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ORIGINAL ARTICLE

Fatal venlafaxine poisonings are associated with a high prevalence of drug interactions

Terhi Launiainen · Ilpo Rasanen · Erkki Vuori · Ilkka Ojanperä

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Abstract Venlafaxine (VEN) is an antidepressant found to possess a higher fatal toxicity index (FTI, i.e., deaths in proportion to consumption) than other newer antidepressants and selective serotonin reuptake inhibitors (SSRIs). The aim of this study was to elucidate using post-mortem cases whether the apparent high toxicity of VEN is associated with adverse drug interactions, pharmacogenetic factors and/or the manner of death. Within a 2-year period, a comprehensive post-mortem database and death certificates were searched for cases with laboratory findings of VEN, findings of other drugs, associated background information and the cause and manner of death. In 123 cases, the concentrations of VEN and its two metabolites, *O*-desmethylvenlafaxine (O-VEN) and *N*-desmethylvenlafaxine (N-VEN), and the *CYP2D6* genotype were determined in post-mortem blood. The median concentrations of VEN, O-VEN and N-VEN were 560, 420 and 49 μg/l, respectively. A prominent feature of the VEN-positive cases was the high abundance of interacting drugs (46%), being more common with higher VEN concentrations. Compared to other common antidepressants, VEN-positive cases showed the highest suicide frequency, but also the proportion of suicidal VEN poisonings of all suicides was substantially higher than that of mirtazapine or SSRIs. Relative CYP2D6 activity did not predispose to high VEN concentrations, and the frequency of the extreme phenotypes followed the general population. In conclusion, the high suicide potential of VEN in combination with the high prevalence of drugs causing adverse interactions could be the reason for the observed high FTI.

Keywords Venlafaxine · Venlafaxine metabolite · Post-mortem database · Pharmacogenetics · CYP2D6

Introduction

Venlafaxine (VEN) is a bicyclic antidepressant that inhibits the reuptake of serotonin, noradrenaline and, to a lesser extent, dopamine [1]. It is metabolized by the cytochrome P450 enzyme CYP2D6 to its major metabolite *O*-desmethylvenlafaxine (O-VEN), while its conversion to the minor metabolite *N*-desmethylvenlafaxine (N-VEN) is catalyzed by CYP3A4 and possibly CYP2C19 and CYP2C9 [2, 3]. Both metabolites are further demethylated to *N,O*-desmethylvenlafaxine [4]. Of the metabolites, only O-VEN has been shown to have similar pharmacological activity to the parent drug. Venlafaxine is administered as a racemic mixture and while the *S*(-)-enantiomer inhibits both noradrenaline and serotonin uptake, the *R*(+)-enantiomer primarily inhibits serotonin reuptake [1]. CYP2D6 also displays stereoselctivity towards the *R*(+)-enantiomer [5].

CYP2D6 is highly polymorphic and substantial differences in its variation between populations have been described [6, 7]. This genetic variation gives rise to considerable phenotypic effects. By traditional classification, the CYP2D6 phenotypes are defined as follows: poor metabolizers (PM) lack the functional enzyme; intermediate metabolizers (IM) possess two decreased-function variants or one in combination with a non-functional variant; extensive metabolizers (EM) have at least one totally functional variant, and ultra-rapid metabolizers (UM) carry an active gene duplication or multiplication in addition to a

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T. Launiainen (✉) · I. Rasanen · E. Vuori · I. Ojanperä
Department of Forensic Medicine, University of Helsinki,
P.O. Box 40, FI-00014 Helsinki, Finland
e-mail: Terhi.Launiainen@helsinki.fi

functional variant [8]. The term heterozygous extensive metabolizer (HEM) has also been introduced to distinguish EMs carrying one functional and one inactive variant [9]. Genetic variation has been identified as an important factor behind the variation in psychiatric drug response [10]. Poor metabolism can cause severe adverse drug reactions (ADR), accumulation of the parent drug and even fatal intoxications. It has been suggested that PMs could be more susceptible to the adverse and toxic effects of VEN [11–13]. On the other hand, ultra-rapid drug metabolism can lead to therapeutic failure [14]. In clinical settings, a correlation between *CYP2D6* genotype and the metabolic ratio of VEN and O-VEN has been found [15, 16], but previous phenotyping studies have usually identified only the subgroup of PMs because considerable overlap exists within and between the other phenotype groups [8].

The apparent phenotype can be altered by pharmacokinetic drug interactions; there are several known CYP2D6 inhibitors that can lead to mimicking of the PM phenotype. PM subjects do not demonstrate CYP2D6-related interactions as there is no enzyme to inhibit, but they might be more susceptible to inhibition or induction of the alternative pathway via CYP3A4 [4]. Pharmacodynamic interactions take place at the receptor level and usually involve an additive or synergistic effect with combined toxicity. Thus, the use of VEN with other serotonergic (and noradrenergic) drugs can expose the patient to additive effects like the serotonin syndrome. As the concentrations will not change, the role of pharmacodynamic interactions is even more difficult to assess than the pharmacokinetic interactions.

Depression as a disease predisposes to suicidal behavior and requires long treatment periods involving extensive medication and consequently a high risk of drug interactions. The multitude of drugs, often together with alcohol, is also a common observation in suicide cases [17]. In several recent studies, VEN has scored a remarkably high fatal toxicity index (FTI), i.e., deaths in proportion to consumption, in comparison with other newer antidepressants and selective serotonin reuptake inhibitors (SSRIs) [18–21]. Adverse outcomes have been reported with VEN use, including cardiac toxicity [22–24], hyponatremia [25, 26], serotonin syndrome and seizures [27, 28] and rhabdomyolysis [29]. A higher risk of discontinuation of VEN therapy due to ADRs has been observed [30–32]. A switch from one SSRI to another has been found to be as efficacious and with fewer ADRs than a switch to VEN [33]. On the other hand, VEN has been suggested to be more effective than SSRIs or tricyclics in the treatment of resistant depression [34]. Furthermore, it has been found that patients with more severe illness and higher susceptibility to suicide are commonly prescribed VEN resulting in apparent greater toxicity [35].

In this study, a comprehensive post-mortem database and the related death certificates within a 2-year period are searched for cases with laboratory findings of VEN, findings of other drugs, associated background information, and the cause and manner of death. For a subgroup of cases with post-mortem blood samples available, the concentrations are determined for VEN and its two metabolites, O-VEN and N-VEN, and the *CYP2D6* genotype is determined. The study focuses on adverse drug interactions (ADI) and the relative metabolic activity of CYP2D6 as these factors are hypothesized to contribute to the failure of antidepressant therapy resulting in subsequent fatal outcomes.

Material and methods

Reference standards

O-desmethylvenlafaxine (HCl) and *N*-desmethylvenlafaxine (HCl) were purchased from SynFine Research Inc. (Ontario, Canada), while venlafaxine (HCl) was donated by Lederle (Pearl River, NY, USA). The internal standards dibenzepin (HCl), ketobemidone (HCl) and desipramine (HCl) were donated by the Finnish National Agency for Medicines, the Finnish National Institute of Health and Welfare, and Geigy (Basel, Switzerland), respectively.

Subjects and blood samples

The subjects were autopsied in Finland during the 2-year study period and all the toxicological analyses were performed at the Department of Forensic Medicine, University of Helsinki. The study included all cases that were previously proven to be VEN positive by a routine quantitative drug screening method for blood samples [36]. These 191 cases were entered into the laboratory database between 30 June 2005 and 29 June 2007. The samples, containing ~1% NaF, were stored at −20°C. The samples were thawed (+4°C) and vortex mixed before genotyping and the quantitative determination of VEN, O-VEN and N-VEN.

Genotyping

DNA was isolated from 250 µl of autopsy blood using an E.Z.N.A. SE Blood kit (Omega Bio-Tek Inc., Doraville, GA, USA). *CYP2D6* genotyping was performed by a previously described method that detected 11 variants of the allele (**2*, **3*, **4*, **5*, **6*, **9*, **10*, **17*, **29*, **39*, **41*) [37]. Alleles not carrying detected mutations were classified as **1*. The detection was carried out using an ABI PRISM SNaPshot™ multiplex kit (Applied Biosystems, Foster

City, CA, USA). Two additional long PCR reactions 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 multiplicity of the duplication could not be determined and hence the duplicated allele is denoted *xN*.

Quantification of venlafaxine, *O*-desmethylvenlafaxine and *N*-desmethylvenlafaxine

Sample preparation

For the preparation of calibration and control samples, ethanolic solutions of the reference standards were carefully evaporated to dryness at +50°C followed by addition of the blank blood matrix: pooled venlafaxine-negative post-mortem blood from five to seven individuals.

Each blood sample (1 g) was mixed with 500 µl of KH_2PO_4 buffer (pH 6.2), followed by addition of 50 µl of the internal standard solution (dibenzepin, ketobemidone and desipramine, each 0.01 mg/ml in water). After addition of 1 ml of 0.5 M Na_2HPO_4 buffer (pH 9.0), the pH was adjusted to 9.2 with 1 M NaOH solution. The sample was extracted with 2.5 ml of butyl acetate for 2 min in a vortex mixer and, after centrifugation, the upper phase was transferred into another test tube and evaporated to dryness at +65°C. One hundred microliter of triethylamine and 100 µl of propionic acid anhydride were added to the residue, followed by incubation at +80°C for 30 min. After cooling, 1.0 ml of 0.5 M Na_2HPO_4 (pH 9.0) was added, and the sample was extracted with 1.5 ml of butyl acetate for 2 min. The upper organic phase was evaporated to dryness, and the residue was dissolved in 300 µl of butyl acetate.

For badly decomposed samples, an additional purification step was performed before derivatization. Of the 0.05 M H_2SO_4, 1.0 ml was added to the butyl acetate phase and the mixture was vortex mixed for 2 min. After centrifugation and discarding the upper organic layer, 1.0 ml of 0.5 M Na_2HPO_4 (pH 9.0) was added to the aqueous phase and the pH adjusted to 9.2. The sample was extracted with 2.0 ml of butyl acetate for 2 min and, after centrifugation, the upper organic phase was submitted for derivatization as described above.

Gas chromatography–mass spectrometry (GC-MS)

The GC-MS unit comprised an Agilent 5975 inert XL mass selective detector coupled to an Agilent 6890 N gas chromatograph equipped with a 7683 injector (Agilent Technologies, Santa Clara, CA, USA). A ZB-5MS capillary column (12 m×0.20 mm i.d. with 0.33 µm film) from Phenomenex (Torrance, CA, USA) was used. The GC-MS was operated using ChemStation software. Helium was used as carrier gas at a constant flow rate of 2 ml min^{-1}. Pulsed flow injection was used in splitless mode with a 2 µl injection volume and an injection pressure of 259 kPa for 0.5 min. The injector port temperature was 250°C and the transfer line temperature 300°C. The oven temperature was initially held at 120°C for 0.5 min and then increased by 30°C min^{-1} to 320°C and held there for 2 min. The GC-MS analysis was performed in selected-ion monitoring mode. An internal standard was assigned for each of the three analytes as follows: dibenzepin (ions m/z *224*, 195) for VEN (propionic anhydride derivative (PA); m/z *202*, 134), ketobemidone (PA; m/z *303*, 246) for O-VEN (2PA; m/z *188*, 244) and desipramine (PA; m/z *322*, 208, 193) for N-VEN (2PA; m/z *134*, 221, 264). The target ions are in italics.

Method validation

The background noise from blood matrix and reagents at the selected ions was studied with eight post-mortem blood samples that were known to be VEN negative. Internal standards were added to two of the samples. To evaluate the matrix effect on the precision of the extraction procedure, another set of ten blank blood samples were spiked with two different concentrations (100 and 1,000 µg/l) of the analytes. The precision expressed as coefficient of variation (CV) ranged from 4.0% to 7.7% for VEN and O-VEN and from 12.0% to 20.0% for N-VEN. The linearity of the method was studied with five parallel samples of eight concentration levels in the range of 10-3,000 µg/l; an additional low concentration level (5 µg/l) was added for the minor metabolite N-VEN. The signal-to-noise ratio was >20 for all analytes at the lowest concentration levels studied. For the linear range precision and accuracy up to ±15% and ±20%, respectively, were accepted. The limit of quantification (LOQ) was 30 µg/l for VEN and O-VEN and 10 µg/l for N-VEN and the linear ranges were 30–3,000 µg/l for VEN and O-VEN and 10–1,000 µg/l for N-VEN, applying three-point linear calibration curves forced to zero. For precision, nine parallel samples were prepared from a single post-mortem sample and analyzed the same day. The CVs were 6.6% (O-VEN), 7.7% (VEN) and 8.1% (N-VEN). For the evaluation of day-to-day precision and stability, a post-mortem sample was repeatedly frozen, thawed, extracted and analyzed in each sample series (n=6) during the 2-week period. The CVs were 6.7% (O-VEN), 13.9% (VEN) and 15.9% (N-VEN). A new blank blood sample, calibration samples and two spiked control samples (100 and 1,000 µg/l for VEN and O-VEN and 30 and 300 µg/l for N-VEN) were prepared for each series and run at the beginning and end of the sequence. For samples that had concentrations above the linear range, a calibration curve with six points from 2,000 to 10,000 µg/l was set up and the samples were diluted with blank blood prior to extraction so that they fitted the curve.

Database analysis

Post-mortem database

The case files in the laboratory post-mortem database included a forensic pathologist's referral, laboratory analysis results and information extracted from the death certificate issued by a forensic pathologist. The referral contained background information from the police, such as a brief description of the circumstances of death, known medications and the main autopsy findings. The analytical data contained analysis results for alcohols, medicines and drugs of abuse, and occasionally for other substances. Information from the final death certificate included the age and gender of the deceased, the cause of death with contributing factors according to the International Classification of Diseases and the manner of death (World Health Organization).

Drug-drug interactions

Drugs possessing pharmacokinetic or/and pharmacodynamic interactions with VEN were identified from the Swedish, Finnish, Interaction X-referencing (SFINX) interaction database [38]. The drug combinations classified as the two most severe (classes C and D) were included in the study. Other known CYP2D6 inhibitors and CYP3A4 inhibitors and inductors were included as well as other antidepressants having a synergistic pharmacodynamic effect. The present study is based on the SFINX database version of 6 November 2008.

Drug consumption

Drug consumption data (including hospital prescriptions) were obtained from the Finnish National Agency for Medicines and expressed as defined daily doses (DDD) per 1,000 inhabitants per day (DDD/1,000 inh/day). The DDDs were defined by the Nordic Council of Medicines. The number of antidepressant deaths was obtained from the laboratory post-mortem database. The FTI was calculated by dividing the number of deaths attributed to a drug by the consumption of the drug over the same period and area (deaths/DDD/1,000 inh/day). For example, there were 18 observed deaths attributed to venlafaxine in Finland during the year 2006 and the sales for 2006 were 4.87 DDD/1,000 inh/day (DDD 100 mg), resulting in the FTI 18/4.87≈3.70 for venlafaxine.

Statistical analysis

MINITAB 13.31 was used for statistical analysis. The allele variants were given an activity score according to their relative functionality as follows: non-functional allele or deletion 0, allele of reduced activity 0.5, allele of wild-type activity 1 and increased activity 2 [39]. The sum for each individual was calculated yielding seven possible genotype groups from 0 to 3 at intervals of 0.5. For each individual, the metabolite ratios (VEN/O-VEN and VEN/N-VEN) were calculated and, after logarithmic transformation, the genotype group median metabolite ratios along with their 95% confidence intervals were plotted against the sum of the genotype. The Mann-Whitney test was used to evaluate the difference between the median metabolite ratios. The test for two proportions was used to compare the allele and suicide frequencies and to evaluate the distribution of the genotypes against the total VEN concentration. A p value of <0.05 was considered to indicate a statistically significant difference.

Results

Overview

Of all 191 cases found positive for VEN in previous blood drug screening, complete reanalysis of blood involving quantification of VEN, O-VEN and N-VEN and *CYP2D6* genotyping was successful in 123 cases. The remaining 68 cases were excluded for the following reasons: in 48 cases, there was not enough blood left for the study; in five badly decomposed samples, genotyping was not successful; and in 15 cases, the quantification of N-VEN was not considered reliable.

The subgroup of 123 cases represented well the whole material of 191 cases in terms of sex ratio (57% males vs. 54% males, respectively), average age (50±16 years vs. 50±15 years), number of alcohol-positive cases (44% vs. 48%) and average blood alcohol concentration (1.62±0.85‰ vs. 1.7±0.91‰), but there was a slight difference in number of nicotine positive cases (43% vs. 55%). Out of the 123 cases, 51 were classified by the forensic pathologist as drug poisonings—either suicides, accidents, or unclear causes. VEN was mentioned in 40 (78%) of the death certificates and 18 of these cases were classified as VEN poisonings, i.e., VEN possessed the highest concentration compared to therapeutic level among all drug findings within that particular case.

Quantification of venlafaxine, *O*-desmethylvenlafaxine and *N*-desmethylvenlafaxine

In the subgroup material, the median concentrations of VEN, O-VEN and N-VEN were 560, 420 and 49 μg/l (2,020; 1,600 and 190 nmol/l), respectively, and the concentration ranges were 50–88,500; 78–34,200 and 10–27,100 μg/l, respectively (Table 1). Below LOQ concen-

Table 1 Post-mortem femoral blood concentration of venlafaxine and its two metabolites (μg/l)

	n	10th percentile	Median	90th percentile
Venlafaxine				
All cases	120	100	560	4,760
Death by drug poisoning	49	160	1,290	12,380
Death by venlafaxine poisoning	18	1,920	6,070	60,140
Others causes of death	71	100	330	1,080
O-desmethylvenlafaxine				
All cases	119	130	420	1,380
Death by drug poisoning	47	56	510	3,770
Death by venlafaxine poisoning	17	120	1,110	11,850
Others causes of death	72	190	400	1,010
N-desmethylvenlafaxine				
All cases	117	11	49	670
Death by drug poisoning	50	15	71	1,590
Death by venlafaxine poisoning	18	160	730	6,030
Others causes of death	67	10	30	280
Total venlafaxine (VEN+O-VEN+N-VEN)				
All cases	123[a]	340	1,140	6,020
Death by drug poisoning	51[a]	420	2,100	18,850
Death by venlafaxine poisoning	18[a]	2,850	7,390	79,070
Others causes of death	72[a]	340	880	2,000

[a] Including cases in which the concentration was below LOQ

trations of VEN, O-VEN and N-VEN were measured in three, four and six cases, respectively. One case did not contain a detectable amount of N-VEN. Figure 1 shows the distribution of the total VEN concentration (sum of VEN, O-VEN and N-VEN) up to 6,750 μg/l. Figure 1 also shows the cumulative distribution of fatal venlafaxine poisonings assessed by forensic pathologists, revealing a median total VEN concentration of 3,250 μg/l (approximately 11,900 nmol/l).

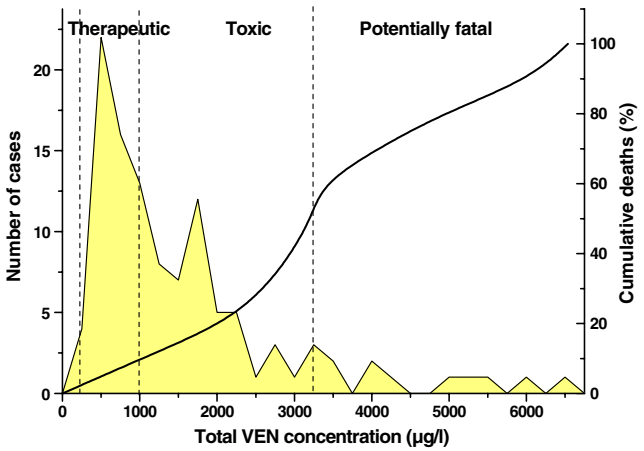

Fig. 1 Distribution of measured total venlafaxine concentrations (VEN+O-VEN+N-VEN, μg/l) in the subgroup of 123 post-mortem cases. The *bold curve* represents the cumulative distribution of fatal venlafaxine poisonings assessed by forensic pathologists and indicates a median fatal concentration of 3,250 μg/l. Ten cases (8,250-150,000 μg/l) were excluded from the figure

Other drugs and adverse drug interactions

The potentially interacting drugs together with their mechanism of interaction and the number of cases are presented in Table 2. Mirtazapine, levomepromazine and tramadol were the most frequent findings with 17, 15 and 11 cases, respectively. One of the tricyclic antidepressants was found in 17 cases. The cases were divided into four different concentration groups by the total VEN concentration as follows: subtherapeutic range (<250 μg/l, approximately <920 nmol/l), therapeutic range (250–1,000 μg/l, approximately 920–3,690 nmol/l), toxic range (1,000–3,250 μg/l, approximately 3,690–11,900 nmol/l) [40] and potentially fatal range (>3,250 μg/l, approximately >11,900 nmol/l). The potentially fatal range was based on the mean median fatal total VEN concentration established above. In 46% of all cases, there was at least one interacting drug, and the proportion increased with the concentration group from therapeutic to potentially fatal (Fig. 2). Only one case could be classified as a pure VEN poisoning, and in this case the total VEN concentration was 48,300 μg/l with no alcohol present.

The cases with CYP2D6 inhibitor findings (N=33) were compared with those without a finding (N=88). The poor metabolisers found were excluded as there should be no effect. Between these groups there was a statistically significant difference in the median total VEN concentration (2,097 vs. 925 μg/l, respectively) and in the VEN/O-VEN ratio (2.69 vs. 0.78). Almost twice as many cases fell

Table 2 Number of findings of drug combinations with potential for severe adverse drug interaction in the subgroup of 123 venlafaxine cases

Drug	Interaction with venlafaxine	Number of cases
Tricyclic antidepressants[a]	Inhibition of CYP2D6, additive serotonergic effect	17
Mirtazapine	Additive serotonergic effect	17
Levomepromazine	Inhibition of CYP2D6	15
Tramadol	Additive serotonergic effect	11
Carbamazepine	Induction of CYP3A4	4
Warfarin	Increased anticoagulation	3
Fluoxetine	Inhibition of CYP2D6, additive serotonergic effect	2
Metoclopramide	Unknown (inhibition of CYP2D6)	2
NSAIDs[b]	Increased risk of GI bleeding by decreased platelet aggregation	2
Verapamil	Inhibition of CYP3A4	2
Citalopram	Additive serotonergic effect	2
Paroxetine	Inhibition of CYP2D6, additive serotonergic effect	1
Bupropion	Inhibition of CYP2D6	1
Salicylic acid	Increased risk of GI bleeding by decreased platelet aggregation	1
Thioridazine	Inhibition of CYP2D6	1
Perphenazine	Inhibition of CYP2D6	1
Amiodarone	Inhibition of CYP3A4	1
Diltiazem	Inhibition of CYP3A4	1
Fluconazole	Inhibition of CYP3A4	1
Phenytoin	Induction of CYP3A4	1

[a] Amitriptyline, trimipramine, doxepin, nortriptyline (alone)

[b] Nonsteroidal anti-inflammatory drugs

Drugs that were included in the study but for which no cases were found: celecoxib, chloroquine, clomipramine, dexamethasone, duloxetine, flecainide, fluphenazine, fluvoxamine, haloperidol, lithium, mianserin, milnacipran, moclobemide, nefazodone, phenobarbital, propafenone, quinidine, reboxetine, sertraline, terbinafine, trazodone, valdecoxib

into the potentially fatal range when an inhibitor was present (27% vs. 15%, p=0.147), but the difference was smaller in the toxic range (45% vs. 33%, respectively).

Genotyping

The observed *CYP2D6* allele frequencies and genotype distribution are presented in Tables S1 and S2 of the Electronic Supplementary material. Only two PM cases (<2%) were found, while 12 cases represented UM (10%). In two cases, the duplication was in combination with an inactive allele variant so they were not counted as UM. In one case, there was duplication of an inactive allele (**4xN*). No IM cases were detected. There was no statistical difference between the observed and expected distributions of genotypes within the previously described concentration groups (p>0.25 for each, data not shown).

The metabolite ratios were plotted against the sum of the *CYP2D6* genotype (Fig. 3). There was a negative correlation between the VEN/O-VEN ratio and the genotype sum. There was a 2.6-fold difference between the median VEN/O-VEN ratios of the HEM (sum group 1) and EM (sum group 2) cases (p<0.05), but no statistical difference was observed between other groups. Neither was any obvious shift towards the *N*-demethylation pathway via CYP3A4 detected, while there was little variation between the genotype groups VEN/N-VEN ratio medians (CV <9%), except for the two cases of PM (sum group 0).

Venlafaxine in comparison with other antidepressants

The FTI for year 2006 was calculated for venlafaxine and other commonly used antidepressants for which there were over 100 detected cases in the post-mortem database during the 2-year study period. As expected, the FTI of venlafaxine (expressed as deaths/DDD/1,000 inh/day) was higher (3.70) than that of mirtazapine or the SSRIs fluoxetine, citalopram and sertraline (0.21–0.99), but much lower than that of the tricyclic antidepressants amitriptyline (12.14) and doxepine (34.41).

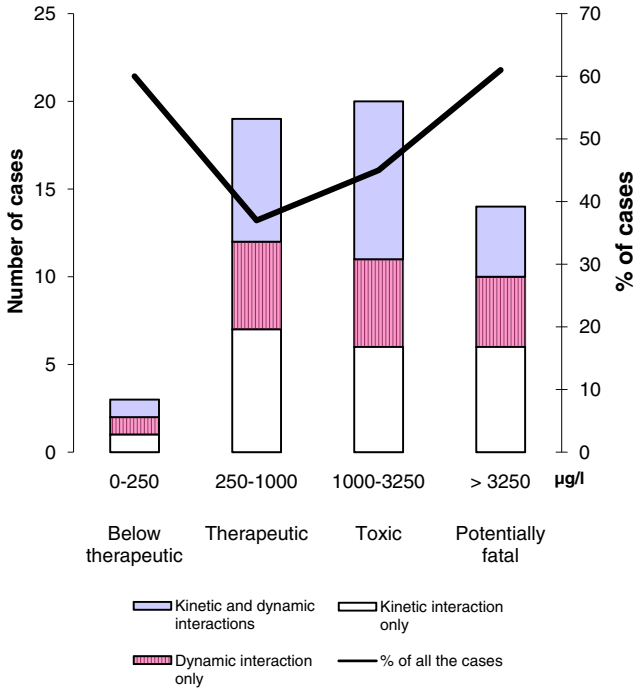

Fig. 2 Distribution of VEN-positive cases containing interacting drugs in the total VEN concentration groups. Cases with a *CYP3A4* inductor are included; there are three cases in the therapeutic concentration group and two in the potentially fatal concentration group

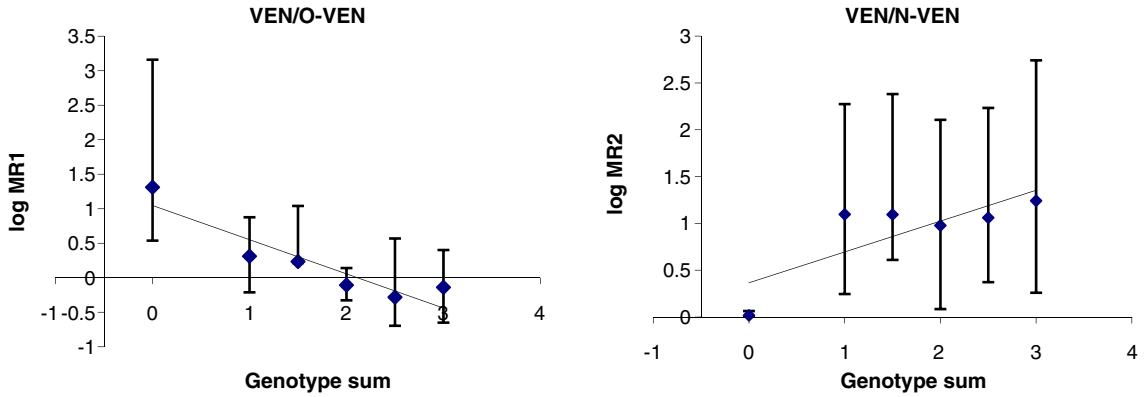

Fig. 3 Metabolite ratios for O-VEN (MR1) and N-VEN (MR2) plotted against the genotype sum of *CYP2D6* alleles. Logarithmic transformations of median metabolite ratios are shown with 95% confidence intervals

During the 2-year study period, the proportion of cases classified as suicides by all methods (hanging, shooting, self-poisoning by any substance, etc.) ranged from 28% for fluoxetine-positive cases to 42% for VEN-positive cases (Table 3). There was a statistical difference between VEN and amitriptyline, fluoxetine or citalopram. The proportion of suicides committed primarily with the specific antidepressant was calculated. The tricyclics had the highest percentages (48–62%) and the SSRIs the lowest (3–8%). Mirtazapine was quite close to the SSRIs (8%) whereas VEN had a significantly higher percentage (29%).

Discussion

The reasons for the high number of fatalities associated with VEN are complex; both the intrinsic toxicity of the drug and the subscription practice, which is biased towards higher-risk patients, has been proposed. The aim of this study was to get an insight into this problem by studying in detail the information that can be extracted from post-mortem laboratory analyses and death certificates. The material available for the study was exceptionally extensive; as the frequency of medico-legal autopsies in Finland was high (more than 20% of all deaths) and toxicological samples were collected in more than 50% of these cases, corresponding to approximately 6,000 cases a year [41]. A comprehensive drug screening was performed for the vast majority of the cases. To our knowledge, this is the most extensive material for which the concentration of N-VEN has been determined. The VEN and O-VEN concentrations shown in Table 1 are similar to previously reported data from post-mortem samples [42–44].

In VEN-positive cases, the frequency of drug combinations that could cause severe ADIs was higher (46%) than that generally found in post-mortem toxicology (0.71%) [45], and the percentage increased along the concentration group from therapeutic to potentially fatal. The presence of CYP2D6 inhibitors elevated both the total VEN concentration and the VEN/O-VEN ratio. Furthermore, the proportion of cases having a potentially fatal concentration of VEN was higher among the cases containing inhibitors.

Table 3 Antidepressant findings and suicides in Finland during 30 June 2005 to 29 June 2007

Drug	Positive cases			Suicides committed primarily with the drug	
	n	Suicides (%)	*p*[a]	*n*	% of all suicides with the same drug detected
Tricyclic antidepressants					
Amitriptyline	334	96 (29)	0.002	46	48
Doxepine	129	52 (40)	>0.05	32	62
SSRIs					
Fluoxetine	146	41 (28)	0.007	2	5
Citalopram	729	211 (29)	0.001	16	8
Sertraline	101	33 (33)	>0.05	1	3
Newer antidepressants					
Mirtazapine	516	192 (37)	>0.05	15	8
Venlafaxine	191	80 (42)		23	29

[a] Test for two proportions vs. venlafaxine

Comparison with other common antidepressants in Finland indicated that the frequency of suicides by all methods was highest among subjects on VEN (42%), doxepine (40%) and mirtazapine (37%). Fatalities specified as suicides with VEN were considerably more numerous than those with mirtazapine (31% vs. 8%, respectively). This could indicate that, as a drug, VEN holds suicide potential approaching that of the tricyclic antidepressants [28, 46].

The subjects on VEN did not stand out from the general population pharmacogenetically. The allele frequencies were compared with the combined results of a study on two Finnish populations (eastern and western Finnish) [6], but no statistical difference was observed. There were only two PMs (<2%), which is less than expected as it has been estimated that 5–10% of the European population is homozygous for a defective *CYP2D6* allele [6, 7], but then again the result was consistent with a previous study on Finnish smokers (2%) [47]. The 5.6% allele frequency observed for the effective duplications (**1xN* and **2xN*) is similar to that reported in a recent Finnish study (4.6%) [6], which indicates a higher CYP2D6-related metabolic rate in Finns than previously estimated for Scandinavian populations (1–2%) [48, 49]. However, it has been estimated that only a fraction of UM phenotypes are explained by genotyping duplicated alleles in Caucasian populations [50].

The relative CYP2D6 activity did not seem to predispose to high VEN concentrations. In both genetic PM cases, the concentration of VEN was equal to the concentration of N-VEN and only minor amounts of O-VEN were detected. These cases emphasize the importance of N-VEN analysis. If only VEN and O-VEN had been determined, different conclusions could have been drawn as the assumed total amount of ingested VEN would have fallen to half. Further, if the genotype was not known, the exceptionally high concentration of N-VEN might suggest pharmacogenetic reasons behind the fatality. A PM genotype has been found to increase the risk of side-effects, possibly due to the slight pharmacological differences between VEN and O-VEN [11, 51]. The contribution of N-VEN has not been determined. Both the wide inter-individual variability within the VEN/O-VEN metabolite ratio and the significant difference between the median metabolite ratios of HEMs and EMs observed in this study have also been found in other studies [9, 11]. Besides the sum of the CYP2D6 genotype, also other factors might contribute to the drug/metabolite ratio such as enzyme saturation at very high doses and survival time after intake [12].

One of the limitations of this study is certainly the relatively small sample size of the subgroup material. Moreover, post-mortem material is known to pose difficulties for the interpretation of toxicology results [52]. In the present study, only femoral venous blood samples were included, which minimizes the risk of post-mortem redistribution [53, 54]. The extent to which the vast number of interacting drugs interferes with the metabolic ratios in relation to genotype remains uncertain, but the result portrays the real situation in patients on VEN. There are also indications that smoking can lower the O-VEN and *N,O*-desmethylvenlafaxine levels [55]. Although all the drugs found are prescription drugs in Finland, it is not known whether they were taken deliberately together. Individuals suffering from depression are likely to have access to a reservoir of drugs from previous therapies. In a study on risk factors for suicide patients on venlafaxine, at least one other antidepressant was prescribed more often (72.5%) than for patients on fluoxetine (27.6%) or citalopram (39.5%) in the year prior to the study [35]. This result is parallel to the present findings, suggesting that ADI is a significant factor contributing to the apparently high toxicity of VEN.

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