Protective Effect of Fleroxacin against the Nephrotoxicity of Isepamicin in Rats

Tomoko YAZAKI,* Yuji YOSHIYAMA,*a Paul WONG,*a Denis BEAUCHAMP,* and Motoko KANKEa
Division of Clinical Pharmacy, Kyoritsu College of Pharmacy,* 1–5–30 Shibakoen, Minato-ku, Tokyo 105–8512, Japan and Research Center for Infectious Diseases, Laval University,a 2705, boul. Ste-Foy, Quebec, G1V 4G2 Canada.

Received September 21, 2001; accepted December 28, 2001

The protective effect of fleroxacin on isepamicin-induced nephrotoxicity was investigated. Wistar rats were administered either fleroxacin 100 mg/kg orally, isepamicin 300 mg/kg subcutaneously, or fleroxacin and isepamicin in combination for 14 d. The animals given 300 mg/kg of isepamicin showed a significant increase in urine N-acetyl-β-D-glucosaminidase (NAG) levels as compared with the control animals which received saline (p<0.01). However, the increase in NAG level was markedly less when isepamicin was administered in combination with fleroxacin (p<0.01). Fleroxacin alone had no effect on urine NAG activity. Serum creatinine and blood urea nitrogen (BUN) levels were significantly higher in animals treated with isepamicin alone than in the control animals (p<0.01) or animals receiving the isepamicin fleroxacin combination (p<0.01). Histopathologically, fleroxacin induced very few cellular alterations, but considerably reduced the manifestation of typical signs of isepamicin nephrotoxicity. This investigation demonstrates that fleroxacin protects animals against isepamicin-induced nephrotoxicity.

Key words fleroxacin; protective effect; nephrotoxicity; isepamicin; combination; rat

Isepamicin is an aminoglycoside used clinically in Japan.1) Its advantages included having low nephrotoxicity and effectiveness with only once daily dosing in the treatment of numerous infections.2,3) Since fleroxacin is often combined with isepamicin, and this combination has been shown to be very effective as a first-line antibiotic combination in the treatment of severe infections, the potential of additive nephrotoxicity in the fleroxacin isepamicin combination regimen is of great clinical importance.

Different compounds or drugs used concomitantly with aminoglycosides may either increase or decrease aminoglycoside toxicity. In fact, drugs such as cisplatinum4) and vancomycin5) increase the nephrotoxicity of aminoglycosides, while recent studies showed that poly-L-aspartic acid,6) daptonycin,7,8) carbenicillin,9) ticarcillin,10) and ceftriaxone11) protected the kidney against aminoglycoside-induced nephrotoxicity.

Fleroxacin is a synthetic broad-spectrum antibiotic belonging to the class of fluoroquinolones.12) It shows good antibacterial activity against many gram-negative bacteria, and less activity against gram-positive bacteria.13,14)

A recent investigation showed that fleroxacin (intraperitoneally) protected against gentamicin-induced nephrotoxicity in rats when both drugs were administered in combination.15) In Japan, fleroxacin is given orally. In rats, fleroxacin reaches peak serum levels within 1 h after oral administration, with a half-life of 2.3 h.16) In contrast with several other quinolones, fleroxacin is not metabolized and is mainly excreted unchanged in the urine.14,17,18) Fleroxacin reaches peak kidney concentrations within 0.5–1 h. In fact, high concentrations of fleroxacin have been measured in the kidney of rabbits19) and in the human kidneys.20) Moreover, the accumulation of fleroxacin was higher in the kidneys of patients with symptomatic complicated urinary tract infections.21)

To our knowledge, there are no data on the effects of fleroxacin (orally) on the nephrotoxicity of administered isepamicin. In view of the particular distribution of fleroxacin within the kidney, we have evaluated the nephrotoxic potential of the fleroxacin (orally) isepamicin combination regimen.

MATERIALS AND METHODS

Adult male Wistar rats weighing between 130—150 g were used. The animals were housed in a light-controlled room (lights on from 0800 to 2000 h) at a room temperature of 24±1 °C and humidity of 60±10% for 1 week. They were acclimated for one week, and they had free access to food and water throughout the experiment.

Isepamicin was dissolved in saline (0.9% NaCl). Fleroxacin was suspended in 0.3% sodium carboxymethyl cellulose (CMC-Na).

For 14 d, animals were given one of the 4 regimens: a single injection of either saline (intraperitoneally) and CMC-Na (orally), saline (intraperitoneally) and fleroxacin 100 mg/kg (orally), isepamicin 300 mg/kg (intraperitoneally) and CMC-Na (orally) or isepamicin (intraperitoneally) with fleroxacin (orally) at the same dose as when used alone. Animals were injected at 1300 h. This dosing schedule was determined from previous studies.11,22,23) Rats were randomly assigned to groups of five and were individually accommodated in metabolism cages to collect urine. Urine samples were collected 24 h prior to the beginning of the administration of drug and every 24 h thereafter. Urine volumes were measured and the urine samples were centrifuged at 3000 rpm for 10 min. The N-acetyl-β-D-glucosaminidase (NAG) activity of the supernatant was determined and expressed as international units per total urine volume collected over 24 h. The activity of NAG was measured by sodio-m-cresolsulfonphthaleinyl N-acetyl-β-D-glucosaminide method (NAG test, Shionogi & Co., Ltd., Osaka, Japan). Rats were sacrificed 24 h after the last injection of isepamicin. Under anesthesia, blood samples were collected from the inferior vena cava for the determination of serum creatinine and blood urea nitrogen (BUN), and kidney samples were removed for histopathological observation. Serum creatinine and BUN levels were measured

* To whom correspondence should be addressed. e-mail: yoshiyama-yyj@kyoritsu-ph.ac.jp © 2002 Pharmaceutical Society of Japan
by the Folin–Wu method and Urease–Indophenol method. Histopathological samples were prepared by standard periodic PAS staining and were evaluated as viewed under a microscope. Statistical analysis of the differences between groups was first performed by analysis of variance. If p values were <0.05, group comparisons were done by the Fisher protected least-significant-difference post hoc test.

RESULTS

Figure 1 shows 24 h urine NAG levels for each group throughout the 14 d of the experiment. Urine NAG activities were significantly higher in the group treated with isepamicin alone than in the groups given saline (control) or fleroxacin (p<0.01). In other words, the extent of increase in urine NAG activity was significantly smaller when fleroxacin was given with isepamicin as compared with isepamicin alone (p<0.01). The serum creatinine and BUN levels of each group are presented in Figs. 2 and 3. Significantly higher levels of serum creatinine and BUN were observed in the group of isepamicin alone compared with groups treated with saline or fleroxacin alone (p<0.01). However, the serum creatinine and BUN levels of the group given isepamicin with fleroxacin were similar to that of the control group. This suggests that the concomitant administration of these two drugs is less toxic than isepamicin alone.

Histopathological analysis of the renal tissue showed tubular necrosis, degeneration, vacuolation of proximal tubular cells, cell infiltration in the interstitium, and hyaline cast formation in the tubular lumen in the group given 300 mg/kg isepamicin alone. The group of fleroxacin alone showed no significant change in kidney histology. In contrast, these abnormalities were significantly reduced, especially the number of necrosed cells, when fleroxacin was administered concomitantly with isepamicin. The highest incidence of histopathologic abnormalities was observed in the group

Fig. 1. Time Course of Urine NAG Activities of Each Treatment Group

○, control; ■, isepamicin (300 mg/kg); □, fleroxacin (100 mg/kg); ●, isepamicin (300 mg/kg) plus fleroxacin (100 mg/kg). Each point represents the mean and standard deviation for five rats. **, significantly different from all other groups (p<0.01).

Fig. 2. Serum Creatinine Levels in Animals Treated with Isepamicin 300 mg/kg Once Daily for 14 d

Isepamicin was administered with saline (NaCl, 0.9%) or with fleroxacin (100 mg/kg). Each point represents the mean and standard deviation (bar) for n=5. **, significantly different from all other groups (p<0.01).

Fig. 3. Blood Urea Nitrogen Levels in Animals Treated with Isepamicin 300 mg/kg Once Daily for 14 d

Isepamicin was administered with saline (NaCl, 0.9%) or with fleroxacin (100 mg/kg). Each point represents the mean and standard deviation (bar) for n=5. **, significantly different from all other groups (p<0.01).
given isepamicin alone. In contrast, the lowest incidence was observed in the group given the fleroxacin isepamicin combination (Fig. 4).

DISCUSSION

The present study shows that fleroxacin protects against isepamicin-induced nephrotoxicity, as shown by lower NAG in urine excretion, lower serum creatinine and BUN levels, as well as fewer histopathological signs in groups treated with the isepamicin fleroxacin combination as compared with isepamicin alone.

The present study also demonstrates that fleroxacin protects proximal tubular cells against isepamicin-induced nephrotoxicity. The results of this study show that fleroxacin alone has no significant nephrotoxic effect. Furthermore, no specific signs or alterations in kidney histology that could be associated with fleroxacin administration were observed.

Some β-lactam antibiotics have also been reported to reduce the nephrotoxic potential of aminoglycosides when administered concomitantly. Data from our laboratory has also shown that latamoxef and ceftriaxone protect against gentamicin-induced nephrotoxicity. Aminoglycosides are frequently used in combination with fleroxacin or other broad-spectrum cephalosporins to treat patients with major infections. These patients are particularly prone to renal injury induced by aminoglycosides. The protective effect of fleroxacin against aminoglycoside toxicity observed in this study might be beneficial in additive or synergic combination of antibiotics in the treatment of seriously ill patients. There may be several mechanisms for the observed protective effect of fleroxacin on renal cells. Beauchamp et al. demonstrated that fleroxacin protected against gentamicin-induced nephrotoxicity when injected intraperitoneally. Fleroxacin significantly reduced the accumulation of gentamicin in the renal cortex of rats treated at 40 mg/kg i.p. compared with animals treated with gentamicin alone at the same dosage. Although the mechanism by which fleroxacin protects against isepamicin-induced nephrotoxicity remains unclear, it might be due, at least in part, to a reduction in the accumulation of aminoglycosides in the kidney. Further investigations are needed to better understand the mechanism and extent of this protective effect.

In summary, the concomitant administration of fleroxacin and isepamicin may help to achieve a chemotherapeutic strategy that reduces the toxic effects of isepamicin while maximizing its therapeutic effectiveness.

Acknowledgements The authors express their gratitude to Setsuko Sakurai, Chikako Ishigami, Chiho Uehara and Miori Wada for their assistance in this study.

REFERENCES


