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Haloperidol is an inhibitor but not substrate for MDR1/P-glycoprotein

Koichi Iwaki, Toshiyuki Sakaeda, Mikio Kakumoto, Tsutomu Nakamura, Chiho Komoto, Noboru Okamura, Kohshi Nishiguchi, Takashi Shiraki, Masanori Horinouchi and Katsuhiko Okumura

Abstract

The involvement of the multidrug resistant transporter MDR1/P-glycoprotein in the penetration of haloperidol into the brain and absorption in the intestine was investigated to examine its role in inter/intra-individual variability, using the porcine kidney epithelial cell line LLC-PK_1 and its MDR1-overexpressing transfectant, LLC-GA5-COL150. The inhibitory effect of haloperidol on other MDR1 substrates was also investigated in terms of the optimization of haloperidol-based pharmacotherapy. The transepithelial transport of [^{3}H]haloperidol did not differ between the two cell lines, and vinblastine, a typical MDR1 substrate, had no effect on the transport, suggesting that haloperidol is not a substrate for MDR1, and it is unlikely that MDR function affects haloperidol absorption and brain distribution, and thereby the response to haloperidol. However, haloperidol was found to have an inhibitory effect on the MDR1-mediated transport of [^{3}H]digoxin and [^{3}H]vinblastine with an IC50 value of 7.84 ± 0.76 and 3.60 ± 0.64 μM, respectively, suggesting that the intestinal absorption, not distribution into the brain, of MDR1 substrate drugs could be altered by the co-administration of haloperidol in the clinical setting, although further clinical studies are needed.

Department of Hospital Pharmacy, School of Medicine, Kobe University, 7-5-2 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan

Koichi Iwaki, Toshiyuki Sakaeda, Mikio Kakumoto, Tsutomu Nakamura, Chiho Komoto, Kohshi Nishiguchi, Takashi Shiraki, Masanori Horinouchi, Katsuhiko Okumura

Department of Clinical Evaluation of Pharmacotherapy, Graduate School of Medicine, Kobe University, 1-5-6, Minatojima-minamimachi, Chuo-ku, Kobe 650-0047, Japan

Noboru Okamura

Correspondence: T. Sakaeda, Department of Hospital Pharmacy, School of Medicine, Kobe University, 7-5-2, Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan. E-mail: sakaedat@med.kobe-u.ac.jp

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Introduction

Haloperidol, a typical antipsychotic drug, is widely used to treat acute and chronic psychoses, including schizophrenia and manic states. There is extensive inter-individual variability in the response to haloperidol and its use can often result in extrapyramidal reactions, including Parkinson-like symptoms, dystonia, akathisia and dyskinesia (Keepers et al 1983; Ulrich et al 1998). Inter-individual variability is not restricted to haloperidol, and the diagnosis of responders/non-responders has been an important clinical issue for pharmacotherapy to treat major disorders of the central nervous system (CNS), including depression, schizophrenia, chronic pain and epilepsy. Variability in pharmacodynamic factors, such as receptor density and affinity, has been considered responsible but recently Siddiqui et al (2003) reported an association between drug-resistant epilepsy and the genotype of a pharmacokinetic factor, the multidrug resistant transporter MDR1/P-glycoprotein, based on reports that antiepileptic agents, including carbamazepine, phenytoin and phenobarbital, are substrates for MDR1 (Schinkel et al 1996; Potschka & Löscher 2001; Potschka et al 2001, 2002).

MDR1 was originally isolated from multidrug resistant cancer cell lines and revealed to pump out anti-cancer agents from cells to reduce drug concentrations (Gottesman & Pastan 1988; Endicott & Ling 1989). Subsequently, it has been shown that MDR1 is expressed in normal tissue (Thiebaut et al 1987, 1989) and plays an important role in drug disposition and drug–drug interaction (Sakaeda et al 2002a, 2003, 2004; Sakaeda 2005). Notably, its role and interaction at the blood–brain barrier (BBB) are thought to be critical for drugs acting in the CNS (Lee & Bendayan 2004; Löscher & Potschka 2005; Pardridge 2005). In addition, because MDR1 plays a role in restricting the absorption of substrates, MDR1 inhibition is thought to raise the blood concentration of substrates for MDR1 (Adachi et al 2003; Dietrich et al 2003). Especially, absorption of substrates for MDR1 and cytochrome P450 (CYP) 3A4 is restricted by their synergistic effect (Wacher et al 2001; Benet et al 2004).

Indeed, overlapping substrates for CYP3A4 and MDR1 have been reported (Wacher et al 1995) and haloperidol is mainly metabolized by CYP3A4 (Kalgutkar et al 2003).

In this study, we clarify the contribution of MDR1 transporting activity to the penetration of haloperidol into the brain so as to examine its role in inter/intra-individual variability, using the porcine kidney epithelial cell lines LLC-PK_1 and its MDR1-overexpressing transfectant, LLC-GA5-COL150. Inhibitory effects of haloperidol on other MDR1 substrates were also investigated in terms of possible drug–drug interaction in haloperidol-based pharmacotherapy.

Materials and Methods

Materials

Colchicine and haloperidol were purchased from Wako Pure Chemical Industries Ltd (Osaka, Japan). Digoxin was obtained from Sigma-Aldrich Co. (St Louis, MO). [^{3}H]Digoxin (595.7 GBq mmol^{-1}), [^{3}H]vinblastine (355.2 GBq mmol^{-1}), and [^{3}H]haloperidol (555 GBq mmol^{-1}) were purchased from PerkinElmer Inc. (Boston, MA). [methoxy-^{14}C]Inulin (303 MBq mmol^{-1}) was provided by Amersham Biosciences Ltd (Little Chalfont, UK). All other chemicals used were obtained commercially or were of the highest grade available.

Culture of LLC-PK_1 and LLC-GA5-COL150 cells

LLC-GA5-COL150 cells were established by transfecting with human *MDR1* cDNA into porcine kidney epithelial LLC-PK_1 cells (Tanigawara et al 1992; Ueda et al 1992). Human MDR1 was expressed at the apical membranes and pumped out MDR1 substrates from cells to the apical side. Both cell lines were maintained in a culture medium consisting of Medium199, including 100 mg L^{-1} of glutamine (Dainippon Pharmaceutical Co. Ltd, Osaka, Japan), supplemented with 10% fetal bovine serum (FBS; Lot No. AGM7413, HyClone, UT, or Lot. No. 2353H, MP Biomedicals, Inc., Aurora, OH) without antibiotics. Only for LLC-GA5-COL150 cells, 150 ng mL^{-1} of colchicine was added to the culture medium. LLC-PK_1 cells (1.0×10^6) and LLC-GA5-COL150 cells (1.5×10^6) were seeded on plastic culture dishes (100 mm diameter) in 10 mL of the culture medium. Monolayer cultures were grown in a humidified atmosphere of 5% CO_2–95% air at 37°C, and subcultured every 4 days for LLC-PK_1 cells and 7 days for LLC-GA5-COL150 cells with 0.02% EDTA–0.05% trypsin solution (Invitrogen Corp., Carlsbad, CA).

Transepithelial transport of [^{3}H]digoxin, [^{3}H]vinblastine and [^{3}H]haloperidol across LLC-PK_1 and LLC-GA5-COL150 cell monolayers

The transepithelial transport of [^{3}H]digoxin, [^{3}H]vinblastine and [^{3}H]haloperidol across LLC-PK_1 and LLC-GA5-COL150 cell monolayers was examined as described previously (Tanigawara et al 1992; Sakaeda et al 2002b, 2005). Basal-to-apical (B-to-A) transport, corresponding to brain-to-blood or serosal-to-mucosal transport, and apical-to-basal (A-to-B) transport, corresponding to blood-to-brain or mucosal-to-serosal transport, were measured independently. Both cell lines were seeded on Transwell (24 mm diameter, 3 μm pore size; Cat. No. 3414, Corning Inc., Acton, MA) at a density of 2.0×10^6 and 2.4×10^6 cells/well for LLC-PK_1 and LLC-GA5-COL150 cells, respectively, and cultured under a humidified atmosphere of 5% CO_2–95% air at 37°C for 3 days. Both lines formed monolayers with the basal membrane attached to the microporous membrane of the Transwell system and the apical membrane facing the culture medium. It has been confirmed that human MDR1 is expressed at the apical membrane to pump out MDR1 substrates from cells to the apical side (Tanigawara et al 1992; Ueda et al 1992). Three hours before the transport experiments, the culture medium was replaced with fresh culture medium without colchicine. The transport experiments were initiated by replacing the culture medium on the donor side (i.e. basal side for B-to-A transport and apical side for A-to-B transport) with 2 mL of fresh medium containing either [^{3}H]digoxin (100 nM, 18.5 kBq mL^{-1}), [^{3}H]vinblastine (100 nM, 18.5 kBq mL^{-1}) or [^{3}H]haloperidol (100 nM, 18.5 kBq mL^{-1}), together with a marker of cell monolayer integrity, [methoxy-^{14}C]inulin (6.0 μM, 1.85 kBq mL^{-1}), and that on the receiver side (i.e. apical side for B-to-A transport and basal side for A-to-B transport) with 2 mL of fresh culture medium alone. The monolayers were incubated at 37°C, and 25-μL samples of the medium were taken from the receiver side at designated time points (60, 120 and 180 min for [^{3}H]digoxin and [^{3}H]vinblastine and 20, 40 and 60 min for [^{3}H]haloperidol). The radioactivity associated with the collected media was determined by liquid scintillation counting (LSC-5100; Aloka Co., Ltd, Tokyo, Japan).

The effect of unlabelled haloperidol on the transport of 100 nM [^{3}H]digoxin or [^{3}H]vinblastine was also investigated using LLC-GA5-COL150 cell monolayers, as well as that of 20 μM unlabelled vinblastine on the transport of 100 nM [^{3}H]haloperidol. Unlabelled drugs were added on both sides of the monolayers at the indicated concentration at 1 h before the start until the end of experiments.

Data analysis

Transport data are presented as a percentage of the initial amount of total radioactivity added in the donor side. The net B-to-A transport ratio was calculated by dividing B-to-A transport by A-to-B transport at the last time point. All data are expressed as the mean ± standard deviation (s.d.) of at least three experiments.

The concentration of haloperidol having a 50% inhibitory effect, the IC50 value, was calculated using WinNonlin software (Pharsight Corp., Mountain View, CA) based on the equation; $v = v_{max} - (v_{max} - v_0)\cdot\{I/(I + IC50)\}$, where v_{max}, v_0 and I are the maximum net B-to-A transport ratio of [^{3}H]digoxin or [^{3}H]vinblastine, its nonspecific portion and concentration of inhibitor (i.e., unlabelled haloperidol), respectively. The statistical analysis of the data was performed using a one-way analysis of variance followed by Scheffe's test (two-tailed), with $P < 0.05$ considered significant.

Results

Figure 1 shows the transepithelial transport of [^{3}H]digoxin, [^{3}H]vinblastine and [^{3}H]haloperidol across LLC-PK$_1$ and LLC-GA5-COL150 cell monolayers and Table 1 shows the net B-to-A transport ratio. The B-to-A transport of [^{3}H]digoxin and [^{3}H]vinblastine, typical substrates for MDR1, was increased and the A-to-B transport was decreased in LLC-GA5-COL150 cells, compared with LLC-PK$_1$ cells, showing that this experimental system is appropriate for the assessment of MDR1-mediated transport. On the other hand, no difference in the transport of [^{3}H]haloperidol was found between LLC-PK$_1$ and LLC-GA5-COL150 cells, suggesting that haloperidol is not a substrate for MDR1, consistent with the results obtained by Schinkel et al (1996) using an MDR1-overexpressing cell line, L-MDR1.

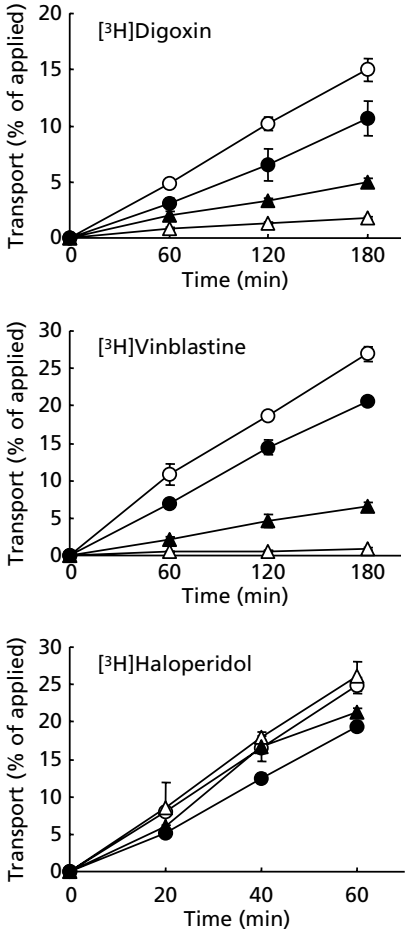

Figure 1 Transepithelial transport of 100 nM [^{3}H]digoxin, [^{3}H]vinblastine and [^{3}H]haloperidol across LLC-PK$_1$ and LLC-GA5-COL150 cell monolayers. Open (circles, triangles) and closed (circles, triangles) symbols show the transepithelial transport across LLC-GA5-COL150 and LLC-PK$_1$ cell monolayers, respectively. Circles (open, closed) show the B-to-A transport, and triangles (open, closed) indicate the A-to-B transport. Each point represents the mean ± s.d., n = 3 or more.

Figures 2 (upper 2 panels) shows the effect of 50 μM haloperidol on the transepithelial transport of [^{3}H]digoxin and [^{3}H]vinblastine across LLC-GA5-COL150 cell monolayers. The B-to-A transport of [^{3}H]digoxin and [^{3}H]vinblastine was decreased, and the A-to-B transport was increased, showing that haloperidol inhibited the MDR1-mediated transport of [^{3}H]digoxin and [^{3}H]vinblastine, consistent with the findings of El Ela et al (2004) using Caco-2 cells. In contrast, as shown in Figure 2 (lower panel), 20 μM vinblastine had no effect on the transport of [^{3}H]haloperidol. Figure 3 shows the relationship between the concentration of co-administered haloperidol and the net B-to-A transport ratio of [^{3}H]digoxin and [^{3}H]vinblastine, giving the IC50 value of 7.84 ± 0.76 and 3.60 ± 0.64 μM, respectively.

Discussion

In general, drugs of relatively small molecular weight and high lipophilicity will be effectively distributed in the brain, but if they are substrates for MDR1, they will be pumped back into the circulating blood after entering the endothelial cells, and by a modification of MDR1 function, their distribution in the brain can be drastically enhanced (Schinkel et al 1994, 1995, 1996). Thus, it is critical for drugs acting in the CNS as to whether they are a substrate for MDR1, because MDR1 transporting activity can affect their distribution in the brain, and thereby the response they evoke. In addition, because MDR1 plays a role in restricting absorption in the intestine, not only for CNS drugs, MDR1 inhibition may raise blood concentrations of substrates for MDR1 (Adachi et al 2003; Dietrich et al 2003). Also, substrates for MDR1 and CYP3A4 synergistically restrict absorption in the intestine (Wacher et al 2001; Benet et al 2004). It is necessary to clarify whether a substrate for CYP3A4 is a substrate for MDR1 or not.

Table 1 Transport rate of digoxin, vinblastine and haloperidol in LLC-PK$_1$ and LLC-GA5-COL150 cells

Compound	LLC-PK$_1$			LLC-GA5-COL150		
	B-to-A[a]	A-to-B[a]	B-to-A/A-to-B[b]	B-to-A	A-to-B	B-to-A/A-to-B
Digoxin	10.7 ± 1.5	4.98 ± 0.36	2.15 ± 0.33	15.0 ± 1.0	1.80 ± 0.13	8.32 ± 0.53**
Vinblastine	20.7 ± 0.1	6.49 ± 0.57	3.20 ± 0.31	26.9 ± 1.0	0.964 ± 0.167	28.4 ± 4.2*
Haloperidol	19.4 ± 0.6	21.3 ± 0.6	0.913 ± 0.042	24.9 ± 1.0	26.0 ± 2.0	0.957 ± 0.044

Data represent means ± s.d., n = 3 or more [a]B-to-A and A-to-B transport are expressed as % of amount applied per 3 h for digoxin and vinblastine and per 1 h for haloperidol. [b]Net B-to-A transport ratios were calculated by dividing B-to-A transport by A-to-B. *P < 0.01, **P < 0.001, compared with LLC-PK$_1$ cells.

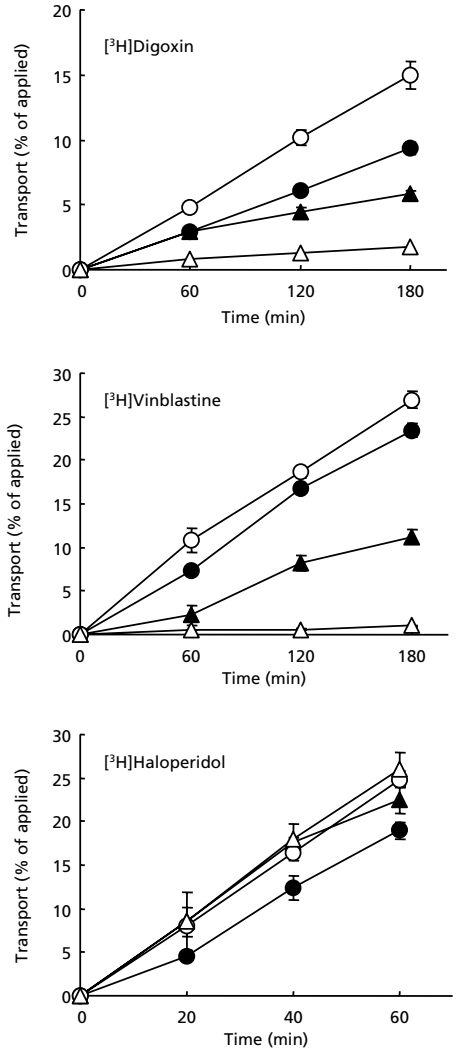

Figure 2 Effect of 50 μM haloperidol on transepithelial transport of 100 nM [^{3}H]digoxin and [^{3}H]vinblastine, and the effect of 20 μM vinblastine on that of 100 nM [^{3}H]haloperidol across LLC-GA5-COL150 cell monolayers. Open (circles, triangles) and closed (circles, triangles) symbols show the transepithelial transport in the absence or presence of inhibitors, respectively. Circles (open, closed) show the B-to-A transport, and triangles (open, closed) indicate the A-to-B transport. Each point represents the mean ± s.d, n = 3 or more.

The antipsychotic drug haloperidol is widely used to treat acute and chronic psychoses, including schizophrenia and manic states. However, there is extensive inter-individual variability in the response to haloperidol and its use can result in extrapyramidal reactions, including Parkinson-like symptoms, dystonia, akathisia and dyskinesia (Keepers et al 1983; Ulrich et al 1998). Variability in receptor density and affinity is understood to affect drugs, such as haloperidol, which target the CNS, but recently Siddiqui et al (2003) have demonstrated an association between drug-resistant epilepsy and the genotype of *MDR1*, raising the possibility that the variability in response is due to that in brain distribution. Although additional clinical research on the effects of *MDR1* genotypes on pharmacodynamics have provided results inconsistent, or only partially consistent, with the report by Siddiqui et al (2003), it is necessary to list the drugs that are substrates for MDR1, especially when haloperidol is mainly metabolized by CYP3A4 (Kalgutkar et al 2003). Here, we investigated the involvement of MDR1 in the transport of [^{3}H]haloperidol using an MDR1-overexpressing cell line, LLC-GA5-COL150, and found that [^{3}H]haloperidol is not a substrate for MDR1, consistent with results of Schinkel et al (1996). Thus, it is unlikely that MDR1 function affects haloperidol absorption and brain distribution, and thereby the response to haloperidol.

Herein, the effects of haloperidol on MDR1-mediated transport were also subjected to investigation. Kataoka et al (2001) have shown that the cytotoxicity of vinblastine was increased in the presence of haloperidol in multidrug resistant cancer cells, suggesting MDR1-mediated drug interaction by haloperidol. As shown in Figure 3, haloperidol has an inhibitory effect on the transport of [^{3}H]digoxin and [^{3}H]vinblastine mediated by MDR1 in a concentration-dependent manner. This is the first study to directly show the inhibition of the MDR1-transporting activity of haloperidol. The IC50 values are 7.84 ± 0.76 and 3.60 ± 0.64 μM for [^{3}H]digoxin and [^{3}H]vinblastine, respectively, which is comparable with the chemosensitizing activity reported by Kataoka et al (2001). Our results suggest that the chemosensitizing by haloperidol shown by Kataoka et al is due to inhibition of MDR1. Because the blood concentration of haloperidol ranges from 1 to 100 nM in the clinical setting, it is unlikely to cause drug–drug interaction at the BBB. Although it is difficult to

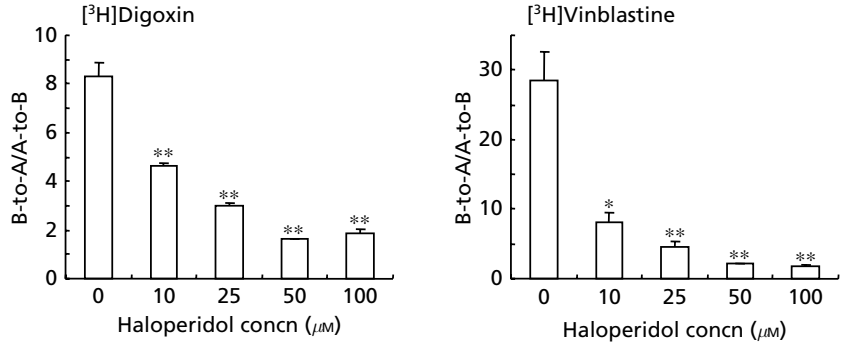

Figure 3 Net B-to-A transport ratio of [^{3}H]digoxin and [^{3}H]vinblastine in the absence or presence of haloperidol in LLC-GA5-COL150 cells. Each bar represents the mean + s.d., n = 3 or more. *$P < 0.01$, **$P < 0.001$, compared with control.

estimate concentration in the intestine, if 1 mg of haloperidol was taken with 250 mL water, concentration of haloperidol could reach over 10 μM. Consequently, it is likely that haloperidol will increase the intestinal absorption of any substrate for MDR1 co-administered orally. In addition, haloperidol has an inhibitory potency for nifedipine metabolism mediated by CYP3A4 with a K_i value of 0.25 μM (Galetin et al 2002). MDR1 and CYP3A4 synergistically work to restrict absorption substrates for MDR1 and CYP3A4 in the intestine (Wacher et al 2001; Benet et al 2004). From these data, we should consider the dosing amount of substrates for MDR1 and CYP3A4 in patients treated with haloperidol.

Conclusion

Haloperidol is not a substrate but an inhibitor of MDR1. It is unlikely that MDR1 function affects haloperidol absorption and brain distribution and, thereby, the response to haloperidol. The intestinal absorption, not distribution into the brain, of MDR1 substrate drugs will be altered by the co-administration of haloperidol in the clinical setting, although further clinical studies are needed.

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