Lipid Peroxidation and Generations of Oxygen Radicals Induced by Cephaloridine in Renal Cortical Microsomes of Rats

Yoshihiro SUZUKI and Jun-ichi SUDO*
Department of Toxicology and Clinical Pharmacology, Faculty of Pharmaceutical Sciences, Higashi-Nippon-Gakuen University, Ishikari-Tobetsu, Hokkaido 061-02, Japan

Accepted October 12, 1989

Abstract—To investigate whether oxygen radicals would be generated by cephaloridine (CER) in the renal cortical microsomes obtained from rats and whether the microsomal lipid peroxidation would be promoted by CER, the microsomes were incubated under a pure oxygen atmosphere in a medium containing the reduced nicotinamide adenine dinucleotide phosphate regenerating system, under various conditions. Generations of superoxide anion and hydrogen peroxide and malondialdehyde formation were all dependent on microsomal protein concentrations, incubation periods and CER concentrations. Scavengers of the microsomal lipid peroxidation induced by CER, (+)-cyanidanol-3, mannitol, sodium benzoate and N-acetyl tryptophan, which are scavengers of hydroxyl free radicals, inhibited the CER-stimulated lipid peroxidation in the microsomes. Histidine, a scavenger of hydroxyl free radicals and singlet oxygen, and alpha-tocopherol, reduced-glutathione and NN'-diphenyl-p-phenylenediamine, the three of which are non-specific antioxidants, also inhibited the CER-stimulated lipid peroxidation in the microsomes. Accordingly, our findings may strongly support that CER generates not only superoxide anions and hydrogen peroxide but also hydroxyl free radicals in the kidney, and these generated oxygen radicals react with the membrane lipids to induce peroxidation and nephrotoxicity.

Materials and Methods

General procedures and isolation of renal cortical microsomes: Male Wistar rats (San-kyo Labo Service Co.; Tokyo, Japan) were housed in ordinary cages under the following conditions: temperature of 22±3°C, relative humidity of 50±10%, and free access to water and standard diet pellets (MF; Oriental Yeast Co.; Chiba, Japan) until the experiments.

* To whom all correspondence should be addressed.
Isolation of renal cortical microsomes was done by the method of Potter et al. (17). Under ether-anesthesia, the abdominal cavity was opened through a ventral incision, and both kidneys were perfused through the abdominal aorta with ice-cold saline. Renal cortices were cut and homogenized with two volumes of 150 mM KCI-20 mM Tris-HCl buffer (pH 7.4) by motor-driven glass/Teflon homogenizers. Then, renal cortical microsomes were isolated by differential centrifugation.

**Protein determination:** The protein amount of the microsomes was determined by the method of Kresze (18), with the modification described by Lowry et al. (19), using bovine serum albumin as the standard.

**Assays of superoxide anion and hydrogen peroxide:** Formation of superoxide anion was measured as the reduction of acetylated cytochrome c, monitored by the absorbance at 550 nm, under a pure oxygen atmosphere by the method of Kakinuma and Minakami (20); and it was calculated as the difference in the cytochrome c reduction in the absence and presence of 4000 U/ml superoxide dismutase (21). Acetylated cytochrome c was prepared by the method of Azzi et al. (22). The composition of the reaction medium was 0 to 50 μg-protein/ml microsomes, 50 μM acetylated cytochrome c, 0 to 50 mM CER, and it contained the reduced nicotinamide adenine dinucleotide phosphate (NADPH) regenerating system: 150 mM KCl-20 mM Tris-HCl buffer (pH 7.4) containing 1.9 mM NADP, 20 mM glucose-6-phosphate, 1.1 U/ml glucose-6-phosphate dehydrogenase and 8.6 mM magnesium chloride (23). Incubation was done in 1 ml of the medium, at 37°C, for periods of 0 to 15 min.

Hydrogen peroxide formed was determined by the methods of Sasame and Boyd (21) and Hildebrandt et al. (24) under a pure oxygen atmosphere. The composition of the reaction medium was 0 to 200 μg-protein/ml microsomes, 0 to 50 mM CER, and the above NADPH regenerating system. Incubation was done in 0.3 ml of the medium, at 37°C, for periods of 0 to 30 min. Immediately after the incubation was terminated, 0.5 ml of 3% (w/v) trichloroacetic acid was added to the medium, mixed and kept in ice for 20 min. The mixture was centrifuged at 22,000×g for 10 min at 2°C, and hydrogen peroxide in its supernatant was determined with ferroammonium sulfate and potassium thiocyanate by the method of Hildebrandt et al. (24), using hydrogen peroxide as the standard.

**Assay of microsomal lipid peroxidation:** Microsomal malondialdehyde (MDA) formation, as one of indices for lipid peroxidation, was determined by the method of Mimnaugh et al. (25), using the thiobarbituric acid reaction. The composition of the reaction medium was 0 to 800 μg-protein/ml microsomes, 0 to 50 mM CER and the NADPH regenerating system described above. Incubation was done under a pure oxygen atmosphere using 0.35 ml of the medium, at 37°C, for periods of 0 to 60 min. After the incubation was stopped by the addition of 0.15 ml of cold 2.0 M trichloroacetic acid-1.7 N HCl solution, the mixture was centrifuged at 10,000×g for 10 min at 4°C. One milliliter of 1.0% (w/v) thiobarbituric acid, followed by 10 μl of butylated hydroxytoluene (final concentration, 0.01%), was added to 250 μl of the supernatant, and the mixture was heated at 95°C for 15 min. Then the formed MDA was measured at 533 nm (26) using authentic MDA as the standard, which was prepared from malonaldehyde bis(dimethyl)acetal by the method of Estebauer and Slater (27).

**Study on the inhibition of microsomal lipid peroxidation by antioxidant enzymes and radical scavengers:** To investigate the abilities of the compounds and enzymes to inhibit microsomal lipid peroxidation, one of the substances was added to the above incubation medium for the MDA assay described above, and the MDA formed was determined in the above manner. The substances used were superoxide dismutase, catalase, reduced-glutathione (+)-cyanidanol-3, sodium benzoate, mannitol, N-acetyl tryptophan, histidine, alpha-tocopherol and NN'-diphenyl-p-phenylenediamine. The concentration(s) of each substance in the incubation medium was selected on the basis of reported concentrations required to inhibit the generations of the oxygen radical species (12–14, 23, 25, 28–33). The activity of superoxide dismutase
used was ascertained by the method of Elstner and Heupei (34); the activity determined by this method is known to be approximately 10 times higher than that determined by the method of McCord and Fridovich (35). The activity of the catalase used was determined by the method of Decker (36).

Percentages of inhibition of microsomal lipid peroxidation were calculated as follows (See Table 1): A baseline value of 14.5±0.2

Table 1. Inhibition of lipid peroxidation in renal cortical microsomes by antioxidant enzymes and scavengers of oxygen radicals

| Inhibitor (Concentration) | MDA formation (nmole/30 min/mg protein) | Percentage of inhibition (%) |
|---------------------------|----------------------------------------|-----------------------------|
| CER-free                  | 14.5±0.2                               |                             |
| CER-stimulated (25 mM)    | 30.8±1.0*                              |                             |
| CER-stimulated (25 mM)    |                                        |                             |
| Superoxide dismutase      | 32.3±2.1                               | 0                           |
| Superoxide dismutase      | 23.5±0.4**                             | 44.8                        |
| Catalase                  | 33.3±0.4                               | 0                           |
| Catalase                  | 16.7±0.7**                             | 86.5                        |
| Superoxide dismutase      | 16.7±0.8**                             | 86.5                        |
| plus Catalase             |                                        |                             |
| Glutathione (0.1 mM)      | 31.3±0.7                               | 0                           |
| Glutathione (1 mM)        | 29.4±2.8                               | 8.6                         |
| Glutathione (10 mM)       | 6.0±0.7**                              | >100.0                      |
| (+)-Cyanidanol-3 (0.1 mM)| 30.6±1.3                               | 1.2                         |
| (+)-Cyanidanol-3 (1 mM)   | 4.8±0.2**                              | >100.0                      |
| (+)-Cyanidanol-3 (10 mM)  | 4.4±0.4**                              | >100.0                      |
| Sodium benzoate (1 mM)    | 34.4±0.7                               | 0                           |
| Sodium benzoate (10 mM)   | 25.5±0.2**                             | 32.5                        |
| Sodium benzoate (100 mM)  | 20.8±0.4**                             | 61.3                        |
| Mannitol                  | 33.0±1.6                               | 0                           |
| Mannitol (10 mM)          | 20.0±0.4**                             | 66.3                        |
| N-Acetyl tryptophan (1 mM)| 32.1±0.6                               | 0                           |
| N-Acetyl tryptophan (10 mM)| 31.9±1.5                             | 0                           |
| N-Acetyl tryptophan (50 mM)| 6.3±0.2**                             | >100.0                      |
| Histidine (10 mM)         | 26.5±1.7                               | 26.4                        |
| Histidine (100 mM)        | 13.0±0.5**                             | >100.0                      |
| alpha-Tocopherol (0.02 mM)| 33.1±0.8                               | 0                           |
| alpha-Tocopherol (0.2 mM) | 17.5±0.4**                             | 81.6                        |
| alpha-Tocopherol (2 mM)   | 17.1±0.4**                             | 84.0                        |
| NN'-diphenyl-p-phenylenediamine (0.002 mM)| 4.2±0.4**                             | >100.0                      |
| NN'-diphenyl-p-phenylenediamine (0.02 mM)| 4.5±0.3**                             | >100.0                      |
| NN'-diphenyl-p-phenylenediamine (0.2 mM)| 4.9±0.3**                             | >100.0                      |

Abbreviations: cephaloridine, CER: malondialdehyde, MDA. Values of MDA formed represent the mean±S.E.M. of 6 experiments. Statistical analyses were done in comparison with the values* of "CER-stimulated and Inhibitor-free". **, P<0.01. The difference (16.3 nmole MDA/30 min/mg microsomal protein) between the values of "CER-free and Inhibitor-free" and those of "CER-stimulated and inhibitor-free" was regarded as the net generation of MDA at 25 mM CER, and it was used for calculation of the percentages of inhibition induced by the antioxidant enzymes and scavengers of oxygen radicals. The values of >100.0 denote that the substance inhibited not only the CER-induced MDA formation but also the MDA formation in the absence of CER.
nmol MDA/30 min/mg-protein was obtained by incubation of the renal cortical microsomes in the absence of CER and inhibitors, and a control value of 30.8±1.0 nmole MDA/30 min/mg-protein was also obtained by incubation in the presence of 25 mM CER without inhibitors. The differences of 16.3 nmole MDA/30 min/mg microsomal protein represented the net generation of MDA at 25 mM CER, and it was used to calculate the percentages of inhibition induced by the detoxifying enzymes and radical scavengers.

In the above spectrophotometric assays, a Hitachi-320 spectrophotometer with a constant temperature cuvette holder (Hitachi Co., Tokyo, Japan) was used.

Statistics: Results were reported as the mean±standard error (S.E.M.). To define statistically significant differences among the groups, the data were subjected to one-way analysis of variance and subsequently to the Bonferroni’s method and Student’s t-test (37); P values of less than 0.05 were considered to be significant.

Results

Generation of superoxide anion by CER in renal cortical microsomes: When incubation with 0 to 50 μg-protein/ml renal cortical microsomes was done in the presence of 25 mM CER for 15 min, superoxide anion generation concentration-dependently increased with increasing microsomal protein concentration in the incubation medium (Fig. 1A). When incubations were carried out for 0 to 15 min in the presence of 25 mM CER and 25 μg/mil microsomal protein in the incubation medium, superoxide anion generation increased time dependently (Fig. 1B). When incubation with 0 to 50 mM CER was done with 25 μg/ml microsomal protein and an incubation time of 15 min, superoxide anion formation increased concentration-dependently with increasing CER concentration in the incubation medium, showing significant differences at CER concentrations higher than 6.25 mM in comparison with the incubation in the absence of CER (Fig. 1C).

Generation of hydrogen peroxide by CER in renal cortical microsomes: When incubation with 0 to 200 μg of microsomal protein/ml was done in the presence of 25 mM CER for 30 min, generation of hydrogen peroxide showed a concentration-dependent increase with increasing microsomal protein concentration (Fig. 2A). When incubation for 0 to 30 min was done in the presence of 25 mM CER and with 100 μg/ml microsomal protein in the incubation medium, generation of hydrogen peroxide increased with increasing incubation time (Fig. 2B). When incubation with 0 to 50 mM was done with 100 μg/ml microsomal protein for 30 min, hydrogen peroxide formation increased in a concentration-dependent manner with increasing CER concentration, showing significant differences at more than 6.25 mM CER in comparison with the incubation in the absence of CER (Fig. 2C).

When the amounts of superoxide anion and hydrogen peroxide produced by CER were compared at 15 min in Fig. 1B and 2B, the produced amounts (nmole/min/mg-protein of microsomes) were 12.5±0.7 in superoxide anion and 11.0±0.2 in hydrogen peroxide; the two were almost equal to one another.

Lipid peroxidation induced by CER in renal cortical microsomes: Lipid peroxidation of the microsomal membranes was determined by using MDA formation as its index. When incubation with 0 of 800 μg microsomal protein/ml was done with 25 mM CER for 30 min, MDA formation concentration-dependently increase with increasing microsomal protein concentration (Fig. 3A). When incubation for 0 to 60 min was done in the presence of 25 mM CER and 400 μg microsomal protein/ml in the incubation medium, MDA formation increased with increasing incubation time (Fig. 3B). When incubation with 0 to 50 mM CER was done with 400 μg microsomal protein/ml for 30 min, the MDA formation increased concentration-dependently with increasing CER concentration in the incubation medium, showing significant differences at more than 12.5 mM CER in comparison with the incubation in the absence of CER (Fig. 3C).

Inhibition of CER-induced microsomal lipid peroxidation by antioxidant enzymes and radical scavengers (Table 1): Incubation of the renal cortical microsomes without inhibitors produced 14.5±0.2 and 30.8±1.0 nmole
Fig. 1. Effects of protein concentrations (A), incubation periods (B) and cephaloridine concentrations (C) in renal cortical microsomes on generation of superoxide anion induced by cephaloridine. Abbreviation: cephaloridine, CER. Microsomes were incubated at 37°C under a pure oxygen atmosphere, in the absence or the presence of CER, by varying the incubation conditions. In Fig. 1A, microsomes (0 to 50 µg-protein/ml) were incubated for 15 min, in the absence or the presence of CER at 25 mM. In Fig. 1B, microsomes (25 µg-protein/ml) were incubated for periods of 0 to 15 min in the absence or the presence of 25 mM CER. In Fig. 1C, microsomes (25 µg-protein/ml) were incubated for 15 min at 0 to 50 mM CER. Other compositions of the incubation medium were described as in the Materials and Methods. The number of experiments was 6. Points and vertical bars represent the mean±S.E.M. Statistical analyses were done for each microsomal protein concentration, incubation period and CER concentration: *, P<0.05; **, P<0.01.

MDA/30 min/mg-protein in the absence and presence of CER (25 mM), respectively.

All the antioxidant enzymes and radical scavengers that were used in this study showed concentration-dependent inhibitory effects on the microsomal lipid peroxidation induced by CER. Superoxide dismutase and catalase, antioxidant enzymes, in high concentrations (40000 and 4000 U/ml, respectively) effectively inhibited the CER-stimulated lipid peroxidation in the microsomes. A combined use of superoxide dismutase (40000 U/ml) and catalase (4000 U/ml) showed inhibition of the same degree as in the
Fig. 2. Effects of protein concentrations (A), incubation periods (B) and cephaloridine concentrations (C) in renal cortical microsomes on generation of hydrogen peroxide induced by cephaloridine. Microsomes were incubated at 37°C under a pure oxygen atmosphere, in the absence or presence of CER, by varying the incubation conditions. In Fig. 2A, microsomes (0 to 200 μg•protein/ml) were incubated for 30 min, in the absence or the presence of 25 mM CER. In Fig. 2B, microsomes (100 μg•protein/ml) were incubated for periods of 0 to 30 min in the absence or the presence of CER at 25 mM. In Fig. 2C, microsomes (100 μg•protein/ml) were incubated for 30 min at 0 to 50 mM CER. Other compositions of the incubation medium were as described in the Materials and Methods. Other explanations are as described in Fig. 1.

Case when catalase (4000 U/ml) was singly used. Reduced-glutathione (10 mM), which possessed reducing action, effectively inhibited the CER-stimulated lipid peroxidation. (+)-Cyanidanol-3 (1 and 10 mM), mannitol (10 mM), sodium benzoate (10 and 100 mM) and N-acetyl tryptophan (50 mM), which are scavengers of hydroxyl free radicals showed effectively inhibited the CER-stimulated lipid peroxidation; their inhibitory activities were in the following decreasing order of effectiveness: (+)-cyanidanol-3, mannitol, sodium benzoate and N-acetyl tryptophan. Histidine (100 mM), which is a scavenger of both hydroxyl free radicals and singlet oxygen, showed significantly inhibited the CER-stimulated peroxidation. Alpha-Tocopherol (0.2 and 2 mM), a scavenger of singlet ox-
Fig. 3. Effects of protein concentrations (A), incubation periods (B) and cephaloridine concentrations (C) in renal cortical microsomes on malondialdehyde formation induced by cephaloridine. Microsomes were incubated at 37°C under a pure oxygen atomosphere, in the absence or the presence of CER, by varying the incubation conditions. In Fig. 3A, microsomes (0 to 800 µg-protein/ml) were incubated for 30 min in the absence or the presence of 25 mM CER. In Fig. 3B, microsomes (400 µg-protein/ml) were incubated for periods of 0 to 60 min in the absence or the presence of 25 mM CER. In Fig. 3C, microsomes (400 µg•protein/ml) were incubated for 30 min at 0 to 50 mM CER. Other compositions of the incubation medium were as described in the Materials and Methods. Other explanations are as described in Fig. 1.

Hydrogen peroxide can be formed from superoxide anion non-enzymatically or through catalysis by superoxide dismutase (30). The hydrogen peroxide formed in these reactions can, in turn, be detoxified to water
by the antioxidant enzymes catalase and glutathione peroxidase or due to the peroxidatic nature of cytochrome P-450 (38, 39). Rapid detoxification of superoxide anion is vital to the cell because of the cytotoxic properties of this anion itself (30, 38, 39).

In addition, if the superoxide anion and hydrogen peroxide generated by the oxygen metabolism are not rapidly removed, these two oxygen metabolites could produce still more highly reactive oxygen species like the hydroxyl free radical (31, 40, 42) and singlet oxygen (41-43). Schraufstätter et al. (44) reported in their in vitro study that the biochemical events important in the lethal injury of cells induced by oxidants were caused in the initial 30 min of exposure of the cells to the oxidant. This fact implied that when the cells could not be protected against the oxidants during this period, the cells could not be rescued from the lethal damage.

Our present in vitro study directly demonstrated that if conditions for the generations of superoxide anion and hydrogen peroxide were sufficient, CER could immediately generate the two radicals and could initiate lipid peroxidation in the renal cortical microsomes (Figs. 1 to 3). This fact was further proved indirectly by our in vitro experiments using superoxide dismutase and catalase (Table 1). In relation to these findings, our previous in vivo studies (16, 45) indicated that renal lipid peroxidation was promoted within 3 hours following intravenous injection of CER (1 g/kg body weight) in rats, and that the renal reduced-glutathione fell during the period, although renal superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glucose-6-phosphate dehydrogenase, the last three of which were the enzymes concerned with the glutathione peroxidase system, hardly changed with the exception of glutathione reductase. This fact suggested that the injected CER generated oxygen radicals in the kidney in the initial 3 hours and that the renal reduced glutathione was spent for scavenging the radicals.

As to the renal generation of the radicals, our present in vitro study to investigate the potentials of scavengers of oxygen radicals in inhibiting the microsomal lipid peroxidation induced by CER, (+)-cyanidanol-3, sodium benzoate, mannitol and N-acetyl tryptophan, all of which are scavengers of hydroxyl free radicals (28, 31, 32, 42), showed inhibitions of the CER-stimulated lipid peroxidation in the microsomes (Table 1): this finding indirectly demonstrated that the formation of hydroxyl free radicals was caused by CER in the microsomes. Also, histidine and alphatocopherol less highly, respectively, inhibited the microsomal lipid peroxidation (Table 1). Although these two possessed an ability to scavenge singlet oxygen, the specificity to the oxygen radicals was obscure (12, 25, 28, 31, 42). Thus, as to whether the singlet oxygen was formed by CER in the microsomes, it remains to be further investigated. Although we failed to demonstrate the generation of singlet oxygen in this study, our present direct and indirect demonstration that superoxide anion, hydrogen peroxide and hydroxyl free radical were generated by CER strongly suggested a possibility that the three radicals participated in promoting the peroxidation of membranous lipid of the microsomes in combination with each other. Furthermore, the fact that reduced-glutathione and NN'-dibenzyl-pphenylenediamine, non-specific antioxidants (12, 14, 15, 33, 46), most effectively inhibited the microsomal lipid peroxidation (Table 1), might support the presence of the above combined effects on the lipid peroxidation by the three radicals.

We will furthermore discuss the renal producing mechanism(s) of the free radicals by CER. CER has a pyridinium substituent on the beta-lactam ring, and this pyridinum ring is similar to paraquat, a pyridinum-containing compound, which is reduced to a free radical by a single electron reduction reaction catalyzed by cytochrome P-450 reductase and NADPH (12, 30). On the basis of this structural similarity, Kuo et al. (12) speculated that CER might also be metabolized through such a redox cycle which would consume NADPH, generate superoxide, and initiate lipid peroxidation. When the CER-producing mechanism(s) of the radicals is considered with the speculation of Kuo et al. (12) and our present results, CER might first produce the superoxide anion under an aerobic condition. The produced superoxide anion would be secondly converted to hydrogen peroxide...
through catalysis by superoxide dismutase or non-enzymatically by contact with reduced substances (40). Our present results that the superoxide anion and hydrogen peroxide were detected in the 3rd and 5th min, respectively, following the addition of CER strongly supported the above speculation. In addition, because it was observed that the produced amount of superoxide anion per microsomal protein per min was nearly equal to that of hydrogen peroxide, one molecule of hydrogen peroxide was considered to be converted not from two molecules of superoxide anions by superoxide dismutase, but from one molecule of superoxide anion through other reactions by contact with reduced substances such as glutathione, ascorbic acid, and so on (30, 44). Also, as to the producing mechanism(s) of the hydroxyl free radical and singlet oxygen, it has been known that Fe$^{3+}$ ions exist in a trace amount in phosphate-buffered incubation medium, and that the Fe$^{3+}$ ion at an extremely low concentration can produce the hydroxyl free radical and singlet oxygen from the superoxide anion and the hydrogen peroxide generated (30, 31, 39, 41, 43). Then, these generated oxygen radicals would react with polyunsaturated lipids of the cell membrane to induce peroxidation of the membrane lipids. Nevertheless, concerning these points, further investigations are needed.

Finally, we will discuss the CER concentration of 25 mM employed routinely in our present in vitro study. Our previous report (47) indicated that following CER injection (1 g/kg body weight, i.v.) in rats, the concentration of CER excreted into the urine during the initial 2 hours was 47.7 mg/ml (112 mM), and that the renal content of CER at the time-point of 20 min, at which the injected CER maximally accumulated in the kidney, was 6.86 mg/g-wet weight of kidney tissue (Providing that 1 g kidney was equal to 1 ml of water and providing that CER distributed homogeneously in the kidney, this value corresponded to 16.4 mM). Thus, the CER concentration of 25 mM was not so far from the concentration in our previous in vivo studies (16, 45, 47). In addition, our present in vitro study indicated that superoxide anions and hydrogen peroxide were generated at concentrations of more than 6.25 mM (Figs. 1C and 2C) and that the lipid peroxidation was promoted at more than 12.5 mM (Fig. 3C). Accordingly, the results of our present in vitro study might strongly support that CER generates not only superoxide anions and hydrogen peroxide but also hydroxyl free radicals in the kidney in vivo, and the generated oxygen radicals react with the membranous lipids to induce peroxidation and nephrotoxicity.

Acknowledgments: This research was supported by a grant from The Special Research Foundation of Higashi-Nippon-Gakuen University (Grant No. 89PB-1). 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.

References

1 Child, K.J. and Dodds, M.G.: Mechanism of urinary excretion of cephaloridine and its effects on renal function in animals. Br. J. Pharmacol. 26, 108-119 (1966)
2 Child, K.J. and Dodds, M.G.: Nephron transport and renal tubular effects of cephaloridine in animals. Br. J. Pharmacol. Chemother. 30, 354-370 (1967)
3 Tune, B.M., Fernholt, M. and Schwartz, A.: Mechanism of cephaloridine transport in the kidney. J. Pharmacol. Exp. Ther. 191, 311-317 (1974)
4 Perkins, R., Apicella, M.A., Lee, I.-S., Cuppage, F.E. and Saslaw, S.: Cephaloridine and cephalotin: Comparative studies of potential nephrotoxicity. J. Lab. Clin. Med. 71, 75-84 (1968)
5 Tune, B.M. and Fravert, D.: Mechanism of cephalosporin nephrotoxicity: A comparison of cephaloridine and cephaloglycin. Kidney Int. 18, 591-600 (1980)
6 Kiguchi, M. and Sudo, J.: Defect of urinary concentration capacity in cephaloridine-administered rats. Chem. Pharm. Bull. (Tokyo) 36, 1857-1864 (1988)
7 Atkinson, R.M., Currie, J.P., Davis, B., Pratt, D.A.H., Sharpe, H.M. and Tomich, E.G.: Acute toxicity of cephaloridine, an antibiotic derived from cephalosporin C. Toxicol. Appl. Pharmacol. 8, 398-406 (1966)
8 Silverblatt, F., Truck, M. and Bulger, R.: Nephrotoxicity due to cephaloridine: A light- and electron-microscopic study in rabbits. J. Infect. Dis. 122, 33-44 (1970)
9 Boyd, J.F., Butcher, B.T. and Stewart, G.T.: The nephrotoxicity and histology of cephaloridine and its polymers in rats. Br. J. Exp. Pathol.
10 Boyd, J.F., Butcher, B.T. and Stewart, G.T.: The nephrotoxic effect of cephaloridine and its polymers. Int. J. Clin. Pharmacol. 7, 307–315 (1973)

11 McMurtry, R.J. and Mitchell, J.R.: Renal and hepatic necrosis after metabolic activation of 2-substituted furans and thiophenes, including furosemide and cephaloridine. Toxicol. Appl. Pharmacol. 42, 285–300 (1977)

12 Kuo, C.-H., Maita, K., Sleight, S.D. and Hook, J.B.: Lipid peroxidation: A possible mechanism of cephaloridine-induced nephrotoxicity. Toxicol. Appl. Pharmacol. 67, 78–88 (1983)

13 Cojocel, C., Laeschke, K.H., Inselmann, G. and Baumann, K.: Inhibition of cephaloridine-induced lipid peroxidation. Toxicology 35, 295–305 (1985)

14 Goldstein, R.S., Pasino, D.A., Hewitt, W.R. and Hook, J.B.: Biochemical mechanisms of cephaloridine nephrotoxicity: Time and concentration dependence of peroxidative injury. Toxicol. Appl. Pharmacol. 83, 261–270 (1986)

15 Goldstein, R.S., Contardi, L.R., Pasino, D.A. and Hook, J.B.: Mechanisms mediating cephaloridine inhibition of renal gluconeogenesis. Toxicol. Appl. Pharmacol. 87, 297–305 (1987)

16 Suzuki, Y. and Sudo, J.: Changes in lipid peroxidation and activities of xanthine oxidase, superoxide dismutase and catalase in kidneys of cephaloridine-administered rats. Japan. J. Pharmacol. 49, 43–51 (1989)

17 Potter, W.Z., Davis, D.C., Mitchell, J.R., Jollow, D.J., Gillette, J.R. and Brodie, B.B.: Acetaminophen-induced hepatic necrosis. III. Cytochrome P-450-mediated covalent binding in vitro. J. Pharmacol. Exp. Ther. 187, 203–210 (1973)

18 Kresze, G.-B.: Methods for protein determination. In Methods of Enzymatic Analysis, 3rd Ed., Edited by Bergmeyer, H.U., Vol. 1, p. 84–99, Verlag Chemie, Weinheim, Deerfield Beach and Basel (1983)

19 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275 (1951)

20 Kakinuma, K. and Minakami, S.: Effects of fatty acids on superoxide radical generation in leukocytes. Biochim. Biophys. Acta 538, 50–59 (1978)

21 Sasame, H.A. and Boyd, M.R.: Superoxide and hydrogen peroxide production and NADPH oxidation stimulated by nitrofurantoin in lung microsomes: Possible implications for toxicity. Life Sci. 24, 1091–1096 (1979)

22 Azzi, A., Montecucco, C. and Richter, C.: The use of acetylated ferricytochrome C for the detection of superoxide radicals produced in biological membranes. Biochem. Biophys. Res. Commun. 65, 597–603 (1975)

23 Mimnaugh, E.G., Trush, M.A. and Grant, T.E.: A possible role for membrane lipid peroxidation in anthracycline nephrotoxicity. Biochem. Pharmacol. 35, 4327–4335 (1986)

24 Hildebrandt, A.G., Roots, I., Tjoe, M. and Heinemeyer, G.: Hydrogen peroxide in hepatic microsomes. Methods Enzymol. 52, 342–350 (1978)

25 Mimnaugh, E.G., Trush, M.A. and Grant, T.E.: Stimulation by Adriamycin of rat heart and liver microsomal NADPH-dependent lipid peroxidation. Biochem. Pharmacol. 30, 2797–2804 (1981)

26 Bernheim, F., Bernheim, M.L.C. and Wilbur, K.M.: The reaction between thiobarbituric acid and the oxidation products of certain lipids. J. Biol. Chem. 174, 257–264 (1948)

27 Esterbauer, H. and Slater, T.F.: The quantitative estimation by high performance liquid chromatography of free malonaldehyde produced by peroxidizing microsomes. IRCS Med. Sci. 9, 749–750 (1981)

28 Misra, H. and Grosky, L.D.: Paraquat and NADPH-dependent lipid peroxidation in lung microsomes. J. Biol. Chem. 256, 9994–9998 (1981)

29 Mimnaugh, E.G., Gram, T.E. and Trush, M.A.: Stimulation of mouse heart and liver microsomal lipid peroxidation by anthracycline anticancer drugs: characterization and effects of reactive oxygen scavengers. J. Pharmacol. Exp. Ther. 226, 806–816 (1983)

30 Bus, J.S. and Gibson, J.E.: Role of activated oxygen in chemical toxicity. In Drug Metabolism and Drug Toxicity, Edited by Mitchell, J.R. and Horning, M.G., p. 21–32, Raven Press, New York (1984)

31 Gutteridge, J.M.C.: Lipid peroxidation initiated by superoxide-dependent hydroxyl radicals using complexed iron and hydrogen peroxide. FEBS Lett. 172, 245–249 (1984)

32 Paller, M.S.: Free radical scavengers in mercuric chloride-induced acute renal failure in the rat. J. Lab. Clin. Med. 105, 459–463 (1985)

33 Ramsammy, L.S., Josepovitz, C., Ling, K.Y., Lane, B.P. and Kaloyanides, G.J.: Effects of diphenyl-phenylendiamine on gentamicin-induced lipid peroxidation and toxicity in rat renal cortex. J. Pharmacol. Exp. Ther. 238, 83–88
34 Elstner, E.F. and Heupei, A.: Inhibition of nitrite formation from hydroxylammonium chloride: A simple assay for superoxide dismutase. Anal. Biochem. 70, 616–620 (1976)

35 McCord, J.M. and Fridovich, I.: Superoxide dismutase. J. Biol. Chem. 244, 6049–6055 (1969)

36 Decker, L.A.: Catalase. In Worthington Enzymes, Enzyme Reagents, Related Biochemicals, Edited by Decker, L.A., p. 63–65, Worthington Biochemical Co., Freehold (1977)

37 Wallenstein, S., Zucker, C.L. and Fleiss, J.L.: Some statistical methods useful in circulation research. Circ. Res. 47, 1–9 (1980)

38 Hildebrandt, A.G., Speck, M. and Roots, I.: Possible control of hydrogen peroxide production and degradation in microsomes during mixed function oxidation reaction. Biochem. Biophys. Res. Commun. 54, 968–973 (1973)

39 Kappus, H. and Sies, H.: Toxic drug effects associated with oxygen metabolism: Redox cycling and lipid peroxidation. Experientia 37, 1233–1241 (1981)

40 Fridovich, I.: Superoxide dismutase. Annu. Rev. Biochem. 44, 147–159 (1975)

41 Kappus, H.: Oxidative stress in chemical toxicity. Arch. Toxicol. 60, 144–149 (1987)

42 Elstner, E.F., Osswald, W. and Konze, J.R.: Reactive oxygen species: Electron donor-hydrogen peroxide complex instead of free OH radicals? FEBS Lett. 121, 219–221 (1980)

43 Kellogg, E.W. and Fridovich, I.: Superoxide, hydrogen peroxide, and singlet oxygen in lipid peroxidation by a xanthine oxidase system. J. Biol. Chem. 250, 8812–8817 (1975)

44 Schraufstätter, I.U., Hinshaw, D.B., Hyslop, P.A., Spragg, R.G. and Cochrane, C.G.: Glutathione cycle activity and pyridine nucleotide levels in oxidant-induced injury of cells. J. Clin. Invest. 76, 1131–1139 (1985)

45 Suzuki, Y. and Sudo, J.: Changes in glutathione peroxidase system and pyridine nucleotide phosphate levels in kidneys of cephaloridine-administered rats. Japan. J. Pharmacol. 51, 181–189 (1989)

46 Sugihara, K., Nakano, S. and Gemba, M.: Effect of cisplatin on in vitro production of lipid peroxides in rat kidney cortex. Japan. J. Pharmacol. 44, 71–76 (1987)

47 Hayashi, T. and Sudo, J.: Relieving effect of saline on cephaloridine nephrotoxicity in rats. Chem. Pharm. Bull. (Tokyo) 37, 785–790 (1989)