Drug Development and Industrial Pharmacy, 31:901–905, 2005

ISSN: 0363-9045 print / 1520-5762 online
DOI: 10.1080/03639040500272058

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The Effects of Competitive Displacement on Haloperidol's Plasma Distribution in Normolipidemic and Hyperlipidemic Plasma

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ABSTRACT *Purpose.* To assess whether dyslipidemia affects haloperidol's overall plasma distribution when it is in the presence of another highly protein bound drug that competes for plasma protein binding sites. *Methods.* We performed in vitro studies in which warfarin sodium was pre-incubated in normolipidemic and hyperlipidemic plasma samples in varying concentrations. Following the pre-incubation with warfarin, [^{3}H]-haloperidol mixed with unlabeled haloperidol was added to the plasma samples. The plasma was separated into its lipoprotein and lipoprotein deficient fractions by density gradient ultracentrifugation and haloperidol distribution was determined. *Results.* Our results indicate that when normolipidemic plasma was pre-incubated with various concentrations of warfarin no significant redistribution of haloperidol was noted among the various plasma lipoprotein fractions. However, in the case of the hyperlipidemic plasma, pre-incubating with warfarin did result in a significant redistribution of haloperidol from the lipoprotein-deficient fraction to the very-low-density and low-density fractions of lipoproteins. *Conclusion.* Understanding how plasma lipoproteins influence competitive displacement interactions would be valuable in helping to 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.

KEYWORDS Haloperidol, Lipids, Triglycerides, Cholesterol

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INTRODUCTION

In general, lipoproteins consist of a hydrophobic core of triglycerides and cholesterol esters surrounded by phospholipids and proteins. These

heterogeneous macromolecular aggregates transport lipids from the intestine to the liver (exogenous pathway) as well as to and from tissues (endogenous pathway) (Davis, 1996; Wasan & Cassidy, 1998). Apart from their predominant function of lipid transportation, they are also involved in several biological activities that include immune reactions, coagulation, tissue repair, and carriers of a number of hydrophobic compounds (Wasan & Cassidy, 1998). With respect to the latter, any disturbance in lipid metabolism leading to changes in an individual's lipoprotein profile could result in a redistribution of these hydrophobic compounds among the different lipoproteins subclasses. In turn, this redistribution could significantly influence the pharmacokinetic and pharmacodynamic properties of the agent.

Previously, our laboratory characterized the plasma protein and lipoprotein distribution of haloperidol using plasma samples representing different lipid profiles (Procyshyn et al., 2003). Our findings revealed that as plasma triglyceride levels increased, haloperidol redistributed from the lipoprotein-deficient fraction into the very-low-density as well as the low-density lipoprotein fractions. Furthermore, an increase in cholesterol levels also resulted in a shifting of haloperidol into the low-density lipoprotein fraction. To further characterize haloperidol's lipoprotein distribution, we would like to take into consideration the fact that haloperidol is highly protein bound (up to 92%) primarily to albumin and α1-acid glycoprotein. As such, it is likely that competitive displacement of haloperidol from plasma proteins in individuals with dyslipidemia could further affect its overall plasma distribution. To this end, the objective of this study is to assess whether dyslipidemia affects haloperidol's overall plasma distribution when it is in the presence of another highly protein bound drug that competes for plasma protein binding sites.

MATERIALS AND METHODS

Warfarin sodium was pre-incubated 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 h at 37°C ($n=6$). In this study, the total cholesterol and triglyceride concentrations were manipulated using purified high density lipoproteins (HDL), low density lipoprotein (LDL), or very low density lipoprotein (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 pre-incubation with warfarin, [^{3}H]-haloperidol (obtained from New England Nuclear; purity greater than 95%) mixed with unlabeled haloperidol (total concentration=18.0 ng/ml) was added to the plasma samples and incubated for 1 h 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). Very low-density lipoprotein (VLDL), low density lipoprotein (LDL), high density lipoprotein (HDL), and the lipoprotein deficient (LPD; which consists primarily of albumin and α_1-acid glycoprotein) fractions were analyzed for [^{3}H]-haloperidol 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 haloperidol was replicated three times.

STATISTICAL ANALYSIS

[^{3}H]-haloperidol recovery in the presence and absence of warfarin were compared by analysis of variance (INSTAT2, GraphPad Inc., San Diego, CA, USA). Critical differences were assessed by Tukey post hoc tests. A difference was considered significant if the probability of chance explaining the results was reduced to less than 5% ($P<0.05$). All data were expressed as a mean±standard deviation.

RESULTS

In the control normolipidemic samples ($n=6$; containing no warfarin), 79.40±1.41% of the haloperidol was recovered in the LPD fraction (Table 1). Haloperidol was also recovered in the other fractions of plasma

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

Warfarin concentration (μg/ml)	Percentage of haloperidol recovery in VLDL (mean±S.D.)	Percentage of haloperidol recovery in LDL (mean±S.D.)	Percentage of haloperidol recovery in HDL (mean±S.D.)	Percentage of haloperidol recovery in LPD (mean±S.D.)
0	1.31±0.08	3.50±0.27	15.79±1.21	79.40±1.41
1	1.81±0.14	3.00±0.27	16.69±1.44	79.13±1.73
5	1.21±0.18	3.28±0.31	15.48±1.50	80.03±1.80
10	1.27±0.17	3.27±0.31	17.47±1.69	78.00±2.07

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.
LPD: lipoprotein deficient fraction.

as follows: 15.79±1.21% in the HDL, 3.50±0.27% in the LDL, and 1.31±0.08% in the VLDL. When this normolipidemic plasma was pre-incubated with various concentrations of warfarin (1, 5, and 10 μg/ml), no significant redistribution of haloperidol among the various plasma fractions was noted (Table 1).

In the control hyperlipidemic samples (n=6; containing no warfarin), 76.36±2.09% of haloperidol was recovered in the LPD fraction (Table 2). Haloperidol was also recovered in the other fractions of plasma as follows: 12.61±1.73% in the HDL, 5.22±0.20% in the LDL, and 5.82±0.23% in the VLDL. Unlike the normolipidemic plasma mentioned above, pre-incubating the hyperlipidemic plasma with warfarin (1, 5, 10 μg/ml) did result in a significant redistribution of haloperidol among the various fractions. For example, plasma samples pre-incubated with 5 μg/ml of warfarin was associated with a significant decrease in the amount of haloperidol recovered within the LPD fraction (69.97±0.90%) when compared to the control (76.36±2.09%, $P<0.05$). This decrease of haloperidol within the LPD fraction coincided with significant increases in haloperidol recovery within the VLDL (6.70±0.46%) and LDL (9.27±0.38%) fractions compared to their controls (5.82±0.23% and 5.22±0.20%, respectively, $P<0.05$). Likewise, significant increases in haloperidol recovery within the VLDL and LDL fractions (compared to their respective controls) were noted when plasma samples were pre-incubated with 10 μg/ml of

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

Warfarin concentration (μg/ml)	Percentage of haloperidol recovery in VLDL (mean±S.D.)	Percentage of haloperidol recovery in LDL (mean±S.D.)	Percentage of haloperidol recovery in HDL (mean±S.D.)	Percentage of haloperidol recovery in LPD (mean±S.D.)
0	5.82±0.23†	5.22±0.20†	12.61±1.73	76.36±2.09†
1	5.29±0.15	5.68±0.28	13.74±1.08	75.29±0.92
5	6.70±0.46*	9.27±0.38*	14.07±1.31	69.97±0.90*
10	10.59±0.39*	10.44±0.37*	14.14±0.74	64.83±0.80*

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.
LPD: lipoprotein deficient fraction.
$^\dagger P<0.05$ for the corresponding value in Table 1.
*$P<0.05$ for that sample compared to control (containing no warfarin).

warfarin. In these cases, the increase in haloperidol recovery within the VLDL and LDL fractions were not significantly different from plasma samples pre-incubated with only 5 μg/ml.

DISCUSSION

Previously, we showed that haloperidol redistributes itself from the LPD fraction to the VLDL and LDL fractions as plasma triglyceride levels increase (Procyshyn et al., 2003). 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 haloperidol in the presence of another highly protein bound drug (warfarin) is dependent on the concentration of lipoproteins within the plasma sample. For example, in the normolipidemic plasma samples (Table 1), increasing the concentration of warfarin did not significantly affect the lipoprotein distribution of haloperidol. Conversely, in the hyperlipidemic samples (Table 2), the presence of warfarin in concentrations greater than 5 μg/ml not only results in a decrease in haloperidol's protein binding within the LPD fraction, but there is also a shift of haloperidol into the VLDL and LDL fractions.

Although these studies were carried out in vitro, some inferences may be made with regards to the competitive displacement of haloperidol in vivo. For example, when haloperidol (or any highly protein bound drug) is competitively displaced by another highly protein bound drug, the initial pharmacokinetic effect would be an increase in the unbound active concentration of haloperidol. This is independent of an individual's lipoprotein profile. Following this, compensatory pharmacokinetic mechanisms begin to operate to deal with the sudden increase in the unbound active haloperidol (discussed in detail previously) (Procyshyn et al., 2005). These compensatory mechanisms are dependent on the apparent volume of distribution as well as the change in unbound fraction of haloperidol. In turn, the apparent volume of distribution and unbound fraction are dependent on lipoprotein profiles. Nevertheless, once these compensatory mechanisms take over, the end result for both scenarios (i.e., normolipidemia and hyperlipidemia) will be the same. That is, the steady state unbound active concentration of haloperidol is expected to return to levels similar to those observed prior to competitive displacement. This being the case, it would be reasonable to assume that no change in clinical status would ensue. However, we believe that this may only apply to the situation in which the plasma triglycerides and/or cholesterol are not elevated. In the presence of elevated plasma triglycerides and/or cholesterol however, it may be that haloperidol's redistribution among the lipoproteins contributes to its effectiveness. This is despite the fact that the steady state unbound active concentration is similar to those individuals with normal lipid profiles.

To explain this somewhat unintuitive situation, one must consider to what effect the redistribution of haloperidol among lipoproteins may have on its ability to infiltrate the blood brain barrier. The blood brain barrier is made of lipophilic endothelial cells and moderates the passage of compounds from the blood into the brain's interstitial tissues. The rate of transport of compounds across this physiological barrier is dependent on molecular size, density, and the lipophilic nature of the agent (Machard et al., 1989). Therefore, it is conceivable that haloperidol's association with the VLDL and LDL fractions of plasma increases its lipophilicity and, thus, its receptivity towards the blood brain barrier. If this is the case, then more haloperidol would reach its pharmacological site of action thereby potentially increasing its pharmacological effectiveness.

This theoretical argument that we make for haloperidol has already been made for clozapine (Procyshyn, 2003; Procyshyn et al., 2001). However, in the case of clozapine, there is some clinical evidence to support our hypothesis that elevated plasma lipoproteins may increase its effectiveness. For example, in a small prospective study of eight treatment-resistant individuals diagnosed with schizophrenia (DSM-IV criteria), Durson et al. measured fasting lipids and BPRS scores at baseline and then again 12 weeks after clozapine treatment (Dursun et al., 1999). Upon completion of the study, the patients were receiving a mean dosage of 352±73 mg per day of clozapine. Endpoint measurements also revealed an 11% increase in triglycerides that coincided with

a significant reduction in the BPRS scores (i.e., baseline BPRS = 43.7±3.1, endpoint BPS = 25.0±3.9). Although the data does not prove contributory cause it certainly implicates an association between the two variables. No significant change was noted in this study for total cholesterol, HDL or LDL.

A second example demonstrating a possible relationship between triglycerides and clozapine's effectiveness comes from a case reported (Pande et al., 2002). In this instance, 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 seven 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 five weeks later the patient recovered. Remarkably, though unfortunate, was the fact that the patient's recovery coincided once again with elevated plasma triglyceride concentrations.

Our study demonstrates that lipoproteins have the ability to influence the plasma distribution of a competitively displaced hydrophobic compound. Although our study is unable to demonstrate is that this redistribution can affect an agent's pharmacological activity, it has been shown that plasma lipoproteins can modify the pharmacokinetics, tissue distribution, and pharmacological activity of lipophilic compounds (Brajtburg et al., 1984; Brunner et al., 1988; Lemaire & Tillement, 1982; Wasan, 1996; Wasan et al., 1990). Hence, our hypothesis that elevated triglycerides and/or cholesterol may increase the effectiveness of haloperidol is not without foundation.

The fact that our study uses warfarin was deliberate and should be representative of any highly protein bound drug. This is particularly relevant to psychiatry in which various classes of psychotropic medication are highly protein bound. Understanding how plasma lipoproteins influence competitive displacement interactions would be valuable in helping to 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.

ACKNOWLEDGMENTS

Funding for the study comes from the Riverview Hospital Research Grant Competition and CIHR Grant #MOP-14484.

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