Change in Pharmacokinetics of Model Compounds with Different Elimination Processes in Rats during Hypothermia

Koyo NISHIDA,*[,a] Madoka OKAZAKI,[a] Ryuichi SAKAMOTO,[a] Natsuko INAOKA,[a] Hideaki MIYAKE,[a] Shintaro FUMOTO,[a] Junzo NAKAMURA,[a] Mikiro NAKASHIMA,[b] Hitoshi SASAKI,[b] Mikio KAKUMOTO,[c] and Toshiyuki SAKAEDA[c]

[a] *Department of Clinical Pharmacy, Graduate School of Biomedical Sciences, Nagasaki University; 1–14 Bunkyo-machi, Nagasaki 852–8521, Japan:* [b] *Department of Hospital Pharmacy, Nagasaki University Hospital of Medicine and Dentistry; 1–7–1 Sakamoto, Nagasaki 852–8501, Japan: and* [c] *Department of Hospital Pharmacy, School of Medicine, Kobe University; 7–5–2 Kusunoki-cho, Chuo-ku, Kobe 650–0017, Japan.*

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We compared the pharmacokinetics of model compounds with different elimination processes between hypothermic and normothermic rats, to obtain basic information concerning drug therapy during hypothermia. Male Wistar rats were anesthetized with sodium pentobarbital and kept at temperatures of 37 °C (normothermic group) by heat lamp, and 32 °C or 28 °C (hypothermic group) by external cooling. We chose phenolsulfonphthalein (PSP), indocyanine green (ICG) and fluorescein isothiocyanate (FITC)-dextran (FD-4, Mw 4400) as model compounds to determine changes in clearance pathways during hypothermia therapy. The plasma concentrations of PSP as biliary, urinary and metabolic elimination type were increased significantly in the hypothermic group (32 °C, 28 °C) after i.v. administration at a dose of 1 mg, compared to the normothermic group (37 °C). Each PSP clearance (bile, urine and metabolites) in the hypothermic group was decreased, suggesting an influence of hypothermia on the active elimination process. The decreasing tendency was marked at a temperature of 28 °C. Moreover, the plasma concentrations of ICG as the biliary excretion type after i.v. administration to the hypothermic rats at a dose of 1 mg were higher with more than 50% decrease in the total body clearance compared to normothermic rats. On the other hand, there was almost no difference in the i.v. pharmacokinetics of FD-4 as the urinary excretion type between 37 °C and 32 °C. However, renal clearance of FD-4 was significantly decreased at a temperature of 28 °C. Accordingly, the change in pharmacokinetics of a drug in the hypothermic group could differ with the elimination processes.

Key words hypothermia; pharmacokinetics; active transport; glomerular filtration

Cerebral ischemia accompanied by head trauma, cerebral and cardiac infarction leads to neuron dysfunction and cell death,[1] and its after-effects such as dementia, perception injury and hemiplegia are serious problems. Excess glutamate released from the neuron, energy disorder from impaired circulation and free radical generation have been clarified as the causes leading to neuron death involved with cerebral ischemia.[2,3] Busto *et al.* reported in 1989[4] that it was possible to prevent neuron dysfunction and cell death in the ischemia by lowering the body temperature of the rats, *i.e.* surface cooling to 32—33 °C. The multiple mechanisms of hypothermia-induced neuroprotection were identified such as reduction in cerebral metabolism, energy depletion and stabilization of cell membranes as reported previously.[4—10] Hypothermia therapy has been increasingly applied to the humans[11] since it was proved useful in rats and dogs to treat experimental ischemic brain injury in the early 1990s.

On the other hand, hypothermia therapy possibly causes side effects such as arrhythmia, impaired immune function and coagulation disorders as reviewed by Schubert.[12] In order to negate these side effects, several kinds of medicines such as antiarrhythmic drugs, antibiotics and anticoagulants are administered to the patients.[12] Moreover, like other critically ill patients, those with severe head injuries typically receive a large number of medications. In addition, although combination pharmacotherapy with an inhibitor of glutamate and calcium might act synergistically by attenuating an ischemia-induced release of neurotoxic glutamate,[2,3] it is possible for undesirable drug-interactions to occur. Although hypothermic patients tend to receive many different pharmacologic agents, most of them are administered according to dosing schedules derived from the normothermic host. These schedules do not take into consideration any changes in drug pharmacokinetics or pharmacodynamics associated with hypothermia therapy. Physiological and biochemical changes may happen during hypothermia therapy related to a reduction in blood flow[13] and energy deficiency.[14] Therefore, pharmacokinetics of a drug during hypothermia should differ largely from normothermic conditions. There have been a few reports that recently investigated the influence of hypothermia on drug pharmacokinetics and pharmacodynamics such as phenytoin,[15] neostigmine[16] and vecuronium.[17] As well, the possibility of side effects and drug-interactions caused by variations in drug pharmacokinetics during hypothermia must be sufficiently understood.

In the present study, we assessed systematically the influence of hypothermia at 32 °C on the pharmacokinetics of several compounds as models with different elimination processes. Moreover, we examined the pharmacokinetic change in hypothermia at 28 °C, aiming to consider the unexpected conditions such as too much cooling and body temperature dependency on pharmacokinetic change of a drug in the patients fully by three different body temperatures.

MATERIALS AND METHODS

Chemicals Phenolsulfonphthalein (PSP) and indocyanine green (ICG) were purchased from Nacalai Tesque, Inc.

* To whom correspondence should be addressed. e-mail: koyo-n@nagasaki-u.ac.jp

(Kyoto, Japan) and Daiichi Pharmaceutical Co., Ltd. (Tokyo, Japan), respectively. Fluorescein isothiocyanate-dextran (FITC-dextran) with an average molecular weight of 4400 (FD-4) was obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

***In Vivo* Experiment** All animal experiments in the present study conformed to the Guidelines for Animal Experimentation at Nagasaki University.

Male Wistar rats (270—300 g) were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and the left femoral artery was cannulated with a polyethylene tube (i.d. 0.25 mm, o.d. 0.8 mm; Dural Plastics, Dural, Australia). After the middle abdomen was cut open about 3 cm, the common bile duct was cannulated with a polyethylene tube (i.d. 0.28 mm, o.d. 0.61 mm, Becton Dickinson & Co., Parsippany, NJ, U.S.A.).

Rats were divided into the three experimental animal groups: (a) a normothermic group in which rectal temperature was maintained at 37 °C by a heat lamp throughout the procedure; a hypothermic group kept at 32 °C (b) or 28 °C (c) in which hypothermia was induced by external cooling for 1 h before the drug administration, with rectal temperatures thereafter being maintained at 32 °C or 28 °C. During the experiment, plastic bag containing crushed ice was placed over the hypothermic rats to maintain body temperature at 32 °C or 28 °C. The animal experiments were started around 9 a.m. to exclude the intraday difference of drug pharmacokinetics.

Intravenous Administration The drug solution (0.1 ml) was injected into the jugular vein. After administration of the drug solution, blood (200 μl) was collected at the selected times from the heparinized cannula inserted into the femoral artery over 4 h. Blood was centrifuged at 15000 rpm for 5 min. Bile samples were collected at appropriate time intervals for 4 h. At 4 h after administration, urine was collected from the bladder directly by a syringe.

Analytical Method The concentrations of model compounds in the plasma, bile and urine were determined as follows.

The concentration of free PSP was determined spectrophotometrically at 560 nm after dilution with 1 M NaOH. The total concentration of free PSP and its metabolite was measured in the same manner after the samples were subjected to acid hydrolysis (1 M HCl at 100 °C for 30 min).[18] The concentration of PSP metabolite was estimated from the difference between these values.

The concentration of ICG was determined spectrophotometrically at 805 nm after proper dilution with saline containing 0.1% (w/v) bovine serum albumin as a stabilizer.

The concentration of FD-4 was determined spectrophotofluorometrically at excitation and emission wavelengths of 489 and 515 nm, respectively.

Calculation of Moment Parameters The plasma concentration profiles of free PSP and ICG, and biliary excretion rate profiles of free PSP, its metabolite and ICG after i.v. administration were analyzed based on the statistical moment theory.[19] Moment parameters for the plasma concentration profile (AUC_p, MRT_p) and biliary excretion rate profiles (AUC_b, MRT_b) were calculated by numerical integration using a linear trapezoidal formula and extrapolating to infinite time based on a monoexponential equation. The total body clearance (CL_{total}) was calculated by dividing administration dose by AUC_p. For PSP, moment parameters for the biliary excretion rate profiles of free PSP ($AUC_{b,f}$, $MRT_{b,f}$) and its metabolite ($AUC_{b,m}$, $MRT_{b,m}$) were calculated independently.

Biliary, Renal and Metabolic Clearance To evaluate the excretion and metabolism capability in the liver or kidney, the biliary excretion clearance (CL_b) of free PSP and ICG, the renal clearance (CL_r) of free PSP and FD-4 and the metabolic clearance (CL_m) of PSP were calculated from Eqs. 1, 2 and 3, respectively.

$$CL_b = \frac{X_b}{AUC_{p,finite}} \quad (1)$$

$$CL_r = \frac{X_u}{AUC_{p,finite}} \quad (2)$$

$$CL_m = \frac{X_m}{AUC_{p,finite}} \quad (3)$$

X_b, X_u and X_m represent the amount of cumulative excretion into the bile and urine of free PSP, FD-4 and ICG, and the total amount of PSP metabolite excreted into the urine and bile until 4 h after i.v. administration, respectively. $AUC_{p,finite}$ is the finite *AUC* of the plasma concentration profile until 4 h.

Statistical Analysis Statistical comparisons were performed by Dunnett's test after examining with analysis of variance (ANOVA). $p<0.05$ was considered to be indicative of statistical significance, compared to control condition (normothermic group at 37 °C). All results were expressed as the mean±standard error (S.E.) of at least five experiments.

RESULTS

Comparison of PSP Pharmacokinetics between the Hypothermic and Normothermic Rats Figure 1 shows the plasma concentration profiles of free PSP after i.v. administration of PSP to rats at a dose of 1 mg under different body temperatures. The PSP plasma concentrations were higher in the hypothermic group (32 °C, 28 °C) than those in the normothermic group (37 °C), and the elimination phase rate constant showed a significant decrease (37 °C, 0.021 min^{-1}; 32 °C, 0.013 min^{-1}; 28 °C, 0.010 min^{-1}).

Figure 2 illustrates the biliary excretion rate profiles of

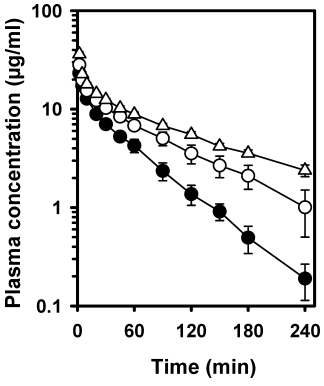

Fig. 1. Plasma Concentration Profiles of Free PSP at a Dose of 1 mg after I.V. Administration to Rats under Body Temperatures of 37 °C, 32 °C and 28 °C

Each point represents the mean±S.E. of six experiments. Key: 37 °C (●), 32 °C (○) and 28 °C (△).

Table 1. Moment Parameters for Plasma Concentration Profiles of Free PSP and Biliary Excretion Profiles of Free PSP and Its Metabolite at a Dose of 1 mg after I.V. Administration to Rats with Body Temperatures under 37 °C, 32 °C and 28 °C

Body temp.	AUC_p (μg·min/ml)	MRT_p (min)	$AUC_{b,f}$ (μg)	$MRT_{b,f}$ (min)	$AUC_{b,m}$ (μg)	$MRT_{b,m}$ (min)
37 °C	788.6±70.9	49.9±4.7	333.6±25.7	49.5±2.9	241.8±34.5	84.5±8.4
32 °C	1487.9±227.0*	95.1±15.7*	482.3±34.6**	88.2±12.1*	216.8±47.1	117.7±18.5
28 °C	2054.1±120.6**	130.6±9.4**	400.3±25.9	112.7±7.3**	264.1±30.4	201.1±27.3**

Each value is the mean±S.E. of six experiments. * $p<0.05$, ** $p<0.01$, significantly different from 37 °C.

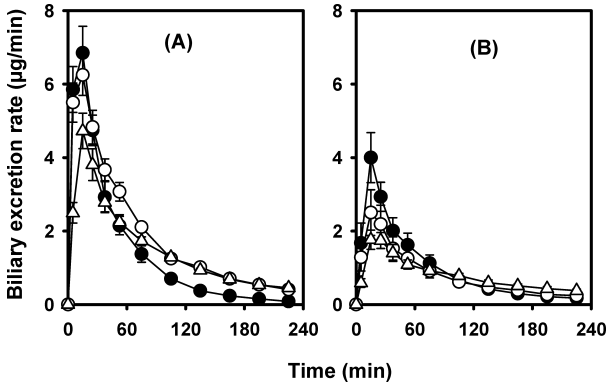

Fig. 2. Biliary Excretion Rate Profiles of Free PSP (A) and Its Metabolite (B) at a Dose of 1 mg after I.V. Administration to Rats under Body Temperatures of 37 °C, 32 °C and 28 °C

Each point represents the mean±S.E. of six experiments. Key: 37 °C (●), 32 °C (○) and 28 °C (△).

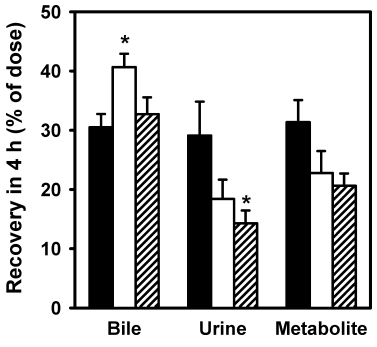

Fig. 3. Recovery in 4 h (% of Dose) of Free PSP (Bile or Urine) and Its Metabolite (Total Recovery in Both Bile and Urine) after I.V. Administration to Rats at a Dose of 1 mg under Body Temperatures of 37 °C (Closed Column), 32 °C (Open Column) and 28 °C (Slashed Column)

Each bar represents the mean±S.E. of six experiments. * $p<0.05$, significantly different from 37 °C.

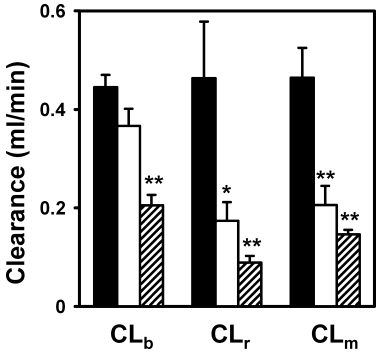

Fig. 4. Biliary (CL_b), Renal (CL_r) and Metabolic Clearance (CL_m) of PSP after I.V. Administration to Rats at a Dose of 1 mg under Body Temperatures of 37 °C (Closed Column), 32 °C (Open Column) and 28 °C (Slashed Column)

Each bar represents the mean±S.E. of six experiments. * $p<0.05$, ** $p<0.01$, significantly different from 37 °C.

free PSP (A) and its metabolite (B) after i.v. administration of PSP to the hypothermic and normothermic rats at a dose of 1 mg. In the hypothermic group, the maximum biliary excretion rates of free PSP and its metabolite were decreased according to the body temperature, although not significant.

Moment parameters for the plasma concentration profiles of free PSP (Fig. 1) and biliary excretion rate profiles of free PSP and its metabolite (Figs. 2A, B) are summarized in Table 1. The AUC_p values of PSP in the hypothermic group were significantly larger than those in the normothermic group. Hypothermic rats (0.87±0.10 and 0.58±0.02 ml/min, 32 °C and 28 °C, respectively) exhibited a significantly smaller CL_{total} of PSP than normothermic rats (1.51±0.15 ml/min, 37 °C). In addition, the MRT_p and $MRT_{b,f}$ of PSP in the hypothermic group were significantly longer than those in normothermic group.

Figure 3 shows the recovery in 4 h (% of dose) of free PSP in the bile and urine, and PSP metabolite in both the bile and urine after i.v. administration of PSP to the normothermic and hypothermic rats at a dose of 1 mg. The amounts of free PSP excreted into the urine and total metabolites in the hypothermic group were approximately two-thirds those of the normothermic group. In contrast, biliary excretion of free PSP was increased at 32 °C (Fig. 3), although not significant at 28 °C. In addition, biliary and urinary recovery rates of PSP metabolite excretion were calculated as 37 °C (66%, 34%), 32 °C (75%, 25%) and 28 °C (83%, 17%), showing a tendency of decrease in urinary contribution by low body temperature.

Figure 4 represents the biliary, renal and metabolic clearances of PSP at a dose of 1 mg after i.v. administration to rats. A marked reduction in CL_r and CL_m of PSP was recognized in the hypothermic group, compared to the case of CL_b.

Comparison of ICG Pharmacokinetics between the Hypothermic and Normothermic Rats Figures 5A and B illustrate the plasma concentration and biliary excretion rate profiles of ICG after i.v. administration to the hypothermic and normothermic rats at a dose of 1 mg, respectively. The plasma concentrations of ICG were much higher in the hypothermic group than in the normothermic group (Fig. 5A). The biliary excretion of ICG was delayed largely in the hypothermic group compared to the normothermic group (Fig. 5B). The high plasma concentration and delayed biliary excretion tendencies were marked in the hypothermic group at 28 °C.

Table 2 summarizes the moment parameters for the plasma

Table 2. Moment Parameters for Plasma Concentration and Biliary Excretion Rate Profiles and Biliary Clearance (CL_b) of ICG at a Dose of 1 mg after I.V. Administration to Rats with Body Temperatures under 37 °C, 32 °C and 28 °C

Body temp.	AUC_p (μg·min/ml)	MRT_p (min)	AUC_b (μg)	MRT_b (min)	CL_{total} (ml/min)	CL_b (ml/min)
37 °C	271.4±53.4	27.0±4.7	921.1±41.1	49.2±5.2	4.83±0.70	4.18±0.63
32 °C	550.0±62.3*	46.4±8.9	932.8±26.0	95.9±3.5	2.28±0.26**	1.94±0.26**
28 °C	1302.2±67.1**	50.8±10.3	1026.2±58.3	376.8±46.0**	0.91±0.05**	0.42±0.04**

Each value is the mean±S.E. of at least six experiments. * $p<0.05$, ** $p<0.01$, significantly different from 37 °C.

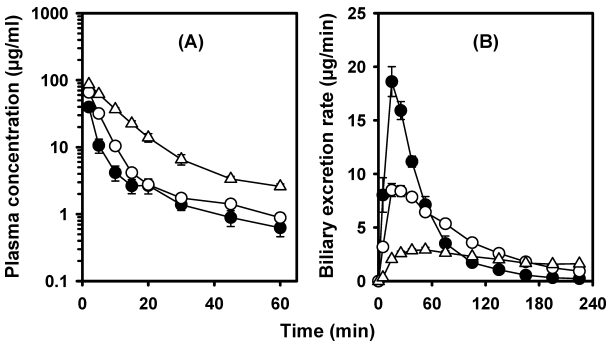

Fig. 5. Plasma Concentration (A) and Biliary Excretion Rate (B) Profiles of ICG after I.V. Administration to Rats at a Dose of 1 mg under Body Temperatures of 37 °C, 32 °C and 28 °C

Each point represents the mean±S.E. of at least six experiments. Key: 37 °C (●), 32 °C (○) and 28 °C (△).

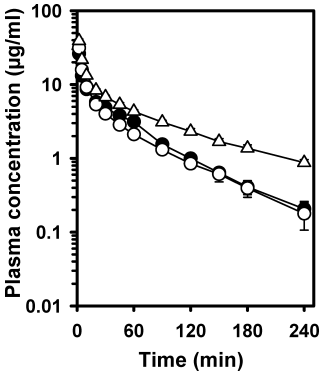

Fig. 6. Plasma Concentration Profiles of FD-4 after I.V. Administration to Rats at a Dose of 1 mg under Body Temperatures of 37 °C, 32 °C and 28 °C

Each point represents the mean±S.E. of at least five experiments. Key: 37 °C (●), 32 °C (○) and 28 °C (△).

concentration and biliary excretion rate profiles of ICG. The AUC_p and MRT_b of ICG were increased, according to decrease in the body temperature. As listed in Table 2, the CL_b of ICG in the hypothermic group decreased significantly to about 50% and 10% of that of the normothermic group, at the temperatures of 32 °C and 28 °C, respectively.

Comparison of FD-4 Pharmacokinetics between the Hypothermic and Normothermic Rats The plasma concentration profiles of FD-4 after i.v. administration to the hypothermic and normothermic rats at a dose of 1 mg are shown in Fig. 6. Table 3 lists the renal clearance of FD-4 after i.v. administration. Plasma concentrations were almost equal at all time points between 37 °C and 32 °C. The CL_r of FD-4 was also almost the same value in the two groups. In contrast, the plasma concentrations of FD-4 were considerably increased at 28 °C, and the CL_r was significantly decreased to about 60% of normothermic group as listed in Table 3.

Table 3. CL_{total} and CL_r of FD-4 at a Dose of 1 mg after I.V. Administration to Rats with Body Temperatures under 37 °C, 32 °C and 28 °C

Body temp.	CL_{total} (ml/min)	CL_r (ml/min)
37 °C	1.88±0.13	1.45±0.05
32 °C	2.10±0.14	1.57±0.09
28 °C	1.07±0.08**	0.86±0.05**

Each value is the mean±S.E. of at least five experiments. * $p<0.05$, ** $p<0.01$, significantly different from 37 °C.

DISCUSSION

PSP, a hydrophilic dye (organic anion), has been clinically used as a renal function test compound in humans, and is excreted into the bile and urine as a free form or conjugative metabolite in rats.[18] We selected PSP because of its capability to evaluate the hypothermia effects on two different elimination routes of excretion and metabolism simultaneously. The plasma disappearance of free PSP after i.v. administration was delayed in the hypothermic rats according to decrease in the body temperature (Fig. 1), and CL_{total} decreased to about 60% and 40% that of the normothermic rats, respectively, at temperatures of 32 °C and 28 °C. There is a report showing that cardiac output in a hypothermic group of piglets kept at 29 °C decreased to about 40% that of a normothermic group.[13] The change in the blood flow rate accompanied by a decrease in the cardiac output was considered to be the reason for the decrease in the clearance of PSP even in the initial phase in the hypothermic rats (Fig. 1).

AUC_p of PSP was increased by about three-times at the temperature of 28 °C (Table 1), whereas $AUC_{b,f}$ and $AUC_{b,m}$ were not so changed in the hypothermic rats. This is probably because final excretion amount of PSP and its metabolite was maintained, even though biliary and metabolism clearance activity of PSP into the bile was decreased in the hypothermic rats at 28 °C.

In the hypothermic group as compared to the normothermic rats, we observed a tendency for the amounts of free PSP excreted into the urine and its metabolite to decrease while the biliary excretion of free PSP was increased at 32 °C (Fig. 3), probably due to compensation for the decrease in urinary excretion and metabolism. The liver and kidney play important roles as main excretory organs, and it was reported that the liver and kidney have a mutual compensation-function in the biliary and urinary excretion processes.[20,21]

ICG has been widely used as a clinical diagnostic index to evaluate liver function, especially hepatic blood flow and biliary excretion. ICG shows a characteristic disposition after

i.v. administration, *i.e.*, ICG distributes throughout the plasma exclusively without extravascular distribution, and is exclusively excreted into the bile without biotransformation *via* an active multi-specific organic anion transport system.[3,22] As shown in Fig. 5A, ICG plasma disappearance was delayed in the hypothermic rats according to decrease in the body temperature after i.v. administration. In the hypothermic group (32 °C, 28 °C), a continuous biliary excretion of ICG was observed (Fig. 5B), and then the MRT_b of ICG was extended about two- and eight-fold at temperatures of 32 °C and 28 °C, respectively (Table 2). This also reveals that biliary excretion of ICG *via* an active multi-specific organic anion transport system[22] in the liver was extended by low body temperature.

The CL_b of ICG in the hypothermic rats was markedly decreased as listed in Table 2. In contrast, CL_b of PSP was not decreased so much (Fig. 4), compared to ICG (Table 2). The differences in temperature-dependent effects should be related to the hepatic extraction ratio of ICG and PSP. Elimination of drugs such as ICG with a high hepatic extraction ratio depends largely upon hepatic blood flow, whereas the clearance of drugs such as PSP[23] with an intermediate or low hepatic extraction ratio is much less dependent on alterations in the hepatic blood flow. Therefore, clearance of a hepatic blood flow-limited type drug such as ICG with a high hepatic extraction ratio would be decreased considerably during hypothermia accompanied by decrease in blood flow.

Consequently, the active transport and metabolism mainly in the liver and kidney would be attenuated at 32 °C and 28 °C according to decrease in the body temperature due to a failure in the energy supply, as confirmed in the present study. During the care of patients undergoing hypothermia, possible modifications are needed in drug dosing, especially in case of drugs eliminated by active transport and metabolism, and their plasma concentrations should be monitored.

As the next step, we assessed the pharmacokinetics of FD-4 excreted mainly by glomerular filtration, in order to study the influence of hypothermia on the elimination process *via* passive diffusion. As shown in Fig. 6, the plasma pharmacokinetics of FD-4 at 32 °C and 37 °C were almost equal. It was thus indicated that the glomerular filtration function would not be affected under hypothermia at 32 °C. Although renal functions are reduced in parallel with a decrease in renal blood flow during hypothermia, glomerular filtration function should be changed to a lesser extent. Since the renal vascular system has an auto-regulation function for homeostasis maintenance, the kidney is able to keep the glomerular filtration function constant in spite of variations by change in the systemic circulation. The hypothermia-inducing temperature of 32 °C set in this study is supposed to be within the range of this auto-regulated renal function. Therefore, the pharmacokinetics of a drug such as the main excretion route of glomerular filtration is considered to be unchanged during hypothermia therapy at 32 °C.

However, it was reported that both the glomerular filtration and renal blood flow rates under a body temperature of 28 °C were decreased to about half of their control values at 37 °C in the rats.[24] In fact, the plasma concentration profile and pharmacokinetic parameters of FD-4 were markedly changed under the hypothermic condition at 28 °C (Fig. 6, Table 3). Especially, the decrease in CL_r at 28 °C should be an important factor for choosing administration dose. In addition, change in the pharmacokinetics of a drug with a high molecular weight larger than FD-4 in a temperature-dependent fashion is of special interest.

In conclusion, the change in drug pharmacokinetics during hypothermia could differ with the elimination processes. The present results might be useful for drug therapy during hypothermia although they were not directly applied to clinic. Further studies are needed to assess the influence of hypothermia intensity and dose on the pharmacokinetics of actual medicines and to examine the underlying mechanisms sensitive to body temperature.

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