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Racemic Ketamine and S(+)-Ketamine Concentrations in Cerebrospinal Fluid after Epidural and Intravenous Administration in Rabbits

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The Pharmacokinetic characteristic of ketamine, particularly the shift from the epidural space to the cerebrospinal fluid (CSF), is still unclear. Furthermore pharmacokinetic differences between racemic ketamine and S(+)-ketamine are not clearly described when administered into the epidural space. We measured plasma and CSF concentrations of racemic ketamine and S(+)-ketamine after 2 mg/kg intravenous or 2 mg/kg epidural injection in 32 rabbits, and calculated pharmacokinetic parameters by the moment analysis method. The elimination half time of S(+)-ketamine was significantly shorter than that of racemic ketamine and the systemic distribution volume of S(+)-ketamine was significantly smaller than that of racemic ketamine in the CSF. Pharmacokinetic parameters in the CSF after epidural injection of racemic versus S(+)-ketamines were: maximum concentration, 0.4 ± 0.1 versus 0.6 ± 0.2 μg/mL (not significant); time to maximum concentration, 9.7 ± 2.1 versus 9.0 ± 3.4 min (not significant); elimination half time, 127.1 ± 25.2 versus 89.3 ± 19.4 min (P = 0.005); area under the curve, 56.4 ± 6.4 versus 56.6 ± 11.0 μg•mL/min (not significant); and distribution volume, 19463.5 ± 3266.1 versus 13613.3 ± 4895.2 mL (P = 0.014), respectively. When injected intravenously, there was no significant difference in these parameters of the CSF between racemic and S(+)-ketamines. Racemic ketamine passed easily through the blood brain barrier when administered intravenously. It also shifted to the CSF through the systemic circulation, even when they were administered epidurally. S(+)-Ketamine had similar movement as racemic ketamine.

Key words: analgesia; cerebrospinal fluid; epidural administratin;ketamine; pharmacokinetics

Ketamine has been used widely as a sole anesthetic when injected intravenously and as an adjuvant analgesic agent with local anesthetics when administered epidurally (Islas et al., 1985; Naguib et al., 1986; Ravat et al., 1987). However, it is still debatable that the experimental and clinical effects of epidural ketamine on the intensity of somatic and visceral pain (Subramaniam et al., 2001). One of the major issues behind this debate is the uncertainty regarding pharmacokinetics, especially the transfer of ketamine into the cerebrospinal fluid (CSF), that is, the pharmacokinetics of transfer

Abbreviations: AUC, area under the curve; BBB, blood brain barrier; BP, blood pressure; Cmax, maxmam concentration; CSF, cerebrospinal fluid; HPLC, high-performance liquid chromatography; HR, heart rate; T1/2β, elimination half life; Tmax, time to maximum concentration; Vdss, distribution volume

from the epidural space to the CSF across the dura mater and from the systemic circulation to the CSF through the blood brain barrier (BBB). Pedraz et al. (1991) reported in the dog model that ketamine easily crosses the dura mater due to its lipid solubility when administered epidurally. However, there is a report that epidurally administered ketamine does not work at a segmental action (Horiuchi et al., 1991). Moreover, there are no reports of a spinal segmental action for epidural ketamine, although epidurally administered lipid-soluble drugs such as fentanyl are known to produce apparent spinal segmental actions (Dos Santos et al., 1996; Schols et al., 1996; Bernards et al., 2003a; 2003b). We hypothesized that epidurally administered ketamine is rapidly absorbed into the systemic circulation and shifts into the intrathecal space through the BBB. This will also explain the non-segmental action of ketamine.

Racemic ketamine consists of 2 optical isomers, S(+)-ketamine and R(-)-ketamine and it is known that analgesic and sedative actions of the S(+)-ketamine is much stronger, resulting in widely clinical use in Europe (Geisslinger et al., 1991; Kharasch et al., 1992; Arendt-Nielsen et al., 1996; Murata et al., 2000). However precise pharmacokinetic study has not be elucidated regarding differences in S(+)-ketamine and R(-)-ketamines when administered in the epidural space. If pharmacokinetic parameters of S(+)-ketamine and R(-)-ketamines are widely different, the local action of epidural ketamines may be segregated. To test these hypothesis, the pharmacokinetics of ketamine in the CSF were studied by intravenous and epidural administrations of racemeic and S(+)-ketamines.

Materials and Methods

Anesthesia

Female Japanese white rabbits weighting 2.6 to 3.5 kg were used. The whole experimental procedure using the animals was approved by the Tottori University institutional animal care committee. Pentobarbital (Abbott, Chicago, IL), 50 mg/kg, was administered through a 24 G stabilized catheter inserted into the ear vein to anesthetize the rabbit. Then, the rabbit was fixed in supine position and a tracheostomy was performed. Anesthesia was maintained with 1 to 2% sevoflurane in an air-oxygen mixture (inspired oxygen concentration = 0.4). The rabbit was ventilated mechanically at a respiratory rate of 40 breaths/min and tidal volume of 10 mL/kg using a ventilator for small animals (rodent ventilator model 683, Harvard Medical Instruments, Cambridge, MA).

Preparation of the rabbit

A 24 G catheter was inserted into the right femoral artery for blood sampling and for monitoring of blood pressure (BP) and heart rate (HR). Another catheter was inserted into the right internal jugular vein for injection o f racemic ketamine or S(+)-ketamine. Lactated Ringer's solution was infused at a rate of 10 ml/kg/h. The rabbit was then turned into prone position and a laminectomy was performed at either C2 or C3 level using the electric scalpel and the drill. A 2 Fr catheter for collection of the CSF was inserted after confirming of the location of the arachnoid membrane according to the method of Tiscot et al. (1995). The epidural space was punctured in the epidural administration group at the interspace between L5 and L6 with a 20 G Touhy needle according to the method of Taguchi et al. (1996) and an epidural catheter was advanced to 3 cm within the epidural space.

Sample collection

After completion of the preparation, we put a 30-min interval to stabilize arterial blood pressure, heart rate and end-tidal carbon dioxide tension within normal limits. The vital signs were continuously monitored and kept within the normal range throughout the study period. Either racemic ketamine or S(+)-ketamine (Alexis biochemicals, Lausen, Switzerland) were administered at 2 mg/kg in a bolus through the epidural catheter in the epidural

groups and injected as a bolus dose via the internal jugular vein in the intravenous groups. The arterial blood (1.5 mL) and CSF (0.2 mL) were simultaneously collected with heparinized syringes at 1, 3, 5, 10, 15, 30, 60 and 120 min after administration of the test drug. Both samples were centrifuged at 3000 rpm at 0°C and the supernatant was stored at –80°C until analysis. Indocyanine green pigment (0.5 ml) was injected through the epidural catheter to confirm an adequate positioning of the epidural catheter in randomly selected rabbits.

Measurement

Racemic ketamine and S(+)-ketamine concentrations in the plasma and CSF were measured by high-performance liquid chromatography (HPLC) (Shimadzu SPD-10A, SCL-10A, LC-10AD, PGU-12A, CTO10A and CR-8A, Kyoto, Japan). Chromatographic conditions were as follows: mobile phase, phosphate buffer:methanol:acetonitorile = 7:2:1; velocity of mobile phase, 1.2 mL/min; temperature, 45°C; wavelength, 215 nm; and column, phenilcolumn. Pharmacokinetic parameters, maximum concentration (Cmax), time to Cmax (Tmax), elimination half time (T1/2β), area under the curve (AUC) and distribution volume (Vdss) were calculated from the plasma and CSF concentrations of both racemic ketamine and S(+) ketamine by the moment analysis method using a pharmacokinetics data analysis software (PK Solutions, version 2.0, Montrose, CO).

Statistics

Parametric data were analyzed by Student's *t*-test. A *P* value less than 0.05 was considered statistically significant. All data were expressed as mean ± SD.

Results

There were no significant differences in the mean body weight among 4 study groups, and that for the epidural racemic ketamine group, epidural S(+)-ketamine group, intravenous racemic ketamine group and intravenous S(+)-ketamine group was 3.0 kg, 3.0 kg, 2.9 kg and 2.8 kg, respectively. There were no significant differences in homodynamic parameters (HR and BP). Figure 1 shows the changes in the plasma and the CSF concentrations over time in both groups of racemic ketamine. Figure 2 also shows changes of concentration over time in both groups of S(+)-ketamine. Plasma concentrations were significantly higher than the CSF concentrations throughout our study period in both racemic ketamine and S(+)-ketamine epidural

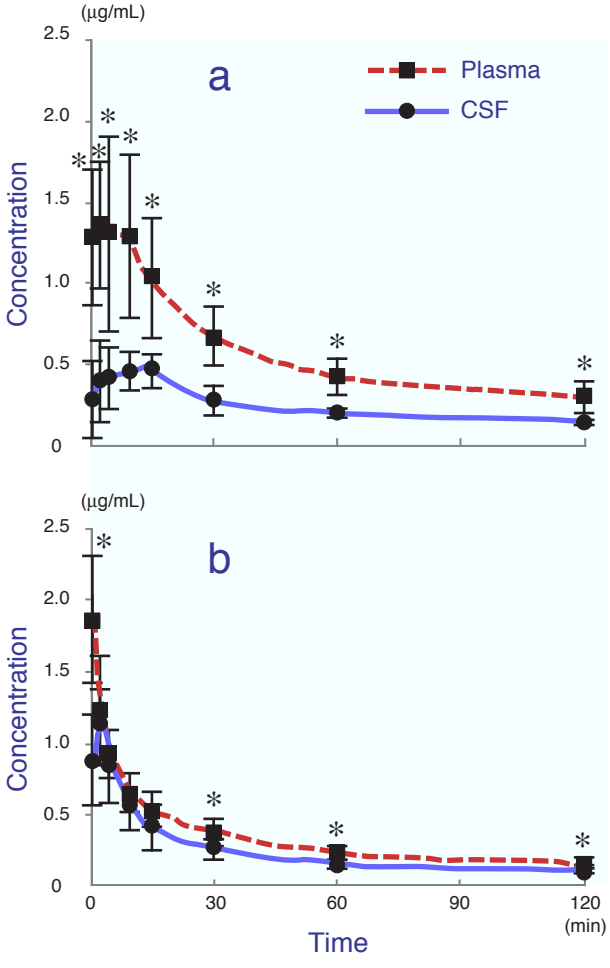
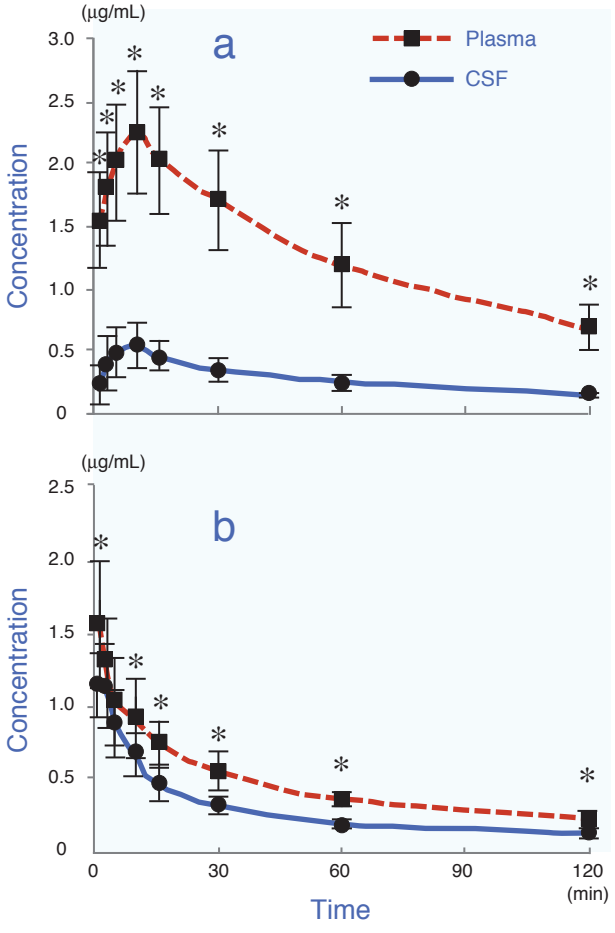


Fig. 1. Time course of concentrations of racemic ketamine in plasma and CSF following a bolus injection of 2 mg/kg epidurally (**a**) and intravenously (**b**). Closed squares represent plasma concentrations. Closed circles represent CSF concentrations. Data are expressed as mean ± SD. These graphs represent concentration curves before pharmacokinetic analyzing. *$P < 0.05$, plasma concentration versus CSF concentration.

Table 1. Pharmacokinetic parameters in epidural administration

			Racemic ketamine	S(+)-Ketamine	*P* value
Plasma	Cmax	(μg/mL)	1.4 ± 0.5	2.1 ± 0.5	0.016*
	Tmax	(min)	6.3 ± 2.8	8.3 ± 2.8	0.167
	T1/2β	(min)	106.4 ± 27.8	85.3 ± 27.6	0.149
	AUC	(μg•min/mL)	111.5 ± 22.8	247.3 ± 61.9	< 0.0001*
	Vdss	(mL)	7754 ± 2172	2992 ± 1055	< 0.0001*
CSF	Cmax	(μg/mL)	0.4 ± 0.1	0.6 ± 0.2	0.055
	Tmax	(min)	9.7 ± 2.1	9.0 ± 3.4	0.595
	T1/2β	(min)	127.1 ± 25.2	89.3 ± 19.4	0.005*
	AUC	(μg•min/mL)	56.4 ± 6.4	56.6 ± 11.0	0.958
	Vdss	(mL)	19463 ± 3266	13613 ± 4895	0.014*

Data are expressed as mean ± SD.
AUC, area under the curve; Cmax, peak concentration; CSF, cerebrospinal fluid; T1/2β, elimination half life; Tmax, time to peak concentration; Vdss, distribution volume.
* $P < 0.05$ for intergroup comparison.



groups. In intravenous groups, plasma concentrations of racemic ketamine were significantly higher than the CSF concentrations at the point of 1, 30, 60 and 120 min. Also plasma concentrations of S(+)-ketamine were significantly higher than the CSF concentration at the point of 1, 10, 15, 30, 60 and 120 min.

Pharmacokinetic parameters

When these agents were administered epidurally, Cmax of racemic ketamine was significantly smaller than that of S(+)-ketamine, AUC of racemic ketamine was significantly smaller than that of S(+)-ketamine and Vdss of racemic ketamine was significantly larger than that of S(+)-ketamine in the plasma. In the CSF, T1/2β of racemic ketamine was significantly longer than that of S(+)-ketamine and Vdss of racemic ketamine was significantly larger than that of S(+)-ketamine (Table 1). On the other hand, when these drugs were injected intravenously, T1/2β of racemic ketamine was significantly

Fig. 2. Time course of concentrations of S(+)-ketamine in plasma and CSF following bolus injection of 2 mg/kg epidurally (**a**) and intravenously (**b**). Closed squares represent plasma concentrations. Closed circles represent CSF concentrations. Data are expressed as mean ± SD. These graphs represent concentration curves before pharmacokinetic analyzing. *$P < 0.05$, plasma concentration versus CSF concentration.

Table 2. Pharmacokinetic parameters in intravenous administration

			Racemic ketamine	S(+)-Ketamine	*P* value
Plasma	Cmax	(μg/mL)	2.4 ± 1.0	1.7 ± 0.4	0.123
	T1/2β	(min)	145.9 ± 56.6	87.6 ± 22.8	0.017*
	AUC	(μg•min/mL)	87.8 ± 24.2	93.9 ± 17.2	0.571
	Vdss	(mL)	11666 ± 4043	7287 ± 1583	0.013*
CSF	Cmax	(μg/mL)	0.6 ± 0.3	0.7 ± 0.2	0.625
	Tmax	(min)	6.0 ± 2.6	5.6 ± 3.8	0.829
	T1/2β	(min)	143.6 ± 34.8	120.1 ± 36.4	0.207
	AUC	(μg•min/mL)	54.9 ± 16.6	56.9 ± 14.3	0.797
	Vdss	(mL)	20523 ± 4762	16064 ± 3925	0.060

Data are expressed as mean ± SD.
AUC, area under the curve; Cmax, peak concentration; CSF, cerebrospinal fluid; T1/2β, elimination half life; Tmax, time to peak concentration; Vdss, distribution volume.
* $P < 0.05$ for intergroup comparison.

Table 3. Comparison of CSF concentrations of racemic ketamine and S(+)-ketamine between epidural and intravenous groups

			Racemic ketamine	S(+)-Ketamine	*P* value
Plasma	Cmax	(μg/mL)	0.4 ± 0.1	0.6 ± 0.3	0.076
	Tmax	(min)	9.7 ± 2.1	6.0 ± 2.6	0.007*
	T1/2β	(min)	127.1 ± 25.0	143.6 ± 34.8	0.294
	AUC	(μg•min/mL)	56.4 ± 6.4	54.9 ± 16.6	0.814
	Vdss	(mL)	19463 ± 3266	20523 ± 4762	0.612
CSF	Cmax	(μg/mL)	0.6 ± 0.2	0.7 ± 0.2	0.302
	Tmax	(min)	9.0 ± 3.4	5.6 ± 3.8	0.081
	T1/2β	(min)	89.3 ± 19.4	120.1 ± 36.4	0.053
	AUC	(μg•min/mL)	56.6 ± 11.0	56.9 ± 14.3	0.966
	Vdss	(mL)	13613 ± 4895	16064 ± 3925	0.288

Data are expressed as mean ± SD.
AUC, area under the curve; Cmax, peak concentration; CSF, cerebrospinal fluid; T1/2β, elimination half life; Tmax, time to peak concentration; Vdss, distribution volume.
* $P < 0.05$ for intergroup comparison.

longer than that of S(+)-ketamine and Vdss of racemic ketamine in the plasma was significantly larger than that of S(+)-ketamine in the plasma (Table 2). Tmax was significantly shorter when administered intravenously than when administered epidurally ($P = 0.007$) in the pharmacokinetic parameters on the shift to the CSF of racemic ketamine (Table 3). After completion of the experiments, the location of epidural catheter tip was confirmed by staining of the epidural space from L1 to L6 level.

Discussion

The major findings were as follows: i) The CSF concentrations of both racemic ketamine and S(+)-ketamine approximated to their plasma concentrations 3 min after intravenous administration. Thereafter their concentration curves over time in the CSF were similar to those in the plasma. ii) The CSF concentration of both racemic ketamine and S(+)-ketamine were significantly lower than their plasma concentration after epidural adminis-

tration. These findings indicate that intravenously administered ketamine rapidly and massively shifts into the CSF through the BBB. Furthermore epidurally administered ketamine is rapidly absorbed into the systemic circulation through the epidural vessels and shifts into the CSF. Increase in the CSF concentrations of racemic ketamine until 10 min after epidural administration is considered to be conducted by the amount of ketamine, which shifted to the CSF across the BBB and penetrated into the CSF across the dura mater. As AUC of racemic ketamine in the plasma is significantly larger than that in the CSF (111.5 μg•min/mL in the plasma versus 56.4 μg•min/mL in the CSF, $P < 0.0001$), amount of ketamine absorbed into the plasma from the epidural space is likely to be more than that absorbed directly into the CSF. Taking rapid shift of racemic ketamine from the plasma to the CSF into consideration, we propose that shift from the plasma to the CSF across the BBB may play an important role in increase of the CSF concentration of racemic ketamine.

It has been still unclear how the intravenously administered ketamine shifts to the CSF across the BBB. Furthermore, our results demonstrated that they shifted rapidly to the CSF across the BBB after an intravenous bolus injection. Their CSF concentrations approximated to their plasma concentrations 3 min after intravenous administration, thereafter, their concentration curves over time in the CSF were similar to those in the plasma. This is the first report to indicate that both racemic ketamine and S(+)-ketamine rapidly get across the BBB.

Horiuchi et al. (1991) reported that racemic ketamine administered epidurally in rabbits showed none of the inhibition of somatosensory evoked eyelid microvibration produced by stimulation of sciatic and ulnar nerves, concluding that racemic ketamine administered into the epidural space did not produce spinal segmental action. If racemic ketamine penetrated into the CSF through the dura mater and increased its concentration in the CSF more than that in the plasma as mentioned previously, somatosensory evoked potential would be inhibited by intrathecal racemic ketamine. Therefore, racemic ketamine unlikely shifts into the CSF through the dura mater in rabbits. Moreover, there is no report on analgesic effect produced by epidural ketamine in dogs to our knowledge. Our results and the above-mentioned phamacodynamic results suggested that the direct penetration to the CSF across the dura mater of racemic ketamine would be less in rabbits than that in dogs previously described by Pedraz (Pedraz et al., 1991).

In the comparison of racemic ketamine with S(+)-ketamine on pharmacokinetic analysis, S(+)-ketamine showed a shorter elimination half time from the plasma and a smaller distribution volume in the plasma than racemic ketamine. There were no significant differences in pharmacokinetic parameters on the CSF between racemic ketamine and S(+)-ketamine. These findings suggest that S(+)-ketamine is a beneficial drug for the patient rather than racemic ketamine to get pharmacological action as well as racemic ketamine in the CSF and to reduce the incidence of adverse effects when administered intravenously. S(+)-ketamine had similar pharmacokinetic parameters to those of racemic ketamine when administered epidurally. The concentrations of S(+)-ketamine in the plasma were significantly more than those in the CSF throughout the study period. Its AUC in the plasma also was larger than that in the CSF indicating that S(+)-ketamine was absorbed to the plasma more than to the CSF. We believed that S(+)-ketamine administered epidurally shifted to the CSF across the BBB rather than by direct penetration through the dura mater as well as racemic ketamine. Interestingly, S(+)-ketamine had a larger AUC and a smaller Vdss compared with racemic ketamine when they had administered epidurally. This indicates that S(+)-ketamine is absorbed from the epidural space to the plasma more than racemic ketamine and attains to higher plasma concentration than racemic ketamine, resulting in a significantly higher Cmax of S(+)-ketamine in the plasma than racemic ketamine. S(+)-ketamine had a significantly shorter T1/2β and a smaller Vdss in the CSF than racemic ketamine. This is the first report that demonstrated different pharmacokinetic characteristics between racemic ketamine and S(+)-ket-

amine. The differences were probably elicited by the difference in structures of racemic mixture and the S(+)-isomer of ketamine. Fast disappearance from the CSF and small drug distribution in the CSF appears to decrease the intensity of adverse effect, suggesting that S(+)-ketamine is a preferable agent to the patient rather than racemic ketamine when administered epidurally.

We set study period on the basis of the results of previous study (Pedraz et al., 1991). However, actual elimination half times in the CSF after intravenous and epidural administration of racemic ketamine were longer than our study period (120 min). The study period longer than 120 min would prolong the elimination half time and increase AUC. However, addition of points for collecting samples beyond 120 min after a bolus injection is not considered to affect our results on pharmacokinetic parameters because concentration curves of racemic ketamine in the plasma and the CSF became very gentle decline from 60 min after administration.

In conclusion, racemic ketamine administered epidurally shifted into the plasma and passed easily through the BBB. And same as racemic ketamine, S(+)-isomer of ketamine were absorbed from the epidural space to the plasma significantly more than to the CSF when administered epidurally. Passage into the CSF across the BBB through the systemic circulation may play a predominant role in the shift of ketamine from the epidural space to the CSF in rabbits.

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