

Verapamil, but not Probenecid, Co-Administration Can Convert Desloratadine to a Sedating Antihistamine in Mice

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Abstract: The possibility that non-sedating antihistamines could elicit sedation in mice due to drug-induced inhibition of brain PgP was evaluated by measuring the ability of desloratadine alone or in combination with verapamil to cause ataxia in mice. Also, the concentrations of desloratadine in plasma and in brain homogenates were measured by liquid chromatography-mass spectrometry. Relative to methylcellulose (control) treatment, verapamil plus desloratadine decreased rotarod performance of mice. Plasma concentrations of desloratadine appeared comparable in the mice treated with either desloratadine or verapamil plus desloratadine, however the rate of decline of desloratadine from brain tissue was slower in mice treated with verapamil plus desloratadine compared to mice treated with desloratadine only. These data suggest that inhibition of brain PgP can convert desloratadine to a sedating antihistamine in mice.

Key Words: PgP, desloratadine, antihistamines, pharmacokinetics, pharmacodynamics.

INTRODUCTION

Symptoms of allergic disorders often benefit from treatment with H1 histamine receptor antagonists (H1-antagonists). The so-called first generation H1-antagonists such as diphenhydramine, an ethanolamine, are known to reach sufficient concentrations in the CNS to cause CNS side effects, most notably sedation [1]. The second generation H1-antagonists such as cetirizine, loratadine, fexofenadine, and desloratadine cause significantly less sedation owing to less accumulation in the CNS. Second generation H1-antagonists are therefore referred to as non-sedating antihistamines. A clear distinction between the CNS effects of loratadine and diphenhydramine in humans relative to their differences in CNS penetrability has been described [2].

The ability of P-glycoprotein (PgP) encoded by human MDR1 (*ABCB1*) and by rodent *mdr1a* and *mdr1b* to limit CNS exposure to numerous drugs by effluxing them from the CNS is well known [3]. PgP was shown to efflux second generation H1 antagonists much more effectively than first generation H1 antagonists in MDCK cells transfected with MDR1 [4]. Brain-to-plasma AUC ratios of second generation antihistamines were higher in *mdr1a/b* knockout mice than wild type mice, whereas for first generation antihistamines the ratios were comparable for both strains [4]. Likewise, brain:plasma ratios of another second generation antihistamine, bepotastine, were higher in *mdr1* knockout mice compared to wild-type mice [5].

In view of the well-characterized requirement of PgP activity to limit brain accumulation of second generation antihistamines, this study was designed to determine whether drug-mediated inhibition of PgP could elicit sedative effects

from a non-sedating antihistamine, thereby giving rise to an otherwise unexpected drug-drug interaction.

EXPERIMENTAL

Chemicals

Desloratadine (>97% purity) was purchased from Sequoia Research Products (Oxford, UK). Diphenhydramine, methylcellulose (MC), probenecid, and verapamil were purchased from Sigma-Aldrich (St. Louis, MO). Methanol, acetonitrile and formic acid (FA) were of HPLC grade and were purchased from Fisher Scientific (New Jersey, USA). HPLC grade water was obtained using a Milli Q system. Mouse plasma was purchased from Pel-Freez[®] Biologicals (Rogers, Arkansas, USA). Liquid nitrogen and argon gas were purchased in high purity (99.998%) from Linde Gas (Toledo, OH, USA).

Animals

Male CBL57 mice weighing ~ 25 g (19.9-36.7) were acquired from Harlan Sprague-Dawley (Indianapolis, IN), and maintained in the main campus vivarium. The study protocol was approved by the Institutional Animal Care and Use Committee of the main campus.

Treatments

All treatments were given by gavage. Drugs were prepared as suspensions in 1% methylcellulose (w/v in distilled water). Doses were prepared fresh daily, and administered in a volume of 10µl/g after an overnight fast.

Animals were treated with either 100 mg/kg probenecid, 100 mg/kg desloratadine, 80 mg/kg diphenhydramine, 30 mg/kg verapamil, desloratadine + verapamil (100 mg/kg + 30 mg/kg, resp), probenecid+desloratadine (100 mg/kg + 100 mg/Kg, resp), or methylcellulose (MC). Verapamil was always administered as two successive 15 mg/kg doses 10 min apart, since bolus 30 mg/Kg doses produced signs of toxicity

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in some animals. Rotarod, plasma pharmacokinetic (PK), and brain PK experiments each used a different group of animals.

Rotarod Experiments

Ataxia, as a measure of sedation, was assessed 30-40 min after drug administration by rotarod performance [6-9]. Mice were placed on a rotarod rotating at 7 rpm, and the time required to fall (up to a maximum time of 90 seconds) was tabulated. Approximately twelve mice (9-15) were used for each treatment.

Pharmacokinetic (PK) Experiments

Plasma and brain PK of desloratadine was assessed in mice treated with either desloratadine (100 mg/kg) or desloratadine + verapamil (100 mg/kg + 30 mg/kg, resp). A separate group of animals was used for brain and plasma PK experiments. Seventy-two animals were divided into four groups of eighteen animals each. Three animals each were dosed for each of six sampling times (10-60 min post dosing at 10 minute intervals) for blood sampling (desloratadine group and desloratadine+verapamil group) and brain sampling (desloratadine group and desloratadine+verapamil group).

Blood (100-250 μ l) was collected by cardiac puncture under CO₂ anesthesia in tubes coated with dipotassium EDTA. Immediately after collection, the tubes were swirled and shaken followed by centrifugation. For brain PK animals were euthanized at 10 min intervals from 10-60 min post dosing. Brains were immediately excised and homogenized in 0.9% saline (1:4 w/v).

Bioanalytical

An Alliance® HT liquid chromatograph (model 2795) from Waters corporation (Milford, MA, USA) and Quattro Micro™ (triple-quadrupole) mass spectrometer from Micromass (Manchester, UK) were used with an XTerra™ MS C₁₈ analytical column (2.1 x 150 mm, 5 μ m) and a guard column (2.1 x 10 mm, 5 μ m) from Waters Corporation (Milford, MA, USA). Chromatography was carried out *via* a gradient system with a flow rate of 300 μ l/min after an injection volume of 10 μ l. The mobile phase consisted of 0.1 % formic acid (FA) in water (A) and acetonitrile (B). The starting eluent was 98 % A and 2 % B for 2.0 min. Then B was increased linearly to 50 % in 1.0 min, held for 3.0 min, increased linearly to 98 % in 1 min, returned to initial composition of 98 % A and 2%B in 1.00 min, and then held for 2.00 min in order to re-equilibrate the column. A 0.2 % FA in methanol-water mixture (50:50, v/v) was used as the needle wash solvent, 0.1 % formic acid in water as the purge solvent, and 100 % methanol as a seal wash solvent. The column and samples were kept at 35 \pm 5 °C and 4 \pm 5 °C, respectively.

Mass spectrometric detection was done using an ESI source in the positive mode. Dissolution and cone gas (N₂) flow rates were 600 L/h and 25 L/h, respectively; the source and dissolution gas temperatures were 140 and 400 °C, respectively. The ESI source tip (capillary) voltage was 3.0 kV; extractor was 2 V; and, ion energy for MS1 was 0.2 and for MS2 was 1.0 V. Argon gas was used as the collision gas:

Cell pressure was approximately 3.1 x 10⁻³ mbar. The dwell time was 100 ms. Data acquisition and processing were carried out using MassLynx software.

Lower limit of detection (LLOD) and lower limit of quantitation (LLOQ) were determined based on signal-to-noise ratio (s/n). A s/n of 3:1 was used to establish the LLOD, and a s/n of 10:1 was used for the LLOQ. Precision was assessed by relative standard deviation (RSD) for intra-day and inter-day, respectively.

Pharmacokinetic Analysis

Concentration vs time data for desloratadine was fitted using non-linear regression. The disappearance rate constants of desloratadine from plasma and brain homogenates were estimated by linear regression from the slopes of plots of the natural logarithms of concentration versus time data. All six time points from 10-60 min were included.

AUC₀₋₆₀ values for brain and plasma were computed by the trapezoidal rule.

Statistical Analysis

Since a maximum time on the rotarod of 90 sec was permitted, a Kruskal-Wallis test was used to determine whether there was a significant difference between any of the treatments on rotarod performance. Differences between diphenhydramine and desloratadine+verapamil were analyzed using a two-sample t-test, since the data for those treatments was normally distributed. Differences between diphenhydramine and MC and between desloratadine+verapamil and MC were evaluated by the Mann-Whitney test using a Bonferonni adjustment that set alpha (α) at 0.0167 (i.e. 0.05/3).

RESULTS

Rotarod Performance

As shown in Fig. (1), all animals remained on the rotarod for 90 seconds except for those treated with diphenhydramine (28.4 \pm 19.1 sec) or verapamil+desloratadine (50.9 \pm 26.1 sec). Differences between diphenhydramine and desloratadine+verapamil did not quite attain statistical significance, since the p value of 0.025 was slightly greater than 0.0167 (i.e. 0.05/3). However, both diphenhydramine and desloratadine+verapamil treated animals performed significantly less well on the rotarod than the MC treated animals (p<0.0001).

Bioanalytical

The standard curve was linear over the entire concentration range. LLOD and LLOQ were 0.5 ng/ml and 1.0 ng/ml, respectively, both in plasma and brain homogenates. Precision of the method was assessed by relative standard deviation (% RSD) from the analysis of three quality control samples (5 ng/ml, 50 ng/ml and 250 ng/ml). Intra- and inter-day precision for all concentrations were less than 6 % in plasma and less than 8 % in brain homogenates. The recovery of desloratadine was also evaluated for three QC samples, and found to be in the range of 105-110 % and 90-110 %, in plasma and in brain homogenates, respectively.

Desloratadine Disappearance from Plasma and Brain

Mean concentrations of desloratadine in plasma and brain homogenates at each sampling time are shown in Fig. (2).

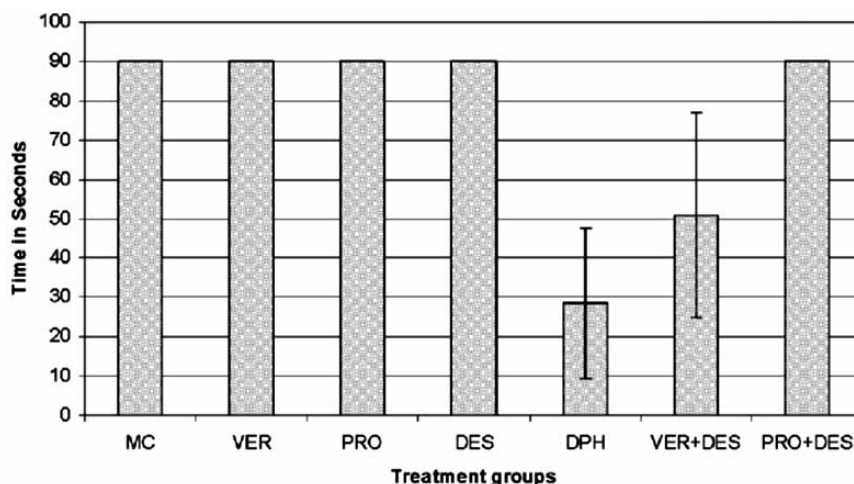


Fig. (1). Effects of all treatments on rotarod performance of mice (seconds). Observations were truncated at 90 sec. Vertical bars denote SDs. Absence of vertical bars signifies that all animals tested remained on the rotarod for 90 sec. Abbreviations are as follows: MC, methylcellulose; DES, desloratadine; VER, verapamil; PRO, probenecid; and DPH, diphenhydramine.

During the sampling period there appeared to be no significant plasma decay in desloratadine concentrations whether desloratadine had been administered alone or with verapamil. Alternatively, while desloratadine concentrations in brain homogenates remained stable during the sampling period for desloratadine + verapamil treated mice, an apparent decline in brain desloratadine levels in the animals treated only with desloratadine was detected with an apparent disappearance

rate constant estimated at 0.017 min^{-1} . However, brain:plasma AUC ratios were not different at 0.33 and 0.28 for desloratadine and desloratadine+verapamil, respectively.

DISCUSSION

It is now widely accepted that differences in CNS transport of first generation H1 antagonists such as diphenhydramine and second generation H1 antagonists such as

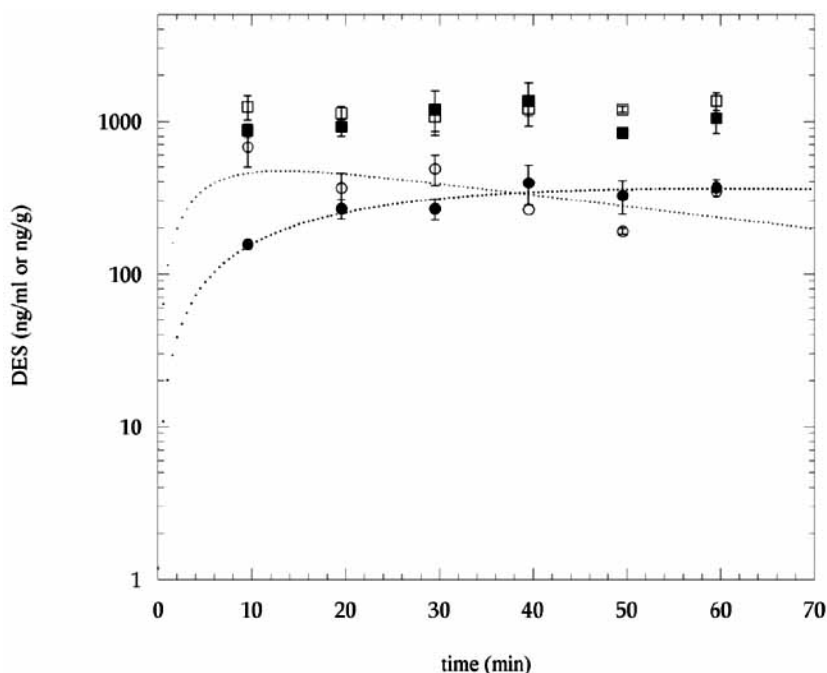


Fig. (2). Plasma and brain levels of desloratadine (DES) in animals treated with either desloratadine 100 mg/Kg, orally or desloratadine + verapamil at doses of 100 mg/kg orally and 30 mg/kg orally, respectively. Open symbols denote DES treatment, whereas closed symbols denote DES+VER treatment. Upper symbols are for plasma concentrations of DES (ng/ml). Lower curves are the concentrations of DES in brain homogenates (ng/g brain). Vertical bars denote SEMs. No error bars are shown at 40 min, since there was only a single observation. No error bars are shown at 60 min, because they fall within the plotting symbol. Plasma AUC values are 65133 and 56845 $\text{ng} \cdot \text{ml}^{-1} \cdot \text{min}$ for DES and DES+VER, respectively. Brain AUC values are 21555 and 15955 $\text{ng} \cdot \text{g}^{-1} \cdot \text{min}$ for DES and DES+VER, respectively.

desloratadine account to a large extent for the greater sedative effects of the first generation agents which tend not to be effluxed by PgP from the CNS [3]. Chen *et al.* very clearly demonstrated the role of PgP in effluxing second generation H1 antagonists such as desloratadine in *mdr1(-/-)* mice [4]. A logical extension of these observations is to predict the potential for inhibitors of brain PgP to slow the efflux of second generation H1 antagonists, thereby permitting their residency in the brain at concentrations high enough and durations long enough to elicit demonstrable CNS depressant effects such as sedation.

After a set of preliminary dose-ranging studies establishing an oral dose of diphenhydramine that would impair rotarod performance in mice (80 mg/Kg) and doses of antihistamines and transporter inhibitors that would not impair performance, we administered diphenhydramine, desloratadine, verapamil, probenecid, and combinations of verapamil+desloratadine and probenecid +desloratadine to mice, and used rotarod performance as a surrogate measure of sedation. Only diphenhydramine alone and the combination of desloratadine+verapamil impaired rotarod performance. Verapamil was selected as an inhibitor of PgP. In MDCKII-MDR1 cells verapamil inhibited PgP transport with an IC50 of ~ 7 μ M [10]. In brain perfusion experiments in wild type mice verapamil inhibited *in situ* vinblastine transport with an IC50 of 23 μ M [11]. Probenecid was selected, because it is an inhibitor of a different brain efflux transporter, MRP2 (ABCC2) [12], thus it was used as a sort of negative control, and also to demonstrate the selectivity of the verapamil effect.

In addition to causing sedation, the addition of verapamil to desloratadine treatment may have slowed the rate of desloratadine disappearance from brain (Fig. 2). It must be noted that neither brain nor plasma levels of desloratadine were measured serially in any given animal. Moreover, verapamil is a relatively weak PgP inhibitor exhibiting IC50s ranging from approximately 1-40 μ M depending upon the method, substrate, or cell line used [13]. Consequently, the interindividual dosing and dispositional characteristics (ADME) of desloratadine, relatively weak inhibitory effect of verapamil on PgP, and rapid elimination of verapamil in mice [14], barred us from establishing significantly different brain:plasma concentrations or AUC ratios. However, only for the desloratadine group of mice was there any evidence of a decay in brain desloratadine levels with a significant slope over the entire sampling period, although the higher 60 min concentration gives evidence that the effect of verapamil dissipates rapidly. The behavioral effects of verapamil+desloratadine were generally compatible with the PK data, though not identical. Behavioral deficits were detected some 30-40 min after dosing, and the biggest differences in brain levels of desloratadine between the desloratadine treated animals and the desloratadine+verapamil treated animals occurred between 40-50 minutes. However, the rotarod experiments were conducted before and separate from the PK experiments and in different groups of mice. The rotarod experiments pre-dated the PK experiments, and we have not yet extended the rotarod experiments out to 50 or 60 minutes post-dosing.

These results are broadly similar to the recently reported verapamil-induced (1 mg/Kg iv) increased CNS uptake of dextromethorphan in rats that was also attributed to PgP inhibition [15]. Although the administration of verapamil and also desloratadine by gavage and at higher doses than those used in earlier studies precludes direct pharmacokinetic comparisons, the general outcomes are comparable. The relatively constant plasma concentrations of desloratadine in the initial 60 minutes post gavage are consistent with the 3-6 hr half-life for desloratadine reported in both *mdr1(-/-)* mice and wild type mice [4,16].

In conclusion, high oral doses of desloratadine+verapamil caused ataxia or sedation in mice, though not as profound as a single oral dose of diphenhydramine. Neither desloratadine alone, probenecid alone, nor verapamil alone affected the rotarod performance of mice. While brain levels of desloratadine remained relatively constant for one hour post dosing of desloratadine+verapamil, they appeared to disappear somewhat faster in mice treated with only desloratadine. These findings are consistent with those of others that have shown that verapamil is a weak inhibitor of brain PgP, and can promote the accumulation of dextromethorphan in rat brains [15]. It would be premature to conclude that verapamil and other inhibitors of brain PgP will convert non-sedating antihistamines to sedating antihistamines in humans. However, these experiments using large oral doses of a PgP substrate and an inhibitor in mice, if not proof-of-principle, are consistent with the notion that non-sedating antihistamines can become at least transiently sedating if given with a PgP inhibitor. Both itraconazole and cyclosporine exhibit lower IC50s for PgP inhibition than does verapamil [10], and cyclosporine A has recently been shown to increase brain:plasma ratios of ¹¹C-verapamil in humans [17]. Looking to the future, it might be prudent to consider this type of a drug-drug interaction outcome as the development of potent PgP inhibitors as adjuncts to cancer chemotherapy gains traction.

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