Protective Effects of a Ginseng Component, Maltol(2-Methyl-3-Hydroxy-4-Pyrone) against Tissue Damages Induced By Oxygen Radicals*

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Abstract□ Maltol(2-methyl-3-hydroxy-r-pyrone), a component known to be present in Korean Ginseng root showed an antioxidant action but its potency as an antioxidant was low; about 1/50th that of other antioxidants such as p-phenylenediamine, BHA and BHT. However, maltol was able to protect the oxidation damages in biological systems such as adriamycin-induced membrane damage in isolated cardiomyocytes, paraquat-induced toxicities in isolated hepatocytes and reperfusion injury in isolated hearts. The antioxidant action of maltol was also shown to be effective *in vivo*. The antioxidant action of this compound was probably due to the removal of hydroxyl radicals. In view of the roles of oxygen radical in various pathological processes, Korean Ginseng root which contains several antioxidants including maltol is expected to have beneficial effects on the oxygen radical-involved processes.

Keywords□ Maltol, Oxygen free radicals, Lipid peroxidation, Reperfusion injury and Korean ginseng

Introduction

Maltol(Fig. 1) was known to be a compound present in *Korean Ginseng* root, and was reported to have antioxidant acton through the observation that the compound reduced malondialdehyde (MDA) level in an ethanol-intoxicated mouse liver[1]. But further evaluation will be required to provide more definite evidence for its antioxidant action not only for development of this compound as a new antioxidant but also for seeking any potential usefulness of this compound as an ingredient of the medicinal plant used long in the Orient. For this purpose, maltol, in this communication, was further studied to confirm its antioxidant action and evaluate its potency as an antioxidant, and also tested for its protective effects against oxidation damages occurring in cells and tissues.

Methods

Microsomes isolated from mouse livers were incubated with varying concentrations of maltol at 37°C for 1 h in 50 mM Tris-HCl, pH 7.4 with the following enzymatic and non-enzymatic peroxidation systems; enzymatic; Fe(III)-ADP/NADPH and paraquat/NADPH, and nonenzymatic; Fe(II)/ascorbate and Cu(II)/H_2O_2. After the reactions, aliquots were assayed for MDA by thiobarbituric acid method[2]. When ethane was measured as a peroxidation index, the reactions were carried out in sealed vials. Air aliquots above reaction mixtures were assayed for ethane by gas chromatograph[3]. To estimate the lipid peroxidation *in vivo*, ethane contained in expired air from rats was measured according to the method described by Lawrence and Cohen[4]. Cardiocytes were isolated from adult Sprague-Dawley rats by Farmer's method[5].

Results and Discussion

As shwon in Fig. 2, MDA was produced when microsomes were treated with each of the four reaction systems. But maltol decreased the MDA production by any of the reaction systems in a dose-dependent manner. Ethane, another peroxidation product of lipids was also produced by all the reac-

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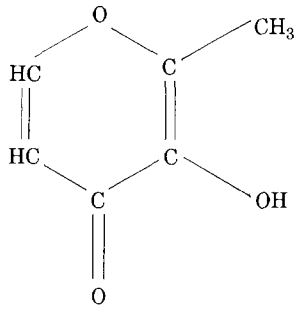

Fig. 1. Structure of maltol

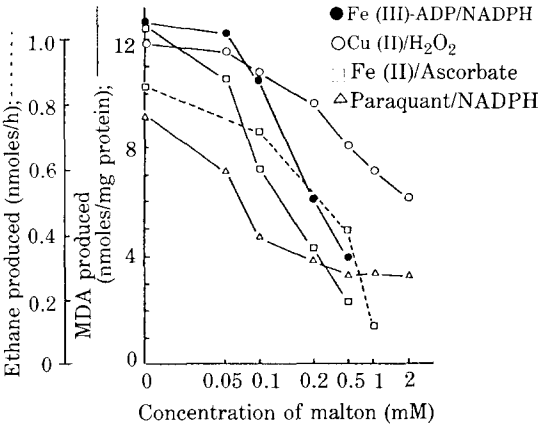

Fig. 2. Effect of maltol on lipid peroxidation of hephatic microsomes induced by various reaction systems. Microsomes (1 mg protein/m*l*) were incubated with each of the following reaction systems; i) 80μM $FeCl_3$, 0.4 mM ADP and 0.4 mM NADPH, ii) 10μM paraquat and 0.5 mM NADPH, , iii) 10μM $FeSO_4$ and 0.1 mM ascorbate and iv) 50 μM $CuSO_4$ and 5 mM H_2O_2. This data was from ref. 6.

tion systems except Cu(II)/H_2O_2 (which produced ethylene as a major hydrocarbon product).

The production of the hydrocarbon gases was also decreased by maltol(Fig. 2 shows only the result observed with Fe(II)/ascorbate, and the data from the other reaction systems are not shown). The maximum effect of maltol was observed at 500-1000 μM and the inhibition to 50% was at about 100 μM(IC50). Based upon the IC50s of maltol and other antioxidants such as p-phenylenediamine (p-PDA), butylated hydroxytoluene(BHT) and butylated hydroxyanisole(BHA), the potency of maltol action was 1/5th-1/170th that of the other antioxidants (Table 1).

Table 1. Comparison of antioxidant potencies between maltol and other antioxidants*

Assays	Reaction systems	Antioxidants			
		Maltol	p-PDA	BHA	BHT
MDA	Paraquat/NADPH	1	26.4	44.1	28.7
	Fe (II)/Ascorbate	1	4.7	23.7	11.6
	Cu (II)/H_2O_2	1	58.4	170.6	71.5
	Fe (III)-ADP/NADPH	1	27.9	135.0	32.2
Ethane	Fe (II)/Ascorbate	1	87.8	49.7	42.3

* Potency was based on concentrations to inhibit the lipid peroxidation to 50% (IC50). The reciprocal of IC50 of maltol was taken as 1. The data was from ref. 6.

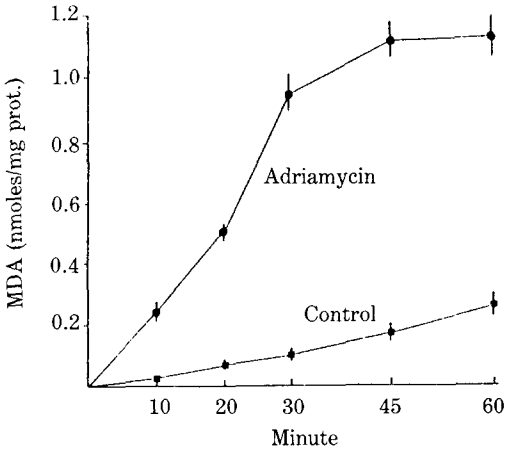

Fig. 3. Adriamycin-induced lipid peroxidation in isolated cardiomyocytes of adult rat. Digitonized isolated cardiomyocyte preparations (10^6 cells/m*l*) were incubated at 37°C in 150 mM KCl, 3 mM $MgCl_2$, 30 mM Tris-HCl (pH 7.4) buffer solution supplemented with 1 mM NADPH and 1 mM NADH. Concentration of adriamycin in the reaction mixture was 100μM. Lipid peroxidation was estimated by measuring malondialdehyde (MDA) concentration by thiobarbituric method.

Maltol was tested to see whether it can prevent the oxidation damages occurring in biological systems. First, the compound was tested on the adriamycin(AM)-induced membrane damage in isolated cardiomyocytes. When added to cardiomyocytes, AM, a redox-cycling compound to generate oxygen radicals in cells[7], induced lipid peroxida-

Table 2. Effects of oxygen radical scavengers on adriamycin-induced lipid peroxidation in isolated cardiomyocytes of adult rats

Experimental system	MDA production** (nmol/30 min/mg prot.)	Percent inhibition
Control*	0.97±0.08	0
-NADPH/NADH	0	-
-Adriamycin	0.18±0.01	-
+SOD (0.02 mg/ml) Catalase (0.02 mg/ml)	0.81±0.07	17
+SOD (2 mg/ml) Catalase (2 mg/ml)	0	100
+Thiourea (50 mM)	0.25±0.05	74

*Digitonized isolated cells (10^6 cells/ml) were incubated at 37°C in a medium containing 150 mM KCl, 3 mM $MgCl_2$, 30 mM Tris-HCl (pH 7.4), 1 mM NADPH/NADH and 100μM adriamycin.
**Mean±S.E. of 6 experiments.

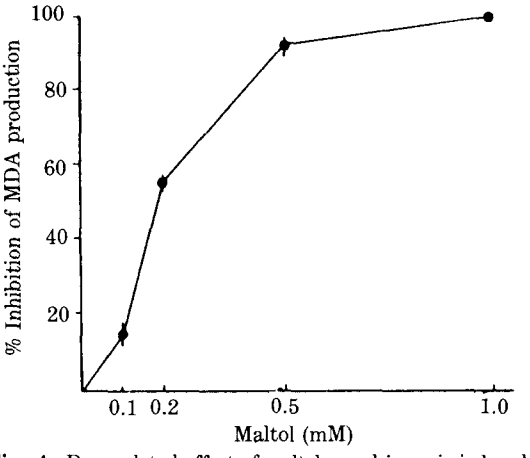

Fig. 4. Dose-related effect of maltol on adriamycin-induced lipid peroxidation in isolated cardiomycete preparation of adult rats. Digitonized isolated cardiomyocyte preparation (10^6 cells/ml) were incubated at 37°C in a medium containing 150 mM $MgCl_2$, 30 mM Tris-HCl (pH 7.4), 1 mM NADPH, 1 mM NADH and 100μM adriamycin. Measurement of MDA production was done as in Fig. 3.

tion. As shwon in Fig. 3, MDA production increased with time to a maximum of 1.11 nmol/mg protein at 45 min. The AM-induced lipid peroxidaiton was inhibited significantly by various oxygen radical scavengers such as superoxide dismutase, catalase and a hydroxyl radical scavenger, thiourea(Table 2), suggesting that the AM-induced membrane damage is mediated by oxygen radicals. But this AM-induced lipid peroxidation was suppressed by maltol as well as α-tocopherol and BHT. As shown in Fig. 4, the maltol effect was dose-dependent; at 1 mM, the compound inhibited MDA production almost completely. Concentration for 50% inhibition was 200 μM. The protective effect of maltol against the cellular oxidation damages was also confirmed in another cellular toxicity system, i.e. paraquat (PQ)-induced toxicity in isolated hepatocytes. Here also maltol could prevent the lipid peroxidation, leakages of lactate dehydrogenase from their cytosol and cell death induced by PQ which is also a well known redox-cycling compound[7] (unpublished data).

Maltol was also tried for the protection against oxidation injury at organ level. We used an oxygen paradox(a phenomenon that reoxygenation exaggerates damages to the tissues which are being ischemic) of heart as a model for organ toxicity by oxidation. The oxygen paradox was demonostrated in isolated rat hearts on Langendorff perfusion apparatus by changing perfusion fluids from N_2-saturated(to make ischemic state) to O_2-saturated one(to reoxygenate). The oxygen paradox in the hearts was confirmed by increased levels of MDA, LDH and creatine phosphokinase in the perfusion fluid. The involvement of oxygen radicals in this reoxygenation injury was indicated by the decrease of their levels in the perfusion fluid by various oxygen radical scavengers. Maltol was also shown to alleviate the oxygen paradox; this compound decreased the release of MDA and the cellular enzymes as other antioxidant such as α-tocopherol and BHT did(unpublished data). Thus, we clearly demonstrated the protective action of maltol against the oxidation damages at tissue level. In a preliminary observation, maltol also decreased the enhanced ethane level in the expired gas of rats which were treated with CCl_4. This result supports that maltol can be effective as an antioxidant *in vivo*.

In order to find an explanation for its antioxidant action, the interaction of maltol with any of the

reactive oxygen species was tested. Maltol showed no effect on the production of adrenochrome from epinephrine treated with xanthine/xanthine oxidase whereas superoxide dismutase completely inhibited the production under the same condition. When H_2O_2 was allowed to react with maltol, the amount of H_2O_2 was not changed. But catalase or Fe^{++} immediately decomposed H_2O_2. Maltol did not also affect the oxidation of NADPH in D_2O by UV-irradiated rose bengal. However, the oxidation was inhibited by diazabicyclo(2,2,2)octane. These results indicate that maltol does not react with any of O_2^-, H_2O_2 or $^1O_2$. On the other hand, maltol inhibited the production of ethylene from methional by Fe^{++}/H_2O_2. At the same time, this compound accelerated the decomposition of H_2O_2 by Fe^{++}. This accelerated decomposition was also observed with several scavengers for HO·. Although maltol was shown to chelate Fe^{++}, this compound, with respect to this reaction, was totally different from other iron chelators such as EDTA and DETAPAC; the latters inhibited the decomposition of H_2O_2. The observed results suggest that maltol interacts with HO· produced from Fe^{++}/H_2O_2. In agreement with the assumption, maltol was observed to be decomposed by Fe^{++}/H_2O_2.

The results obtained in this study indicate that maltol, although its potency was low compared to that of other known antioxidants, has an antioxidant action strong enough to suppress the oxidative damaging processes in biological systems. Although it can chelete Fe ions, the removal of HO· may be a possible mechanism for its antioxidant action. In view of the roles of oxygen radicals in various pathological processes such as carcinogenesis, ischemic heart diseases and aging, Korean Ginseng which contains several antioxidants including maltol is expected to have beneficial effects on the oxygen radical-involved processes.

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