JPP Journal of Pharmacy and Pharmacology
ROYAL PHARMACEUTICAL SOCIETY

JPP Journal of Pharmacy and Pharmacology

JPP 2011, 63: 58–64

Received April 5, 2010
Accepted July 5, 2010
DOI
10.1111/j.2042-7158.2010.01176.x
ISSN 0022-3573

Research Paper

Evaluation of the pharmacokinetic interaction of midazolam with ursodeoxycholic acid, ketoconazole and dexamethasone by brain benzodiazepine receptor occupancy

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Abstract

Objectives To clarify whether alterations in midazolam pharmacokinetics resulting from changes in cytochrome P450 3A (CYP3A) activity lead to changes in its pharmacodynamic effects, benzodiazepine receptor occupancy was measured in the brain of rats after oral administration of midazolam.

Methods Receptor occupancy was measured by radioligand binding assay in rats pretreated with ursodeoxycholic acid (UDCA), ketoconazole and dexamethasone, and the plasma concentration of midazolam was simultaneously determined.

Key findings There was a significant increase in the apparent dissociation constant and decrease in the maximum number of binding sites for specific [^{3}H]flunitrazepam binding after oral administration of midazolam at pharmacologically relevant doses, suggesting that midazolam binds significantly to brain benzodiazepine receptors. Pretreatment with UDCA significantly enhanced the binding. This correlated well with significant enhancement by UDCA of the plasma midazolam concentration. The brain benzodiazepine receptor binding of oral midazolam was significantly enhanced by pretreatment with ketoconazole, a potent inhibitor of CYP3A, whereas it was significantly reduced by treatment with dexamethasone, an inducer of this enzyme. These effects paralleled changes in the plasma concentration of midazolam.

Conclusions The results indicate that pharmacokinetic changes such as altered CYP3A activity significantly influence the pharmacodynamic effect of midazolam by affecting occupancy of benzodiazepine receptors in the brain. They also suggest in-vivo or ex-vivo time-dependent measurements of receptor occupancy by radioligand binding assay to be a tool for elucidating the pharmacokinetic interaction of benzodiazepines with other agents in pre-clinical and clinical evaluations.

Keywords benzodiazepine receptor; dexamethasone; ketoconazole; midazolam; ursodeoxycholic acid

Introduction

The pharmacological effects of drugs are determined by both pharmacokinetic and pharmacodynamic processes. Pharmacokinetics includes the absorption, distribution, excretion and metabolism of drugs, while pharmacodynamics includes receptor occupancy, signal transduction and homeostatic mechanisms.[1] To evaluate the efficacy and safety of drugs, it is important to clarify the relationship between pharmacokinetics and pharmacodynamics.

The benzodiazepines are psychoactive drugs with broad therapeutic applications as anxiolytics, anticonvulsants, muscle relaxants and hypnotics,[2] several of which, such as midazolam, undergo extensive hepatic and gastrointestinal metabolism by cytochrome P450 3A (CYP3A) isoforms.[3–5] Thus, midazolam is commonly used for monitoring CYP3A activity in pre-clinical and clinical evaluations.[6] There are pre-clinical and clinical studies to indicate that the pharmacokinetics of midazolam is significantly influenced by changes in CYP3A activity.[6–8] However, it has not been clarified whether the

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pharmacokinetic alteration of midazolam is responsible for significant pharmacodynamic effects in the central nervous system. The benzodiazepines exert most of their pharmacological actions thorough specific interactions with benzodiazepine receptors.[9,10] Our previous studies have demonstrated in-vivo or ex-vivo receptor binding of drugs to be very useful in predicting or characterizing pharmacological specificity in relation to pharmacokinetics for preclinical and clinical evaluations.[11,12]

Ursodeoxycholic acid (UDCA) is a component of human and rat bile, and is present in very low amounts as secondary bile acid, formed by 7β-epimerization of the primary bile acid chenodeoxycholic acid.[13] UDCA has been used to prevent cholestasis by dissolving gallstones in the treatment of some cholestatic liver diseases such as primary biliary cirrhosis.[14–16] Studies have indicated that UDCA significantly affects hepatic CYP3A activity in human primary cultured hepatocytes,[17] mouse liver[18] and patients with cholesterol gallstone disease.[19] More recently, we have shown that a single oral administration of UDCA to rats led to a significant increase in CYP3A1 and CYP3A2 activity in the liver, suggesting an induction of CYP3A expression by UDCA, possibly through the activation of nuclear receptors such as pregnane X receptor. On the other hand, the bioavailability of midazolam was elevated by a single oral administration of UDCA, possibly because of the enhancement of midazolam absorption by the incorporation of midazolam into bile micelles and the downregulation of the CYP3A9 mRNA level in the small intestine.[6] Thus, UDCA may play a different role in the regulation of CYP3A isoforms in the rat. In either case, co-administration of UDCA with CYP3A substrates may influence not only their pharmacokinetic profiles but also their pharmacodynamic effects. However, the effect of UDCA on the pharmacodynamics of CYP3A metabolized drugs has yet to be examined.

The aim of the present study was to determine whether alterations in midazolam pharmacokinetics resulting from changes in CYP3A activity lead to changes in its pharmacodynamic effects in the brain by measuring benzodiazepine receptor occupancy. The receptor occupancy by midazolam was measured by radioligand binding assay in the brain of rats pretreated with UDCA. In addition, the effects of ketoconazole and dexamethasone (a typical inhibitor and inducer of CYP3A, respectively) on the receptor occupancy by midazolam were investigated.[20,21] The plasma concentration of midazolam in these rats was also determined.

Materials and Methods

Materials

[Methyl-^{3}H]flunitrazepam ([^{3}H]flunitrazepam, 3.01 TBq/mmol) was purchased from PerkinElmer Life Sciences, Inc (Boston, MA, USA). UDCA and midazolam were purchased from Wako Pure Chemical Ltd (Osaka, Japan). 1′-Hydroxymidazolam and 4-hydroxymidazolam were obtained from UFC Ltd (Manchester, UK). Diazepam and flunitrazepam were purchased from Sigma Aldrich (St Louis, MO, USA). All other reagents were of the highest purity available from commercial sources.

Animals

Male Sprague-Dawley rats, 8 weeks old (Japan SLC Inc., Shizuoka, Japan) were housed under a 12-h light/dark cycle in a room with controlled temperature and humidity. Food and water were provided *ad libitum*. This study was conducted in accordance with the Guidelines for Care and Use of Laboratory Animals adopted by the US National Institutes of Health. All the animal experiments were approved by the Institutional Animal Care and Use Committee of the University of Shizuoka.

Oral administration of drugs

Rats fasted overnight were treated orally with UDCA (300 mg/kg) or dexamethasone (80 mg/kg) 24 h before, or ketoconazole (10 mg/kg) 1 h before, midazolam treatment. The drugs were dissolved in 1% carboxymethylcellulose as a solvent. Control rats received the vehicle only. The rats then received an oral administration of midazolam (15 mg/kg), and 15, 60 and 180 min later the benzodiazepine receptor binding was measured. For the measurement of the plasma concentration of midazolam, blood was taken from a vein 5–180 min after the administration.

Radioligand binding assay

Rats received vehicle or midazolam with or without pretreatment with UDCA, dexamethazone and ketoconazole; they were then killed by decapitation and the brain was immediately removed. The tissue was homogenized by a Kinematica Polytron homogenizer (Kinematica Inc, Bohemia, NY, USA) in 39 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.4). The homogenate was centrifuged at 48 000*g* for 15 min at 4°C. The pellet was resuspended in the same buffer for the binding assay. The radioligand binding assay for benzodiazepine receptors was performed using [^{3}H]flunitrazepam as described by Regan *et al*.[22] All experimental steps were conducted quickly. In saturation experiments, brain homogenate (65–88 μg protein/assay) was incubated with various concentrations of [^{3}H]flunitrazepam (0.03–3.0 nM) in 50 mM Tris-HCl buffer (pH 7.4). In the competition experiment, tissue homogenate was incubated with [^{3}H]flunitrazepam (0.3 nM) in the presence of midazolam, 1′-hydroxymidazolam, 4-hydroxymidazolam, diazepam, flunitrazepam, UDCA, dexamethasone and ketoconazole. Incubation was carried out for 90 min at 4°C, and the reaction was terminated by rapid filtration (Cell Harvester; Brandel Co, Gaithersburg, MD, USA) through Whatman GF/B glass filters (Whatman, Japan K.K., Tokyo, Japan). The filters were then rinsed three times with 3 ml of ice-cold 50 mM Na^+/K^+ phosphate buffer (pH 7.4). Tissue-bound radioactivity was extracted from the filters overnight in scintillation fluid and the radioactivity was determined by a liquid scintillation counter. Specific binding of [^{3}H]flunitrazepam was determined experimentally from the difference between counts in the absence and presence of 10 μM diazepam. Protein concentrations were measured using a BCA Protein Assay Kit (Pierce Chemical, Rockford, IL, USA).

Determination of midazolam

The concentration of midazolam was measured by high-performance liquid chromatography (HPLC) as described

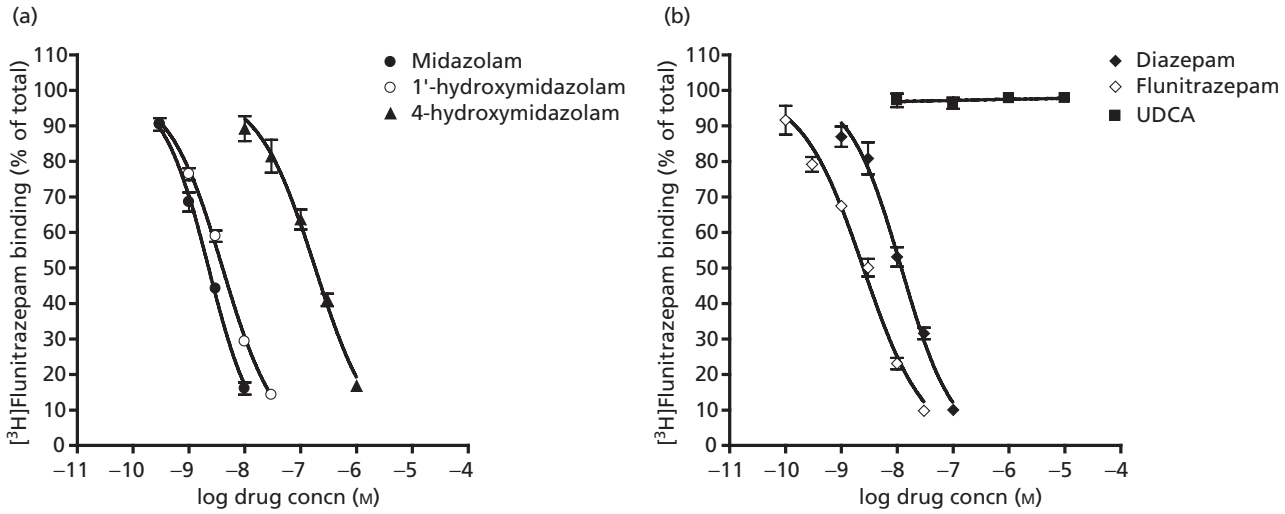

Figure 1 Competitive inhibition of specific [^{3}H]flunitrazepam binding in rat brain homogenate. [^{3}H]Flunitrazepam binding was measured in the presence of midazolam (0.3–10 nM), 1′-hydroxymidazolam (0.3–30 nM), 4-hydroxymidazolam (10 nM–1 μM), diazepam (1–100 nM), flunitrazepam (0.1–30 nM) and ursodeoxycholic acid (UDCA; 10 nM–10 μM). Each point represents mean ± SE for four to five rats.

previously but with minor modifications.[23] Plasma samples (0.1 ml) containing nitrazepam (1 μg) as an internal standard were added to 1 ml of 1 M sodium phosphate buffer (pH 9.0) and 3 ml of diethyl ether/methylene chloride (7 : 3). After being shaken, the mixture was centrifuged at 3000*g* for 5 min. The organic phase was transferred to a glass tube and evaporated under a stream of nitrogen gas at 40°C in a water bath. The dry residue was dissolved in 200 μl of mobile phase (acetonitrile/methanol/0.1% sodium acetate/tethrahydrofuran, 14 : 24 : 60 : 2, v/v). HPLC was performed using an HPLC analytical column (CAPCELL PAK C18 MGII, 4.6 mm × 150 mm, 5 μm particle size; Shiseido Co. Ltd, Tokyo, Japan) at 40°C and a flow rate of 1.0 ml/min. The sample was detected at a wavelength of 254 nm. The limit of quantification for midazolam was 50 nM in plasma. The inter-assay coefficients of variation for this method were less than 14.0%.

Statistical analysis

The apparent dissociation constant (K_d) and maximum number of binding sites (B_{max}) for [^{3}H]flunitrazepam were estimated by a nonlinear regression analysis of the saturation data using GraphPad Prism 4.03 (GraphPad Software, Inc., San Diego, CA, USA). The ability of non-labelled agents to inhibit the specific binding of the radioligand was estimated from IC50 values, defined as the molar concentration of agents necessary to displace 50% of specific binding (determined by log probit analysis). The inhibition constant, K_i, was calculated from the equation $K_i = IC50 / (1 + L / K_d)$, where L is the concentration of [^{3}H]flunitrazepam.

Statistical analysis of the data was performed using the Student's *t*-test or a one-way analysis of variance, followed by Dunnett's test for multiple comparison. The data were expressed as mean ± SE. Statistical significance was accepted at $P < 0.05$.

Results

Inhibitory effects on specific [^{3}H]flunitrazepam binding

Specific binding of [^{3}H]flunitrazepam (0.03–3.0 nM) to crude membranes from the rat brain was saturable, reaching a plateau at around 2.5 nM. The estimated K_d and B_{max} values were 1.69 ± 0.04 nM and 2365 ± 45 fmol/mg protein, respectively. As shown in Figure 1, midazolam (0.3–10 nM), 1′-hydroxymidazolam (0.3–30 nM), 4-hydroxymidazolam (10–1000 nM), diazepam (1–100 nM) and flunitrazepam (0.1–30 nM) inhibited [^{3}H]flunitrazepam binding (0.3 nM) in the rat brain in a concentration-dependent manner. From the K_i values, the order of affinity was midazolam, flunitrazepam > 1′-hydroxymidazolam > diazepam >> 4-hydroxymidazolam (Table 1). Thus, midazolam displayed about 76-fold greater affinity for [^{3}H]flunitrazepam than 4-hydroxymidazolam. UDCA (0.01–10 μM), ketoconazole (0.1–1 μM) and dexamethasone (0.01–1 μM) exerted little inhibitory effect on brain [^{3}H]flunitrazepam binding.

Effect of pretreatment with UDCA, ketoconazole and dexamethasone on the binding of midazolam in brain benzodiazepine receptors

The effect of oral administration of midazolam on benzodiazepine receptor binding in the rat brain was examined. As shown in Table 2, there was significant (1.50 and 1.43-fold, respectively) increase in the K_d values for [^{3}H]flunitrazepam binding in the rat brain 15 and 60 min after the oral administration of midazolam (15 mg/kg), compared with control (vehicle-treated) rats. Simultaneously, the B_{max} values for [^{3}H]flunitrazepam were slightly (14 and 20%, respectively) but significantly decreased by the midazolam treatment. The changes were no longer observed at 180 min. The oral administration of a higher dose (30 mg/kg) of midazolam further

Table 1 Inhibition constants and Hill coefficients for competitive inhibition of specific [^{3}H]flunitrazepam binding in rat brain homogenate

Drug	K_i (nM)	nH
Midazolam (0.3–10 nM)	1.96 ± 0.19	1.13 ± 0.05
1′-Hydroxymidazolam (0.3–30 nM)	3.49 ± 0.26	0.84 ± 0.02
4-Hydroxymidazolam (10–1000 nM)	149 ± 21	0.83 ± 0.07
Diazepam (1–100 nM)	10.1 ± 1.8	0.98 ± 0.08
Flunitrazepam (0.1–30 nM)	1.94 ± 0.23	0.75 ± 0.02
Ursodeoxycholic acid (0.01–10 μM)	NC	NC
Dexamethasone (0.01–1 μM)	NC	NC
Ketoconazole (0.1–1 μM)	NC	NC

K_i, inhibition constant; nH, Hill coefficient; NC, not calculated. [^{3}H]Flunitrazepam binding in rat brain was measured in the presence of midazolam, 1′-hydroxymidazolam, 4-hydroxymidazolam, diazepam, flunitrazepam, ursodeoxycholic acid, dexamethasone and ketoconazole. Each value represents mean ± SE of four to five determinations.

increased (2.38-fold at 60 min) the K_d values for [^{3}H]flunitrazepam binding.

We examined the effects of pretreatment with UDCA, dexamethasone or ketoconazole on the binding of midazolam to benzodiazepine receptors in the rat brain. The pretreatment with UDCA (300 mg/kg, p.o.) significantly enhanced (3.33 and 2.11-fold, respectively) the K_d values for specific [^{3}H]flunitrazepam binding at 15 and 60 min after oral administration of midazolam (15 mg/kg) (Table 2). The increase was significantly greater than the enhancement in midazolam-treated rats without UDCA pretreatment. Similarly, the K_d value for brain [^{3}H]flunitrazepam binding 60 min after the administration of midazolam (15 mg/kg) was significantly (2.96-fold) increased by pretreatment with ketoconazole (10 mg/kg, p.o.) and decreased by pretreatment with dexamethasone (80 mg/kg, p.o.) (Table 2). Thus, the enhancement by midazolam of the K_d values of ketoconazole-pretreated rats was statistically significant compared with that without ketoconazole pretreatment, and the enhancement of the K_d values by midazolam was almost completely inhibited by dexamethasone pretreatment. The B_{max} values for brain [^{3}H]flunitrazepam binding 60 min after the oral administration of midazolam (15 mg/kg) in UDCA, ketoconazole or dexamethasone-pretreated rats were not significantly different compared with the values in control rats.

Relationship between the increase in K_d values for [^{3}H]flunitrazepam and plasma concentration of midazolam

Figures 2 and 3 illustrate the enhancement of K_d values for brain [^{3}H]flunitrazepam binding and plasma drug concentration in rats after oral administration of midazolam. As shown in Figure 2, the UDCA (300 mg/kg, p.o.) pretreatment increased the plasma concentration of midazolam at 5–180 min after the administration of midazolam (15 mg/kg). In these rats, the increase at 15 and 60 min correlated with the enhancement of K_d values for specific [^{3}H]flunitrazepam binding in the rat brain, although the correlation was on only two time points. The plasma concentration of midazolam in rats 60 min after oral administration of this agent (15 mg/kg) was significantly increased by pretreatment with ketoconazole (10 mg/kg, p.o.) as well as UDCA, whereas it was markedly decreased by dexamethasone (80 mg/kg, p.o.) (Figure 3). The enhancement by UDCA and ketoconazole correlated closely with the significant increase by both agents of K_d values for brain [^{3}H]flunitrazepam binding in midazolam-treated rats.

Discussion

The effect of pretreatment with UDCA, ketoconazole and dexamethasone on the binding of midazolam to benzodiazepine receptors in the rat brain was examined. Specific [^{3}H]flunitrazepam binding in the rat brain was saturable and of high affinity, and displayed pharmacological specificity that characterized selective binding of benzodiazepine receptors (Table 1). The results showed that the receptor binding affinity of 1′-hydroxymidazolam compared with the parent compound was slightly weaker, while that of 4-hydroxymidazolam was markedly (about 76-fold) weaker. A similar observation was reported previously in the rat brain.[24] It was also demonstrated that 4-hydroxymidazolam had about half the potency of 1′-hydroxymidazolam in behavioural pharmacological tests in mice.[25] In addition, Mandema *et al*. [26] evaluated the pharmacological effects of midazolam and 1′-hydroxymidazolam by monitoring electroencephalography and saccadic eye movement in humans. In that study, the pharmacological action of 1′-hydroxymidazolam was weaker than that of midazolam. Our data support these functional observations. UDCA, ketoconazole and dexamethasone displayed little direct binding of benzodiazepine receptors.

There was a significant increase in K_d values for specific [^{3}H]flunitrazepam binding in the rat brain 15 and 60 min after the oral administration of midazolam at pharmacologically relevant doses (15 or 30 mg/kg) compared with control rats. Given that an increase in the K_d values for a radioligand in drug-pretreated tissues in the radioreceptor assay usually indicates that the agent is competing with the radioligand for the same binding sites,[11,12,27] the current result strongly suggests that orally administered midazolam binds significantly to benzodiazepine receptors in the rat brain. This was also supported by a significant decrease in B_{max} values for [^{3}H]flunitrazepam 15 and 60 min after the administration of midazolam. Furthermore, the time course of the increase in K_d values for [^{3}H]flunitrazepam appeared to correlate with that of the plasma concentration of midazolam (Figure 2), suggesting a close relationship between the pharmacokinetics and pharmacodynamics of midazolam.

Pretreatment with UDCA significantly promoted the binding of benzodiazepine receptors in the rat brain after oral administration of midazolam (Table 2). This correlated well with the significant enhancement by UDCA of plasma midazolam concentrations (Figures 2 and 3). Bile acids enhance the absorption of lipophilic compounds by the incorporation of midazolam into bile micelles.[28] Moreover, we previously indicated that a single oral treatment with UDCA led to a significant decrease in the level of CYP3A9 mRNA in the small intestine.[6] Thus, it is likely that UDCA treatment increases the oral bioavailability of midazolam, resulting in a higher concentration of this agent in the brain and leading

Table 2 Effect of oral administration of midazolam on the apparent dissociation constant and maximum number of binding sites of [^{3}H]flunitrazepam binding in rat brain

Drug administration	Time (min)	K_d (nM)	B_{max} (fmol/mg protein)
Control (vehicle)		1.69 ± 0.04 (1.00)	2365 ± 45 (1.00)
Vehicle + midazolam (15 mg/kg)	15	2.55 ± 0.17 (1.50)**	2024 ± 79 (0.86)*
	60	2.41 ± 0.04 (1.43)**	1896 ± 55 (0.80)**
	180	1.70 ± 0.04	2149 ± 65
Vehicle + midazolam (30 mg/kg)	60	4.02 ± 0.25 (2.38)**,††	2066 ± 139
Ursodeoxycholic acid + midazolam (15 mg/kg)	15	5.64 ± 0.65 (3.33)**,††	2030 ± 120
	60	3.56 ± 0.14 (2.10)**,††	2199 ± 87
	180	1.89 ± 0.12	2212 ± 132
Ketoconazole + midazolam (15 mg/kg)	60	5.01 ± 0.54 (2.96)**,†	2010 ± 165
Dexamethasone + midazolam (15 mg/kg)	60	1.94 ± 0.16	2145 ± 148

B_{max}, maximum number of binding sites; K_d, apparent dissociation constant. Each value represents the mean ± SE of four to eight rats. Values in parentheses represent the fold-increase in K_d and B_{max} values relative to control. *$P < 0.05$, **$P < 0.01$, significantly different compared with control values; †$P < 0.05$, ††$P < 0.01$, significantly different compared with values at the same time after oral administration of vehicle + midazolam (15 mg/kg) (Student's *t*-test with Bonferroni–Holm test).

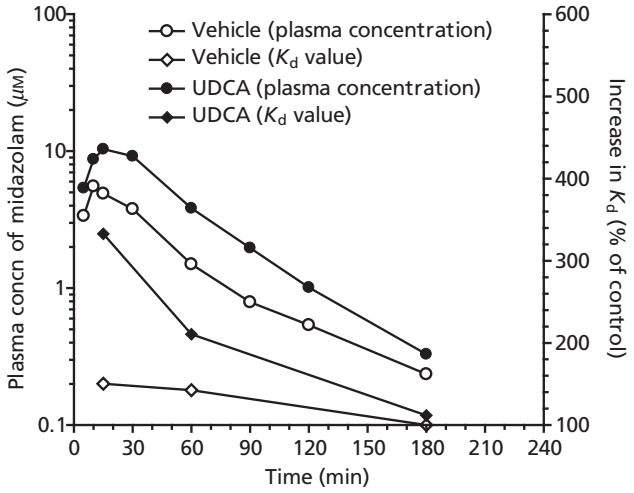

Figure 2 Influence of ursodeoxychoilc acid on midazolam pharmacokinetics and pharmacodynamics. Plasma concentration–time curves of midazolam and brain benzodiazepine receptor binding (increase in K_d values for specific [^{3}H]flunitrazepam binding) after the oral administration of midazolam (15 mg/kg) following a single oral administration of ursodeoxycholic acid (UDCA; 300 mg/kg) and vehicle in rats. Each point represents the mean for four to seven rats.

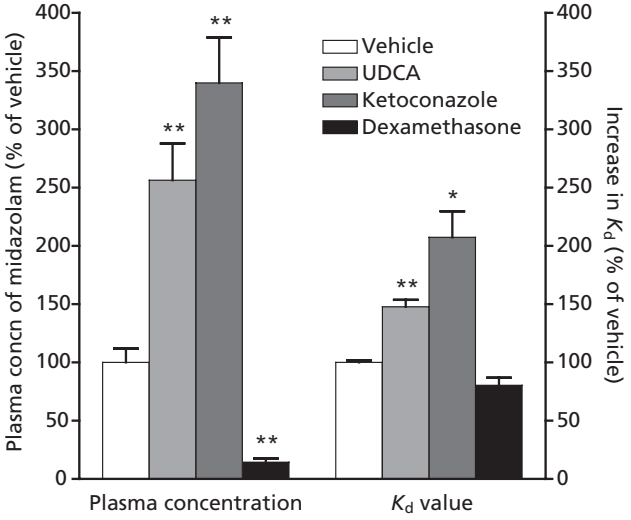

Figure 3 Alteration of midazolam pharmacokinetics and pharmacodynamics. Plasma concentration and brain benzodiazepine receptor binding (increase in K_d values for specific [^{3}H]flunitrazepam binding) of midazolam at 60 min after its oral administration (15 mg/kg) following a single oral administration of vehicle, ursodeoxycholic acid (UDCA; 300 mg/kg), dexamethasone (80 mg/kg) and ketoconazole (10 mg/kg) in rats. Each point represents the mean ± SE for four to seven rats. *$P < 0.05$, **$P < 0.01$, significant difference compared with control (vehicle) values by Student's *t*-test (plasma concentration) and Student's *t*-test with the Bonferroni–Holm test (K_d value).

to an enhancement of benzodiazepine receptor occupancy. However, it has been shown that repeated administration of UDCA for 7 days abolished the alteration of midazolam pharmacokinetics that was observed with a single treatment of UDCA,[6] and so further studies will be required to investigate the effect of chronic treatment of UDCA on the pharmacodynamics of midazolam in rats.

CYP3A isoforms convert midazolam to 1′-hydroxymidazolam and 4-hydroxymidazolam in humans and rodents.[29] Among the CYP3A subfamily in rats, CYP3A1 and CYP3A2 are predominantly expressed in the liver, and CYP3A9 is highly expressed in the intestine.[30,31] Although 1′-hydroxylation is the major metabolic pathway in humans, midazolam is mainly metabolized to 4-hydroxymidazolam rather than 1′-hydroxymidazolam by CYP3A1, CYP3A2 and CYP3A9 in rats.[32] Since the pharmacological activity of 4-hydroxymidazolam is less potent than that of midazolam and 1′-hydroxymidazolam,[24] the in-vivo pharmacodynamic effect of midazolam may not take into account the influence of its metabolites. In other words, the alteration of midazolam pharmacokinetics by modulating CYP3A activity in rats could be directly responsible for its pharmacodynamics. In the present study, the significance of changes in CYP3A activity on the pharmacodynamics of midazolam was examined in rats after oral administration of midazolam following pretreatment with ketoconazole and dexamethasone. Ketoconazole inhibits

CYP3A with a K_i in the nanomolar range in rat and human liver microsomes.[8,20] Previous investigation showed that the C_{max} value of ketoconazole, administered intraperitonealy (10 mg/kg), reached approximately 9 μg/ml (17 μM) in rats.[33] It is therefore likely that the dose of ketoconazole used in the present study produced almost complete inhibition of midazolam metabolism *in vivo*. The binding of brain benzodiazepine receptors by midazolam was significantly enhanced by pretreatment with ketoconazole, which paralleled the increase in the plasma concentration of midazolam (Figure 3). In line with our results, Kotegawa *et al*.[8] showed that the coadministration of midazolam and ketoconazole produced a rightward shift in the relationship of concentration versus electroencephalography effect of midazolam, and nearly doubled the EC50 value of midazolam in rats. Thus, our findings further confirmed the influence of ketoconazole on the pharmacodynamic effect of midazolam with regard to benzodiazepine receptor binding in the brain.

Dexamethasone is a potent inducer and substrate of CYP3A.[21,34] Previous reports demonstrated that dexamethasone markedly increased the levels of mRNA for CYP3A1 and CYP3A2 in the liver, and CYP3A9 in the small intestine via pregnane X receptors and glucocorticoid receptors.[6,31] Dexamethasone, which markedly reduced the plasma concentration of midazolam, caused a decrease in the brain receptor binding due to the oral midazolam. This result is in agreement with the previous finding that dexamethasone shortened the sleeping time induced by midazolam, in addition to significant decreases in the plasma concentration of midazolam in rats.[35] Taken together, these results indicate that pharmacokinetic changes by the inhibition or induction of CYP3A activity may significantly influence the pharmacodynamic effect of midazolam in the central nervous system. The concentrations of midazolam in the plasma and brain after systemic administration may be significantly influenced by the transporter expressed in the intestine, liver, kidney and/or brain.[36] Thus, the present measurement of brain benzodiazepine receptor binding activity could be used to evaluate the agents affecting midazolam pharmacokinetics via not only CYP enzymes but also transporters.

Conclusions

This is the first in-vivo study to indicate that pharmacokinetic changes of midazolam by UDCA, ketoconazole and dexamethazone may significantly influence central nervous system effects of the drug by modulating the occupancy of brain benzodiazepine receptors in the brain. The results may reveal in-vivo or ex-vivo time-dependent measurement of brain receptor occupancy using the radioligand binding assay to be a tool for elucidating the pharmacokinetic interaction of benzodiazepines with other agents in pre-clinical and clinical evaluations.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Acknowledgements

The authors thank Ms Misato Sugiura and Ms Chikoto Ohta for excellent technical assistance.

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