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Inter-Strain Differences of Exogeneous Creatinine Disposition in Rats and Mice[1)]

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Inter-strain differences of exogeneous creatinine disposition in rats and mice were assessed by comparing the plasma or whole blood level-time data following intravenous administration to rats (Wistar, Sprague–Dawley, F344, ACI and Donryu strains) and mice (ddY and C57BL/6 strains).

Almost all pharmacokinetic parameters were about the same among all the strains of rats studied, and this was also the case for mice. However, non-negligible and statistically significant differences were observed in $(V_d')_{extrap}$ values. For example, the $(V_d')_{extrap}$ value for F344 rats ($4.62 \times 10^3 \pm 7.38 \times 10^2$ ml/kg) was about 1.7 times that for Donryu rats ($2.75 \times 10^3 \pm 2.99 \times 10^2$ ml/kg) ($p < 0.01$). Similarly, the value for C57BL/6 mice ($2.77 \times 10^3 \pm 5.04 \times 10^2$ ml/kg) was about twice that for ddY mice ($1.35 \times 10^3 \pm 4.63 \times 10^2$ ml/kg) ($p < 0.01$). This result for mice was consistent with the whole-body autoradiographic data. These findings suggest that the distribution properties of creatinine vary among different strains of rats and mice.

Keywords——inter-strain difference; rat; mouse; creatinine; plasma level; whole blood level; whole-body autoradiography; distribution volume; total body clearance

Many species of experimental animals have been used in the life science studies, though various strains of rats and mice are the most common experimental animals. Experimental data are frequently compared among the different strains, and therefore, basic biopharmaceutical data on differences among the strains, *e.g.*, in absorption, distribution, metabolism and excretion of drugs or other chemical substances, are considered to be necessary. In this paper, in order to provide basic information for biopharmaceutical studies, creatinine, which is water-soluble and metabolically inert and whose renal clearance is generally accepted as an index of renal function, was selected as a model chemical, and the disposition of creatinine was examined by plasma or whole blood analyses in various strains of rats (Wistar, Sprague–Dawley, F344, ACI and Donryu strains) and mice (ddY and C57BL/6 strains). The whole-body autoradiographic technique was also used to assess the muscle distribution of creatinine in mice.

Materials and Methods

Animals——Male rats of Wistar, Sprague–Dawley, Donryu, F344 and ACI strains and male mice of ddY and C57BL/6 strains were purchased from Shizuoka Agricultural Cooperative for Laboratory Animals, Hamamatsu, Japan. These rats and mice were used for experiments at 6 weeks old and 6 to 8 weeks old, respectively. All rats were chronically cannulated into the left external jugular vein by the method of Upton.[2)]

Chemicals——[carbonyl-^{14}C]Creatinine hydrochloride (specific activity, 12.0 mCi/mmol) was purchased from Amersham Japan, Tokyo, Japan, and dissolved in saline. The radiochemical purity was more than 98%. All other chemicals were of analytical grade and were used without further purification.

Plasma or Whole Blood Levels Following Intravenous Administration——[^{14}C]Creatinine was administered

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intravenously to rats through the cannula (10 μCi/kg) and to mice into the tail vein (100 μCi/kg). The dose as creatinine was 0.94 mg/kg in each case. In the case of rats, blood samples (about 250 μl) were withdrawn periodically from the cannula into small heparinized tubes. Plasma samples were obtained by centrifuging the tubes at 3000 rpm for 15 min. An aliquot (100 μl) of the plasma was used for scintillation counting. On the other hand, in the case of mice, blood samples (5 μl) were taken periodically with Microcaps (Drummond Scientific Co., Broomal, Pa., U.S.A.) by wounding the tail vein with a razor, and were subjected to scintillation counting. The above plasma or whole blood samples were dissolved in 200 μl of Soluene-350 (Packard Instrument Co., Downers Grove, Ill., U.S.A.). Hydrogen peroxide (50 μl) was used for the decolorization of whole blood samples.

Whole-Body Autoradiography Following Intravenous Administration to Mice——Mice were given 100 μCi/kg of [^{14}C]creatinine (0.94 mg/kg as creatinine) into the tail vein, and sacrificed 2 min after the administration by soaking them in dry-ice acetone (−78 °C) without anesthesia. Sections (40 μm) were obtained with a microtome (Yamato 1111, Tokyo, Japan) at about −25 °C, and attached to Salotape (Hisamitsu Pharmaceutical Co., Ltd., Tosu, Japan). After being dried in a freeze-dryer for a few days, the sections were placed in contact with X-ray films (No. 150, Fuji Photo Film Co., Ltd., Tokyo, Japan) for 20 d at 4 °C. A densitometer (PDA-11, Konishiroku, Tokyo, Japan) was used for determining the optical density of autoradiograms.

Radioactivity Measurement——The radioactivity was determined in a Mark II liquid scintillation spectrometer (Nuclear-Chicago Corporation, Des Plaines, Ill., U.S.A.). All samples were determined with 10 ml of toluene–Triton X-100 liquid scintillator (2,5-diphenyloxazole (PPO) 5 g, 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP) 300 mg, toluene 700 ml, Triton X-100 300 ml). The counting efficiencies were automatically determined by the ^{133}Ba external standard ratio method and cpm was converted to dpm.

Results and Discussion

Plasma Levels Following Intravenous Administration to Rats

Creatinine is not metabolized in rats[3] (though the microflora in the gastrointestinal tract can metabolize creatinine[4]) and it is quantitatively excreted into the urine following *i.v.* administration to rats.[4] Therefore, all of the radioactivity in plasma obtained following *i.v.* administration of [^{14}C]creatinine was regarded as originating from [^{14}C]creatinine.

The plasma level–time data (Fig. 1) following *i.v.* administration to Wistar, Sprague–Dawley, F344, ACI and Donryu rats were analyzed on the basis of a two-compartment open

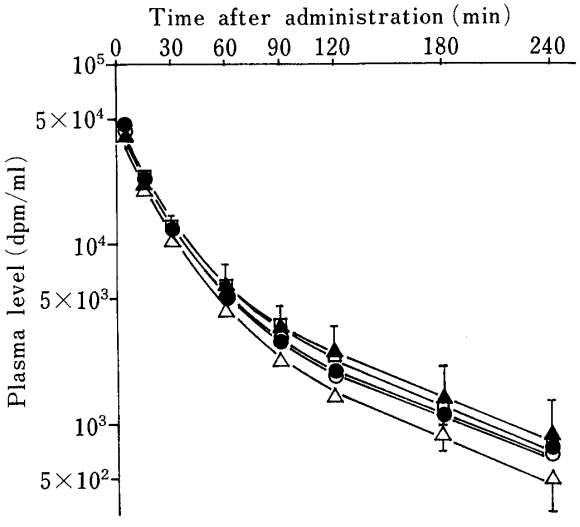

Fig. 1. Plasma Levels Following Intravenous Administration of [^{14}C]Creatinine to Various Strains of Rats

Each point and vertical bar represent the mean ± S.D. for three rats. ○, Wistar rats; ●, Sprague–Dawley rats; △, F344 rats; ▲, ACI rats; □, Donryu rats.

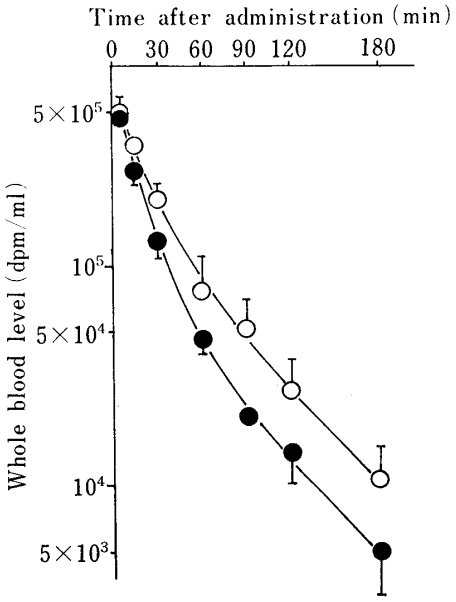

Fig. 2. Whole Blood Levels Following Intravenous Administration of [^{14}C]Creatinine to ddY and C57BL/6 Mice

Each point and vertical bar represent the mean ± S.D. for four to six mice. ○, ddY mice; ●, C57BL/6 mice.

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TABLE I. Pharmacokinetic Properties Following Intravenous Administration of [^{14}C]Creatinine to Various Strains of Rats (Value for Parameter ± Standard Error[a])

Parameter	Strain				
	Wistar ($n=8$)[b]	Sprague–Dawley ($n=8$)	F344 ($n=8$)	ACI ($n=8$)	Donryu ($n=8$)
A (dpm/ml)	$4.52\times10^4 \pm 4.00\times10^3$	$5.00\times10^4 \pm 4.51\times10^3$	$4.52\times10^4 \pm 4.82\times10^3$	4.05×10^4[c] $\pm 3.00\times10^3$	$4.56\times10^4 \pm 3.91\times10^3$
B (dpm/ml)	5.85×10^3[d] $\pm 8.91\times10^2$	5.85×10^3[d] $\pm 7.77\times10^2$	4.81×10^3[d] $\pm 7.68\times10^2$	$7.69\times10^3 \pm 7.20\times10^2$	$8.08\times10^3 \pm 8.78\times10^2$
α (min^{-1})	$5.61\times10^{-2} \pm 5.56\times10^{-3}$	$6.09\times10^{-2} \pm 5.72\times10^{-3}$	$6.12\times10^{-2} \pm 6.49\times10^{-3}$	$6.05\times10^{-2} \pm 5.32\times10^{-3}$	$6.23\times10^{-2} \pm 6.09\times10^{-3}$
β (min^{-1})	$9.27\times10^{-3} \pm 8.37\times10^{-4}$	8.93×10^{-3}[d] $\pm 7.51\times10^{-4}$	$9.63\times10^{-3} \pm 8.98\times10^{-4}$	9.31×10^{-3}[c] $\pm 5.35\times10^{-4}$	$9.99\times10^{-3} \pm 6.26\times10^{-4}$
k_{10} (min^{-1})	$3.55\times10^{-2} \pm 2.51\times10^{-3}$	3.78×10^{-2}[c] $\pm 2.71\times10^{-3}$	4.04×10^{-2}[d] $\pm 3.32\times10^{-3}$	3.22×10^{-2}[c] $\pm 1.90\times10^{-3}$	$3.49\times10^{-2} \pm 2.34\times10^{-3}$
V_1' (ml/kg)	435 ± 36	398 ± 34	444 ± 45	461[d] ± 31	413 ± 32
V_2' (ml/kg)	452 ± 10	488 ± 100	483 ± 12	530 ± 94	453 ± 91
$(V_d')_{extrap}$ (ml/kg)	3790[d] ± 557	3790[d] ± 503	4620[d] ± 738	2890 ± 271	2750 ± 299
CL_{tot} (ml/min/kg)	15.4 ± 1.7	15.0 ± 1.7	17.9[d] ± 2.4	14.8 ± 1.3	14.4 ± 1.5

a) W. E. Deming, "Statistical Adjustment of Data," John Wiley and Sons, Inc., New York, 1946. *b*) The number of input data, each of which is the mean for three rats. *c*) Significantly different from the value for Donryu rats at $p<0.05$. *d*) Significantly different from the value for Donryu rats at $p<0.01$.

TABLE II. Pharmacokinetic Properties Following Intravenous Administration of [^{14}C]Creatinine to ddY and C57BL/6 Mice (Value for Parameter ± Standard Error[a])

Parameter	Strain	
	ddY ($n=8$)[b]	C57BL/6 ($n=8$)
A (dpm/ml)	4.28×10^5[c] $\pm 5.65\times10^4$	$5.17\times10^5 \pm 2.80\times10^4$
B (dpm/ml)	1.65×10^5[c] $\pm 5.66\times10^4$	$8.02\times10^4 \pm 1.46\times10^4$
α (min^{-1})	$5.20\times10^{-2} \pm 1.17\times10^{-2}$	$6.13\pm10^{-2} \pm 4.97\times10^{-3}$
β (min^{-1})	$1.53\times10^{-2} \pm 2.17\times10^{-3}$	$1.54\times10^{-2} \pm 1.21\times10^{-3}$
k_{10} (min^{-1})	3.12×10^{-2}[c] $\pm 2.30\times10^{-3}$	$4.38\times10^{-2} \pm 1.96\times10^{-3}$
V_1' (ml/kg)	374 ± 28	372 ± 19
V_2' (ml/kg)	156 ± 81	196 ± 43
$(V_d')_{extrap}$ (ml/kg)	1350[c] ± 463	2770 ± 504
CL_{tot} (ml/min/kg)	11.7[c] ± 1.2	16.3 ± 1.1

a) W. E. Deming, "Statistical Adjustment of Data," John Wiley and Sons, Inc., New York, 1946. *b*) The number of input data, each of which is the mean for four to six mice. *c*) Significantly different from the value for C57BL/6 mice at $p<0.01$.

model, and the estimated pharmacokinetic parameters are summarized in Table I. Almost all pharmacokinetic parameters including the total body clearance (CL_{tot}) were about the same among the various species. Therefore, the inter-strain differences of kidney function were considered to be negligible, because the total body clearance of creatinine is almost equal to the sum of the clearance due to glomerular filtration and tubular secretion in rats.[5] On the other hand, quite large differences were observed in the $(V'_d)_{extrap}$ values. For example, the $(V'_d)_{extrap}$ value for F344 rats ($4.62\times10^3 \pm 7.38\times10^2$ ml/kg) was about 1.7 times that for Donryu rats ($2.75\times10^3 \pm 2.99\times10^2$ ml/kg) ($p<0.01$). This result suggests that the distribution behavior of creatinine may vary from strain to strain of rats.

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Whole Blood Levels Following Intravenous Administration to Mice

In line with the case of rats, creatinine intravenously administered to mice is quantitatively excreted into the urine in unchanged form,[6] and the radioactive substance in blood was regarded as [^{14}C]creatinine.

Whole blood level–time data (Fig. 2) following *i.v.* administration to ddY and C57BL/6 mice were analyzed on the basis of a two-compartment open model, and the estimated pharmacokinetic parameters are summarized in Table II. The total body clearance was significantly different between ddY (11.7 ± 1.2 ml/min/kg) and C57BL/6 (16.3 ± 1.1 ml/min/kg) mice ($p < 0.01$), suggesting that glomerular filtration and/or tubular secretion of creatinine may be enhanced in C57BL/6 mice as compared with ddY mice. Further, the $(V'_d)_{extrap}$ value for C57BL/6 mice ($2.77 \times 10^3 \pm 5.04 \times 10^2$ ml/kg) was about twice that for ddY mice ($1.35 \times 10^3 \pm 4.63 \times 10^2$ ml/kg) ($p < 0.01$). Distribution of creatinine from blood to tissues is assumed to be enhanced in C57BL/6 mice. The whole-body autoradiograms in the two strains

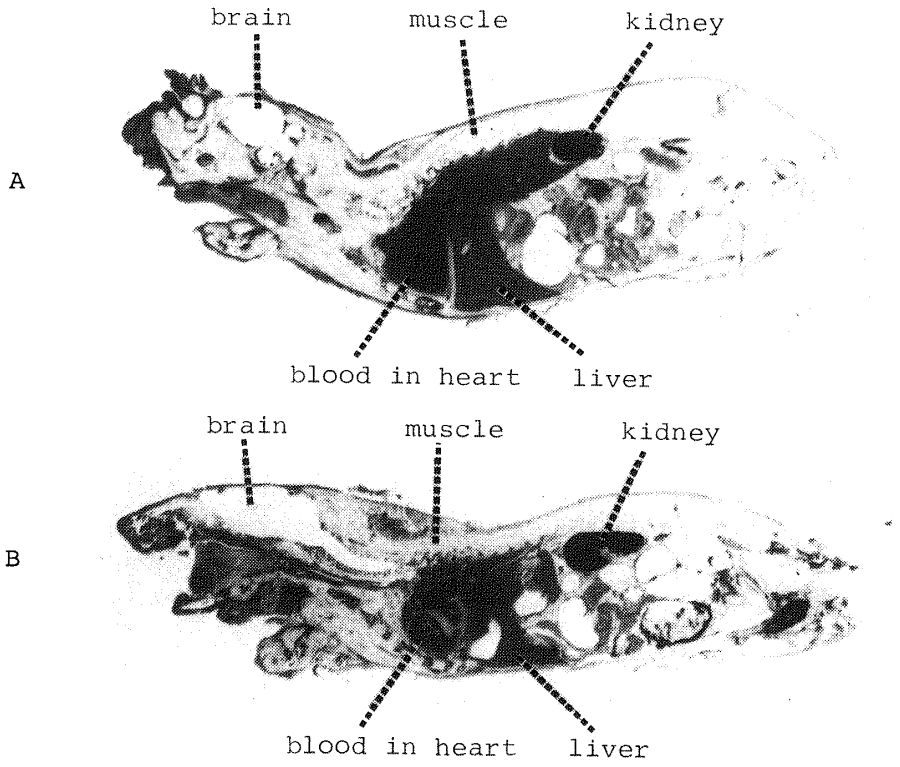

Fig. 3. Whole-Body Autoradiograms Showing the Distribution of Radioactivity (Dark Areas) at 2 min Following Intravenous Administration of [^{14}C]Creatinine to ddY and C57BL/6 Mice

A, ddY mice; B, C57BL/6 mice.

TABLE III. Optical Densities of Muscle and Heart Blood in the Autoradiograms at 2 min after Intravenous Administration of [^{14}C]Creatinine to ddY and C57BL/6 Mice

Tissue	Strain	
	ddY	C57BL/6
Muscle	0.04	0.05
Heart blood	0.36	0.29
Muscle/heart blood	0.11	0.17

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were compared.

Whole-Body Autoradiograms Following Intravenous Administration to Mice

Figure 3 shows the whole-body autoradiograms at 2 min after *i.v.* administration to ddY (Fig. 3-A) and C57BL/6 (Fig. 3-B) mice. No difference between the two autoradiograms is apparent by visual observation. For a more objective evaluation, the optical densities of the muscle, which is considered to be a representative tissue belonging to a peripheral compartment, and heart blood in the autoradiograms were determined with a densitometer (Table III). Though only one experiment was done, the optical density ratio of muscle to heart blood was larger in C57BL/6 mouse (0.17) than in ddY mouse (0.11), suggesting that the transfer of creatinine from blood to muscle might be enhanced in C57BL/6 mice. This result is consistent with the above discussion on the $(V'_d)_{extrap}$ value. The optical densities of other tissues, however, were not determined since the tissues were to small for accurate evaluation.

In this work, creatinine, which is water-soluble and metabolically inert and whose renal clearance is generally accepted as an index of renal function, was selected as a model chemical in order to obtain basic data for biopharmaceutical studies. As a next step, it should be interesting to study a lipid-soluble compound.

References and Notes

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