Differential Effects of Ubiquinone Q\textsubscript{7} and Ubiquinone Analogs on Macrophage Activation and Experimental Infections in Granulocytopenic Mice

Summary: Several ubiquinone derivatives differing from each other only in the number of isoprenic moieties are known to enhance phagocytosis of mouse macrophages and to protect mice from experimental bacterial infections. Two synthetic ubiquinone analogs were compared to ubiquinone Q\textsubscript{7} with respect to several biological parameters. It was found that the two analogs were equivalent to ubiquinone Q\textsubscript{7} in their ability to enhance the clearance of carbon particles in mice. Unlike ubiquinone Q\textsubscript{7}, however, they did not prolong the survival of granulocytopenic mice with experimental bacterial infections. A possible explanation for this discrepancy lies in the fact that ubiquinone Q\textsubscript{7} causes the secretion of colony stimulating activity in vitro and in vivo while the two synthetic ubiquinone analogs do not have such effects. These findings are discussed in the context of present concepts of macrophage activation and unspecific immunity.

Zusammenfassung: Unterschiedliche Einflüsse von Ubichinon Q\textsubscript{7} und Ubichinon Analoga auf die Makrophagen-Aktivierung und experimentelle Infektionen bei granulozytopenischen Mäusen. Eine Reihe von Ubichinon-Derivaten, die sich voneinander nur in der Anzahl ihrer Isopren-Reste unterscheiden, stimuliert die Phagozytose von Makrophagen und schützt Mäuse vor experimentellen bakteriellen Infektionen. Zwei synthetische Analoga des Ubichinons wurden in der vorliegenden Studie mit Ubichinon Q\textsubscript{7} verglichen. Dabei wurden verschiedene biologische Parameter untersucht. Es fand sich, daß die beiden analogen Verbindungen in ihrer Fähigkeit, die Elimination von Kohlepartikeln aus dem Blutkreislauf von Mäusen zu stimulieren, dem Ubichinon Q\textsubscript{7} äquivalent sind. Im Gegensatz zu Ubichinon Q\textsubscript{7} verursachten diese Substanzen jedoch keine Verlängerung der Überlebenszeit granulozytopenischer Mäuse mit experimentellen bakteriellen Infektionen. Eine mögliche Erklärung für diese Diskrepanz liegt in der Tatsache, daß Ubichinon Q\textsubscript{7} in vitro und in vivo die Sekretion von koloniestimulierendem Faktor (CSF) bewirkt, während die beiden synthetischen Ubichinon-Analoga keine derartigen Wirkungen zeigen. Die vorgelegten Befunde werden im Zusammenhang mit aktuellen Konzepten der Makrophagen-Aktivierung und der unspezifischen Immunität diskutiert.

Introduction

Ubiquinone Q\textsubscript{8} as well as other ubiquinones differing among each other only in the number of isoprenic residues have recently been shown to enhance phagocytosis in mouse macrophages and to exert protective effects in experimentally infected mice (1). In view of these findings, it appeared warranted to chemically modify the structure of naturally occurring ubiquinones in order to arrive at compounds with even higher efficacy and – if possible – other favourable properties like water solubility. For reasons of availability and efficacy, ubiquinone Q\textsubscript{7} was chosen as the prototype for this series of experiments. In this paper we report on the biological effects of ubiquinone Q\textsubscript{7} and two of its analogs which carry naphthoquinone systems instead of a methoxybenzoquinone ring. The analogy between ubiquinone Q\textsubscript{7} and the two synthetic compounds (2, 3) lies mainly in the fact that these two ring systems have similar steric requirements. All these compounds were shown to stimulate the phagocytosis of carbon particles and sheep red blood cells by mouse macrophages. However, while ubiquinone Q\textsubscript{7} exerted clear-cut protection against the lethal effects of a number of bacterial model infections in mice, both of the analogs were inactive in this respect. A tentative explanation for this discrepancy may lie in the fact that in granulocytopenic mice only ubiquinone Q\textsubscript{7} induces the secretion of colony-stimulating activity while the two semisynthetic ubiquinone analogs did not.

Materials and Methods

Animals

Mice of the hybrid strain B6D2F\textsubscript{1} weighing 20–22 g were used. The animals, obtained from a commercial breeder, were kept at a temperature of 20–22° C in the Sandoz Forschungsinstitut (SFI) animal care division. The mice were housed in plastic cages, and fed standardized mouse pellets and water ad libitum.

Immune Modulating Drugs

Ubiquinone Q\textsubscript{8} was obtained from Takeda Chemical Industries Ltd., Osaka, Japan. PSK, a protein bound polysaccharide from Coriolus versicolor was obtained from Kureha Co. Ltd., Tokyo, Japan. An extract of the mycelia of the fungus Flammulina velutipes was prepared by extraction with NaOH and precipitation with (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4}. Afterwards dialysis and lyophilization was performed. The brownish powders (PSK, Flammulina velutipes) were dissolved in saline and injected intraperitoneally at a dose of 120 mg/kg.

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Preparation of Ubiquinone Analogs

3-Methyl-2-(3,7,11-trimethyl)-2,6,10-dodecatrieny1,4-naphthoquinone (85,725) (2). 5 g of naphthohydroquinone and 0.8 ml of boron trifluoride ether were dissolved in 30 ml of absolute Dioxan. To this solution 2.2 g Farnesol dissolved in 30 ml Dioxan were slowly added. After 24 h the reaction mixture was partitioned between 5% aqueous sodium bicarbonate and diethyl ether. The organic phase was then dried with sodium sulfate. The residue resulting from this procedure was taken up in petrol ether, separated from unsoluble particles and applied to a Silicagel column which was subsequently eluted with diethyl ether/hexane 3:1. 1 g of the a. m. compound (30% of the theoretical yield) could be isolated in this way. NMR spectrum (CDCl₃) (δ, ppm) 7.6 – 8.2, M, 4H aromatic 4.9 – 5.2, M, 3H olefinic 3.4, J=6.5 Hz, D, 2H methylene 2.2, s, 3H CH₃-quinone 1.85–2.15, M, 8H methylene 1.5 – 1.75 12H, 4-methyl.

2-Methyl-3-(3,7,11-trimethyl-2,6-10-dodecatrieny1)1,4 naphthoquinone 1-monoacetate (85,730) (3) was prepared in an analog fashion from Monoacetylnaphthohydroquinone and Farnesol. NMR spectrum (CDCl₃) (δ, ppm) 8.0 – 8.2, M, 1H aromatic 7.35– 7.7, M, 3H aromatic 5.8 br 1H OH 5.0 – 5.3, M, 3H H olefinic 3.45 D (J=6.5 Hz) 2H methylene 2.48 S 3H Ac 2.56 S 3H methyl 1.9 – 2.2, M, 6H methylene 1.6 – 1.85, M, 12H 4 methyl

The structures of ubiquinone Q₇ and the two ubiquinone analogs are shown in Fig. 1.

Test for Endotoxin Contamination

In order to exclude contamination of our ubiquinone Q₇ preparation with endotoxin, the limulus amebocyte lysate gel test was performed. Ubiquinone Q₇ was suspended in sterile, pyrogen-free water at 5 mg/ml and filtered through 0.45 or 0.22 µm type Millipore nitrocellulose filters. After appropriate dilution, the filtrates were tested for endotoxin activity using the limulus amebocyte lysate reagent kit (Millipore Corp., Freehold, New Jersey).

Microorganisms

Pseudomonas aeruginosa (ATCC 12), Escherichia coli (ATCC 120), Klebsiella pneumoniae (ATCC 476), Staphylococcus aureus (ATCC 47) and Candida albicans (ATCC 124) were obtained from the SFI collection. The bacteria were grown overnight in Trypticase Soy Broth. Candida albicans was grown in Sabouraud Broth. The cultures were aliquoted in 1 ml portions in vials and stored frozen in liquid nitrogen. Before use, cultures were thawed at 37 °C and diluted with broth to the appropriate concentrations. The numbers of colonies formed were counted after plating further dilutions of bacterial inocula in duplicates on agar plates and incubating them for 24 h at 37 °C.

Tests for Immune Parameters

Carbon clearance test for in vivo phagocytosis: The test was based on the work of Biozzi (4) and was performed as described earlier (5). Briefly, a suspension of carbon particles (Indian Ink, Günther Wagner, Hannover, FRG) was injected intravenously. Blood samples were obtained by puncturing the retroorbital plexus at different times. The blood was lysed in distilled water and the amount of carbon determined spectrophotometrically.}

The regression coefficient of the elimination curve was determined by a computer program.

Preparation of peritoneal cell suspensions for erythrophagocytosis. For erythrophagocytosis and for testing the supernatants for colony stimulating activity (CSA) peritoneal cells (PC) were harvested 72 h after the intraperitoneal injection of 50 mg/kg ubiquinone Q₇ or of its analogs. Control mice received saline. The peritoneal cavity was rinsed by injecting 3 ml MEM. After gentle massage the fluid was withdrawn with a Pasteur pipette. Preparation of macrophage culture supernatants for testing of CSA: 2.1 x 10⁷ peritoneal cells were incubated in 30 ml MEM containing 10% FCS in tissue culture flasks (Nunc, Denmark) in the presence of ubiquinone Q₇ or its analogs at a concentration of 100 µg/ml for 24 h. Supernatants were collected by centrifugation at 1,000 g for 10 min and stored frozen at –20 °C for further testing on colony stimulating activity.

Figure 1: Structures of ubiquinone Q₇ and the two ubiquinone analogs 85,725 and 85,730.