al. 2007).

3.3.1 Drug toxicity

In animal experiments, the conventional way to study acute lethal toxicity is to measure LD_{50} (Lethal Dose, 50%), the amount of drug required to kill 50% of a given population. Obviously this is not an acceptable way to assess toxicity in humans. Animal testing might even be a futile exercise, as there may be considerable differences in drug metabolism between humans and the common experimental animals. The number of intoxication cases treated in hospitals and the proportion of survivals could help to estimate the relative toxicity in humans, but the outcome is also related to the timing and effectiveness of treatment and the existence of antidotes. Moreover, drug-related fatalities often take place beyond the reach of medical help.

3.3.2 Fatal toxicity index

A practical measure of relative drug toxicity is the fatal toxicity index (FTI), which is calculated by relating the number of fatalities attributed to a drug to the consumption of the drug over the same period and area (King and Moffat 1981; King and Moffat 1983). Drug consumption can be expressed as the number of prescriptions, kilograms or defined daily doses (DDDs) dispensed. DDD is the assumed average maintenance dose per day for a drug used for its main indication in adults, also including over-the-counter drugs (WHO Collaboration Centre for Drug Statistics Methodology). A fatal poisoning case rarely contains only a single drug, which makes the compiling of drug fatality statistics difficult. Deaths can be classified according to the most important finding, e.g.

the highest 'concentration-to-therapeutic-concentration' ratio (Vuori et al. 2009). The FTI can be expressed as deaths/DDD/1,000 inhabitants/day. It must be noted that the frequency of poisonings is not only dependent on the availability and inherent toxicity of the drug, but it can also be related to the indications and manner of use. Prescription drugs that have anxiolytic or euphorogenic properties, like benzodiazepines and opioids, are often abused, and "sleeping pills" might be considered a serene way to take one's own life. Antidepressants are mostly prescribed to depressed patients with elevated risk of suicide, but also for other less risky conditions. There are also indications that different antidepressants are prescribed to different age groups (White et al. 2008). Drug deaths can be caused by either intentional or accidental overdoses, and sometimes the intention is unclear. For illicit drugs, fatal toxicity can be evaluated by relating the number of associated deaths to measures of availability such as seizures by law enforcement agencies (King and Corkery 2010).

The FTI has been used to compare the toxicity of individual drugs and classes of drugs. The old group of tricyclic antidepressants (TCAs) has been associated with a higher FTI than the newer antidepressant drugs introduced after 1973 (Cassidy and Henry 1987). Selective serotonin reuptake inhibitors (SSRIs) have been shown to cause fewer fatal intoxications than TCAs in proportion to their consumption (Buckley and McManus 2002; Cheeta et al. 2004; Morgan et al. 2004; Koski et al. 2005a). The selective serotonin and norepinephrine reuptake inhibitor (SNRI) venlafaxine is a newer antidepressant that has scored a higher FTI than other serotonergic agents in many published studies (Buckley and McManus 2002; Whyte et al. 2003; Cheeta et al. 2004; Morgan et al. 2004; Koski et al. 2005a; Flanagan 2008). It has been suggested that FTI can also depend on prescribing practices: for example, venlafaxine might be prescribed to patients whose depression has been resistant to SSRIs and thus carry a higher suicide risk (Egberts et al. 1997; Heerdink et al. 2003; Mines et al. 2005). However, it has been concluded that inherent toxicity is a crucial factor of a high FTI (Farmer and Pinder 1989; Buckley and McManus 2004). Despite continuing uncertainty over the underlying reasons, database studies have influenced the prescribing information issued by the US manufacturer of venlafaxine (Deshauer 2007). Combined information on venlafaxine concentration, CYP2D6 status, interacting drugs, abused substances and background data would all be required to study the causes of the fatalities.

AIMS OF THE STUDY

The aims of the study were:

I To evaluate the overall prevalence of adverse combinations of common drugs and to assess the potential role assigned to adverse drug interactions in fatalities.

II To establish the prevalence and nature of adverse combinations of the problematic anticoagulant warfarin, especially with NSAIDs, paracetamol and tramadol.

III To elucidate whether the apparent high toxicity of the new-generation antidepressant venlafaxine is associated with adverse drug interactions, pharmacogenetic factors and/or the manner of death.

IV To obtain reliable information on smoking and drug use frequency through a population-based study of deceased young adults and to compare smokers and non-smokers in terms of alcohol, drug and drug-of-abuse findings and the manner of death.

MATERIALS AND METHODS

1 Autopsy cases

All cases were Finnish medico-legal autopsies from the years 2000-2009. All autopsy samples taken for forensic toxicology were analyzed at the Laboratory of Toxicology, Department of Forensic Medicine, University of Helsinki.

1.1 Samples

All concentration data was acquired from femoral venous blood taken at autopsy (**I-IV**). The samples, containing 1% NaF to prevent microbial alteration, were stored at +4°C until analysis, after which they were preserved at -20°C. The qualitative drug analysis of urine samples was performed from urine samples without the preservative (**IV**).

1.2 Post-mortem database

A referral from the forensic pathologist, the results of toxicological analyses, and information from the final death certificate were coded into the Laboratory Information Management System (LIMS) (Access 2000). The referral contained background information from the police and forensic pathologist, such as name, age, gender, place of residence, known occupation, a brief description of the circumstances of death, known medications and the main autopsy findings. The analytical data contained results for alcohols, drugs and drugs of abuse, and for other substances, such as volatiles and carbon monoxide, when requested. Information from the final death certificate included the cause-of-death with contributing factors according to the 10th revision of the International Classification of Diseases and the manner of death (ICD-10, WHO).

2 Data refining

2.1 SFINX

The Swedish, Finnish, INteraction X-referencing (SFINX) database was used for identification of adverse drug combinations (Strandell et al. 2008; Böttiger et al. 2009; SFINX 2009). SFINX is a regularly updated commercial drug interaction database introduced in December 2005. Two-compound adverse drug interactions are ranked by their clinical status from probably insignificant (A) to severe (D) and the level of documentation is classified from sparse (0) to well established (4). The result page lists the brand names of available preparations, a short description of the ADIs, recommended measures concerning combined administration, and related references in the literature. Additive or synergistic pharmacodynamic interactions of medicines having similar

pharmacological effects, e.g. two CNS depressants, are not flagged in the SFINX database. The database was accessed through the National Library of Health Sciences in Finland.

2.2 Case selection criteria

I. The study series consisted of autopsy cases from a seven-year period (1 January 2000 - 31 December 2006), for which femoral blood analysis results were available (37,367 cases). Twenty-four primary (i.e. index) medicines were chosen for the study on the basis of their high frequency as findings in post-mortem blood, as the principal finding in fatal poisonings (highest 'concentration-to-therapeutic-concentration'-ratio) or generally known ADIs. The interacting medicines were identified from the SFINX query results and only the most severe (class D) interactions were taken into account. Pairs in which the interacting drug was not in the laboratory's analysis arsenal during 2000-2006 were excluded (e.g. warfarin and NSAIDs). Also excluded were combinations causing decreased concentrations or effect, and additive or synergistic pharmacodynamic effects of drugs possessing similar pharmacological mechanisms.

II. The cases included a twelve-month series of autopsy cases from 1 February 2007 to 31 January 2008. The drugs possessing pharmacokinetic and/or pharmacodynamic interactions with warfarin were identified from the SFINX database, and the drug combinations classified as the most severe (class D) and the second most severe (class C) were included in the study. For each warfarin-positive case (n=328), a control case was extracted by selecting the next matching case in numerical order with the following criteria: same gender, age ± 5 years, and, if alcohol was present in the warfarin case, ±0.5‰ blood alcohol concentration (otherwise no alcohol was accepted) and no interacting drugs detected. Cases positive for paracetamol or tramadol alone were extracted from the post-mortem toxicology database to compare the bleeding frequency.

III. The cases were entered in the laboratory database during the two-year period 30 June 2005 to 29 June 2007. The study included all cases previously found to be positive for venlafaxine by a quantitative drug screening method for blood samples (n=191) (Rasanen et al. 2003). Venlafaxine was compared with other antidepressants with over 100 positive cases during the study period.

IV. The cases comprised autopsies from the three-year period 1 November 2006 to 31 October 2009. The study focused on young adults of 15-34 years of age (n=2,154). Nicotine exposure was determined qualitatively by the detection of nicotine and/or one of its main metabolites (cotinine, *trans*-3'-hydroxycotinine) using a screening method for

urine samples (n=1,623) (Pelander et al. 2003; Ojanperä et al. 2006). Cases where none of these three analytes were detected were defined as controls (non-nicotine users).

3 Analysis methods

A multi-technique approach was used to perform a comprehensive toxicological analysis of blood and urine samples. Urine samples were screened with the laboratory's routine qualitative drug screening methods by immunoassay and liquid chromatography - time-of-flight mass spectrometry (LC-MS-TOF) (Pelander et al. 2003; Ojanperä et al. 2006). Besides approximately 700 drugs, the method included nicotine, cotinine and *trans*-3'-hydroxycotinine (**IV**). Simultaneously, blood samples were quantitatively monitored for 200 drugs by three methods: gas chromatography with nitrogen phosphorus detection (GC-NPD) for acidic/neutral drugs (Ojanperä et al. 1991) and from February 2006 on by gas chromatography - mass spectrometry (GC-MS) (**II**), GC with electron capture detection (GC-ECD) for benzodiazepines (Rasanen et al. 2000), and GC-NPD for basic drugs (Rasanen et al. 2003). Confirmation and additional determinations were carried out using GC-MS and LC coupled to triple quadrupole MS/MS in both urine and blood. Digoxin was routinely determined for all known users and all deceased individuals 65 years or older by a quantitative immunological method (limit of quantification (LOQ) 0.64 nmol/l). Ethanol (along with other volatile alcohols) was determined in blood samples by headspace GC. The blood alcohol concentrations were reported in mass per mass units as parts per thousand (‰) and the LOQ for ethanol was 0.2‰. The screening procedure covers the majority of psychotropic drugs available on the licit and illicit markets in Finland, with special emphasis on abused substances. The Laboratory has acted in the capacity of Testing Laboratory T 115 since 1997, accredited by the Finnish Centre for Metrology and Accreditation (FINAS).

3.1 Analysis of acidic and neutral drugs

A new GC-MS based screening method was developed and validated for quantitative blood screening. The new method included quantification of 40 acidic and neutral drugs such as antiepileptics, paracetamol, warfarin and NSAIDs (**II**). The analytes were extracted into ethyl acetate, followed by a derivatization step and separation on a DB-5MS capillary column (Agilent Technologies, Santa Clara, CA, USA). The full-scan spectral data obtained was purified using an Automated Mass Spectral Deconvolution and Identification system (NIST, USA), version 2.1 (AMDIS 2006), and the analytes were quantitated using three-point linear calibration.

3.2 Metabolite analysis

Venlafaxine and its two major metabolites were determined by GC-MS (**III**). *O*-desmethylvenlafaxine (HCl) and *N*-desmethylvenlafaxine (HCl) were purchased from SynFine Research Inc. (Ontario, Canada) and venlafaxine (HCl) was donated by Lederle (Pearl River, NY, USA). The analytes were determined in blood samples with a LOQ of 30 µg/l for VEN and O-VEN, and 10 µg/l for N-VEN. The analytes were extracted into butyl acetate, followed by GC separation and detection by MS using selected ion monitoring (SIM). The metabolite ratios were calculated as the concentration of venlafaxine per concentration of metabolite.

(-)-Nicotine (hydrogen tartrate, Sigma), (-)-cotinine (Sigma) and *trans*-3'-hydroxycotinine (Toronto Research Chemicals Inc) were added to the qualitative routine LC-MS-TOF method in November 2006 (**IV**). The limit of detection (LOD) was 100 ng/l for nicotine, 75 ng/l for cotinine and 200 ng/l for *trans*-3'-hydroxycotinine. Because a reported cotinine concentration measured after low-level exposure to nicotine mimicking passive smoking is much smaller (4-5 ng/l in urine) (Benowitz et al. 2009) than the LOD, it can be hypothesized that the method detected mainly active nicotine use.

4 Genotyping

CYP2D6 genotyping was performed using a method that detected 11 variants of the allele: **2, *3, *4, *5, *6, *9, *10, *17, *29, *39* and **41* (**III**) (Sistonen et al. 2005). If none of these mutations were detected the allele was classified as **1*. E.Z.N.A. SE Blood kit DNA was used to isolate DNA from 250 µl of autopsy blood (Omega Bio-Tek Inc., Doraville, GA, USA). The detection was performed using an ABI PRISM SNaPshot™ multiplex kit (Applied Biosystems, Foster City, CA, USA). Two additional long polymerase chain reactions (PCRs) were used to analyze whole-gene deletion and duplication, and the phase of gene duplication in heterozygous genotypes was defined from the SNaPshot™ result. The duplicated allele is denoted *xN* because the multiplicity of the duplication could not be determined with the method used.

4.1 CYP2D6 allele activity scores, genotype classes and phenotypes

The allele variants were scored according to their relative functionality as follows: 0 for a non-functional allele (**3, *4, *6, *4xN*) or deletion (**5*), 0.5 for an allele of reduced activity (**9, *10, *17, *29, *41, *10xN, *41xN*), 1 for an allele of wild type activity (**1, *2, *39*) and 2 for increased activity (**1xN, *2xN*) (Gaedigk et al. 2008). To compare the metabolite ratio and the predicted enzyme activity, the sum for each individual genotype

was calculated yielding seven possible genotype groups from 0 to 3 at intervals of 0.5. CYP2D6 phenotypes were predicted from genotypes (Table 2). The prediction of enzyme activity of each haplotype was based on results of previous studies (for reference, see Home Page of the Human Cytochrome P450 (*CYP*) Allele Nomenclature Committee 2008).

5 Statistical methods

MINITAB 13.31 software (Minitab Inc., State College, PA, USA) was used for calculation of confidence intervals (CIs), means, medians, test of two proportions, and Mann-Whitney test (**I**, **II**, **III**). A *p*-value of <0.05 was considered to indicate a statistically significant difference. Both frequentist and Bayesian inference were used to analyze the data with online software OpenBUGS, version 3.1.1 (**IV**) (Ntzoufras 2009; OpenBUGS 2010).

RESULTS

1 Prevalence of adverse drug combinations

0.71% of 37,367 cases showed a drug combination possessing a potentially severe interaction (**I**). The total number of possible ADI cases in which the interaction mechanism matched the background information and analytical data was 62, comprising 23% of all ADI hits and 0.17% of all cases analyzed. The prevalence of at least one adverse drug combination among warfarin users was 33% (**II**). The prevalence was 46% among venlafaxine users (**III**).

1.1 Drug combinations with a pharmacodynamic interaction mechanism

Pharmacodynamic ADIs were the more common type of possible ADIs with 52 out of 62 cases (**I**). The most prominent types of pharmacodynamic ADIs were serotonin toxicity and combinations of a β_1-blocker and either of the calcium antagonists verapamil or diltiazem. A NSAID was detected in 6 out of 328 warfarin-positive cases (**II**). The most common pharmacodynamic combinations with warfarin were paracetamol, tramadol and citalopram. The drugs most frequently found to interact with venlafaxine were tricyclic antidepressants (TCAs) (also possessing a pharmacokinetic effect), mirtazapine and tramadol (**III**).

1.2 Drug combinations with a pharmacokinetic interaction mechanism

Only 10% of the possible pharmacokinetic ADI cases (10 of 98) had an elevated target compound concentration (**I**). Approximately half of all possible pharmacokinetic ADI cases involved digoxin in combination with the calcium antagonist verapamil, but only one case met the set ADI criteria and exhibited a probably fatal level of digoxin (Bauman et al. 2006). The effect of adverse warfarin combinations was judged only from the observed fatal bleeding instead of increased warfarin concentration (**I**, **II**). Among the cases that included a kinetic warfarin combination, the most common interacting drugs were fluconazole, metronidazole and fluvoxamine (**I,** n=14) and trimethoprim, amiodarone, fluconazole and phenytoin (**II,** n=18). Carbamazepine, which has a diminishing effect on anticoagulation, was observed in 7 cases (**II**). The most prominent interacting drugs detected along with venlafaxine were TCAs, levomepromazine and fluoxetine (**III**).

1.3 Death certificates

In the 57 death certificates for the 62 possible ADIs, the forensic pathologist had included the studied adverse combinations of 2 or more drugs as a contributing factor of death

in 19 certificates (33%) (**I**). In 8 of these cases the forensic pathologist had highlighted the same specific 2-compound ADI as recognised in the study. Serotonin toxicity was concluded to be the cause of death in two fatalities, both involving citalopram and moclobemide. The manner of death distribution of the suspected ADI cases showed no deviation from all cases analyzed during the study period.

2 Concomitant use of warfarin and paracetamol

Paracetamol was the most frequently co-used drug (n=53) capable of interacting with warfarin (328 cases) (**II**). Among cases where these two drugs were detected together the prevalence of fatal bleeding was 4.6 and 2.7 times higher compared with cases of only paracetamol or warfarin findings, respectively. Bleeding was most commonly intracranial and there was no statistical difference in terms of its aetiology (spontaneous or traumatic). There were not enough cases to obtain reliable results on the bleeding risk due to combined tramadol and warfarin use.

2.1 Post-mortem warfarin concentrations

66 % of all cases studied had a warfarin concentration below the LOQ of 0.5 mg/l in whole blood (**II**). The LOD was 0.1 mg/l. The plasma/blood ratio was studied *in vitro* using fresh blood samples (n=3) from two individuals and the result was 1.63 -2.01.

3 Venlafaxine findings and fatalities

3.1 Pharmacogenetics

Genotyping of *CYP2D6* was successful in 123 of the 128 cases of available post-mortem blood samples that were found to be venlafaxine positive in routine screening (**III**).

3.1.1 Allele and phenotype frequencies

The observed CYP2D6 allele frequencies of venlafaxine users did not differentiate them from the general population. Most of the VEN users (89%) were EMs. Only two PMs were identified (2%) and 10% of the VEN-positive cases were identified as UMs. The relative CYP2D6 activity did not seem to predispose to high venlafaxine concentrations as the expected and observed distributions of phenotypes into four concentration groups were similar (see **III** for details).

3.1.2 CYP2D6 and venlafaxine metabolite ratios

There was a negative correlation between the VEN/O-VEN ratio and the genotype sum of functional alleles; a 2.6-fold difference in the median VEN/O-VEN ratio was observed between HEMs and EMs, but other groups did not differ from each other to a statistically

significant extent. Except for the two PMs, no shift towards the N-methylation pathway was observed.

3.2 Concentrations

The median concentrations of VEN, O-VEN and N-VEN in the 123 cases that were successfully quantitated were 560, 420 and 49 µg/l, respectively. Concentrations of VEN, O-VEN and N-VEN were below the LOQ in 3, 4 and 6 cases, respectively. One case did not have a detectable amount of N-VEN. The distribution of concentration sums of VEN, O-VEN and N-VEN was plotted together with the cumulative distribution of fatal venlafaxine poisonings (assessed by the forensic pathologist) to reveal a median total VEN concentration of 3,250 µg/l in post-mortem whole blood (Figure 3).

3.3 Drug interactions

A high frequency of interacting drugs (46%) was typical of the VEN-positive cases and the presence of these drugs was shown to be more common with higher VEN concentrations. Only one case of poisoning due to VEN alone was detected. The presence of a CYP2D6 inhibitor was related to an elevated median combined concentration of VEN and metabolites and VEN/O-VEN ratio.

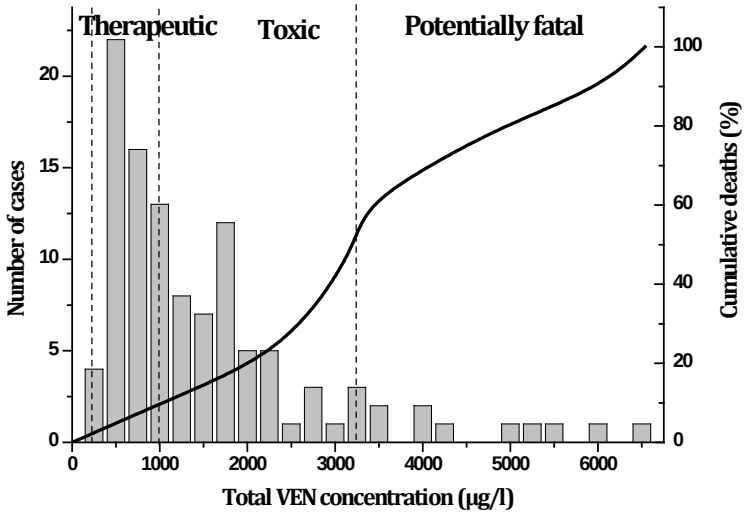

Figure 3. Sum concentration (VEN + O-VEN + N-VEN) distribution of venlafaxine-positive cases (**III**). The cumulative distribution of fatal poisonings is indicated with a bold curve.

Table 4. Venlafaxine in comparison with other common antidepressants in Finland

	Year 2006				Two-year study period**				
	DDD	Sales	Observed			Positive cases			Suicides committed primarily with the drug
Drug	mg	DDD/1000 inhab./day	deaths^	**FTI**	n	suicides (**%**)	p*	n	**% of all suicides**
Tricyclics									
Amitriptyline	75	3.87	47	**12.14**	334	96 (**29**)	0.002	46	**48**
Doxepin	100	0.93	32	**34.41**	129	52 (**40**)	>0.05	32	**62**
SSRIs									
Citalopram	20	22.74	17	**0.75**	729	211 (**29**)	0.001	16	**8**
Fluoxetine	20	5.42	5	**0.92**	146	41 (**28**)	0.007	2	**5**
Sertraline	50	4.87	1	**0.21**	101	33 (**33**)	>0.05	1	**3**
Newer antidepressants									
Mirtazapine	30	7.06	7	**0.99**	516	192 (**37**)	>0.05	15	**8**
Venlafaxine	100	4.87	18	**3.7**	191	80 (**42**)		23	**29**

DDD defined daily dose, *FTI* fatal toxicity index

^ from femoral venous blood only

* test for two proportions vs. venlafaxine

**30 June 2005 - 29 June 2007

3.4 Venlafaxine in comparison with other antidepressants

The fatal toxicity index (FTI) was calculated for venlafaxine and other commonly used antidepressants for 2006 and is expressed as deaths/DDD/1000 inhabitants/day (Table 4). VEN-positive cases had overall the highest suicide frequency (all suicide methods included), but the proportion of suicides committed with VEN was substantially higher (29%) than that with mirtazapine (8%) or SSRIs (3-8%).

4 Nicotine in deceased young adults

4.1 Prevalence of nicotine findings

The overall prevalence of nicotine use in the post-mortem toxicology database was 55% (all ages) and among young adults (15-34 years) 75% (**IV**). The study material covered approximately 60% of all deceased young adults in Finland during the study period.

4.2 Nicotine and substance abuse

A blood alcohol concentration higher than 0.2‰ was found twice as often in the smokers group (60%) than in the non-smokers group (30%) (Table 5). The median concentration of ethanol was higher in nicotine users (1.6‰) than in non-nicotine users (1.3‰) (mean difference 0.23, 0.07-0.39, 95% CI for the difference). In total, the number of illicit drug findings (n=462) was smaller than the number of abused prescription drug findings (n=585) (e.g. opioids, opioid substitutes and pregabalin). There were 509 cases with abuse of at least one of the illicit or prescription drugs studied, and these cases

represented 24% of all young adults and 31% of the cases where routine urine screening was done. Amphetamine and cannabis were the most common illicit drug findings in the study material. Nicotine had been used by 85% of the drug abusers and drug abuse was twice as common among nicotine users (36%) than among non-nicotine users (18%).

4.3 Nicotine and psychopharmaceuticals

Nicotine use was studied among cases of young adults who were positive for drugs used for psychopharmacotherapy. Over half of the study population of young adults (66%, Table 5) were positive for at least one psychopharmaceutical drug. Nicotine users formed the majority of the cases in each drug group: 70% of antipsychotic positive cases (posterior prevalence; CI 65-75% as 95% highest posterior density interval), 72% of antidepressants (CI 68-75%), 76% of other selected drugs (CI 67-82%, propranolol, orfenadrine, tizanidine), 78% of anxiolytics (CI 75-81%) and 79% of hypnotics and sedatives (CI 76-82%). However, psychopharmaceuticals were positive more often in the non-nicotine users group (76%) than in the nicotine users group (63%) (Table 5).

4.4 Nicotine and manner of death

The distribution of manner of death was compared between the nicotine users and non-nicotine users. No statistical differences were observed except that there were fewer natural deaths in the smokers group than in the non-smokers group.

Table 5. Findings of nicotine, alcohol, abused drugs and psychopharmaceuticals in 15-34 year old deceased

	All young adults n=1623	Nicotine users n=1215	Non-nicotine users n=408
Blood ethanol ≥0.2‰	849 (52%)	726 (60%)	123 (30%)
Drug abuse positive	509 (31%)	434 (36%)	75 (18%)
Psychopharmaceuticals positive	1073 (66%)	761 (63%)	312 (76%)

DISCUSSION

1 Methodological considerations

1.1 The Finnish post-mortem toxicology database

In Finland, a sudden or unexpected death leads to a medico-legal investigation conducted by the police. If considered necessary, the police launch a cause-of-death investigation that is performed by a forensic pathologist. An investigation is always conducted when a fatality is not known to be caused by a disease, or is known to be related to an accident, crime, suicide, poisoning, occupational illness, medical procedure or war (as are deaths that do not occur under medical care or are otherwise sudden or unclear). Even reasonable doubt about the causes mentioned above is enough for an investigation and no permission from the next-of-kin is needed. Approximately 50,000 Finns die each year and a medico-legal autopsy is performed in almost one case in four. Toxicological samples are collected after the decision of the forensic pathologist. All toxicological analyses are performed in one laboratory at the Department of Forensic Medicine of Hjelt Institute, University of Helsinki. This leads to toxicological analysis of over 6,000 cases a year, which represents over 10% of all deaths nationwide.

The samples undergo screening and quantification analysis for legal and illicit drugs and poisonous substances. The results are stored in the laboratory's toxicology database. These, together with the autopsy results, are evaluated by the forensic pathologist to exclude, confirm or reveal the reasons behind the fatality. Finally a death certificate, including cause and manner of death, is issued by the forensic pathologist and a copy is sent to the Laboratory of Toxicology, where the data is integrated into the toxicology database. This, in combination with the high autopsy rate and validated up-to-date analysis methods, leads to a large, constantly accumulating and commensurable nationwide databank that can be used for epidemiological studies.

1.2 Post-mortem pharmacology

Besides the multiple factors that can affect pharmacotherapy in clinical pharmacology, the post-mortem setting poses considerable additional difficulties in interpreting the toxicological results (Pounder and Jones 1990; Drummer 2007; Ferner 2008). After death the active processes that have created concentration differences in the body cease. This may lead to a post-mortem redistribution of drugs and hence the concentration in the blood (and other sample matrixes) may change dramatically, as has been proven to happen with digoxin (Koren and MacLeod 1985; Yarema and Becker 2005).

Depending on the properties of the drug, the concentration can increase or decrease or the drug itself can be altered by microbiological activity or post-mortem changes in the environment (Ferner 2008). Substantial differences have been observed between concentrations measured from peripheral and central sites of the body (Leikin and Watson 2003), and toxicologically important substances like ethanol can be generated after death by microbes (Zumwalt et al. 1982; Vuori et al. 1983; Petkovic et al. 2005). Appropriate sampling of blood from femoral veins and use of a preservative are crucial for a representative quantitative analysis result (Flanagan et al. 2005). These standard procedures are applied throughout autopsy rooms in Finland. A negative result in a post-mortem setting can be a consequence of long survival time (interval between drug intake and death) or post-mortem degradation. Furthermore, there are numerous substances like opioids that do not have a difference between safe, therapeutic, toxic and fatal concentrations (Drummer and Odell 2001). Background data is critical in evaluating tolerance, and toxicological results should always be interpreted in their individual context.

Plasma and serum are the preferred sample matrixes in living patients, but post-mortem analysis is performed on haemolysed whole blood. Most therapeutic concentration ranges for drugs given in the literature are for plasma or serum and therefore cannot be applied directly to post-mortem cases as the concentration ratio between whole blood and plasma varies from drug to drug (Skopp 2004). This particularly influences drugs that are highly protein bound like benzodiazepines (Jones and Larsson 2004) and warfarin (**II**). Furthermore, the response to a drug is dependent on the free drug concentration, but a post-mortem result is a sum of both free and protein-bound drug. The finding that the post-mortem concentrations of warfarin were frequently below the established therapeutic range for plasma (1–3 mg/l) (Schulz and Schmoldt 2003) and also below the limit of quantification (0.5 mg/l), could be explained, at least to some extent, by the plasma/whole blood ratio experiments (**II**). The plasma samples had almost twice the concentration of the whole blood samples. Due to the individual variation in the response to warfarin therapy, the qualitative result is more important than the post-mortem concentration of warfarin. However, the expected concentration range is important in method development.

To make any conclusions on a single case, post-mortem concentrations should be viewed against data accumulated from post-mortem samples that have undergone similar sampling, storage and analysis and whose background data is available.

Venlafaxine has been shown to undergo post-mortem redistribution (Levine et al. 1996; Rodda and Drummer 2006). A recent study involving determination of venlafaxine and its metabolites from both plasma and whole blood showed no major concentration differences between the matrixes (Kingbäck et al. 2010). The concentration distribution from a two-year series of venlafaxine cases was shown together with the accumulation curve for fatal VEN intoxications (**III**). In cases with causes of death other than drug poisoning, the measured median concentrations of 330 µg/l for VEN and 400 µg/l for O-VEN were higher than those reported in a large therapeutic drug monitoring database study (50 and 140 µg/l in serum, respectively, for 150 mg daily dose (Reis et al. 2009)). However these results (**III**) are in agreement with a previously suggested therapeutic range of 250-750 µg/l for total drug and metabolites in serum (Moffat et al. 2004), and overall the results were comparable with those from previous studies on post-mortem material (Jaffe et al. 1999; Goeringer et al. 2001; Reis et al. 2007).

Multi-drug use and substance abuse are common in post-mortem cases and make the interpretation of the results challenging (Jones et al. 2010). A fatality can seldom be attributed purely to a single substance and, besides factors like tolerance, the possibility of drug–drug interactions, drug-alcohol interactions and drug-disease interactions should be taken into account. For example, commonly used benzodiazepines are often found together with alcohol (Koski et al. 2002; Holmgren and Jones 2003). Substantial tolerance can develop toward both substances, but they do possess an additive interaction as they both act as CNS depressants. In order to handle the large study material some rough generalizations had to be made for exclusion of predominant poisonings where the contribution of ADIs to the fatality was no longer relevant (**I**). Predominant alcohol poisonings were excluded by setting the cut-off limit (2.5‰) lower than a published post-mortem median blood alcohol concentration (3.6‰) (Jones and Holmgren 2003) because the fatal blood alcohol concentration might be lower in combined drug-alcohol poisonings (Koski et al. 2002; Koski et al. 2003; Koski et al. 2005a; Koski et al. 2005b).

Awareness of the complete medical profile, the possibility of interactions, and the intentions of the prescribing doctor and patient could not be elucidated from the data available. The strength of a post-mortem toxicology database study is that the drugs, including many over-the-counter drugs, are actually detected in the blood rather than just in prescription databases, where the timing range of dispensing can vary and concomitant use might be impossible to confirm (Heikkilä et al. 2006). The figures presented here are probably underestimates for the overall prevalence of ADIs. Drugs

remain that are not included in the laboratory analysis repertoire, such as antibiotic, antifungal, and antiviral drugs and drugs used in cancer treatment. These drugs are commonly used in hospitals and by outpatients and they possess potential for serious ADIs but rarely cause fatal poisonings in forensic settings (Howard et al. 2002; Zwart-van Rijkom et al. 2009). Another issue is that ADIs that lead to diminished concentration or effect cannot be identified with certainty. Also, pharmacodynamic effects (e.g. serotonin toxicity and cardiac symptoms) that do not leave an easily detectable sign, like bleeding after warfarin interactions (**I**, **II**), are much more challenging to evaluate than pharmacokinetic interactions. It must be noted that the gender distribution of the Finnish post-mortem toxicology database is skewed: men consistently make up approximately 75% of the cases in the database (**I**, unpublished result; **IV**). Gender differences in inappropriate drug use have been reported (Johnell et al. 2009), and there might be differences in the frequency of reporting and in the experiencing of adverse drug reactions and interactions (Leone et al. 2010; Strandell and Wahlin 2011).

1.3 Drug selection

The medicines and drugs of abuse investigated in this thesis were chosen on the basis of their commonness as findings in post-mortem cases or as a cause of fatal poisonings in Finland during the study period (Vuori et al. 2006; Vuori et al. 2009). The poisoning and substance abuse scene is constantly changing and new substances frequently emerge; however, despite the introduction of new drugs like SSRIs, old TCAs like amitriptyline remain on the drug market and continue to cause fatal poisonings. The detection and quantification of new drugs is further challenged by the often poor availability of reference substances (Laks et al. 2004). The internet provides easy access to (presumed) prescription drugs and abused substances, such as opioids and anabolic agents, but there is no guarantee for the ingredients of these products. Also, the contents of street drugs might come as a surprise to the end-user (Vallersnes et al. 2009; Cole et al. 2011). Tourists and foreigners might have used drugs that are not available in Finland. Use of over-the-counter drugs is hard to monitor, but they do include drugs such as paracetamol, salicylic acid and NSAIDs, which can cause ADRs. The new GC-MS method has greatly improved the ability to screen for this often neglected group of drugs (**II**). In conclusion, rather than a target analysis, post-mortem toxicology demands a general unknown approach and adaptable methods. Without comprehensive drug screening procedures that are routinely and identically applied to all samples, comparable results cannot be produced for epidemiological studies. A recent publication of two suicides committed with nicotine (Corkery et al. 2010) illustrates the importance of including

commonly used but usually nonfatal substances like nicotine and caffeine (Thelander et al. 2010) in the analyte repertoire.

Warfarin and venlafaxine were given special attention in this thesis because they are both commonly used prescription drugs related to a high number of ADRs and fatalities. Haemorrhages of the gastrointestinal tract and CNS are among the most common fatal ADRs, with antithrombotic drugs and NSAIDs as the suspects (Sihvo et al. 2000; Wester et al. 2008; Strandell and Wahlin 2011). The metabolism of venlafaxine can be affected by several drugs acting on CYP2D6 or CYP3A4 (Holliday and Benfield 1995; Ereshefsky and Dugan 2000), which can alter the outcome of drug therapy, although the contribution of these interactions to fatalities has not been systematically studied.

1.4 Genotyping

Pharmacogenetics in a post-mortem setting is a relatively new area of research, one that helps to reduce the number of cases where the cause-of-death remains undetermined (Sajantila et al. 2010). DNA in post-mortem blood samples can be difficult to extract and amplify because of decomposition. The samples might be of poor quality and contain impurities that can inhibit the enzymes used, while DNA may have degraded into small fragments. In 5 out of 128 cases genotyping for *CYP2D6* was not successful (**III**). The methods used were chosen according to the existing equipment and the procedures developed in-house (Sistonen et al. 2005), but other approaches such as pyrosequencing (Zackrisson and Lindblom 2003), real-time PCR (Eriksson et al. 2002) and nanochip technology (Heller et al. 2006) can also be applied.

The VEN/O-VEN metabolic ratio has been proposed as a way to phenotype patients for CYP2D6 status (PM or EM) (Nichols et al. 2009; Lobello et al. 2010; Preskorn 2010), but it is doubtful whether this could be applied to post-mortem samples. Only two PMs led to a wide CI, and the other extreme CYP2D6 phenotype, UMs, could not be distinguished from the study sample on the basis of the metabolite ratio (**III**). Furthermore, post-mortem samples are often a far cry from clinical samples with their steady-state therapeutic concentrations, as they may involve overdose concentrations and subsequent enzyme saturation.

2 Prevalence of adverse drug combinations

The general prevalence of the use of drug combinations that can lead to severe ADIs is hard to study. The published estimates of incidences vary because of the definition

of 'severe/serious/hazardous/clinically important/significant', the drug selection, the source of the defined ADIs and finally the study population. Previous studies have concluded that overall ADIs are frequent but mostly not clinically significant or fatal (Egger et al. 2003; Glintborg et al. 2005; Reis and Cassiani 2011). Some examples of ADI incidence studies are presented in Table 6. As might be expected, the number is higher in hospital studies than in outpatient settings. The 0.71% prevalence of severe ADI combinations is in agreement with these previous results (**I**). Not everyone exposed to ADIs eventually suffers an adverse outcome. In a recent Italian study based on individual case safety reports, 22% of exposed patients experienced an associated ADR (Leone et al. 2010), which is similar to the 23% proportion of the detected ADIs that were considered to have a possible role in the fatality (**I**).

The forensic pathologists seldom mentioned the specific role of the ADI (**I**) studied in the death certificate as an underlying or contributing factor, but the drugs studied were mentioned in the death certificates in one third of the possible ADI cases. It has been proposed that drug-related deaths are underreported by forensic pathologists because other obvious conditions mask the contribution of the drugs to the fatality (Pilgrim et al. 2010a). Serotonin toxicity is likely to be underreported as a cause of death despite the frequent use of serotonergic drugs (Pilgrim et al. 2010c). It was mentioned in only two death certificates (**I**), even though the combined serotonin toxicity was among the most abundant possible ADI type (**I**, **III**). The situation might be similar with drug combinations causing adverse cardiovascular reactions like the combined use of a β_1-blocker and calcium antagonists (**I**). However, it must be pointed out that serotonin toxicity is a clinical diagnosis (Sternbach 1991; Isbister et al 2007) and post-mortem toxicology results must be backed up by background information in order to make such conclusions on the fatality. The use of two serotonergic drugs has been contraindicated,

Table 6. Incidences of individual cases with a severe adverse drug-drug combination

Study	Setting/study sample	Incidence (%)
Chen et al. 2005	General practice medical records	0.04
Heikkilä et al. 2006	Pharmacy dispensing database	0.4
Study I	Post-mortem toxicology database	0.71
Peng et al. 2003	Ambulatory patients	<1
Åstrand et al. 2006	Pharmacy dispensing database	1.5
Hosia-Randell et al. 2008	Elderly nursing home patients	4.8
Grönroos et al. 1997	Hospital patients	6.8
Reis et al. 2011	Intensive care unit patients	7
Egger et al. 2003	Hospital discharge patients	8.8

but recently it has been suggested that combining antidepressant medications, especially mirtazapine with other agents, could be beneficial to drug therapy (Blier et al. 2010). Computer-based registries, software and databases are essential if health care professionals and scientists are to keep up with the constantly accumulating data on ADIs. ADI databases should be constantly updated and the search results should be restricted to clinically significant ADIs in order to prevent an overload of warnings, which can have a numbing effect on users (Grönroos et al. 1997; Indermitte et al. 2007; Böttiger et al. 2009; Zwart-van Rijkom et al. 2009). Drug-drug interactions were the most frequently overridden type of drug safety alerts in a study conducted in a Dutch university medical centre (van der Sijs et al. 2009).

The prevalence of drug combinations able to cause severe ADIs was worrying for both venlafaxine (46%, **III**) and warfarin (33%, **II**). Most warfarin users are of a more advanced age and thus prone to multi-drug use (Hovstadius et al. 2010). The reported prevalences of adverse warfarin co-prescribing vary widely (33-82%) (Wittkowsky et al. 2004; Gasse et al. 2005; Feldstein et al. 2006; Snaith et al. 2008). Warfarin is among the most frequently reported drugs in the WHO database of spontaneous ADI reports (Strandell and Wahlin 2011), and recently it was stated that potentially interacting drugs were used in more than 50% of warfarin-associated bleeding reported to the Norwegian spontaneous reporting system (Narum et al. 2011). The combined use of NSAIDs and warfarin was rare (2%, **II**) in comparison with previous studies from Scotland (26.3%, primary care practice) (Snaith et al. 2008), from US (24.3%, dispensing database) (Malone et al. 2005) and Norway (16-23%, Norwegian prescription database) (Håkonsen et al. 2009). The low incidence was surprising, especially as warfarin + NSAIDs have previously been reported to be at the top of serious interaction online surveillance alerts in studies by a Finnish community pharmacy (1st place with 37% of alerts (Heikkilä et al. 2006)) and a university central hospital (3rd place in number of prescriptions (Grönroos et al. 1997). In this thesis, the origin of the NSAIDs (over-the-counter or prescription) was not known. Use of the new selective COX-2 inhibitors (oxicames and coxibs) has also been reported to increase the risk of bleeding when combined with warfarin therapy (Battistella et al. 2005), but these prescription drugs were rarely observed in the in this study.

Documented reports of the role of ADIs in venlafaxine-related ADRs and fatalities are more rare. In an Australian study, inappropriate combinations of serotonergic drugs were relatively frequent in post-mortem material, especially tramadol and SSRIs or

SNRIs and the concomitant use of multiple serotonergic antidepressants (Pilgrim et al. 2010a). Other antidepressants have been shown to be prescribed more often to patients on venlafaxine than to those on fluoxetine or citalopram (Mines et al. 2005; Rubino et al. 2007).

It is known that tobacco has interactions with drugs (e.g. olanzapine, clozapine, opioids), but the mechanism is often metabolic induction and a decrease in the effect (Kroon 2007). Despite their clinical significance, these interactions were not studied in this thesis as they are nearly impossible to evaluate from post-mortem material (**IV**).

3 Safety of warfarin and paracetamol combination

In several studies of drugs co-prescribed with warfarin paracetamol-containing products were the most common findings (Wittkowsky et al. 2004; Gasse et al. 2005; Feldstein et al. 2006; Snaith et al. 2008). Paracetamol and tramadol were also the most frequently found analgesics in the post-mortem study material (**II**). The proportion of fatal bleeding was higher among cases where warfarin and paracetamol were found together than in cases where paracetamol or warfarin was used alone or in the age, gender and alcohol-matched control cases where the drugs studied were not found. This supports previous clinical evidence of the possible adverse nature of this drug combination. Since the publication of Study **II**, a randomized controlled trial has reported that paracetamol (even 2g/day) enhanced the anticoagulant effect of warfarin in patients on stable therapy (Zhang et al. 2010).

The concentrations of the drugs studied were not taken into account as the optimal warfarin concentration is very patient specific (**II**). One notable advantage of using post-mortem study material is that the use of alcohol can be reliably included in the control case selection criteria. The number of cases is unfortunately relatively small, representing only one year of screened cases. In addition, it could be argued that those individuals using paracetamol as well as warfarin might have been in somewhat poorer health because for some reason they were using an analgesic. Nevertheless, the result suggests the need for more caution and close monitoring of warfarinized patients who use paracetamol concomitantly. This ADI might become even more important in the future as the sales of both drugs are increasing each year (Figure 4). The safety of the combined use of tramadol and warfarin could not be evaluated due to the inadequate number of cases. Findings of anticoagulants, NSAIDs, paracetamol and salicylic acid can be crucial to the cause-of-death investigation not only as an individual cause of fatal poisoning or ADR, but also because of their severe ADIs.

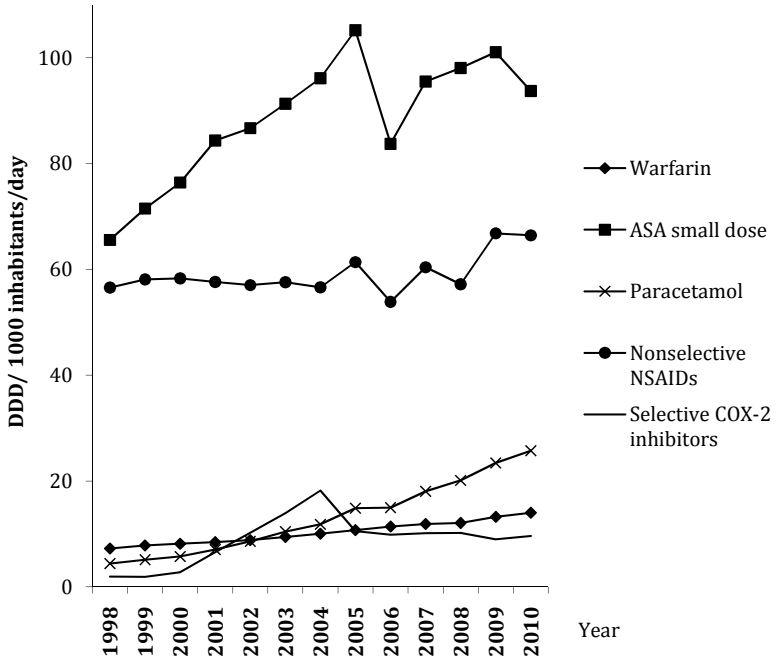

Figure 4. Sales of warfarin, NSAIDs and paracetamol in Finland 1998-2010. (National Agency for Medicines/Finnish Medicines Agency). (*ASA* acetylsalicylic acid)

4 Venlafaxine toxicity

Venlafaxine has repeatedly been shown to have a FTI lower than that of TCAs but higher than SSRIs (Buckley and McManus 2002; Whyte et al. 2003; Cheeta et al. 2004; Morgan et al. 2004; Koski et al. 2005a; Flanagan 2008) (**III**). Furthermore, venlafaxine has been placed similarly when ranked by toxicity in overdoses using the relative toxicity index (rate of deaths by suicide or undetermined intent compared to rate of non-fatal self-poisonings) (Hawton et al. 2010). A retrospective cohort study comparing patients who deliberately poisoned themselves with venlafaxine or SSRIs found that venlafaxine self-poisoners exhibited a higher degree of serious suicidal intent (Chan et al. 2010). Venlafaxine is reported to be prescribed for and taken in overdose by patients with a more severe clinical picture of depression and psychiatric disorders and history of self-harm (Egberts et al. 1997; Mines et al. 2005; Bergen et al. 2010). The VEN-positive cases had a high suicide rate and there were more suicides committed with VEN than SSRIs or mirtazapine (**III**).

As both extreme CYP2D6 phenotypes (PMs and UMs) may lead to problems in drug therapy (Rau et al. 2004), their frequency among the venlafaxine-positive cases was

expected to be elevated. However, there were only 2 PMs (2%), whereas the estimate for the European population is 5-10% (Bradford 2002; Sistonen et al. 2009). In a study conducted among Finnish smokers the general frequency of PMs was also 2% (Saarikoski et al. 2000). A low occurrence of PMs has previously been reported among patients with persistent mood disorders (Kawanishi et al. 2004), in a study of 53 Swedish autopsy cases involving citalopram (Holmgren et al. 2004), and among fatal intoxication cases (Zackrisson et al. 2004). Recently CYP2D6 UM phenotype has been associated with a higher suicidality (Stingl Formerly Kirchheiner and Viviani 2011) and suicide completion (Zackrisson et al. 2010). Among depressed patients with failed CYP2D6 substrate drug therapy, the frequency of CYP2D6 gene duplication was reported to be tenfold higher than in healthy Swedes (Kawanishi et al. 2004). The frequency of effective allele duplications (5.6%, **II**) was higher than has been estimated for a Scandinavian population (1-2%) (Dahl et al. 1995; Kawanishi et al. 2004; Ingelman-Sundberg et al. 2007) but similar to the results of a Finnish study (4.6%) (Sistonen et al. 2009). This accentuates the importance of knowledge of inter-population variation of CYP2D6 when interpreting results.

Ultrarapid metabolism can lead to an insufficient blood concentration and subsequent failure of drug treatment or to high concentrations of active/toxic metabolites. Minor metabolites can also become major ones if the dominant pathway is restricted, as in the case of CYP2D6 PMs, where the amount of N-VEN was equal to VEN (with only traces of O-VEN) resulting from an apparent shift towards the N-methylation pathway via CYP3A4 (**III**). Besides the accumulation of VEN (Wijnen et al. 2009) slight pharmacological differences have been proposed as the reason behind the observed increased risk of adverse side-effects in PMs (Hendset et al. 2006; Shams et al. 2006). As the sum of parent and active metabolite has been measured to be similar in both PM and EM phenotype patients (Fukuda et al. 2000), it has even been suggested that the polymorphism has minor clinical importance (Gardiner and Begg 2006). The contribution of N-VEN to the ADRs of venlafaxine in PMs has not been considered. Inclusion of N-VEN analysis in addition to the parent drug and active metabolite could give a better estimate of the total venlafaxine concentration in PM cases, and a high N-VEN concentration could suggest a need for genotyping in both clinical tests and cause-of-death investigations.

The channelling of venlafaxine to more troubled patients might play a part in the high toxicity indexes. There was also an indication that VEN possesses intrinsic suicide potential approaching that of TCAs (Table 4). The relative CYP2D6 activity did not

predispose to high venlafaxine concentrations, but the co-existence of a drug possessing pharmacological interactions did. This result is in agreement with a Swedish post-mortem study of citalopram, in which the authors suggested that pharmacokinetic interactions were likely to play a more important role than pharmacogenetic deficiencies in drug metabolism (Holmgren et al. 2004). Compared with other new antidepressants, venlafaxine is associated with a greater risk of poisoning when taken in combination with alcohol (Koski et al. 2005a). The vulnerability of venlafaxine to drug and alcohol interactions is a notable factor in the fatalities. A multitude of drugs and alcohol are common in suicides (Ohberg et al. 1996), but unintentional ADIs are a problem that demands preventive measures. Nevertheless, it must be kept in mind that venlafaxine does work well for most patients (Gutierrez et al. 2003). A meta-analysis compared the effect of venlafaxine with that of other antidepressants and placebo and concluded that venlafaxine appeared to be more effective than TCAs or SSRIs in treatment-resistant depression (Bauer et al. 2009). In a study of risk factors for the sudden unexplained death of psychiatric inpatients, venlafaxine was associated with a reduced odds ratio (Windfuhr et al. 2010). Also, the cardiac toxicity associated with venlafaxine has recently been questioned in a large population-based study (Martinez et al. 2010). The pros and cons of VEN therapy should be evaluated for each patient and the possibility of drug and alcohol interactions should be considered more carefully.

5 Nicotine and substance abuse

The prevalence of daily smoking among adults in Finland has been estimated to be 22% for men and 16% for women by population-based surveys (Helakorpi 2010), which is similar to the estimates for the white non-Hispanic population in the United states (23% and 18%, respectively) (CDC, Centers for Disease Control and Prevention 2009). The prevalence of nicotine findings among deceased young adults was roughly three times higher, 75% (**IV**). Smoking has been shown to be most common among individuals with a low level of education and low income (Giskes et al. 2005; Helakorpi et al. 2008), and persons with mental health issues (Lasser et al. 2000; Leonard et al. 2001; Lawrence et al. 2009). There are also indications that nicotine users have a higher risk of suicide (Hughes 2008; Hawton and van Heeringen 2009; Berlin et al. 2010). However, the proportion of deaths classified as suicides did not differentiate between the nicotine-user and non-nicotine user groups; there were fewer deaths classified as natural in the smokers group but no other differences were observed (**IV**). Based on the analysis results over half (66%) of all the study cases included drugs that are used to treat

mental problems, 52% were positive for alcohol, and 31% of the cases were abusers of illicit and/or prescription drugs. The nicotine use prevalence among cases positive for psychopharmaceuticals varied between 70% and 79% (posterior mean of prevalence) among drug groups including antipsychotics, antidepressants, anxiolytics and hypnotics and sedatives.

Smoking and alcohol use have been found to aggregate in the same users (Istvan and Matarazzo 1984). This was also evident from the post-mortem analysis results, which showed that positive blood alcohol (>0.2‰) was twice as common among the nicotine users (mean 60%, 95% highest posterior density interval (HPDI) 57%-63%) as among non-nicotine users (mean 30%, 95% HPDI 26%-35%). Previously, a high frequency of smoking has also been observed among opioid addicts in methadone treatment (Elkader et al. 2009). Buprenorphine has been more common in the treatment of opioid dependence than methadone in Finland (Alho et al. 2007). Although the therapeutic use of these drugs is carefully controlled, it does not seem to be effective in preventing their abuse (**IV**). Other prescription drugs were also popular among drug abusers, especially opioids such as fentanyl, oxycodone and tramadol. Amphetamine and cannabis were the most frequent illicit drug findings. Overall, of all the drug abuse cases, 85% were also positive for nicotine use. Recently, signals have surfaced about the abuse liability of pregabalin (Vuori et al. 2009; Schwan et al. 2010), and the post-mortem data supports this concern: the 42 cases of abuse represented 62% of all positive findings (n=68) (**IV**). Pregabalin is a drug used for the treatment of epilepsy, neuropathic pain and generalized anxiety disorder, but the abuse potential might not be acknowledged by prescribing doctors.

Post-mortem studies of nicotine and smoking have so far been few in number and limited to drug deaths (Hafezi et al. 2001) or concentrations in suicide cases and controls (Moriya and Hashimoto 2004; Moriya et al. 2007). Tobacco use or smoking might not be considered a relevant factor in a cause-of-death investigation, but recently two suicides in the UK were reported with nicotine extracted from tobacco using internet-derived instructions (Corkery et al. 2010). No such cases were observed in the study sample of young adults (**IV**). The cases represented approximately 60% of all deceased young adults nationwide during the study period. Even though confined to deceased persons, the series can be considered representative and population-based. The results provide information that can complement the picture obtained from the traditional survey responders and help to estimate the true prevalence of nicotine use in society. It also offers unique and reliable proof of the interconnection of nicotine and substance abuse.

CONCLUSIONS

The overall prevalence of adverse combinations of common drugs was relatively low in the post-mortem toxicology database. Although adverse drug interactions do not seem to be a major problem in cause-of-death investigations in general, their detection and identification in an individual case can be crucial, albeit difficult. Background information, autopsy findings and toxicological analysis results are all needed for evaluating the role of ADIs in fatalities. Drug interaction databases are of great assistance in keeping up with the growing knowledge of ADIs.

Pharmacogenetic variation poses further challenges for the interpretation of toxicological results. However in the case of the new-generation antidepressant venlafaxine, which has been previously related to an unexpectedly large number of fatalities, the genotypic variation at CYP2D6 did not seem to be decisive. Instead, the presence of interacting drugs was a prominent feature that was likely to contribute more to the fatalities. Among suicides by antidepressants, venlafaxine was more often involved than SSRIs or mirtazapine.

The observed increase in bleeding frequency in cases containing both paracetamol and warfarin compared with their use alone supports the clinical evidence of an adverse interaction. These results call for attention from medical professionals and possibly for a re-evaluation of the presumed safety of this combination. The contraindicated use of NSAIDs in warfarinized individuals was rare.

The presence of nicotine in 15-34 year old deceased young adults was roughly three times more common than the smoking frequency estimates for the living population. The toxicological findings in these cases supported previous observations about the interconnection of nicotine and substance abuse and mental disorders, but not elevated risk of suicide. The trend in prescription drug abuse was evident in this age group, especially for buprenorphine, methadone, opioids and pregabalin.

A high medico-legal autopsy rate, frequent toxicological sampling, comprehensive analytical procedures and the availability of referral and death certificate information from the forensic pathologist create an excellent basis for toxicological and pharmacoepidemiological research. The results derived from this constantly accumulating data source are applicable for both clinical and post-mortem work.

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