# Intranasal Administration of Milnacipran in Rats: Evaluation of the Transport of Drugs to the Systemic Circulation and Central Nervous System and the Pharmacological Effect

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Recently, transnasal drug delivery has attracted a great deal of attention as an administration route to deliver drugs directly to the central nervous systems (CNS) and drug targeting of the CNS is expected to increase. In the present study, we investigated the possibility of using a transnasal delivery system for milnacipran, a serotonin–noradrenaline reuptake inhibitor (SNRI), by evaluating the transport to the systemic circulation and cerebrospinal fluid (CSF) and the pharmacological effect after intranasal (i.n.) administration. Moreover, the effect of chitosan as a bioadhesive material on the transport to the systemic circulation and CSF and the pharmacological effect after i.n. administration were evaluated. As a result, i.n. administration of milnacipran was found to produce a higher direct delivery to the CNS as well as to the systemic circulation, suggesting that this is a promising route of administration and an alternative to peroral (p.o.) administration. Furthermore, the i.n. co-administration with chitosan led to increased plasma and CSF concentrations and an enhanced pharmacological effect, evaluated by means of the forced swimming test. The results suggested that chitosan produced a long residence time of milnacipran in the nasal cavity due to its bioadhesive effect, leading to the enhanced transport of milnacipran from the systemic circulation to the CNS *via* the blood–brain barrier by an increase in systemic absorption as well as direct transport to the CNS, resulting in a higher antidepressant effect compared to that with p.o. administration.

**Key words**    intranasal administration; central nervous systems; milnacipran; forced swimming test; chitosan

The number of patients with depression has increased in recent years and this condition has become an important social problem. According to the World Health Organization (WHO), there are about 121 million people in the world presently suffering from depression. It is expected that this number will increase and depression is projected to reach number 2 in the ranking of contributors to the global burden of disease in 2020.[1] The introduction of various drugs, such as tricyclic antidepressants, has led to a more effective treatment of depression. Recently, newer antidepressants that are selective serotonin reuptake inhibitors (SSRIs), such as paroxetine and fluvoxamine, and serotonin–noradrenaline reuptake inhibitors (SNRIs), *i.e.* milnacipran, have been developed and these agents exhibit fewer side effects than the tricyclic antidepressants.[2—4] Currently, one of the recommended first-line treatments for major depression (mild to moderate, single or recurrent nonpsychotic episodes) is the use of SSRIs or SNRIs.[5] However, despite fewer side effects than tricyclic antidepressants, it has been reported that both SSRIs and SNRIs can cause gastrointestinal damage.[6] Moreover, the concentration of the SSRIs, such as fluvoxamine, in the brain was reported to be in proportion to the total dose, but not the daily dose; that is, the increase in concentration was extremely slow, taking more than one week for the concentration to reach steady state.[7] Therefore, enhancement of the delivery of antidepressants to the brain is needed because a sufficient increase in the brain concentration is needed for the appearance of the antidepressant effect.

Transnasal drug delivery is an alternative route to the injection of various drugs. In our previous studies, we have reported that the absorption of model drugs, such as fluorescein isothiocyanate-labeled dextrans with different molecular weights (MW, *ca.* 4, 12.0, 38.3, 50.7, 167.0 kDa, FD-4, FD-10, FD-40, FD-70, FD-150), salmon calcitonin (MW, *ca.* 3.5 kDa), $\alpha$-human atrial natriuretic peptide ($\alpha$-hANP, MW, *ca.* 3.0 kDa) and recombinant human granulocyte colony-stimulating factor (rhG-CSF, MW, 18.8 kDa), from the nasal cavity to the systemic circulation is increased by using poly-L-arginine as an enhancer in rats.[8—10] Thus, this system is a promising alternative route to injection for water-soluble macromolecular drugs such as peptides and proteins. Moreover, recently, direct delivery of drugs from the nasal cavity to the central nervous system (CNS) *via* the olfactory pathway is attracting increasing attention in order to target the drugs directly to the CNS for the treatment of schizophrenia, meningitis, Parkinson's disease and Alzheimer's disease as well as depression; this approach can also lead to a reduction in systemic effects.[11—13] Following peroral (p.o.) and intravenous (i.v.) administration, the drugs must pass through the blood–brain barrier (BBB) and blood–cerebrospinal fluid barrier (BCSFB) for their delivery to the brain. However, most drugs do not easily cross the BBB and BCSFB.[14] Therefore, the drug concentrations required for treatment of the above diseases are not achieved in the brain and cerebrospinal fluid (CSF). In contrast, there may be a possibility of targeting these drugs to the CNS using intranasal (i.n.) administration.[15,16] Because the olfactory neuroepithelium in the nasal cavity is the only part of the CNS where dendritic processes of first order neurons are exposed directly to the environment in the upper nasal passage, and bundles of neu-

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ronal axons project through pores in the cribiform plate of the ethmoid bone to synaptic glomeruli in the olfactory bulb; the nose has the potential to act as a portal for direct delivery of drugs to the CNS.[17,18] Considerable evidence now exists in the literature that drugs administered nasally to animals and humans can be transported directly to the CNS.[18] Also, in the case of i.n. administration, a number of drug transport pathways to the CNS are known, such as the systemic pathway, in which the drug is absorbed across the nasal cavity into the systemic circulation and then across the BBB into the brain; the olfactory pathway, in which the drug passes through the olfactory epithelium (paracellularly and extracellularly) into the olfactory bulb and further into the brain tissue or into the CSF as mentioned above; also there is the trigeminal pathway, in which the drug is transported *via* the nervous system.[18,19] In addition, drug absorption (especially paracellular transport) *via* the olfactory system has been shown to be very fast, with the drug being detected in the CSF and brain tissue at 1 min post-dosing.[18] Hence i.n. administration could have an important impact on the treatment and management of acute conditions such as migraine, panic attacks, nausea, breakthrough and acute pain, and other conditions that would benefit from a very rapid therapeutic effect. Considering the advantages of transnasal drug delivery systems, it may be possible to solve at least some of the problems in the treatment of depression with SSRIs and SNRIs mentioned above. Therefore, the development of a transnasal delivery system for milnacipran, as SNRI, could be significant in the treatment of depression.

Chitosan is a naturally existing polysaccharide composed of glucosamine and *N*-acetylglucosamine residues and can be derived by partial deacetylation of chitin, which is generally obtained from crustacean shells.[20] Chitosan is known to be biocompatible, bioadhesive, with low toxicity and low immunogenicity and degradable by enzymes.[21—23] Chitosan has a cationic charge and has been widely used in many drug delivery applications.[24,25] Many researchers have studied the use of chitosan for i.n. administration.[11—13] Chitosan has been shown to have bioadhesive characteristics and to be retained in the nasal cavity for prolonged periods.[18] Chitosan has also been proven to be an efficient absorption enhancer that can transiently open tight junctions between epithelial cells and thereby significantly enhance the absorption of even large molecular weight polar drugs.[26,27]

In the present study, we investigated the possibility of a transnasal delivery system for milnacipran in rats. The delivery of milnacipran to the systemic circulation and CSF directly after i.n. administration was evaluated. The pharmacological effect of milnacipran after p.o. and i.n. administration was also determined by means of the forced swimming test which is generally used in the development of antidepressants. Moreover, the effect of chitosan as a bioadhesive material for the transport of milnacipran to the systemic circulation and the CSF, and the pharmacological effect after i.n. administration were also evaluated.

## MATERIALS AND METHODS

**Materials** Milnacipran hydrochloride (MW 282.81, p$K_a$ 9.7, partition coefficient (Pc) 1.2 (chloroform/water, 25 °C, pH 7.1), $F$ (%) by p.o. route 85%) was kindly donated by Asahi Kasei Pharma Corporation (Tokyo, Japan). Chitosan (MW 200 kDa) and urethane were purchased from Sigma-Aldrich Co. (St. Louis, MO, U.S.A.). All other reagents were of the highest grade available.

**Animals** Male Wistar rats (8 weeks old, weight 250—300 g) were supplied by Saitama Experimental Animals Supply Co., Ltd. (Saitama, Japan). Animals were allowed food and water *ad libitum*, and were kept under a 12/12 h light/dark cycle with at least 7 d of local vivarium acclimatization before experiments were carried out. All the experiments were performed in accordance with the guidelines for animal use in the Institutional Animal Care and Use Committee, Life Science Research Center, Josai University.

**Preparation of Milnacipran Hydrochloride in Physiological Saline Containing 0.5% Chitosan** Chitosan (100 mg) was dispersed in physiological saline (9.4 ml). Hydrochloric acid (1 mol/l, 0.6 ml) was added to the suspension. After heating the solution in a water bath at 90 °C, 1.0% (w/v) chitosan gel was obtained. The gel was mixed with milnacipran hydrochloride in physiological saline at a ratio of 1 : 1 and then milnacipran hydrochloride in physiological saline containing 0.5% (w/v) chitosan was prepared.

**Intravenous Bolus Injection** Milnacipran hydrochloride in physiological saline (10, 20, 60 mg/kg; 1.0 ml/kg; 10, 20, 60 mg/ml, respectively) was injected into the right jugular vein of the anesthetized rats (urethane, 25% (w/v), 1.0 g/kg i.p.), and the animals underwent the same surgical procedure as in the i.n. administration study.

**Intraduodenal Administration** The intraduodenal (i.d.) administration study was performed by the *in situ* closed-loop method. Briefly, the intestine of anesthetized rats (urethane, 25% (w/v), 1.0 g/kg i.p.) was exposed through a midline abdominal incision. After cannulating the bile duct (bile fistula) to remove the bile, an intestinal loop 5 cm in length (the duodenal loop is up to 5 cm from the pyloric region) was made. The intestinal contents were washed out with physiological saline at 37 °C. After milnacipran hydrochloride in physiological saline (20 mg/kg; 4 ml/kg; 5 mg/ml) was administered into the loop, the end of the loop was ligated.

**Intranasal Administration** Closed System: Anesthetized rats (urethane, 25% (w/v), 1.0 g/kg i.p.) were treated using the method of Hirai *et al.*[28] Briefly, a cannula was inserted into the trachea to maintain respiration and the esophagus was occluded by another cannula inserted in the direction of the throat. The nasopalatine duct was sealed with medical adhesive (medical Aron Alpha A® superglue, Daiichi Sankyo Co., Ltd., Tokyo, Japan) to prevent any test solution escaping into the buccal cavity. Milnacipran hydrochloride in physiological saline with or without 0.5% chitosan (20 mg/kg; 0.2 ml/kg; 100 mg/ml) was administered to the left nostril *via* a flexible polyethylene tube attached to a microsyringe. The length of the tube inserted into the nostril was 8 mm.

Open System: Rats with or without insertion of a cannula into the jugular vein were anesthetized by inhalation of diethyl ether. Milnacipran hydrochloride in physiological saline with or without 0.5% chitosan (10, 20, 30 mg/kg; 0.2 ml/kg; 50, 100, 150 mg/ml, respectively) was administered intranasally *via* a flexible polyethylene tube attached to a microsyringe in the same way as described above.

**Peroral Administration** Rats with or without insertion

of a cannula into the jugular vein were anesthetized by inhalation of diethyl ether. Milnacipran hydrochloride in physiological saline (10, 20, 30, 60 mg/kg; 2 ml/kg; 5, 10, 15, 30 mg/ml, respectively) was administered perorally *via* a sonde probe. The top of the sonde was placed in the cardiac region of the rat stomach.

**Pharmacological Study Using the Forced Swimming Test**   The forced swimming test was carried out on rats according to the method of Porsolt.[29] Briefly, rats were individually placed in a cylindrical glass container (40 cm in height, 18 cm in diameter) filled to a level of 18 cm with water (25±1 °C). At first, all the rats were forced to swim for 15 min as a conditioning session, and tested for another 5 min after 24 h as a test session. All rats were anesthetized by inhalation of diethyl ether and administered milnacipran perorally or intranasally as described above after a conditioning session and 20 or 60 min before the test session. A group of rats receiving only physiological saline with or without 0.5% chitosan was used as a control. The test sessions were recorded by an observer. The total duration of immobility during 5 min of the test session was recorded. The immobility time was defined as the time spent by the rats floating in the water without struggling, and making only those movements necessary to keep their heads above the water.

**Collection of Plasma**   Blood samples (0.2 ml) were collected from the jugular vein using a heparinized syringe and needle or *via* a cannula using a heparinized syringe at predetermined times after administration, and plasma samples (0.1 ml) were obtained by centrifugation (17860 *g*, 4 °C, 5 min).

**Collection of CSF**   Sampling of the CSF was performed by cisternal puncture as previously reported by Chou and Levy.[30] Briefly, an incision was made in the skin over the occipital bone and the first layer of the muscle was cut. A 22 gauge needle (Terumo Corp., Tokyo, Japan) was cut and the blunt end of the needle was directly connected to a piece of silicone tubing (20 cm long, SILASCON®, i.d.; 0.5 mm, o.d.; 1.0 mm, Kanaka Medix Corp., Osaka, Japan). The needle was then carefully inserted into the cisterna magna and the CSF was withdrawn into the tube using a 1 ml disposable syringe (Terumo Corp., Tokyo, Japan). Collection was terminated as soon as blood appeared in the tubing, and only those CSF samples with a volume more than 150 μl were accepted. The CSF samples were centrifuged at 17860 *g* for 5 min at 4 °C to obtain supernatants (100 μl) to determine the drug concentration. The collection of CSF was performed once for each rat. Then a population pharmacokinetic analysis was performed.

**Sample Analysis**   Three hundred microliters of NH₄Cl (pH 9.5) were added to plasma or CSF samples (100 μl) and mixed for 30 s. Five hundred microliters of chloroform/2-isopropanol/*n*-heptane (60/14/26) were added to the mixture followed by shaking for 2 min. After centrifugation (17860 *g*, 4 °C, 5 min), the chloroform layer was separated. The chloroform was evaporated in a heating block under a nitrogen stream (MG-2200, Tokyo Rikakikai Co., Ltd., Tokyo, Japan) and the extracted samples were reconstituted in 100 μl mobile-phase solution. Thirty microliters of these samples were injected into a high performance liquid chromatography (HPLC) system to determine the milnacipran concentration. The HPLC system used involved a pump (LC-9A), a UV detector (SPD-6A), an integrator (C-R6A), a column oven (CTO-6A), a system controller (SCL-6B), an auto injector (SIL-6B) (Shimadzu Corp., Kyoto, Japan), and a reverse-phase column (CAPCELL C18, Type UG80, 5 μm, 4.6 mm i.d. −250 mm, Shiseido Co., Ltd., Tokyo, Japan). The mobile phase was acetonitrile : 50 mM phosphate buffer (pH 2.8) (30 : 70) and the flow rate was 1.0 ml/min. The UV detector was operated at 200 nm and the column temperature was maintained at 40 °C.

**Data Analysis**   Plasma and CSF data were analyzed by a non-linear least squares regression program (Algorithm: Damping Gauss–Newton method).[31] The $C_{max}$ and $T_{max}$ values were obtained from the milnacipran concentration–time curves, and the area under the milnacipran concentration *versus* time curve (*AUC*) value was calculated by moment analysis. The plasma and CSF concentrations of milnacipran after i.v. (10, 20, 60 mg/kg), i.d. (20 mg/kg), p.o. (20 mg/kg) and i.n. (20 mg/kg) administration were extrapolated exponentially to infinite time (∞). The elimination kinetics of milnacipran fitted a two-compartment model following i.v. administration at three doses. The $AUC_{i.v.\infty}$ was proportional to the dose administered and linear relationships between the $AUC_{i.v.\infty}$ and the administered dose were obtained (data not shown). At these doses, the elimination kinetics were linear and the kinetic parameters were also almost identical for the three doses. The $k_{12}$ (min⁻¹), $k_{21}$ (min⁻¹), $k_{10}$ (min⁻¹), $Vd_{ss}$ (ml/kg) and total clearance ($CL_{tot}$, ml/min/kg) for the elimination parameters obtained from the i.v. data were 0.0892±0.0379, 0.0609±0.0184, 0.0274±0.0031, 2913.6± 211.4 and 35.38±2.00, respectively. The bioavailability (%) of milnacipran following i.d., p.o. and i.n. administration based on the period 0—∞ ($F_\infty$) was calculated.

The absorption profiles of milnacipran after i.d., p.o. and i.n. administration were calculated using a deconvolution method applied to the i.d., p.o., i.n. and i.v. data. The absorption rate constant ($k_a$) of milnacipran after i.d., p.o. and i.n. administration was calculated.

The $AUC_{CSF}/AUC_{plasma}$ ratio for i.v., i.d. and i.n. administration was calculated to evaluate the efficiency of the delivery of milnacipran to the CSF. The Drug Targeting Efficiency (DTE) and Direct Transport Percentage (DTP) after i.n. administration were calculated as follows (Eqs. 1—3):

$$\text{DTE} = \frac{(AUC_{CSF}/AUC_{plasma})_{i.n.}}{(AUC_{CSF}/AUC_{plasma})_{i.v.}} \tag{1}$$

$$\text{DTP (\%)} = \frac{B_{i.n.} - B_x}{B_{i.n.}} \times 100 \tag{2}$$

$$B_x = (B_{i.v.}/P_{i.v.}) \times P_{i.n.} \tag{3}$$

where $AUC_{CSF}$ and $AUC_{plasma}$ are the areas under the concentration of milnacipran *versus* time in CSF and plasma, respectively. $(AUC_{CSF}/AUC_{plasma})_{i.n.}$ and $(AUC_{CSF}/AUC_{plasma})_{i.v.}$ are the ratio of $AUC_{CSF}$ and $AUC_{plasma}$ after i.n. and i.v. administration, respectively. $B_x$ is $AUC_{CSF}$ caused by the transfer to the CSF from the systemic circulation through the BBB after i.n. administration. $B_{i.v.}$ and $B_{i.n.}$ are the $AUC_{CSF}$ after i.v. and i.n. administration, respectively. $P_{i.v.}$ and $P_{i.n.}$ are the $AUC_{plasma}$ after i.v. and i.n. administration, respectively.

The data are represented as the mean or mean±S.E. Statistical analysis was performed by one-way analysis of variance

(ANOVA) followed by Tukey–Kramer test. A $p$-value of less than 0.05 was considered statistically significant.

## RESULTS AND DISCUSSION

**Transfer of Milnacipran to the Systemic Circulation after i.n. Administration** Figure 1 shows the plasma concentration of milnacipran after i.v., i.d. and i.n. administration in rats. Table 1 summarizes the pharmacokinetic parameters of milnacipran calculated from the plasma data. Following i.v. administration, the pharmacokinetics of milnacipran fitted a typical 2-compartment model (Fig. 1). Non-linear elimination of milnacipran was not observed over the range of doses used in this study. Following i.d. administration, the $C_{max}$, $T_{max}$, $F_{\infty}$ and $k_a$ were $3074.8\pm98.7$ ng/ml, 60 min, 70.8% and $0.054\,min^{-1}$, respectively, and the absorption of milnacipran through the duodenum was slow. In contrast, milnacipran was absorbed rapidly after i.n. administration, and the $T_{max}$ was only 22.5 min and the $k_a$ was $0.161\,min^{-1}$. The $C_{max}$ and $F_{\infty}$ after i.n. administration were higher than those after i.d. administration ($C_{max}$ 5131.6±284.4 ng/ml, $F_{\infty}$ 85.1%) (Table 1). The absorption profile obtained by deconvolution indicated that the absorption of milnacipran was almost complete after about 120 min following i.d. administration, and after 20—30 min following i.n. administration (data not shown). Therefore, the systemic absorption of milnacipran markedly increased and was almost complete a short time after i.n. administration. These results suggest that i.n. administration is a promising route for the administration of milnacipran.

**Transfer of Milnacipran to the CSF after i.n. Administration** Figure 2 shows the CSF concentration of milnacipran after i.v., i.d. and i.n. administration in rats. Table 2 summarizes the pharmacokinetic parameters of milnacipran

calculated from the CSF data. Following i.v. administration, milnacipran was found in the CSF and the $C_{max}$ was 2216.2 ng/ml. Interestingly, the $T_{max}$ was only within 10 min, and 27.8% of the amount of milnacipran in plasma transferred to the CSF after i.v. administration. Following i.n. administration, the $C_{max}$ was about 2- and 4-fold higher than that obtained by i.v. and i.d administration, respectively. In addition, the $T_{max}$ was only 20 min following i.n. administration and was early compared with that after i.d. administration ($T_{max}$ 60 min). The area under the CSF concentration–time curve ($AUC_{CSF\infty}$) following i.n. administration was also about 2-fold higher than that after i.v. and i.d. administration. Although the $AUC_{CSF\infty}/AUC_{plasma\infty}$ ratio for i.d. administration (0.309) was almost equal to that following i.v. administration (0.278), the $AUC_{CSF\infty}/AUC_{plasma\infty}$ ratio for i.n. administration (0.574) was approximately 2-fold higher than that following i.v. administration (Table 2). Van der Berg *et al.* have reported that the value of the $AUC_{CSF}/AUC_{plasma}$ ratio is smaller than that after i.v. administration, indicating a mainly systemic transport of the drug to the CNS, and the value of the ratio is larger than that after i.v. administration, indicating a mainly direct transport of drug from the nose to the CNS.[32] Furthermore, the Drug Targeting Efficiency (DTE) and Direct Transport Percentage (DTP) after i.n. administration were 2.1 and 51.5%, respectively and milnacipran transferred to CSF from the nostril *via* systemic circulation was 48.5%, suggesting that about half of milnacipran transferred to the CSF was due to direct transport from the nostril to the CSF. These results suggest that i.n. administration is a promising route for the delivery of drugs to the CSF. Charlton *et al.* have reported that i.n. administration of a model drug, a glycine receptor antagonist and angiotensin antagonist, results in greater delivery to the olfactory lobes and brain com-
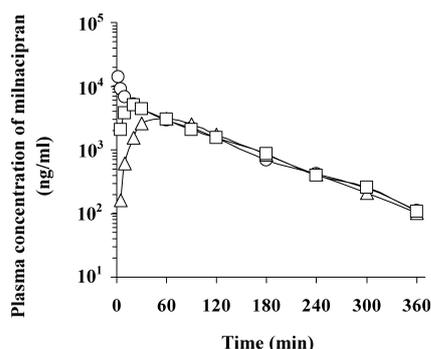


Fig. 1. Plasma Concentration of Milnacipran after i.v., i.d. and i.n. Administration in Rats

○: i.v., △: i.d., □: i.n. administration. The dose was 20 mg/kg for i.v., i.d. and i.n. administration. Each data point represents the mean±S.E. ($n=4$).
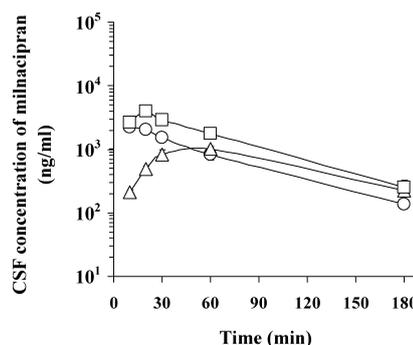


Fig. 2. CSF Concentration of Milnacipran after i.v., i.d. and i.n. Administration in Rats

○: i.v., △: i.d., □: i.n. administration. The dose was 20 mg/kg for i.v., i.d. and i.n. administration. Each data point represents the mean±S.E. ($n=3$).

Table 1. Pharmacokinetic Parameters of Milnacipran Calculated from Plasma Data after i.v., i.d. and i.n. Administration in Rats

| | $C_{max}$ (ng/ml) | $T_{max}$ (min) | $k_a$ (min$^{-1}$) | $AUC_{plasma\infty}$ (ng·min/ml) | $F_{\infty}$ (%) |
|---|---|---|---|---|---|
| i.v. | — | — | — | 592912.4±33284.7 | — |
| i.d. | 3074.8±98.7 | 60 | 0.054 | 420011.3±14780.4 | 70.8 |
| i.n. | 5131.6±284.4** | 22.5 | 0.161 | 504462.0±14151.5** | 85.1 |

The dose was 20 mg/kg for i.v., i.d. and i.n. administration. ** $p<0.01$ compared with i.d. administration. Each data represents the mean or mean±S.E. ($n=4$).

Table 2. Pharmacokinetic Parameters of Milnacipran Calculated from CSF Data after i.v., i.d. and i.n. Administration in Rats

| | $C_{max}$ (ng/ml) | $T_{max}$ (min) | $AUC_{CSF\infty}$ (ng·min/ml) | $\dfrac{AUC_{CSF\infty}}{AUC_{plasma\infty}}$ | DTE | DTP (%) |
|---|---|---|---|---|---|---|
| i.v. | 2216.2 | 10 | 165064.9 | 0.278 | — | — |
| i.d. | 1005.5 | 60 | 129963.5 | 0.309 | — | — |
| i.n. | 4019.1 | 20 | 289425.0 | 0.574 | 2.1 | 51.5 |

The dose was 20 mg/kg for i.v., i.d. and i.n. administration. Each data represents the mean ($n=3$).

pared with i.v. administration, and it has been demonstrated by autoradiography that drugs administered intranasally are transferred to the brain and the CSF *via* the olfactory pathway.[33] Their results corresponded with our results in this study. They have also indicated that the three glycine receptor antagonists were transported to the CNS to differing degrees although they had similar molecular structures and similar physicochemical characteristics (DTP 51.95—99.99%). Sakane *et al.* investigated the relationship between the physicochemical characteristics and the direct drug transport from the rat nasal cavity to the CSF, and reported that the degree of the direct transport to the CSF was dependent on the lipophilicity (partition coefficient (Pc)), the dissociation and the molecular weight (possible up to at least 20000 Da) of the drugs.[34—36] In our present study, milnacipran was used as a model drug, and the direct transport from the nasal cavity to the CSF might be influenced by the physicochemical characteristics of milnacipran (MW 282.81, $pK_a$ 9.7, Pc 1.2).

**Pharmacological Effect 60 min after i.n. Administration of Milnacipran** Figure 3 shows the effects of treatment with milnacipran on the immobility time in the forced swimming test in rats. In the case of p.o. and i.n. administration of milnacipran in physiological saline, the immobility time of rats was reduced significantly compared with controls, depending on the dose ($p < 0.05$, Figs. 3a, b). Moreover, the pharmacological effect after i.n. administration was higher than that after p.o. administration at each dose and there were significant differences between the immobility times for both administrations at 20 and 30 mg/kg ($p < 0.05$). This result is due to the higher $C_{\max \text{ plasma}}$, $C_{\max \text{ CSF}}$, $AUC_{\text{plasma}\infty}$, $AUC_{\text{CSF}\infty}$ and $AUC_{\text{CSF}\infty}/AUC_{\text{plasma}\infty}$ ratio after i.n. administration compared with those obtained by i.d. administration as shown in Tables 1 and 2. However, the pharmacokinetics in this pharmacological study differed from that in the pharmacokinetic study, because of the difference between experimental conditions in both studies. Therefore, the plasma and CSF concentrations of milnacipran in rats under the conditions in the pharmacological study are not clear. So, we evaluated the pharmacokinetics of milnacipran in rats under the conditions in the pharmacological study further.

**Plasma Concentration of Milnacipran after i.n. Administration in Rats under the Conditions in the Pharmacological Study** Figures 4a and b show the plasma concentration of milnacipran after i.d., p.o. and i.n. administration in rats under different conditions. Table 3 summarizes the pharmacokinetic parameters of milnacipran calculated from the plasma data. The $AUC_{0—60\,\text{min plasma}}$ after i.n. administration of milnacipran in rats under open system (141539.0 ng·min/ml) was higher than that after p.o. administration (78895.6 ng·min/ml) (data not shown). The CSF concentration after i.n. administration was due to the transport from systemic circulation to CNS *via* BBB as well as the direct transport from nasal cavity to CNS, because the amount of milnacipran transferred to CSF *via* the BBB was determined by the $AUC$. As a result, the pharmacological effect 60 min after i.n. administration was higher than that after p.o. administration (Fig. 3). However, the $C_{\max}$, $AUC_{\text{plasma}\infty}$, $k_a$ and $F_\infty$ in the pharmacological study (p.o. and i.n. administration in rats under open system) were lower than those in the pharmacokinetic study (i.d. and i.n. administration in rats under closed system) and the $T_{\max}$ was higher than that in the pharmacokinetic study (Table 3). Following p.o. administration, drug was transferred to stomach and degraded by digestive en-
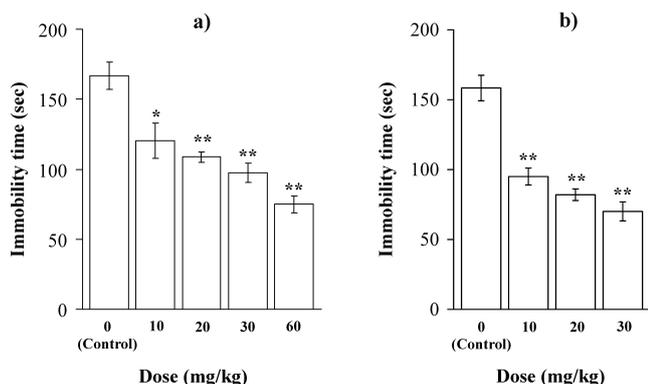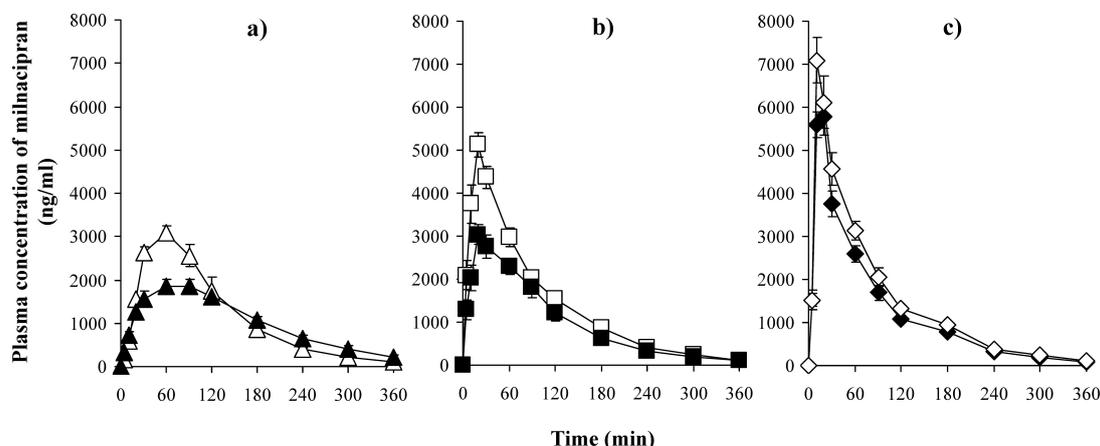


Fig. 3. Effects of Treatment with Milnacipran on the Immobility Time in the Forced Swimming Test in Rats

a) p.o. administration, b) i.n. administration. Control group received physiological saline only. ∗$p < 0.05$, ∗∗$p < 0.01$ compared with the control group. The immobility time was measured 60 min after administration. It was reduced significantly depending on the dose for both administrations of milnacipran in physiological saline. Each data column represents the mean±S.E. ($n = 3$—5).



Fig. 4. Plasma Concentration of Milnacipran after i.d., p.o. and i.n. Administration in Rats under Different Conditions

a) △: i.d., ▲: p.o. administration, b) i.n. administration (□: closed system, ■: open system), c) i.n. co-administration with 0.5% chitosan (◇: closed system, ◆: open system). The dose was 20 mg/kg for i.d., p.o. and i.n. administration. Each data point represents the mean±S.E. ($n = 4$).

Table 3. Pharmacokinetic Parameters of Milnacipran Calculated from Plasma Data after i.d., p.o. and i.n. Administration in Rats under Different Conditions

| | $C_{max}$ (ng/ml) | $T_{max}$ (min) | $k_a$ (min$^{-1}$) | $AUC_{plasma\,\infty}$ (ng·min/ml) | $F_\infty$ (%) | $F_o/F_c$ [a] (%) |
|---|---|---|---|---|---|---|
| i.d. | 3074.8±98.7 | 60 | 0.054 | 420011.3±14780.4 | 70.8 | — |
| p.o. | 1889.9±155.0 | 67.5 | 0.038 | 395268.8±29761.2 | 66.7 | — |
| i.n. (closed system) | 5131.6±284.4 | 22.5 | 0.161 | 504462.0±14151.5 | 85.1 | — |
| i.n. (open system) | 3044.8±254.2 | 30 | 0.092 | 365081.4±38521.6 | 61.6 | 72.4 |
| i.n. (+Chi, closed system) | 7615.7±410.0 | 12.5 | 0.227 | 532526.3±25090.9 | 89.8 | — |
| i.n. (+Chi, open system) | 6348.9±200.2 | 15 | 0.197 | 446342.5±23634.8 | 75.3 | 83.8 |

The dose was 20 mg/kg for i.d., p.o. and i.n. administration. Chi: 0.5% chitosan. a) $F_o/F_c$ (%)=$F_{open\,system}/F_{closed\,system}$×100. Each data represents the mean or mean±S.E. ($n$=4).

zymes. Following i.n. administration in rats under open system, drug solution was transferred from the nasal cavity to the esophagus, because there was no surgical operation and mucociliary clearance, therefore, this led to a reduced pharmacological effect as predicted from the pharmacokinetic data. Thus, pharmaceutical ways of improving the drug elimination by mucociliary clearance, and longer residence in the nasal cavity are required in order to obtain greater pharmacological effects.

**Effect of 0.5% Chitosan on the Systemic Absorption after i.n. Administration of Milnacipran in Rats** Figure 4c shows the plasma concentration after i.n. co-administration of milnacipran with 0.5% chitosan in rats under various conditions. Table 3 summarizes the pharmacokinetic parameters of milnacipran calculated from the plasma data. After i.n. co-administration of milnacipran with 0.5% chitosan in rats under open system, the $C_{max}$ and $k_a$ were higher than those after i.n. administration in rats under closed system and the $T_{max}$ was only 15 min. The $AUC_{plasma\infty}$ and $F_\infty$ were higher than those after i.n. administration in rats under open system and nearly those after i.n. administration in rats under closed system. The relative % residence of milnacipran in nasal cavity ($F_o/F_c$) after i.n. administration was improved to 83.8% from 72.4% by co-administering 0.5% chitosan. These results suggest that i.n. co-administration with 0.5% chitosan leads to a long residence time in the nasal cavity, resulting in enhanced absorption of the drug to the systemic circulation. Furthermore, it is expected that the transport of drug to the CNS directly and *via* the BBB will also be enhanced from the data in Figs. 1, 2 and Tables 1, 2. Soane *et al.* evaluated the clearance characteristics of two bioadhesive nasal delivery systems in the form of chitosan microspheres and chitosan solution, from the nasal cavity of conscious sheep. They demonstrated that the bioadhesive chitosan delivery systems were cleared at a slower rate from the sheep nasal cavity, with half-times of clearance (time taken for 50% clearance $t_{50\%}$) of 43 min and 115 min, for the solution and microsphere formulations, respectively, compared with that of a control solution (15 min).[37] Charlton *et al.* demonstrated that the formulations containing low methylated pectins (LM-5 and LM-12) as a bioadhesive material or chitosan G210 as a bioadhesive material and as an absorption enhancer were able to reach the olfactory region in the nasal cavity of human volunteers when delivered using a simple nasal drop device, and displayed a significantly increased residence time on the epithelial surface.[11] Their results supported our result that the absorption of milnacipran increased
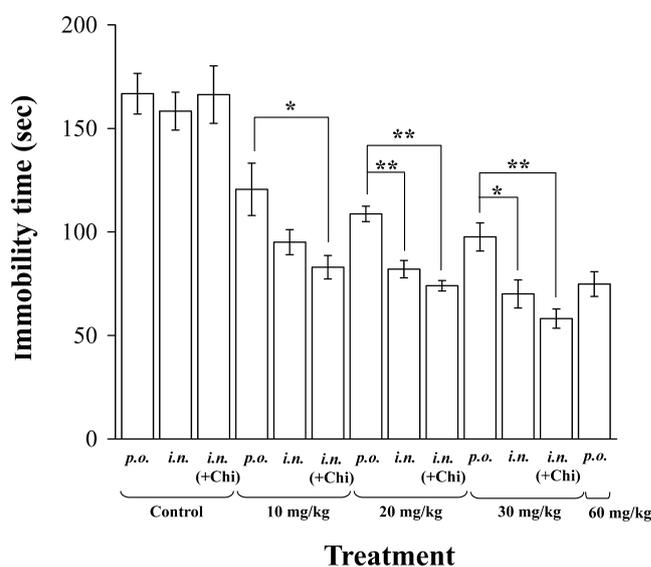


Fig. 5. Effects of 0.5% Chitosan on the Pharmacological Effect after i.n. Administration of Milnacipran in Rats

∗$p$<0.05, ∗∗$p$<0.01 compared with the p.o. administration group. Chi: 0.5% chitosan. Control group received physiological saline with or without 0.5% chitosan only. The immobility time was measured 60 min after administration. It was reduced significantly depending on the dose for all administrations. In addition, the immobility time after i.n. co-administration with chitosan was reduced significantly compared with that obtained by p.o. administration ($p$<0.05). Each data column represents the mean±S.E. ($n$=3—5).

following i.n. co-administration with chitosan.

**Effect of 0.5% Chitosan on the Pharmacological Effect after i.n. Administration of Milnacipran in Rats** Figure 5 shows the effect of 0.5% chitosan on the pharmacological effect after i.n. administration of milnacipran in rats. After i.n. co-administration of milnacipran with 0.5% chitosan, the immobility time of rats was reduced significantly depending on the dose ($p$<0.01, Fig. 5). Moreover, the immobility time after i.n. co-administration with 0.5% chitosan was reduced significantly compared with that obtained by p.o. administration at each dose ($p$<0.05, Fig. 5). The i.n. administration of milnacipran at 20 mg/kg with 0.5% chitosan or 30 mg/kg had almost the same effect as the p.o. administration of 60 mg/kg, indicating that the antidepressant effect after i.n. administration was obtained at lower doses compared with p.o. administration. These results suggest that the i.n. co-administration of milnacipran with chitosan produces a higher antidepressant effect compared to that with p.o. administration.

The antidepressant effect increased with increasing plasma concentration as shown in Fig. 4, but this might be due to the
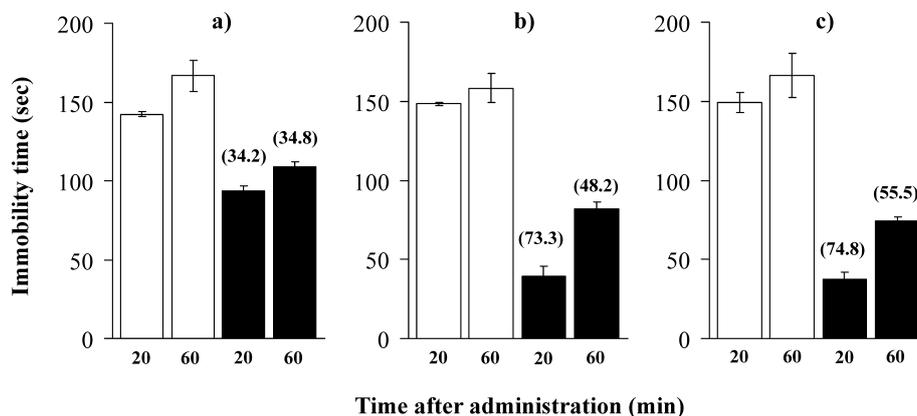
Fig. 6.   The Immobility Time 20 and 60 min after Administration of Milnacipran in Rats

a) p.o. administration, b) i.n. administration, c) i.n. co-administration with 0.5% chitosan. □: Control, ■: Treatment group. The dose was 20 mg/kg for p.o. and i.n. administration. Control group received physiological saline with or without 0.5% chitosan only. The number in parentheses indicates the antidepressant effect (%). It was calculated as follows; antidepressant effect (%)=(immobility time in control−immobility time in treatment)/immobility time in control×100. Each data column represents the mean±S.E. ($n$=3—5).
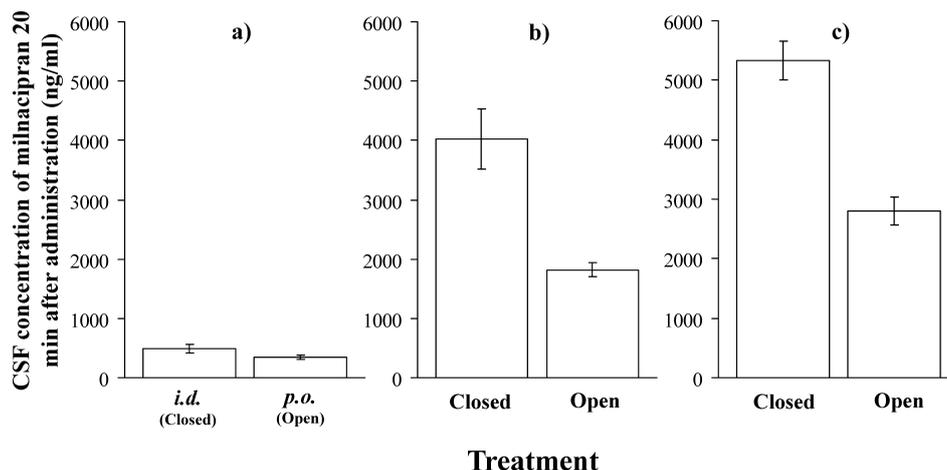


Fig. 7.   CSF Concentration of Milnacipran 20 min after i.d., p.o. and i.n. Administration in Rats under Different Conditions

a) i.d. and p.o. administration, b) i.n. administration, c) i.n. co-administration with 0.5% chitosan. Closed: Closed system, Open: Open system. The dose was 20 mg/kg for i.d., p.o. and i.n. administration. Each data column represents the mean±S.E. ($n$=3).

enhanced transport of milnacipran from the systemic circulation to CSF *via* the BBB by increase in systemic absorption. Also, it is expected that a higher effect would be obtained at about 15—30 min after i.n. administration, because the time is close to the $T_{max}$ (Table 3).

**Pharmacological Effect 20 min after i.n. Administration of Milnacipran**   Figure 6 shows the pharmacological effect 20 and 60 min after various administrations of milnacipran. The antidepressant effect 20 min after p.o. administration was 34.8% and equal to that obtained 60 min after administration (34.2%). In contrast, the effect 20 min after i.n. administration with or without 0.5% chitosan was higher than that obtained 60 min after administration. The antidepressant effect increased with increasing plasma concentration of milnacipran (Fig. 4), but the effect 20 min after i.n. co-administration with 0.5% chitosan was equal to that obtained 20 min after i.n. administration, despite the large difference (2-fold) between the respective plasma concentrations of milnacipran. This may be due to a time lag in the appearance of the antidepressant effect. However, the effect 60 min following i.n. co-administration with chitosan was higher than that obtained 60 min following i.n. administration

without chitosan and the effect had a tendency to prolong. These results suggest that i.n. administration of milnacipran produces a higher antidepressant effect and early compared with p.o. administration.

**CSF Concentration 20 min after i.n. Administration of Milnacipran**   Figure 7 shows the CSF concentration of milnacipran 20 min after i.d., p.o. and i.n. administration in rats under different conditions. In each administration, the CSF concentration of rats under open system was lower than that obtained in rats under closed system as was seen with the plasma concentration. In particular, the CSF concentration of rats under closed system was 2.2-fold higher than that obtained in rats under open system in i.n. administration. However, these CSF concentrations of milnacipran 20 min after administration in rats under open system corresponded with the antidepressant effects. The effect increased with increasing CSF concentration of milnacipran as well as the plasma concentration. For the drug to exert its effects, the brain concentration may be more important for milnacipran than CSF concentration, but the brain concentration increases with increasing CSF concentration, because milnacipran has comparatively higher lipophilicity and the distribution of drug to

the brain depends on its lipophilicity. These results suggest that i.n. co-administration with chitosan leads to enhanced transport of milnacipran from the systemic circulation to the CNS *via* the BBB by increase in systemic absorption as well as direct transport to the CNS, resulting in a higher CSF concentration compared to that with p.o. administration.

CONCLUSION

The i.n. administration of milnacipran produces a higher direct delivery to the CNS as well as the systemic circulation, suggesting that it is a promising route of administration and an alternative to p.o. administration. The antidepressant effect after i.n. administration of milnacipran was higher than that after p.o. administration. Furthermore, the i.n. co-administration of milnacipran with chitosan led to a greater antidepressant effect. This result suggests that chitosan produces a long residence time of milnacipran in the nasal cavity due to its bioadhesive properties, leading to the enhanced transport of milnacipran from the systemic circulation to the CNS *via* the BBB by increase in systemic absorption as well as direct transport to CNS, resulting in a higher antidepressant effect compared to that with p.o. administration.

REFERENCES

1) Kendell R. E., "Depressive Disorders," ed. by Maj M., Sartorius N., Second Edition, John Wiley & Sons Ltd., Chichester, West Sussex, 2002, pp. 52—54.
2) Slattery D. A., Hudson A. L., Nutt D. J., *Fundam. Clin. Pharmacol.*, **18**, 1—21 (2004).
3) Nestler E. J., Barrot M., DiLeone R. J., Eisch A. J., Gold S. J., Monteggia L. M., *Neuron*, **34**, 13—25 (2002).
4) Cooper J. R., Floyd E. B., Roth R. H., "The Biochemical Basis of Neuropharmacology," 7th ed., Oxford University Press, New York, 1996, pp. 340—343.
5) Janicak P. G., Davis J. M., Preskorn S. H., Ayd F. J. Jr., "Principles and Practice of Psychopharmacotherapy," Third ed., Lippincott Williams & Wilkins, Philadelphia, 2001.
6) Delimi-Stula A., *Hum. Psychopharmacol.*, **15**, 255—260 (2000).
7) Komoroski R. A., Newton J. E., Cardwell D., Sprigg J., Pearce J., Karson C. N., *Magn. Reson. Med.*, **31**, 204—211 (1994).
8) Miyamoto M., Natsume H., Iwata S., Ohtake K., Yamaguchi M., Kobayashi D., Sugibayashi K., Yamashina M., Morimoto Y., *Eur. J. Pharm. Biopharm.*, **52**, 21—30 (2001).
9) Miyamoto M., Tsukune T., Hori S., Hayashi T., Natsume H., Sugibayashi K., Morimoto Y., *Biopharm. Drug Dispos.*, **22**, 137—146 (2001).
10) Miyamoto M., Natsume H., Satoh I., Ohtake K., Yamaguchi M., Kobayashi D., Sugibayashi K., Morimoto Y., *Int. J. Pharm.*, **226**, 127—138 (2001).
11) Charlton S. T., Jones N. S., Davis S. S., Illum L., *Eur. J. Pharm. Sci.*, **30**, 295—302 (2007).
12) Charlton S. T., Davis S. S., Illum L., *J. Controlled Release*, **118**, 225—234 (2007).
13) Charlton S. T., Davis S. S., Illum L., *Int. J. Pharm.*, **338**, 94—103 (2007).
14) Pardridge W. M., *Pharm. Sci. Technol. Today*, **2**, 49—59 (1999).
15) Illum L., *Drug Discov. Today*, **7**, 1184—1189 (2002).
16) Illum L., *J. Controlled Release*, **87**, 187—198 (2003).
17) Hilger P. A., "Otolaryngology, A Textbook of Ear, Nose and Throat Diseases," W. B. Saunders, Philadelphia, 1989, pp. 177—195.
18) Illum L., *J. Pharm. Pharmacol.*, **56**, 3—17 (2004).
19) Thorne R. G., Pronk G. J., Padmanabhan V., Frey W. H. II, *Neuroscience*, **127**, 481—496 (2004).
20) Illum L., *Pharm. Res.*, **15**, 1326—1331 (1998).
21) Kumar M. N., Muzzarelli R. A., Muzzarelli C., Sashiwa H., Domb A. J., *Chem. Rev.*, **104**, 6017—6084 (2004).
22) Varum K. M., Myhr M. M., Hjerde R. J., Smidsrod O., *Carbohydr. Res.*, **299**, 99—101 (1997).
23) Rao S. B., Sharma C. P., *J. Biomed. Mater. Res.*, **34**, 21—28 (1997).
24) Alonso M. J., Sanchez A., *J. Pharm. Pharmacol.*, **55**, 1451—1463 (2003).
25) Lee K. Y., *Macromol. Res.*, **15**, 195—201 (2007).
26) Illum L., *Eur. J. Pharm. Sci.*, **11**, 1—18 (2000).
27) Smith J. M., Dornich M., Wood E. J., *Biomaterials*, **26**, 3269—3276 (2004).
28) Hirai S., Yashiki T., Miwa H., *Int. J. Pharm.*, **9**, 165—172 (1981).
29) Porsolt R. D., Le Pichon M., Jalfre M., *Nature* (London), **266**, 730—732 (1977).
30) Chou R. C., Levy G., *J. Pharmacol. Exp. Ther.*, **219**, 42—48 (1981).
31) Yamaoka K., Tanigawara Y., Nakagawa T., Uno T., *J. Pharmacobio-Dyn.*, **4**, 879—885 (1981).
32) Van der Berg M. P., Merkus P., Romeijn S. G., Verhoef J. C., Merkus F. W. H. M., *J. Drug Target*, **11**, 325—331 (2003).
33) Charlton S. T., Whetstone J., Fayinka S. T., Read K. D., Illum L., Davis S. S., *Pharm. Res.*, **25**, 1531—1543 (2008).
34) Sakane T., Akizuki M., Yamashita S., Nadai T., Hashida M., Sezaki H., *Chem. Pharm. Bull.*, **39**, 2456—2458 (1991).
35) Sakane T., Akizuki M., Yamashita S., Sezaki H., Nadai T., *J. Pharm. Pharmacol.*, **46**, 378—379 (1994).
36) Sakane T., Akizuki M., Taki Y., Yamashita S., Sezaki H., Nadai T., *J. Pharm. Pharmacol.*, **47**, 379—381 (1995).
37) Soane R. J., Hinchcliffe M., Davis S. S., Illum L., *Int. J. Pharm.*, **217**, 183—191 (2001).