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Role of lycopene in recovery of radiation induced injury to mammalian cellular organelles

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Whole body exposure of male rats to 7 Gy gamma irradiation increased lipid peroxidation in the liver resulting in biomembrane damage of subcellular structures and release of their enzymes. This is evidenced by increase of thiobarbituric acid-reactive substances (TBARS) in mitochondria, lysosomes and microsomes. This was associated with a decrease in activity of the enzymes specific for each subcellular fraction; namely, mitochondrial glutamate dehydrogenase (GDH), lysosomal β-glucuronidase and microsomal glucose-6-phosphatase. This was paralleled by an increased activity of these enzymes in the cytosol. Rats were supplemented with lycopene, a carotenoid present in tomatoes (5 mg/kg weight/day), by gavage, for 7 days before exposure to 7Gy gamma irradiation. This resulted in diminishing amount of TBARS recorded for each subcellullar structure in the liver of irradiated animals. Significant amelioration in the decrease recorded for the activity of mitochondrial glutamate dehydrogenase, lysosomal β-glucuronidase and microsomal glucose 6-phosphatase was observed. This was associated with significant amelioration in the increase recorded for the activity of these enzymes in the cytosol. It is postulated that lycopene could play an important role in the recovery of the integrity of biological membranes of the liver after radiation injury.

1. Introduction

Lipid peroxidation of biological membranes contributes significantly to the development of radiation induced cell injury, because these cellular elements play a decisive role in the functional organization of the cell. Humans are endowed with antioxidant defense systems that scavenge and minimize the formation of active oxygen species. However, these systems are not always fully operative. Therefore, diet derived antioxidants become particularly important in diminishing cumulative oxidative damage [1]. Interest in lycopene, a carotenoid found almost exclusively in tomatoes and tomato products, is growing rapidly following the recent publication of epidemiological studies implicating lycopene in the prevention of cardiovascular disease and cancer of the prostate or gastrointestinal tract [2, 3]. It is also thought that a low level of plasma lycopene contributes to chronic renal failure [4]. Other outstanding features are that, in contrast to other carotenoids, the serum values of lycopene are not usually reduced by smoking or alcohol consumption, but by increasing age [5].

Lycopene is the pigment responsible for the red color of ripe tomatoes, watermelon and other fruits. It has unique structural and chemical features [2]. It is an isomer of the carotenes but no provitamin A. Lycopene accounts for about 50% of carotenoids in human blood (approximately 0.5 μmol/l plasma), its tissue levels vary from 1 nmol/g wet weight in adipose tissue to up to 20 nmol/g wet weight in adrenal and testes [6]. It was found that a decrease in the plasma concentration of lycopene compromises the antioxidant status and increases oxidative stress [7]. Lycopene reacts with peroxynitrite, a powerful oxidizing and nitrating agent generated *in vivo* by the combination of $NO^{\bullet}$ and superoxide radical anion $O_2^{\bullet -}$ [8].

The experiments presented here reveal the effect of lycopene supplementation in rats on the radiation induced changes, indicated as the amount of thiobarbituric acid-reactive substances (TBARS) in mitochondria, lysosomes and microsomes of the mammalian liver. In parallel, the activities of glutamate dehydrogenase, a marker enzyme for mitochondria, β-glucuronidase for lysosomes and glucose-6-phosphatase for microsomes [9] were determined in the cytosol. An attempt to correlate these findings with the role of lycopene in the recovery of the integrity of biological membranes is discussed.

2. Investigations, results and discussion

Lipid peroxidation of biological membranes is an important field of investigation, since the cellular elements perform a decisive role in the functional organization of mammalian cells. Plasma membranes define the periphery of cells separating its contents from surroundings and are a barrier to the free passage of inorganic ions and most other charged or polar compounds. Moreover, cell cytoplasm is also permeated by membrane-bound bodies that play central roles in cell metabolism. Mitochondria, with a double membrane, are the principal producers of ATP. Lysosomes are spherical vesicles with a single membrane contain hydrolytic enzymes which, if not confined in lysosomal membranes, would be harmful to the organism. The endoplasmic reticulum, a system of internal membranes rich in ribosomes, which itself may be continuous with the external membrane. Microsomes, particles derived from the endoplasmic reticulum, consist mainly of ribonucleic acids (RNA). The peroxidation of biological membranes lipids by Reactive Oxygen Species (ROS) results in structural changes of membranes and release of their contents.

The results presented here show that exposure of rats to radiation results in a significant decrease in the activity of glutamate dehydrogenase in the mitochondria, β-glucuronidase in the lysosomes and glucose-6-phosphatase in the microsomes (Table 1). This was accompanied by an increase in their activity in the cytosol (Table 1), probably due to the release of these enzymes from their organelles to the cytosol through damaged membranes. When living organisms are exposed to ionizing radiation (γ-rays) oxidizing agents such as $^{\bullet}OH$, $HO_2^{\bullet}$, H_2O_2, are generated in the aqueous medium in the living cells. These reactive oxygen species interact with vital molecules, causing their alteration and destruction [10, 11]. The polyunsaturated fatty acids (PUFA) of cell membrane phospholipids are major targets of the highly reactive $^{\bullet}OH$ attack [12]. Damage of the membranes, of subcellular organelles, results from the peroxidation of the lipid portion of membranes.

Radiation induced lipid peroxidation of biological membranes is demonstrated by the increase in the amount of thiobarbituric acid-reactive substances (TBARS) recorded in the organelles studied (Table 2).

Table 1: Variations in the enzyme activities in the subcellular organelles of different animal groups

Glutamate dehydrogenase activities (U/g protein) in liver mitochondria and cytosol

	Times post irradiation					
Animal groups	Mitochondria			Cytosol		
	10 days	20 days	30 days	10 days	20 days	30 days
Group 1	2240 ± 171	2260 ± 182	2390 ± 191	10.9 ± 10.9	9.5 ± 9.5	9.5 ± 9.5
Group 2	2230 ± 190	2290 ± 188	2370 ± 189	9.5 ± 9.5	10.9 ± 10.9	9.5 ± 9.5
Group 3	1344 ± 107 a	1197 ± 110 a	1381 ± 125 a	38.6 ± 2.7 a	40.4 ± 2.9 a	30.1 ± 1.5 a
Group 4	1680 ± 134 ab	1558 ± 146 ab	1836 ± 126 ab	9.5 ± 9.5 ab	15.2 ± 1.8 ab	14.1 ± 1.6 ab

β-glucuronidase activities U/g protein in liver lysosomes and cytosol

	Times post irradiation					
Animal groups	Mitochondria			Cytosol		
	10 days	20 days	30 days	10 days	20 days	30 days
Group 1	1.50 ± 0.09	1.65 ± 0.98	1.59 ± 0.10	0.19 ± 0.01	0.20 ± 0.01	0.20 ± 0.01
Group 2	1.53 ± 0.11	1.55 ± 0.12	1.62 ± 0.11	0.20 ± 0.01	0.19 ± 0.01	0.20 ± 0.01
Group 3	0.99 ± 0.07 a	0.94 ± 0.06 a	1.11 ± 0.06 a	0.42 ± 0.11 a	0.45 ± 0.11 a	0.34 ± 0.10 a
Group 4	1.25 ± 0.74 ab	1.42 ± 0.86 ab	1.37 ± 0.71 ab	0.21 ± 0.13 ab	0.24 ± 0.02 ab	0.25 ± 0.03 ab

Glucose-6-phosphatase activities (μmol consumed glucose-6phosphate/mg protein/min) in liver microsomes and cytosol

	Times post irradiation					
Animal groups	Mitochondria			Cytosol		
	10 days	20 days	30 days	10 days	20 days	30 days
Group 1	1.88 ± 0.12	1.89 ± 0.11	1.93 ± 0.11	0.16 ± 0.01	0.16 ± 0.03	0.17 ± 0.02
Group 2	1.91 ± 0.12	1.98 ± 0.13	1.89 ± 0.14	0.15 ± 0.01	0.16 ± 0.02	0.17 ± 0.04
Group 3	1.16 ± 0.10 a	1.08 ± 0.10 a	1.37 ± 0.10 a	0.28 ± 0.07 a	0.31 ± 0.06 a	0.30 ± 0.07 a
Group 4	2.01 ± 0.13 ab	1.53 ± 0.11 ab	1.61 ± 0.12 ab	0.18 ± 0.04 ab	0.20 ± 0.03 ab	0.21 ± 0.05 ab

Each value represents the mean of 6 observations ± S. E.
a: Significant difference when comparing with the corresponding value of control rats
b: Significant difference when comparing with the corresponding value of irradiated rats
Group 1: Control rats, Group 2: Lycopene treated rats, Group 3: Irradiated rats, Group 4: Lycopene treated rats prior to irradiation

In rats receiving lycopene before irradiation, an increase was recorded in the activity of the enzymes in the subcellular fractions (Table 1). This could be attributed to the role of lycopene in controlling lipid peroxidation of biological membrane, as demonstrated by the reduced amount of thiobarbituric acid-reactive substances (TBARS) in mitochondria, lysosomes and microsomes (Table 2).

From the results obtained, it could be postulated that the protective role of lycopene relies on the series of conjugated double bonds. The additional energy produced by exposure to radiation can be transferred to lycopene. Consequently, lycopene would prevent the alteration and destruction of vital molecules.

Lycopene prevents the propagation of lipid peroxidation and terminates this process [2, 13]. Dietary supplementation of lycopene resulted in a significant increase in serum lycopene levels and diminished the amounts of thiobarbituric acid-reactive substances (TBARS) [14]. It was reported that intake of lycopene from tomatoes to 6 male (60 mg/day) for a period of 3 months resulted in a significant decrease in their plasma cholesterol concentration [15].

Lycopene is a strong reducing agent [2, 16], and it is remarkable that it has an important role in terminating peroxidative reactions. Because of its high singlet oxygen quenching capacity [5, 17] and its interaction with peroxyl radicals [6, 18], lycopene protects the biological membranes lipids against oxidation [13] and consequently inhibits the accumulation of thiobarbituric acid-reactive substances (TBARS) [19].

It is concluded that by suppressing radiation induced lipid peroxidation, lycopene retains the integrity of biological membranes. As a consequence of the role of lycopene in minimizing biological membrane damage, its role in regulation of enzyme activity is expected by preventing enzyme releases from their corresponding organelles.

3. Experimental

Male Swiss albino rats, (100–120 g), obtained from the animal farm of the Egyptian Organization for Vaccine and Biological Products at Helwan, Cairo, Egypt, were used. The animals were maintained under standard conditions of ventilation, temperature and humidity. Food as standard pellets, containing all the nutritive elements, and liberal water intake were available. Animals were divided into 4 equal groups of 18 animals each:

Group 1: Control rats not supplemented with lycopene and not irradiated.

Group 2: Lycopene treated rats supplemented with lycopene in a concentration of 5mg/kg body weight/day for a period of 7 consecutive days.

Table 2: Thiobarbituric acid-reactive substances (TBARS) concentrations (nmol/mg protein) in different liver subcellular fractions of different animal groups

Mitochondrial thiobarbituric acid-reactive substances (TBARS)

Animal groups	Times post irradiation		
	10 days	20 days	30 days
Group 1	19.45 ± 3.50	19.86 ± 3.66	20.21 ± 3.89
Group 2	19.01 ± 4.21	18.35 ± 3.78	19.14 ± 4.11
Group 3	41.08 ± 7.88 a	46.36 ± 8.51 a	38.44 ± 8.14 a
Group 4	20.22 ± 4.33 b	20.36 ± 4.82 b	23.11 ± 4.99 b

Lysosomal thiobarbituric acid-reactive substances (TBARS)

Animal groups	Times post irradiation		
	10 days	20 days	30 days
Group 1	85.16 ± 14.47	83.61 ± 13.89	87.17 ± 13.81
Group 2	81.36 ± 16.12	80.69 ± 15.12	83.11 ± 14.83
Group 3	116.9 ± 18.70 a	121.4 ± 20.01 a	128.81 ± 19.89 a
Group 4	88.24 ± 14.41 b	90.21 ± 14.06 b	89.46 ± 14.11 b

Microsomal thiobarbituric acid-reactive substances (TBARS)

Animal groups	Times post irradiation		
	10 days	20 days	30 days
Group 1	31.11 ± 4.91	29.38 ± 4.73	33.18 ± 4.88
Group 2	31.02 ± 4.79	32.08 ± 4.81	32.33 ± 4.69
Group 3	42.94 ± 6.81 a	40.68 ± 6.54 a	45.69 ± 6.87 a
Group 4	33.14 ± 4.89 b	31.13 ± 4.69 b	34.53 ± 4.37 b

Each value represents the mean of 6 observations ± S. E.
a: Significant difference when comparing with the corresponding value of control rats
b: Significant difference when comparing with the corresponding value of irradiated rats
Group 1: Control rats, Group 2: Lycopene treated rats, Group 3: Irradiated rats, Group 4: Lycopene treated rats prior to irradiation

Group 3: Irradiated rats received a whole body exposure of 7 Gy at a dose rate of 0.667 Gy/min.
Group 4: Rats supplemented with lycopene prior to irradiation as in group 3 were then exposed to 7 Gy whole body radiation.
Irradiation was carried out using a ^{137}Cs source (Gamma cell-40) at the National Center for Radiation Research and Technology (NCRRT, Cairo, Egypt).
Lycopene, purchased from Sigma, was dissolved in pure sesame oil and supplemented by gavage using an oral stomach tube.
Six rats were sacrificed from each group on days 10, 20 and 30 after irradiation. A 20% liver homogenate was prepared in 0.25 M sucrose. Subcellular fractions of liver homogenate were obtained by differential centrifugation: mitochondria: 10 min at 8000 × g; lysosomes: 10 min at 15,000 × g; microsomes and cytosol: 60 min at 100,000 × g [9].
Glutamate dehydrogenase was determined according to Plummer [9], β-glucuronidase according to Stahl and Touster [20], glucose-6-phosphatase activity according to Swanson [21]. Total protein contents of each subcellular fraction were determined according to the Folin Lowry methods of protein assay [22].
The polyunsaturated fatty acids of cellular membrane are peroxidized to malondialdehyde (MDA). To determine the extent of lipid peroxidation, the content of malondialdehyde (MDA) was estimated as thiobarbituric acid-reactive substances (TBARS). A 10% liver homogenate was prepared in 0.15 M KCl and subcellular fractionation performed as described by Plummer [9]; the level of thiobarbituric acid-reactive substances (TBARS) for each subcellular fraction was measured as described by Yoshioka et al. [23].
Student's t-test was applied for the statistical analysis of results (Kish) [24].

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