《 Original Article 》

Hypnotic doses of benzodiazepine-$GABA_A$ receptor agonists may not only be associated with the intrinsic receptor binding affinity, but also with pharmacokinetic parameters of drug exposure in the brain

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Although the difference between the lowest and highest approved oral doses of benzodiazepine (BZD) hypnotics is almost 30-fold, it remains unclear whether the intrinsic potency of the BZD-gamma-aminobutyric acid type A ($GABA_A$) receptor agonist is solely responsible for this variability. We conducted the present study to examine this issue. In previous studies, we determined the half-maximum inhibitory concentration of the $GABA_A$ receptor-chloride ion channel complex in human brain (hIC_{50}) for seven BZDs and two non-BZD drugs with agonistic action on $GABA_A$ receptors. Hypnotic doses of these drugs and the biomarkers of brain exposure of these drugs [plasma concentration-time curve (AUC) and maximum drug concentration (C_{max})] were retrieved from the official prescribing information provided by pharmaceutical companies. We also calculated the AUC and C_{max} of the unbound drug (AUC_u and $C_{max,u}$, respectively) as biomarkers of drug brain exposure, by multiplying AUC and C_{max} by the plasma unbound drug fraction (fu). In addition, we estimated the liposolubility (e.g., $logD_{7.4}$) of the drugs by using a computer software (Marvin Sketch®). There was a significant correlation between log hIC_{50} and log nitrazepam-equivalent hypnotic doses of BZD-$GABA_A$ receptor agonists ($r = 0.91$, $p < 0.01$). A significant correlation was observed between hIC_{50} and AUC_u ($r = 0.84$, $p < 0.01$), between hIC_{50} and $C_{max,u}$ ($r = 0.94$, $p < 0.01$), and between $logD_{7.4}$ and log hIC_{50} ($r = -0.95$, $p < 0.001$). We concluded that the differences in the hypnotic doses of BZDs may be largely accounted for by the variability of hIC_{50}, and to some extent by the differences in volume of distribution (Vd) and fu, as $Cmax,u = fu \cdot D/Vd$. In addition, hIC_{50} may be affect the lipophilicity of drugs ($logD_{7.4}$), because drug transfer to the lipid-rich brain tissue is another factor associated with the potency of BZDs in the brain neurons.

Key words; benzodiazepine-$GABA_A$ receptor agonist, hypnotics, IC_{50}, lipophilicity, plasma unbound drug

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1. Introduction

Since the prototype benzodiazepine (BZD) drugs, chlordiazepoxide and diazepam, were introduced into clinical practice in the 1960s, more than 30 BZDs have been introduced into clinical use. Previous studies performed on animals (e.g., rodents) revealed that the hypnotic effects of BZDs may be associated with their agonistic effects on the gamma-aminobutyric acid type A ($GABA_A$) receptor-chloride ion channel complex in cerebral neurons. The binding of BZDs to $GABA_A$ receptors promotes the opening of the chloride ion channels and the influx of chloride ions into neurons, thereby leading to hyperpolarization of the neuronal membranes and reducing their liability to depolarization by external stimuli. These are considered the molecular mechanisms associated with the sedative, hypnotic, and anxiolytic effects of BZDs[1,2].

There is an approximately 30-fold difference in the oral doses of BZDs approved as hypnotics in Japan. A previous *in vitro* study of post-mortem human brain tissues demonstrated that there was an approximately 1,000-fold difference in the half maximal inhibitory concentration (hIC_{50}) of the $GABA_A$ receptor binding for BZDs[3]. In addition, it was also shown that there was a significant correlation between hIC_{50} values and their clinical doses[3,4]. These findings appear to support the hypothesis that clinical doses of BZDs are largely associated with the differences in the pharmacological effects at the sites of action (e.g., hIC_{50}). However, the variability in the pharmacokinetics of BZDs may also be associated with differences in the therapeutic doses of BZDs. For example, there are large differences in the elimination half-lives and plasma protein binding of BZDs. Owing to the differences in the pharmacokinetics, the cumulative exposure of BZDs in brain tissue [i.e., the area under the plasma drug concentration-time curve (AUC)] or the maximum exposure of drugs in the brain tissue [i.e., maximum drug concentrations (C_{max})] after the administration of the same dose may differ considerably between BZDs. In this report, we attempt to explain the apparent differences in the hypnotic doses of BZDs from the perspective of intrinsic $GABA_A$ receptor agonistic potency (i.e., hIC_{50}), as well as the pharmacokinetic biomarkers of BZDs.

2. Methods

At present, 12 BZDs are available as hypnotics in Japan. Excluding BZDs with active metabolites or prodrugs, we analyzed seven BZD drugs (brotizolam, estazolam, etizolam, flunitrazepam, lormetazepam, nitrazepam, and triazolam) and two non-BZD hypnotic (zopiclone and zolpidem) with a similar mechanism of action to BZD drugs. Nitrazepam-equivalent hypnotic doses of each drug were obtained from a previous report[2]. We retrieved approved hypnotic doses, AUC of plasma total (unbound plus bound) drug concentrations (AUC) and maximum plasma total drug concentration after a single oral dose (C_{max}) from the corresponding prescribing information provided by pharmaceutical companies[5-14]. In addition, data on plasma unbound fraction (fu) of the drugs were retrieved from the prescribing information. AUC and C_{max} expressed as plasma unbound concentrations were calculated by multiplying AUC and C_{max} by fu of the respective drugs. As plasma

drug concentrations and related pharmacokinetic parameters are expressed as non-SI unit (e.g., μg/mL) in most of the prescribing informations used in the present study, they were converted to SI units (e.g., mole/L).

We retrieved hIC_{50} values of the eight BZDs from a previous report where they were measured in post-mortem brain tissues using ^{3}H-flunitrazepam as a ligand of the $GABA_A$ receptors[3]. As the hIC_{50} for zolpidem was not available from this report, it was estimated from the relationship between half-maximum inhibitory concentrations of BZDs obtained from Sprague Dawley rats (rIC_{50}) using ^{3}H-flumazenil as a ligand of the $GABA_A$ receptors[15] and hIC_{50} for the corresponding BZDs, as in the study of Kobayashi et al.[3]. Eight drugs (alprazolam, brotizolam, clonazepam, diazepam, flunitrazepam, lorazepam, triazolam, and zopiclone) were used for the correlation analysis.

The physicochemical properties (e.g., molecular weight, lipophilicity, and solubility) of the BZDs were estimated by using the MarvinSketch® software (version 17.13). Specifically, the V-g method [16] was used for estimating liposolubility of drugs with log D values at pH 7.4 (log $D_{7.4}$), and the formula proposed by Yalkowsky et al. was used to estimate aqueous solubility of drugs with log S values at pH 7.4 (log $S_{7.4}$)[17]. An index of the hydrophilic-lipophilic balance (HLB) was estimated with the Davies' formula[18].

A correlation between hIC_{50} and rIC_{50} was analyzed with an exponential model ($y=a \cdot x^{b}$). A relationship between the log nitrazepam-equivalent doses of the BZDs and the log hIC_{50} was also analyzed with the exponential model. Relationships between log AUC or AUC_u and log hIC_{50} and those between log C_{max} or $C_{max,u}$ and log hIC_{50} were analyzed with the exponential model. All statistical analyses were performed with JMP® Pro 11.0.0 (SAS Institute Japan) and values of $p<0.01$ were considered statistically significant.

3. Results

Table 1 summarizes the $GABA_A$ receptor binding parameters such as hIC_{50}, pharmacokinetic parameters such as AUC and C_{max}, and approved hypnotic doses, and log$D_{7.4}$ for the nine BZDs that were currently approved in Japan for clinical use. There was an approximately two-order difference in the receptor binding affinity assessed by rIC_{50} and hIC_{50} among the BZDs studied. In addition, there was approximately 40-fold differences in nitrazepam-equivalent dose of BZDs. Nevertheless, there was an excellent correlation between the two binding parameters: $hIC_{50} = 1.34 \cdot rIC_{50}^{1.16}$ ($r= 0.97$, $p<0.01$, $n=8$). When the rIC_{50} value of 14.0 nmoles/L for zolpidem[15] as substituted into the equation, we were able to estimate that hIC_{50} was be 28.9 nmoles/L. As no values for hIC_{50} are reported in literature, we used this value and undertook further analysis as described below.

As for pharmacokinetic parameters of BZDs (AUC and C_{max}) of the BZDs at the respective approved doses, there were also large differences (120- and 54-fold) among the drugs.

For Log$D_{7.4}$, there was an eight-fold difference among the drugs. Despite these large between-drug differences in BZD receptor binding affinity, approved doses, pharmacokinetic parameters that are associated with drug exposure to the brain, and physicochemical properties, there were significant correlations between the parameters. For example, there was a significant correlation between the log

Table 1 *In vitro* affinity of BZDs to BZD-$GABA_A$ receptor binding in human brain (hIC_{50}) and their pharmacokinetic parameters of drug exposure in the brain.

Compound	hIC_{50} [3] (nmoles/L)	Nitrazepam-equivalent dose [2] (mg)	Nitrazepam-equivalent dose [2] (μmoles)	AUC [5-14]		C_{max} [5-14]		$LogD_{7.4}$ [16]
				total conc. (nmoles/L*h)	unbound conc. (nmoles/L*h)	total conc. (nmoles/L)	unbound conc. (nmoles/L)	
Brotizolam	1.2	0.25	0.6	76.3	7.6	9.5	0.9	3.1
Estazolam	28.9	2.0	6.8	11813.3	2362.7	381.4	76.3	2.51
Etizolam*	4.6	1.5	4.4	560.0	39.2	62.6	4.4	3.4
Flunitrazepam	5.9	1.0	3.2	512.0	109.6	51.1	10.9	2.58
Lormetazepam	2.0	1.0	3.0	182.0	15.7	16.7	1.4	3.26
Nitrazepam	24.8	5.0	17.8	4874.3	731.1	141.9	21.3	2.67
Triazolam	1.7	0.25	0.7	39.2	4.3	7.2	0.8	3.31
Zopiclone	103.6	7.5	19.3	1365.6	423.3	183.3	56.8	0.41
Zolpidem**	28.9	10	13.1***	1597.3	63.9	390.4	15.6	3.08

hIC_{50} = half-maximum inhibitory concentrations of BZDs to the BZD-$GABA_A$ receptor complex binding in the human brain tissues, AUC= area under the plasma concentration-time curves, C_{max} = maximum plasma concentration after a single oral administration

*For AUC and C_{max} of etizolam, those obtained after an oral administration of 1.0 mg were multiplied by a factor of 1.5, as no data were available for those after the administration of 1.5 mg.

**The hIC_{50} value for zolpidem was estimated from the equation $hIC_{50} = 1.34 \cdot rIC_{50}^{1.16}$ and the rIC_{50} value of 14 nmoles/L obtained from the study[15]. rIC_{50}= half-maximum inhibitory concentrations of BZDs to the BZD-$GABA_A$ receptor complex binding in the rat brain tissues.

***The molecular weight of zolpidem tartrate that is contained in the commercially available formula is 764.87. The pharmacokinetic parameters were calculated using the weight of the zolpidem bas

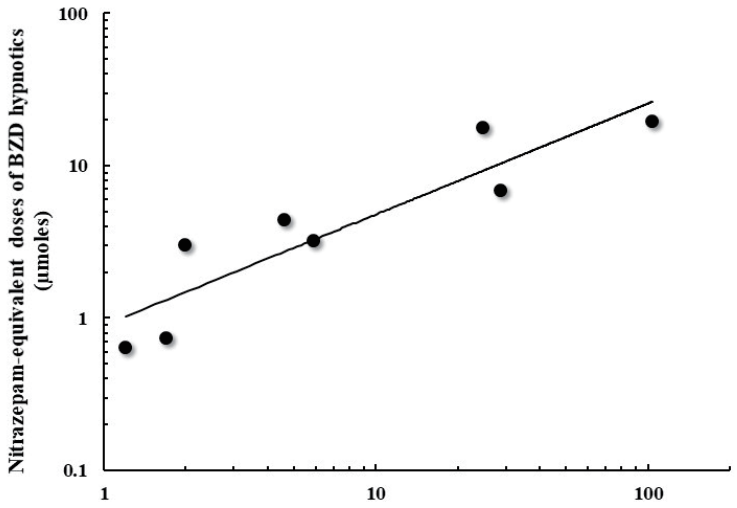
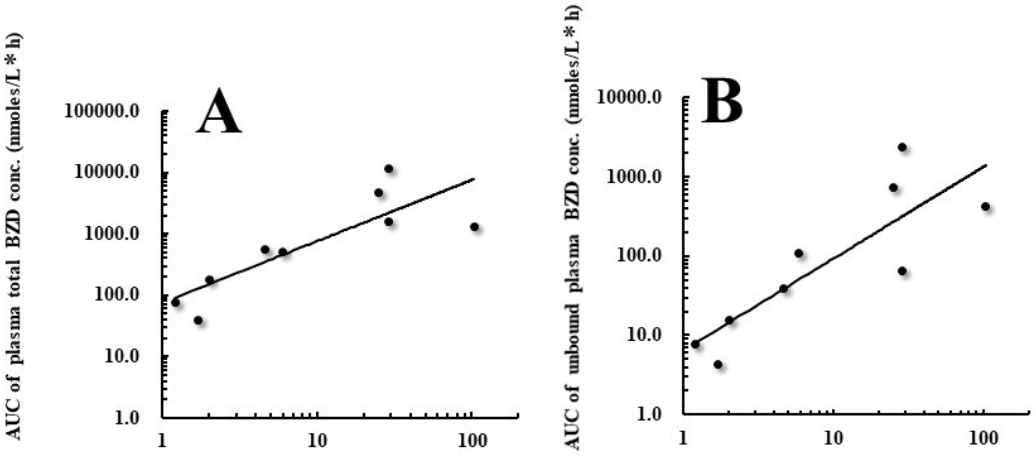
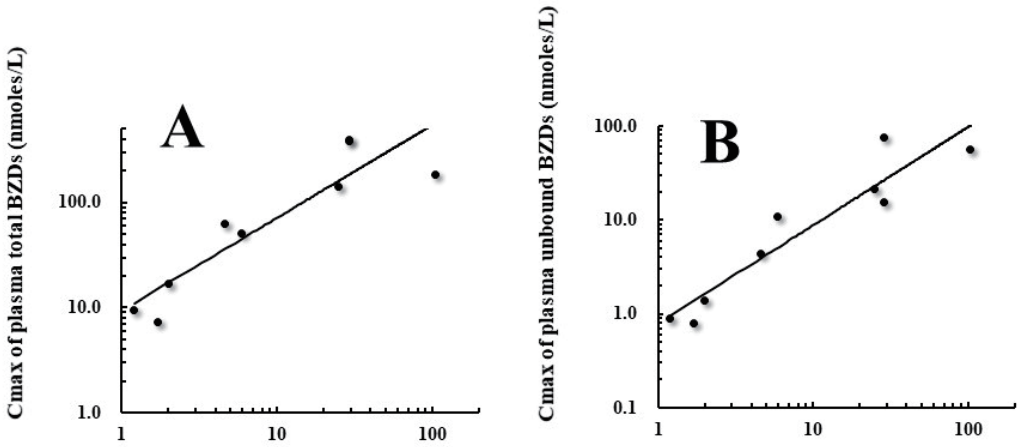


Figure 1 Correlation between the log nitrazepam-equivalent doses of BZD hypnotics and the log half-maximum inhibitory concentrations of BZDs to the BZD-$GABA_A$ receptor complex binding in the human brain tissues. Micromoles equivalent to 5 mg of nitrazepam (17.8 μmole) were plotted on the y axis. There was a significant correlation between the two parameters: $y = 0.90 \cdot x^{0.73}$, $r=0.91$, $p<0.01$.



Half-maximum inhibitory concentrations of BZDs to the BZD-GABAA receptor complex binding in human brain tissue (nmoles/L)

Figure 2 Correlation between the log AUC of total plasma concentrations of BZDs and the log half-maximum inhibitory concentrations of BZDs to the BZD-$GABA_A$ receptor complex binding in human brain tissue. (A): $y = 76.00 \cdot x^{1.00}$, $r = 0.83$, $p < 0.01$. Correlation between the log AUC of plasma unbound BZDs and the log half-maximum inhibitory concentrations of BZDs to the BZD-$GABA_A$ receptor complex binding in human brain tissue. (B): $y = 6.56 \cdot x^{1.15}$, $r = 0.84$, $p < 0.01$.



Half-maximum inhibitory concentrations of BZDs to the BZD-GABAA receptor complex binding in human brain tissue (nmoles/L)

Figure 3 Correlation between the log C_{max} of plasma total BZDs and the log half-maximum inhibitory concentrations of BZDs to the BZD-$GABA_A$ receptor complex binding in human brain tissue. (A): $y = 9.37 \cdot x^{0.88}$, $r = 0.91$, $p < 0.01$. Correlation between the log C_{max} of plasma unbound BZDs and the log half-maximum inhibitory concentrations of BZDs to the BZD-$GABA_A$ receptor complex binding in human brain tissue. (B): $y = 0.79 \cdot x^{1.04}$, $r = 0.94$, $p < 0.01$.

nitrazepam-equivalent doses of BZDs (y) and log hIC_{50} (x): $y = 0.90 \cdot x^{0.73}$, r=0.91, p<0.01 (Figure 1). Furthermore, there were significant correlations between log AUC (y) and log hIC_{50} (x) and between the log AUC_u (y) and the log hIC_{50} (x): $y = 76.00 \cdot x^{1.00}$, r=0.83, p<0.01 and $y = 6.56 \cdot x^{1.15}$, r=0.84, p<0.01, respectively (Figures 2A and 2B). Similarly, there were significant correlations between the log C_{max} (y) and the log hIC_{50} (x) and between the log $C_{max,u}$ (y) and log hIC_{50} (x): $y = 9.37 \cdot x^{0.88}$, r=0.91, p<0.01 and $y = 0.79 \cdot x^{1.04}$, r= 0.94, p<0.01, respectively (Figures 3A and 3B).

There were significant (p<0.01) correlations between the log hIC_{50} and the respective physicochemical parameters: for log $D_{7.4}$, log $S_{7.4}$, and HLB; correlation coefficients were r=-0.95, 0.97, and 0.90, respectively (data are not shown).

4. Discussion

In the present study, we found that there was a significant correlation (r=0.91, p<0.01) between hIC_{50} values obtained from *in vitro* ligand binding study and nitrazepam-equivalent hypnotic doses of BZD-$GABA_A$ receptor complex agonists for the therapeutic doses were established via clinical trials. This finding was consistent with our understanding that differences in the hypnotic doses of BZDs were largely accounted for by their intrinsic potency at the site of action. In addition, there were significant correlations between hIC_{50} and pharmacokinetic biomarkers of drug exposure to the brain tissue (AUC and C_{max}). Furthermore, the correlation coefficients for the relationship between hIC_{50} and the above drug exposure biomarkers expressed as unbound plasma drug concentrations (AUC_u and $C_{max,u}$) were equal to or greater than those for AUC and C_{max}. Overall, the relationship between $C_{max,u}$ and hIC_{50} showed the highest r value (0.94) of the pharmacokinetic biomarkers of drug exposure to the brain. As there was 3–5-fold difference in plasma protein binding of BZDs (Table 1), not only hIC_{50}, but also fu is associated with the variability of the hypnotic doses of BZDs. Collectively, for a newly developed BZD drug, its hypnotic $C_{max,u}$ may be predicted by hIC_{50}, and the corresponding hypnotic dose after a single dose may be approximated in accordance with the basic pharmacokinetic equations, as $D = C_{max,u} \cdot V_{d,u}$ or $C_{max} \cdot V_d$.

There were significant correlations between the hIC_{50} values and the physicochemical parameters of BZDs (i.e., log $D_{7.4}$, log $S_{7.4}$, and HLB). These physicochemical properties were associated with lipophilicity and liposolubility of BZDs. As the brain tissue is lipid-rich, BZDs with higher liposolubility may have higher concentration at the binding sites. However, it remains unclear whether this principle is applicable to other hypnotic drugs with non-BZD structures. Further studies are required to answer this question.

There present study has limitations. As only nine hypnotic drugs of BZD-$GABA_A$ receptor agonists are available in Japan, our analysis on the relationship between hIC_{50} and clinical doses and the pharmacokinetic biomarkers of brain drug exposure was performed on only a small number of drugs. As a result, further studies are required to confirm the external validity with use of other BZDs approved as hypnotics in other countries.

Recently, there has been a surge of interest regarding a formulary system of drugs by all stakeholders (e.g., physicians and pharmacists) of healthcare in order to establishing policies for the economical and safe use of drugs[19]. The formulary

system may be useful for recommending a small number of drugs among those with similar indications. As the implementation of a formulary system would require pharmacists to estimate the equivalent doses of drugs, the approach proposed in the present article based upon the information available in the prescribing information and interview forms may be useful for pharmacists working in both hospitals and community pharmacies.

In conclusion, we revealed that *in vitro* pharmacodynamic potency of BZDs (hIC_{50}) and *in vivo* pharmacokinetic biomarkers representing drug exposure to the brain (fu, AUC_u, and $C_{max,u}$) may collectively contribute to the variability of hypnotic doses of the BZDs. Our preliminary findings on the relationship between physicochemical properties of BZDs and hIC_{50} should be studied in greater depth in the future.

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Conflicts of Interest

The authors have no conflict of interest to be declared.

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