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Communication

Distribution of domperidone into the rat brain is increased by brain ischaemia or treatment with the P-glycoprotein inhibitor verapamil

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Abstract

Domperidone (DOM), a peripheral dopamine D2 receptor antagonist, is a substrate of P-glycoprotein (P-gp). Therefore, the transport of DOM into the brain may be restricted by P-gp function at the blood–brain barrier, and when the function of P-gp is impaired by ATP depletion under conditions of brain ischaemia (e.g. cerebral thrombosis), side-effects may be induced as a result of increased distribution of DOM into the brain. In this study, we investigated the effects of brain ischaemia and verapamil, a P-gp inhibitor, on the permeability coefficient–surface area product (PS values) of DOM across the blood–brain barrier by using an in-situ rat brain perfusion technique. The PS values of DOM were increased 3.4- and 3.6-fold after brain ischaemia for 10 and 20 min, respectively. Furthermore, co-administration of verapamil significantly increased the PS values of DOM by 42.6- and 43.3-fold in the normal and ischaemia groups, respectively. Brain vascular volume was not affected by ischaemia or verapamil. These results show that the transport of DOM into the brain is restricted by P-gp and that the brain distribution of DOM can be increased by cerebral ischaemia or co-administration of a P-gp inhibitor.

Introduction

Domperidone (DOM), a selective dopamine D2 receptor antagonist (Kohli et al 1983), possesses gastrointestinal effects and anti-emetic properties. Although DOM is a very potent inhibitor of dopamine D2 receptor with a K_D value of 0.26 nM (Hall & Wedel 1986) in-vitro, it rarely causes extrapyramidal side-effects in-vivo, unlike other dopamine D2 receptor antagonists such as metoclopramide (O'Shea 1980). The most plausible explanation for the lack of central nervous system (CNS) effects is that the transport of DOM into the brain is restricted by P-gp at the blood–brain barrier in-vivo. Indeed, DOM is a substrate of human MDR1-P-glycoprotein (P-gp) and mouse mdr1a-P-gp in-vitro (Schinkel et al 1996).

Recently, it was reported that concomitant administration of loperamide, a P-gp substrate and a well-tolerated stegnotic, and quinidine, a P-gp inhibitor, causes adverse CNS effects (Sadeque et al 2000). As P-gp is an ATP-dependent efflux transporter (Sakata et al 1994), its function may be impaired by ATP depletion resulting from brain ischaemia, and adverse CNS effects may appear as a result of increased distribution of DOM into the brain.

In fact, it was reported that DOM caused neuroleptic-like side-effects in *mdr1a* knockout mice (*mdr1a* (−/−)) (Schinkel et al 1996), which lack efflux transport of

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DOM from the brain by P-gp. However, there appeared to be no difference in brain distribution of DOM between *mdr1a* (−/−) and wild-type mice (Schinkel et al 1996). This apparent contradiction is explained by the fact that they analysed not only unchanged DOM, but also its metabolites (Schinkel et al 1996), which are formed rapidly (Heykants et al 1981).

In this study, we aimed to investigate the effects of brain ischaemia and verapamil, a P-gp inhibitor, on the uptake of unchanged DOM into the brain by using an in-situ brain perfusion technique (Takasato et al 1984), in which the influence of peripheral metabolism and plasma protein binding is avoided, and by using a high-performance liquid chromatography (HPLC) system (Yamamoto et al 1998), which can selectively determine unchanged DOM.

Materials and Methods

Animals and reagents

Male Wistar rats (7–8 weeks old, 200–250 g; Seac Yoshitomi, Ltd, Fukuoka, Japan) were used in the experiments. The rats were housed under controlled environmental conditions (general food and water were freely available) and treated according to the Guiding Principles for the Care and Use of Laboratory Animals approved by the Japanese Pharmacological Society.

DOM was supplied by Kyowa Hakko Kogyo Co., Ltd (Tokyo, Japan). Verapamil and Clear-sol I were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Pentobarbital sodium and Solvable were obtained from Dainippon Pharmaceutical Co., Ltd (Osaka, Japan) and Packard BioScience (Groningen, The Netherlands), respectively. All other chemicals used in the experiments were of analytic grade.

Surgical procedure of brain ischaemia and in-situ brain perfusion

Rats were anaesthetized with pentobarbital sodium (50 mg kg^{-1}, i.p.) and subjected to either ischaemic surgery (Pulsinelli & Brierley 1979) or sham operation. For ischaemic surgery, the occipital region of the head was incised and both vertebral arteries were coagulated by a coagulator (Janus Bipolar Coagulator J-45; Keisei Medical Industrial, Tokyo, Japan).

At 48 h after occlusion or incision, rats were anaesthetized with pentobarbital sodium (50 mg kg^{-1}, i.p.). Both of the common carotid arteries were ligated for 10 or 20 min in the ischaemia groups, or 20 min in the verapamil-treated ischaemic group. After this procedure, brain perfusion (Takasato et al 1984) was carried out as described below. The occipital and superior thyroid arteries were coagulated and cut, and the right pterygopalatine artery was ligated. Then a polyethylene tube (SP-10; Natume, Tokyo, Japan) filled with heparinized saline was inserted into the right external carotid artery. The right carotid artery was prepared for ligation by encircling the artery with surgical thread. The criteria for brain ischaemia were bilateral ptosis (Pulsinelli & Brierley 1979), raising the forelegs, and dark eyes.

Krebs-Henseleit buffer, consisting of (in mM) 118.0 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 25 $NaHCO_3$ and 2.5 $CaCl_2$, was used as perfusion fluid. Just before the experiment, 10 mM D-glucose, 2.5 $\mu g\ mL^{-1}$ DOM and 500 μM verapamil were added. The perfusate was oxygenated for 10 min with 95% O_2/5% CO_2, adjusted to pH 7.4 with 1 M HCl, and kept at 37°C. Immediately after completion of the surgical procedure, perfusion fluid was infused into the right external carotid artery at a constant rate of 4.98 mL min^{-1} (Takasato et al 1984) by an infusion pump (55-1111; Harvard Apparatus, MA, USA), and the right common carotid artery was immediately ligated. The perfusion was continued for 35 s (including 5 s for the perfusate to reach the brain) and terminated by decapitation. The right hemisphere was immediately removed and perfusion fluids were collected after decapitation. The brain samples and perfusion fluid samples were kept at −20°C and 4°C, respectively. The determination of DOM was performed within 24 h.

Determination of brain vascular volume and distribution of DOM

The brain vascular volume was measured by using [^{14}C]sucrose, according to the procedure previously reported (Murakami et al 2000). After perfusion, the right cerebral hemisphere was immediately excised, weighed and placed in a scintillation vial. In addition, a 20-μL sample of perfusate was placed in a scintillation vial and prepared for scintillation counting by addition of 15 mL of scintillation cocktail. The brain samples were shaken at 50°C in 1.5 mL Solvable for 2 h, and then treated with 200 μL of 30% H_2O_2. After digestion, the solution was neutralized by addition of 100 μL of 6 M HCl and prepared for scintillation counting by addition of 15 mL of scintillation cocktail (Clear-sol I). The radioactivity of the brain and perfusate samples was measured with a liquid scintillation counter (LS6500; Beckman Instrument, Inc., CA, USA).

The concentration of DOM in brain was measured by HPLC with fluorescence detection by use of the reported method (Yamamoto et al 1998) with minor modifications. Homogenates of brain samples were prepared in 9-fold vols (w/v) of ice-cold methanol. To 1 mL of each homogenate, 10 μL of propranolol (3 μg mL^{-1}) was added. The mixture was shaken with a vortex mixer for 60 s and centrifuged at 13000 g at 4°C for 10 min. The supernatant (600 μL) was transferred to another tube and evaporated to dryness under a nitrogen stream. The dried residue was dissolved in 200 μL of mobile phase, which consisted of 0.02 M phosphate buffer (pH 3.5) and methanol (55:45, v/v), and further centrifuged at 13000 g at 4°C for 2 min. Then, 100 μL of the supernatant was applied onto the HPLC system.

The HPLC system consisted of a pump (LC-10AD; Shimadzu, Kyoto, Japan) and a spectrofluorometric detector (RF550; Shimadzu) set at 282 nm (excitation) and 328 nm (emission). The column was Mightysil RP-8 GP (150 mm, 4.6 mm i.d., 5 μm particle size; Kanto Chemical Co., Ltd, Tokyo, Japan) and the guard cartridge was Cosmosil 5C18-MS-II (Nacalai Tesque, Inc., Kyoto, Japan). The detection limit for brain samples was 200 ng (g brain)$^{-1}$. The extraction recovery was evaluated by comparing the peak area with that obtained after the direct injection of a known amount of DOM.

Calculation of brain vascular volume, K_p values and PS values

The concentration of DOM in the brain assessed by the HPLC system includes the amount of DOM remaining in the brain vascular vessels. Therefore, the real brain concentration of DOM was estimated by applying equation 1:

$$C^*_{brain} = (C_{brain} - v.v. \times C_b) / 1 - v.v. \quad (1)$$

where C^*_{brain} is the real concentration of DOM in the brain (μg (g brain)$^{-1}$); C_{brain} is the concentration of DOM in the brain assessed by HPLC (μg (g brain)$^{-1}$); v.v. is the brain vascular volume of 0.013 mL (g brain)$^{-1}$ (Murakami et al 2000); and C_b is the concentration of perfusate (μg mL^{-1}).

We confirmed in the preliminary experiment that the K_p value (μL (g brain)$^{-1}$), the ratio of tissue to perfusate concentration, increased in proportion to the lapse of time up to 30 s (data not shown). Therefore, we obtain equations 2 and 3 (Takasato et al 1984):

$$K_p(t) = (C^*_{brain}(t)) / C_b \quad (2)$$

$$PS = -F_{pf} \times \ln (1 - (K_p(t))/ T \times F_{pf}) \quad (3)$$

where PS is the permeability coefficient–surface area product (μL s^{-1} (g brain)$^{-1}$); T is perfusion time (s); and F_{pf} is the cerebral perfusion rate of 0.129 (mL s^{-1} (g brain)$^{-1}$) calculated in our laboratory (Murakami et al 2000) using [^{14}C]diazepam, which is freely diffusible through the blood–brain barrier.

Data analysis

Analysis of variance followed by Bonferroni's test was performed to analyse the differences between the mean values. A value of $P < 0.05$ was considered statistically significant.

Results

Effects of brain ischaemia and verapamil on the brain vascular volume

The brain vascular volumes of the control, 10-min ischaemia, and 20-min ischaemia groups were 13.0±1.1, 13.6±1.6, and 11.9±1.3 μL (g brain)$^{-1}$, respectively. Those of the non-ischaemia group and 20-min ischaemia group in the presence of verapamil were 9.9±1.2 and 11.3±0.8 μL (g brain)$^{-1}$, respectively (mean±s.e., n = 3–9). There were no significant differences in brain vascular volume among these groups.

Effects of brain ischaemia and verapamil on K_p values and PS values

The K_p value and PS value of the control group were 85.1±6.5 and 2.9±0.2 μL s^{-1} (g brain)$^{-1}$, respectively (Table 1). The PS values of the 10-min ischaemia and 20-min ischaemia were 10.0±1.0 and 10.6±2.8 μL s^{-1} (g brain)$^{-1}$, respectively (Table 1). The PS values of the 10-min ischaemia and 20-min ischaemia

Table 1 Effects of brain ischaemia and co-administration of verapamil on the permeability coefficient–surface area product (PS values) of domperidone.

Group	PS values (μL s^{-1} (g brain)$^{-1}$)
Control (n = 4)	2.9±0.2
10-min ischaemia (n = 3)	10.0±1.0*
20-min ischaemia (n = 3)	10.6±2.8*
Verapamil (n = 3)	127.1±15.2*
Verapamil +20-min ischaemia (n = 3)	129.4±8.7*

Each value represents the mean±s.e. *$P < 0.01$ significantly different compared with control (analysis of variance and Bonferroni's test).

groups were significantly increased by 3.4- and 3.6-fold over the control value, respectively. Further, the PS values of DOM in the presence of verapamil without ischaemia and with 20-min ischaemia were 127.1±15.2 and 129.4±8.7 μL s^{-1} (g brain)$^{-1}$, respectively (mean±s.e., n = 3–4) (Table 1). In the presence of verapamil, ischaemia did not significantly alter the PS value of DOM. The extraction recovery of DOM was 88.6±6.6% (mean±s.e.).

Discussion

DOM, a peripherally acting selective dopamine D2 receptor antagonist, does not readily cross the blood–brain barrier. It has been reported that quinidine, a P-gp inhibitor, evoked an adverse CNS reaction to loperamide, conceivably via an increase in the brain distribution of loperamide (Sadeque et al 2000). Similarly, distribution of DOM into the brain may be increased in-vivo when the extruding function of P-gp is impaired.

In the present study, brain ischaemia increased the brain distribution of DOM approximately 3-fold without affecting the brain vascular volume measured with [^{14}C]sucrose, showing that the integrity of the blood–brain barrier was well preserved. Therefore, the elevated brain distribution of DOM is presumably attributable to the impaired function of P-gp, an ATP-dependent transporter, resulting from the depletion of ATP during ischaemia. The brain distribution of DOM was increased over 40-fold by 500 μM verapamil, which has been shown to completely inhibit the function of P-gp (Chikhale et al 1995). The above results demonstrate that the brain distribution of DOM is restricted by P-gp in-vivo.

Although these increases in the brain distribution of DOM were statistically significant by analysis of variance followed by Bonferroni's test, we also performed a non-parametric comparator test (Kruskal–Wallis test). Again, there was a significant difference between the treatments ($P = 0.0099$). In the subsequent Dunn's post test, the effect of verapamil remained statistically significant ($P < 0.05$). However, the effect of ischaemia did not attain statistical significance. Nevertheless, considering that brain ischaemia increased the brain distribution of DOM approximately 3-fold, its influence cannot be ignored.

It is possible that the function of other ATP-dependent efflux transporters (ABC transporters) was also impaired by brain ischaemia. Moreover, the contributions of other transporters to the distribution of DOM into the brain remain uncertain. The degree of decrease in the ATP content is also uncertain because no study has been performed to analyse the ATP content of brain vascular endothelial cells, although the ATP content in the whole brain certainly decreases after ischaemia (Pulsinelli & Duffy 1983). One reason why the PS values in the ischaemia groups were much lower than that of the verapamil-treated group in this study may be that significant amounts of ATP remained in microvessel endothelial cells, allowing P-gp to partially maintain its barrier function.

Multidrug resistance-associated proteins (MRPs), which belong to the ABC transporter family, may also participate in the transport of DOM across the blood–brain barrier. Seven homologues of the transporters, MRP1–MRP7, have been identified to date. MRP1 and MRP2 were found to be expressed in rat brain vascular vessels (Regina et al 1998; Miller et al 2000). However, it remains unclear whether DOM is a substrate of MRP1 and/or MRP2. With regard to MRP1, the cerebrovascular expression is low (Regina et al 1998), and its localization and direction of transport are established. On the other hand, MRP2 is not inhibited by verapamil (Miller et al 2000). Therefore, we considered that the efflux transport of DOM is primarily attributable to the function of P-gp.

In conclusion, DOM was confirmed to be a substrate of P-gp in-vivo, and this may be the reason why the drug can act as a peripherally selective dopamine D2 receptor antagonist without adverse CNS effects. Brain ischaemia and verapamil were each found to increase the brain distribution of DOM in this study. Therefore, factors that increase the distribution of DOM into the brain (e.g. cerebral hypoxia, cerebrovascular disease, concomitant treatment with P-gp inhibitors) may result in the appearance of adverse CNS effects of DOM in the clinical setting.

References

Chikhale, E. G., Burton, P. S., Borchardt, R. T. (1995) The effect of verapamil on the transport of peptides across the blood–brain barrier in rats: kinetic evidence for an apically polarized efflux mechanism. *J. Pharmacol. Exp. Ther.* **273**: 298–303

Hall, H., Wedel, I. (1986) Comparisons between the in vitro binding of two substituted benzamides and two butyrophenones to domamine-D2 receptors in the rat striatum. *Acta. Pharmacol. Toxicol.* **58**: 368–373

Heykants, J., Knaeps, A., Meuldermans, W., Michiels, M. (1981) On the pharmacokinetics of domperidone in animals and man. I. *Eur. J. Drug Metab. Pharmacokinet.* **6**: 27–36

Kohli, J. D., Glock, D., Goldberg, L. I. (1983) Selective DA2 versus antagonist activity of domperidone in the periphery. *Eur. J. Pharmacol.* **89**: 137–141

Miller, D. S., Nobmann, S. N., Gutmann, H., Toeroek, M., Drewe, J., Fricker, G. (2000) Xenobiotic transport across isolated brain

microvessels studied by confocal microscopy. *Mol. Pharmacol.* **58**: 1357–1367

Murakami, H., Takanaga, H., Matsuo, H., Ohtani, H., Sawada, Y. (2000) Comparison of blood–brain barrier permeability in mice and rats using in situ brain perfusion technique. *Am. J. Physiol.* **279**: H1022–H1028

O'Shea, M. (1980) A double-blind comparison of domperidone and metoclopramide in the treatment of postprandial dyspeptic symptoms. *Curr. Ther. Res.* **28**: 367–370

Pulsinelli, W. A., Brierley, J. B. (1979) A new model of bilateral hemispheric ischemia in the unanesthetized rat. *Stroke* **10**: 267–272

Pulsinelli, W. A., Duffy, T. E. (1983) Regional energy balance in rat brain after transient forebrain ischemia. *J. Neurochem.* **40**: 1500–1503

Regina, A., Koman, A., Piciotti, M., Hafny, B. E., Center, M. S., Bergmann, R., Couraud, P., Roux, F. (1998) Mrp1 multidrug resistance-associated protein and P-glycoprotein expression in rat brain microvessel endothelial cells. *J. Neurochem.* **71**: 705–715

Sadeque, A. J. M., Wandel, C., He, H., Shah, S., Wood, A. J. J. (2000) Increased drug delivery to the brain by P-glycoprotein inhibition. *Clin. Pharmacol. Ther.* **63**: 231–237

Sakata, A., Tamai, I., Kawazu, K., Deguchi, Y., Ohnishi, T., Saheki, A., Tsuji, A. (1994) In vivo evidence for ATP-dependent and P-glycoprotein-mediated transport of cyclosporin A at the blood–brain barrier. *Biochem. Pharmacol.* **48**: 1989–1992

Schinkel, A. H., Wagenaar, E., Mol, C. A. A., Deemeter, L. V. (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

Takasato, Y., Rapoport, S. I., Smith, Q. R. (1984) An in situ brain perfusion technique to study cerebrovascular transport in the rat. *Am. J. Physiol.* **247**: H484–H493

Yamamoto, K., Hagino, M., Kotaki, H., Iga, T. (1998) Quantitative determination of domperidone in rat plasma by high-performance liquid chromatography with fluorescence detection. *J. Chromatogr. B.* **720**: 251–255