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Half-life of ubiquinone-9 in rat tissues

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The half-life of ubiquinone-9 in various rat tissues was determined. Rats were injected intraperitoneally with [^{3}H]mevalonate and the decay of radioactivity incorporated into ubiquinone-9 was followed using reverse-phase HPLC. The half-life varied between 49 h (testis) and 125 h (kidney).

Ubiquinone, CoQ, Isoprenoid turnover; Mevalonate

1. INTRODUCTION

Ubiquinone has a broad distribution in animal tissues and the amount present on a weight basis in different tissues varies to only a limited extent [1–3]. Upon subfractionation this compound is recovered in all membranes, being particularly enriched in Golgi, lysosomal and inner mitochondrial membranes [4].

The terminal portion of ubiquinone biosynthesis occurs exclusively in Golgi vesicles and it thus appears that this lipid is transported from this location to the various other organelles through the cytoplasm [5]. The major established function of ubiquinone is its participation in the mitochondrial respiratory chain as a redox component, but this function does not explain its presence in most other membranes [6]. Increasing evidence obtained in recent years indicates that ubiquinone in the reduced form plays an important role as a cellular antioxidant [7,8]. Available reports suggest that for quenching free radicals and reactive oxygen species, cells prefer ubiquinol to α-tocopherol [9].

The level of ubiquinone is altered considerably in connection with various physiological and pathophysiological conditions, indicating the importance of this lipid in cellular life [10,11]. Both in humans and in rat the amount of ubiquinone in most tissues increases after birth, while later in life this amount decreases [12]. A high-cholesterol diet lowers the amount of this component in liver, whereas treatment with clofibrate, *N*-nitrosodiethylamine or, in particular, di(2-ethylhexyl)phtalate increases its cellular concentrations [13]. The level of ubiquinone in hyperplastic nodüli is increased, but in tumors only half of the original content of this lipid remains [14]. Human studies have demonstrated the presence of low ubiquinone concentrations in cardiomyopathies and various muscle diseases [15,16].

In vivo and in vitro studies have demonstrated that biosynthesis of ubiquinone occurs in all tissues, but the rates of biosynthesis and breakdown of this lipid in different tissues are not yet known. Uptake of dietary ubiquinone occurs only to a limited extent and, consequently, most of this lipid is provided by de novo synthesis. The high rate of its synthesis is an indirect indication for a well functioning mechanism for breakdown.

The aim of the present study was to investigate the half-life of ubiquinone and examine possible variations in its turnover in various types of tissues. This was achieved by monitoring the decay of radioactivity incorporated into this lipid from a labeled precursor.

2. MATERIALS AND METHODS

Male Sprague–Dawley rats weighing 180 g at the beginning of the experiment were used in all cases. These animals were injected intraperitoneally with 1 mCi RS-5-[^{3}H]mevalonolactone (30 Ci/mmol) dissolved in 0.9% NaCl. Synthesis of labeled mevalonolactone was performed as described earlier [17].

Tissues were homogenized using a Turrax ultra blender. The homogenates were supplemented with 4 μg ubiquinone-6 (Sigma) as an internal standard and thereafter extracted twice with chloroform:methanol, 2:1, without alkaline hydrolysis. After washing this extract with the theoretical upper phase (chloroform:methanol:water, 3.48:47) the chloroform fraction was placed onto a silica column to remove charged lipids. The chloroform effluent was collected and the solvent evaporated. The residue was then dissolved in 30 μl *n*-hexane and analyzed by HPLC using a Hewlett Packard hypersil ODS (C_{18}) 3 μm reverse-phase column. A linear gradient was used from the initial 2-propanol:methanol:hexane, 40.60:5, in pump system A to hexane.2-propanol, 70.30, in pump system B, with a flow rate of 1.5 ml/min and a program time of 35 min. The absorption of the eluate was monitored at 275 nm and radioactivity was measured using a radio flow detector (Radiomatic, Tampa, FL).

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3. RESULTS AND DISCUSSION

Effective labeling of isoprenoid lipids is difficult to achieve using initial precursors, such as acetate, because of the many metabolic pathways which utilize these compounds. [^{3}H]Mevalonate is employed primarily for studying the biosynthesis of cholesterol, but, in addition, labeled mevalonate is also incorporated into isoprenoids covalently bound to proteins, into dolichols and into ubiquinone [18].

It is quite important in the present type of study to attain complete extraction and effective purification of ubiquinone by HPLC. Quantitative extraction is possible even without alkaline hydrolysis and ubiquinone may subsequently be isolated by reverse-phase HPLC. The major form of this lipid in rat tissues was found to contain 9 isoprene residues, but in certain tissues small amounts of ubiquinone-10 were also present (Fig. 1).

In this study the labeling behavior of ubiquinone-9 was monitored. The half-lives of ubiquinone-9 isolated from various tissues were investigated by following the decay in radioactive labeling after intraperitoneal injection of [^{3}H]mevalonate. Fig. 2 illustrates the pattern obtained with heart tissue, as an example. The initial time-point was chosen as 23 h after the intraperitoneal injection. After this period free [^{3}H]mevalonate could no longer be detected in the cytoplasm of various tissues (not shown). Using a semilogarithmic plot, the decay in radioactivity was followed for 160 h; during this period the decay was found to be linear.

The amount of ubiquinone-9 in tissue varies between 20 and 170 μg/g wet weight, the lowest values being found for testis, spleen and thymus and the highest for heart, liver and kidney (Table I). The half-life of this lipid displays quite limited variation among the tissues measured. In heart, muscle, testis, thyroid, intestine, colon and spleen the $t_{1/2}$ values were between 50 and 60 h, while in stomach, liver and pancreas the corresponding values were between 70 and 90 h. The half-lives in thymus and kidney were somewhat longer i.e. 104 and 125 h, respectively.

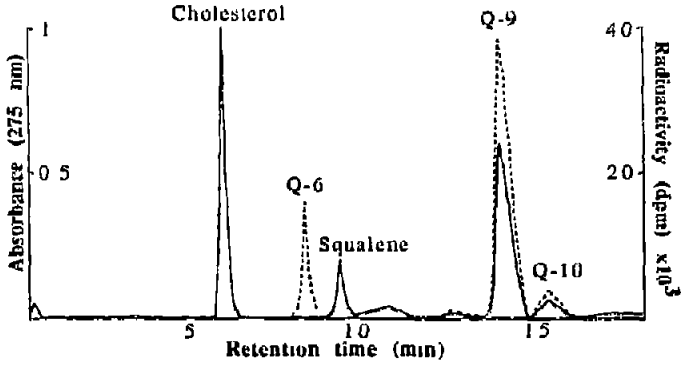

Fig. 1. Separation of lipids extracted from rat heart by HPLC Cholesterol and squalene were identified using standards which comigrated with the radioactive peaks. Dashed line, absorbance at 275 nm; solid line, radioactivity in dpm Q-6, ubiquinone-6 (internal standard); Q-9 and -10, ubiquinone-9 and -10.

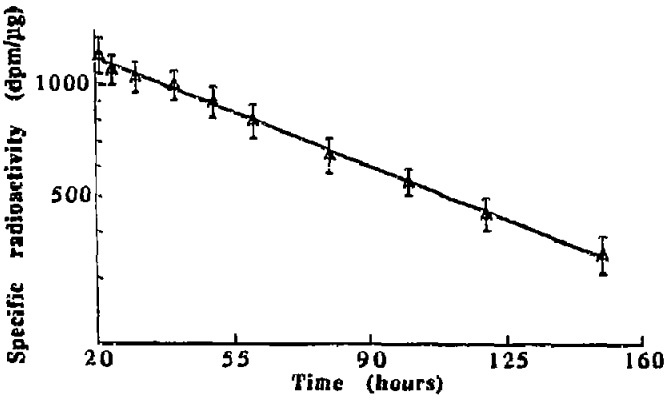

Fig. 2 Decay of specific radioactivity in heart ubiquinone-9. Ubiquinone-9 was isolated from heart homogenate at various time-points after intraperitoneal injection of [^{3}H]mevalonate, and its level of radioactive labeling determined. The values are the means of six experiments ± S.D.

The half-life values obtained in these experiments were most probably not affected by re-utilization of the radioactive label. In contrast to amino acids the mevalonate molecule is modified on its way to appearing in isoprenoid lipids. There are no data available concerning the route of breakdown of isoprenoid lipids in animal tissues. However, even if acetate appears as a final end-product of this catabolic pathway, utilization of this labeled precursor for synthesis of ubiquinone is probably insignificant. Nor can the relatively long half-life of ubiquinone in kidney be explained by accumulation of radioactive precursor in this organ, since the mevalonate pool in kidney is small [19].

The half-lives of phospholipids are around 100 h, of cholesterol 150 h and of dolichol 65–140 h in rat liver [20,21]. Thus, ubiquinone demonstrates a similar range

Table I

Amounts and half-life of ubiquinone-9 in various rat tissues

Tissue	Amount (μg/g wet weight)	Half-life (h)
Liver	171 ± 11.3	79
Heart	162 ± 10.2	59
Kidney	133 ± 7.9	125
Stomach	63.4 ± 4.7	72
Thyroid	52.6 ± 6.1	49
Colon	46.5 ± 3.1	54
Muscle	46.2 ± 3.8	50
Intestine	40.1 ± 1.9	54
Pancreas	32.0 ± 2.2	94
Testis	23.6 ± 1.4	50
Thymus	21.7 ± 1.8	104
Spleen	20.3 ± 2.0	64

1 mCi [^{3}H]mevalonate was injected intraperitoneally into the rats and after various time periods tissues were extracted and the specific labeling of ubiquinone-9 determined by HPLC. The values are the means ± S.D of six experiments

or even somewhat shorter half-life. The rate of turnover of ubiquinone in individual tissues is of considerable interest, because their supply is provided mainly or exclusively by endogenous metabolism and is not influenced by dietary or circulatory levels of ubiquinone, in contrast to cholesterol.

The small amount of ubiquinone present in blood is not involved in the redistribution, but probably has a specific function, such as preventing oxidation of LDL cholesterol [9]. This antioxidant function of ubiquinone makes it particularly important to determine both the amounts and the turnover of this lipid in various tissues. Another important factor in this context is the capacity of each tissue to maintain ubiquinone in the reduced form necessary for its antioxidant action.

The amounts of ubiquinone in intracellular membranes varies, and both organellar and cellular levels vary widely in connection with different physiological and pathophysiological conditions. Because of its significance for cellular function it will be of importance in the future to study the regulation of the biosynthesis and breakdown of ubiquinone at different locations.

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