【Full title】

Benzodiazepine concentrations in the breastmilk and plasma of nursing mothers: Estimation of relative infant dose

【Running title】

Benzodiazepine concentrations in the breastmilk

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【Abstract】

Benzodiazepines are common therapies for mental illness and insomnia and are used during pregnancy and lactation. Although benzodiazepines have been shown to be transferred into breast milk, the amount transferred is small and compatible with breastfeeding. However, information is not available for all drugs. Therefore, we aimed to determine the milk to plasma (M/P) ratio and relative infant dose (RID), which are used as indicators of drug transfer to breast milk, to determine the safety of such drugs for lactating women and breastfeeding infants. The study comprised 11 pregnant women who visited the obstetrics department of Hokkaido University Hospital and Tenshi Hospital for childbirth. The samples were analyzed using liquid chromatography-tandem mass spectrometry, and the M/P ratio and RID were calculated. The condition of the mother and baby at 1 month after delivery was determined from the clinical information. The target benzodiazepines were alprazolam, brotizolam, clonazepam, clotiazepam, etizolam, ethyl loflazepate, flunitrazepam, and lorazepam. For all drugs, the M/P ratios were <1 and remained constant over time. For drugs other than ethyl loflazepate, the RID values were less than 10%, which are considered safe; however, even with ethyl loflazepate, it was only slightly over 10%. No abnormalities were found in breastfeeding infants whose mothers were receiving these medications. The RID results of this study suggest that drug exposure through breast milk is small; thus, maternal drug treatment and breastfeeding are compatible.

【Introduction】

Mental healthcare for perinatal women has emerged as an important area of research. Depression is one of the most common complications during the perinatal period [1, 2]; panic disorder and anxiety are also common [3, 4]. Anxiety and stress during pregnancy may have various adverse effects, including morphological abnormalities of the baby, impaired fetal development, complications of labor, and altered mental development of the newborn [5-10]. Therefore, drug therapies are considered necessary for anxiety symptoms that occur or worsen during pregnancy; however, it is necessary to examine the risk of congenital abnormalities, perinatal complications, and side effects [11-13]. In addition, most patients with postpartum depression and anxiety experienced depression and anxiety during pregnancy, respectively [14], suggesting that continued drug therapy may be necessary after birth. Maternal depression causes a decrease in infant milk intake [15,16].

On the other hand, the benefits of breastfeeding to the mother and the infant are well established [17]. The benefits for the mother are the reduction of physical damage, such as by the promotion of uterine involution and the lengthening of the birth interval. Long-term breastfeeding has also been reported to reduce the risk of developing several diseases, including breast cancer, ovarian cancer, and metabolic diseases [18]. The benefit to infants is that breast milk is a source of nutrition and immunity that will prevent future illness [19-21]; furthermore, improved neurological prognosis has been reported [20,21]. Valproic acid use by the mother was reported to lower the IQ of the offspring; however, this decrease was smaller

in infants fed breast milk [22]. Given these benefits, breastfeeding should be recommended, except in special cases in which breastfeeding is contraindicated. However, the available information on the compatibility between drug use and breastfeeding is insufficient. Hence, it is not uncommon for people to give up breastfeeding.

Benzodiazepines are often prescribed for anxiety symptoms in pregnant women [23,24]. Drugs used during pregnancy are likely to continue to be needed during lactation. Several benzodiazepines, namely, alprazolam, clonazepam, diazepam, and lorazepam, are reported to be transferred to breast milk. Recently, the relative infant dose (RID) has been used as a parameter to indicate drug migration to infants through breast milk. The RID is calculated by dividing the infant's dose via breast milk in "mg/kg/day" by the maternal dose in "mg/kg/day" multiplied by 100. If this value is less than 10%, it is considered unlikely to have a clinical effect on the infant [25]. In a study of eight lactating women who took a single dose of 0.5 mg alprazolam [26], the M/P ratio was 0.36 and the RID was 3%. Based on the reported data, Hale et al. calculated the RID of 8.5% as a corrected value using an average maternal weight of 70 kg and an average infant feeding volume of 150 mL/kg/day [25]. One study reported an M/P ratio for clonazepam of 0.33 [27]. The RID was reported to be 2.5% following the oral administration of clonazepam 2 mg [28], and Hale et al. estimated it to be 2.8% [25]. After administration of a high dose (80 mg) of diazepam, the M/P ratio was 0.2, and the RID was 4.7% [29]. Calculations based on the previous report by Hale et al. showed that the M/P ratio was 0.2–2.7 and the RID was 0.88%–7.14% [25]. For lorazepam, the M/P ratio was

reported to be 0.15–0.26, based on data from four patients who received 3.5 mg [30]. Following the administration of 2.5 mg twice daily, it was reported that the concentration in breast milk was 12 μg/L; consequently, the RID was estimated to be 2.6%–2.9% [25, 31].

Benzodiazepines and CNS-acting drugs taken by mothers do not appear to pose a significant risk of direct adverse effects on breastfeeding infants [32, 33]. However, neonates are more susceptible to the effects of CNS depressants; thus, these drugs should be taken with caution [34]. Therefore, information and evaluation of individual drugs, rather than analogies based on similar drugs, is necessary. The purpose of this study was to calculate the M/P ratio and RID in eight benzodiazepines, and to establish if mothers taking these drugs can safely breastfeed.

【Methods】

Ethics

The study subjects were pregnant women who were visiting or were admitted to the Obstetrics Department of Hokkaido University Hospital and Tenshi Hospital and met the following conditions: (1) 20 years of age or older at the time of obtaining consent; (2) were taking benzodiazepines before delivery; and (3) after receiving sufficient explanation of the study and understanding the study, freely gave their consent to participate. This study was approved by the Hokkaido University Hospital Clinical Research Ethics Committee (approval number: 017-0131) and Tenshi Hospital Ethics Committee (approval number: 103).

Sample collection

Maternal blood samples and breast milk samples were collected 3–6 days after delivery, at the time when the plasma concentration peaked and before the drug administration, and at any time during the 1-month post-examination screening. According to the package insert information, the time of the peak plasma concentration was 2 h for alprazolam, 1–1.5 h for brotizolam, 2 h for clonazepam, 1 h for clotiazepam, 1 h for etizolam, 1 h for ethyl loflazepate, 2 h for lorazepam, and 2 h for flunitrazepam. Breast milk samples were manually collected from one breast with the help of a nurse. The plasma samples were centrifuged at 2,300 × *g* for 10 min at 15ºC; the isolated plasma and breast milk were stored frozen at -30ºC until analysis.

Quantification of benzodiazepines in breast milk and plasma

The concentration of benzodiazepines in breast milk and plasma was quantified using liquid chromatography-electrospray ionization tandem mass spectrometry (LC-MS/MS), as previously described [35]. Briefly, sample preparation was performed by liquid-liquid extraction with ethyl acetate. The benzodiazepines were separated on a C18 column using a gradient elution of acetonitrile in aqueous ammonium acetate solution and were detected in the positive ion electrospray mode with multiple reaction monitoring. As ethyl loflazepate is immediately and completely metabolized to an unstable metabolite after intestinal absorption [36], we measured the ethyl loflazepate metabolite, CM7116, in the

present study. The lower limit of quantification (LLOQ) in breast milk was 0.25 ng/mL for alprazolam, brotizolam, clotiazepam, flunitrazepam, and CM7116 and 0.5 ng/mL for clonazepam, etizolam, and lorazepam. The LLOQ in plasma was 0.25 ng/mL for brotizolam; 0.5 ng/mL for alprazolam, clonazepam, clotiazepam, etizolam, flunitrazepam, and lorazepam; and 1.0 ng/mL for CM7116.

Analysis of breast milk concentration

The M/P ratio was calculated by dividing the total breast milk concentration by the total plasma concentration.

RID was calculated from the following formula:

$$\text{RID (\%)} = \frac{\text{Peak concentration in breastmilk (mg/mL)} \times \text{Infant intake of breastmilk (mL/kg/day)}}{\text{Maternal dose (mg/kg/day)}} \times 100$$

Infant intake of breastmilk (mL/kg/day) is the theoretical value (150 mL/kg/day), and the measured value is [single breast milk intake (mL) × number of nursing episodes per day/infant weight (kg)].

Estimation of maternal mental condition

Maternal mental status was evaluated from the medical records. We used the results of drug administration, childcare/lactation, nursing records, psychiatric evaluations immediately after delivery and 1 month after delivery, and the Edinburgh Postnatal Depression Scale at 1 month after delivery

【Results】

The concentration of eight benzodiazepines in breast milk was measured in 11 patients. The patients' demographics are shown in Table 1. The median age was 34 years of age (range, 31–36), and the complications included panic disorder, anxiety, depression, dissociative disorder, and personality disorder. In 7 patients (64%), this was their first delivery. Vaginal delivery occurred in five patients and six patients underwent cesarean section. The median gestation period was 39 weeks 2 days (38 weeks 1 day–40 weeks 0 days). Preterm birth occurred in one patient.

Plasma and breast milk were collected 3–6 days after delivery, before drug administration and at the time of peak plasma concentration after administration, and at any time during the 1-month checkup.

The drug concentrations, M/P ratios, and RIDs determined for plasma and breast milk collected 3–6 days after delivery are shown in Table 2. For lorazepam and brotizolam, the trough concentration in milk was below the LLOQ. For other drugs, the concentration was within the measurable range. The calculated M/P ratios at peak concentrations were 0.35–0.49 for alprazolam, 0.12 for brotizolam, 0.40 for clonazepam, 0.15 for clotiazepam, 0.17 for etizolam, 0.11–0.13 for ethyl loflazepate, 0.47–0.85 for flunitrazepam, and 0.21–0.26 for lorazepam. At the trough concentrations, the M/P ratios were 0.35–0.52 for alprazolam, 0.37 for clonazepam, 0.21 for etizolam, 0.10–0.17 for ethyl loflazepate, 0.49–0.89 for flunitrazepam, and 0.17 for lorazepam; M/P ratios were not calculable for brotizolam and clotiazepam. The drug concentration in breast milk was lower than that in plasma, and the peak and trough values were

similar.

For all drugs, the RID calculated from the measured value was smaller than the RID calculated from the theoretical value. The calculated RID (theoretical amount) was 3.8%–9.3% for alprazolam, 2.1%–4.4% for lorazepam, 2.3% for brotizolam, 4.6% for clonazepam, 2.5% for clotiazepam, 0.6% for etizolam, and 1.6%–2.5% for flunitrazepam; these were less than 10%, which is considered to be safe. The RID of the ethyl loflazepate metabolite CM7116 (molecular weight: 288.7 g/mol) was 11.9% and 11.4%, and when converted from the mass ratio to ethyl loflazepate weight (molecular weight: 360.8 g/mol), it was 12.9% and 12.4%, respectively; hence, these values were slightly above 10%.

M/P ratio and RID of each of the drugs at the 1-month checkup are shown in Table 3. M/P ratio for brotizolam, the value 3 days after delivery was 0.12, which was different from the value at the 1-month checkup (0.59). Alprazolam, lorazepam, flunitrazepam, and the ethyl loflazepate metabolite CM7116 were not very different at the 1-month checkup compared with the values at 3–6 days after delivery.

The changes in breastfeeding status and maternal mental status from the 3 to 6 days after delivery at the1-month checkup are shown in Table 4. Some studies suggest that symptoms of postpartum depression and anxiety tend to reduce the period of breastfeeding. Therefore, we investigated the mental conditions of the mother from the medical records in addition to breastfeeding status. There was an increase in drug dosage in two patients. There was one patient for whom the dose was reduced. Drug

dosage was unchanged in eight patients. Four of the 11 patients had stopped breastfeeding by the time of the 1-month checkup, for the following reasons: neonatal death (1 patient); insufficient milk production and deterioration of mental symptoms (1 patient); a desire to switch to formula milk from breast milk because of maternal fatigue (2 patients). Some of the infants had mild postnatal withdrawal symptoms, but there were no side effects of the drug via breast milk. No children were found to have any abnormalities at the 1-month checkup. There were no issues with weight gain (average 34.3 [22.4–46.9] g/day; data not shown).

【Discussion】

Benzodiazepines are used for the treatment of anxiety symptoms and insomnia and can even be used during pregnancy [23, 24]. In many cases, drugs used during pregnancy continue to be required even after delivery. Given the recent public health messaging surrounding the importance of breastfeeding, information about drug transfer to breast milk is needed. Some studies have analyzed the transfer of benzodiazepines to breast milk [25-31]. However, among benzodiazepines, the M/P ratio and RID parameters have been reported only for some drugs, and values for other drugs were inferred from these data. Therefore, we tried to calculate these parameters for benzodiazepines that may be used by lactating women.

The previously reported alprazolam M/P ratio was 0.36 and RID was 3%–8.5% [25, 26]. For the two

cases in this study, values were almost the same as those previously reported. The previously reported M/P ratio for lorazepam was 0.15–0.26, and the RID was 2.6%–2.9% [25, 30, 31]. The three cases in this study had similar M/P ratios; the RID was slightly higher in one case but the same as previously reported in the other two cases. These results suggested the validity of this measurement system.

The M/P ratios of all eight benzodiazepines were below 1, which suggested no enrichment in breast milk. In addition, it has been reported that the time-dependent changes in plasma concentration and breast milk concentration differ depending on the fat-solubility of the drug, and that these concentrations alter in parallel for highly lipophilic drugs [37]. The product information indicates that benzodiazepines are highly lipophilic. In a case report describing the plasma and breast milk concentrations of alprazolam over time, parallel changes in concentration were observed [26]. In this study, the peak and trough M/P ratios of each drug were almost the same, suggesting that plasma and breast milk concentrations were in parallel. This result was clinically significant, because it showed that it was possible to estimate the concentration in human milk from the plasma concentration at any time after taking it, even if the concentration in breast milk could not be directly measured.

However, in this study, the M/P ratio was calculated using a single point because of the burden of blood collection on the mother and the ethical considerations of exploiting breast milk to feed the child. The M/P ratio should be expressed as the area under the concentration-time curve (AUC) ratio or the average concentration ratio of breast milk and maternal plasma concentrations sampled over time.

Based on previous studies and the results of this study, it is suggested that breast milk and plasma concentrations move in parallel for benzodiazepines. Therefore, the calculated M/P ratio should not result in a significant error; however, this is a limitation of this study and further studies are needed in the future.

In this study, the RID was calculated from the measured and theoretical intake (150 mL/kg/day). As the measurements were taken 3–6 days after delivery, the feeding volume was low and the calculated RID values were small. In this period, an infant's intake of breast milk increases every day; therefore, it is considered that the RID obtained from the theoretical breastfeeding volume is suitable for the general evaluation of risk. To estimate the maximum amount of drug intake by infants via breast milk, the peak concentrations of the drugs in the breast milk were used to calculate the daily drug intake in infants and were used to calculate the RID. With the exception of ethyl loflazepate, the RID determined from the theoretical feeding value in this study was below 10%, the safe threshold, and we considered that taking these drugs and breastfeeding were compatible.

The RID of ethyl loflazepate exceeded the safe threshold. However, it was only slightly above the threshold. Therefore, due to the high potency and long elimination half-life of the drug, it is necessary to be careful of potential side effects arising from accumulation in the infant, but breastfeeding should not be prohibited. Although follow-up was continued to approximately 1 month after delivery, in the two subjects receiving ethyl loflazepate, no adverse events, such as somnolence, were observed in the infants,

and there were no issues with weight gain. However, as ethyl loflazepate undergoes a first-pass effect during passage through the intestinal tract and in the liver and no unchanged drug is detected in blood or breast milk, the amount of metabolites should be measured. Therefore, for an accurate evaluation of the RID, it is necessary to study the gastrointestinal absorption of this metabolite in infants.

Of breast milk samples approximately 1 week after birth and approximately 3–4 weeks after birth, the latter has a slightly lower protein content and a higher fat content. Brotizolam had a high M/P ratio at the 1-month checkup, but no changes were observed in the other drugs. As the number of cases was small, it was not possible to conclude whether this difference in breast milk composition altered the M/P ratio. In addition, as the sampling method does not take into account the difference in the composition of foremilk and hindmilk, further investigation is necessary regarding the relationship between the transferability of the drug to milk and the milk composition, including fat concentration.

Some reports suggest that postpartum depression and anxiety symptoms tend to reduce the period of breastfeeding. It has also been reported that breastfeeding is less likely to start, and formula supplementation is more likely to occur. [38,39]. These tendencies were also observed in this study. Postpartum-specific anxiety is associated with low oxytocin and low prolactin, which may interfere with the milk reflex and subsequent milk production [40]. It is theorized that these mechanisms have an adverse effect on breastfeeding. It is well-known that breastfeeding is beneficial to mothers and infants, and it is important to prolong the lactation period as much as possible. Therefore, stabilization of maternal

psychological symptoms is necessary, and drug treatment may continue during breastfeeding. In addition, it is reported that more than 90% of women in Japan wish to breastfeed [41], and breastfeeding should not, as a rule, be stopped because of the use of drugs to treat this disease. In contrast, the risk to the infant due to the drug should be avoided, and to evaluate the risk and the benefit correctly, it is important to identify objective parameters, including the migration of the drug to breast milk.

【Conclusions】

For alprazolam, lorazepam, brotizolam, clonazepam, clonazepam, etizolam, and flunitrazepam, RID was less than 10%, which is considered an appropriate safety margin. Although the RID of ethyl loflazepate was higher than 10%, it was not large enough to be considered to induce adverse events in infants, and breastfeeding does not need to be stopped if the mother is taking this drug. For lactating women who take benzodiazepines, there is little information to judge whether breastfeeding is safe; therefore, judgement is often based on limited information and experience. As empirical judgments can be biased, we considered that this research, which measures parameters that are objective and have a universal understanding, such as RID, is meaningful. However, the number of cases for each drug is very low; hence, further data are needed to draw firm conclusions.

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Table1 Maternal characteristics

No	Age	Weight, kg	Disorder	Drug	Parity	Delivery	Gestational age, wk.d
1	34	59.7	Panic disorder	Alprazolam	P0	Vaginal delivery	41wk 2d
2	36	51.3	Anxiety	Clotiazepam	P0	Caesarean section	27wk 6d
3	36	60.4	Depression	Ethyl loflazepate	P1	Vaginal delivery	40wk 1d
4	29	60.9	Bipolar affective Panic disorder	Alprazolam Flunitrazepam	P3	Caesarean section	39wk 2d
5	24	50.5	Dissociative disorder	Clonazepam Flunitrazepam	P0	Vaginal delivery	39wk 5d
6	31	52.3	Borderline personality disorder	Etizolam	P0	Vaginal delivery	39wk 4d
7	34	41.5	Mood disorder Neuropsychiatric SLE	Lorazepam	P0	Caesarean section	38wk 5d
8	31	56.1	Panic disorder	Lorazepam Ethyl loflazepate	P1	Caesarean section	37wk 0d
9	31	66.7	Depression	Flunitrazepam	P1	Vaginal delivery	37wk 6d
10	41	66.7	Dissociative disorder	Lorazepam	P0	Caesarean section	39wk 5d
11	36	64.7	Personality disorder	Brotizolam	P0	Caesarean section	38wk 3d
Value for Characteristic (IQR : interquartile range)	34 (31-36)	59.7 (51.8-62.8)			Primiparity 64%	Vaginal delivery/Caesarean section 5 / 6	39wk 2d (38wk 1d - 40wk 0d)

Table 2 Plasma and breast milk concentrations of benzodiazepines, M/P ratio, and RID (time after delivery : 3-6 days)

Drug	Patient No.	Maternal dose (mg/day)	Total concentration (ng/ml) Peak		Total concentration (ng/ml) Trough		M/P ratio Peak	M/P ratio Trough	Daily volume of intake breastmilk (ml/kg/day)	RID (%) measured value	RID (%) theoretical value
			Plasma	Breast Milk	Plasma	Breast Milk					
Alprazolam	1*	0.8	6.95	3.4	5.36	2.78	0.49	0.52	77.24	2.0	3.8
	4	2.4	69.5	24.5	68**	23.8**	0.35	0.35	117.1	7.3	9.3
Lorazepam	7	0.5	8.15	1.72	1.32	< LLOQ	0.21	-	78.95	1.1	2.1
	8	0.5	7.15	1.64	4.92	0.826	0.23	0.17	78.66	1.5	2.7
	10	0.5	7.55	1.98	1.65	< LLOQ	0.26	-	106.67	1.0	4.4
Brotizolam	11	0.25	5.03	0.589	1.2	< LLOQ	0.12	-	12.79	0.7	2.3
Clonazepam	5	1	15	6.07	12.1	4.53	0.40	0.37	89.02	2.7	4.6
Clotiazepam	2	5	109	16.3	-	-	0.15	-	138.33	2.4	2.5
Etizolam	6	1	4.63	0.77	4.31	0.90	0.17	0.21	85.71	0.3	0.6
Flunitrazepam	5	2	8.62	4.09	3.05	1.48	0.47	0.49	89.02	0.9	1.6
	9	1	2.93	2.48	0.588	0.524	0.85	0.89	95.58	1.6	2.5
Ethyl loflazepate	3	0.5	48.9	6.55	46.7	8.1	0.13	0.17	-	-	11.9 (12.9***)
	8	1	123	13.6	88.2	11.4	0.11	0.13	78.66	6.0 (6.5***)	11.4 (12.4***)

* This data is from Reference [35]

** 4 hr after intake

*** Compared with the mass CM7116/ethyl loflazepate＝0.922

Table 3 Plasma and breast milk concentrations of benzodiazepines, M/P ratio, and RID (time after delivery : 1 month)

Drug	Patient No.	Maternal intake dose (mg/day)	Time after oral administration	Total concentration (ng/ml)		M/P ratio	RID (%) measured value	RID (%) theoretical value
				Plasma	Breast milk			
Alprazolam	1*	1	1.5	13.3	5.42	0.41	5.3	4.6
Lorazepam	7	0.5	15.5	6.78	1.39	0.21	1.3-3.1	1.7
	8	0.5	2.5	7.94	1.5	0.19	0.7-1.0	2.7
	10	0.5	12	7.79	1.27	0.16	1.9	2.7
Brotizolam	11	0.125	11	0.458	0.272	0.59	0.2	1.8
Flunitrazepam	9	2	11.75	1.73	1.19	0.69	-	0.6
Ethyl loflazepate	8	1	14.5	141	13.7	0.1	3	12.3

* This data is from Reference [35]

Table 4 Changes in breastfeeding status and maternal mental status

No	Drug	Maternal intake dose (mg/day)		Feeding Method		Maternal mental condition	
		Intrapartum period	1 month after delivery	Immediately after delivery	1 month after delivery	Immediately after delivery	1 month after delivery
1	Alprazolam	0.8	1.6	Breast milk	Breast milk	Good	Good
2	Clotiazepam	5	No change	Breast milk	*	Good	Good
3	Ethyl loflazepate	0.5	No change	Breast milk & formula milk	Formula milk	Good	Worsening
4	Alprazolam Flunitrazepam	2.4	No change	Breast milk & formula milk	Formula milk	Good	Good
5	Clonazepam Flunitrazepam	1	No change	Breast milk & formula milk	Breast milk	Good	Good
6	Etizolam	1	1.5	Breast milk	Breast milk	Good	Worsening
7	Lorazepam	0.5	No change	Breast milk & formula milk	Breast milk & formula milk	Good	Worsening
8	Lorazepam Ethyl loflazepate	0.5	No change	Breast milk	Breast milk & formula milk	Good	Good
9	Flunitrazepam	1	No change	Breast milk	Breast milk	Good	Good
10	Lorazepam	0.5	No change	Breast milk & formula milk	Breast milk & formula milk	Good	Good
11	Brotizolam	0.25	0.125	Breast milk & formula milk	Formula milk (main)	Good	Good

* Neonatal death