

## EVALUATION OF THE INTERACTION OF LORATADINE AND DESLORATADINE WITH P-GLYCOPROTEIN

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### ABSTRACT:

The absorption of many drugs is affected by their interaction with ATP-binding cassette (ABC) transporters. The most extensively studied of these ABC transporters is the proein product of MDR1 (multidrug resistance) that encodes a 170-kDa integral plasma membrane phosphorylated glycoprotein known as P-glycoprotein (P-gp). The purpose of this study was to determine, using two different methods, whether the nonsedating antihistamine loratadine (L) and its active metabolite desloratadine (DL) interact with P-gp. MDR cells presenting human P-gp were incubated with the fluorescent P-gp substrate daunorubicin with or without L, DL, and several positive controls. The  $IC_{50}$  of loratadine ( $\sim 11 \mu M$ ) was  $\sim 160$  times the maximum observed plasma concentration ( $C_{max}$ ) following a dose of 10 mg. The  $IC_{50}$  of desloratadine ( $\sim 43 \mu M$ ) was  $\sim 880$  times the  $C_{max}$  following a dose of 5 mg. The positive control,

cyclosporin A, had an  $IC_{50}$  of  $\sim 1 \mu M$ . ATP hydrolysis activity was measured in the membrane fraction prepared from MDR cells presenting P-gp, which were exposed to various concentrations of test compounds. Known substrates of P-gp demonstrated clear, repeatable, concentration-dependent increases in ATP hydrolysis activity. L caused an increase in ATPase activity above basal levels. L had a  $V_{max}$  about 200% basal activity and  $K_m$  of  $\sim 3 \mu M$  for P-gp. In contrast, DL had no significant effect on baseline ATP hydrolysis. L inhibited human P-gp much less than verapamil or cyclosporin A. DL inhibited human P-gp significantly less than L (4 times). DL therefore is not a significant inhibitor of P-gp and should not cause clinical drug interactions with agents that are P-gp substrates.

Histamine  $H_1$ -receptor antagonists are effective first-line therapeutic agents in the management of allergic rhinitis, a condition affecting approximately 45 million Americans with a trend toward a larger afflicted population. Loratadine (L)<sup>1</sup> is a widely prescribed, selective, nonsedating, peripheral histamine  $H_1$ -receptor antagonist that is not associated with performance impairment and has an excellent safety record (Barnett et al., 1984; Bradley and Nicholson, 1987; Gaillard et al., 1988; Ramaekers et al., 1992; Kay et al., 1997; Slater et al., 1999; Philpot 2000; Prenner et al., 2000; Salmun et al., 2000). Due to the high incidence of allergic rhinitis across the full range of the population, antihistamines are often administered concurrently with other drugs. Because drug disposition and exposure can drastically change when a coadministered drug inhibits an avenue of elimination or disposition (drug-drug interaction), the elevated exposure to one or more drugs can lead to potentially grave consequences.

Mammalian cells possess a natural battery of defense mechanisms against xenobiotic assault. A particular class of proteins actively transports an extensive array of structurally unrelated large lipophilic compounds from the cell, providing what is often known as multiple drug resistance (MDR) (Pastan and Gottesman, 1987). Multidrug resistance is characterized by active efflux or pumping of xenobiotics

and pharmaceuticals via transmembrane proteins acting as hydrophobic "vacuum cleaners" (Gottesman and Pastan, 1993; Gottesman et al., 1996). The protein product of the MDR1 gene encodes a 170-kDa integral plasma membrane phosphorylated glycoprotein, P-glycoprotein (P-gp), which is the best known and most extensively studied among these transporters and thus far appears to have the largest substrate list. The gross structural features of P-gp appear to be shared by a large family of membrane transporters known as ATP-binding cassette (ABC) transporters, which evidently act as ATP-driven pumps that remove xenobiotics from the interior of cells. Expression of P-gp in normal human tissues, particularly within the cellular membranes of the gastrointestinal tract, liver, blood-brain barrier, adrenals, and kidneys, suggests that the protein plays a role in cellular protection as well as in secretion (Gottesman and Pastan, 1993). While the primary function of this protein is unknown, its ability to confer resistance to a wide variety of structurally and chemically unrelated compounds remains impressive. Indeed, the substrate list for this transporter reveals that P-gp shares a similar tolerance or acceptance as cytochrome P450 3A4 (CYP3A4), the predominant intestinal and hepatic cytochrome P450 oxygenase enzyme, and may even prove to be more extensive in its substrate recognition and as an avenue of drug elimination (Fisher et al., 1996).

It is becoming evident that drug interactions ostensibly mediated by the cytochrome P450 3A4 oxidative pathway are also the result of P-gp inhibition (Siegsmond et al., 1994; van Asperen et al., 1996; Lown et al., 1997). Given the enormous number of substrates now known to be recognized by P-gp, and a binding site that is evidently hydrophobic, the substrates for CYP3A4 and P-gp clearly overlap.

<sup>1</sup> Abbreviations used are: L, loratadine; MDR, multidrug resistance; P-gp, P-glycoprotein; ABC, ATP-binding cassette; DL, desloratadine.

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Among the more grave examples of clinical drug interactions are the  $H_1$ -receptor antagonist terfenadine with ketoconazole and erythromycin (Monahan et al., 1990), as well as simvastatin with itraconazole and mibefradil (Neuvonen et al., 1998); all are substrates/inhibitors of P-gp (Siegmund et al., 1994; Ambudkar et al., 1999). Another  $H_1$ -receptor antagonist, fexofenadine, also interacts with ketoconazole and erythromycin. A report implicates active transporters as a major factor in the disposition of fexofenadine (Cvetkovic et al., 1999). Additionally, P-gp polymorphisms may cause wide-ranging reactions to treatment with P-gp substrates. If a polymorphic gene product of MDR1 has inferior selectivity toward a therapy, increased systemic exposure to that erstwhile P-gp substrate could be expected (Kioka et al., 1989; Mickley et al., 1998; Declèves et al., 2000; Hoffmeyer et al., 2000).

This report quantifies the interactions of L and its active metabolite DL with the substrate binding site of the ubiquitous ABC transporter P-gp by using two different methods. DL has significantly less potential for interaction with P-gp than the most commonly prescribed antihistamine loratadine, an agent known for its safety profile.

### Materials and Methods

**Chemicals.** Loratadine and desloratadine were from Schering-Plough compound resources. Daunorubicin, verapamil, colchicine, cyclosporin A, mannitol, dithiothreitol, ATP disodium, ammonium molybdate, ascorbic acid, sodium *meta*-arsenite, aprotinin, leupeptin, EGTA, EDTA, HEPES, ouabain, phenylmethylsulfonyl fluoride, and tris(hydroxymethyl)aminomethane base were purchased from Sigma Chemical Co. (St. Louis, MO). Hanks' balanced salt solution, alpha minimum essential medium, Dulbecco's modified Eagle's medium, penicillin/streptomycin, fetal bovine serum, and trypsin-EDTA were obtained from Life Technologies, Inc. (Rockville, MD). Sodium orthovanadate was purchased from Pfaltz & Bauer Inc. (Waterbury, CT). Microplates (Costar 96-well), plastic tubes, and cell culture flasks (75 cm<sup>2</sup>) were purchased from Corning Inc. (Corning, NY). All other reagents were of the highest grade commercially available.

**Cell Lines.** CR1R12 cell line, provided by Dr. Alan Senior (University of Rochester, Rochester, NY), was maintained as described previously (Wang et al., 2000a). The 3T3 G185 cell line presenting the gene product of human MDR1 was licensed from National Institutes of Health and maintained in Dulbecco's modified Eagle's medium.

**Fluorescence-Activated Cell Sorter Flow Cytometry.** A direct functional assay was performed with the flow cytometer as described previously (Wang et al., 2000a).

**ATP Hydrolysis and Phosphate Release.** The consumption of ATP was determined by the liberated inorganic orthophosphate, which forms a color complex with molybdate (Wang et al., 2000b).

### Results

Inhibition of marker efflux (Wang et al., 2000a) was used in this study to characterize the interaction potential of L, DL, and some known substrates with MDR1. Vanadate, a known, very potent inhibitor of MDR1 efflux function, served as a positive control. Inclusion of adequate concentrations of vanadate in the incubation media inhibited daunorubicin efflux dye and resulted in a dramatic increase in retained fluorescence. This condition was considered to represent total inhibition.

L caused a concentration-dependent increase in fluorescence retention during the efflux phase. Maximum inhibition by L was approximately 43% of that observed with vanadate (total inhibition). The concentration dependence of inhibition displayed a sigmoidal response curve (Fig. 1), a consequence of cooperativity, with the Hill equation for allosteric interaction therefore providing a significantly better fit to the data:  $v = V_{max}S^n/(K' + S^n)$  (Wang et al., 2000c). The  $IC_{50}$  for L in the NIH 3T3-G185 cell line (overexpressing the cloned human MDR1 gene product) on this passage was  $\sim 11 \mu M$ , less potent

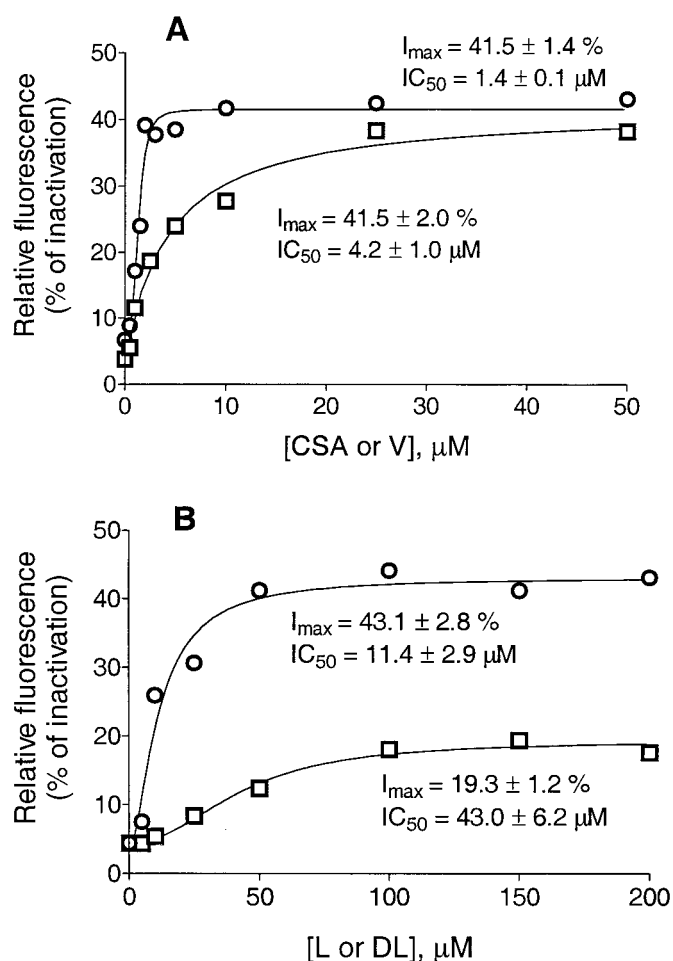


FIG. 1. Intracellular retention of daunorubicin in G185 cells versus competing verapamil (A; squares), cyclosporine A (A; circles), loratadine (B; circles), and desloratadine (B; squares) concentration.

Fluorescence intensity is expressed as relative fluorescence. The efflux phase or incubation was 30 min in all cases. The average number of cells per assay was 10,000. The function for the line through the data is the Hill equation:  $v = V_{max}S^n/(K' + S^n)$ . The parameters  $IC_{50}$  and the maximum inhibition ( $I_{max}$ ) along with the standard deviation are shown on the respective graphs.

than the positive controls verapamil and cyclosporin A (Fig. 1A) with an  $IC_{50}$  of about 4 and 1  $\mu M$ , respectively. For L the  $IC_{50}$  was  $\sim 160$  times the maximum observed plasma concentration ( $C_{max}$ ) following the recommended dose (10 mg; Salmun et al., 2000).

DL caused significantly less functional inhibition, achieving a maximum equivalent to only 19% total inhibition (Fig. 1B). Moreover, the  $IC_{50}$  of  $\sim 43 \mu M$  was about 4-fold greater than that for L. For DL, the  $IC_{50}$  was  $\sim 880$  times the maximum observed plasma concentration ( $C_{max}$ ) following the recommended dose (5 mg; Banfield et al., 2001a,b). The results described here using cells with the human MDR1 transporter are similar to those using a rodent cell and transporter (data not shown).

If a compound is a substrate of P-gp the hydrolysis of ATP is required as the driving force. As ATP is consumed at a purported rate of about one or two per transport event, the hydrolysis of ATP represents transport rate or activity assay of function (Eytan et al., 1996; Ambudkar et al., 1997; Stein, 1997; Shapiro and Ling, 1998; Sauna and Ambudkar, 2000, 2001; Wang et al., 2000b). As exemplified by the known P-gp substrate nifedipine, there was a clear concentration dependence with activity rising to a maximum at  $\sim 44$  nmol/min/mg (positive control, data not shown). By fitting the data to

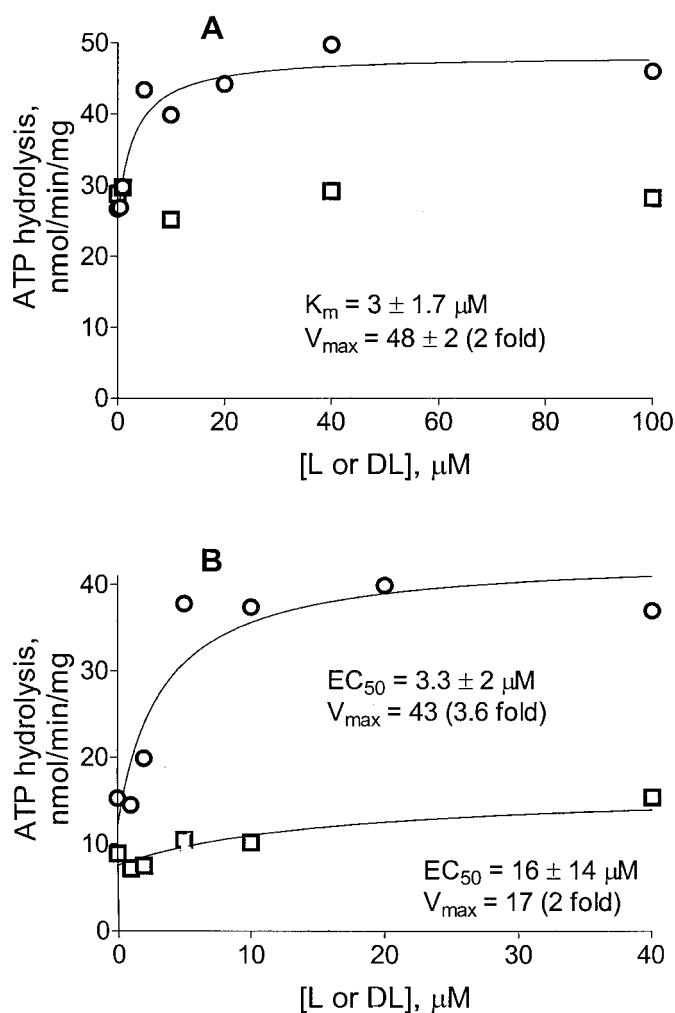


Fig. 2. A, P-gp-mediated ATP hydrolysis rates versus loratadine (circles) or desloratadine (squares) concentration.

The data for loratadine is fit to a hyperbola and the  $V_{max} = 48 \pm 7$  nmol/min/mg of membrane protein with a  $K_m = 3 \pm 1.7 \mu\text{M}$ . B, P-gp-mediated ATP hydrolysis rates versus loratadine or desloratadine in the presence of  $10 \mu\text{M}$  H33342 (to lower the baseline activity). The data are fit to a hyperbola and for loratadine (circles) the  $V_{max} = 42 \pm 7$  nmol/min/mg of membrane protein with an  $EC_{50} = 3.3 \pm 2 \mu\text{M}$  (instead of a parameter  $K_m$  it is called an  $EC_{50}$  for "effective concentration" as the conditions include an alternative substrate). For desloratadine (squares) the  $V_{max} = 17 \pm 4$  nmol/min/mg of membrane protein with an  $EC_{50} = 16 \pm 14 \mu\text{M}$ .

a modified form of the Michaelis-Menten equation (added constant accounting for baseline activity) using nonlinear regression, a  $K_m$  of about  $13 \mu\text{M}$  and a  $V_{max}$  of about 240% of control activity was determined. It was noted that in the absence of a substrate, the enzyme was able to hydrolyze ATP to produce a basal level of phosphate release. Therefore, activity data are presented as a percentage of the basal or control activity that is probably due to the transport of endogenous substrate(s). Any change in the rate of ATP hydrolysis represents the sum of the basal activity and the contribution of the exogenous substrate to ATP hydrolysis. Therefore, a substrate with a rate similar to basal activity may not exhibit altered ATP hydrolysis activity due to masking by the basal activity. As often reported (Wang et al., 2000b, and references therein), known substrates of P-gp have demonstrated a clear repeatable concentration-dependent increase in ATP hydrolysis activity.

As shown in Fig. 2A, L caused an increase in ATP hydrolysis activity above basal levels with a classic Michaelis-Menten relationship to concentration. L appears to be a faster substrate (transport rate

higher than many other substrates) with a  $V_{max}$  that is  $\sim 200\%$  above basal activity and a  $K_m$  of  $3 \mu\text{M}$ . DL has no significant effect on ATP hydrolysis under the conditions of the assay (Fig. 2A). Since the transporter exhibits basal activity, however, the addition of an exogenous transporter substrate may not change the ATP hydrolysis rate if its rate of transport is similar to the basal rate. The Hoechst compound H33342, known to reduce ATPase activity below basal activity, was used to reveal effects on ATP hydrolysis that would otherwise be masked by basal activity. For example, repeating the above-mentioned experiments in the presence of  $10 \mu\text{M}$  H33342 (for all assays) lowers the basal activity and changes the assay or baseline reference point. Under these conditions, L increases ATP hydrolysis rates with an  $EC_{50}$  (as conditions are contrived, the parameter is designated  $EC_{50}$ ) similar to the  $K_m$  determined under assay conditions without H33342 added (Fig. 2B). DL caused a slight increase in hydrolysis above the suppressed (with  $10 \mu\text{M}$  H33342) activity; this increase occurred, however, only at very high concentrations compared with L (Fig. 2B). The interaction of DL with P-gp is 4-fold less than that of L, a result in agreement with the comparative results from the direct transport inhibition in whole cell described above.

## Discussion

In this report we show by two different methods that the interaction of DL with the ubiquitous ABC transporter P-gp is significantly less than that of L, a widely prescribed antihistamine with a prodigious safety record. This result supports some structure-activity relationship studies showing that less lipophilic (hydrophobic) compounds are often less likely to interact with the substrate binding site of P-gp (Klopman et al., 1997; Litman et al., 1997). As DL is the descarboethoxy oxidized L, it is less lipophilic, and hence more soluble, and would therefore be expected to have a lower affinity for the binding site of the MDR1 gene product P-gp. Indeed, the functional inhibition of P-gp by DL was much less than that by L, as measured by both extent and affinity (4 fold) despite suggestions of a significant interaction with P-gp (Hwang et al., 2000). In other words, the maximum extent of DL-mediated inhibition was still less than half that achieved by other positive compounds, including L. Moreover, L itself, with an  $IC_{50}$  of about  $11 \mu\text{M}$ , would not be expected to exhibit significant interactions at clinically relevant doses, which indeed is the case. The  $IC_{50}$  values represent about 160 times the highest observed  $C_{max}$  of L and 880 times the highest observed  $C_{max}$  of DL. Hence, DL would be expected to exhibit no clinical interaction with compounds transported by P-gp even at the higher concentrations expected in intestinal mucosal. Indeed, clinical studies show that DL exhibits no significant interactions with typical test drugs (Banfield et al., 2001a,b). The pharmacokinetic profiles of many drugs that are substrates for P-gp would therefore not be affected via this mechanism when coadministered with DL. The lack of interaction with P-gp will mean more predictable pharmacokinetics of desloratadine when used in the treatment of allergic rhinitis and other allergic diseases.

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