Evaluation of the Contribution of the Nasal Cavity and Gastrointestinal Tract to Drug Absorption Following Nasal Application to Rats

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Drugs applied to the nose in *in vivo* physiologic condition undergo absorption from the nasal cavity and the gastrointestinal (GI) tract because drug solution in the nasal cavity, together with mucus layer, is cleared to pharynx and then to the GI tract by coordinated beat of the cilia on nasal epithelial cells. The purpose of this study was to develop evaluate the contribution of the nasal cavity and the GI tract to drug absorption following nasal application and to clarify the relation to the transepithelial permeability of the drug (the permeability to Caco-2 monolayer, $P_{\text{Caco-2}}$). Male Wistar rats received intravenous, nasal, and oral drug administration and drug concentration–time profiles in plasma were determined. Fractional absorption after nasal application (F_{n}) and oral administration (F_{po}) were calculated from the area under the curve following intravenous injection (AUC_{iv}), nasal application (AUC_{n}), and oral administration (AUC_{po}) as $AUC_{\text{n}}/AUC_{\text{iv}}$ and AUC_{po}/AUC_{iv}, respectively. Fractional absorption from the nasal cavity (F_{NC}) and the GI tract (F_{GI}) following nasal application was calculated as $(F_{\text{n}}-F_{po})/(1-F_{po})$ and $F_{po}(1-F_{\text{NC}})$, respectively. The shape of the curve between F_{NC} and $P_{\text{Caco-2}}$ was similar with the one observed in the case of oral bioavailability except the curve shifted right. It is noteworthy that the relation between F_{GI} and $P_{\text{Caco-2}}$ showed a bell-shaped curve with peak at 10^{-6} cm/s of $P_{\text{Caco-2}}$. Highly permeable drug is primarily absorbed through the nasal mucosa before it is cleared to the GI tract. With the decrease in $P_{\text{Caco-2}}$, the larger amount of the drug is cleared to the GI tract and absorption from the GI tract is increased. Poorly permeable drug, on the other hand, was absorbed neither from the nasal was nor the GI tract. These findings suggest that the primary absorption site of drug after nasal application is decided by mucociliary clearance and absorption through the nasal mucosa.

Key words nasal application; mucociliary clearance; fractional absorption; Caco-2 permeation; gastrointestinal tract

Nasal administration has gained much attention by many researchers within the last few decades because of its great potential utility for rapid drug delivery.[1—3] It offers an attractive alternative for drugs that have limited oral bioavailability, are destroyed by gastrointestinal (GI) fluid, or are highly susceptible to hepatic first pass or gut-wall metabolism.[4] Nasal drug delivery also offers the convenience and safety of noninvasiveness. In addition, nasal drug administration results in quick onset of action as compared with oral and transdermal administrations.

The respiratory epithelium is covered with a mucous layer. Some respiratory epithelial cells possess cilia on their surface. These cilia beat in coordinated fashion to transport the mucous layer to the nasopharynx, where it is swallowed.[5,6] The combined action of mucus layer and cilia is called mucociliary clearance (MC). It is an important nonspecific defense mechanism of the respiratory tract to protect the body against noxious inhaled materials. Due to MC, drugs applied to the nasal cavity are cleared to the nasopharynx and, thereafter, to the GI tract, together with the mucus layer. Some fraction of nasally administered drug undergoes absorption from the GI tract. Although the contribution of the nose and GI tract to drug absorption after nasal application has been reported,[7] no information on its relation to membrane permeability of the drug is available at present.

Hirai *et al*. reported a series of studies on nasal drug absorption.[8—10] In their reports, rats were investigated in the supine position under anesthesia with the esophagus ligated to avoid clearance of drug from the nasal cavity. Since their reports, much research has been done utilizing the same surgical procedure. This procedure is reasonable in studies aimed at clarifying the barrier characteristics of the nasal mucosa. However, this experimental situation is quite different from the physiologic condition. It is not feasible to investigate MC in rats in which the esophagus is ligated. Additionally, the position of the animal during the absorption study is likely important. Movement of the drug by mucociliary clearance may differ in the supine position as compared with that in the normal prone position.

The aim of this research was to evaluate nasal and intestinal absorption following nasal drug administration. For this purpose, drug absorption following nasal application was investigated in the normal physiologic condition. The relation of the fractional absorption of the drug from the nasal cavity and from the GI tract to Caco-2 permeability ($P_{\text{Caco-2}}$) was clarified and discussed here in.

THEORY AND CALCULATION

Bioavailability of the drug after nasal and oral administration is calculated as follows

$$F_{\text{n}}=AUC_{\text{n}}/AUC_{\text{iv}}$$
$$F_{po}=AUC_{po}/AUC_{\text{iv}}$$

where F_{n} and F_{po} is the bioavailability after nasal and oral administration, respectively. AUC_{n}, AUC_{po}, and AUC_{iv} are the area under the concentration–time profile following nasal, oral, and intravenous administration of the drug. *AUC* was calculated according to the trapezoidal rule up to the last sampling point and extrapolation.

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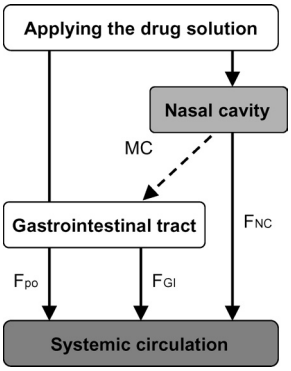

Fig. 1. Drug Absorption and Disposition after Nasal and Oral Administration

Model drugs that likely undergo no degradation and metabolism in the nasal cavity were selected for simplification. Inulin and mannitol are non-degradable markers of paracellular transport.[11—13] The elimination route of methotrexate,[14] acyclovir,[15] and possibly sulfanilic acid[16] is urinary excretion and metabolism of these drugs in the liver and nasal cavity might be negligible. These model drugs are assumed to follow linear kinetics. Therefore F_n is the sum of F_{NC}, the fractional absorption from the nasal cavity, and F_{GI}, fractional absorption from the GI tract after nasal administration.

$$F_n = F_{NC} + F_{GI} \quad (1)$$

The fractional clearance of the drug to the GI tract by MC is defined as $1-F_{NC}$. Since the drug cleared from the nasal cavity is absorbed from GI tract at the fraction of F_{po}, F_{GI} can be calculated according to Eq. (2).

$$F_{GI} = F_{po}\,(1-F_{NC}) \quad (2)$$

Substitution of Eq. (2) into Eq. (1) and rearrangement of the equation result in the following equation.

$$F_{NC} = (F_n - F_{po})/(1-F_{po}) \quad (3)$$

Based on Eq. (1), F_{GI} is calculated as

$$F_{GI} = F_n - F_{NC} = F_n - (F_n - F_{po})/(1-F_{po}) \quad (4)$$

From *AUC*s obtained in animal studies, F_{GI} and F_{NC} were calculated according to Eqs. (3) and (4).

MATERIALS AND METHODS

Materials Acyclovir, [8-^{3}H], inulin-methoxy, [methoxy-^{14}C], and mannitol, D-[1-^{14}C], were the product of American Radiolebeled Chemicals Inc. (St. Louis, MO, U.S.A.). [3′, 5′, 7-^{3}H] methotrexate, sodium salt was from Amersham Biosciences Limited (Piscataway, NJ, U.S.A.). These radioactive materials were purchased from Japan Radioisotope Association. Sulfanilic acid was purchased from Nacalai Tesque Inc. (Kyoto, Japan). Caco-2 cell was obtained from Dainippon Pharmaceuticals Co. (Osaka, Japan). Reagents and the medium used for Caco-2 culture and preparation of the monolayer were purchased from Sigma-Aldrich (St Louis, MO, U.S.A.), Gibco Laboratories (Lenexa, KS, U.S.A.). All the other chemicals were of reagent grade and commercially available.

Animal Study All animal studies were previously approved by the Committee of the Animal Care of Shujitsu University and conducted under the Guideline. Male Wistar rats (B.W. 200—260 g) were used in animal experiments. The rats used for nasal and oral absorption studies were fasted overnight.

Intravenous Bolus Injection: Under intraperitoneal sodium pentobarbital (50 mg/kg, Nembutal, Abbott Laboratories, Abbott Park, IL, U.S.A.) anesthesia, the right femoral artery was cannulated with polyethylene tubing (SP-31, Natsume, Tokyo, Japan) for collection of blood samples. Drug solution (0.1 ml/kg B.W. of physiological saline) was injected into the left femoral vein. Blood samples were collected in heparinized tubes at predetermined time intervals for 60 min. The blood was centrifuged to obtain the plasma.

Nasal Administration: Under light ether anesthesia, the right femoral artery was cannulated with polyethylene tubing. Drug dissolved in 5 μl of physiological saline was instilled at 1 cm depth from the nostril by microsyringe. The surgical procedure and nasal application of the drug were done under light ether anesthesia. Animals were kept in their cage (KN-326-III, Natsume, Tokyo, Japan) thereafter throughout the experiment. Animals usually became completely conscious 5—10 min after instillation. Blood samples were collected for 360 min after drug administration. During this period, the animal was allowed free access to water.

The method criteria to apply the drug to the nasal cavity of the rat was decided as follows. The volume of the nasal cavity and the total surface area of the nasal epithelium in human are 16—18 ml and 180 cm^2, respectively.[17—21] Those in the rat are 0.4 ml and 10 cm^2, which are 2—5% of the human. In most human studies, the volume instilled to the nasal cavity was 50—200 μl. Based on these parameters, 1—10 μl was considered reasonable as the volume for the rat. When 10 μl of solution was instilled to the rat, the solution was sometimes blown out by a sneeze-like behavior of the rat even under light ether anesthesia. When the volume was decreased to 5 μl, no leakage of the solution was observed. Additionally, the location in the nasal cavity at which the solution is instilled is important. The preliminary experiment showed that when the solution is instilled at 1 cm depth from the nostril, the drug is likely located in the center of the nasal cavity. Consequently, 5 μl of the dosing solution was instilled at 1 cm depth from the nostril by microsyringe.

Ether may enhance or inhibit drug absorption through the nasal mucosa. Therefore the change in nasal drug absorption caused by ether anesthesia was examined comparing the profiles of mannitol in the plasma after nasal administration under intraperitoneal pentobarbital and continuous ether exposure. No change was observed between the profiles under pentobarbital anesthesia and ether exposure (data not shown).

Oral Administration: Under light ether anesthesia, the right femoral artery was cannulated with polyethylene tubing as described above. After the recovery of the rat from ether anesthesia, the drug solution (1 ml) was orally applied to the rat. Animals were kept in their cage thereafter throughout the experiment. Blood samples were collected for 300 min. The collected blood was treated as described above.

Culture of Caco-2 and Preparation of Caco-2 Monolayers Caco-2 cells were grown in Dulbecco's modified Eagle

medium supplemented with 10% fetal bovine serum, 1% L-glutamine, 1% non-essential amino acid, and 5% antibiotic-antimycotic solution in a culture flask.[22] Caco-2 monolayers were prepared according to the short-term culture method[23] using the culture kit, BIOCOAT® HTS Caco-2 Assay System (Beckton Dickinson Bioscience, Bedford, MA, U.S.A.).

***In Vitro* Study on Transepithelial Transport** Hank's balanced salts solution (HBSS) pH 7.4 supplemented with 15 mM glucose was used for transport studies. Caco-2 monolayers were preincubated with drug-free transport medium at 37 °C for 10 min. Transport medium of both sides was replaced with transport medium (0.8 ml) containing the drug (1 mM) on the apical side and drug-free transport medium (2.0 ml) in basolateral side. Thereafter, aliquot of the sample was taken from basolateral side for 60 min. The permeability [apparent permeability coefficient, $P_{\text{Caco-2}}$ (cm/s)] of the drug was calculated according to the following equation:

$$P_{\text{Caco-2}}=dQ/dt/(A\cdot C_0)$$

where dQ/dt is the appearance rate of drugs in the basolateral side (%dose/s), C_0 is the initial drug concentration in the apical side (%dose=1), and A is the surface area of the monolayer (0.9 cm^2).

Drug Assay Radioactive Drugs (Acyclovir, Inulin, Mannitol, and Methotrexate): The plasma (100 μl) was transferred to the counting vial and treated with 0.5 ml of Soluene 350 (Perkin-Elmer, Wellesley, MA, U.S.A.). No treatment was done on the sample from *in vitro* transepithelial transport study. The scintillation cocktail, 10 ml of Clearsol II (Nacalai Tesque, Kyoto, Japan), was added. The radioactivity in the sample was determined by liquid scintillation counter, LSC3500 (Aloka, Tokyo, Japan).

Sulfanilic Acid: Methanol (1200 μl) was added to the plasma (100 μl) for deproteinization and the mixture centrifuged. The supernatant was taken for the analysis with LC/MS system (API1100, Agilent Technology, Palo Alto, U.S.A.) equipped with the reversed-phase column (YMC-Pack Pro C18 RS, 150×4.6 mm, YMC Co., Ltd., Kyoto, Japan). The mobile phase consisted of acetonitrile–0.1% formic acid with a gradient from 6 to 10% acetonitrile at the flow rate of 0.6 ml/min. Sulfanilic acid in the sample from the *in vitro* transepithelial transport study was assayed with HPLC (LC-2010C HT, Shimadzu, Kyoto, Japan) equipped with the reversed-phase column (YMC-Pack Pro C18 RS, 150×4.6 mm, YMC Co., Ltd., Kyoto, Japan). The mobile phase was 20 mM sodium phosphate monobasic at the flow rate of 0.5 ml/min. The absorbance was monitored at 254 nm.

RESULTS AND DISCUSSION

Transepithelial Transport Profiles of the Drugs Figure 2 indicates transepithelial transport profiles of model drugs. Caco-2 cell was used in this study since Caco-2 is very popular and much information is available on the comparison with the oral drug absorption from the literature. The amount of drug transported to basolateral solution showed a linear increase during the transport experiment. The permeability of inulin to Caco-2 monolayer was the lowest, and methotrexate showed the highest. Acyclovir had moderate membrane permeability.

Fractional Absorption and Permeability to Caco-2 of the Drugs Table 1 lists $P_{\text{Caco-2}}$, *AUC*s, and fractional absorption. The model drugs showed better absorption following nasal application than that after oral administration. Inulin is a highly hydrophilic compound and is mainly absorbed through the paracellular route.[12,13] Inulin also has been used as a marker of paracellular transport. Therefore F_{po} of inulin was very low. However, F_{n} of inulin was significantly larger than F_{po}. This result is in good agreement with a report that pore transport is developed in nasal mucosa.[24,25] F_{po} of methotrexate and sulfanilic acid was 0.508 and 0.339, respectively. F_{NC} of these drugs showed 0.963 and 0.738, respectively, which are approximately 2-fold larger in comparison with F_{po}.

F_{NC} of methotrexate was 0.963, indicating that it was completely absorbed from the nasal cavity before clearance to the GI tract by MC. F_{NC} of mannitol and acyclovir was 0.087 and 0.369, respectively. F_{NC} decreased with the decrease in $P_{\text{Caco-2}}$ of drugs. On the other hand, F_{GI} of acyclovir, which has moderate $P_{\text{Caco-2}}$, was highest among all drugs.

Sakagami *et al.*[7] investigated fractional contributions of lung, nose, and GI absorption following nose-only aerosol

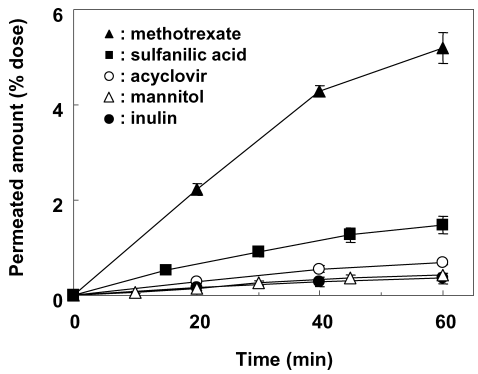

Fig. 2. Transport Profiles of 5 Drugs across Caco-2 Monolayer
Data are expressed as mean with S.E. of 3—4 experiments.

Table 1. Parameters Obtained in *in Vitro* Transepithelial Transport ($P_{\text{Caco-2}}$) and *in Vivo* Animal Study (F_{n}, F_{po}, F_{NC}, and F_{GI})

Compounds	Caco-2 permeability ($\times 10^{-6}$ cm/s)	*AUC*s (%dose·min/ml)			Fractional absorption			
	$P_{\text{Caco-2}}$	AUC_{n}	AUC_{po}	AUC_{iv}	F_{n}	F_{po}	F_{NC}	F_{GI}
Inulin	0.34±0.07	5.63±0.93	3.74±0.66	45.56±3.58	0.124±0.020	0.082±0.014	0.046	0.078
Mannitol	0.92±0.15	25.18±1.60	23.03±2.08	47.58±6.04	0.529±0.034	0.484±0.044	0.087	0.442
Acyclovir	1.37±0.21	16.29±1.46	14.96±2.50	18.58±2.76	0.877±0.079	0.805±0.135	0.369	0.508
Sulfanilic acid	3.69±0.40	51.79±5.61	21.22±1.53	62.63±5.39	0.827±0.090	0.339±0.024	0.738	0.089
Methotrexate	5.95±0.29	10.16±1.41	5.26±0.01	10.35±1.66	0.982±0.136	0.508±0.010	0.963	0.019

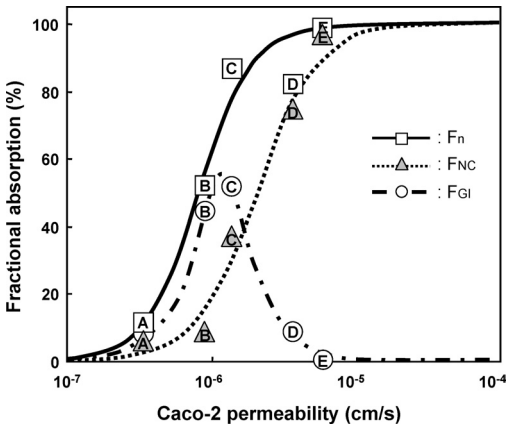

Fig. 3. Correlation of F_n, F_{NC}, and F_{GI} to Caco-2 Permeability; A: Inulin, B: Mannitol, C: Acyclovir, D: Sulfanilic Acid, E: Methotrexate

The fitted lines of F_n and F_{NC} were obtained from Hill's sigmoidal equation.

exposure of fluorescein. In their study, oral active charcoal (0.1 mg/kg BW) was used to diminish GI absorption of fluorescein. The active charcoal adsorbs the free fluorescein in GI tract to inhibit absorption. However, inhibition of GI absorption by active charcoal is not complete (92.6%). The degree of inhibition may be dependent on the drug. Active charcoal in the GI tract may enhance systemic elimination of the drug through GI exsorption. The method developed in this study is based on the kinetic theory and applicable to any drugs if fractional absorption after nasal and oral drug administration is available.

Relation of F_n, F_{NC}, and F_{GI} to Caco-2 Permeability

Figure 3 indicates the relation of F_n, F_{NC}, and F_{GI} to $P_{Caco\text{-}2}$. The correlations of F_n and F_{NC} to $P_{Caco\text{-}2}$ showed a sigmoid curve as was reported in the relation of the orally absorbed fraction to $P_{Caco\text{-}2}$.[26] The shape of the curve between F_{NC} and $P_{Caco\text{-}2}$ shifted right. The right shift of the curve corresponds to GI absorption after clearance from the GI tract by MC.

It is noteworthy that the relation between F_{GI} and $P_{Caco\text{-}2}$ showed a bell-shaped curve with peak at 10^{-6} cm/s of $P_{Caco\text{-}2}$. The curve suggested that highly permeable drugs are primarily absorbed from the nasal cavity for a short period of time after nasal application. Consequently, F_{GI} is determined by the rates of MC and absorption through the nasal mucosa. The fractional contribution of F_{NC} to F_n is dependent on $P_{Caco\text{-}2}$. The contribution of F_{NC} in methotrexate is 98.1% and is decreased with the decrease in $P_{Caco\text{-}2}$. Nasal drug application has both advantage and disadvantage over oral application. The advantage is that small volume of drug solution can be spread widely over the mucosal surface, which results in rapid drug absorption, whereas the disadvantage is the short residence time in the nasal cavity. The small contributions of F_{NC} to F_n in mannitol (16%) and inulin (37%) are a consequence of these factors, *i.e.* rapid absorption and short residence time in the nasal cavity for nasal application and slow absorption and long residence time in GI tract for oral application.

CONCLUSIONS

Drug absorption following nasal application was examined under physiologic condition in rats and fractional absorption from the nasal cavity and GI tract after nasal administration calculated. Drug absorption following nasal application is better than oral application and a sigmoid curve was observed between the fractional absorption and the permeability to Caco-2. A bell-shaped curve was shown between fractional absorption from the GI tract after nasal administration and the permeability to Caco-2. It is important to take into consideration that the drug is absorbed both from the nasal cavity and GI tract after nasal administration and that the primary absorption site of the drug after nasal application is decided by both the mucociliary clearance and absorption through the nasal mucosa, when nasal drug delivery is estimated and optimized.

REFERENCES

1) Bechgaard E., Gizurarson S., Hjortkjaer R. K., *J. Pharm. Pharmacol.*, **49**, 747—750 (1997).
2) Hussain A., Hirai S., Bawarshi R., *J. Pharm. Sci.*, **69**, 1411—1413 (1980).
3) O'Hagan D. T., Illum L., *Crit. Rev. Ther. Drug Carrier Syst.*, **7**, 35—97 (1990).
4) Illum L., *J. Control. Release*, **87**, 187—198 (2003).
5) Schipper N. G. M., Verhoef J. C., Merkus F. W. H. M., *Pharm. Res.*, **8**, 807—814 (1991).
6) van de Donk H. J., Merkus F. W., *J. Pharm. Sci.*, **71**, 595—596 (1982).
7) Sakagami M., Kinoshita W., Sakon K., Makino Y., *Arch. Toxicol.*, **77**, 321—329 (2003).
8) Hirai S., Yashiki T., Matsuzawa T., Mima H., *Int. J. Pharm.*, **7**, 317—325 (1981).
9) Hirai S., Yashiki T., Mima H., *Int. J. Pharm.*, **9**, 165—172 (1981).
10) Hirai S., Yashiki T., Mima H., *Int. J. Pharm.*, **9**, 173—184 (1981).
11) Hilgendorf C., Spahn-Langguth H., Regardh C. G., Lipka E., Amidon G. L., Langguth P., *J. Pharm. Sci.*, **89**, 63—75 (2000).
12) Ranaldi G., Marigliano I., Vespignani I., Perozzi G., Sambuy Y., *J. Nutr. Biochem.*, **13**, 157—167 (2002).
13) Stewart B. H., Chan O. H., Lu R. H., Reyner E. L., Schmid H. L., Hamilton H. W., Steinbaugh B. A., Taylor M. D., *Pharm. Res.*, **12**, 693—699 (1995).
14) Kuroda T., Namba K., Torimaru T., Kawashima K., Hayashi M., *Biol. Pharm. Bull.*, **23**, 334—338 (2000).
15) de Miranda P., Krasny H. C., Page D. A., Elion G. B., *J. Pharmacol. Exp. Ther.*, **219**, 309—315 (1981).
16) Hanna R. M., Borchard R. E., Schmidt S. L., *J. Vet. Pharmacol. Ther.*, **11**, 121—129 (1988).
17) Daley-Yates P. T., Baker R. C., *Br. J. Clin. Pharmacol.*, **51**, 103—105 (2001).
18) Fattinger K., Benowitz N. L., Jones R. T., Verotta D., *Eur. J. Clin. Pharmacol.*, **56**, 305—310 (2000).
19) Gudmundsdottir H., Sigurjonsdottir J. F., Masson M., Fjalldal O., Stefansson E., Loftsson T., *Pharmazie*, **56**, 963—966 (2001).
20) Hardy J. G., Lee S. W., Wilson C. G., *J. Pharm. Pharmacol.*, **37**, 294—297 (1985).
21) Humbert H., Cabiac M. D., Dubray C., Lavene D., *Clin. Pharmacol. Ther.*, **60**, 265—275 (1996).
22) Hidalgo I. J., Raub T. J., Borchardt R. T., *Gastroenterology*, **96**, 736—749 (1989).
23) Yamashita S., Konishi K., Yamazaki Y., Taki Y., Sakane T., Sezaki H., Furuyama Y., *J. Pharm. Sci.*, **91**, 669—679 (2002).
24) Donovan M. D., Flynn G. L., Amidon G. L., *Pharm. Res.*, **7**, 863—868 (1990).
25) Fisher A. N., Brown K., Davis S. S., Parr G. D., Smith D. A., *J. Pharm. Pharmacol.*, **39**, 357—362 (1987).
26) Chong S., Dando S. A., Soucek K. M., Morrison R. A., *Pharm. Res.*, **13**, 120—123 (1996).