# Nasal Absorption of Zidovudine and Its Transport to Cerebrospinal Fluid in Rats

Toshinobu SEKI,* Norihito SATO, Tetsuya HASEGAWA, Takeo KAWAGUCHI, and Kazuhiko JUNI

*Faculty of Pharmaceutical Sciences, Josai University, 1–1 Keyakidai, Sakado, Saitama 350–02, Japan.*
Received December 1, 1993; accepted April 6, 1994

**The nasal absorption of zidovudine (AZT) and its subsequent transport to cerebrospinal fluid (CSF) was examined in rats. Both rapid absorption and a high CSF concentration were observed after the nasal application. Plasma and CSF concentrations of AZT increased when probenecid was coadministered with AZT. Thus, this nasal coadministration of AZT and probenecid could be useful for the treatment of AIDS patients with neuropathies.**

**Keywords** zidovudine; AZT; nasal absorption; cerebrospinal fluid; probenecid; rat

Zidovudine (3′-azido-3′-deoxythymidine, AZT), an inhibitor of the reverse transcriptase of the human immunodeficiency virus , has primarily been administered orally. However, oral administration is not appropriate for the treatment of AIDS patients with central nervous system (CNS) dysfunctions, since frequent oral administration to these afflicted patients is difficult. Non-oral and non-parenteral dosage forms for AIDS treatments have therefore been researched to improve patient compliance and therapeutic efficacy: for example, transdermal delivery systems[1] and suppositories.[2] In this study, we report on nasal application, since nasal dosage forms can be given to patients by health care individuals and do not require specific techniques; the applied drug is immediately absorbed through the nasal mucous membrane, largely avoiding first-pass effects.[3] Moreover, a part of the absorbed drug is distributed directly to the CNS as well as *via* blood flow.[4] Plasma and cerebrospinal fluid (CSF) concentration profiles of AZT in rats after the nasal application of AZT with or without probenecid were studied in this paper to determine the efficiency of nasal delivery of AZT.

## MATERIALS AND METHODS

**Materials** AZT was purchased from Yamasa Shoyu Co. (Chiba, Japan). Probenecid was purchased from Sigma Chemical (St. Louis, MO). All other chemicals were of reagent grade and were used as received.

**Animal Preparation** Male Wistar rats weighing 180—260 g were anesthetized intraperitoneally with pentobarbital (50 mg/kg), and the femoral artery was cannulated with polyethylene tubing (SP-31). A surgical procedure for the *in vivo* nasal absorption study was carried out as described by Hirai *et al.*[3a]

**Nasal Absorption of Drugs** An aqueous suspension of AZT (200 μmol/ml), or a suspension of AZT and probenecid (200 μmol/ml of each) was applied to the nasal cavity through the nostril (100 μl/kg). The nostril was closed with an adhesive agent immediately after the administration. Blood samples were taken at 5, 15, 30, 45 and 60 min thereafter. To measure the concentration of drug in CSF, the experiments were stopped at 15 min and CSF was taken by a cisternal puncture[5]; in all evaluations, more than 150 μl of CSF was taken without any blood contamination. The CSF was separated as shown in Fig. 1.[4a] The initial 50 μl of CSF is referred to as fraction S and the last 50 μl as fraction D. For comparison with the nasal applications, AZT (20 μmol/kg) was also infused intravenously for 1 h to other rats which were surgically operated on and treated similarly to those used in the nasal absorption studies.

**Determination of AZT and Probenecid in Plasma and CSF** An HPLC system used to determine the presence of AZT and probenecid in plasma and CSF was composed of a Shimadzu LC-6A pump, a Shimadzu SPD-6A UV detector, a Rheodyne 7125 injector, and a reversed-phase column (LiChrospher RP-18e, 250 × 4 mm). The samples

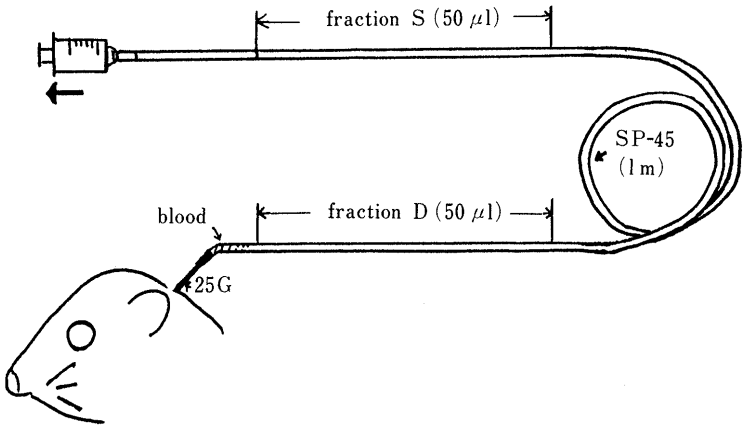


Fig. 1. Collection and Division of CSF

(100 μl of plasma or 50 μl of CSF) were mixed with the same volume of acetonitrile containing the internal standards (5 μg/ml of 7-β-hydroxypropyltheophyline for AZT and 5 μg/ml of *n*-butyl *p*-hydroxybenzoate for probenecid). After centrifugation at 14000 rpm, the supernatants (20 μl) were subjected to HPLC twice. For AZT analysis, the UV detector was operated at 265 nm, and a mobile phase containing 84.9% water, 15% acetonitrile and 0.1% acetic acid flowed at 1 ml/min. For probenecid analysis, the UV detector was operated at 244 nm, and a mobile phase containing 49.9% water, 50% acetonitrile and 0.1% acetic acid flowed at 1 ml/min.

## RESULTS AND DISCUSSION

To determine the nasal absorption of AZT, the plasma concentrations were evaluated for 1 h after the nasal application with or without probenecid, and the results are shown in Table I. Plasma concentrations of AZT in the i.v. infused rats are also shown in Table I. The plasma level of AZT increased immediately after the nasal application. The absolute bioavailability after the nasal application of AZT cannot be calculated because the value of $AUC_{60-\infty}$ is unknown for both the nasal application and the i.v. infusion. Since the elimination of AZT is affected by the anesthetization with pentobarbital (the values of elimination half-life in rats are 1.0 and 0.25 h with and without the anesthetization, respectively),[1,2] the actual plasma concentration profiles of AZT after 60 min cannot be predicted. The absolute bioavailability after the nasal application (69%) was estimated based on the assumption that the plasma concentration at 60 min divided by the elimination rate constant in the anesthetized rats gives $AUC_{60-\infty}$.

The value of $AUC_{0-60}$ obtained from the plasma concentration profile of AZT after the simultaneous application of AZT and probenecid was significantly higher than that of AZT alone. The increase in plasma level could be due to a decrease in the renal clearance of AZT caused by the coadministered probenecid.[6] The plasma concentration profile of probenecid is similar to that of the coexisting AZT.

Table II shows the plasma and CSF concentrations of the drugs at 15 min and the concentration ratio of CSF/plasma. The CSF/plasma ratio of AZT at 60 min after i.v. infusion is also shown. Since the concentration in CSF at 1 h after nasal application was too low to determine, the concentration following nasal application was evaluated after 15 min. The difference in the CSF concentrations of AZT between fractions S and D following i.v. infusion was not significant, and the values of the CSF/plasma ratio in the fractions were 0.091 ± 0.006 (± S.D.) and 0.116 ± 0.032, respectively. The direct transport of several drugs from the nasal cavity to CSF has been reported by Sakane *et al.*[4a,b] Our results showed that the CSF/plasma ratio at 15 min of AZT without probenecid, AZT with probenecid, and probenecid itself in fraction D, which contains the CSF in the vicinity of the nasal cavity, were 0.361 ± 0.276, 0.471 ± 0.251 and 0.472 ± 0.408, respectively, each value in fraction D being higher than that in fraction S. Although the differences in the ratio between the two fractions are not statistically significant, except in the case of AZT coadministered with probenecid, and the CSF/plasma ratios at 15 min following nasal application does not represent an equilibrium distribution, the high CSF/plasma ratio in fraction D

TABLE I. Plasma Concentrations of AZT and Probenecid

| | Plasma concentration (μM) Time (min) | | | | | $AUC_{0-60}$ (μM h) |
|---|---|---|---|---|---|---|
| | 5 | 15 | 30 | 45 | 60 | |
| Nasal (20 μmol/kg) | | | | | | |
| AZT | 12.57 ± 2.73[a] | 10.94 ± 1.70 | 7.61 ± 1.70 | 5.05 ± 1.80 | 4.93 ± 1.53 | 7.63 ± 0.57 |
| AZT[b] + probenecid | 18.83 ± 4.81 | 16.50 ± 5.08 | 12.84 ± 3.70 | 10.31 ± 3.57 | 8.79 ± 3.89 | 12.77 ± 3.67 |
| Probenecid[c] + AZT | 20.85 ± 3.66 | 20.81 ± 9.82 | 9.48 ± 8.34 | 4.75 ± 6.33 | 1.83 ± 2.54 | 10.82 ± 6.48 |
| i.v. infusion (20 μmol/kg/h) | | | | | | |
| AZT | 3.18 ± 0.74 | 5.61 ± 2.03 | 7.00 ± 1.17 | 10.00 ± 2.63 | 9.95 ± 4.87 | 7.06 ± 1.96 |

*a*) Mean ± S.D. *b*) AZT values following coadministration of AZT (20 μmol/kg) and probenecid (20 μmol/kg). *c*) Probenecid values following coadministration of AZT (20 μmol/kg) and probenecid (20 μmol/kg).

TABLE II. Plasma and CSF Concentrations of AZT and Probenecid

| | Concentration at 15 min (μM) | | | CSF/plasma ratio | |
|---|---|---|---|---|---|
| | Plasma | Fraction S | Fraction D | Fraction S | Fraction D |
| Nasal | | | | | |
| AZT | 12.67 ± 3.72[a] | 1.24 ± 0.50 | 4.60 ± 3.39 | 0.097 ± 0.022 | 0.361 ± 0.276 |
| AZT[b] + probenecid | 17.80 ± 2.71 | 3.34 ± 1.88 | 8.27 ± 4.81 | 0.200 ± 0.128 | 0.471 ± 0.251 |
| Probenecid[c] + AZT | 13.10 ± 7.09 | 4.14 ± 3.45 | 8.15 ± 8.39 | 0.268 ± 0.147 | 0.472 ± 0.408 |
| i.v. infusion[d] | | | | | |
| AZT | 9.95 ± 4.87 | 0.89 ± 0.41 | 1.08 ± 0.34 | 0.091 ± 0.006 | 0.116 ± 0.032 |

*a*) Mean ± S.D. *b*) AZT values following coadministration of AZT and probenecid. *c*) Probenecid values following coadministration of AZT and probenecid. *d*) Values were observed at 60 min.

observed after the nasal application may suggest the direct transport of AZT from the nasal cavity to CSF. Probenecid increased the CSF concentration of AZT not only in fraction D but also in fraction S. The enhanced distribution of AZT intravenously coadministered with probenecid into CSF has been reported.[6a,7] Probenecid can act as an inhibitor for the efflux of AZT from CSF to plasma. The high CSF concentration of AZT following the nasal coadministration of AZT and probenecid could be attributed to the high distribution of probenecid in CSF. The effect of probenecid on the direct transport of AZT to CSF is not clear in this study.

Although the direct distribution of AZT into the CSF after nasal application has not been fully proved, the nasal application of AZT coadministered with probenecid by this means shows promise as a possible treatment for AIDS patients with CNS dysfunctions. An adhesive preparation to the olfactory epithelium could be useful for the efficient and continuous delivery of AZT to CSF. Further study on such a preparation should be required.

## REFERENCES

1) T. Seki, C. Toeda, T. Kawaguchi, K. Sugibayashi, Y. Morimoto, *Chem. Pharm. Bull.*, **38**, 3086 (1990); T. Seki, T. Kawaguchi, K. Sugibayashi, K. Juni, Y. Morimoto, *Int. J. Pharmaceut.*, **57**, 73 (1989); T. Seki, T. Kawaguchi, K. Juni, *Pharm. Res.*, **7**, 948 (1990).
2) T. Kawaguchi, T. Hasegawa, K. Juni, T. Seki, *Int. J. Pharmaceut.*, **77**, 71 (1991).
3) *a*) S. Hirai, T. Yashiki, T. Matsuzawa, H. Mima, *Int. J. Pharmaceut.*, **7**, 317 (1981); *b*) H. Char, S. Kumar, S. Patel, D. Piemontese, K. Igbal, A. W. Malick, R. A. Salvador, C. R. Behl, *J. Pharm. Sci.*, **81**, 750 (1992); *c*) M. A. Sarkar, *Pharm. Res.*, **9**, 1 (1992).
4) *a*) T. Sakane, M. Akizaki, M. Yoshida, S. Yamashita, T. Nadai, M. Hashida, H. Sezaki, *J. Pharm. Pharmacol.*, **42**, 449 (1991); *b*) T. Sakane, M. Akizaki, S. Yamashita, T. Nadai, M. Hashida, H. Sezaki, *Chem. Pharm. Bull.*, **39**, 2456 (1991); *c*) T. C. Kumar, G. F. X. David, A. Sakaranarayanan, V. Puri, K. R. Sundram, *Proc. Natl. Acad. Sci. U.S.A.*, **79**, 4185 (1982).
5) R. C. Chou, G. Levy, *J. Pharmacol. Exp. Ther.*, **219**, 42 (1981).
6) *a*) M. A. Hedaya, R. J. Sawchak, *J. Pharm. Sci.*, **78**, 716 (1989); *b*) M. A. Heyada, W. F. Elmquist, R. J. Sawchak, *Pharm. Res.*, **7**, 411 (1990); *c*) M. Qian, T. S. Finco, M. Mehta, C. T. Viswanathan, J. M. Gallo, *J. Pharm. Sci.*, **80**, 1007 (1991).
7) R. J. Sawchak, M. A. Hedaya, *Pharm. Res.*, **7**, 332 (1990).