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Competitive Displacement of Clozapine from Plasma Proteins in Normolipidemic and Hyperlipidemic Plasma Samples: Clinical Implications

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ABSTRACT *Objective.* This study assesses whether competitive displacement of clozapine by warfarin affects clozapine's overall plasma distribution. *Methods.* Warfarin sodium was preincubated in normolipidemic and hyperlipidemic plasma samples in varying concentrations. Following the preincubation with warfarin, [^{3}H]clozapine mixed with unlabeled clozapine was added to the plasma samples. The plasma was separated into its lipoprotein and lipoprotein-deficient fractions by density gradient ultracentrifugation, and clozapine distribution was determined. *Results.* When normolipidemic plasma was preincubated with various concentrations of warfarin, no significant redistribution of clozapine was noted among the various plasma lipoprotein fractions. However, in the case of the hyperlipidemic plasma, preincubating with warfarin did result in a significant redistribution of clozapine from the lipoprotein-deficient fraction to the very-low-density and low-density fractions of lipoproteins. Based on pharmacokinetic principles, the steady-state unbound concentration of clozapine in normolipidemic and hyperlipidemic plasma is not expected to change. *Conclusion.* Although no change in the steady-state unbound (active) concentration of clozapine would predict no change in clinical status, it is possible that this may only apply to the individuals with a normal lipid profile. We believe clozapine's association with lipoproteins (particularly triglycerides) may actually increase clozapine's effectiveness.

KEYWORDS Clozapine, Lipids, Triglycerides, Cholesterol

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INTRODUCTION

A drug's net pharmacological activity can be conceptualized as the sum of all its pharmacokinetic and pharmacodynamic processes. Within this

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framework, the degree to which a drug is bound to plasma proteins will contribute to its net pharmacological activity. Of the plasma proteins involved in binding drugs, albumin and alpha$_1$-acid glycoprotein are considered to be the most significant, having preferential affinities for acidic and basic drugs, respectively (Koch-Weser & Sellers, 1976). Although drugs do bind to other plasma proteins, it has been generally accepted that this represents only a small fraction of the total drug binding. However, this prevailing perception is being challenged and the pharmacological significance of drug binding to other plasma proteins (i.e., lipoproteins) is being acknowledged.

The function of plasma lipoproteins in influencing the biological activity of hydrophobic drugs is emerging in the literature, and its potential clinical relevance is being realized (Wasan, 1996; Wasan & Cassidy, 1998). For example, several lines of evidence indicate that increases in plasma low-density lipoprotein (LDL) cholesterol concentrations are associated with increases in amphotericin B, but decreases in cyclosporine-induced kidney toxicity (Brunner et al., 1988; Lemaire & Tillement, 1982; Wasan et al., 1990). With specific interest to psychiatry are psychotropic drug-induced changes in plasma lipid and lipoprotein concentrations that have been associated with increased clinical response and remission in psychiatric disorders (Diebold et al., 1998). Along these lines are reports documenting significant clinical improvement associated with increases in triglyceride levels secondary to clozapine therapy (Dursun et al., 1999; Pande et al., 2002). Offering an explanation for this phenomenon were data from our laboratory showing that a rise in triglyceride levels produced a significant shift in clozapine's plasma distribution (Procyshyn et al., 2001). More specifically, clozapine redistributed from the lipoprotein-deficient (LPD) fraction of plasma to the very-low-density lipoprotein (VLDL) fraction of the plasma. Based on these findings, we hypothesized that clozapine's association with the VLDL fraction of plasma may be, in part, responsible for its clinical effectiveness. If this is the case, then competitive displacement of clozapine from plasma proteins in individuals with dyslipidemia could also affect its overall plasma distribution, and consequently, its pharmacological activity. Because clozapine is highly bound to protein (95%), competitive displacement by another highly protein-bound drug could potentially alter its plasma distribution. To this end, the objective of this study is to assess whether competitive displacement of clozapine by warfarin (a highly protein-bound drug) affects clozapine's overall plasma distribution.

EXPERIMENTAL PROCEDURES

Warfarin sodium was preincubated in normolipidemic (Total cholesterol = 160 mg/dL; Triglycerides = 175 mg/dL) and hyperlipidemic (Total cholesterol = 280 mg/dL; Triglycerides = 310 mg/dL) plasma samples in varying concentrations (1, 5, 10 μg/mL) for 24 hours at 37°C ($n = 6$). In this study, the total cholesterol and triglyceride concentrations were manipulated using purified high-density lipoproteins (HDL), LDL, or VLDL obtained from pooled human plasma. The plasma used in this study was obtained from the Canadian Blood Services and constitutes pooled normolipidemic plasma from healthy volunteers tested for hepatitis B and C, as well as for the human immunodeficiency virus.

Following the preincubation with warfarin, [^{3}H]clozapine mixed with unlabeled clozapine (total concentration = 1.0 μg/mL) was added to the plasma samples and incubated for 1 hour at 37°C. The plasma samples were then cooled to 4°C and separated into their lipoprotein and lipoprotein-deficient fractions by density gradient ultracentrifugation, as previously described elsewhere (Wasan et al., 1999). VLDL, LDL, HDL, and the LPD (which consists primarily of albumin and α_1-acid glycoprotein) fractions were analyzed for [^{3}H]-clozapine against external standard calibration curves (corrected for quenching and luminescence). Total cholesterol, triglyceride, and protein concentrations were determined by enzymatic assay kits as previously described (Wasan et al., 1999). The plasma distribution of clozapine was replicated three times. Correlation analysis between [^{3}H]-clozapine recovery and cholesterol and triglyceride concentrations was determined using the Pearson correlation coefficient test and considered significant if $p < .05$.

RESULTS

In the control normolipidemic samples ($n = 6$; containing no warfarin), 79.03 ± 0.43% of the clozapine

TABLE 1 [^{3}H]Clozapine Recovery in Normolipidemic Plasma with Various Concentrations of Warfarin

Warfarin concentration (μg/mL)	Percentage of clozapine recovery in VLDL (mean ± SD)	Percentage of clozapine recovery in LDL (mean ± SD)	Percentage of clozapine recovery in HDL (mean ± SD)	Percentage of clozapine recovery in LPD (mean ± SD)	Percentage of clozapine bound to protein in LPD (mean ± SD)
0	0.75 ± 0.04	3.23 ± 0.21	16.99 ± 0.52	79.03 ± 0.43	86.02 ± 0.10
1	1.00 ± 0.06	5.43 ± 0.50	17.10 ± 0.51	76.47 ± 0.55	81.11 ± 0.80*
5	0.92 ± 0.07	6.47 ± 0.21	17.38 ± 0.68	75.23 ± 0.70	84.23 ± 1.20
10	0.81 ± 0.08	5.62 ± 0.31	18.98 ± 0.43	74.60 ± 0.46	77.90 ± 1.60*

Total cholesterol = 160 mg/dL; total triglycerides = 175 mg/dL.
N = 6 for each treatment group.
VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; LPDP, lipoprotein-deficient fraction.
*$p < .05$ for that sample compared with control (containing no warfarin).

was recovered in the LPD fraction (Table 1). Clozapine was also recovered in the other fractions of plasma as follows: 16.99 ± 0.52% in the HDL, 3.23 ± 0.21% in the LDL, and 0.75 ± 0.04% in the VLDL. When this normolipidemic plasma was preincubated with various concentrations of warfarin (1, 5, and 10 μg/mL), no significant redistribution of clozapine among the various plasma fractions was noted (Table 1). However, the increase in warfarin concentration did result in a significant decline in the proportion of clozapine bound to protein within the LPD fraction of plasma (Table 1). To this end, when the plasma concentration of warfarin was 1 μg/mL, clozapine was found to be 81.11 ± 0.8% bound to protein. This was an almost 5% decrease in protein binding of clozapine from baseline (86.02 ± 0.1%). At warfarin concentrations of 5 and 10 μg/mL, the proportion of clozapine bound to protein was 84.23 ± 1.2% and 77.90 ± 1.6% respectively (Table 1).

In the control hyperlipidemic samples ($n = 6$; containing no warfarin), 72.28 ± 0.39% of clozapine was recovered in the LPD fraction (Table 2). Clozapine was also recovered in the other fractions of plasma as follows: 11.17 ± 0.52% in the HDL, 9.98 ± 0.32% in the LDL, and 6.57 ± 0.63% in the VLDL. Unlike the normolipidemic plasma mentioned previously, preincubating the hyperlipidemic plasma with warfarin (1, 5, 10 μg/mL) did result in a significant redistribution of clozapine among the various fractions. For example, plasma samples preincubated with 1 μg/mL of warfarin was associated with a significant decrease in the amount of clozapine recovered within the LPD fraction (61.76 ± 1.33%) when compared with the control (72.28 ± 0.39%, $p < .05$). This decrease of clozapine within the LPD fraction coincided with significant increases in clozapine recovery within the VLDL (11.10 ± 1.11%) and LDL (15.52 ± 0.89%) fractions compared with their

TABLE 2 [^{3}H]Clozapine Recovery in Hyperlipidemic Plasma with Various Concentrations of Warfarin

Warfarin concentration (μg/mL)	Percentage of clozapine recovery in VLDL (mean ± SD)	Percentage of clozapine recovery in LDL (mean ± SD)	Percentage of clozapine recovery in HDL (mean ± SD)	Percentage of clozapine recovery in LPD (mean ± SD)
0	6.57 ± 0.63†	9.98 ± 0.32†	11.17 ± 0.52	72.28 ± 0.39†
1	11.10 ± 1.11*	15.52 ± 0.89*	11.62 ± 0.61	61.76 ± 1.33*
5	13.01 ± 0.88*	13.50 ± 0.77*	11.62 ± 0.55	61.87 ± 1.87*
10	14.96 ± 1.47*	15.02 ± 0.61*	10.62 ± 0.79	59.41 ± 1.20*

Total cholesterol = 280 mg/dL; total triglycerides = 310 mg/dL.
N = 6 for each treatment group.
VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; LPDP, lipoprotein-deficient fraction.
†$p = .05$ for the corresponding value in Table 1.
*$p < .05$ for that sample compared with control (containing no warfarin).

controls (6.57 ± 0.63% and 9.98 ± 0.32%, respectively, $p < .05$). Likewise, significant increases in clozapine recovery within the VLDL and LDL fractions (compared with their respective controls) were noted when plasma samples were preincubated with 5 or 10 μg/mL of warfarin. However, in these cases, the increase in clozapine recovery within the VLDL and LDL fractions was not significantly different from plasma samples preincubated with only 1 μg/mL.

DISCUSSION

From our previous work, we showed that clozapine redistributes itself from the LPD fraction to the VLDL fraction as plasma triglyceride levels increase (Procyshyn et al., 2001). Comparing the normolipidemic controls (Table 1) to the hyperlipidemic controls (Table 2) in this study, we confirm this observation. However, apart from validating our previous work, the main finding of this study is that the plasma redistribution of clozapine in the presence of another highly protein-bound drug (warfarin) depends on the concentration of lipoproteins within the plasma sample. For example, in the normolipidemic plasma samples (Table 1), the presence of warfarin produces in a decrease in clozapine's protein binding without any redistribution among the lipoprotein fractions. In contrast, in the hyperlipidemic samples (Table 2), the presence of warfarin not only results in a decrease in clozapine's protein binding, but there is also a shift of clozapine into the VLDL and LDL fractions. Based on pharmacokinetic principles and our previous studies, we want to speculate on the clinical implications of these results. However, to make clinical inferences using our in vitro data, we must make the assumption that our findings are reflective/representative of in vivo conditions in which warfarin would competitively displace clozapine from plasma proteins.

To begin, we must first recognized that hepatic clearance of protein-bound drugs depends on hepatic blood flow, intrinsic clearance, and protein binding (Eq. 1):

$$Cl_h = Q[f_u Cl'_{int}/Q + f_u Cl'_{int}] \quad (1)$$

where Q is hepatic blood flow, f_u is the fraction of unbound drug in the blood, and Cl'_{int} is the intrinsic clearance of unbound drug (in this case, clozapine). Because clozapine has a low hepatic extraction ratio,[a] the intrinsic clearance (Cl'_{int}) would be very small in comparison to hepatic blood flow (Q). As such, the hepatic clearance of clozapine (Cl_h) would become equal to the product of the fraction of unbound clozapine and the intrinsic clearance of clozapine (Eq. 2):

$$Cl_h = f_u Cl'_{int} \quad (2)$$

If clozapine binding to protein is constant (i.e., in the absence of warfarin), then the hepatic clearance of clozapine becomes equal to the intrinsic clearance of clozapine itself (Eq. 3). However, if clozapine competitively is displaced by another highly protein-bound drug (e.g., warfarin), this will result in a proportional change in the hepatic clearance of clozapine as a function of the increase in the fraction of unbound clozapine (f_u) (Eqs. 2 and 3):

$$Cl_h = f_u Cl'_{int} = Cl_{int} \quad (3)$$

With these concepts in mind, let us consider the clinical implications of the two scenarios presented by our data.

Scenario 1: Competitive Displacement of Clozapine by Another Highly Protein-Bound Drug in Normolipidemic Plasma

This scenario is based on the data in Table 1 where warfarin competitively displaces clozapine in normolipidemic plasma. As indicated by the data, there is a significant decrease in clozapine recovery within the LPD fraction that does not correlate with a significant increase in clozapine recovery within any of the lipoprotein fractions. However, this decrease in clozapine recovery within the LPD fraction does correlate positively with a decrease in the percentage of clozapine bound to protein within the LDP fraction. This suggests that warfarin has competitively displaced clozapine from protein-binding sites, subsequently

[a]Extraction ratio may be expressed as 100% of the drug entering the liver less the relative concentration of drug, which is removed by the liver.

increasing both the unbound fraction of clozapine and the unbound concentration of clozapine (two very different entities). At this point in vivo, two compensatory mechanisms immediately begin to operate to deal with the sudden increase in unbound (active) clozapine. First, the apparent volume of distribution (V_d) increases because it depends on the ratio of the fraction of unbound clozapine in blood (which increased) and tissue (Eq. 4):

$$V_d = V_B + f_u/f_{ut} V_T \quad (4)$$

where V_B is the volume of the blood, V_T is the volume of the tissue, f_u is the fraction of unbound clozapine in blood, and f_{ut} is the fraction of unbound clozapine in tissue. This increase in V_d happens within minutes to hours, thereby resulting in clozapine's quick redistribution. A second compensatory mechanism that would be operating is the proportional increase in the hepatic clearance of clozapine as the fraction of unbound clozapine (f_u) increases (Eq. 2). In other words, when the fraction of unbound clozapine increases (as a consequence of competitive displacement and redistribution), total clozapine is eliminated more rapidly until the unbound (active) steady-state concentration returns to the initial point. [Note: The clearance of unbound drug is not changed, so the unbound concentration (C_{uss} = dose rate/Cl'_{int}) falls back to the initial value over 3–5 half-lives]. The end result is a decrease in total clozapine concentration, an increase in the unbound fraction of clozapine, no change in unbound clearance, and no change in the steady-state unbound (active) concentration of clozapine. The clinical implications of this scenario are inconsequential because there should be no change in therapeutic response. Furthermore, the dose of clozapine should not be increased even though the total plasma concentration of clozapine has decreased.

Scenario 2: Competitive Displacement of Clozapine by Another Highly Protein-Bound Drug in Hyperlipidemic Plasma

This scenario is based on the data in Table 2 where warfarin competitively displaces clozapine in hyperlipidemic plasma. As indicated by the data, a significant decrease in clozapine recovery occurs within the LPD fraction and correlates with an increase in recovery of clozapine within the VLDL and LDL fractions of lipoproteins. This confirms our previous findings (Procyshyn et al., 2001) and is suggestive that competitive displacement of clozapine results in the redistribution/partitioning of clozapine into the VLDL and LDL fractions. As in the previous scenario, competitive displacement of clozapine from protein-binding sites would initially result in an increase in both the unbound fraction of clozapine and the unbound concentration of clozapine. However, on the immediate redistribution of clozapine (from the plasma proteins to lipoproteins) the unbound fraction of clozapine would decrease, thereby resulting in a reduction in V_d (Eq. 4). Furthermore, as the fraction of unbound clozapine decreases (as a consequence of redistribution), the elimination of total clozapine is proportionately reduced until the unbound (active) steady-state concentration returns to the initial point. Remember that the clearance of unbound drug was not changed and will fall back to the initial value over 3–5 half-lives. The end result would be an increase in total clozapine concentration, a decrease in the unbound fraction of clozapine, no change in unbound clearance, and no change in the steady-state unbound (active) concentration of clozapine. In this case, if we are guided by tradition pharmacokinetic principles, then the dosage of clozapine should not be changed even though the total plasma concentration of clozapine has increased.

In both scenarios, no change in the steady-state unbound (active) concentration of clozapine is expected, and as a consequence, no change in clinical status should be noted. However, we believe this may only apply to the first and not to the second scenario. We believe clozapine's association with lipoproteins (particularly triglycerides) may actually increase clozapine's effectiveness. Based on our previous work, we hypothesized that elevated triglycerides observed in clozapine-treated patients (Dursun et al., 1999; Gaulin et al., 1999; Ghaeli & Dufresne, 1996; Henderson et al., 2000; Spivak et al., 1999) may facilitate the passage of clozapine across the blood–brain barrier, thereby rendering it more bioavailable (Procyshyn et al., 2001). Clinical data (some circumstantial) supporting our hypothesis can be found in the current

literature. For example, Leadbetter et al. (1992) noted significant improvement in brief psychiatric rating scale (BPRS) total scores and composite negative symptoms scores for patients with marked clozapine-induced weight gain. Although the investigators did not examine lipoprotein profiles, one could surmise that with significant weight gain comes a high probability of increased plasma triglycerides. More definitive data correlating serum triglycerides with clinical response to clozapine come from a small prospective study of eight treatment-resistant individuals diagnosed with schizophrenia (Dursun et al., 1999). In this study, the investigators measured fasting lipids and BPRS scores at baseline, and then again after 12 weeks of clozapine treatment. Upon completing the study, the patients were receiving a mean dosage of 352 ± 73 mg per day of clozapine. End point measurements revealed an 11% increase in triglycerides, which coincided with a significant reduction in the BPRS scores (i.e., baseline BPRS $= 43.7 \pm 3.1$, end point BPRS $= 25.0 \pm 3.9$). Although the data do not prove contributory cause, they certainly implicate an association between the two variables. No significant change was noted in this study for total cholesterol, HDL, or LDL.

We have also reported on a case in which serum triglycerides concentrations positively correlated with clinical response to clozapine (Pande et al., 2002). In this particular case, a patient who had shown a remarkable clinical response to clozapine was found to have significantly elevated plasma triglycerides. As such, a lipid-lowering agent was prescribed. Within 7 weeks the triglyceride levels had fallen significantly, and the patient relapsed into a psychotic episode consisting of auditory hallucinations and delusions. At this time, the lipid-lowering agent was discontinued, and 5 weeks later the patient recovered. Remarkably, although unfortunate, was the fact that the patient's recovery coincided once again with elevated plasma triglyceride concentrations.

The interpretations of these two scenarios must be considered in view of the limitations of this study. Obviously, the biggest limitation is that we have extrapolated our in vitro data and applied them to in vivo pharmacokinetic principles. Furthermore, we have made the assumption that our in vivo model represents in vitro competitive drug displacement. The fact that our study uses warfarin was deliberate and should be representative of any highly protein-bound drug. In the field of psychiatry, this may include drugs from various classes, including antidepressants, antipsychotics, and mood stabilizers. Not only will these compounds competitively displace clozapine from plasma proteins, they may also (if hydrophobic) be prone to redistribution among the plasma lipoproteins. Thus, understanding how plasma lipoproteins influence competitive displacement interactions would be valuable in helping explain (and perhaps predict), pharmacokinetic parameters that may affect clinical outcome. The clinical significance of competitive displacement of drugs in patients with dyslipidemia requires further study.

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