Effect of Latamoxef on Platelet Function and Prothrombin Time in Partially Nephrectomized Rats

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Abstract—Latamoxef (daily 100 and 300 mg/kg, i.v.) was injected once a day for 8 days to 75% and 90% nephrectomized rats kept on a vitamin K-sufficient diet (500–600 ng/g) or a vitamin K-deficient diet (30–50 ng/g), and changes in ADP-induced platelet aggregation and prothrombin time were examined. The half-life of latamoxef was markedly prolonged and plasma latamoxef and *N*-methyl-tetrazolethiol (NMTT) concentrations increased, resulting in a delay of the total body clearance of the compounds. The ADP-induced platelet aggregation increased after nephrectomy, and latamoxef slightly but inconsistently decreased the aggregation. Prothrombin time did not change even in the 90% nephrectomized rats kept on an ordinary diet, but increased dose-dependently in the vitamin K-deficient nephrectomized rats, with the 90% nephrectomized animals showing larger increases of prothrombin time. These data suggest that NMTT or NMTT-containing antibiotics cause no hypoprothrombinemia even in partially nephrectomized rats when they are fed an ordinary diet containing vitamin K, but these compounds enhance the manifestation of hypoprothrombinemia in vitamin K deficiency. Further renal failure promotes the manifestation by increasing drug concentration in the blood. However, platelet aggregation in these animals is not significantly affected at the doses examined.

Renal failure is considered to enhance the manifestation of side-effects of drugs by prolonging high drug levels in the blood. In the case of antibiotics, renal dysfunction is a risk factor for developing coagulopathy (1–3).

Recently, latamoxef (moxalactam) has been reported to decrease ADP-induced platelet aggregation (4) and produce hypoprothrombinemia in vitamin K-deficient patients (5, 6) and experimental animals (7, 8). The hypoprothrombinemia is produced not only by latamoxef but also by the other antibiotics which possess *N*-methyl-tetrazolethiol (9–15) or methylthiadiazolethiol (16) in their structures, since such side-chains interfere with the reuse of vitamin K by inhibiting vitamin K-epoxide reductase (17).

In the present experiment, we examined the effect of latamoxef on ADP-induced

platelet aggregation and prothrombin time in partially nephrectomized rats which show markedly decreased glomerular filtration rate (GFR) (18) and creatinine clearance (19).

Materials and Methods

Sprague-Dawley male rats, 8 weeks old, obtained from Japan Clea were kept in an air-conditioned room (25±1°C, 50–60% humidity) lighted 12 hr a day (8 a.m. to 8 p.m.). Commercially available diet (JCL-CA-1, Japan Clea Co., Ltd., Tokyo) was used as a vitamin K-sufficient diet containing 500–600 ng/g of vitamin K₁. A vitamin K-deficient diet containing 30–50 ng/g of vitamin K₁ was prepared with vitamin K-free casein (18.0%), sucrose (67.6%), a mixture of rapeseed and soybean oils (8.0%), salt mixture (Hegsted salt) (4.0%), fibers (1.5%) and adequate amounts of vitamins

except vitamin K.

Partial nephrectomy of the rats was performed by the method of Ormrod and Miller (18). Briefly, about 50% (or 80%) of the tissue weight of one kidney was cut off using a razor blade under sodium methylhexabital anesthesia (125 mg/kg, i.p.) and one week after the operation, the contralateral kidney was removed under anesthesia to obtain a 75% (or 90%) nephrectomized animal. Latamoxef was injected i.v. once a day at 100 mg/kg or 300 mg/kg for 8 days at 3 weeks after the second operation for the 75% nephrectomized rats, and after 2 weeks for the 90% nephrectomized rats. At 3 hr after the last injection, the animal was placed under sodium methylhexabital anesthesia (125 mg/kg, i.p.), and blood was withdrawn from the abdominal aorta with a disposable syringe containing 1/10 volume of 3.8% sodium citrate solution.

Plasma and urine creatinine levels were determined and creatinine clearance was calculated (19). Plasma urea-nitrogen, albumin and total protein levels were determined by the Technicon Auto Analyzer SMA PLUS MICRO (New York), and urea-nitrogen clearance was estimated in a similar manner.

Plasma latamoxef and NMTT levels were determined using a high performance liquid chromatograph (HPLC) with a Varian liquid chromatograph Model LC-5000 equipped with a stainless steel column, 15 cm×6.0 mm I.D., packed with YMC A-312 (5 μ m) octadecylsilane reverse phase (Yamamura Chemical Laboratory Corp., Kyoto), and a

Model 100 UV detector (at 254 nm wavelength). A Rheodyne 7125 loop injector was used with a 500- μ l sample loop. All chromatograms were recorded and calculated with a Shimadzu C-R2AX data processor. The mobile phase was prepared by mixing 250 ml of methanol with 750 ml of phosphate buffer (pH 6.0, ionic strength 0.075 M) containing 5 mM of tetrabutylammonium hydroxide. The solvent flow rate was 1.0 ml/min. The detection limits were 2 μ g/ml of rat plasma or latamoxef and 0.5 μ g/ml for NMTT.

Platelet aggregation was examined by the method of Born (20) using a Type AUTO-RAM 61 aggregometer (Rika-Denki Co., Ltd., Tokyo). Prothrombin time was determined with a CLOTEK apparatus (Hyland Division Travenol Laboratories, Inc., Costa Mesa, California). Adenosine diphosphate (ADP) was obtained from P-L Biochemical Inc. (Milwaukee) and latamoxef (disodium salt) from Shionogi & Co., Ltd. (Osaka).

Results

Table 1 shows the creatinine clearance and urea-nitrogen clearance in the partially nephrectomized rats. The creatinine clearance decreased to about 60% of the control in the 75% nephrectomized rats and to about 30% in the 90% nephrectomized rats. The urea-nitrogen clearance decreased respectively to 40–50% and to 20–30% in these animals. These values were almost constant regardless of the time after operation.

The plasma creatinine and urea-nitrogen levels increased in the 75% nephrectomized

Table 1. Creatinine and urea-nitrogen clearances in sham-operated (control) and partially nephrectomized rats

	Groups	Weeks after operation			
		1	2	3	4
Creatinine clearance (ml/100 g/24 hr)	Control	663±29 ^a (100)	777±37 (100)	510±25 (100)	468±33 (100)
	75% Nephrectomy	397±18* (60)	441±25* (57)	274±20* (54)	281±23* (60)
	90% Nephrectomy	228±14* (34)	214±15* (28)	148±18* (29)	138±20* (30)
Urea-nitrogen clearance (ml/100 g/24 hr)	Control	476±41 (100)	466±36 (100)	556±46 (100)	483±41 (100)
	75% Nephrectomy	230±14* (48)	260±17* (56)	219±14* (40)	215±18* (44)
	90% Nephrectomy	153±11* (32)	140±9* (30)	107±9* (19)	110±16* (23)

^a Mean±S.E. in over 5 rats. * Statistically significant compared with the control ($P<0.05$). () Percentages of the control.

Table 2. Plasma creatinine, urea-nitrogen, albumin and total protein levels in partially nephrectomized rats

	Control	75% Nephrectomy	90% Nephrectomy
(I) 2 Weeks after operation			
No. of rats	5	7	6
Creatinine (mg/100 ml)	0.60±0.04 ^a	1.13±0.08*	2.07±0.19*
Urea-nitrogen (mg/100 ml)	26.4±1.0	71.0±6.6*	130.0±11.0*
Albumin (g/100 ml)	3.26±0.05	3.11±0.06	3.02±0.07*
Total protein (g/100 ml)	6.08±0.07	5.81±0.11	5.77±0.06*
(II) 4 Weeks after operation			
No. of rats	5	8	6
Creatinine (mg/100 ml)	0.66±0.06	1.15±0.07*	2.27±0.19*
Urea-nitrogen (mg/100 ml)	21.4±1.6	71.5±5.9*	133.0±8.0*
Albumin (g/100 ml)	3.40±0.06	3.08±0.07*	2.67±0.07*
Total protein (g/100 ml)	6.70±0.35	5.89±0.12*	5.35±0.16*

^a Mean±S.E. * Statistically significant compared with the control ($P<0.05$).

Table 3. Changes of ADP-induced platelet aggregation in 75% nephrectomized rats

Weeks after nephrectomy	Control			Nephrectomized		
	ADP (μ M)			ADP (μ M)		
	1	3	5	1	3	5
3	16.5±3.2 ^a	52.6±1.4	61.1±1.9	26.0±4.1	54.4±4.2	64.5±2.9
4	21.1±1.6	55.0±1.0	64.0±0.6	43.4±3.7*	68.7±1.1*	74.0±1.0*
6	26.4±2.1	52.9±1.1	60.7±0.5	32.2±1.7*	58.4±1.3*	65.2±1.1
8	13.2±3.4	47.0±2.4	56.8±1.8	27.4±4.9	57.8±2.2*	64.2±1.8*

^a Mean±S.E. in 5 rats. Values represent maximal aggregations in percentage. * Statistically significant compared with the paired-control ($P<0.05$).

rats (Table 2), concomitantly with the decreases of these clearance values (Table 1). The plasma albumin and total protein concentrations also decreased progressively with time in the nephrectomized rats. The maximum decrease in plasma protein concentration was 20%, as shown in Table 2.

Figure 1 shows the disappearance curves of latamoxef in the control and 90% nephrectomized rats, when 20 mg/kg of latamoxef had been injected i.v. The half-life of latamoxef was 17.3 min in the control rats, but it was 63.2 min in the nephrectomized rats. Thus, the elimination rate constant K_{10} decreased (0.0368 vs. 0.01147/min). The area under the curve was increased (5.23 vs. 1.54 mg·min/ml), and the total body clearance was decreased (3.94 vs. 13.15 ml/min/kg) in the nephrectomized rats.

Time course changes in the ADP-induced platelet aggregation are given in Table 3. ADP-induced platelet aggregation was rather

Fig. 1. Plasma latamoxef concentration in sham operated control and 90% nephrectomized rats given 20 mg/kg i.v. of latamoxef. Values and bars represent the mean and S.E. in 6 rats.

enhanced after nephrectomy. This enhancement, however, was not related to the period after operation.

Table 4 shows the effect of latamoxef

treatment on ADP-induced platelet aggregation and prothrombin time in the partially nephrectomized rats kept on an ordinary diet. The platelet aggregation slightly

Table 4. Effect of latamoxef on ADP-induced platelet aggregation and prothrombin time in sham-operated and partially nephrectomized rats (ordinary diet)

		No. of rats	Platelet aggregation (%)			Prothrombin time (sec)
			ADP (μM)			
			1	3	5	
Sham-operated rats						
Control	Saline	6	36.9±2.0 ^a	67.3±1.9	72.4±1.2	13.4±0.2
Latamoxef	100	5	37.0±3.9	67.7±1.1	73.7±0.5	13.2±0.4
	300	6	41.9±1.7	68.6±1.1	73.9±1.4	13.4±0.3
Nephrectomized rats (75%)						
Control	Saline	4	34.2±5.8	66.6±2.4	72.5±2.1	12.9±0.1
Latamoxef	100	4	27.2±5.8	61.3±3.4	68.3±2.6	13.4±0.2
	300	4	30.1±1.9	61.2±1.3	68.9±2.5	13.4±0.2
Nephrectomized rats (90%)						
Control	Saline	5	48.7±3.8	73.3±3.1	77.7±2.7	13.3±0.2
Latamoxef	100	5	49.5±2.5	71.4±0.7	76.4±1.6	13.1±0.2
	300	5	34.9±2.0*	62.3±0.8*	67.6±0.7*	13.4±0.5
Intact rats	No treatment	5	45.4±3.2	69.9±1.9	75.7±1.5	13.1±0.2

^a Mean $\pm$ S.E. * Statistically significant compared with the control ($P<0.05$). Latamoxef was injected i.v. once a day for 8 days from the 3rd week for the 75% nephrectomized rats and from the 2nd week for the 90% nephrectomized rats.

Table 5. Effect of latamoxef on ADP-induced platelet aggregation and prothrombin time in sham-operated and partially nephrectomized rats (vitamin K-deficient diet)

	Daily dose (mg/kg)	No. of rats	Platelet aggregation (%)			Prothrombin time (sec)
			1	ADP (μ M) 3	5	
Sham-operated rats						
Control	Saline	6	29.3 $\pm$ 2.2 ^a	56.4 $\pm$ 2.1	64.6 $\pm$ 2.1	13.2 $\pm$ 0.4
Latamoxef	100	6	23.8 $\pm$ 3.0	55.1 $\pm$ 2.6	65.2 $\pm$ 1.2	13.9 $\pm$ 0.4
	300	6	28.4 $\pm$ 2.2	58.3 $\pm$ 2.6	65.1 $\pm$ 2.5	14.7 $\pm$ 0.6
Nephrectomized rats (75%)						
Control	Saline	5	37.1 $\pm$ 1.7	63.5 $\pm$ 1.2	69.7 $\pm$ 1.1	11.8 $\pm$ 0.04
Latamoxef	100	5	33.0 $\pm$ 2.0	62.8 $\pm$ 1.0	69.7 $\pm$ 0.7	13.5 $\pm$ 0.9
	300	5	16.4 $\pm$ 4.3*	49.9 $\pm$ 4.5*	58.8 $\pm$ 4.1	18.1 $\pm$ 1.5*
Nephrectomized rats (90%)						
Control	Saline	5	31.4 $\pm$ 3.0	62.9 $\pm$ 1.9	71.1 $\pm$ 2.4	12.4 $\pm$ 0.4
Latamoxef	100	6	32.7 $\pm$ 2.2	62.9 $\pm$ 2.0	70.2 $\pm$ 1.3	16.2 $\pm$ 0.7*
	300	6	26.1 $\pm$ 2.2	57.2 $\pm$ 1.6	64.7 $\pm$ 1.3	23.9 $\pm$ 4.4*
Intact rats (Ordinary diet)		4	31.2 $\pm$ 2.8	62.6 $\pm$ 1.1	68.4 $\pm$ 0.9	13.5 $\pm$ 0.4

^a Mean $\pm$ S.E. * Statistically significant compared with the control ($P<0.05$). Latamoxef was injected i.v. once a day for 8 days from the 3rd week for the 75% nephrectomized rats and from the 2nd week for the 90% nephrectomized rats.

Table 6. Plasma concentrations of latamoxef and NMTT after i.v. injection of latamoxef to sham-operated and nephrectomized rats

Diet		Daily dose (mg/kg)	Plasma concentration ($\mu\text{g/ml}$)	
			Latamoxef	NMTT
Ordinary diet	Sham-operated rats			
	Latamoxef	100	<2	1.19 $\pm$ 0.16 ^a
		300	<2	1.76 $\pm$ 0.17
	Nephrectomized rats (90%)			
	Latamoxef	100	54.0 $\pm$ 5.9 ^a	10.8 $\pm$ 1.5
		300	222.8 $\pm$ 19.4	36.4 $\pm$ 4.6
Vitamin K-deficient diet	Sham-operated rats			
	Latamoxef	100	<1	0.58 $\pm$ 0.04 ^a
		300	<1–3.52	1.41 $\pm$ 0.12
	Nephrectomized rats (90%)			
	Latamoxef	100	45.9 $\pm$ 6.9 ^a	15.4 $\pm$ 1.6
		300	191.5 $\pm$ 17.0	33.0 $\pm$ 1.8

NMTT: *N*-Methyltetrazoethiol. ^a Mean $\pm$ S.E. in 5–6 rats.

decreased in the 90% nephrectomized rats treated with a higher dose of latamoxef, but not in the 75% nephrectomized rats. On the other hand, prothrombin time was not changed by the treatment with latamoxef even in the 90% nephrectomized rats. Similar experiments were performed with rats kept on a vitamin K-deficient diet for the last one week (Table 5). The ADP-induced platelet aggregation slightly decreased after the treatment in the 75% nephrectomized rats but not in the 90% nephrectomized rats. However, prothrombin time dose-dependently increased after the treatment with latamoxef, and the increase was greater in the 90% nephrectomized rats.

Table 6 shows the plasma concentrations of latamoxef and NMTT in the animals shown in Tables 4 and 5. Since the animals were sacrificed 3 hr after the last injection, the plasma latamoxef concentrations were less than 2 $\mu\text{g/ml}$ in all the sham-control rats. However, the concentrations were about 50 $\mu\text{g/ml}$ and 200 $\mu\text{g/ml}$ in the 90% nephrectomized rats receiving 100 and 300 mg/kg of latamoxef, respectively. The plasma NMTT concentrations were also less than 2 $\mu\text{g/ml}$ in the control rats, but they were 10–15 $\mu\text{g/ml}$ and 33–36 $\mu\text{g/ml}$ in the nephrectomized rats, respectively.

Discussion

The present data demonstrate that latamoxef causes no hypoprothrombinemia even in partially nephrectomized rats when they are fed a regular diet containing sufficient amounts of vitamin K, but latamoxef enhances the manifestation of hypoprothrombinemia in vitamin K-deficient rats and renal failure promotes its manifestation, probably by increasing the plasma drug concentration. These data suggest that this latamoxef effect in vitamin K deficiency can be prevented by vitamin K feeding even in 90% nephrectomized rats, where creatinine clearance is only 30% of that in the control animals. The vitamin K-deficient diet which we prepared contained 30–50 ng/g of vitamin K₁, a marginal dose which prevents hypoprothrombinemia in male rats (21). When male rats are fed a diet which contains lesser amounts of the vitamin, marked hypoprothrombinemia manifests itself within a few days (21).

Vitamin K is absorbed in the quinone form, reduced to the biologically active dihydroquinone form by vitamin K reductase mainly in the liver, converted to vitamin K epoxide coupled with the γ -glutamylcarboxylation reaction, and then reduced to the original

quinone form by vitamin K epoxide reductase (22). This sequence is known as the vitamin K cycling. Although NMTT or NMTT-containing antibiotics including latamoxef have been reported to inhibit γ -glutamyl-carboxylation (23), we have previously demonstrated that NMTT or latamoxef does not inhibit γ -glutamylcarboxylase (24), even under conditions in which prothrombin time is increased and plasma clotting factors such as prothrombin and factor VII are markedly decreased (21). Recently, Bechtold et al. (25, 26) demonstrated that cephalosporins including latamoxef increase plasma vitamin K epoxide levels, and Suttie and his colleagues (15, 17) have proposed that NMTT or NMTT-containing antibiotics inhibit vitamin K epoxide reductase. These results suggest that NMTT or NMTT-containing antibiotics do not inhibit γ -glutamylcarboxylase but inhibit vitamin K epoxide reductase, resulting in impairment of the vitamin K cycling. This hypothesis fits well with many phenomena observed with the use of these antibiotics, since the drug-induced hypoprothrombinemia manifests itself only with vitamin K deficiency (3, 7, 8) and is easily overcome by treatment with vitamin K (3, 8, 27, 28).

The hypoprothrombinemia observed in the vitamin K-deficient and partially nephrectomized rats seems to be due to interruption of the vitamin K cycling caused by delayed excretion of the drug. The total body clearance of latamoxef in the 90% nephrectomized rats was markedly decreased (3.94 vs. 13.15 ml/min/kg).

Our data suggest that ADP-induced platelet aggregation increases in the partially nephrectomized rats (Table 3). Bang et al. (29) reported that ADP- and collagen-induced platelet aggregations are enhanced in patients with nephrosis. This enhancement may be related to the decrease in plasma albumin concentration, since a decrease in binding of arachidonic acid to albumin may increase thromboxane A_2 synthesis (30). Thus, the observed enhancement of platelet aggregation may be linked to the decreased albumin concentration also observed in our nephrectomized rats. However, platelet function is decreased in advanced uremic patients (31). The increase of platelet ag-

gregation in our 75% nephrectomized rats was observed even 8 weeks after the operation (Table 3), suggesting that uremia was not severe in these animals. Latamoxef slightly decreased the aggregation at a higher dose (Tables 4 and 5). This inhibition also seems to be related to the drug concentration in blood, but is inconsistent with our experimental results. Bang et al. (4) previously demonstrated that latamoxef treatment decreases ADP-induced platelet aggregation in human volunteers. This decrease is dose-dependent; daily treatment with 12 g or 6 g of latamoxef for one week decreased the aggregation but that with 3 g did not. The mechanism of this inhibition is not known. Many beta-lactam antibiotics, including carbenicillin, latamoxef and cefotaxime, inhibit ADP-induced platelet aggregation in vitro but at extremely high concentrations like 1000 μ g/ml or more (4). It is not yet known whether the in vivo inhibition occurs by the same mechanism as in the in vitro type, but if it is the same, the drug concentration in the blood may be related to the manifestation of the in vivo inhibition of platelet aggregation. NMTT does not inhibit rat platelet aggregation either in vivo or in vitro at a final concentration of 10 mM (K. Uchida and T. Ishigami, unpublished data). Therefore, the inhibition of platelet function may be related to the beta-lactam ring or surrounding structures.

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