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Effects of renal insufficiency and aging on the pharmacokinetics of a phenethylamine class α_{1A}-adrenoceptor agonist NS-49

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Abstract

Effects of renal insufficiency and of aging on the pharmacokinetics of NS-49, a newly developed phenethylamine class α_{1A}-adrenoceptor agonist eliminated mainly by renal excretion, were investigated in rats after a single administration of ^{14}C-NS-49. The gromerular filtration rate (GFR) in the partially nephrectomized rats was about half that in the sham-operated rats, and the plasma creatinine concentration in the former was well above the normal limit. Plasma concentrations of radioactivity after intravenous or oral administration of ^{14}C-NS-49 were much higher in the nephrectomized rats than in the intact and sham-operated rats. As a result, the areas under the plasma concentration-time curve ($AUC_{0-\infty}$) after intravenous and oral administrations respectively increased 5-fold and 7-fold after partial nephrectomy. The elimination half-life ($t_{1/2,\beta}$) was increased about 2-fold by partial nephrectomy. The systemic availability for the partially nephrectomized rats remained unchanged, indicative that partial nephrectomy does not affect the absorption of NS-49. Plasma concentrations of radioactivity after intravenous or oral administration of ^{14}C-NS-49 to 88-week-old rats were higher than in 7-week-old rats, the $AUC_{0-\infty}$ value for the aged rats being about two times higher. The aged rats, unlike the nephrectomized rats, showed no marked difference in the $t_{1/2,\beta}$, value, whereas their V_{ss} value was about half that for the young rats. These findings are considered to be caused by physiologic age-related changes; decrease in renal function and loss of body water. Systemic availability in the aged rats did not differ from that in the young, indicative that aging has no effect on the extent of absorption of this drug.

Keywords: Aging; NS-49; Rat; Renal excretion; Renal insufficiency

1. Introduction

NS-49, (*R*)-(−)-3′-(2-amino-1-hydroxyethyl)-4′-fluoromethanesulfonanilide hydrochloride, a newly developed phenethylamine class α_{1A}-adrenoceptor agonist (Obita et al., 1995; Muramatsu et al., 1995; Taniguchi et al., 1997), increases intraurethral pressure with little effect on blood pressure (Taniguchi et al., 1996). It therefore should be useful for treating stress incontinence. Its pharmacokinetics studied in rats,

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rabbits, and dogs after a single administration of ^{14}C-NS-49 showed that the drug was well absorbed with negligible first-pass metabolism and mainly eliminated by renal excretion (Mukai et al., 1999a,b). Because renal failure or aging is known to affect the pharmacokinetics of drugs eliminated mainly by renal excretion (Shono et al., 1994; Mizuno et al., 1987; Dawling and Crome, 1989), we investigated the pharmacokinetics after a single administration of ^{14}C-NS-49 to rats with chronic renal insufficiency induced by partial nephrectomy and to aged rats.

2. Materials and methods

2.1. Compounds and reagents

NS-49 and ^{14}C-NS-49 (6.76 MBq/mg, shown in Fig. 1) were prepared as described elsewhere (Mukai et al., 1999a). ^{3}H-Inulin was purchased from New England Nuclear Research Products (Boston, MA, USA). Its specific activity was 8.1–11.3 MBq/mg. The other reagents used, of guaranteed reagent grade, were obtained commercially.

2.2. Animals

Male Sprague-Dawley rats (7 weeks) (Slc, Japan SLC, Hamamatsu, Japan) were used. Renal insufficiency was induced by partial nephrectomy using the methods of Ormrod and Miller (1980), and Sterner (1979).

Under diethyl ether anesthesia, an incision was made along the abdominal midline, and two-thirds of the left kidney removed. The right kidney was removed 1 week later after the animals had recovered. Two weeks after the first incision, the plasma creatinine concentration was measured, and the drug administered. In the sham operations, the left kidney was exposed, and 7 days later the right kidney was surgically exposed. This procedure duplicated surgical trauma but did not affect renal function. Eighty-seven-week-old male Sprague-Dawley rats purchased from Japan SLC (Hamamatsu, Japan) comprised the aged rats. The animals were acclimated to conditions of 23 ± 2°C and 55 ± 5% relative humidity for at least a week before use and allowed water and pellet food (F-2, Funabashi Farms, Funabashi, Japan) ad libitum. Food was withdrawn for 24 h before the experiments.

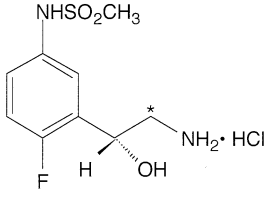

Fig. 1. Chemical structure of ^{14}C-NS-49 (*,^{14}C- labeled position).

2.3. Drug preparation and dose

For intravenous use, ^{14}C-NS-49 was dissolved in saline, and the solution adjusted to 0.1 mg/ml. Rats were administered a 0.1 mg/kg dose via the jugular veins. For the oral dose, ^{14}C-NS-49 was diluted appropriately with unlabeled NS-49 then dissolved in a 0.5% aqueous solution of methyl cellulose. The preparation (0.5 mg/ml) was administered orally through a stomach tube at a dose of 1 mg/kg.

For determination of the gromerular filtration rate (GFR) and of the renal clearance (CLre) of NS-49, ^{3}H-inulin and ^{14}C-NS-49 were dissolved in saline containing 10% D-mannitol to the respective concentrations of 3.3–4.6 μg/ml (0.037 MBq/ml) and 2 μg/ml (0.014 MBq/ml). The test solution was infused through the jugular vein at the rate of 5 ml/h.

2.4. Determination of the plasma radioactivity concentration

At designated times after the intravenous or oral administration of ^{14}C-NS-49, blood samples were drawn from the jugular veins into heparinized test tubes. Plasma was obtained after centrifugation (3000 rpm, 10 min) of the blood samples. The radioactivity concentration in the plasma was measured with a liquid scintillation counter (LSC-3500, Aloka, Tokyo, Japan) after the addition of 10 ml of emulsifying liquid scintil-

lator (Emulsifier-scintillator 299, Packard Instrument, Downers Grove, IL, USA). Counting efficiencies were corrected automatically by the external standard ratio method.

2.5. Determination of the plasma creatinine concentration

A Creatinine-test Wako Kit (Wako pure chemical industries, Kyoto, Japan) was used to measure the plasma creatinine concentration.

2.6. Renal clearance method

Rats were anesthetized with sodium pentobarbital (40 mg/kg i.p.). After the mid-abdominal incision was made, the bladder was cannulated with a polyethylene catheter (Intramedic PE-100, Division of Becton Dickinson, Parsippany, NJ, USA) for urine collection. ^{14}C-NS-49 and ^{3}H-inulin were infused intravenously, and 1 h was allowed to elapse between the start of infusion and the initial urine and plasma collections to ensure stable plasma concentrations of both drugs. Simultaneous determinations of the CLre values of NS-49 and inulin (i.e., GFR) were made every 10 min. Urine was collected during each 10-min period, and blood samples were obtained from the jugular veins at the midpoint in the urine collection period. The CLre value was calculated by $(U/P) \times V$, where U and P are the respective concentrations of the drug in the urine and plasma, and V is the urine flow rate. Radioactivity in the plasma and in the urine was measured after the addition of 10 ml of emulsifying liquid scintillator (Emulsifier-scintillator 299, Packard Instrument).

2.7. Pharmacokinetic parameter values

Parameter estimation was accomplished using the nonlinear least squares regression analysis program MULTI (Yamaoka et al., 1981). The inverse value of each data point was used as the weighting value in the least-squares method. A two-compartment open model was fitted to the plasma radioactivity concentrations for individual animals. The maximum plasma concentration

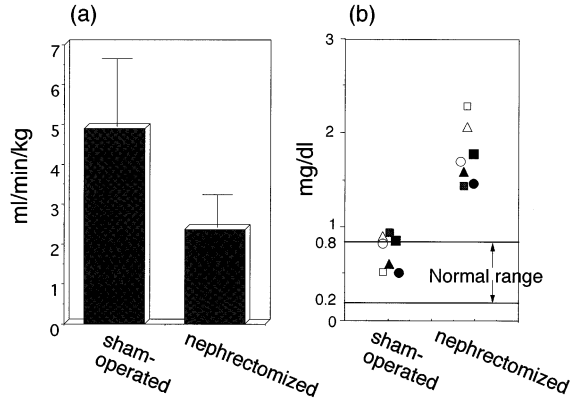

Fig. 2. Glomerular filtration rate (a) and plasma creatinine concentration (b) for partially nephrectomized and sham-operated rats. Bars in panel (a) represent means ± S.D. ($n = 7–8$). The points in panel (b) represent individual values.

(C_{max}), time (T_{max}) at which C_{max} occurred, elimination half-life ($t_{1/2,\beta}$), apparent volume of distribution at steady state (V_{ss}) and area under the plasma concentration-time curve ($AUC_{0-\infty}$) were derived from the model-dependent parameters. Total body clearance (CL_{tot}) for the intravenous administration was determined from the Dose/$AUC_{0-\infty}$.

2.8. Statistical analysis

Data were analyzed statistically using the Student's t-test.

3. Results

3.1. Effects of renal insufficiency

3.1.1. Renal function in rats following partial nephrectomy

The GFR and plasma creatinine concentrations for the partial nephrectomized and sham-operated rats are shown in Fig. 2. The GFR of the nephrectomized rats was less than half that of the sham-operated rats.

Plasma creatinine concentrations in the nephrectomized rats were markedly above the normal limit (< about 0.8 mg/dl), whereas those in the sham-operated rats were almost within that limit.

3.1.2. Radioactivity concentration in the plasma

The plasma radioactivity concentrations after intravenous or oral administration of ^{14}C-NS-49 to partially nephrectomized, sham-operated, and normal rats are shown in Fig. 3. Values of the pharmacokinetic parameters are given in Table 1. After intravenous administration, the plasma radioactivity concentration in each group decreased biexponentially. $t_{1/2,\beta}$ for the partially nephrectomized rats was significantly longer than for the sham-operated and normal rats. The $AUC_{0-\infty}$ value for the nephrectomized rats was about 5-fold that for the normal rats, whereas the value for the sham-operated rats was slightly larger than that for the normal rats. After oral administration to the sham-operated and normal rats, the plasma radioactivity concentrations reached similar maximums, about 0.2 μg/ml at 1 h. The nephrectomized rats, however, had a maximum plasma concentration of 0.47 μg/ml about 3 h after administration. The $AUC_{0-\infty}$ value was increased about 7-fold by partial nephrectomy. The systemic availability was high in all the groups (82% for the normal rats, 68% for the sham-operated rats, and 104% for the nephrectomized rats).

3.1.3. Correlation between plasma creatinine concentration and $AUC_{0-\infty}$

A good correlation was found between the plasma creatinine concentration and the $AUC_{0-\infty}$ value of radioactivity after oral administration of ^{14}C-NS-49 to nephrectomized and sham-operated rats (Fig. 4). This indicates that the change in the AUC of NS-49 caused by decreased renal function can be estimated from the plasma creatinine concentration, a good index of renal function.

3.2. Effects of aging

3.2.1. Renal function of aged rats

The GFR, CL_{re} of NS-49 and plasma creatinine concentrations of the 88-week-old rats are shown in Fig. 5. The GFR and CL_{re} of NS-49 are about half those for the 7-week-old rats. The plasma creatinine concentrations for the aged rats are above the normal limit.

3.2.2. Radioactivity concentration in plasma

Plasma concentrations of radioactivity after intravenous and oral administrations of ^{14}C-NS-49 to the aged rats are shown in Fig. 6, and the pharmacokinetic parameter values in Table 1. After intravenous administration, the plasma concentrations decreased biexponentially with a $t_{1/2,\beta}$ of 2.35 h, similar to the value for young rats. The $AUC_{0-\infty}$ was increased about 2-fold in the aged rats, and the V_{ss} was decreased to half the value for the young rats. After oral administration, the plasma radioactivity concentrations in the aged rats reached a maximum of 0.278 μg/ml at 1.4 h. The $AUC_{0-\infty}$ value was twice that for the young rats. Systemic availability for the aged rats was

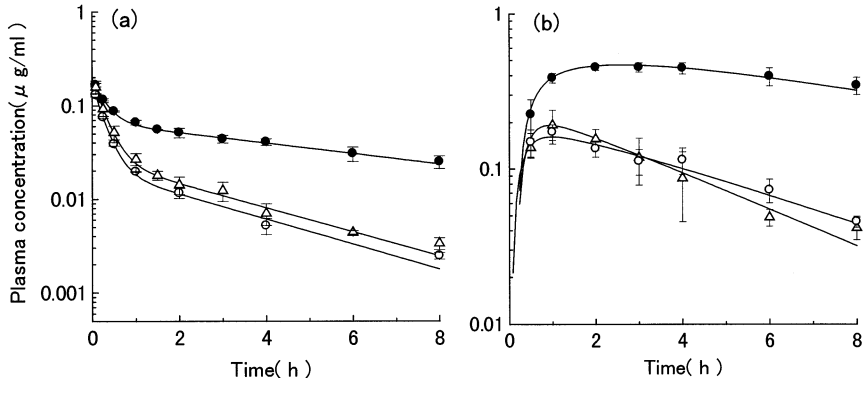

Fig. 3. Effect of partial nephrectomy on the plasma concentration of radioactivity after intravenous (a; 0.1 mg/kg) or oral (b; 1.0 mg/kg) administration of ^{14}C-NS-49 to rats. ●, nephrectomized; △, sham-operated; ○, control. Points represent means ± S.D. ($n = 3$–4).

Table 1
Effects of partial nephrectomy and aging on the pharmacokinetic parameter values of radioactivity after intravenous (0.1 mg/kg) or oral (1.0 mg/kg) administration of ^{14}C-NS-49 to rats

Route	Parameter	Control	Nephrectomized	Sham-operated	Aged
i.v.	A (μg/ml)[a]	0.151 ± 0.009	0.130 ± 0.010	0.176 ± 0.029	0.244 ± 0.037[c]
	B (μg/ml)[a]	0.021 ± 0.002	0.067 ± 0.003[d]	0.027 ± 0.006	0.044 ± 0.000[d]
	α (h^{-1})[a]	3.96 ± 0.45	3.46 ± 0.43	3.67 ± 0.18	3.40 ± 0.99
	β (h^{-1})[a]	0.309 ± 0.003	0.130 ± 0.019[d]	0.297 ± 0.035	0.300 ± 0.047
	V_1 (l/kg)[a]	0.582 ± 0.038	0.509 ± 0.032	0.502 ± 0.096	0.351 ± 0.042[d]
	V_{ss} (l/kg)[b]	2.03 ± 0.03	1.30 ± 0.04[d]	1.71 ± 0.44	1.06 ± 0.07[d]
	$t_{1/2,\beta}$ (h)[b]	2.25 ± 0.02	5.43 ± 0.87[d]	2.35 ± 0.29	2.35 ± 0.40
	$AUC_{0-\infty}$ (μg h/ml)[b]	0.107 ± 0.006	0.563 ± 0.095[d]	0.139 ± 0.018	0.223 ± 0.044[c]
	CL_{tot} (l/h/kg)[b]	0.940 ± 0.050	0.181 ± 0.028[d]	0.728 ± 0.098[c]	0.460 ± 0.082[d]
p.o.	k_a (h^{-1})[a]	0.646 ± 0.282	0.581 ± 0.258	0.480 ± 0.040	0.394 ± 0.112
	T_{lag} (h)[a]	0.163 ± 0.190	0.169 ± 0.137	0.046 ± 0.091	0.095 ± 0.165
	C_{max} (μg/ml)[b]	0.190 ± 0.049	0.471 ± 0.045[d]	0.164 ± 0.027	0.278 ± 0.028[c]
	T_{max} (h)[b]	1.02 ± 0.06	2.89 ± 1.05[c]	0.990 ± 0.104	1.44 ± 0.20[d]
	$AUC_{0-\infty}$ (μg h/ml)[b]	0.915 ± 0.110	6.63 ± 2.11[d]	1.01 ± 0.10	2.02 ± 0.07[d]
	F[a]	0.818 ± 0.109	1.04 ± 0.25	0.676 ± 0.080	0.845 ± 0.099

[a] Estimated by nonlinear least squares fitting of two-compartment model equations to the plasma radioactivity concentrations for the individual animals. Each value is a mean ± S.D. ($n = 3$–4).
[b] Calculated from the model-dependent parameters.
[c] $P < 0.05$.
[d] $P < 0.01$, significantly different from the control value.

85%, indicative of there being no age-related effect on the extent of absorption.

4. Discussion

To examine the effects of renal insufficiency and aging on the pharmacokinetics of NS-49 which is eliminated mainly through the kidneys, we determined the radioactivity concentrations in the plasma and urine after a single administration of ^{14}C-NS-49 to rats with surgically induced renal insufficiency and 88-week-old rats. We calculated the CL_{re} value from the radioactivity concentration because most of the radioactivity in rat plasma and urine has been shown to be due to unchanged NS-49 (Mukai et al., 1999a).

The surgically induced renal failure model was chosen because of its selectivity, tissue destruction being confined to the kidney. The partially nephrectomized rats had a GFR decrease of about one half and a notable increase in the plasma creatinine concentration, therefore they were accepted as an experimental model of chronic renal insufficiency. After intravenous administration of ^{14}C-NS-49 to the partially nephrectomized rats, the plasma radioactivity concentration was higher than the concentrations in the sham-operated and normal rats, and the CL_{tot} value was much lower. Partial nephrectomy induced an increase in $t_{1/2,\beta}$. These findings are considered to be the results of reduced renal function caused by partial nephrectomy because this drug is poorly metabolized, and CL_{re} accounted for about 80% of the CL_{tot} in the rats (Mukai et al., 1999a). Similar findings were obtained after oral administration of ^{14}C-NS-49 to the nephrectomized rats, indicative of the same cause-and-effect relation. There was no marked change in systemic availability owing to partial nephrectomy, which means that it did not affect the absorption of NS-49.

There was a good correlation between the plasma creatinine concentration and the $AUC_{0-\infty}$ value after oral administration of ^{14}C-NS-49 to nephrectomized rats. The plasma creatinine concentration depends on GFR and is a good index

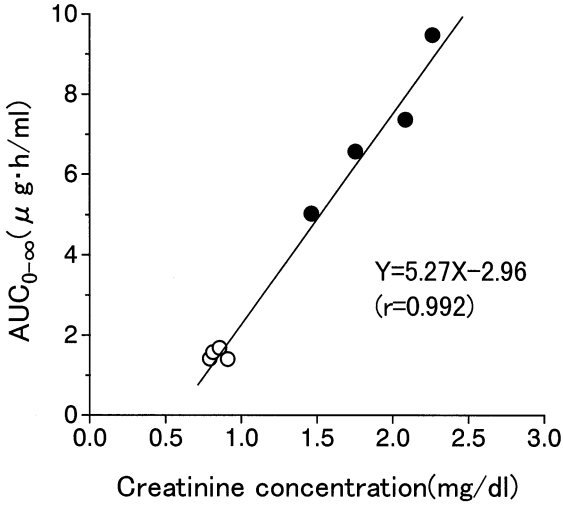

Fig. 4. Correlation between the plasma creatinine concentration and the $AUC_{0-\infty}$ of radioactivity after oral administration of ^{14}C-NS-49 (1.0 mg/kg) to rats. ●, nephrectomized; ○, sham-operated.

of renal function (Knochel and Selden, 1981). We found that the pharmacokinetics of NS-49 depends on renal excretion with tubular secretion in rats (Mukai et al., 1999a) and humans (unpublished). The same correlation therefore should be obtained for patients with impaired renal function.

Age-related differences in the pharmacokinetics of NS-49 were investigated in 88-week-old rats. Renal function in humans decreases by about

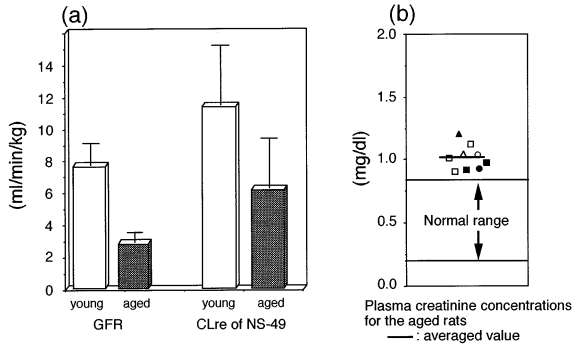

Fig. 5. Age-associated changes in the glomerular filtration rate (a), CL_{re} of NS-49 (a) and plasma creatinine concentrations (b). Bars in panel (a) represent means ± S.D. ($n = 4$). Points in panel (b) represent individual values.

1–2% annually after age 50, and normal creatinine clearance in an 80-year-old individual is about 40% less than that in a young subject (Dawling and Crome, 1989; Schmucker, 1979). The aged rats used in this study showed similar changes in renal function; the GFR and the CL_{re} of NS-49 decreased to about half the values for young rats. This indicates that GFR and renal tubular secretion decreased similarly in the aged rats and supports the intact nephron theory that posits that the number of intact nephrons decreases with age (Schmucker, 1985). Plasma creatinine concentrations in the aged rats were slightly above the normal limit but not as high as those in the nephrectomized rats despite the similar degree of decrease in the GFR. There is no age-related increase in the serum creatinine concentration because aging also results in the loss of muscle mass thereby reducing creatinine synthesis (Ewy et al., 1969; Nielsen et al., 1971). For geriatric patients estimation of the AUC value of NS-49 from the plasma creatinine concentration is of limited value because it is not a good index of renal function.

The plasma radioactivity concentrations after intravenous or oral administration of ^{14}C-NS-49 were higher in the aged than in the young rats because of the decrease in CL_{re} discussed above. Aging, unlike partial nephrectomy, did not affect the $t_{1/2,\beta}$, but it lowered the V_{ss} to about half the value for the young rats. The decrease in V_{ss} is considered to correspond to the age-associated increase in body fat (20–40%) at the expense of lean mass and the decrease in body water (10–15%) (Novak, 1972; Shock et al., 1953) because NS-49 is a hydrophilic drug. The $t_{1/2,\beta}$, which is obtained with $0.693 \times V_{\beta}/CL_{tot}$ (in which V_{β} is the distribution volume of post-distributive phase), may not have shown age-related change because the distribution volume and CL_{tot} decreased similarly in the aged rats. There were no age-related differences in the systemic availability after the oral administration, evidence that the extent of absorption is unaffected by aging.

In conclusion, the plasma radioactivity concentrations after ^{14}C-NS-49 was administered to nephrectomized or aged rats were much higher than in the control rats. This was due mainly to the reduced renal function induced by partial

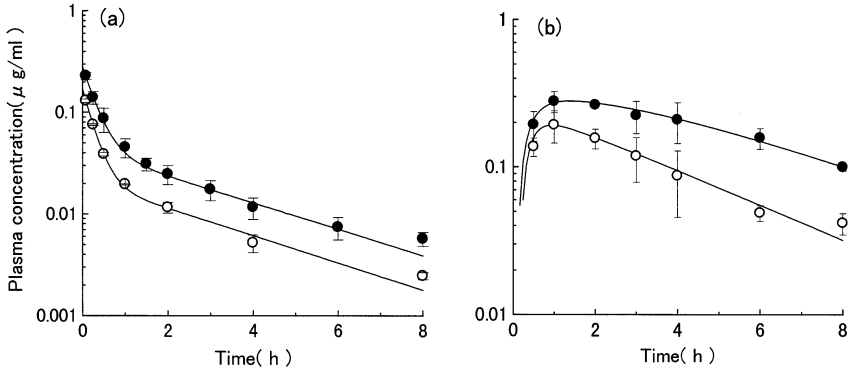

Fig. 6. Effect of aging on plasma concentrations of radioactivity after intravenous (a; 0.1 mg/kg) or oral (b; 1.0 mg/kg) administration of ^{14}C-NS-49 to rats. ●, aged; ○, young. Points represent means ± S.D. ($n = 3–4$).

nephrectomy or age-associated physiological changes.

Similar phenomena should be shown clinically because the elimination of this drug in humans also depends mainly on renal excretion.

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