Contribution of Active Oxygen to the Production of Methylguanidine Using Alloxan

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Dear Sir,

Foster [1], Giovannetti et al. [2] and Cohen et al. [3] have demonstrated that the production of methylguanidine (MG) is increased markedly under conditions of renal failure. Many studies on the precursor of MG have been carried out so far [4-8], and the hypothesis that creatinine (Cr) is the precursor of MG is now predominant. On the other hand, Aoyagi and co-workers [9-10] have demonstrated under in vitro conditions and in isolated liver cells that active oxygen is involved in the production of MG from Cr, and have proposed a route of oxidization from Cr. We have also examined the mechanism of MG production in an in vitro experiment using the Fenton reaction (Haber-Weiss reaction), which is widely used for the generation of hydroxyl radical, and demonstrated that Cr decreased gradually with time, while MG increased gradually [11,12], thus indicating the importance of the role of hydroxyl radicals in MG production. However, there has been no report describing the role of active oxygen in the production of MG in the living body. In the present study, we investigated this issue, using alloxan, a substance known to produce active oxygen.

Male rats of the LWH:Wistar strain, with a body weight of about 200 g, were placed in metabolic cages at $23 \pm 1^\circ\text{C}$ under a 12-hour dark/light cycle. A laboratory pellet chow (obtained from CLEA Japan Inc., Tokyo, Japan; protein 24.0%, lipid 3.5%, carbohydrate 60.5%) and water were given ad libitum. The animals were given alloxan intraperitoneally at a dose of 100 or 300 mg/kg body weight.

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Fig. 1. Urinary excretion of methylguanidine in rats administered alloxan intraperitoneally at a dose of 100 or 300 mg/kg body weight.

Urine was collected for 48 h following the administration of alloxan, and deproteinized by addition of trichloroacetic acid (final concentration 10%). The supernatant obtained by centrifugation at 3,000 rpm for 10 min was injected into a Japan Spectroscopic liquid chromatograph using a step-gradient system. A fluorescence spectrometer, model FP-210

(excitation 365 nm, emission 495 nm; Japan Spectroscopic Co., Tokyo, Japan) was used for detection of the MG on the column.

The urinary level of MG in normal rats was 2.54 µg at 24 h and 6.91 µg at 48 h. In 300 mg

normal rats given alloxan at a dose of 100 mg/kg, the urinary level of MG was only slightly higher than the control level until 12 h after administration (fig. 1). However, the urinary excretion of MG began to increase at 24 h, reaching a level about 1.8-fold higher than the control level at 48 h. After administration of a higher dose, 300 mg/kg, the urinary excretion of MG began to increase markedly at 24 h, reaching a level about 5.2-fold higher than the control level at 48 h.

Alloxan is used widely as an agent for inducing diabetes, probably through inhibi-

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tion of insulin synthesis by severing the DNA of beta cells in the Langerhans' islets of the pancreas. With regard to the mechanism of DNA cleavage, LeDoux et al. [13], Heikkila et al. [14], Cohen and Heikkila [15], Yamamoto et al. [16] and Asayama et al. [17] have reported that the administered alloxan is reduced to dialuric acid, which produces a group of active oxygen species, i.e. hydrogen peroxide, superoxide radical and hydroxyl radical, in the process of autoxidation. Among these active oxygen species, the hydroxyl radical is highly reactive and cleaves DNA. Production of active oxygen has been observed in liver cells and erythrocytes as well as beta cells in Langerhans' islets. Thus, this substance seems to produce active oxygen in many organs including the pancreas [18]. In the present study, the urinary MG level was increased markedly in rats 24 h after administration of alloxan at 100 or 300 mg/kg body weight. The level was further increased at 48 h. Since it has been ascertained that in normal rats almost all of the MG produced in the body is excreted rapidly, the results of the present study appear to confirm that active oxygen is involved in MG production.

References

Foster NB: The isolation of a toxic substance from the blood of uremic patients. *Trans Assoc Am Physicians* 1915;30:305.

Giovannetti S, Biagini M, Balestri PL, et al: Uraemia-like syndrome in dogs chronically intoxicated with methylguanidine and creatinine. *Clin Sci* 1969;36:445-452.

Cohen BD, Handelsman DG, Pai BN: Toxicity arising from the urea cycle. *Kidney Int* 1975;7: s-285-s-287.

Perez G, Faluotico R: Creatinine: A precursor of methylguanidine. *Experientia* 1973;29: 1473-1474.

Gonella M, Barsotti G, Lupetti S, et al: Factors affecting the metabolic production of methylguanidine. *Clin Sci Mol Med* 1975;48:341-347.

Orita Y, Tsubakihara Y, Ando A, et al: Effect of arginine or creatinine administration on the urinary excretion of methylguanidine. *Nephron* 1978;22:328-336.

Mikami H, Orita Y, Ando A, et al: Metabolic pathway of guanidino compounds in chronic renal failure; in Lowenthal A, Mori A, Marescau B (eds): *Urea Cycle Diseases*. New York, Plenum Press, 1982, pp 449-458.

Nagase S, Aoyagi K, Narita M, et al: Biosynthesis of methylguanidine in isolated rat hepatocytes and in vivo. *Nephron* 1985;40:470-475.

Nagase S, Aoyagi K, Narita M, et al: Active oxygen in methylguanidine synthesis. *Nephron* 1986;44:299-303.

10 Aoyagi K, Nagase S, Narita M, et al: Role of active oxygen on methylguanidine synthesis in isolated rat hepatocytes. *Kidney Int* 1987;32:S-229-S-233.

Nakamura K, Ienaga M, Yokozawa T, et al: Production of methylguanidine from creatinine via creatol by active oxygen species: Analyses of the catabolism in vitro. *Nephron* 1991;58:42-46.

Oura H, Yokozawa T: Free radical and crude drugs. *Med Drug J* 1990;26:2815-2820.

LeDoux SP, Hall CR, Forbes PM, et al: Mechanisms of nicotinamide and thymidine protection from alloxan and streptozocin toxicity. *Diabetes* 1988;37:1015-1019.

Heikkila RE, Winston B, Cohen G: Alloxan-induced diabetes. Evidence for hydroxyl radical as a cytotoxic intermediate. *Biochem Pharmacol* 1976;25:1085-1092.

Cohen G, Heikkila RE: The generation of hydrogen peroxide, superoxide radical, and hydroxyl radical by 6-hydroxydopamine, dialuric acid, and related cytotoxic agents. *J Biol Chem* 1974;249:2447-2452.

Yamamoto H, Uchigata Y, Okamoto H: Streptozotocin and alloxan induce DNA strand breaks and poly(ADP-ribose) synthetase in pancreatic islets. *Nature* 1981;294:284-286.

Asayama K, English D, Slonim AE, et al: Chemiluminescence as an index of drug-induced free radical production in pancreatic islets. *Diabetes* 1984;33:160-163.

Asayama M, Nyfeler F, English D, et al: Alloxan-induced free radical production in isolated cells. *Diabetes* 1984;33:1008-1011.

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