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The role of ubiquinones in the regulation of lipid metabolism in rat thymocytes

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The effect of ubiquinones Q-1, Q-2, Q-8 and Q-9 on lipid metabolism in rat thymocytes in vitro was studied. The cells were incubated in a medium containing ubiquinones within the concentration range from 1 to 100 μM. A 2-fold decreased cholesterol synthesis was observed in thymocytes incubated with ubiquinone Q-9 at a concentration of exogenous ubiquinone of no less than 40 μM. Incubation of thymocytes with ubiquinones UQ-1 and UQ-2 that are characteristic of rats (40 μM and 100 μM) resulted in a decrease of cholesterol synthesis. Ubiquinone-8 had a tendency to inhibit the cholesterogenesis in rat thymocytes.

Lipid metabolism; Ubiquinone; Cholesterol; Thymocyte

1. INTRODUCTION

It is well known that various pathological states such as radiation disease, carcinogenesis and others, are accompanied by strong disturbances in energy processes in cells. The disturbances manifest themselves as a change in the electron transport rate along the mitochondrial chains, activation of free radical formation and stimulation in lipid peroxidation in membranes.

Ubiquinones, like other nontoxic inhibitors of oxidative processes (both naturally occurring and synthetic antioxidants), have long been used to inhibit such kinds of pathology [1–3]. Ramasarma and co-workers [4–6] revealed the regulatory role of ubiquinones in squalene and cholesterol synthesis in rat liver. We showed that coenzyme Q-9 produced a similar effect on phospholipid metabolism in rat liver, spleen and intestine mucosa. In particular, it was found that ubiquinone-induced accumulation or depletion of some phospholipids in rat organs is mainly determined by a change in the rate of phospholipid degradation [7]. It was also shown that the fatty acid composition of some bacteria depends on the concentration of ubiquinones [8]. Ubiquinone-9, acting as a regulator of the metabolism of some isoprenoid compounds, changes the rate of cholesterol and phospholipid synthesis in rat liver, thus normalizing the lipid composition of microsomal membranes and lipid metabolism in irradiated animals [9]. Besides, protective and therapeutic effects of ubiquinones were revealed in animals exposed to ionizing radiation [10].

The concentration of ubiquinones is one of the factors regulating the protein content in the tissues of animals in their life-span [11]. Moreover, it was found that the state of the immune system depends on the level of ubiquinones in the animal tissues [12]. There is reason to believe that ubiquinones are activators of the immune processes. Thus, studies of the regulatory role of ubiquinones in lipid metabolism in thymocytes, which are directly involved in the immunological reactions, seem promising.

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2. MATERIALS AND METHODS

Male Wistar rats weighing 150–180 g were used. Thymocytes were obtained by pressing thymus tissue through a nylon grid (200 μM). Pooled thymocytes of five rats were used in each experiment. The isolated cells were suspended in a medium containing 140 mM NaCl, 5 mM KCl, 0.5 mM $MgSO_4$, 1.2 mM $CaCl_2$, 10 mM glucose, 1 mM Na_2HPO_4, 2.5 mM $NaHCO_3$, 10 mM Hepes-NaOH (pH 7.4). An incubation medium of 5 ml contained about 5×10^7 cells per 1 ml and 20 μCi/ml of [2-^{14}C]sodium acetate. Ubiquinones were added as a water-alcohol emulsion (ubiquinones Q-8 and Q-9) or water-alcohol solution (ubiquinones Q-1 and Q-2). In all cases, the final concentration of ethanol did not exceed 1%. The cells were incubated for 120 min at 37°C with periodic shaking. Cell suspensions were routinely examined microscopically for trypan blue exclusion. A small portion of cells (0.1 ml of the incubation mixture) was washed 3 times with 20 vols of buffer mixture. The cells were treated with 1% SDS. Radioactivity was determined in a liquid scintillation counter SL-7.

To measure the rate of total RNA synthesis, 1-ml aliquots of cell suspension were incubated for 20, 60 and 120 min at 37°C with 20 μCi/ml of [6-^{3}H]uracil. The reaction was stopped by adding 0.3 ml of 40% TCA solution. The cells were centrifuged, washed 3 times with 15 vols of buffer mixture, mixed with SDS and left overnight. Radioactivity was measured with a liquid scintillation counter SL-20. The protein content was determined by the Lowry method. Total lipid extracts were prepared according to the method of Folch et al. [13]. The lipid extracts were resolved into fractions by thin-layer chromatography on silica gel L 5/40 μ using hexane/diethyl ether/acetic acid (73:25:2, v/v) as the developing solvent. The content of free cholesterol and cholesterol esters was determined in aliquots by the Liebermann-Burchard reaction [14]. Lipids were dried in a rotor evaporator; toluene, PPO and POPOP were added and radioactivity was determined in a liquid scintillation counter SL-20.

3. RESULTS

Thymocytes from male Wistar rats were incubated for 2 h with and without alcohol at a concentration of no more than 1% and ubiquinones were added as a water-alcohol emulsion or water-alcohol solution, and then stained with trypan blue. The amount of the stained cells did not exceed 5–7%. This means that all measurements were performed with living cells. It has been recently shown that a 4-h incubation of rat thymocytes at 37°C does not induce cell death [15]. Another criterion of cell viability was the kinetics of the total RNA synthesis measured in thymocytes incubated with [6-^{3}H]uracil for 2 h (fig.1). It was shown that the incorporation of the labelled uracil into thymocyte RNA in vitro was

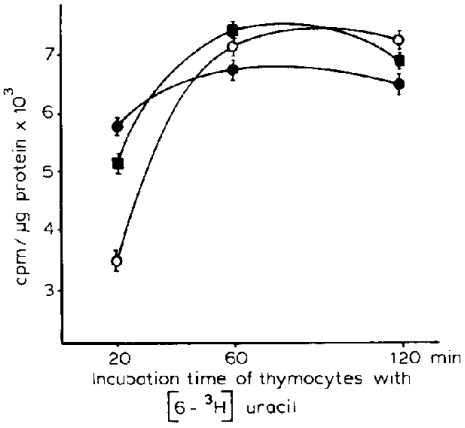

Fig.1. Incorporation of [6-^{3}H]uracil into thymocyte RNA in vitro. (○) Control cells; (■) thymocytes incubated in the presence of 100 μM ubiquinone Q-9; (●) thymocytes incubated with 100 μM ubiquinone Q-1. Each value represents the mean ± SD of four independent determinations. The error bars represent ± SD. Similar results were obtained when incubating thymocytes with ubiquinones Q-2 and Q-8.

the same both for control thymocytes and cells incubated with ubiquinones for 2 h.

The addition of ubiquinones and alcohol to the

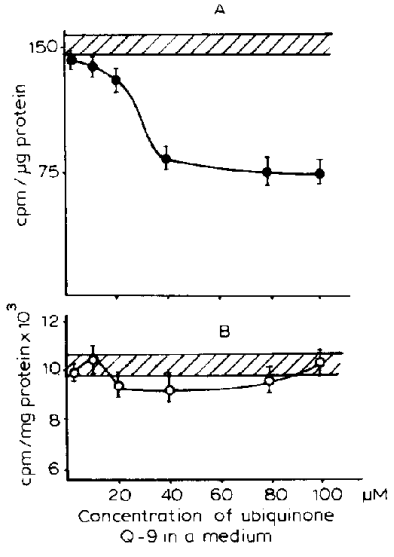

Fig.2. Incorporation of [2-^{14}C]acetate into cholesterol (A) and total lipid fraction (B) of thymocytes incubated with ubiquinone Q-9 for 120 min. Each value represents the mean ± SD of four or five independent determinations. The error bars represent ± SD. Shaded zones, control (without ubiquinones added).

Table 1

Incorporation of [2-^{14}C]acetate into lipid fractions of rat thymocytes incubated in the presence of ubiquinones Q-1, Q-2 and Q-8

Ubiquinone	Number of experiments	Incorporation of [2-^{14}C]acetate into cholesterol		Incorporation of [2-^{14}C]acetate into the total lipid fraction[b]
Control	8	157 ± 12		10200 ± 650
40 μM Q-1	4	69 ± 9.1	$p < 0.001$	10700 ± 820
100 μM Q-1	4	75 ± 13	$p < 0.001$	11127 ± 580
40 μM Q-2	5	64.7 ± 8.5	$p < 0.001$	10715 ± 790
100 μM Q-2	4	62.8 ± 7.9	$p < 0.001$	11050 ± 575
40 μM Q-8	4	126 ± 15.1	n.s.	10620 ± 620
100 μM Q-8	4	114 ± 18.2	$p < 0.05$	9980 ± 680

[a] Values are expressed as cpm·μg^{-1} cholesterol
[b] Values are expressed as cpm·μg^{-1} protein

Each value represents the mean ± SD. Statistical analysis was by paired *t*-tests between control cells and ubiquinones loading thymocytes; n.s., insignificant

incubation medium did not affect the permeability of thymocytes for the labelled precursor, and the pool of [2-^{14}C]acetate inside the cells was unchanged. Marked inhibition of cholesterol synthesis in thymocytes was observed when the level of the exogenous ubiquinone-9 was no less than 4×10^{-5} M (fig.2). The incorporation of [2-^{14}C]sodium acetate into the total lipid fraction of thymocytes after loading the mixture with UQ-9 at various concentrations did not markedly differ from the control. Recently, we have observed a similar effect for rat hepatocytes [16]. Therefore, it can be suggested that the inhibitory effect of ubiquinones is exhibited only with regard to steroids and is not extended to fatty acid metabolism.

It was interesting to study the effect of ubiquinones that are not characteristic of rats with both short (UQ-1 and UQ-2) and long (UQ-8) side chains on cholesterol metabolism in thymocytes. Since the inhibitory effect of UQ-9 was exhibited only when its concentration in the medium was no less than 40 μM, we used UQ-1, UQ-2 and UQ-8 at concentrations of 40 and 100 μM. The incubation of thymocytes with UQ-1 and UQ-2 resulted in a considerable (about 2.5-fold) inhibition of cholesterol synthesis (see table 1), while the effect of coenzyme Q-8 was not so pronounced. Probably, this can be explained by low permeability of thymocytes for ubiquinone-8.

4. DISCUSSION

Thus, a correlation has been observed between the rate of cholesterol synthesis in rat thymocytes and concentration of ubiquinones in the medium. It is noteworthy that ubiquinones which are not inherent in rats even in tracer amounts, such as UQ-1 and UQ-2, exhibit an inhibitory effect of cholesterol synthesis in thymocytes. The minimal concentration of UQ-9 which decreases the rate of cholesterol synthesis (4×10^{-5} M) is considerably lower than the physiological concentration of this ubiquinone in heart (3×10^{-4} M [17]), kidneys and liver (1.5×10^{-4} M [17,18]) of rats. In other tissues of mammals (muscle, spleen, lungs, adrenal gland, pancreas), the concentration of coenzyme Q-9 is lower and varies from 4×10^{-5} to 2×10^{-4} M [18,19]. At present, the level of ubiquinone-9 in thymus of animals is unknown. Due to the ability of ubiquinones to inhibit cholesterol synthesis, they can be used for treating diseases accompanied by a sharp increase in the rate of cholesterol synthesis. Recently, we have shown the activation of cholesterol synthesis in thymocytes of rats exposed to ionizing radiation [20]. The protective effect of ubiquinones may well be explained by their ability to inhibit cholesterogenesis.

Apparently, the effect of ubiquinones on cells is not only confined to modifying the lipid-synthesizing activity. Ubiquinones, by virtue of their

antioxidant properties, can directly affect the structure of membranes. A relationship between the susceptibility of biomembranes for the process of radiation-induced lipid peroxidation and the concentration of antioxidants was shown in [21]. The effect of ubiquinones on membrane structure is still poorly understood. It is assumed that ubiquinones, contained in the Golgi apparatus at high concentrations, function as a barrier for the transfer of free radicals [22]. Thus, studies of ubiquinones which are likely to play an important role in the functioning of biological membranes seem to be promising.

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