Brain and Cerebrospinal Fluid Distributions of Metoprolol in Rats

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The distribution of metoprolol tartrate (MPL) into the brain and cerebrospinal fluid (CSF) from plasma was determined at 2 different steady-state plasma concentrations (2 and 20 nmol/ml) and following intravenous bolus administration (100 nmol/kg) in rats. Under steady-state condition, no difference in the brain and CSF distributions of MPL was observed between 2 different plasma MPL concentrations, and the value of brain- or CSF-to-plasma partition coefficients were 5.7 and 1.5, respectively. Following intravenous administration, MPL distributed into CSF very rapidly and its initial uptake phase was not detected. On the other hand, MPL distribution into the brain was relatively slow with the uptake phase. Thus, the brain uptake of MPL from the plasma was pharmacokinetically analyzed using a modified 2-compartment model and deconvolution method, whereas the CSF distribution of MPL was not adequate for kinetic analysis because of the lack of uptake phase. The analysis of brain uptake of MPL by both compartment model and deconvolution gave the same results and the calculated brain K_p value of MPL was almost comparable with that of observed K_p value under steady-state plasma concentration. The distribution of MPL into CSF was considered mainly depending on the pH difference between the plasma and CSF, and the mutual transfer of MPL between CSF and the brain tissue was considered to be negligible from an *in vitro* study.

Keywords — metoprolol; brain; cerebrospinal fluid; distribution; central nervous system; pharmacokinetics; deconvolution; rat

Introduction

Brain uptake of hydrophilic acidic compounds existing as an ionized form in the plasma and of macromolecular compounds such as protein are restricted by blood-brain (B.B.B.) and blood-cerebrospinal (B.CSF.C.) barriers. On the other hand, many weakly basic compounds such as tricyclic antidepressants existing as an undissociated form in the plasma penetrate partially through the B.B.B and/or B.CSF.B depending on their lipophilic properties.^{1,2)}

This manuscript treats with the distribution of metoprolol tartrate (MPL) into brain and cerebrospinal fluid from plasma. MPL, a basic compound having a pK_a value of 9.7, is a cardioselective β_1 -adrenergic antagonist³⁾ which is efficiently absorbed from the gastrointestinal tract in humans⁴⁾ and rats⁵⁾ and distributes to such organs as brain, lung, and kidney.^{6,7)} Side effects of MPL are not severe but the common ones are fatigue, headache, dizziness, insomnia,

and hypertension.⁸⁻¹⁰⁾ With respect to the distribution of MPL into the central nervous system (CNS), it has been reported that the extent of brain uptake of MPL is less than that of propranolol, but the uptake of MPL into CSF is greater than that of atenolol in beagle dogs.¹¹⁾ The CNS uptake of MPL is also reported in human patients suffering from central nervous injury or hypertension. However, in those previous reports,^{6,7,11-13)} the pharmacokinetic properties of MPL for MPL distributions into the brain and CSF are not yet clarified, since data of CNS distribution of MPL under steady-state plasma concentrations and following intravenous bolus administration are lacking.

In the present study, the distribution of MPL into the brain and CSF from plasma under steady-state plasma concentrations and following intravenous bolus administration were determined in rats. The transfer of MPL into the brain from CSF was also examined in an *in vitro* study. Based on these data, brain distribution of MPL

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after intravenous administration was pharmacokinetically analyzed.

Materials and Methods

Materials — Metoprolol ($\pm$) tartrate (MPL) and blue dextran were purchased from Sigma Chemical Co., (St. Louis, MO, U.S.A.). ^3H -Inulin was purchased from Amersham International Ltd., Buckinghamshire, U.K. and was stored at -20°C until use. Clearsol I as a scintillation cocktail was purchased from Nacalai Tesque Ltd., Kyoto, Japan. All other reagents were of reagent grade and purchased from Wako Pure Chemical Industries, Ltd., Osaka or Nacalai Tesque Ltd., Kyoto, Japan.

Animal Studies — Male Wistar rats, 200–300 g, were anesthetized by an intraperitoneal injection of sodium pentobarbital (Nembutal sodium solution, Abbott Lab., U.S.A.) at a dose of 30 mg/kg. Cannulation with polyethylene tubing (PE 50) was made in a femoral artery and/or vein for taking blood and for the administration of drug, respectively. The anesthetized rats were fixed supine on a surface kept at 37°C , or were mounted on a stereotaxic frame in a temperature-controlled cabinet kept at 37°C , depending on the experimental purpose. During the experiments, the rectal temperature of rats was monitored and was kept at above 36°C .

Intravenous Bolus Administration of MPL: MPL dissolved in saline at a concentration of 100 $\mu\text{mol/ml}$ was intravenously administered from the tail vein at a dose of 100 $\mu\text{mol/ml/kg}$. Arterial blood was withdrawn *via* a cannula inserted into a femoral artery at appropriate time intervals. Blood was centrifuged at 10 000 rpm for 5 min to separate plasma, which was stored at -30°C until analysis.

CNS Distribution of MPL under Steady-State Plasma Concentration: Animal study was made in a temperature-controlled cabinet kept at 37°C to maintain the normal rectal temperature of rats above 36°C . An anesthetized rat cannulated with polyethylene tubing in a femoral artery and vein was mounted on a stereotaxic frame in a prone position. After exposing the skull, a small hole (diameter 2.05 mm) was opened on the skull using a dental drill (Leutor mini gold, Natsume

Seisakusyo) not so as to damage dura mater encephali. MPL was administered intravenously *via* cannula at the femoral vein at a dose of 8.35 or 83.5 $\mu\text{mol/kg}$, followed by constant rate infusion of MPL at a rate of 0.175 or 17.5 $\mu\text{mol/min/kg}$, respectively. Thus, steady-state MPL plasma concentration of 2 or 20 nmol/ml was established 90 min after the initiation of constant rate infusion. Blood was withdrawn *via* polyethylene tubing inserted into the femoral artery to separate plasma. CSF sample (more than 50 μl) in the cisterna magna was taken with polyethylene tubing (PE 10) connected to a syringe through the exposed dura mater encephali without damaging the cerebellum. Immediately after collecting CSF sample, the rat was exsanguinated to isolate the whole brain. The brain tissue, CSF, and plasma samples were kept at -30°C until analysis.

CNS Distribution of MPL after Intravenous Bolus Administration: Anesthetized rats were mounted on a stereotaxic frame and a hole was made on the skull in the same manner as described previously. Rats received MPL intravenously at a dose of 100 $\mu\text{mol/kg}$. Rats were killed at scheduled time intervals after administration of MPL to obtain plasma, CSF and brain tissue. The weight of the isolated brain tissue was measured and samples were kept at -30°C until analysis.

Transfer of MPL into the Brain from CSF *in Vitro*: The transfer of MPL from CSF into the brain was examined in an *in vitro* study. Whole brain tissue (about 1.5 g) was freshly isolated from a control rat. The brain tissue was preincubated in a 1.5 ml of an artificial CSF solution at 37°C for 1 min, and the brain was transferred into another artificial CSF solution containing MPL (100 nmol/ml) and blue dextran (1%), toluidine blue O (0.03%), or ^3H -inulin (25 nCi/ml) for incubation. The composition of the artificial CSF solution (adjusted at pH 7.30 at 37°C after bubbling with 95% oxygen and 5% CO_2 mixture gas) was as follows: 122 mM NaCl, 15 mM NaHCO_3 , 10 mM glucose, 3 mM KCl, 1.4 mM CaCl_2 , 1.2 mM MgSO_4 , 0.4 mM K_2HPO_4 , and 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.¹⁴⁾ The incubation was carried out at a rate of 20 time

strokes/min. At appropriate time intervals, 50 μ l of the medium was collected over for 2 h to determine the remaining concentration of drugs in the medium.

Analytical Method — The concentration of MPL in the plasma, CSF, artificial CSF and the brain was determined as reported by Gengo *et al.*¹⁵⁾ with minor modification. Briefly, brain was homogenized with a 6-fold volume of ice-cold water. Plasma (100 μ l), CSF (50 μ l), artificial CSF (50 μ l) or brain homogenate (500 μ l) samples were deproteinized with a 5% trichloroacetic acid solution (400 μ l for plasma, 200 μ l for CSF and artificial CSF, 1 ml for brain homogenate). After centrifuging, 1 N NaOH solution (600, 300 μ l and 1 ml for plasma, CSF and artificial CSF, and brain homogenate, respectively) was added to each supernatant (400, 200 μ l, or 1 ml, respectively). Each solution was vigorously shaken with 7 ml of dichlorethane, and the organic phase separated by centrifugation was evaporated to dryness under reduced pressure. The residue was dissolved with 100 μ l of methanol containing propranolol as an internal standard, and 20 μ l of the solution was injected on ODS-80 TM column (TSK gel, Toyo Soda, Tokyo) of high pressure liquid chromatography (HPLC). The HPLC apparatus (Shimadzu, Kyoto) was equipped with a fluorometric detector (RF-500, Shimadzu, Kyoto). The mobile phase was a mixture of 1% acetic acid and acetonitrile (75:25 v/v) containing 0.05 M sodium 1-heptanesulfonate, and the flow rate was 1 ml/min. Detection of MPL and internal standard was made at 282 nm for emission and 301 nm for excitation. Blue dextran and toluidine blue O were spectrophotometrically determined at 620 and 634 nm, respectively, after

dilution with an appropriate amount of water. Radioactivity of ^3H -inulin in artificial CSF solution was counted on a liquid scintillation counter (LSC-903, Aloka, Tokyo) after mixing with 3 ml of scintillation cocktail.

Data Analysis — A pharmacokinetic analysis was made by the use of MULTI or MULTILUNGE, a non-linear least square analysis program for microcomputer developed by Yamaoka *et al.*¹⁶⁾ with minor modification. The Student's *t*-test was used to estimate a significance with $p=0.05$ as the minimal level of significance.

Results and Discussion

CNS Distribution of MPL at Steady-State Plasma Concentration

The brain and CSF distribution of MPL was determined at two different steady-state plasma concentrations, 2 (low) and 20 (high) nmol/ml. The steady-state MPL plasma concentration was established at least 90 min after initiation of constant rate infusion. To ensure the steady-state plasma condition, the plasma, CSF, and brain samples were taken 2 h after initiation of constant rate infusion. Results are summarized in Table I. No difference in the K_p value, tissue-to-plasma partition coefficient, of MPL in both the brain and CSF was observed between two different MPL plasma concentrations. These findings indicate that MPL distributes into the brain and CSF in a linear fashion at least in these plasma concentration ranges examined. The K_p value of MPL in the brain was 3–4 times greater than that in CSF. The marked difference in the K_p values of MPL for the brain and CSF is also reported in human patients with brain surgery,

TABLE I. Plasma, Brain and CSF Concentrations and K_p Values of Metoprolol under Different Steady-State Plasma Concentrations in Rats

C _{ss} concn. (nmol/ml)			K_p value	
Plasma	Brain	CSF	Brain/plasma	CSF/plasma
1.36 $\pm$ 0.17	7.53 $\pm$ 0.73	2.15 $\pm$ 0.25	5.56 $\pm$ 0.34	1.58 $\pm$ 0.20
24.15 $\pm$ 2.66	140.5 $\pm$ 15.4	34.18 $\pm$ 3.31	5.82 $\pm$ 0.32	1.42 $\pm$ 0.09

Each value represents the mean $\pm$ S.E.M. ($n=4$).

The constant rate infusion of MPL were at a rate of 0.175 or 17.5 μ mol/min/kg.

Fig. 1. Plasma, Brain and CSF Concentrations of Metoprolol Following Intravenous Bolus Administration in Rats

Dose of metoprolol was $100 \mu\text{mol/kg}$. $\circ$, brain; Δ , CSF; $\bullet$, plasma. Solid lines for brain and plasma were calculated by a modified 2-compartment model (Fig. 4). Each point represents the mean $\pm$ S.E.M. ($n=3-11$).

and their K_p values are 12 and 1, respectively.¹⁴⁾ CNS Distribution of MPL after Intravenous Bolus Administration

Time courses of the concentration of MPL in the plasma, CSF and brain were determined by one point method after intravenous bolus administration at a dose of $100 \mu\text{mol/kg}$. The time courses of MPL concentrations in the brain, CSF

Fig. 2. Time Course of Apparent K_p Values of Metoprolol in the Brain and CSF Following Bolus Intravenous Administration in Rats

Dose of metoprolol was $100 \mu\text{mol/kg}$. $\bullet$, brain; $\circ$, CSF. Solid line represents the mean of K_p value. Each point represents the mean $\pm$ S.E.M. of 3–11 trials.

and plasma are shown in Fig. 1. In Fig. 2, the apparent K_p values in the brain and CSF are plotted against the time following intravenous bolus administration. The plasma disposition parameters of MPL analyzed using the conventional 2-compartment model was the same to those in the time sampling method in each individual rat. As shown in Fig. 1 and 2, MPL was uptake into the CNS very rapidly, and the peak MPL concentration in the CSF and brain was observed at 1 and 3–10 min, respectively. In the case of CSF distribution of MPL, no initial uptake phase was detected in the present study in which the initial sampling was 1 min after the drug administration. The concentration of MPL or the apparent K_p value in the brain was far greater than that in CSF at each sampling time. **Transfer of MPL from an Artificial CSF into the Brain *in Vitro***

The transfer of MPL from the artificial CSF into the brain was determined *in vitro*. Blue dextran (M_r 2 000 000) and inulin (M_r 5 200) were used as extracellular space markers.^{17,18)} Toluidine blue O was used as a permeable model compound into the brain. Results are shown in Fig. 3. The disappearance of toluidine blue O was greater than that of MPL and/or ^3H -inulin, and MPL disappeared from the medium at almost the same rate with ^3H -inulin. Similar disappearance rates of MPL and ^3H -inulin will indicate that the transfer of MPL from the artificial CSF into the brain cell will be negligible, whereas MPL distributes into the brain extracellular space like inulin. On the other hand, no dis-

Fig. 3. Brain Uptake of Metoprolol, Inulin, Blue Dextran and Toluidine Blue O from Artificial CSF *in Vitro*

$\circ$, blue dextran; $\bullet$, ^3H -inulin; Δ , metoprolol; $\square$, toluidine blue O. Each points the mean $\pm$ S.E.M. ($n=4$).

Fig. 4. Modified 2-Compartment Model Used for Analysis of Brain Uptake of Metoprolol

k_{12} , k_{21} ; Transfer rate constant between central and brain compartments. k_{13} , k_{31} ; Transfer rate constant between central and CSF compartments. k_{10} ; Elimination rate constant from central compartment.

appearance was observed in the case of blue dextran, indicating that a macromolecule like blue dextran can hardly penetrate the brain extracellular space, because of low diffusibility in the extracellular aqueous space. This negligible transfer of MPL into the brain cell from the artificial CSF solution in an *in vitro* study may suggest that the mutual transfer of MPL between the brain cell and CSF *in vivo* is negligible.

Kinetic Analysis of MPL for CNS Distribution

The brain distribution of MPL shown in Fig. 1 was pharmacokinetically analyzed with a modified 2-compartment model (Fig. 4) and by deconvolution. In the model (Fig. 4), the transfer of MPL from plasma into the brain and vice versa was assumed not to affect the plasma concentration of MPL, since the amount of MPL uptaken by the brain was fairly small compared to the amount in the plasma. Also, the mutual transfer of MPL between CSF and the brain was assumed to be negligible as described above. The

Fig. 5. Time Course of the Weight Function Value, $G(\theta)$, Calculated from the Deconvolution between Plasma and Brain Levels of Metoprolol after Intravenous Bolus Administration

TABLE II. Pharmacokinetic Parameters of Metoprolol Analyzed Using Modified 2-Compartment Model and Deconvolution

Parameter	Compartment model	Deconvolution
k_{12} (min^{-1})	1.318	1.188
k_{21} (min^{-1})	0.209	0.186
k_{13} (min^{-1})	0.352	
k_{31} (min^{-1})	0.178	
k_{10} (min^{-1})	0.090	
V_1 (l/kg)	1.049	
K_p (brain) ^{a)}	6.306	6.387

Pharmacokinetic analysis was made using the mean value obtained from 3—11 trials for each point. ^{a)} $K_p(\text{brain})$ was calculated by k_{12}/k_{21} .

modified 2-compartment model is the same as one used in pharmacokinetic analysis of caffeine in CNS which was previously reported.¹⁹⁾

The differential equations employed for the calculation were as follows:

$$\begin{aligned}
 dX(1)/dt &= k_{31} \times X(3) - (k_{13} + k_{10}) \times X(1) \\
 dX(2)/dt &= k_{12} \times X(1) - k_{21} \times X(2) \\
 dX(3)/dt &= k_{13} \times X(1) - k_{31} \times X(3)
 \end{aligned}$$

Where, $X(1)$, $X(2)$, and $X(3)$ denote the amount of MPL in the central compartment, brain, CSF and peripheral compartments, respectively. When $C(1)$ and V_1 were defined as the drug concentration and the distribution volume in the central compartment, respectively, $X(1)$ at time 0 is the dose ($C(1) \times V_1$). $X(2)$ and $X(3)$ at time 0 are 0, respectively. In this calculation using a microcomputer, the parameters of MPL in plasma disposition were fixed as the same value as those obtained by both time sampling method and one point method after intravenous bolus administration, and only the transfer rate constants between plasma and brain were estimated. The fitting curves are shown in Fig. 1 with the observed MPL concentrations in the plasma and brain.

The transfer of MPL into the brain and CSF from the plasma was also analyzed by deconvolution method.²⁰⁾ In this analysis, the plasma concentration was dealt with as a sole input function, since the transfer of MPL between the brain

and CSF was neglected in this analysis. The MPL concentration in the brain was dealt with as output function, and the weight function, $G(\theta)$, between plasma and brain was estimated. That is, $G(\theta) = K_1 \times \exp(-K_2 t)$, where, K_1 is the transfer rate constant of MPL from the plasma into the brain, and K_2 is the counter rate constant. The time course of the weight function calculated by deconvolution between plasma and the brain levels of MPL after intravenous bolus administration is shown in Fig. 5. Parameters in the brain distribution of MPL obtained by two methods are listed in Table II, and those parameters obtained by the 2 different methods were in fairly good agreement.

Under equilibrium conditions and supposing that MPL is not metabolized in the brain, the following equations will be derived.

$$K_1 \times C_p = K_2 \times C_b, \text{ or } C_b/C_p = K_1/K_2$$

Where, C_p , and C_b represent the MPL concentrations in the plasma and brain under equilibrium condition, respectively. Thus, C_b/C_p denotes the K_p value for the brain under equilibrium conditions. The above equation will also be applicable to the compartment model analysis by substituting k_{12} and k_{21} for K_1 and K_2 , respectively. The calculated K_p values are also listed in Table II. For the brain, the K_p values of MPL calculated by the compartment model and the deconvolution were 6.3 and 6.4, respectively, and these values were almost comparable with that (5.7) obtained by the direct determination under steady-state plasma condition. In this analysis method using the compartment model, only the concentrations of MPL in various regions were used, not a mass balance equation. However, the brain distribution of MPL was well explained by this model, and the transfer rate of MPL between the plasma and the brain, and the K_p value estimated at a steady-state were just comparable with that obtained by the deconvolution method. In contrast to the data for the brain, the data for the CSF were not well explained by this analysis method. The reason for this is not clear in the present study, however, the discrepancy will indicate that another process for the disposition and/or up-

take of MPL is necessary. It is generally accepted that the concentration of compounds in the CSF is different among different regions in the brain; for example: among the lateral ventricle, cisterna magna and area of arachnoid villi. Also, in the present study, we didn't examine the contribution of the transfer of MPL into the CSF from the brain *in vitro* or *in vivo* study, since it was difficult to examine it without damage on ependyme *in vitro* and/or without a radiolabeled compound *in vivo*. Further investigation will be necessary to make clear the time profiles of MPL concentration in the CSF.

In the present study, however, the distribution of MPL into CSF was not analyzed because of its extremely rapid distribution without showing the initial uptake phase as shown in Fig. 1. Assuming that the mutual transfer of MPL between CSF and the brain is negligible *in vivo* as observed *in vitro* (Fig. 3), it will be considered that the transfer of MPL into CNS takes place through B.B.B. and B.CSF.B., separately, and, the barrier function of B.CSF.B. for MPL is fairly low, since MPL distributes into CSF very rapidly. The CSF distribution of MPL *in vivo* is considered to be mainly due to the pH difference between the plasma and CSF as follows; distribution ratio (R) of MPL (CSF/plasma) by pH difference will be theoretically estimated by the following equation. $R = (1 + 10^{pK_a - \text{pH}(\text{CSF})}) / (1 + 10^{pK_a - \text{pH}(\text{plasma})})$. Since pK_a of MPL is 9.7, $\text{pH}(\text{CSF}) = 7.3$,²¹⁾ and $\text{pH}(\text{plasma}) = 7.4$, the R value will be calculated as 1.27. This value is almost comparable to the value of 1.5 obtained *in vivo*. The marked difference in K_p value observed between CSF and brain may result from the barrier of ependyma for MPL mutual transfer between them and possible existence of some special binding sites for MPL in the brain,²²⁾ since the lipophilic beta blocker, propranolol, has been shown to be actively sequestered by brain tissue *via* saturable cytoplasmic binding.^{23,24)} Also, Pardridge *et al.* suggested that lipophilic amines such as propranolol and lidocaine are actively transported by the brain.²⁵⁾ As another possible mechanism, the phospholipids binding of MPL, especially to acidic phospholipids such as phosphatidylserine, may also participate in its brain distribution, since

many basic compounds such as propranolol bind preferentially to those acidic phospholipids in various tissues and are accumulated in the tissue.²⁶⁾ Although no saturable CNS uptake of MPL was observed in the present study, further investigations such as the barrier function of ependyma for MPL transfer between the brain and CSF, uptake mechanism of MPL through B.B.B. and/or, B.CSF.B., and specific binding of MPL to the brain constituents will be necessary to make clear the distribution characteristic of MPL into the CNS.

In the present study, the CNS distribution of MPL was determined under 2 different steady-state plasma concentrations and following intravenous bolus administration in rats. The brain uptake of MPL after intravenous bolus administration was well analyzed by both the compartment model and the deconvolution analysis methods, and the calculated brain K_p value of MPL was almost comparable with that of observed K_p value under steady-state plasma concentration. The distribution of MPL into CNS was considered to depend mainly on the pH difference between the plasma and CSF, and the mutual transfer of MPL between CSF and the brain cell was considered to be negligible by an *in vitro* study.

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