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# Olanzapine Penetration into Brain is Greater in Transgenic *Abcb1a* P-glycoprotein-Deficient Mice than FVB1 (Wild-Type) Animals

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The transmembrane energy-dependent efflux transporter P-glycoprotein (P-gp) limits a range of drugs from penetrating cells and deposits them into the extracellular space. P-gp is highly expressed in several normal tissues, including the luminal surface of capillary endothelial cells in the brain of humans. In this study, we tested whether olanzapine distribution to tissues highly expressing P-gp or devoid of this transporter was similar in *Abcb1a* (−/−) mice lacking P-gp and control animals. At 1 h following the intraperitoneal injection of 2.5 μg olanzapine/g mouse, olanzapine concentrations were statistically and significantly higher in brain (three-fold), liver (2.6-fold), and kidney (1.8-fold) of *Abcb1a* (−/−) mice than those of the control FVB *Abcb1a* (+/+) mice, and not statistically different in plasma, spleen, or penile tissue. Similar differences were also found for the ratios of organ:plasma and organ:spleen between the two groups. This is the first report that the presence of the *Abcb1a* gene is an important factor controlling brain access to olanzapine. The finding that the brain penetration of olanzapine is limited by P-gp implies that the highly prevalent functional polymorphisms of *ABCB1* in humans may be a factor contributing to variability in dose requirements for this antipsychotic drug.
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## INTRODUCTION

The adenosine triphosphate (ATP)-binding cassette (ABC) superfamily of efflux transport proteins contains subfamily B, often referred to as the multidrug-resistant subfamily (MDR), and subfamily C, also known as the multidrug-resistant related protein subfamily (MRP) (Germann, 1996; Carson *et al*, 2002). The most extensively studied ABC transporter is P-glycoprotein (P-gp). P-gp belongs to subfamily B and is a 170-kDa transmembrane protein encoded by the human gene *ABCB1*. Formerly known as *MDR1* or *PGY1*, *ABCB1* maps to chromosome 7q21.1 (Dean *et al*, 2001). It was the first ABC transporter cloned and characterized by its ability to confer a multidrug resistance phenotype to cancer cells that had become resistant to chemotherapy (Riordan *et al*, 1985; Roninson *et al*, 1986). In addition to expression in tumor cells, the *ABCB1* protein (P-gp) is widely localized in normal tissues, including the apical membranes of the gastrointestinal tract, the biliary canalicular membranes of hepatocytes, the luminal membranes of proximal tubular epithelial cells in the kidney, and the luminal membranes of endothelial cells in cerebral capillaries forming the blood–brain barrier (Cordon-Cardo *et al*, 1989; Yu, 1999; Silverman, 2000).

The biological function of P-gp appears to be a protective role for major organs by limiting cellular uptake of xenobiotics by excreting these compounds into bile, urine, the intestinal lumen, and limiting accumulation in brain. This function as a drug efflux pump plays an important role in drug absorption, distribution, and elimination (Lin and Yamazaki, 2003). A large number of structurally diverse drugs from various pharmacologic classes are actively transported by P-gp (Schinkel *et al*, 1996; Schuetz *et al*, 1996; Mahar Doan *et al*, 2002; Weiss *et al*, 2003). P-gp is highly polymorphic and its expression in the endothelial cells of the blood–brain barrier limits substrate access to the central nervous system (CNS), thereby influencing the therapeutic consequences of psychoactive drugs (Thompson *et al*, 2000). A P-gp inhibitor was shown to increase the extracellular fluid concentration of morphine in the brain of

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rats, while chronic treatment with morphine leading to tolerance of the antinociceptive effect was shown to be mediated by a morphine-induced increase in the brain P-gp (Aquilante *et al*, 2000).

Several *in vitro* cell systems and a transgenic *Abcb1a/b*(−/−) mouse model have been used to study the functional activity of the protein in the blood–brain barrier (Lin and Yamazaki, 2003; Schwab *et al*, 2003). Drug-stimulated ATPase activity can be determined by monitoring the release of inorganic phosphate (Sarkadi *et al*, 1992; Urbatsch *et al*, 1994). Alternatively, fluorescent indicators can be monitored where the inhibition of P-gp leads to cellular accumulation of rhodamine-123 or calcein AM (Eytan *et al*, 1997; Tiberghien and Loor, 1996). The *Abcb1a*(−/−) 'knockout' mouse model exhibits functional deficiency of P-gp in the blood–brain barrier (Cordon-Cardo *et al*, 1989; Schinkel *et al*, 1996, 1997; Silverman, 2000), and therefore has provided considerable insight into the role and importance of P-gp in the brain entry of drugs (Kim *et al*, 1998; Schinkel *et al*, 1996, 1997; Letrent *et al*, 1999; Aquilante *et al*, 2000). Mice lacking the *Abcb1a* gene display no anatomical, biochemical, or other abnormalities (Schinkel *et al*, 1994, 1997).

Data documenting transport of antipsychotic drugs with P-gp are sparse. No previous reports could be found using the *Abcb1a* (−/−) mouse to test antipsychotic drugs for transport by P-gp. Previous reports using *in vitro* methods have found that levomeprazine and risperidone may be substrates, while chlorpromazine, clozapine, haloperidol, chlorprothixene, and perphenazine may not (Mahar Doan *et al*, 2002; Schinkel *et al*, 1997). The data on chlorpromazine are conflicting with positive transport using the human CCRF-CEM leukemia cells (Syed *et al*, 1996) and low transport by multidrug resistance-transfected MDCK cells (Mahar Doan *et al*, 2002). Using ATPase activity as a marker for P-gp binding affinity, we previously found a rank order of activity from verapamil (positive control) > quetiapine > risperidone > olanzapine > chlorpromazaine > haloperidol = clozapine (Boulton *et al*, 2002). These results suggested that P-gp may influence the access to the brain of the antipsychotics to various degrees.

Verapamil, quetiapine, and risperidone each are partially metabolized by CYP3A4 (Kroemer *et al*, 1993; DeVane and Nemeroff, 2001a, b). Olanzapine is predominantly biotransformed by CYP1A2 and glucuronyl transferases, yet a small contribution by CYP3A4 has been implicated (Ring *et al*, 1996; Markowitz *et al*, 2001). An inter-relationship in substrate specificity between cytochrome *P450* (CYP) 3A4 and P-gp has been proposed (Kim *et al*, 1999). The *ABCB1* and *CYP3A* genes are located on the same chromosome with proximal gene loci in humans (Tsui *et al*, 1995); however, many drugs that are CYP3A4 substrates (midazolam, triazolam, alprazolam) are not transported by P-gp and vice versa (digoxin, fexofenadine; Perloff *et al*, 1999; Schuetz *et al*, 1996; von Moltke *et al*, 1999). Nevertheless, the overlap in CYP3A4 and P-gp substrates is consistent with our previous *in vitro* results based on ATPase activity, suggesting that quetiapine among the antipsychotics had the greatest degree of affinity for P-gp (Boulton *et al*, 2002). Olanzapine has the least evidence as a P-gp substrate. To further assess the role of P-gp in limiting brain entry of the antipsychotics, we compared the tissue distribution of olanzapine in *Abcb1a*(−/−) transgenic mice with that in the FVB control *Abcb1a*(+/+) mice.

## MATERIALS AND METHODS

### Chemicals

Olanzapine and RS0244 (internal standard) were obtained from Eli Lilly Inc. (Indianapolis, ID, USA). Hank's balanced salt solution (HBSS) was purchased from Gibco BRL (Carlsbad, CA, USA). Other chemicals and reagents were the purest grade available and were obtained from Fisher Scientific Co. (Fairlawn, NJ, USA).

### Experimental Animals

Male *Abcb1a*(−/−) mice (FVB/TacfBR-[KO]MDR1aN7) and genetically matched male *Abcb1a*(+/+) mice (FVB/MTtacfBR) 9–10 weeks of age weighing 20–30 g were obtained from Taconic (Germantown, NY, USA). The founding of this colony has been described previously (Schinkel *et al*, 1994). Animals were housed individually and maintained at a 12:12 h light/dark cycle. The animals were cared for in accordance with the US Public Health Service policy for the Care and Use of Laboratory Animals, and the experimental studies were approved by the Medical University of South Carolina Animal Care Committee.

### Experimental Procedures

Olanzapine was dissolved in 0.9.% saline with minimal use of 1 N HCL (1‰, v/v). A dose of 2.5 μg olanzapine/g mouse was administered intraperitoneally (i.p.). The entire volume injected was 10 μl/g mouse. At 1 h after the injection, the mice were anesthetized with halothane and decapitated. Trunk blood was collected in EDTA-coated tubes and centrifuged at 3000*g* for 20 min to determine plasma concentrations of olanzapine.

The brain (brain tissue plus meninges), liver, kidney, spleen, and penis were dissected and weighed, and then homogenized in five-fold volume of an HBSS and 0.02 M HEPES buffer, pH 7.2. The homogenates were frozen at −70°C until high-performance liquid chromatography (HPLC) analysis.

### HPLC Analysis of Olanzapine Plasma and Tissue Concentrations

The plasma and tissue samples obtained at 1 h following administration of olanzapine were extracted and determined using HPLC method previously developed and validated in our laboratory and modified for tissue samples (Boulton *et al*, 2001; Llorca *et al*, 2001). Briefly, olanzapine was extracted from 100 or 200 μl of plasma or organ samples after the addition of 50 μl of internal standard working solution (RS0244 10 μg/ml), 0.2 ml of 1 M bicarbonate buffer (pH 11), and 5 ml of a mixture of hexane–isoamyl alcohol (98:2, v/v). The mixture was shaken for 15 min and centrifuged at 3000*g* for 5 min. The organic layer was transferred to another tube and back-extracted with 0.2 ml 0.2 M sulfuric acid. After shaking and centrifugation, the aqueous layer was re-extracted with 0.2 ml of 1 M bicarbonate

buffer. The organic phase was evaporated under nitrogen at 45°C. The residue was dissolved in 100 µl of mobile phase (65% methanol:35% water containing 0.2% triethylamine and was adjust to pH 7.0 with 85% phosphoric acid), and 50 µl was injected into the chromatographic system. Olanzapine and internal standard were separated by a Luna 5u C18(2) column (250 × 4.6 mm; Phenomenex), and the effluent was monitored at a UV wavelength of 273 nm and at a flow rate of 1 ml/min at ambient temperature. The detection limit of olanzapine was 5 ng/ml. The intraday and interday coefficients of variation were lower than 7% at relevant concentrations ($n=7$). The plasma and tissue extraction recoveries ranged from 83 to 98%.

### Data Analysis

Drug quantitation was performed by comparing peak height ratios of olanzapine to the internal standard with ratios derived from calibration curve of standards containing known amounts of drug extracted from plasma or tissues as described above. Final concentrations of olanzapine were expressed as either µg/ml for plasma or µg/g for tissues.

An unpaired *t*-test was used to compare the differences between the concentrations of olanzapine in the *Abcb1a*(−/−) mice and control mice and their tissue:plasma and tissue:spleen ratios. A two-tailed *P*-value was used and the level of statistical significance was $P<0.01$.

## RESULTS

The plasma and organ concentrations of olanzapine 1 h after the i.p. injection of 2.5 µg olanzapine/g mouse are shown in Table 1 and Figure 1. Substantially and statistically higher olanzapine concentrations were found in the brain (three-fold), liver (2.6-fold), and kidney (1.8-fold) of *Abcb1a*(−/−) mice than those of the control mice (Table 1, Figure 1). Consistently higher ratios were also found for the organ:plasma and the organ:spleen concentrations of olanzapine in the *Abcb1a*(−/−) mice than in the control mice (Table 1, Figure 2). However, there was no statistically significant difference for concentrations of olanzapine in the plasma, spleen, and penis between the two groups, nor for the tissue:plasma ratios of spleen and penis (Table 1, Figures 1 and 2).

**Table 1** Tissue Concentrations and Tissue:Plasma Ratios of Olanzapine in *Abcb1a(−/−)* ($n=6$) and Wild-Type Mice ($n=6$) after 1 h i.p. Injection of Olanzapine 2.5 µg/g Mouse

| | ***Abcb1a*(−/−) (mean ± SD)** | **Wild type (mean ± SD)** | **Ratio[a]** | ***P*-value (unpaired *t*-test)** |
|---|---|---|---|---|
| Plasma | 0.6 ± 0.1 | 0.6 ± 0.1 | 1.1 | 0.5 |
| Brain | 1.3 ± 0.3 | 0.5 ± 0.2 | 3.0 | 0.0006* |
| Liver | 3.5 ± 0.6 | 1.5 ± 0.6 | 2.6 | 0.0002* |
| Kidney | 2.8 ± 0.7 | 1.5 ± 0.2 | 1.8 | 0.002* |
| Spleen | 3.1 ± 0.9 | 3.1 ± 0.3 | 1 | 0.9 |
| Penis | 0.5 ± 0.3 | 0.6 ± 0.3 | 0.9 | 0.5 |
| Brain:plasma | 2.0 ± 0.5 | 0.9 ± 0.4 | 2.6 | 0.002* |
| Liver:plasma | 5.5 ± 1.2 | 2.5 ± 0.8 | 2.4 | 0.0005* |
| Kidney:plasma | 4.4 ± 1.3 | 2.6 ± 0.1 | 1.7 | 0.006* |
| Spleen:plasma | 4.7 ± 1.0 | 5.2 ± 1.0 | 0.9 | 0.5 |
| Penis:plasma | 0.8 ± 0.5 | 1.0 ± 0.3 | 0.9 | 0.4 |
| Brain:spleen | 0.5 ± 0.2 | 0.2 ± 0.1 | 2.6 | 0.009* |
| Liver:spleen | 1.2 ± 0.5 | 0.5 ± 0.2 | 2.4 | 0.007* |
| Kidney:spleen | 1.0 ± 0.4 | 0.5 ± 0.1 | 1.7 | 0.02* |
| Penis:spleen | 0.2 ± 0.1 | 0.2 ± 0.05 | 0.9 | 0.9 |

[a]Calculated as the average of the ratios from each individual animal.
*Statistical significance ($P<0.01$) by two-tailed unpaired *t*-test.

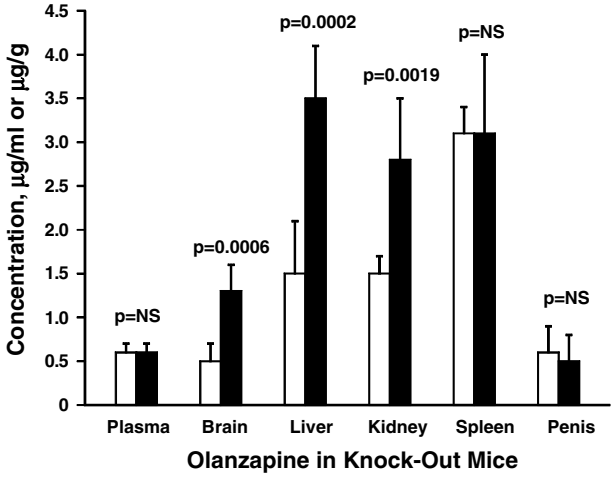


**Figure 1** Plasma (µg/ml) and tissue (µg/g) concentrations of olanzapine in *Abcb1a*(−/−) (solid bars) and *Abcb1a*(+/+) mice (open bars) 1 h after the i.p. injection of 2.5 µg olanzapine/g mouse. Values are shown as mean ± SD ($n=6$). The *P*-values shown were calculated by two-tailed unpaired *t*-test.

## DISCUSSION

The current findings demonstrate that the brain penetration of olanzapine is limited by P-gp. The three-fold difference in the brain concentration of olanzapine between the P-gp-deficient *Abcb1a*(−/−) mice and the FVB control *Abcb1a*(+/+) mice were most likely due to the functional activity of P-gp residing in the blood–brain barrier. This conclusion is also in good agreement with the 2.6-fold difference observed in brain:plasma and brain:spleen ratios of olanzapine between the two groups, indicating that the difference in the brain olanzapine concentration between the two groups was not caused by differences of plasma olanzapine concentration that were identical. Similarly, differences in concentration in the spleen were absent, since the expression of P-gp in the spleen is very low (Cordon-Cardo *et al*, 1989; Schinkel *et al*, 1997). These findings of no drug concentration differences in organs with low P-gp expression (plasma and spleen) are consistent with the previous results of Uhr *et al* (2000) using amitriptyline.

Previously, the absence of P-gp in *Abcb1a*(−/−) mice has been shown as a pervasive influence on the tissue distribution and pharmacokinetics of drugs such as indinavir, nelfinavir, saquavir, loperamide, ondansetron, ivermectin, vinblastin, cyclosporin A, amitriptyline, morphine, some endogenous steroid hormones, citalopram, trimipramine, carbamazepine, phenobarbital, lamotrigine,

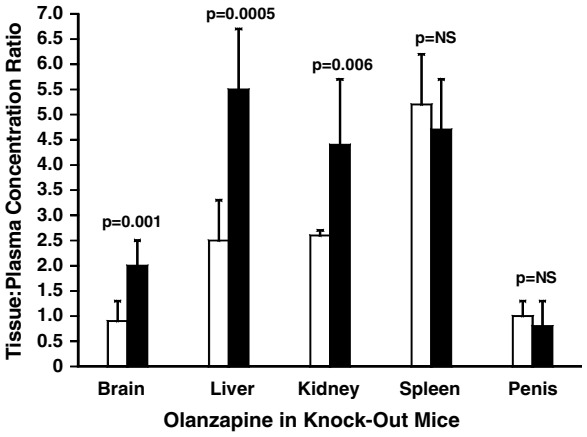


**Figure 2** Organ:plasma ratios of olanzapine in *Abcb1a*(−/−) (solid bars) and *Abcb1a*(+/+) mice (open bars) 1 h after the i.p. injection of 2.5 μg olanzapine/g mouse. Values are shown as mean ± SD ($n = 6$). The *P*-values shown were calculated by two-tailed unpaired *t*-test.

and felbamate (Kim *et al*, 1998; Schinkel *et al*, 1996, 1997; Letrent *et al*, 1999; Aquilante *et al*, 2000; Uhr *et al*, 2000, 2002; Fromm, 2000; Potschka *et al*, 2001, 2002; Uhr and Grauer, 2003). The brain concentrations of these drugs were remarkably higher in *Abcb1a*(−/−) mice than that of *Abcb1a* (+/+) mice. However, differences in the liver and kidney were smaller compared to those in the brain or not statistically significant. In the present study, similar ratios of olanzapine to plasma were found for the brain, liver, and kidney (3.0, 2.6, 1.8, respectively, Table 1) in *Abcb1a*(−/−) compared to *Abcb1a*(+/+) mice. When studies have found large differences in the brain concentration of drugs transported by P-gp in *Abcb1a*(−/−) animals compared to controls, the liver and kidney differences in drug concentration were frequently small, but for some drugs (experimental anticonvulsants and doxorubicin; Van Asperen *et al*, 1999; Cox *et al*, 2002) such differences have been substantial. The exact mechanism(s) for this inconsistency between study results is unclear, but it suggests that the tissue distribution of drugs transported by P-gp in the two animal groups is highly dependent upon the specific substrate involved. Nevertheless, there is consistency in our results that statistically significant differences in drug concentrations were found in tissues normally containing P-gp (brain, liver, kidney), while no differences in concentrations were found in those tissues devoid of P-glycoprotein (plasma, spleen, and penis).

Olanzapine is one of the most widely used antipsychotic agents that has been marketed post clozapine. It is a serotonin/dopamine antagonist that has proven efficacy against both the positive and negative symptoms of schizophrenia, and is widely used in other psychotic disorders (Bhana *et al*, 2001). It has a markedly better side-effect profile than older conventional agents such as haloperidol and chlorpromazine (Beasley *et al*, 1996). However, even with these improvements, not all patients respond adequately to olanzapine and dosage requirements vary widely. Approximately 20–30% of patients with schizophrenia show an inadequate efficacy and 5–10% are intolerant to olanzapine therapy (Bhana *et al*, 2001).

The exact reason for refractory symptoms in olanzapine-treated patients is unknown. However, recent findings of functional polymorphisms of *ABCB1* in humans may provide an insight into one mechanism (Hoffmeyer *et al*, 2000). Single-nucleotide polymorphisms (SNP) in exon 26 (C3435T) and exon 21 (G2677T) have been associated with the functional activity of P-gp. The homozygous T/T genotype mutant alleles were associated with more than two-fold lower intestinal P-glycoprotein expression levels and higher bioavailability of digoxin, a prototype P-gp probe (Kurata *et al*, 2002; Nakamura *et al*, 2002). High frequencies of the mutant alleles are present in the population (19% for the T/T 3435 allele and 28% for the T/T2677 allele; Lin and Yamazaki, 2003). These functional SNPs of P-gp in humans may represent a major source of interindividual variability in the therapeutic efficacy and toxicity of P-gp substrates. This effect may be even more remarkable for psychoactive drugs, which need to penetrate through the P-gp residing in the blood–brain barrier to exert their desirable pharmacological effects. Quite recently, Siddiqui *et al* (2003) reported that the ABCB1 C3435T polymorphism was associated with drug responses of epilepsy. Patients with drug-resistant epilepsy were more likely to have the CC genotype at ABCB1 3435 than the TT genotype compared with patients with drug-responsive epilepsy. In addition, the homozygosity for 3435T alleles of ABCB1 has been suggested as a risk factor for the occurrence of nortriptyline-induced postural hypotension, a CNS side effect related to the protective role of P-gp in the brain penetration of nortriptyline (Roberts *et al*, 2002). Accordingly, it is conceivable that patients who have a high P-gp expression in their blood–brain barrier may have low brain concentrations of olanzapine contributing to therapeutic resistance or failure, and patients who have a low P-gp expression in their blood–brain barrier may have a relatively high brain olanzapine concentration, resulting in adverse events, toxicity, or beneficial therapeutic effects with lower than usual daily drug doses.

Previous studies have indicated that the modulation of P-gp-mediated drug transport can dramatically increase or decrease drugs' penetration into the CNS and affect their therapeutic efficacy (Letrent *et al*, 1999; Aquilante *et al*, 2000). For example, the analgesic effect of morphine in rats was enhanced through the use of a specific P-gp inhibitor (Letrent *et al*, 1999). Accordingly, the current experimental findings provide a rational for the potential use of P-gp modulators with olanzapine or other P-gp substrates, since a reversal of the functional activity of P-gp may result in improved therapeutic efficacy by increasing CNS drug concentration. However, the inhibition of P-gp activity in humans would likely increase both plasma and brain concentrations of olanzapine and other orally administered substrates, since P-gp is also present in the gastrointestinal tract (Fricker and Miller, 2002; Verstuyft *et al*, 2003; Becquemont *et al*, 2001; Hennessy *et al*, 2002). This is not necessarily a deterrent for the use of safe P-gp inhibitors. By artificially increasing the brain:plasma drug concentration ratio through the inhibition of P-gp, smaller oral substrate doses could be used resulting in lower plasma drug concentration, while maintaining an equivalent or higher concentration in the CNS. Adverse events related to systemic drug exposure should be decreased as a result of

using lower oral doses while promoting increased efficacy at the site of action as a result of increased brain drug concentration. Such an approach is currently being investigated to improve the therapeutic efficacy of the protease inhibitors (Choo *et al*, 2000). A requisite step to applying this approach in neuropsychopharmacology is the identification of relevant P-gp substrates.

By measuring P-gp ATPase activity, we previously reported that the antipsychotics have various degrees of binding affinity with P-gp *in vitro*, with rank order of affinity (by measuring $V_{max}/K_m$) as: quetiapine (1.7) > risperidone (1.4) > olanzapine (0.8) > chlorpromazine (0.7) > haloperidol (0.3) = clozapine (0.3) (Boulton *et al*, 2002). Accordingly, olanzapine was identified as an intermediate affinity substrate among the antipsychotics (Boulton *et al*, 2002). Consistent with the present findings, a previous report using monolayer efflux in multidrug resistence-transfected cells identified that cellular uptake of risperidone is mediated by P-gp (Mahar Doan *et al*, 2002). In another study, the conventional antipsychotic chlorpromazine has been shown to be actively transported by P-gp in cancer-derived cells (Syed *et al*, 1996). Unfortunately, binding studies and *in vitro* transport measurements in monolayer cell systems under-represent the complexity of the *in vivo* situation. Inconsistencies have been noted (Polli *et al*, 2001). This is the first report using an *in vivo* model to identify that the brain entry of olanzapine is limited by P-gp. Unfortunately, no chemical, clinical, or pharmacological properties appear to allow accurate predictions within or between drug classifications of those compounds that are transported by P-gp. Even the widely used *in vitro* methods to evaluate transport have produced conflicting findings with approximately half of the studied compounds displaying significant variation in P-gp activity dependent on the specific assay used (Adachi *et al*, 2001; Yamazaki *et al*, 2001). The single and double knockout mouse model appears to produce the most unequivocal results (Schinkel *et al*, 1996).

In conclusion, the present results showed that the expression of P-gp in the blood–brain barrier significantly limits the penetration of olanzapine into the CNS. Functional polymorphisms of P-gp in humans may represent an unrecognized source of interindividual variability in the therapeutic efficacy of olanzapine. The recognition of brain entry of olanzapine is limited by P-gp offering intriguing possibilities for manipulating its therapeutic utility through the use of adjunctive P-gp modulators.

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## REFERENCES

Adachi Y, Suzuki H, Sugiyama Y (2001). Comparative studies on *in vitro* methods for evaluating *in vivo* function of MDR1 P-glycoprotein. *Pharmacol Res* **18**: 1660–1668.

Aquilante CL, Letrent SP, Pollack GM, Brouwer KL (2000). Increased brain P-glycoprotein in morphine tolerant rats. *Life Sci* **64**: 47–51.

Beasley Jr CM, Tollefson G, Tran P, Satterlee W, Sanger T, Hamilton S (1996). The Olanzapine HGAD Study Group. Olanzapine *versus* placebo and haloperidol: acute phase results of the North American double-blind olanzapine trial. *Neuropsychopharmacol* **14**: 111–123.

Becquemont L, Verstuyft C, Kerb R, Brinkmann U, Lebot M, Jaillon P *et al* (2001). Effect of grapefruit juice on digoxin pharmacokinetics in humans. *Clin Pharmacol Ther* **70**: 311–316.

Bhana N, Foster RH, Olney R, Plosker GL (2001). Olanzapine: an updated review of its use in the management of schizophrenia. *Drugs* **61**: 111–161.

Boulton DW, DeVane CL, Liston HL, Markowitz JS (2002). Human recombinant P-glycoprotein specificity for atypical antipsychotics. *Life Sci* **71**: 163–169.

Boulton DW, Markowitz JS, DeVane CL (2001). Olanzapine assay by high performance liquid chromatography using ultraviolet detection. *J Chromatogr* **759**: 319–323.

Carson SW, Ousmanou AD, Hoyler SL (2002). Emerging significance of P-glycoprotein in understanding drug disposition and drug interaction in psychopharmacology. *Psychopharmacol Bull* **36**: 67–81.

Choo EF, Leake B, Wandel C, Imamura H, Wood AJ, Wilkinson GR *et al* (2000). Pharmacological inhibition of P-glycoprotein transport enhances the distribution of HIV-1 protease inhibitors into brain and testes. *Drug Metab Dispos* **28**: 655–660.

Cordon-Cardo C, O'Brien JP, Casals D, Rittman-Grauer L, Biedler JL, Melamed MR *et al* (1989). Multidrug-resistance gene (P-glycoprotein) is expressed by endothelial cells at blood–brain barrier sites. *Proc Natl Acad Sci USA* **86**: 695–698.

Cox DS, Scott KR, Gao H, Eddington ND (2002). Effect of P-glycoprotein on the pharmacokinetics and tissue distribution of enaminone anticonvulsants: analysis by population and physiological approaches. *J Pharmacol Exp Ther* **302**: 1096–1104.

Dean M, Rzhetsky A, Allikmets R (2001). The human ATP-cassette (ABC) transporter superfamily. *Genome Res* **11**: 1156–1166.

DeVane CL, Nemeroff CB (2001a). Clinical pharmacokinetics of quetiapine an atypical antipsychotic. *Clin Pharmacokinet* **40**: 509–522.

DeVane CL, Nemeroff CB (2001b). Evaluation of risperidone drug interactions. *J Clin Psychopharmacol* **21**: 408–416.

Eytan GD, Regev R, Oren G, Hurwitz CD, Assaraf YG (1997). Efficiency of P-glycoprotein-mediated exclusion of rhodamine dyes from multidrug-resistant cells is determined by their passive transmembrane movement rate. *Eur J Biochem* **248**: 104–112.

Fricker G, Miller DS (2002). Relevance of multidrug resistance proteins for intestinal drug absorption *in vitro* and *in vivo*. *Pharmacol Toxicol* **90**: 5–13.

Fromm MF (2000). P-glycoprotein: a defense mechanism limiting oral bioavailability and CNS accumulation of drugs. *Int J Clin Pharmacol Ther* **38**: 69–74.

Germann UA (1996). P-glycoprotein–a mediator of multidrug resistance in tumour cells. *Eur J Cancer* **32A**: 927–944.

Hennessy M, Kelleher D, Spiers JP, Barry M, Kavanagh P, Back D *et al* (2002). St Johns wort increases expression of P-glycoprotein: implications for drug interactions. *Br J Clin Pharmacol* **53**: 75–82.

Hoffmeyer S, Burk O, von Richter O, Arnold HP, Brockmoller J, Johne A *et al* (2000). Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity *in vivo*. *Proc Natl Acad Sci USA* **97**: 3473–3478.

Kim RB, Fromm MF, Wandel C, Leake B, Wood AJ, Roden DM *et al* (1998). The drug transporter P-glycoprotein limits oral absorp-

tion and brain entry of HIV-1 protease inhibitors. *J Clin Invest* **101**: 289–294.

Kim RB, Wandel C, Leake B, Cvet Kovic M, Fromm MF, Dempsey PJ *et al* (1999). Interrelationship between substrates and inhibitors of human CYP3A and P-glycoprotein. *Pharmaceut Res* **16**: 408–414.

Kroemer HK, Gautier JC, Beaune P, Henderson C, Wolf CR, Eichelbaum M (1993). Identification of P450 enzymes involved in metabolism of verapamil in humans. *Naunyn Schmiedebergs Arch Pharmacol* **348**: 332–337.

Kurata Y, Ieiri I, Kimura M, Morita T, Irie S, Urae A *et al* (2002). Role of human MDR1 gene polymorphism in bioavailability and interaction of digoxin, a substrate of P-glycoprotein. *Clin Pharmacol Ther* **72**: 209–219.

Letrent SP, Pollack GM, Brouwer KR, Brouwer KL (1999). Effects of a potent and specific P-glycoprotein inhibitor on the blood–brain barrier distribution and antinociceptive effect of morphine in the rat. *Drug Metab Dispos* **27**: 827–834.

Lin JH, Yamazaki M (2003). Role of p-glycoprotein in pharmacokinetics: clinical implications. *Clin Pharmacokinet* **42**: 59–98.

Llorca PM, Coudore F, Corpelet C, Buyens A, Hoareau M, Eschalier A (2001). Integration of olanzapine determinations in a HPLC-diode array detection system for routine psychotropic drug monitoring. *Clin Chem* **47**: 1719–1721.

Mahar Doan KM, Humphreys JE, Webster LO, Wring SA, Shampine LJ, Serabjit-Singh CJ *et al* (2002). Passive permeability and P-glycoprotein-mediated efflux differentiate central nervous system (CNS) and non-CNS marketed drugs. *J Pharmacol Exp Ther* **303**: 1029–1037.

Markowitz JS, DeVane CL, Liston HL, Boulton DW, Risch SC (2001). Probenecid effects on the disposition of olanzapine and risperidone in healthy volunteers. *Clin Pharmacol Ther* **71**: 30–38.

Nakamura T, Sakaeda T, Horinouchi M, Tamura T, Aoyama N, Shirakawa T *et al* (2002). Effect of the mutation (C3435T) at exon 26 of the MDR1 gene on expression level of MDR1 messenger ribonucleic acid in duodenal enterocytes of healthy Japanese subjects. *Clin Pharmacol Ther* **71**: 297–303.

Perloff MD, von Moltke LL, Cotreau MM, Greenblatt DJ. (1999). Unchanged cytochrome *P450* 3A (CYP3A) expression and metabolism of midazolam, triazolam, and dexamethasone in *mdr*(−/−) mouse liver microsomes. *Biochem Pharmacol* **57**: 1227–1232.

Polli JW, Wring SA, Humphreys JE, Huang L, Morgan JB, Webster LO *et al* (2001). Rational use of *in vitro* P-glycoprotein assays in drug discovery. *J Pharmacol Exp Ther* **299**: 620–628.

Potschka H, Fedrowitz M, Loscher W (2001). P-glycoprotein and multidrug resistance-associated protein are involved in the regulation of extracellular levels of the major antiepileptic drug carbamazepine in the brain. *Neuroreport* **12**: 3557–3560.

Potschka H, Fedrowitz M, Loscher W (2002). P-glycoprotein-mediated efflux of phenobarbital, lamotrigine, and felbamate at the blood–brain barrier: evidence from microdialysis experiments in rats. *Neurosci Lett* **327**: 173–176.

Ring BJ, Catlow J, Linday TJ, Gillespie T, Roskos LK, Cerimele BJ *et al* (1996). Identification of the human cytochromes P450 responsible for the *in vitro* formation of the major oxidative metabolites of the antopsychotic agent olanzapine. *J Pharmacol Exp Ther* **276**: 658–666.

Riordan JR, Deuchars K, Kartner N, Alon N, Trent J, Ling V (1985). Amplification of P-glycoprotein genes in multidrug-resistant mammalian cell lines. *Nature* **316**: 817–819.

Roberts RL, Joyce PR, Mulder RT, Begg EJ, Kennedy MA (2002). A common P-glycoprotein polymorphism is associated with nortriptyline-induced postural hypotension in patients treated for major depression. *Pharmacogenom J* **2**: 191–196.

Roninson IB, Chin JE, Choi K, Gros P, Housman DE, Fojo A *et al* (1986). Isolation of human mdr DNA sequences amplified in multidrug-resistant KB carcinoma cells. *Proc Natl Acad Sci USA* **83**: 4538–4542.

Sarkadi B, Price EM, Boucher RC, German UA, Scarborough GA (1992). Expression of the human multidrug resistance cDNA in insect cells generates a high activity drug-stimulated membrane ATPase. *J Biol Chem* **267**: 4854–4858.

Schinkel AH, Mayer U, Wagenaar E, Mol CA, van Deemter L, Smit JJ *et al* (1997). Normal viability and altered pharmacokinetics in mice lacking mdr1-type (drug-transporting) P-glycoproteins. *Proc Natl Acad Sci USA* **94**: 4028–4033.

Schinkel AH, Smit JJ, van Tellingen O, Beijnen JH, Wagenaar E, van Deemter L *et al* (1994). Disruption of the mouse mdr1a P-glycoprotein gene leads to a deficiency in the blood–brain barrier and to increased sensitivity to drugs. *Cell* **77**: 491–502.

Schinkel AH, Wagenaar E, Mol CA, van Deemter L (1996). P-glycoprotein in the blood–brain barrier of mice influences the brain penetration and pharmacological activity of many drugs. *J Clin Invest* **97**: 2517–2524.

Schuetz EG, Beck WT, Schuetz JD (1996). Modulators and substrates of P-glycoprotein and cytochrome *P4503*A coordinately up-regulate these proteins in human colon carcinoma cells. *Mol Pharmacol* **49**: 311–318.

Schwab D, Fischer H, Tabatabael A, Poli S, Huwyler J (2003). Comparison of *in vitro* P-glycoprotein screening assays: recommendations for their use in drug discovery. *J Med Chem* **46**: 1716–1725.

Siddiqui A, Kerb R, Weale ME, Brinkmann U, Smith A, Goldstein D *et al* (2003). Association of multidrug resistance in epilepsy with a polymorphism in the drug-transporter gene ABCB1. *N Engl J Med* **348**: 1442–1448.

Silverman JA (2000). P-Glycoprotein. In: René L (eds). *Metabolic Drug Interactions*. Lippincott-Raven Press: Philadelphia, PA. pp 135–144.

Syed SK, Christopherson RI, Roufogalis BD (1996). Chlorpromazine transport in membrane vesicles from multidrug resistant CCRF-CEM cells. *Biochem Mol Biol Int* **39**: 687–696.

Thompson JS, Koszdin K, Bernadsrs CM (2000). Opiate-induced analgesia is increased and prolonged in mice lacking P-glycoprotein. *Anesthesiology* **92**: 1392–1399.

Tiberghien F, Loor F (1996). Ranking of P-glycoprotein substrates and inhibitors by a calcein-AM fluorometry screening assay. *Anticancer Drugs* **7**: 568–578.

Tsui LC, Donis-Keller H, Grzeschik KH (1995). Report of the second international workshop on human chromosome 7 mapping 1994. *Cytogenet Cell Genet* **71**: 2–21.

Urbatsch IL, al-Shari MK, Senior AE (1994). Characterization of the ATPase activity of purified Chinese hamster P-glycoprotein. *Biochemistry* **33**: 7069–7076.

Uhr M, Grauer MT (2003). abcb1ab P-glycoprotein is involved in the uptake of citalopram and trimipramine into the brain of mice. *J Psychiatry Res* **37**: 179–185.

Uhr M, Holsboer F, Muller MB (2002). Penetration of endogenous steroid hormones corticosterone, cortisol, aldosterone and progesterone into the brain is enhanced in mice deficient for both mdr1a and mdr1b P-glycoproteins. *J Neuroendocrinol* **14**: 753–759.

Uhr M, Steckler T, Yassouridis A, Holsboer F (2000). Penetration of amitriptyline, but not of fluoxetine, into brain is enhanced in mice with blood–brain barrier deficiency due to mdr1a P-glycoprotein gene disruption. *Neuropsychopharmacology* **22**: 380–387.

Van Asperen A, van Tellingen O, Tijssen F, Schinkel AH, Beijnen JH (1999). Increased accumulation of doxorubicin and doxorubicinol in cardiac tissue of mice lacking mdr1a P-glycoprotein. *Br J Cancer* **79**: 108–113.

Verstuyft C, Strabach S, El-Morabet H, Kerb R, Brinkmann U, Dubert L *et al* (2003). Dipyridamole enhances digoxin bioavailability via P-glycoprotein inhibition. *Clin Pharmacol Ther* **73**: 51–60.

Von Moltke LL, Granda BW, Grassi JM, Greenblatt DJ (1999). Multiple site interaction of triazolam and ketoconazole in mice: role of P-glycoprotein. *Clin Pharmacol Ther* **65**: 143.

Weiss J, Sven-Maria GD, Martin-Facklam M, Kerpen CJ, Ketabi-Kiyanvash K, Haefeli WE (2003). Inhibition of P-glycoprotein by newer antidepressants. *J Pharmacol Exp Ther* **305**: 197–204.

Yamazaki M, Neway WE, Ohe T *et al* (2001). *In vitro* substrate identification studies for P-glycoprotein-mediated transport: species difference and predictability of *in vivo* results. *J Pharmacol Exp Ther* **296**: 723–735.

Yu DK (1999). The contribution of P-glycoprotein to pharmacokinetic drug–drug interactions. *J Clin Pharmacol* **39**: 1203–1211.