JPP Journal of Pharmacy and Pharmacology

JPP 2008, 60: 1149–1154

Received March 10, 2008
Accepted April 25, 2008
DOI 10.1211/jpp.60.9.0005
ISSN 0022-3573

Pharmacokinetics of tramadol in rat plasma and cerebrospinal fluid after intranasal administration

Yan Zhao, Tao Tao, Jinjin Wu, Jiaxin Pi, Ning He, Xuyu Chai and Qinghua Chen

Abstract

We have evaluated the potential of intranasal administration of tramadol. The pharmacokinetic behaviour of tramadol in rat plasma and cerebrospinal fluid (CSF) after intranasal administration was determined and compared with those after intravenous and oral administration. Serial plasma and CSF samples were collected for 6 h, and the drug concentrations were assayed by an HPLC-fluorescence method. The plasma absolute bioavailability values of tramadol after intranasal and oral administration were 73.8% and 32.4%, respectively, in conscious rats. The C_{max} (maximum concentration) value after the intranasal dose was lower ($P < 0.05$), and the MRT (mean retention time) was longer ($P < 0.05$) than the values obtained after intravenous administration. A pharmacokinetic study of tramadol in plasma and CSF was undertaken in anaesthetized rats. The absolute bioavailability values in plasma and CSF after intranasal administration were 66.7% and 87.3%, respectively. The C_{max} values in plasma and CSF after a nasal dose were lower ($P < 0.05$) than after the intravenous dose. The values of C_{max} and $AUC_{0\rightarrow 6\,h}$ in plasma and CSF after intranasal administration were higher than after the oral dose. The mean drug-targeting efficiency after intranasal administration was significantly greater than after the oral dose. In conclusion, intranasal administration of tramadol appeared to be a promising alternative to the traditional administration modes for this drug.

Introduction

Increased attention has been paid to the intranasal (i.n.) administration of analgesics due to its intrinsic advantages over traditional administration, such as more rapid drug absorption, more rapid onset of pain relief compared with oral dosing, and good compliance without complicated administration method or medical service. Some nasal delivery systems of analgesics such as fentanyl, morphine and ketorolac have undergone clinical trials (reported on the official website of the National Institutes of Health). Pharmacokinetic studies in volunteers for nasal administration of fentanyl, alfentanil and sufentanil reported that the mean t_{max} (time to achieve maximum concentration) in serum was less than 10 min, and the mean bioavailability varied from 65 to 78% (Dale et al 2002). Furthermore, intranasal administration was found to be a potential access to the central nervous system (CNS) via the olfactory mucosa. Many substances have shown direct entry to the CNS through the nasal cavity such as vasoactive intestinal peptide, methotrexate, insulin-like growth factor-I, indometacin, interferon beta, zolmitriptan, stavudine, huperzine A, and morphine (Dufes et al 2003; Wang et al 2003; Thorne et al 2004; Dontas et al 2004; Ross et al 2004; Yang et al 2005; Vyas et al 2005; Tao et al 2006; Westin et al 2006).

Tramadol hydrochloride is a synthetic, centrally-acting analgesic, and clinically it is used widely for moderate-to-severe acute and chronic pain. Its chemical name is (±)*cis*-2-((dimethylamino)methyl)-1-(3-methoxyphenyl)cyclohexanol hydrochloride and it has a molecular weight of 299.8 (Figure 1). It is very soluble in water, is freely soluble in ethanol, and has a pK_a of 9.41. The n-octanol/water log partition coefficient (log P) is 1.35 at pH 7.

Among the traditional administration modes, intravenous injection usually requires medical service and oral administration appears to have a slow analgesic response due to absorption. Therefore, an effective therapeutic strategy of tramadol with a quick onset of action, high bioavailability and good compliance needs exploration.

Division of Pharmaceutics, Shanghai Institute of Pharmaceutical Industry, Zhong Shan Bei Yi Road 1111, Shanghai 200437, China

Yan Zhao, Tao Tao, Jinjin Wu, Jiaxin Pi, Ning He, Xuyu Chai, Qinghua Chen

Correspondence: T. Tao Division of Pharmaceutics, Shanghai Institute of Pharmaceutical Industry, Zhong Shan Bei Yi Road 1111, Shanghai 200437, China.
E-mail: taotaosipi@hotmail.com

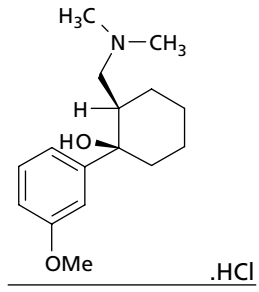

Figure 1 The molecular structure of tramadol hydrochloride.

Fisher et al (1987) reported that aqueous channel mechanisms functioned for the nasal absorption of water-soluble compounds, and the log molecular weight gave a good linear correlation with the log per cent intranasally absorbed (increasing absorption with lower molecular weight). Nasal administration of tramadol hydrochloride could be a promising administration mode, based on its having a small molecular weight and being water soluble.

We have investigated the pharmacokinetic behaviour of tramadol in plasma and cerebrospinal fluid (CSF) to determine whether nasal administration would have quick onset of action and high bioavailability.

Materials and Methods

Chemicals and animals

Tramadol hydrochloride was purchased from Zhejiang Haixiang Pharmaceutical Co. Ltd (China). HPLC-grade acetonitrile was purchased from Tedia Company (US). CP grade ethyl carbamate, analytical grade diethylamine and phosphoric acid were purchased from Sinopharm Chemical Reagent Co., Ltd (China). Water was distilled.

The protocol for animal use and care was approved by the institutional Animal Care and Use Committee of Shanghai Institute of Pharmaceutical Industry. Male Sprague–Dawley rats, with an average weight of approximately 300 g (range 280–320 g), were housed at 25 ± 2°C with free access to water and standard laboratory food.

Three different tramadol hydrochloride solutions were prepared for the three different routes of administration. For intranasal administration tramadol hydrochloride was dissolved in water (60 mg mL^{-1}), and the pH was adjusted to 6.5 with 1 mol L^{-1} NaOH. For intravenous administration, a solution of tramadol hydrochloride (5 mg mL^{-1}) was prepared in saline. A solution (5 mg mL^{-1}) in water was used for oral dosing.

Plasma pharmacokinetics in conscious rats

Rats were anaesthetized for several minutes with ether before intranasal administration of tramadol hydrochloride (5.2 mg kg^{-1}). A PE-10 tube was inserted 1 cm into the left nostril, and the other end of the tube was attached to a microlitre syringe containing the drug solution. For intravenous administration, the tramadol hydrochloride solution (5.2 mg kg^{-1}) was delivered through the caudal vein. Oral gavage of tramadol hydrochloride (5.2 mg kg^{-1}) was performed by attachment of a stainless steel feeding needle to a 1-mL syringe containing the drug solution. At 5, 15, 30, 45, 60, 90, 120, 180, 240 and 360 min after intranasal or oral administration blood was collected from the tail vein. Blood was collected from the retro-orbital plexus at 2, 5, 15, 30, 45, 60, 120, 180, 240 and 360 min after intravenous injection.

Plasma and CSF pharmacokinetics in anaesthetized rats

Rats were anaesthetized with an intraperitoneal dose of 30% (w/v) ethyl carbamate (1 g kg^{-1}) and kept under anaesthesia throughout the experiment. Tramadol hydrochloride (5.2 mg kg^{-1}) was administered by three different routes of administration. Blood was collected from the tail vein at the same sampling time as in the conscious rat study. CSF samples were collected from cannulation of the cisterna magna (Yue et al 2007). The animal was fixed onto a stereotaxic apparatus (Jiangwan I-C, Shanghai, China). The skin overlying the occipital bone was incised, and the underlying muscle and tissue were bluntly dissected so that the atlanto-occipital (a–o) membrane was identified and freed from other tissues. A 25-gauge needle connected to a 10 cm PE-10 tube was punctured into the cisterna magna through the a-o membrane. Once CSF flowed into the tube due to the inner pressure, the mucilage was used to fasten the needle with the membrane. A 100-μL microsyringe was connected to the other end of the PE-10 tube, and 20 μL CSF was withdrawn at the same time points as the blood sampling.

Blood samples were anticoagulated with heparin and centrifuged at 5000 *g* for 10 min to obtain the plasma. The samples of plasma and CSF were stored in a deep freezer at −20°C until HPLC analysis.

Analytical procedure

Acetonitrile (200 μL) was added to 100-μL plasma samples. After vortex-mixing for 5 min, the mixture was centrifuged at 5000 *g* for 10 min. Samples (20 μL) of the supernatant were injected onto the HPLC system. CSF samples were allowed to thaw and were centrifuged at 20000 *g* for 10 min before HPLC analysis. The injection volume was 15 μL.

The HPLC system consisted of a LC-10AD VP delivery system, RF-10AXL fluorescence detector, and CLASS-VP chromatographic integrator (Shimadzu, Japan). The separation was performed on an Inertsil ODS-3 (250 mm × 4.6 mm, 5 μm) with a mobile phase of acetonitrile:water:triethanol-amine: phosphoric acid (22:77:0.1:0.14) with a 1 mL min^{-1} flow rate. Detector excitation at 275 nm and emission at 296 nm were used.

Pharmacokinetic calculations

The C_{max} (maximum concentration) and t_{max} (time to achieve maximum concentration) values were obtained from the concentration–time profile. The area under the concentration–time curve ($AUC_{0\rightarrow t}$) was calculated by the trapezoidal rule. AUC_{0-t} t½ (half life) and MRT (mean retention time) were calculated with Kinetica4.4. The absolute oral or nasal

bioavailability (F) of tramadol hydrochloride was calculated as the ratio of $AUC_{i.n.}$ (AUC_{oral}) to $AUC_{i.v}$:

$$F_{i.n.} = (AUC_{i.n.} \times Dose_{i.v.})/(AUC_{i.v.} \times Dose_{i.n.}) \times 100\%$$

$$F_{oral} = (AUC_{oral} \times Dose_{i.v.})/(AUC_{i.v.} \times Dose_{oral}) \times 100\%$$

Drug targeting efficiency (DTE) (Vyas et al 2005) was calculated to evaluate the brain targeting of the drug via intranasal and oral administration routes. DTE and S_{DTE} (the standard deviation of DTE) were estimated by the following formulas:

$$DTE_{i.n./oral} = (AUC_{CSF}/AUC_{plasma})_{i.n./oral}/(AUC_{CSF}/AUC_{plasma})_{i.v.}$$

$$S_{DTE} = DTE\sqrt{\left(\frac{S_{(AUC_{CSF}/AUC_{plasma})_{i.n./oral.}}}{(AUC_{CSF}/AUC_{plasma})_{i.n./oral}}\right)^2 + \left(\frac{S_{(AUC_{CSF}/AUC_{plasma})_{i.v.}}}{(AUC_{CSF}/AUC_{plasma})_{i.v.}}\right)^2}$$

Statistical analysis

All data were reported as mean ± s.d. and the error bars in the figures represent the standard deviation. Statistical significance was assured by analysis of variance performed in one-way analysis of variance followed by the Tukey test with SPSS 13.0 for Windows. A difference resulting in $P < 0.05$ was considered statistically significant.

Results and Discussion

Plasma pharmacokinetics in conscious rats

After intravenous administration, the drug was quickly eliminated, and at 2 h the concentration in plasma had decreased to one-tenth of the maximum concentration; for oral dosing, the drug concentration in plasma was always low and the $AUC_{0\rightarrow t}$ was small, and the time to achieve the C_{max} was approximately 1 h (Figure 2). Tramadol hydrochloride was absorbed and eliminated quickly after intranasal administration, and the drug concentration in plasma was unexpectedly high at 5 min after the dose. It indicated that nasal tramadol had rapid absorption and apparent onset of effect, maybe because tramadol hydrochloride has a small molecular weight and is easily absorbed through the nasal mucosa directly into the systemic circulation, thus bypassing the first-pass metabolism. Additionally, in the nasal cavity the mucus is transported (mostly towards the pharynx, after which it will be swallowed) by coordinated ciliary motions. Therefore, some drops of the drug solution were trapped in the mucus, and then absorbed into plasma through the gastrointestinal tract. This resulted in the plasma concentration–time curve of a biphasic nature for intranasal tramadol (Figure 2).

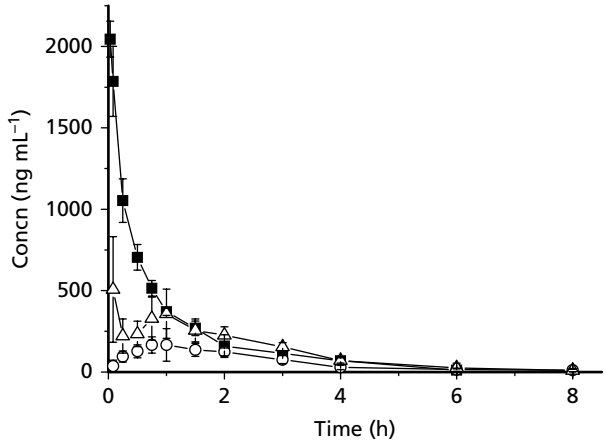

Figure 2 Mean plasma concentration–time curves of tramadol hydrochloride after a single dose of 5.2 mg kg^{-1} to conscious male rats via three routes (n = 6)(■, intravenous; ○, oral; △, intranasal).

Plasma pharmacokinetic parameters after a single dose of 5.2 mg kg^{-1} to male rats via three routes were calculated. Since the intranasal administration of tramadol resulted in significantly ($P < 0.05$) lower C_{max} and longer MRT values than after intravenous injection (Table 1), it suggested that nasal delivery might achieve lower toxicity and longer residence in plasma than after intravenous administration. The AUC after intranasal administration was significantly higher ($P < 0.05$) than after oral dosing. This indicated that intranasal administration of tramadol gave higher bioavailability than after oral administration.

Plasma and CSF pharmacokinetics in anaesthetized rats

The plasma and CSF drug concentration–time curves via three routes are shown in Figures 3 and 4, respectively. The drug concentrations in plasma and CSF were relatively high at 10 min after the intranasal dose, indicating quick onset of action for the nasal route. During 0–6 h after dosing, CSF and plasma drug concentrations after intranasal administration

Table 1 Plasma pharmacokinetic parameters after a single dose of tramadol hydrochloride (5.2 mg kg^{-1}) to conscious rats by three routes

	t_{max} (h)	C_{max} (ng mL^{-1})	AUC (ng mL^{-1} h)	t½ (h)	MRT (h)	F%
Intranasal	0.39 ± 0.53	524.66 ± 303.43[a]	1040.69 ± 289.03[b]	1.32 ± 0.10	2.35 ± 0.33[a]	73.8
Oral	1.00 ± 0.52	190.23 ± 89.77[a]	456.28 ± 72.80[a,c]	1.58 ± 0.70[a]	2.76 ± 0.93[a]	32.4
Intravenous	0.033 ± 0.000	2044.09 ± 110.73[b,c]	1409.84 ± 217.70[b]	0.99 ± 0.11[b]	1.22 ± 0.09[b,c]	100

Values are mean ± s.d., n = 6. [a]$P < 0.05$ significantly different compared with intravenous; [b]$P < 0.05$ significantly different compared with oral; [c]$P < 0.05$ significantly compared with intranasal.

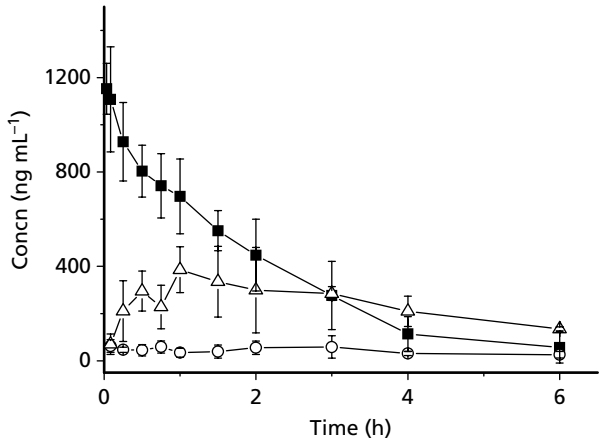

Figure 3 Mean plasma concentration–time curves of tramadol hydrochloride after a single dose of 5.2 mg kg^{-1} to anaesthetized male rats (■, intravenous; ○, oral; △, intranasal).

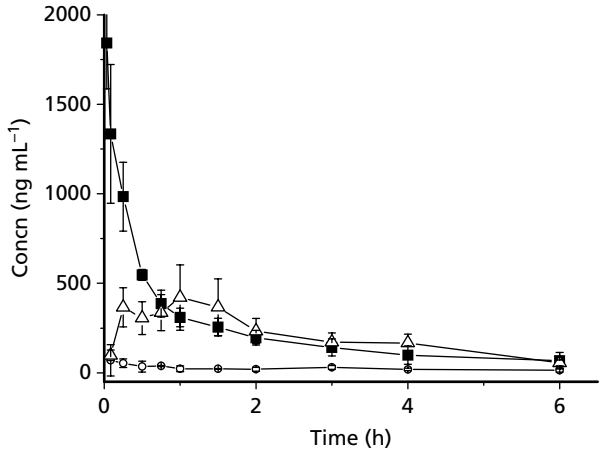

Figure 4 Mean CSF concentration–time curves of tramadol hydrochloride after a single dose of 5.2 mg kg^{-1} to anaesthetized male rats (■, intravenous; ○, oral; △, intranasal).

were higher than those for oral administration. As for the intravenous dose, the concentration–time curves showed that the drug was eliminated more quickly in CSF than in plasma. In addition, there was no absorption or elimination phase shown in the concentration–time curve after oral dosing, indicating that drug absorption through the gastrointestinal tract would be affected greatly by the anaesthetized state.

The pharmacokinetic parameters are shown in Table 2. There was no elimination phase in the concentration–time curve after oral dosing (Figures 3–4), and so the values for t½ and MRT could not be estimated. The values for C_{max} in CSF and plasma after intranasal administration were significantly lower ($P<0.05$) compared with those after intravenous administration, indicating that the nasal route could have a lower toxicity than the intravenous route. After intranasal administration, the MRT in plasma was significantly longer ($P<0.05$) than after intravenous administration, suggesting that nasal delivery gave longer residence of the drug in plasma than after intravenous delivery. The results were consistent with those from conscious animals. The AUC_{plasma} after nasal delivery was significantly lower ($P<0.05$) than after intravenous administration, however, the AUC_{CSF} by the nasal route was not statistically different ($P>0.05$) from the intravenous route. The C_{max} and AUC in CSF and plasma after nasal administration were significantly higher ($P<0.05$) than those after the oral dose. It was concluded that nasal administration resulted in a higher bioavailability in CSF and plasma than after oral administration.

After intravenous administration, the drug $AUC_{CSF\ 0\to6\,h}$ was 64% of $AUC_{plasma\ 0\to6\,h}$, which meant that tramadol could pass into the CNS across the blood–brain barrier (BBB). For oral dosing, the drug $AUC_{CSF\ 0\to6\,h}$ was approximately 58% of $AUC_{plasma\ 0\to6\,h}$, which was similar to that after the intravenous dose, meaning that the drug went into the CNS across the BBB. By intranasal administration, the ratio of $AUC_{CSF\ 0\to6\,h}$ to $AUC_{plasma\ 0\to6\,h}$ was 97%, which was higher than either the intravenous or oral routes. It confirmed that there was a direct nose–brain pathway for tramadol to enter the CNS besides penetration across the BBB by intranasal delivery.

DTE of intranasal administration of tramadol

Tramadol is a centrally-acting analgesic, so its pharmacokinetic behaviour in the CNS is important. DTE was used to measure whether tramadol could pass into the CNS directly via the nasal cavity. If the DTE was larger than 1 it would suggest that some drug could pass into the CNS via the nose–brain passage rather than through the BBB. The DTE values

Table 2 Plasma and CSF pharmacokinetic parameters after a single dose of tramadol hydrochloride (5.2 mg kg^{-1}) to anaesthetized rats by three routes

		t_{max} (h)	C_{max} (ng mL^{-1})	$AUC_{0\to6\,h}$ (ng mL^{-1} h)	t½ (h)	MRT (h)	F%
Plasma	Intranasal	1.25 ± 0.35	403.50 ± 25.41[a,b]	1406.63 ± 55.10[a,b]	2.49 ± 0.47[a]	4.27 ± 0.63[a]	66.70
	Oral	1.46 ± 1.30	94.39 ± 21.21[a,c]	241.76 ± 54.49[a,c]	–	–	11.46
	Intravenous	0.03 ± 0.00	1152.79 ± 107.79[b,c]	2108.85 ± 323.10[b,c]	1.15 ± 0.58[c]	1.89 ± 0.72[c]	100
CSF	Intranasal	1.25 ± 0.35	440.97 ± 28.83[a,b]	1224.62 ± 37.59[b]	1.63 ± 0.26	2.83 ± 0.24	87.33
	Oral	1.02 ± 1.35	81.01 ± 60.23[a,c]	140.43 ± 29.00[a,c]	–	–	10.01
	Intravenous	0.03 ± 0.00	1842.62 ± 255.65[b,c]	1402.21 ± 195.07[b]	1.84 ± 0.62	2.30 ± 0.88	100

[a]$P<0.05$ significantly different compared with intravenous; [b]$P<0.05$ significantly different compared with oral; [c]$P<0.05$ significantly compared with intranasal.

Table 3 Drug targeting efficiency of intranasal and oral dosing of tramadol hydrochloride (5.2 mg kg^{-1}) to rats

Time (h)	Drug targeting efficiency	
	Intranasal	Oral
0.083	1.22 ± 0.59	0.75 ± 0.67
0.25	1.40 ± 0.49	0.70 ± 0.41
0.5	1.38 ± 0.37	0.82 ± 0.30
0.75	1.48 ± 0.33	0.87 ± 0.33
1	1.63 ± 0.33	0.97 ± 0.47
1.5	1.72 ± 0.27	1.10 ± 0.48
2	1.71 ± 0.21	0.92 ± 0.39
3	1.55 ± 0.08	0.86 ± 0.28
4	1.55 ± 0.17	0.93 ± 0.30
6	1.36 ± 0.20	0.90 ± 0.32
x	1.50 ± 0.37	0.88 ± 0.42

during 0–6 h after the intranasal dose were more than 1 (1.22~1.72), while those after the oral dose were 0.70~1.10 (Table 3). It meant that intranasal administration would exhibit brain targeting and some part of the drug could enter the CNS via a direct nose–brain pathway.

Comparison of plasma pharmacokinetic behaviour of tramadol in conscious and anaesthetized rats

The plasma pharmacokinetic behaviour of tramadol in anaesthetized rats was compared with conscious rats (Figure 5). After intravenous administration drug elimination was slower in anaesthetized than conscious rats. After nasal administration, the anaesthetized rats showed a slower absorption and elimination than conscious rats. In conscious rats, the AUC_{plasma} after intranasal administration was not significantly different from after intravenous, but in anaesthetized rats it was significantly lower ($P < 0.05$) compared with after intravenous administration (Tables 1 and 2). This should be attributed to the reduced absorption in anaesthetized rats. The anaesthetized state would have a greater effect on drug absorption and elimination, especially for the oral dose. The plasma absolute bioavailability of the oral tramadol dose in anaesthetized rats was only one-third of that in conscious rats and the plasma concentration–time curve did not give the notable C_{max} in anaesthetized rats. These results might have been due to the irregular decreased absorption through the gastrointestinal tract in the anaesthetized state. The effect on drug pharmacokinetics of anaesthesia should not be neglected. It is necessary to find a proper method of anaesthesia or animal model

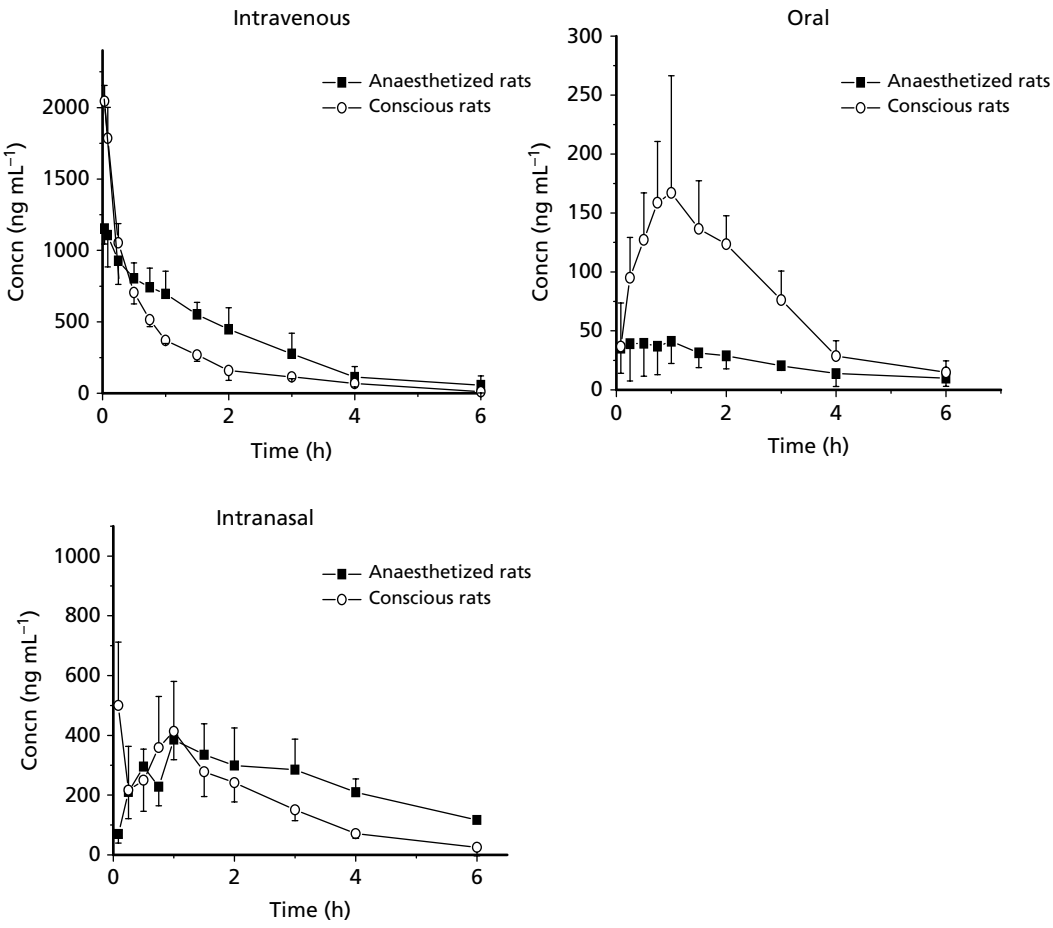

Figure 5 Mean plasma concentration–time profiles of tramadol after three administration routes to conscious or anaesthetized male rats.

to study the drug pharmacokinetics in plasma and CSF simultaneously. Even though the pharmacokinetic behaviour of the oral tramadol dose was significantly affected by anaesthesia, the DTE was believable.

Conclusion

Tramadol hydrochloride was absorbed and eliminated quickly after intranasal administration. The drug concentration in plasma and CSF was relatively high at 15 min after the intranasal dose. The absolute bioavailability in plasma of the nasal tramadol solution for conscious and anaesthetized rats was 73.8% and 66.7%, respectively. The mean DTE after intranasal administration was greater than one, indicating the existence of a direct nose–brain pathway for tramadol. In addition, mucociliary clearance transported drops of the drug solution from the nasal cavity. This limited the time available for absorption (Merkus et al 1998). It was reported that several preparations such as in-situ gel (Tao et al 2006) and microspheres (Sankar et al 2001; Rajinikanth et al 2003; Mao et al 2004) could be prepared to prolong the drug's residence in the nasal cavity and thus increase the bioavailability. Therefore, further studies are necessary to optimize formulations of nasal tramadol to achieve a higher bioavailability and to achieve a sustained drug release.

It could be concluded that intranasal administration of tramadol would have a quick onset of action and high bioavailability. Nasal administration of tramadol could be a promising alternative to its traditional administration modes.

References

Dale, O., Hjortkjaer, R., Kharasch, E. D. (2002) Nasal administration of opioids for pain management in adults. *Acta Anaesthesiol. Scand*. **46**: 759–770

Dontas, I., Sokolis, D. P., Giamarellos-Bourboulis, E. J., Tzonou, A., Giamarellou, H., Karayannacos, P. E. (2004) The influence of indomethacin co-administration on ofloxacin levels in plasma and cerebrospinal fluid in rats. *Int. J. Antimicrob. Agents* **23**: 371–376

Dufes, C., Olivier, J. C., Gaillard, F., Gaillard, A., Couet, W., Muller, J. M. (2003) Brain delivery of vasoactive intestinal peptide (VIP) following nasal administration to rats. *Int. J. Pharm*. **255**: 87–97

Fisher, A. N., Brown, K., Davis, S. S., Parr, G. D., Smith, D. A. (1987) The effect of molecular size on the nasal absorption of water-soluble compounds in the albino rat. *J. Pharm. Pharmacol*. **39**: 357–362

Mao, S., Chen, J., Wei, Z., Liu, H., Bi, D. (2004) Intranasal administration of melatonin starch microspheres. *Int. J. Pharm*. **272**: 37–43

Merkus, F. W., Verhoef, J. C., Schipper, N. G., Marttin, E. (1998) Nasal mucociliary clearance as a factor in nasal drug delivery. *Adv. Drug Deliv. Rev*. **29**: 13–38

Rajinikanth, P. S., Sankar, C., Mishra, B. (2003) Sodium alginate microspheres of metoprolol tartrate for intranasal systemic delivery: development and evaluation. *Drug Deliv*. **10**: 21–28

Ross, T. M., Martinez, P. M., Renner, J. C., Thorne, R. G., Hanson, L. R., Frey, W. H. (2004) Intranasal administration of interferon beta bypasses the blood-brain barrier to target the central nervous system and cervical lymph nodes: a non-invasive treatment strategy for multiple sclerosis. *J. Neuroimmunol*. **151**: 66–77

Sankar, C., Rani, M., Srivastava, A. K., Mishra, B. (2001) Chitosan based pentazocine microspheres for intranasal systemic delivery: development and biopharmaceutical evaluation. *Pharmazie* **56**: 223–226

Tao, T., Zhao, Y., Yue, P., Dong, W. X., Chen, Q. H. (2006) Preparation of huperzine A nasal in situ gel and evaluation of its brain targeting following intranasal administration. *Yao Xue Xue Bao* **41**: 1104–1110

Thorne, R. G., Pronk, G. J., Padmanabhan, V., Frey, W. H. (2004) Delivery of insulin-like growth factor-I to the rat brain and spinal cord along olfactory and trigeminal pathways following intranasal administration. *Neuroscience* **127**: 481–496

Vyas, T. K., Babbar, A. K., Sharma, R. K., Misra, A. (2005) Intranasal mucoadhesive microemulsions of zolmitriptan: preliminary studies on brain-targeting. *J. Drug Target*. **13**: 317–324

Wang, F., Jiang, X., Lu, W. (2003) Profiles of methotrexate in blood and CSF following intranasal and intravenous administration to rats. *Int. J. Pharm*. **263**: 1–7

Westin, U. E., Boström, E., Gråsjö, J., Hammarlund-Udenaes, M., Björk, E. (2006) Direct nose-to-brain transfer of morphine after nasal administration to rats. *Pharm. Res*. **23**: 565–572

Yang, Z., Huang, Y., Gan, G., Sawchuk, R. J. (2005) Microdialysis evaluation of the brain distribution of stavudine following intranasal and intravenous administration to rats. *J. Pharm. Sci*. **94**: 1577–1588

Yue, P., Tao, T., Zhao, Y., Ren, J., Chai, X. (2007) Huperzine A in rat plasma and CSF following intranasal administration. *Int. J. Pharm*. **337**: 127–132