Contribution of Hydroxyl Radical to the Production of Methylguanidine from Creatinine

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Dear Sir,

Methylguanidine (MG) is a probable uremic toxin demonstrated to have a strong association with the induction of uremia. The idea that creatinine (Cr) is a precursor of MG is currently dominant. Giovannetti et al. [1], Perez and Faluotico [2], Gonella et al. [3], Orita et al. [4] and Nagase et al. [5] have reported a positive correlation between the blood Cr level and the degree of MG production, indicating that Cr is very probably a precursor of MG. Our previous study using normal rats and rats with renal failure also demonstrated that MG production increased in proportion to an increase in the level of Cr in the blood [6]. We also examined temporal changes in MG production after Cr loading, and found that the MG produced was excreted rapidly into the urine in normal rats [6]. On the other hand, the production of MG from Cr seems to be increased by factors which promote MG synthesis and inhibit MG degradation. Recently, Aoyagi et al. [7] observed an increased in vitro production of MG from Cr in the presence of active oxygen. Furthermore, in an experiment using isolated rat hepatocytes, they also demonstrated the inhibition of MG production by dimethyl sulfoxide (DMSO), a scavenger of hydroxyl radical, suggesting the involvement of active oxygen in MG production [7]. Data indicating histological damage due to active oxygen in patients with renal failure have been reported by Fillit et al. [8], Giardini et al. [9], Kuroda et al. [10] and Flament et al. [11]. On the basis of these reports, it is considered that, in a state of renal failure, active oxygen increases in quantity and reacts with Cr, resulting in enhanced MG production. However, it remains unclear to what extent active oxygen contributes to the in vivo production of MG from Cr. In this connection, the involvement of hydroxyl radical was investigated using radical scavengers under conditions of increased MG production after Cr loading.

Table 1. Effect of hydroxyl radical scavengers on MG production from Cr

Scavenger	Dose	MG
$\mu\text{g}/3\text{h}$		
DMTU0		13.07 ± 0.80
$\text{mg}/\text{kgB.W.}$	20	$8.47 \pm 0.29^*$
100		$6.86 \pm 0.28^*$
500		$6.34 \pm 0.40^*$
DMSO0		13.81 ± 0.92

ml/kg B.W. 0.16 7.18 ± 0.75*

0.80 6.14 ± 0.30* 4.00 5.39 ± 0.46*

* p < 0.001; significance of differences from the value in rats given no Cr.

Male rats of the LWH: Wistar strain, with a body weight of about 200 g, were placed in metabolic cages at 23 ± 1°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 Cr intraperitoneally at a dose of 1,000 mg/kg body weight, and the urine was collected for 3 h after administration. N,N'-Dimethyl thiourea (DMTU) or DMSO was administered intraperitoneally 30 min before and 30 min after Cr administration. The urine obtained was used for determination of MG. The urine was 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.,

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Tokyo, Japan) was used for detection of the MG on the column. The urinary excretion of MG in the non-treated rats was about 0.5 µg/3 h. Six rats were used for each experimental group. Values were expressed as means ± SE.

Table 1 shows the effect of hydroxyl radical scavengers on the urinary excretion of MG. In rats given an intraperitoneal dose of 1,000 mg Cr/kg body weight, the level of urinary MG excretion at 3 h was about 13 µg/3 h. When DMTU was administered twice, i.e., before and after Cr administration, the level of urinary MG excretion at 3 h decreased in a dose-dependent manner, as shown in table 1. In rats given a total DMTU dose of 500 mg/kg body weight, the level was 51% lower than in the control group. The results in rats given DMSO (specific gravity, 1.105) before and after Cr administration are shown in table 1. In rats given total doses of 0.16, 0.8 and 4 ml/kg body weight, MG levels were 48, 56 and 61% lower than the control level, respectively. Aoyagi et al. [7] have proposed a probable oxidizing route for the production of MG from Cr. However, Nakamura et al. [13] and our group have demonstrated in vitro that creatol is involved as an intermediate in the conversion of MG from Cr [12]. In addition, we have 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 creatol and MG increased gradually [12, 13], thus indicating the importance of the role of hydroxyl radical in MG production. In the present study, the urinary excretion of MG was markedly decreased in all experimental groups given DMTU, also suggesting the involvement of hydroxyl radical. In order to confirm the role of hydroxyl radical, the effects of DMSO were then examined, and results similar to those with DMTU were obtained. These results indicate that hydroxyl radical is involved in the process of in vivo production of MG from Cr and are consistent with the findings of the in vitro study. It has been reported by Ames et al. [14, 15] and Kasai et al. [16, 17] that hydroxyl radical damages bases and sugars in DNA molecules and cuts the DNA chain, inducing cell necrosis or mutation. As adducts of hydroxyl radical, thymine glycol, thymidine glycol and 8-hydroxydeoxyguanosine have recently been isolated from urine and DNA. The present results seem to suggest that the amount of hydroxyl radical produced in the living body can be estimated indirectly from the amount of MG.

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