Increase in Kidney 8-Hydroxydeoxyguanosine Level with the Progression of Renal Failure

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Dear Sir,

It has recently become apparent that active oxygen produces damage to biological membranes, protein and DNA, playing a role in aging, carcinogenesis and the onset and pathological features of rheumatism and other diseases [1, 2]. Although fragmentation of the DNA chain and alteration of thymine glycol and thymidine glycol by active oxygen were reported previously by Okamoto [3], Yamamoto et al. [4] and Cathcart et al. [5], little is known about damage to purine bodies. However, Kasai and Nishimura [6, 7] recently demonstrated that deoxyguanosine (dG) in the DNA molecule is oxidized by oxygen radical to 8-hydroxydeoxyguanosine (8-OH-dG), and concluded that 8-OH-dG can be used as a new indicator of injury to DNA bases by oxygen radical. On the other hand, in some studies carried out by Paller et al. [8], Shah and Walker [9], Diamond et al. [10] and Rehan et al. [11], renal failure induced by renal ischemia or administration of glycerol, puromycin aminonucleoside or antglomerular basement membrane was ameliorated by treatment with scavengers of hydroxyl radical, superoxide anion and hydrogen peroxide. On the basis of these findings, they suggested the involvement of active oxygen in the onset of renal failure. Fillit et al. [12], Giardini et al. [13], Kuroda et al. [14] and Flament et al. [15] have also reported findings suggestive of histological damage by active oxygen in patients with renal failure, suggesting the possibility that patients with renal failure are generally under oxidative stress. However, the extent to which active oxygen contributes to the progression of renal failure at the organ level remains unclear. In order to investigate this issue, we studied the amount of 8-OH-dG in rat renal DNA.

Male Wistar rats (body weight approx. 200 g) were used. Animals with renal failure were prepared by feeding them on an 18% casein diet containing 0.75% adenine (dosage of adenine approx. 350-360 mg/kg body weight) for 10, 20 or 30 days. Normal animals were fed on an 18% casein diet for 10 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 [16-23]. The level of serum constituents in experimental rats were as follows: in rats fed on the adenine diet, urea nitrogen levels were 3.2 times (50.5 ± 1.5 mg/dl) those in normal rats on the 10th experimental day, and 8.6 times higher on the 30th experimental day. An

abnormally high value of about 130 mg/dl was noted on day 30. Creatinine levels were significantly increased to 1.9 times (1.59 ± 0.13 mg/dl) those in normal rats on the 10th experimental day, and 4.4 times (3.66 ± 0.31 mg/dl) those levels on the 30th experimental day. On the 10th, 20th or 30th day of the experimental diet, the animals were sacrificed by decapitation. Kidneys obtained on each occasion were frozen at -80°C for DNA isolation. Defrosted tissue was gently homogenized in a Teflon homogenizer for a minimum time (10-20 s). DNA was then isolated by the method of Marmur [24] and dissolved in 200 μl of 20 mM sodium acetate buffer (pH 4.8), digested with 20 μg of nuclease P1 (United States Biochemical Corporation, USA) at 37°C for 30 min and then treated with 1.3 units of Escherichia coli alkaline phosphatase (Sigma Chemical Co., USA) in 0.1 M Tris-HCl buffer (pH 7.5) for 1 h. The resulting deoxynucleoside mixture was injected into an HPLC apparatus coupled with an electrochemical (EC) detector: apparatus, Shimadzu LC-6A; column, Beck-man Ultrasphere ODS 5 μm (4.6 mm $\times$ 25 cm); eluent, 8% aqueous methanol containing 10 mM NaH_2PO_4 ; flow rate, 1 ml/min; UV detector, Shimadzu SPD-6A, 290 nm; EC detector, Coulochem Model 5100A. Conditions used for digestion, HPLC analysis and 8-OH-dG quantitation were as reported previously [25]. The molar ratio of 8-OH-dG to dG in each DNA sample was determined based on the peak height of authentic 8-OH-dG using the EC detector and the UV absorbance of dG at 290 nm. Statistical analysis of the data in figure 1 was performed using Student's t test.

As shown in figure 1, the content of 8-OH-dG increased as the period of adenine administration continued. The ratio of 8-OH-dG/105 dG levels in DNA purified from renal tissue was 3.81 in normal rats, whereas the level was increased significantly, by 68%, in rats given the adenine diet for 10 days. The level was further increased in rats given the adenine diet for 20 days, and it was as high as 5.7 times the normal level in rats after 30 days of adenine administration. Changes in the amount (picogram) of 8-OH-dG per micro-gram of DNA and per gram of kidney tissue were similar to the above results, showing a marked increase with the course of adenine administration.

With regard to the mechanism of damage to DNA by active oxygen, Kasai and Nishimura [6,7] found that active oxygen hydroxylated dG at the C-8 position to produce 8-OH-

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0-j 1 -i
0 10 20 30

Adenine-feeding period, days

Fig. 1. Changes in 8-OH-dG in kidney DNA following adenine administration. Values are means $\pm$ SE for 5-6 rats. Statistical significance: * $p < 0.05$, ** $p < 0.001$.

dG. 8-OH-dG is currently the only altered purine body detectable in intracellular DNA. An HPLC apparatus fitted with an EC detector is a highly sensitive method for detecting this substance in enzymatically decomposed DNA [25]. In the present study, the content of 8-OH-dG in DNA purified from kidney tissue increased markedly with the course of adenine administration. The content of this substance in rats given the adenine diet for 30 days reached a level 5.7 times higher than that in normal rats. We have previously demonstrated by physiologic biochemistry and histology that renal failure progressed with the course of adenine

administration [16-23]. Therefore, the increase in the level of 8-OH-dG with the course of adenine administration seems to reflect the amount of active oxygen produced in the kidney during the progression of renal failure. On the other hand, the phenomenon observed in the kidney was also found in the liver. Changes in 8-OH-dG in hepatic DNA with the progression of renal failure were similar to those in renal DNA, indicating that damage to DNA was not restricted to the kidney (data not shown). This suggests that the general regulatory and repair mechanism of the body becomes disordered along with the disturbance of renal function. Kuchino et al. [26] have reported that DNA replication using DNA containing 8-OH-dG as a template resulted in misreading of the base sequence before and after the appearance of 8-OH-dG, suggesting that the production of 8-OH-dG by active oxygen induces various pathological conditions in the living body, including carcinogenesis and aging. From these findings, it seems that 8-OH-dG serves not only as an index for estimating the amount of oxygen radical produced in the body but also as an important clue for elucidating the mechanism of onset of various diseases including renal disease.

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