CHRONOTOXICAL STUDY OF GENTAMICIN INDUCED NEPHROTOXICITY IN RATS

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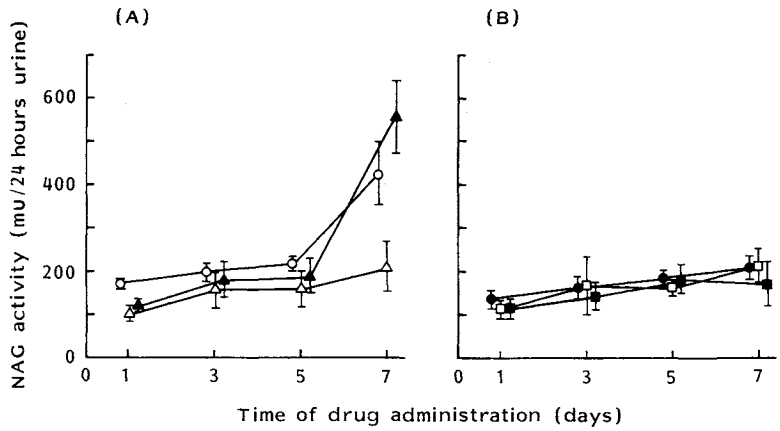
(Received for publication September 21, 1991)

Gentamicin (GM) is an important antibiotic for the treatment of many serious infections with Gram-negative bacteria. Although GM has strong antibacterial effects, both ototoxicity and nephrotoxicity are important and serious side effects of its use. Therefore, the clinical use of GM is difficult for general physicians in certain situations. The nephrotoxicity of GM has been widely investigated in various experimental models. GM produces varying degrees of toxicity depending on the time of administration[1,2]. This study was performed to determine the existence of circadian variation of nephrotoxicity induced by GM in rats.

Male Wistar rats (7 weeks old, weighing 190 to 210 g) were used. Rats were housed in a light-controlled room (lights on from 7:00 am to 7:00 pm) at a room temperature of 24 ± 1°C and humidity of 60 ± 10%, with food and water *ad libitum*. Rats were randomly assigned to 7 groups of 5 rats each and were housed individually in metabolic cages to collect urine. Groups of 35 rats each received GM 60 mg/kg sc alone, or GM 60 mg/kg sc with latamoxef (LMOX) 1,000 mg/kg ip at the midlight (1:00 pm) or at the middark (1:00 am) for 8 consecutive days. Urine samples were collected 24 hours prior to the begining of drug treatment, and every 24 hours urine thereafter 8 days. After the urine volumes were measured, urine samples were centrifuged at 3,000 rpm for 10 minutes. *N*-Acetyl-β-D-glucosaminidase (NAG) activity was measured in the supernatant and expressed as international units per total urine collected for 24 hours. Rats were sacrificed 30 minutes after the 8th day of administration. Under anesthesia, blood samples were obtained from the inferior *vena cava* and kidney samples were taken for the determination of GM concentrations in the serum and kidney. GM levels in serum and kidney were determined by fluorescence polarization immunoassay (TDX; Dinabot Co., Ltd., Tokyo, Japan). The detection limit was 0.27 μg/ml. Kidney samples were then prepared for histopathological

Fig. 1. The time course of urinary *N*-acetyl-β-D-glucosaminidase activity in rats after administration of gentamicin (GM) 60 mg/kg sc alone or gentamicin 60 mg/kg sc with latamoxef (LMOX) 1,000 mg/kg ip at midlight (1:00 pm) or at middark (1:00 am).

Each point represents mean (±SD) for 5 rats. (A) Midlight (1:00 pm) administration, ○ GM (1:00 pm), △ GM (1:00 pm)+LMOX (1:00 pm), ▲ GM (1:00 pm)+LMOX (1:00 am), (B) Middark (1:00 am) administration, ● GM (1:00 am), □ GM (1:00 am)+LMOX (1:00 pm), ■ GM (1:00 am)+LMOX (1:00 am).



observations by the standard operations hematoxylin and eosin staining. Histopathological sections of kidney were evaluated independently by a pathologist who was unaware of the regimens used. Statistical evaluation was performed by analysis of variance (ANOVA) and STUDENT's *t* test was used to determine the significance of difference between midlight and middark dosing.

Although urine volume is known to increase significantly during the day and decrease during the night among day-active individuals, there were no significant differences in 24-hours urinary volumes for the various groups. The effect of time-of-day of drug administration on the time course of urinary NAG activity in rats is shown in Fig. 1. Changes in NAG activity in the urine are considered to be more reliable since NAG is specific to the kidneys, and such changes appear to be more useful in practice. Urinary NAG activity following midlight (1:00 pm) dosing showed a gradual increase with the passage of time. However, the elevation of urinary NAG activity was significantly suppressed ($P<0.05$) following middark (1:00 am) dosing. There was significant variability in the urinary NAG activity depending on the time of administration. When GM was administered in midlight (1:00 pm) together with LMOX (1:00 pm) concomitantly or LMOX (1:00 am) 12-hours interval, the NAG remained unchanged at concomitantly coadministration or increased at 12-hours interval coadministration. Renal tissue specimens obtained 30 minutes after the 8th day of administration were examined histologically. Rats given GM in midlight (1:00 pm) showed: tubular necrosis, degeneration, dilation together with regeneration of proximal tublar cells, cell infiltration in interstitium, and hyaline cast formation in the tubular lumen. Following middark (1:00 am) dosing or in combination with LMOX, these abnormalities were significantly reduced, especially, the number of necrosed cells (Photo 1). Levels of GM in both kidney and serum were significantly reduced following middark dosing, as compared with these of midlight dosing (Table 1). Levels of GM in the kidney was significantly reduced by the concomitant administration of LMOX to the same levels as seen following middark dosing.

There was a marginally significant variation in the pharmacokinetics of GM which depended on the time of administration. The effect of circadian rhythms on GM was first demonstrated in the susceptibility of mice[2]. In the present study, the significant circadian rhythm of GM-induced renal toxicity was demonstrated in rats housed in a light-dark cycle under an *ad libitum* feeding schedule. Severe renal toxicity was found in rats following midlight administration compared with the middark. A similar significant circadian rhythm was shown for plasma and renal GM concentrations 30 minutes after drug administration. BARZA *et al.* reported a close relationship between the accumulation of aminoglycosides in the kidney and the extent of nephrotoxicity[3]. We determined the GM levels in the kidney, at midlight and at middark. GM levels were significantly reduced at middark dosing. These circadian stage-dependent changes in GM kinetics seems to well correlate to GM induced renal toxicity

Photo 1. Light micrograph of rat kidney.

Magnification, × 200. Gentamicin 60 mg/kg sc was administered at the middark (1:00 am) or at the midlight (1:00 pm) for 8 days. (A) middark, (B) midlight.

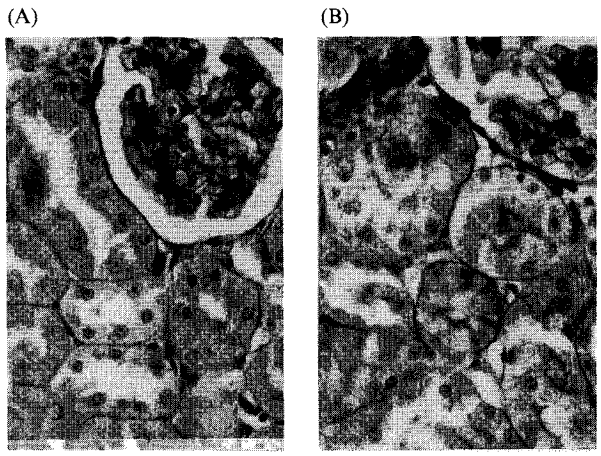


Table 1. Time-dependent changes in plasma gentamicin levels (μg/ml) following administration of gentamicin alone (60 mg/kg) or gentamicin (60 mg/kg) with latamoxef (1,000 mg/kg) in rats.

	Plasma gentamicin levels			Renal gentamicin levels		
	Time of administration		Statistical significance	Time of administration		Statistical significance
	1:00 am	1:00 pm		1:00 am	1:00 pm	
Gentamicin (60 mg/kg)	99.2±21.8	148.1±28.8	$P<0.01$	476.7±77.9	583.2±78.5	$P<0.05$
Gentamicin (60 mg/kg)+ latamoxef (1,000 mg/kg)	102.1±25.6	154.4±28.0	$P<0.01$	424.7±79.2	470.7±76.2	N.S.
Statistical significance	N.S.	N.S.		N.S.	$P<0.05$	

Rats received a dose of 1,000 mg/kg of latamoxef (LMOX) sc and then received a dose of 60 mg/kg of gentamicin sc (GM+LMOX).

Values are expressed as the mean±SD of 5 rats.

Statistical significance by STUDENT's *t* test.

Plasma and renal gentamicin levels were measured 30 minutes after administration.

rhythm in mice. In addition, GM levels were significantly reduced in combination with LMOX following midlight dosing. This reduction may have resulted in the suppression of the GM induced nephrotoxicity. Protective effects of LMOX and piperacillin on aminoglycoside nephrotoxicity in rats have been reported[4,5]. Pharmacologic actions are depended not only on the drug-receptor sensitivity, but also on kinetic variables. Therefore, a circadian rhythm in drug action or toxicity may be due to kinetic alterations. The variation of GM induced renal toxicity seems to be, at least in part, due to the circadian rhythm in the pharmacokinetics of GM.

Acknowledgments

The authors gratefully acknowledge the wonderful cooperation of Dr. TAKASHI SUGIYAMA, Mr. HIROYUKI MIYAZAKI, Dr. SHIGEHIRO OHDO and Dr. NOBUYA OGAWA.

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