Determination of Radical Species in the Kidney of Rats with Chronic Renal Failure by the Spin Trapping Method

Dear Sir,

The importance of active oxygen and related free radicals has been recognized since they were found to be involved in the renal disorder induced by paraquat intoxication or administration of adriamycin. However, the involvement of these radicals in the development of general renal diseases such as nephritis, nephrotic syndrome and renal failure remained unclear until 1984, when Paller et al. [1] found that the xanthine oxidase inhibitor allopurinol protected the kidney from these conditions. In addition, Re-han et al. [2, 3] demonstrated that active oxygen was responsible for the glomerular disorder induced by phorbol myristate, a neutrophil activator, and that active oxygen was involved in the occurrence of Masugi nephritis. Following their reports, various active oxygen scavengers (superoxide dismutase, desferrioxamine, thiourea, etc.) were reported to improve nephritis.

On the other hand, we have been studying the mechanisms of production of a probable uremic toxin, methylguanidine, which increases markedly in the body under conditions of uremia, and have isolated from urine 5-hydroxycreatinine, which is probably a hydroxyl radical-adducted intermediate between creatinine and methylguanidine [4-8]. This suggested that free radicals might be involved in the occurrence of uremia. We also demonstrated that active oxygen plays a certain role in the development of renal failure [9], observing that 8-hydroxy-deoxyguanosine, a hydroxyl radical-adducted deoxyguanosine in DNA reported by Kasai and Nishimura [10, 11], was markedly increased in the kidneys of rats with ade-nine-induced chronic renal failure.

In the present study, in view of the lack of available data on active oxygen species in the kidney, we determined radical species in the rat kidney using electron spin resonance (ESR) spectroscopy combined with spin trapping with 5,5-dimethyl-1-pyrroline-N-oxide (DMPO). Kidney active oxygen species in chronic renal failure were compared with those detected in the normal kidney in order to obtain direct evidence for production of radical species under conditions of renal failure.
Male Wistar rats (body weight approximately 200 g) were used for the experiment. The animals were kept in a wire-bottomed cage under a conventional lighting regimen with a dark night. The room temperature (about 23 °C) and humidity (about 60%) were controlled automatically. Normal animals were fed on an 18% casein diet for 25 days. Animals with chronic renal failure were obtained by feeding them an 18% casein diet containing 0.75% adenine (dosage of adenine approximately 350-360 mg/kg body weight) for 25 days. In rats given adenine, it had been confirmed previously both histologically and biochemically that renal failure progressed as the period of adenine feeding was prolonged [12, 13]. The levels of serum constituents in the rats used for this experiment were as follows: in rats fed an adenine diet, the urea nitrogen value was 5.9 times (105.4 ± 3.0 mg/dl) that of normal rats, and a high value of about 4.23 mg/dl was noted for creatinine. On the 25th day of the experimental diet, the animals were sacrificed by decapitation. The kidneys were removed quickly, cooled on ice, and weighed rapidly. Fresh tissues were homogenized with 10 vol of ice-cold saline solution at 0°C. The homogenate was then used for the determination of radical species. Twenty microliters of DMPO were added to 200 µl of the homogenate and stirred for 10 s. The ESR spectra of DMPO adducts were measured on a JEOL FE-2XG spectrometer (JEOL, Tokyo, Japan; X band, 100 kHz modulation) at 30 °C. The g-factor of each radical was estimated from the signal of external manganese dioxide at g = 1.981 and g = 2.034. Microwave power and sweep time for the ESR measurement were set at 8 mW and 0.5 min, respectively.

Six strong lines, shown in figure 1A, resembled the reported [14] signals of the DMPO adduct of the carbon-centered radical (DMPO-C). The g-factor (g ≈ 2.006) and one hyperfine splitting constant [a(N) = 1.58 mT] agreed well with those reported previously [14]. However, the other hyperfine splitting constant [a(ßH) = 2.42 mT] was a little greater than the reported value [14], suggesting the production of carbon radicals possessing high spin densities. Figure 1B and C shows the formation of the radical assignable to the DMPO adduct of the hydroxyl radical [DMPO-OH; g = 2.006, a(N) = 1.49 mT and a(ßH) = 1.49 mT] as well as DMPO-C. In addition, weak signals of the DMPO adduct of the hydrogen radical (DMPO-H; g = 2.006, a(N) = 1.66 mT, and a(ßH) = 2.25 mT) were present, although not all the peaks were detectable. The location of the remaining peaks, shown particularly in figure 1C, was predicted by the calculated hyperfine splitting pattern using g = 2.006, a(N) = 1.43 mT, and a(ßH) = 1.15 mT. These signals may be safely assigned to the DMPO adduct of the peroxy radical (DMPO-OOH) despite the lack of hyperfine splitting by γH, because the values of g, a(N), and a(ßH) agree with the reported ones [14] and because much stronger signals of DMPO-OOH obtained by Hatano et al. [15] showed an insufficient hyperfine splitting pattern (lack of splitting by γH).

The ESR spectrum of the normal rat kidney (fig. 1 A) is the sum of that of DMPO-C (strong), DMPO-OH (medium), and DMPO-H (weak). In this case, radical species in the left kidney were found to be the Mn2+.

g = 2.034

Fig. 1. ESR spectra of the homogenate of the right kidney of a normal rat (A), and the right (B) and left kidney (C) of a rat with chronic renal failure. Hyperfine splitting patterns of DMPO-C (D) DMPO-OH (E), DMPO-OOH (F) and DMPO-H (G) are shown.
left kidney with chronic renal failure. That is, formation of DMPO-OOH was predominant in the
left kidney and formation of DMPO-OH in the right. Eventually, there was a difference in radical
species between the normal kidney and that with chronic renal failure. In addition, the distinction
in the distribution of DMPO-C, DMPO-OH, DMPO-H and DMPO-OOH between the normal
kidney and that with chronic renal failure suggests that these radical species are closely related to
the etiology and pathology of chronic renal failure. Further investigation to determine where in
the kidney the radical species are produced is desirable in order to clarify the mechanism of
occurrence and progression of renal diseases.

same as those in the right. In the right kidney of rats with chronic renal failure (fig. 1B), the
amount of DMPO-C was decreased to about 35% of that in normal rats, whereas the production
of DMPO-OH was increased. In addition, small amounts of DMPO-OOH and DMPO-H were
detected. In the left kidney of rats with chronic renal failure (fig. 1C), the amount of DMPO-C
was reduced to about 25% of that in normal rats, whereas the production of DMPO-OOH was
markedly increased. In addition, DMPO-OH and a small amount of DMPO-H were observed. It
is worth noting that DMPO-OOH appeared only in the kidney with chronic renal failure and was
undetectable in the normal kidney. In our study, there was a difference in the radical species
between the right and

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Announcement
Charles E. Culpeper Foundation Scholarships in Medical Science
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384