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Can valproic acid be an inducer of clozapine metabolism?

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Abstract

Introduction—Prior clozapine studies indicated no effects, mild inhibition or induction of valproic acid (VPA) on clozapine metabolism. The hypotheses that 1) VPA is a net inducer of clozapine metabolism, and 2) smoking modifies this inductive effect were tested in a therapeutic drug monitoring study.

Methods—After excluding strong inhibitors and inducers, 353 steady-state total clozapine (clozapine plus norclozapine) concentrations provided by 151 patients were analyzed using a random intercept linear model.

Results—VPA appeared to be an inducer of clozapine metabolism since total plasma clozapine concentrations in subjects taking VPA were significantly lower (27% lower; 95% confidence interval, 14% to 39%) after controlling for confounding variables including smoking (35% lower, 28% to 56%).

Discussion—Prospective studies are needed to definitively establish that VPA may 1) be an inducer of clozapine metabolism when induction prevails over competitive inhibition, and 2) be an inducer even in smokers who are under the influence of smoking inductive effects on clozapine metabolism.

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Conflict of interest

No commercial organizations had any role in the writing of this paper for publication. The contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH. For his participation in the study reported in this article, or in the writing of this article, Dr. Diaz did not receive any salary or payment from the other authors or from grants awarded to them. In the past three years, Dr. Diaz had no conflict of interest.

Keywords

clozapine; pharmacokinetics; metabolism; enzyme induction; drug interactions; therapeutic drug monitoring; valproic acid; smoking; tobacco; random-effects linear model

INTRODUCTION

The pharmaceutical company that developed clozapine has published very limited information on clozapine metabolism [1]; most of our knowledge is limited to the N-demethylation of clozapine to norclozapine [2]. Also, there is limited information on the metabolism to clozapine-N-oxide, which is partially accounted for by flavin-containing monooxygenase (FMO) [3], and on the metabolism to the glucuronides by the UDP glucuronosyltransferases (UGTs) [4]. In average subjects not exposed to inducers or inhibitors and not having unusual genetic profiles, it is currently believed that clozapine is mainly metabolized by CYP1A2, with lesser roles for CYP2C19, CYP3A4 and CYP2D6 [2]. A recent study suggested that CYP2C19 may have a greater role in clozapine metabolism than expected since CYP2C19 poor metabolizers have increased plasma clozapine and norclozapine concentrations [5].

Fluvoxamine is a powerful inhibitor of clozapine metabolism [6]. This is not surprising since fluvoxamine is a strong CYP1A2 inhibitor, but other CYP inhibition may be relevant to clozapine metabolism since fluvoxamine is also a strong inhibitor of CYP2C19, a moderate inhibitor of CYP2C9 and CYP3A4, and a weak inhibitor of CYP2D6 [7]. Fluvoxamine inhibitory effects may vary from individual to individual, with increases in clozapine concentrations up to five to ten times [2,8]. A recent study indicates that saturation of CYP inhibition may occur with plasma fluvoxamine concentrations in the range of 50–150 ng/ml [5]. Therefore, interindividual variation in serum clozapine concentrations in patients taking fluvoxamine is probably explained by the different plasma fluvoxamine concentrations, and by different CYP genotypes which contribute to a different relevance of CYP1A2 versus non-CYP1A2 inhibitory pathways of clozapine metabolism. Paroxetine and fluoxetine are mild inhibitors of clozapine metabolism but they can be detected by controlling confounding variables [6,7]. Ciprofloxacin is another clinically relevant CYP1A2 inhibitor that can decrease clozapine metabolism [8].

Smoking and some antiepileptic drugs including carbamazepine, phenytoin and phenobarbital are powerful clozapine inducers [2,8–10]. The different enzymes involved in clozapine metabolism have different levels of vulnerability to induction. Concerning CYP isoforms, CYP2D6 cannot be induced. CYP2C19 is less susceptible to inductive effects than CYP3A4 [11]. The limited information available comparing CYP1A2 versus CYP3A4 induction indicates that carbamazepine has greater effects on CYP3A4 substrates than on CYP1A2 substrates [12]. In summary, there is a limited understanding of the relative role of different enzymes on clozapine metabolism in situations of induction but the different levels of CYP1A2, CYP2C19, CYP3A4 and UGT inductions are probably relevant. The lack of drug-drug interaction (DDI) studies on how inducers change the relative role of different enzymes is a gap not only in clozapine research but in all drugs research [13].

Due to our limited knowledge of clozapine metabolism and the assumption that in most circumstances and most patients norclozapine is the main clozapine metabolite, it is not surprising that most therapeutic drug monitoring (TDM) studies have focused on plasma clozapine and norclozapine concentrations [14]. Prior studies [15,16] suggest that total plasma clozapine concentration, calculated by adding plasma clozapine and norclozapine concentrations, may be a better indicator of overall clozapine metabolism than plasma clozapine concentration alone. Obviously a better total clozapine concentration would also include the plasma concentrations of clozapine-N-oxide and glucuronides. Total plasma concentrations as defined in this article (by adding clozapine and norclozapine) are influenced by inducers and inhibitors in a way that is consistent with known pharmacological mechanisms [15,16]. Similarly, the norclozapine/clozapine ratio has occasionally been used in the literature, but this ratio: 1) is not a good measure of CYP1A2 activity [17], and 2) has very high within-subject variability, even under the same clozapine dose [18]. CYP1A2 contributes to the simultaneous formation and destruction of norclozapine while other CYPs and renal elimination contribute to norclozapine elimination [19–21]. Using a norclozapine/dose ratio makes little sense from a pharmacological point of view because it is difficult to quantitatively predict how increments or reductions in CYP1A2 activity influence this ratio.

Valproic acid (VPA) is metabolized by UGTs (40%), and by β-oxidation as a fatty acid (30%), with minor CYP-dependent metabolism (including CYP2C9, CYP2C19 and CYP2A6). At low doses, β-oxidation may be the most important pathway, while at therapeutic doses glucuronidation may be more important [22]. Until 2008 (see Table 1 for a study review) [23–29], the literature had provided contradictory results concerning the effects of VPA on clozapine metabolism. Studies with different designs indicated no effects [23,26,28], mild inhibition [26,29] or mild induction [24,25,27]. In 2008, Diaz et al [16] developed a statistical random-effects linear model using an Italian clozapine sample that combined unpublished TDM data (N=83) with data from several published DDI studies (N=172), including one study focused on concomitant VPA treatment [26]. After adjusting for potential confounding variables (Table 1), VPA appeared to have inhibitory effects in non-smokers whereas VPA appeared to induce clozapine metabolism in smokers. Based on this finding, Diaz et al. [16] proposed that VPA may have differential effects on plasma clozapine concentrations depending on whether or not the patient is a smoker. The VPA-clozapine DDI is probably very complex. Besides the issue of VPA exhibiting both inductive and inhibitory effects on clozapine metabolism, another finding of the Italian clozapine TDM study [16] was that VPA appeared to potentiate smoking inductive effects on clozapine metabolism since smoking alone produced a 20% reduction in plasma clozapine concentrations [95% confidence interval CI, (−31, −7)], whereas smoking and VPA together produced a 41% reduction (−56, −21).

The lack of smoking control in study designs may explain the inconsistent results from prior studies of VPA and clozapine metabolism. In a recent case report of a smoker [30], the inductive VPA's effects appeared to be dose-related.

Traditionally, VPA has been considered a broad-spectrum metabolic enzyme inhibitor because 1) it inhibits UGT enzymes (UGT1A4 and UGT2B7), 2) it competitively inhibits

CYP2C9, and 3) it weakly inhibits CYP2C19 and CYP3A4 [22,31–33] (Table 2). On the other hand, progressively more literature [34–39] indicates that in the right circumstances VPA can have net inductive effects (Table 2). VPA may not be the only case. This is obviously a complex situation since oxcarbazepine or topiramate can have net inhibitory effects, net inducer effects or no net effects for the same specific substrate. As an example, topiramate in low doses may be a mild inducer of VPA metabolism but, at high doses, it may be an inhibitor [40]. These complex effects depend on the specific metabolism mechanisms, and are frequently influenced by the dosing of the drug that has a mixed inhibitor-inducer profile [41,42].

When discussing VPA effects on clozapine metabolism, some lessons can be learned by paying attention to olanzapine which has a metabolic pathway similar to that of clozapine [2,8]. Olanzapine appears to be mainly metabolized by CYP1A2 and UGT [2,8]. Although the VPA-olanzapine DDI has not been well studied [43], it is important to note that three studies with different designs indicated that VPA may be an olanzapine inducer [37–39]. Moreover, one of these three VPA-olanzapine studies used a prospective design [39] that indicated that, in reality, VPA may have a mixed profile: VPA may have the potential of being both an inhibitor and inducer of olanzapine metabolism. In fact, VPA exhibits net inhibiting effects, net inductive effects or no net effects, depending on VPA concentrations. At low VPA concentrations, VPA appeared to be an olanzapine competitive inhibitor, and at higher VPA concentrations VPA inductive effects appeared to overcome its competitive inhibitory effects [see Table 2, footnote g].

In this study, we examined the hypotheses generated in the Italian clozapine study that, under some conditions, VPA may be a net inducer of clozapine metabolism, and that smoking may modify this inductive effect, using a sample of Swiss patients on clozapine TDM. It is a well-established fact that smoking is an inducer of clozapine metabolism but in this study we also searched for a confirmation of the new finding that the inductive effects of smoking may be augmented by VPA treatment.

MATERIAL AND METHODS

Subjects

This study examined the data of 158 patients who both were on clozapine treatment, and provided measurements of trough steady state plasma clozapine and norclozapine concentrations for TDM during their treatment, at the Hospital of Cery, University of Lausanne, Switzerland. Trough concentrations were defined as those measured after a time ≥10 hours but ≤24 hours elapsed from last clozapine dose. This is a sample of participating patients who provided data for clinical purposes. The Lausanne University Hospital has the general permission from its institutional review board to use for research purposes anonymized data collected in routine clinical work.

Laboratory analysis

Samples were analyzed for plasma clozapine and norclozapine concentrations, measured in ng/mL, by high performance liquid chromatography-mass spectrometry (HPLC-MS) using a

previously published method [44]. Plasma VPA concentrations were obtained by using Fluorescence Polarization Immunoassays (Cobas Integra® 400 Plus, Roche).

Samples

Total clozapine concentrations, defined as the sum of trough steady state plasma clozapine and norclozapine concentrations, were computed. Any available total clozapine concentration was used for the current study if and only if it satisfied two criteria: 1) complete information on clozapine dose, comedications and smoking status was on record at the time the concentration was obtained; and 2) the concentration was obtained at a time when the patient was not taking a known well-established inducer [2,8,10] or inhibitor [2,8,10,16] of clozapine metabolism. A total of 353 total clozapine concentrations, provided by 151 of the 158 patients satisfied both of the above two criteria, and these are the observations analyzed in this article. The number of plasma clozapine concentrations excluded due to co-prescription of well-established inducers was 2 (both under carbamazepine), and due to well-established inhibitors was 12 (fluvoxamine, 3; fluoxetine,5; paroxetine, 2; and ciprofloxacin, 2). Blood samples from patients taking strong inducers or inhibitors at the moment of sampling were excluded due to their small numbers and because there were uneven distributions of well-established inducers and inhibitors across groups with and without VPA.

The average number of total clozapine concentrations provided by the 151 subjects was 2.3, and each subject provided from 1 to 27 total concentrations. Forty-eight percent (73/151) of the patients were males (they provided a total of 167 total plasma clozapine concentrations); 48% (73/151) were smokers (178 concentrations); and 19% (28/151) of the patients provided total clozapine concentrations under VPA (102 concentrations).

Total plasma clozapine concentrations were classified into whether or not they were measured under a potential mild inhibitor of clozapine metabolism (see Table 3, footnote a) [2,8,10,16, 45], and also into whether or not they were measured under a potential mild inducer of clozapine metabolism (Table 3, footnote a) [2,8,10,16,45]. Fifty-five of the 353 investigated total plasma clozapine concentrations were measured when the patient was taking at least one potential mild inhibitor; these 55 measures belonged to 31 patients. Also, 55 total plasma clozapine concentrations, which belonged to 23 patients, were measured under at least one potential mild inducer.

Statistics

A random intercept linear model of the log of total plasma clozapine concentrations was built, similar to the model used by Diaz et al. [16]. This model has been found to represent very accurately the steady-state measures of a substantial number of drugs and patient samples [46–49]. The model was built by using all 353 investigated total plasma clozapine concentrations. The dependent variable of the model was the log of total plasma clozapine concentration, and the log of clozapine dose was included as an independent variable. Other independent variables examined were male gender (1=male, 0=female), smoking (1=smoker, 0=non-smoker), taking VPA (1=taking, 0=not taking), taking potential mild inhibitors of clozapine metabolism (1=taking, 0=not taking), taking potential mild inducers

of clozapine metabolism (1=taking, 0=not taking), and weight (in Kg). The interaction between smoking and taking VPA, defined as the mathematical product of the variables smoking and taking VPA, was also examined as a possible term of the model. Covariate effect sizes were computed as in Diaz et al. [16] and their interpretation is further explained in Diaz et al. [48]. A negative effect size indicates induction of clozapine metabolism, and a positive effect size indicates inhibition, with greater effect sizes in absolute value indicating induction or inhibition, respectively. The final model reported in Table 3 fitted well according to residual analyses.

An additional random intercept linear model of the log of total plasma clozapine concentrations was built by using only concentrations that were measured when 1) the patient was taking VPA and provided a measurement of plasma VPA concentration and 2) the patient was not taking any well-established inhibitor or inducer of clozapine metabolism. A total of 51 total plasma concentrations provided by 18 subjects satisfied this condition and was therefore used to fit this second model. In addition to the log of clozapine dose, this model also included plasma VPA concentrations (in μg/mL) as an independent variable. Other variables examined as potential independent variables of this model were weight and smoking, as well as the interaction between smoking and VPA plasma concentrations which was defined as the product of the two variables.

RESULTS

Variables influencing total plasma clozapine concentrations

Table 3 shows the obtained model of total plasma clozapine concentrations built with all 353 concentrations. Gender, taking VPA, smoking, and clozapine dosage affected significantly total clozapine concentrations (Table 3). After adjusting for these significant variables, the following variables did not significantly affect total clozapine concentrations and were not included in the final model: body weight, taking potential mild inhibitors of clozapine metabolism, and taking potential mild inducers of clozapine metabolism (Table 3). After controlling for potential confounders, subjects taking VPA had significantly lower total plasma clozapine concentrations than patients not taking VPA ($p<0.001$). Specifically, total plasma clozapine concentrations in patients taking VPA were significantly lower than those in patients not taking it (27% lower; Table 3). Also, after controlling for potential confounders, total plasma clozapine concentrations in smokers were significantly lower than in non-smokers (35% lower; Table 3).

After adjusting for gender and clozapine dosage, there was not a significant interaction between smoking and taking VPA ($p=0.7$). Therefore, taking VPA did not significantly modify the effect of smoking on total plasma clozapine concentrations, and smoking did not significantly modify the effect of VPA.

Variables affecting total plasma clozapine concentrations in patients taking VPA

In the model for 18 patients taking VPA who provided VPA levels, only clozapine dose had a significant effect on total plasma clozapine concentrations ($p<0.001$). None of the other

independent variables in this model, including gender, weight, VPA levels and smoking were significant.

DISCUSSION

Can VPA be an inducer of clozapine metabolism?

The results from the total sample suggest that VPA may be an inducer of clozapine metabolism since, after controlling for potential confounders, total plasma clozapine concentrations in subjects taking VPA were significantly lower than those in patients not taking it (Table 3).

Can VPA also be a competitive inhibitor of clozapine metabolism?

Table 4 compares the results of the current study with our prior study which used an Italian sample [16]. An inconsistency appears to exist since the Italian sample appeared to indicate that VPA may be an inhibitor in non-smokers and an inducer in smokers, whereas the Swiss sample appears to suggest that VPA is only an inducer regardless of smoking status.

Unfortunately this Swiss study and the prior Italian study did not control for the duration of VPA treatment. Time may also be an important variable to control for, as suggested by the VPA-olanzapine study [39], because the relative importance of the inhibitory and inductive effects of VPA may change over time before steady state for both induction and competitive inhibition is reached. On the other hand, the Italian sample [16] provided only "circumstantial" evidence that VPA may also be a competitive inhibitor. VPA plasma concentrations and smoking may both interact to determine whether VPA effects are inductive or inhibitory on clozapine metabolism. So, it is possible that uncontrolled and unbalanced time effects on induction and inhibition may have contaminated the data in both samples.

Can VPA modify the inductive effects of smoking on clozapine metabolism?

The Italian study [16] indicated the possibility that VPA may potentiate the inductive effects of smoking, since the negative effect size of smoking on plasma clozapine concentrations in patients taking VPA (−41%) was significantly greater in absolute value than that in patients not taking VPA (−20%) (Table 4). In the total Swiss sample, the average effect size of smoking was −35%, but no significant difference was observed between patients taking and not taking VPA regarding this effect size. Therefore, the Swiss sample demonstrated that VPA had an inductive effect in smokers beyond smoking inductive effects, but did not replicate the result of the Italian study that there is an interaction between smoking and taking VPA. In particular, the current study did not confirm that VPA may potentiate the inductive effects of smoking.

Gender

In our prior US clozapine study [46] using a random intercept linear model, male gender was associated with higher drug clearance. A similar result was obtained in the Swiss study described in this article. It is possible that female sexual hormones may contribute to decrease clozapine metabolism. Certainly, oral contraceptives appear to be inhibitors of

clozapine metabolism [50, 51] and their use was most likely underreported in these TDM patients.

Limitations

The major limitation of this study is that this is not a prospective study designed to test whether or not VPA is an inducer of clozapine metabolism. The Swiss investigators collected this sample for clinical purposes and were unaware of the hypotheses suggested by the Italian clozapine study [16] and the prospective VPA-olanzapine study [39]. However, this lack of influence of the hypotheses on data collection may have its positive side of not biasing the collection procedures.

In our study, the number of total clozapine concentrations provided on any patient varied from 1 to 27. The statistical model used in this study, a mixed model, is known to very efficiently separate inter- and intra-patient variabilities [48] and to appropriately handle different numbers of observations across subjects [52]. Thus, it is not expected a priori that the different observations across subjects biased the estimates of model parameters and, therefore, our conclusions. On the other hand, we have to acknowledge that the lack of enough repeated measures of clozapine concentrations from patients taking VPA before and after starting VPA probably reduced the model's ability to detect both VPA's inductive effects and a possible interaction between VPA and smoking, interaction that was found in the Italian sample [16]. Moreover, the larger the number of available repeated measures, the more likely the mixed model will be able to control for the noise introduced by inter- and intra-patient variability. This gives us confidence in the results reported in Table 3, since our study used 353 observations from 151 patients. In a relatively large naturalistic sample like this one, however, we can expect to find unusual results due to lack of compliance, unusual genetic profiles and/or laboratory errors. Although standard residual analyses did not identify outliers in our data, we cannot rule out the existence of some of these issues.

The lack of availability of plasma clozapine-N-oxide concentrations is unfortunate. The conversion of clozapine to clozapine-N-oxide is probably mainly explained by CYP3A4 and FMO3 [3], and is reversible [53]. The possible influence of VPA inhibition and/or induction on clozapine oxidation to clozapine-N-oxide has not been studied. On the other hand, a study of the major FMO3 polymorphism in Caucasians found no clinically relevant effects on clozapine metabolism [54]. As indicated in the introduction, CYP1A2 contributes to the simultaneous formation and destruction of norclozapine. This implies that using norclozapine/dose ratios measured during routine TDM may not help to disentangle these two simultaneous processes. In fact, a controlled, non-observational longitudinal design may be needed to build a dynamic pharmacological model of these processes. Weekly repeated measures in the same patients under controlled conditions, as well as baseline measures prior to VPA treatment, would be required to establish VPA greater or lower inductive effects on clozapine versus norclozapine.

Any TDM clozapine study is limited by the complete lack of control of variables. Intake of St. John's wort was not recorded. St. John's wort is not expected to have major inductive effects on clozapine since it is not an inducer of theophylline, a CYP1A2-dependent drug, but it may have minor effects since it is described as an inducer of CYP2C9, CYP2C19 and

CYP3A4 [55]. In the other hand some potential clinically-relevant clozapine inhibitors such as caffeine intake [8] and presence of inflammation [8,15] were not assessed in this Swiss TDM study. The other side of the coin is that if we were able to detect the VPA effect using this type of design, it means that the effect should be strong enough to influence clinical practice.

Ideally, one would like to use a prospective design that disentangles the effect of valproate treatment duration, as in the prospective study of the effects of VPA on olanzapine metabolism [39]. That elegant prospective study [39] established that VPA is an inducer of olanzapine metabolism and provided important clues indicating that VPA may also be a competitive inhibitor of olanzapine metabolism that is dependent on VPA levels. Interestingly, our statistical approach did not demonstrate an inductive effect of VPA treatment on olanzapine metabolism when reanalyzing Italian olanzapine data from TDM and DDI studies that included 163 patients who provided 360 plasma samples [56]. In contrast, the relatively small prospective VPA-olanzapine study with 18 patients and 3 samples per patient did demonstrate it [40].

Clinical investigators do not have the abundant resources of pharmaceutical companies to complete large prospective studies lasting for several months. A pharmaceutical company has completed many well-controlled randomized clinical trials combining VPA and clozapine but has never looked into possible DDIs between these two products [43]. The Italian sample and this Swiss clozapine sample had limitations and provided just “circumstantial” evidence supporting the hypothesis of an inductive effect of VPA on clozapine metabolism, but these were relatively inexpensive studies with no external funding for data collection. The use of a sophisticated statistical random-effects linear model [45–48] has converted these inexpensive and unfunded collected samples into great opportunities to explore new and revolutionary ideas about DDIs between clozapine and VPA.

Ideally, a prospective randomized study on the effects of VPA on clozapine metabolism should: 1) stratify by smoking status; 2) provide a comprehensive measure of clozapine pharmacokinetics including at least clozapine, norclozapine and clozapine-N-oxide concentrations and even consider glucuronidation metabolites; and 3) provide a comprehensive measure of VPA pharmacokinetics, including at least total and free VPA and albumin concentrations. This extensive pharmacological information may help to identify the pharmacological mechanism involved in the VPA inductive and/or inhibitory effects on clozapine metabolism. Until that study is completed one can hypothesize that VPA inductive and competitive inhibitory effects may be mediated through UGTs since VPA is partly metabolized by UGTs and is definitively an inhibitor of some UGTs. VPA is not known to induce or competitively inhibit CYP1A2. VPA may be only a mild inhibitor of CYP2C19 and CYP3A4, and is not thought to be an inducer. There are no studies of VPA effects on FMO metabolism.

Conclusion

The Italian clozapine study results [16], the partial replication of these results using the current Swiss sample, and other clozapine [24,25,27,30] and pharmacokinetic literature [34–

39] suggest that VPA may be an inducer of some metabolic pathways and, in particular, of clozapine metabolism. Prospective studies are needed to definitively establish this hypothesis. Prospective studies need to consider that it is possible that VPA may also be a competitive inhibitor of clozapine metabolism [16, 42]. The net effect of VPA on clozapine metabolism may be the result of a combination of these two opposite drug metabolic actions, induction and inhibition, and may be influenced by smoking status and VPA levels, and possibly by VPA treatment duration. This study also highlights the importance of controlling for smoking and VPA levels in order to compare two different studies of the effects of VPA on clozapine metabolism.

A second new idea supported by the Italian [16] and the Swiss samples is that VPA may be an inductor in clozapine patients whose clozapine metabolism has already been induced by smoking. Other studies need to replicate this hypothesis, too.

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Table 1

Review of studies of the effect of VPA on plasma clozapine concentrations.

Author	Study design[a]	Smoking	Correction factor[b]	Net effect
Centorrino et al. 1994[23]	11 pts on versus 17 off VPA	Not described	No effect[c]	No effect
Finley & Warner, 1994[24]	4 pts on/off VPA	Not described	1.8 (1.3–2.1)[d]	Inducer
Longo & Salzman,1995[25]	7 pts on/off clozapine	Not described	1.7[e]	Inducer
Facciola et al. 1998[f][26]	15 patients with/22 without	Not described	No effects	No effect
	6 pts off/on VPA	Not described	Variable effects[g]	Inhibitor
Conca et al. 2000 [27]	1 pt off/on VPA	Smoker	2.1[h]	Inducer
Ulrich et al. 2003[28]	90 Cs on VPA versus 205 Cs off[i]	63% were smokers[j]	No effects[k]	No effect
Wong et al. 2006 [29]	51 pts[l]	59% were smokers[j]	0.85[m]	Inhibitor [n]
Diaz et al. 2008 [16]	45 Cs (37 pts) VPA from 415 Cs (255 pt)	67 smokers	1.3 (0.97, 1.7)	Inducer
		188 non-smokers	0.95 (0.83, 1.08)	Inhibitor
Riesseleman et al. 2013 [30]	1pt 10 Cs	Smoker	1.6[o]	Inducer/No

C = concentration; D = dose; pt= patient; VPA= valproic acid.

[a] Studies with patients on versus patients off used a parallel design (two different groups of patients were compared). Studies with on/off (or off/on) patients used an intra-subject design (the same patient sequentially provided levels with and without the medication or without and with the medication).

[b] A clozapine dose correction factor indicates the dose modification needed to correct for the drug clearance change in an average patient on clozapine who starts taking VPA. The clozapine dose-correction factors were calculated by using changes in serum/plasma clozapine concentration to dose (C/D) ratios [8,10,16]. Correction factors > 1 indicate an increase in drug clearance (e.g., inducer). Correction factors < 1 indicate a decrease in drug clearance (e.g., inhibitor).

[c] The article described no effects. Our calculation of total clozapine C/D ratios were 1.78 for controls and 1.75 for VPA patients. Total clozapine concentration was calculated by adding clozapine, norclozapine and clozapine-N-oxide concentrations.

[d] The article described mild induction. Our calculation of clozapine C/D ratio change is presented in table.

[e] The article described mild induction. Our calculation of total clozapine C/D ratio change is presented in table. Total clozapine concentration was calculated by adding clozapine and norclozapine concentrations.

[f] These patients were incorporated in Diaz et al. [16].

[g] Using change in total plasma concentrations (clozapine+norclozapine+clozapine-N-oxide), correction factors at week 4 were: patient 1=0.88, patient 2=1.11, patient 3=0.94, patient 4=0.85, patient 5=1.06 and patient 6=1.01. The article described VPA as a mild inhibitor.

[h] The article described mild induction. Our calculation of the mean clozapine C/D ratio change is presented in table.

[i] Many patients provided more than one clozapine concentration.

[j] This percentage of smokers is relative to the total sample size. The study did not report the percentage of smokers in VPA patients.

[k] The statistical analysis was not described.

[l] Unclear how many on VPA versus off.

[m] Our calculation of mean total clozapine C/D ratio change at week 12 is presented in table. No correction was made for smoking since data was not provided. Mean total plasma concentrations were calculated by adding mean clozapine and norclozapine concentrations.

[n] The difference in plasma clozapine concentrations was significant at week 12 (p=0.03). This test for differences may be limited by two major weaknesses in the statistical analysis: the authors compared plasma clozapine concentrations without correcting for clozapine dose, and other confounding factors such as smoking were not taken into account.

[o] During the equivalent of high dose VPA (aspiring 81 mg/d potentiated the effects of 1000 mg/d of VPA), the patient had a median total C/D=0.345 from 6 concentrations. During low dose VPA (1000 mg/d with no aspirin) or no VPA, the patient had a median total C/D=0.55 from 3 concentrations. Two concentrations were not entered in calculation since they were measured when inductive effects were disappearing. Adding aspirin is equivalent to increasing VPA dose since aspirin increased total VPA concentration and increased free VPA concentration even more by releasing VPA from serum proteins. On high VPA dose (VPA low dose + aspirin), VPA was an inducer. On low VPA dose (no aspirin) or no VPA, there was no induction.

Table 2

Review of non-clozapine literature on VPA inhibitory versus inductive effects.

	INHIBITORY EFFECTS
Traditional view [31,32]	VPA inhibitory at CYP2C9, UGTs and epoxide hydroxilase
More recent [33]	Inhibitor for paliperidone clearance:[a] unknown mechanism
	INDUCTIVE EFFECTS
Prospective volunteer study [34][b]	Mild auto-inducer of its own metabolism at low doses
In vitro study [35]	Inducer of CYP3A4 and P-glycoprotein gene expression
Prospective patient study [36][c]	Inducer of aripiprazole metabolism[d]
Case series [37][e]	Inducer of olanzapine metabolism
Therapeutic drug monitoring [38][f]	Inducer of olanzapine metabolism
	INDUCTIVE AND INHIBITORY EFFECTS
Prospective study [39]	Net inducer of olanzapine metabolism But can behave as net inhibitor in low VPA concentrations VPA concentration, time and smoking influence net effects[g]

VPA= valproic acid.

[a] This pharmaceutical company study has not been published. The prescribing information [30] described that co-administration of a single paliperidone dose of 12 mg with divalproex sodium extended-release tablets (two 500 mg tablets once daily) resulted in an increase of approximately 50% in the peak concentration and area under the curve of paliperidone. No pharmacological mechanism is provided to explain this inhibition.

[b] A prospective clinical study [34] in 12 young male volunteers taking low VPA doses for 3 weeks, reporting that VPA may induce its own metabolism by inducing β-oxidation.

[c] A pharmaceutical company prospective study [36] in 10 patients treated with VPA for 3 weeks. VPA appeared to be a mild inducer of aripiprazole metabolism. There was a decrease of 26% in the aripiprazole peak concentration and of 24% in the aripiprazole area under the curve with minimal effects on the active metabolite of aripiprazole.

[d] Although the mechanism of VPA inductive effects on aripiprazole are not well understood, induction of p-glycoprotein may be a possible mechanism [10].

[e] A case series of four patients stabilized on olanzapine concluding that VPA decreased olanzapine levels by 50% [37].

[f] A large olanzapine TDM study included 92 VPA patients with 166 plasma olanzapine concentrations and 205 control patients with 247 plasma olanzapine concentrations. The olanzapine concentration/dose ratio decreased by 32% indicating that VPA is a mild olanzapine inducer [38].

[g] A prospective study [40] in 18 patients that found that higher VPA plasma concentrations were associated with statistically significant, small decreases in plasma olanzapine concentrations that depended on VPA treatment duration (2 or 4 weeks). Findings at the 4th treatment week indicated that VPA, besides inducing olanzapine metabolism, may also be a competitive inhibitor. At low VPA concentrations VPA appeared to be an olanzapine competitive inhibitor, and at higher VPA concentrations VPA inductive effects appear to overcome its competitive inhibitory effects. The VPA concentration that led to change from net inhibitory effects to net inductive effects was different in smokers and non-smokers. By using the statistical model developed in this olanzapine study [37], the VPA concentrations producing a sufficiently high inductive effect on olanzapine metabolism that compensates for VPA inhibitory effects can be predicted for week 4. Specifically, 1) if the patient is a 40-year-old smoker taking 10 mg/day of olanzapine, a VPA level of 13.3 μg/mL at week 4 (usual therapeutic ranges for epilepsy are 50–100 μg/mL and for mania 50–125 μg/mL) will neutralize VPA inhibitory effects, and higher VPA concentration will produce a net induction of olanzapine metabolism at week 4. And 2) if the patient is a comparable non-smoker, the neutralization will occur at VPA concentrations of 41.8 μg/mL, with a net induction occurring at higher levels.

Table 3

Random intercept linear model of the natural log of total plasma clozapine concentrations of Swiss patients.[a]

Variable	B[b]	95% CI	p-value	E[c]	95% CI	Correction factor[d]	95% CI
Male gender	−0.18	(−0.35, −0.013)	0.034	−17%	(−29, −1)	1.20	(1.01, 1.42)
Taking valproic acid	−0.32	(−0.49, −0.15)	<0.001	−27%	(−39, −14)	1.38	(1.16, 1.63)
Smoking	−0.42	(−0.56, −0.28)	<0.001	−35%	(−43, −25)	1.53	(1.33, 1.75)
Log(clozapine dose)	0.91	(0.82, 1.003)	<0.001				

[a]The sample consisted of N=151 patients who provided a total of 353 measures of total clozapine concentrations when they were not being co-prescribed with known strong inducers (carbamazepine) or well established inhibitors (fluvoxamine, paroxetine, fluoxetine or ciprofloxacin) of clozapine metabolism. After reviewing the literature [2,8,10,16,45], the authors agreed in a list of potentially mild inhibitors of clozapine metabolism that included amlodipine (number of total clozapine concentrations measured under this comedication, N=1), amitriptyline (N=1), bupropion (N=1), clomipramine (N=2), darifenacine (N=1), diltiazem (N=1), doxycycline (N=1), estradiol (N=2), felodipine (N=1), fenofibrate (N=1), haloperidol (N=13), levomepromazine (N=9), methadone (N=3), metoclopramide (N=1), nifedipine (N=14), perphenazine (N=1), pioglitazone (N=1), propranolol (N=18), and sertraline in doses ≥ 150 mg/day (N=3). The variable “taking potentially mild inhibitors” was not significant in the model. After reviewing the literature [2,8,10,16,45], the authors also agreed in a list of potentially mild inducers of clozapine metabolism that included esomeprazole (N=49), lansoprazole (N=4), omeprazole (N=2) and prednisone (N=1). The variable “taking potentially mild inducers” was not significant in the model.

[b]Regression coefficient.

[c]Effect size on total plasma clozapine concentrations.

[d]A clozapine dose correction factor indicates the dose modification needed to correct for the drug clearance change in an average patient. As only clozapine concentrations contribute to efficacy and norclozapine concentrations do not, a random intercept linear model of the natural log of plasma clozapine concentrations may be the best way to calculate clozapine dose correction factors. A plasma clozapine concentration model (without norclozapine) yielded dose correction factors that were essentially the same as those reported in this table, except for that of valproic acid which was lower, 1.15. To illustrate correction factors, if an average non-smoker starts smoking during clozapine treatment, his/her clozapine dose should be multiplied by 1.53 in order to maintain the same steady-state clozapine concentrations. This approach may not work well in non-average patients, however, and dose estimation should be made on an individual basis following a more elaborate approach that takes full advantage of the random intercept linear model [47, 48].

Table 4

Comparison of effect sizes of smoking and VPA treatment on total plasma clozapine concentrations in Italian versus Swiss patients.

	Italian patients[a]		Swiss patients	
Variable	**Effect size[b]**	**95% CI**	**Effect size[c]**	**95% CI**
Taking VPA			−27%[d]	(−39, −14)
-Non-smokers	+6%	(−7, 20)		
-Smokers	−22%	(−40, 3)		
Smoking			−35%[e]	(−43, −25)
-Not taking VPA	−20%	(−31, −7)		
-Taking VPA	−41%	(−56, −21)		

CI= confidence interval; VPA = Valproic acid.

[a] Results reported in Diaz et al [16].

[b] Effect sizes were adjusted for significant variables: gender, other co-medications and clozapine dose.

[c] Effect sizes were adjusted for significant variables: gender and clozapine dose as shown in Table 3.

[d] There was not a significant interaction between smoking and taking VPA (Table 3); thus, the reported effect size of VPA treatment was not stratified by smoking.

[e] There was not a significant interaction between smoking and taking VPA (Table 3); thus, the reported effect size of smoking was not stratified by taking or not taking VPA.