Changes in Lipid Peroxidation and Activities of Xanthine Oxidase, Superoxide Dismutase and Catalase in Kidneys of Cephaloridine-Administered Rats

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Accepted September 19, 1988

Abstract—To elucidate the toxic and protective mechanisms responsible for cephaloridine (CER)-induced nephrotoxicity, changes in renal formation of malondialdehyde and renal activities of xanthine oxidase, superoxide dismutase and catalase were mainly investigated for 15 days in rats that received single intravenous injections of CER in doses of 100 and 1,000 mg/kg body weight. In the 100 mg/kg group, the above items determined remained within the control levels. In the 1,000 mg/kg group, renal formation of malondialdehyde was observed to be accelerated with the following two stages: highly in the early stage (the 3rd hour to the 2nd day, especially at the 3rd hour) and more highly in the late stage (the 2nd to the 7th day). Concerning the other items determined, significantly different changes were hardly observed in the 1,000 mg/kg group within the 12th hour of the early stage, while the rises in renal activities of xanthine oxidase and falls in renal activities of superoxide dismutase and catalase were observed in the late stage. These results suggested that the increment in malondialdehyde formation in the late stage might be explained enzymatically by both the rises in the activities of xanthine oxidase and the declines in the activities of superoxide dismutase and catalase and that those in the early stage did not relate directly to the above renal enzymatic systems.

Cephaloridine (CER), one of the cephalosporins, is known to produce renal injury in humans and laboratory animals, and its main toxic effect is considered to be on the proximal tubules (1, 2). As regards to the CER nephrotoxicity in experimental animals, the following findings have been reported: suppression of the renal organic anion transport system, reduced glomerular filtration rates, glucosuria, proteinuria and others, which were observed in physiological studies (3, 4); inhibition of gluconeogenesis, inhibition of respiration in mitochondria and decrement in reduced glutathione content in the renal cortex, as observed by biological studies (5–7); nuclear pyknosis, cytoplasmic disintegration, hydropic changes and necrosis in the proximal tubular cells, and patchy loss of the brush border membranes in the proximal tubules, as determined by histopathological studies (1, 2). Several biochemical mechanisms responsible for the CER-nephrotoxicity have been proposed (5, 8–12), while its precise toxic mechanisms remain to be investigated. Among these, lipid peroxidation ascribable to oxygen radicals produced in the kidney has been proposed to be one of the major mechanisms for CER-nephrotoxicity (5, 12).

Based on the above hypothesis that the oxygen radicals might participate in the etiology of CER-induced nephrotoxicity, we investigated the change in malondialdehyde formation and changes in the activities of xanthine oxidase, superoxide dismutase and catalase, as enzymatic factors that produce or are scavengers of oxygen radicals, in the kidneys of CER-administered rats. In this paper, we describe and discuss the results.

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Materials and Methods

Male Wistar rats weighing 210–230 g (Sankyo Labo Service Co., Tokyo, Japan) were kept in ordinary cages, and they were allowed free access to water and standard diet pellets (MF; Oriental Yeast Co., Chiba, Japan). In order to avoid diurnal changes, drug administration and other operations performed on the rats were done between 10:00 a.m. to 11:00 a.m. The day when CER was administered to the rats was designated as the 0th day (hour). The rats received single intravenous injections of CER (Keflodin; Shionogi Co., Ltd., Osaka, Japan) in an equivalent volume of saline (4 ml/kg body weight) through the tail vein, with the doses being 100 and 1,000 mg/kg body weight. The control rats received the same volume of saline.

In the 0th, 3rd, 6th and 12th hours, and the 1st (the 24th hour), 2nd, 3rd, 4th, 5th, 6th, 7th, 10th and 15th days after the CER-administration, operations to prepare kidney homogenate and plasma were carried out. Under ether-anesthesia, the abdominal cavity was opened through a ventral incision. Immediately after ligating the right renal artery and vein, the right kidney was removed and homogenized by a glass-teflon Potter homogenizer with 1.15% potassium chloride containing 3 mM ethylene diamine tetraacetic acid (EDTA) disodium salt; the presence of EDTA prevented any possible lipid peroxidation during the homogenization (13). The right kidney homogenate was used for the determination of malondialdehyde formation. Following removal of the right kidney, blood was taken from the abdominal aorta, put into a heparinized tube, and centrifuged (1,700 x g, 10 min, 4°C) to obtain the plasma. After taking the blood, the left kidney was perfused through the abdominal aorta with ice-cold saline, and then it was removed. The left kidney was homogenized by a glass-teflon Potter homogenizer with 0.25 M sucrose, in order to eliminate the possibility that EDTA might have an effect on the enzyme determinations described below. The left kidney homogenate was used to determine the contents of hypoxanthine and xanthine and the activities of xanthine oxidase, superoxide dismutase and catalase. In the preparations of the right and left kidney homogenates, the volume was adjusted by adding the above solutions so that 10 ml of homogenate contained one kidney.

Plasma urea nitrogen was determined by the method of Chaney and Marbach (14). The analyses described below were done as reported previously (15). Renal protein content was measured by the method of Lowry et al. (16). Renal malondialdehyde formation, as an index in lipid peroxidation, was determined by the method of Uchiyama and Mihara (17) using the thiobarbituric acid reaction. Malondialdehyde, as a standard, was prepared from malonaldehyde bis(dimethyl)-acetal by the method of Esterbauer and Slater (18). For the determination of the renal activities of xanthine oxidase, superoxide dismutase and catalase, the homogenate was treated by Triton X-100 (1% v/v, final concentration). Xanthine oxidase activity with xanthine as the substrate was determined by the method of Fried (19). For determination of superoxide dismutase activity, the Triton-X-100-treated homogenate was sonicated according to the method of Stein et al. (20). Superoxide dismutase activity was determined by the method of Elstner and Heupei (21), and it was calculated using logit paper (22). Catalase activity with hydrogen peroxide as the substrate was determined by the method of Decker (23), and the activity was represented in international units. The contents of hypoxanthine and xanthine were determined by the high-performance liquid chromatographic method of Putterman et al. (24).

Results are given as the mean±standard error (S.E.). Statistical analyses of the data were performed as previously described (25). The values in the groups were first analyzed on variance by the Bartlett method. When an equal variability was present, the values were secondly analyzed by one-way analysis of variance. When significencies were recognized, the values were then examined by the Dunnett method using the mean values.

If an equal variability in the Bartlett method was absent, the values were analyzed by the H-test of Kruskal-Wallis. When significancies were recognized, the values were examined by the Dunnett method using the mean values.
Levels of less than 5% on both sides of the statistical distributions were considered to be significant.

Results

As one of the popular indices of renal failure, changes of plasma urea nitrogen were investigated (Fig. 1). In comparison to the control group, the plasma urea nitrogen in the 100 mg/kg group showed no significantly different changes throughout the whole process. In contrast, the plasma urea nitrogen in the 1,000 mg/kg group rose from the 1st day with a significant difference as compared to the control group and reached the maximal level on the 3rd day, with the value being approximately 10 times the control level. Thereafter, it began to revert toward the control level, but was still higher than the control level until the 7th day. From the 10th day, it returned almost to the control level.

Changes of malondialdehyde formation were investigated in the right kidneys (Fig. 2). The malondialdehyde formation in the administered groups dose-dependently showed higher values as compared with the control group only at the 6th hour in the 100 mg/kg group and from the 3rd hour to the 7th day in the 1,000 mg/kg group. In particular, the malondialdehyde formation in the 1,000 mg/kg group was at a high level from the 3rd to the 5th day, rather than from the 3rd hour to the 2nd day, and it reverted almost to the control level later than the 10th day.

Changes in wet weights, contents of protein, hypoxanthine and xanthine and activities of xanthine oxidase, superoxide dismutase and catalase were investigated in the left kidney.

In terms of the changes in wet weights (Fig. 3, upper figure), the control and the 100 mg/kg groups showed no significant differences from each other throughout the whole process. Those in the 1,000 mg/kg group showed significant rises from the 1st day, as compared with the control group.

Fig. 1. Changes of plasma urea nitrogen following single intravenous injections of cephaloridine in rats. The rats received single intravenous injections of cephaloridine in doses of 100 and 1,000 mg/kg body weight. The day when the injections were carried out was designated as the 0th day (hour). The number of rats was 6 for each time point. Sampling was done in the 0th, 3rd, 6th and 12th hours and the 1st (the 24th hour), 2nd, 3rd, 4th, 5th, 6th, 7th, 10th and 15th days following the injections. The points and bars represent the mean±S.E. The asterisks denote significant differences in comparison to the control group at each time point: *, P<0.05; **, P<0.01. Abbreviation: CER, cephaloridine.

In terms of the total protein contents (Fig. 3, lower figure), the control and the drug-administered groups showed no significantly different changes among each other at each time point throughout the whole process.

Contents of hypoxanthine and xanthine were determined in the left kidneys (Fig. 4). In the 1,000 mg/kg group, a fall in hypoxanthine was observed at the 3rd hour (upper figure), and a rise in xanthine observed at the 12th hour (lower figure). At the other time points, no significantly different changes in hypoxanthine and xanthine were found in the administered groups.
administered groups in comparison to the control group.

Figure 5 shows the changes of total activities of xanthine oxidase in the left kidneys. The activities in the control and the administered groups showed no significantly different changes among each other at each time point earlier than the 24th hour. The 100 mg/kg group showed no significantly different changes in comparison to the control group throughout the whole process. After the 2nd day, the activities in the 1,000 mg/kg group rose, and the higher level, about 2-times the control level, was maintained as a plateau from the 4th to the 10th day. The activities were still higher than the control level on the 15th day.

Figure 6 shows the changes of total activities of superoxide dismutase in the left kidneys. The 100 mg/kg group showed no significantly different changes in comparison to the control group throughout the whole process. The activities in the 1,000 mg/kg group began to fall from the 12th hour, and the lowest activity was observed to be approximately 60% of the control value on the 3rd day. The falls were observed until the 10th day, and the activities reverted to the control level on the 15th day.

Figure 7 shows the changes of total activities of catalase in the left kidneys. The
100 mg/kg group showed no significantly different changes in comparison to the control group. The activities in the 1,000 mg/kg group began to fall from the 1st day. It remained to be lower until the 10th day as compared to the control: in particular, the activities were approximately 30% of the control value during the 3rd to the 6th day. Thereafter, the activities approached the control level.

**Discussion**

It has been reported that the LD$_{50}$ value of CER in rats was 1.3 to 1.4 g/kg body weight in single intravenous administration (2, 26) and that the ND$_{50}$ (nephrotoxic dose) value was 1.0 g/kg body weight in single subcutaneous administration (2). Based on these and other reports (2, 10, 12, 27) which referred to the CER-nephrotoxicity, the doses of CER and periods for sampling were set up.

Our results indicated that the parameters determined in the 100 mg/kg group ranged within the control levels throughout the whole process, whereas the 1,000 mg/kg group showed significantly different changes in comparison to the control group. Thus, this discussion will be mainly based on the results obtained from the 1,000 mg/kg group.

As to the etiology of the nephrotoxicity induced by CER, oxygen radical production has been proposed as one of the major mechanisms in relation to lipid peroxidation (6, 12, 28). In a biological system, oxygen radicals are formed through several production systems (29). Among those, we chose the (hypo)xanthine/xanthine oxidase system as a cytotoxic superoxide radical generator (30), and we investigated the changes of renal activity of xanthine oxidase and the renal contents of hypoxanthine and xanthine as its substrates.

The primary defense against cytotoxic oxygen radicals is provided by antioxidants and enzymes that scavenge the intermediates of oxygen reduction (30–32). In terms of the enzymatic detoxification, the superoxide radical is converted to hydrogen peroxide plus oxygen by superoxide dismutase, and the hydrogen peroxide is furthermore converted to water plus oxygen by catalase (30, 33, 34). Based on these biochemical views, we chose
superoxide dismutase and catalase as enzymatic factors that are scavengers of oxygen radicals, and we investigated the changes of renal activities of these two enzymes.

A main finding in our in vivo study was that single intravenous injections of CER accelerated renal malondialdehyde formation with the following two stages: the early stage between the 3rd hour to the 2nd day and the late stage between the 2nd to the 7th day (Fig. 2).

Relevant to the acceleration in the renal malondialdehyde formation in the early stage, it has been reported that in an in vivo study, lipid peroxidation was increased in the renal cortex several hours after the intraperitoneal injection of CER (2,000 mg/kg) to rats (12), and it was similarly increased in renal cortical slices and microsomes in several to 60 min following the in vitro treatment with the CER (5, 35). Furthermore, Silverblatt et al. (1) reported that in their histopathological study, the rabbits that intramuscularly received CER (200 mg/kg) developed structural alterations in the proximal tubular cells as early as one hour after the dosing. Thus, our finding on the renal elevation in malondialdehyde formation in the early stage was in good accordance with these reports with regard to the time course of renal lipid peroxidation, and the renal injury induced by CER seemed likely to occur within at least 3 hr following the administration under our experimental conditions.

During the initial period of the 3rd to the 6th hour in this early stage, in which malondialdehyde formation was relatively high in the 1,000 mg/kg group, no significantly different changes were observed in the renal activities of xanthine oxidase, superoxide dismutase and catalase among the control and the administered groups (Figs. 2, 5, 6 and 7). This finding might indicate that in the early stage, the acceleration in the renal malondialdehyde formation cannot be attributed to the changes in renal activities of xanthine oxidase, superoxide dismutase and catalase, but may be due to changes in other renal systems that produce and/or scavenge the oxygen radicals. With regard to the systems, endogenous antioxidants and radical scavengers such as glutathione and vitamin E, and other enzymes including glutathione peroxidase, aldehyde oxidase, and NADPH-cytochrome c reductase may be proposed to play important roles in producing and scavenging the oxygen radicals (31, 32, 36, 37). In addition, CER has been reported to cause inhibition of gluconeogenesis, inhibition of respiration in mitochondria, a decrement in reduced glutathione content, and a loss of the high-energy phosphate pool in the kidney, within several hours following its treatment in in vivo and in vitro studies (5–7, 12, 38). Accordingly, the renal elevation of malondialdehyde formation in the early stage of our study might be ascribable to complicated combinations of the above factors.

In addition, the following changes in the renal contents of hypoxanthine and xanthine were observed in our study (Fig. 4): a fall in hypoxanthine at the 3rd hour and a rise in xanthine at the 12th hour. It is generally known that the adenine nucleotides are metabolized into hypoxanthine, and the hypoxanthine is furthermore oxidized, in turn, to xanthine and to uric acid by xanthine oxidase (39). In addition, renal ischemia has been reported to inhibit mitochondrial respiration, to decrease the adenosine triphosphate level, and to increase the hypoxanthine and xanthine levels in the kidney (40, 41). Also CER-administration has been known to inhibit mitochondrial respiration in the renal cortex (7). Accordingly, the CER-administration can be readily speculated to increase the hypoxanthine and xanthine levels in the kidney. Nevertheless, our finding indicated that the renal level of hypoxanthine fell during this period. As to the fall in renal hypoxanthine level, we speculated as follows: conversion of hypoxanthine from adenine nucleotides was accelerated in the kidney, whereas the hypoxanthine was excessively oxidized into xanthine by xanthine oxidase with a speed far beyond the above production of hypoxanthine. Also, the rise in renal level of xanthine could be considered as a result of the excessive oxidation of hypoxanthine to xanthine. As oxygen radicals might be formed through this oxidation reaction (29, 30), our finding suggests that the production of oxygen radicals was promoted through an unknown mechanism(s) in spite of a constant level of
xanthine oxidase activity in the kidney.

Next, we will discuss the changes observed in the late stage. It was in this late stage that most of items determined showed significantly different changes as compared with the control group. The malondialdehyde formation was higher in the late stage in comparison to the early stage. In accordance with the time course of the malondialdehyde formation, the rise in activity of xanthine oxidase and the falls in activities of superoxide dismutase and catalase were markedly observed (Figs. 2, 5, 6 and 7). In this period, renal contents of hypoxanthine and xanthine, which are substrates of xanthine oxidase, remained within the control levels (Fig. 4). As regards to this dissociation in time courses of the changes of the enzyme and the substrates, the following was speculated as its cause: not only were the cellular injuries progressively aggravated, but simultaneously, cellular restoration was initiated and progressed during this period. Namely, repair of the injured renal mitochondria where the level of the high-energy phosphate pool is adjusted may have been initiated similar to what was reported in the reflow of the ischemic kidney (40, 41). As to the renal contents of hypoxanthine and xanthine, these levels were quantitatively sufficient enough to be oxidized by xanthine oxidase. Therefore, it was speculated that the ability to produce cytotoxic oxygen radicals would be elevated in the kidneys.

As to the distributions of xanthine oxidase and superoxide dismutase at the nephron level, the two enzymes were reported to be highly localized in the proximal tubules, especially in the pars convoluta (42, 43). Accordingly, though the distribution of catalase at the nephron level remains to be investigated, the rise in activity of xanthine oxidase and the declines of superoxide dismutase and catalase might imply that the increase in ability to produce superoxide radicals as well as the decrease in the ability to scavenge the radicals was caused in the kidney, especially in the proximal tubules. Thus, the nephrotoxic mechanism of CER in the late stage might be explained enzymatically through the lipid peroxidation. In addition, our results showed that when the renal activities of xanthine oxidase, superoxide dismutase and catalase returned to the control levels, the renal malondialdehyde formation reverted to the control level (Figs. 2, 5, 6 and 7). These coincidences in their time courses also strongly supported our speculation in relation to the etiology of the CER-nephrotoxicity.

Acknowledgments: This research was supported by grants from The Special Research Foundation of Higashi-Nippon-Gakuen University (Grant No. 85-PA-3 and 86PA-4) and from The Research Foundation of Hokkaido Prefecture. The authors wish to thank Guest Professor Dr. Tsuneyoshi Tanabe, Higashi-Nippon-Gakuen University, who gave us many important and useful suggestions during this work.

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