



ANTIOXIDANT EFFECTS OF LYCOPENE AND UBIQUINOL-10 ON THE OXIDATIVE STRESS IN RAT HEPATOCYTES INDUCED BY TERT-BUTHYL HYDROPEROXIDE

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Abstract

Free radicals especially reactive oxygen metabolites can damage DNA, protein, enzymes, and membrane lipids. Lipid peroxidation in hepatocyte membrane may be involved in hepatic diseases. Antioxidants may inhibit this reaction. Due to oxidant-antioxidant imbalance, free radicals may cause destructive effects. For several years, scientists tried to find antioxidant compounds. In this study, the effects of lycopene and ubiquinol-10 on the oxidative stress in rat hepatocytes induced by t-buthyl hydroperoxide was determined. First, rat hepatocytes were isolated and then incubated in the presence of tert-buthyl hydroperoxide and the amount of malondialdehyde, as a marker of lipid peroxidation, was determined. Then, this reaction was performed in the presence of various concentrations of each lycopene and ubiquinol-10, and the malondialdehyde level was determined.

The results of this study showed that in the presence of various concentrations of lycopene and ubiquinol-10 the levels of lipid peroxidation products significantly decreased ($P < 0.05$). Thus, lycopene and ubiquinol-10 have inhibitory effects on lipid peroxidation reaction. This study showed the potential utility of lycopene and ubiquinol-10 in prevention of hepatic dysfunction.

INTRODUCTION

Hepatocytes, which make up the majority of the liver structure, are very active in the metabolism of exogenous chemicals, and this is one of the major reasons why the liver is a target for toxic substances (1). In recent studies, aging and related diseases, such as cancer and coronary heart disease and neurodegenerative disorders such as Alzheimer's disease were found to be related to oxidative and free radical mediated reactions (2). One of the most important of free radicals, reactive oxygen species (ROS) are generated which cause oxidative stress (3). The tipping of the balance towards prooxidant status in oxidative stress induced damage to cellular and extra-cellular macromolecules, such as proteins, lipids and nucleic acids, and affects the immune function (4). Endogenous or dietary factors play a major role in the antioxidative defense of the organism against the ROS generated during normal cellular aerobic respiration (5). Therefore, it is generally assumed that increased intake of dietary antioxidants may help to shift the balance towards an adequate antioxidant status. The epidemiological data support this reputed benefit of dietary antioxidants.

It was postulated that incubation of lipophylic agent with rat hepatocytes, may prevent against oxidative damage. To test this hypothesis, lycopene and ubiquinol-10 were used. These compounds are highly lipophylic and operate in membranes or lipoproteins (6). Lycopene is a carotenoid found in high quantities in tomatoes and tomato-rich products and a potent donor antioxidant (reductant), which increases the lipoprotein resistance against the oxidative modification. In lipid solutions and dispersions, it inhibits formation of radicals linearly with time until consumed in the

process (7). Ubiquinol-10, the reduced form of ubiquinone-10 (coenzyme Q10), is a well-known proton-electron carrier in inner mitochondria membrane and a potent lipophylic antioxidant in different cell membranes. It is well established that both ubiquinols and ubiquinones are active against lipid peroxidation in mitochondria and liposomes but that the quinols are much more powerful antioxidants than the corresponding quinones (8). Recently, we reported that both compounds can protect human lipoprotein more efficiently against lipid peroxidation than antioxidant vitamins (9, 10). The aim of the present study was to investigate the effects of lycopene and ubiquinol-10 on liver injury induced by tert-butyl hydroperoxide (t-BHP), a short-chain analogue of lipid peroxide in rat livers

MATERIALS AND METHODS

Chemicals: tert-Butyl hydroperoxide (t-BHP), thiobarbituric acid (TBA), lycopene, ubiquinol-10 and other chemical substances were prepared from Merck, Germany.

Animals: In this study, male Wister rats (with a body weight of 250 ± 20 g) were purchased from Pasteur Institute Tehran, Iran. These animals were housed in laboratory cages and maintained on a 12 hr light-dark cycle with free access to food and water throughout the study.

Isolation and culture of rat hepatocytes: Rat hepatocytes were prepared by collagenase perfusion, as described previously (11). First, rats were anesthetized with ether. The liver were then removed, minced and homogenized at 4°C in 3ml per g liver, of a buffer made of 20mM Tris-chloride, 0.15m NaCl, 1mM CaCl₂ and 1m PMSF (phenyl methyl sulfonyl fluoride) with pH=7.4. The samples were centrifuged at 800g for 10min. The supernatant was filtered through Miracloth (Calbiochem-Behring). The resulting filtrate was quickly frozen in liquid nitrogen and can be stored at -70 oC for performing the other experiments.

In vitro hepatocytes treatment: First, to the primary cultured rat hepatocytes, various doses of each : lycopene or ubiquinol-10 (0, 10, 20 and 50 μ mol/L) were added to the rat hepatocytes for 4 hr at 37°C. The media were replaced by phosphate-buffered saline. Then, hepatocytes were incubated with t-BHP (1.5Mm) for 30 min at 37°C (12).

Lipid peroxidation assay: Hepatocytes were pretreated with lycopene and ubiquinol-10, and then with t-BHP as described above. The lipid peroxidation product was assayed according to an improved TBA method by spectrophotometer at 532 nm wavelength absorption, using 1,1,3,3-tetra-methoxypropane as a standard (13). The results were expressed as nmol of malondialdehyde (MDA) equivalents per mg protein.

Statistical analysis: The results obtained were expressed as a mean \pm SD. The Student's t-test was used to perform the statistical comparison between the group by one-way analysis of variance. Significant differences were taken as $p < 0.05$.

RESULTS

Cytotoxicity of t-BHP: The TBA method showed that t-BHP expressed toxicity effects to the primary cultures of rat hepatocytes in 0.5, 1 and 1.5 mM concentrations, and in 1.5 mM concentration the highest toxicity effect was observed (Table 1).

Table 1. Cytotoxicity effects of tert-butyl hydroperoxide (t-BHP) assessed by the MDA assay in primary cultured rat hepatocytes. Hepatocyte cultures were treated with various doses of t-BHP for 30 min at 37°C. Data represent mean \pm SD (n=3).

t-BHP (mM)	MDA (nmol/mg protein)
0	104 \pm 15
0.5	111 \pm 17
1	138 \pm 19*
1.5	201 \pm 21**

* $p < 0.05$, ** $p < 0.01$, compared with the group non-treated with t-BHP

Effects of lycopene on t-BHP-induced hepatotoxicity: The effects of lycopene on the cytotoxicity induced by t-BHP in the primary cultured hepatocytes were expressed by the MDA formation. As shown in Table 2, the MDA formation caused by t-BHP (1.5 mM for 30 min) was significantly suppressed by lycopene pretreatment (51% for 50 µmol/L , p<0.001, 46% for 20 µmol/L, p<0.001, and 20% for 10 µmol/L lycopene, p<0.01).

Table 2. Effect of lycopene on the lipid peroxidation induced by tert-butyl hydroperoxide (t-BHP) in 1.5 mM concentration for 30 min in primary cultured rat hepatocytes. Lipid peroxidation was evaluated by malondialdehyde formation. Data represent mean ± SD (n=3).

Lycopene (µmol/L)	MDA (nmol/mg protein)
0	201 ± 21
10	161 ± 22*
20	110 ± 18**
50	100 ± 15**

* p<0.01, ** p<0.001, compared with the group treated with t-BHP alone

Effects of ubiquinol-10 on t-BHP-induced hepatotoxicity: The effects of ubiquinol-10 on the cytotoxicity induced by t-BHP in the primary cultured hepatocytes were expressed by the MDA formation. As shown in Table 3, the MDA formation caused by t-BHP (1.5 mM for 30 min) was significantly suppressed by ubiquinol-10 pretreatment (40% for 50 µmol/L , p<0.001, 26% for 20 µmol/L, p<0.01, and 10% for 10 µmol/L ubiquinol-10, p<0.01).

Table 3. Effect of ubiquinol-10 on the lipid peroxidation induced by tert-butyl hydroperoxide in 1.5 mM concentration for 30 min in primary cultured rat hepatocytes. Lipid peroxidation was evaluated by MDA formation. Data represent mean ± SD (n=3).

Ubiquinol-10 (µmol/L)	MDA (nmol/mg protein)
0	201 ± 21
10	182 ± 19*
20	150 ± 16*
50	122 ± 20**

* p<0.01, ** p<0.001, compared with the group treated with t-BHP alone

DISCUSSION

We employed t-BHP, a short-chain analog of lipid peroxides to induce acute oxidative damage in cultured rat hepatocytes. The model of t-BHP induced acute hepatic damage in animals has been used in several studies, and the capacity of a test compound to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms which have been disturbed by t-BHP is the index of its hepato-protective effect (1, 14).

Reactive oxygen species (ROS; .OH, O₂⁻, RO, ROO, NO) can originate from a number of internal and external sources, such as metabolic reactions, infections, dietary intake and cigarette smoking. The body, however, possesses defense mechanisms to reduce the oxidative damage, and such mechanisms use both enzymes and antioxidant nutrients or medicine to arrest the damaging properties of excited oxygen species. Only when the normal protective mechanism breaks down, or when the effectiveness of antioxidant sources is reduced, which leads to the excessive amount of free radicals in the body, the irreversible oxidative damage may occur. A great number of studies have suggested that antioxidant nutrients and/or medicines play a protective role for the human health (3, 15).

Two distinctive pathways are involved in the metabolism of t-BHP in hepatocytes. The first employs the microsomal cytochrome P-450 system leading to the production of ROS that initiate lipid peroxidation (16); while the second concerns a reaction involving glutathione peroxidase and its substrate glutathione (GSH). Decreased GSH is considered to be a major event in t-BHP- induced toxicity (17).

In the hepatotoxicity and lipid peroxidation experiments, lycopene and ubiquinol-10 were shown to possess a high antioxidative and protective capacity against the injury induced by t-BHP, as reflected in the decreased formation of

MDA. Lycopene and ubiquinol-10 are known to be a highly efficient lipid-soluble antioxidants in the protection of lipids in a variety of biological and model systems including lipoproteins and liposomes (18, 19). It is generally assumed that they may exhibit their protective effects by preventing the formation of lipid free radicals and/or by eliminating them. On the other hand, the possibility that the inhibition of lipid peroxidation is related to their interactions on the lipid structure of the hepatocytes membranes needs further investigation. Lycopene and ubiquinol-10 contain shielding methyl (-CH₃) groups and are optimally positioned in the membranes by their phytol side-chain. Also, the inhibition of photosensitized oxidants of lipids and lipoproteins by both compounds mediated by singlet oxygen have been demonstrated (20). Incubation of lycopene and ubiquinol-10 with hepatocytes over a long period of time may enrich their membranes sufficiently to make them less susceptible to oxidative reactions.

In the present study, lycopene and ubiquinol-10 were observed to inhibit the oxidative damage in in vitro cultured intact cells. This provides biological evidence supporting the use of both compounds for managing liver disorders.

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