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Factors influencing the tissue distribution of coenzyme Q_{10} intravenously administered in an emulsion to rats: emulsifying agents and lipoprotein lipase activity

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The tissue distribution of coenzyme Q_{10} (CoQ_{10}) administered intravenously in an emulsion prepared with egg yolk phosphatidylcholine (PC), egg yolk sphingomyelin (SPM) or a combination of PC and a polyoxyethylene derivative of hydrogenated castor oil (HCO-60) (PC + HCO-60) was investigated. The disappearance from the plasma of CoQ_{10} administered in three different emulsions of lipid particle size less than 0·5 μm varied with the particular emulsifer. Its disappearance occurred most rapidly from the PC emulsion; with the addition of HCO-60, its disappearance was much slower. In the reticuloendothelial system, the concentration of CoQ_{10} was higher in the spleen, for both the SPM and PC + HCO-60 emulsions than for the PC emulsion. HCO-60 reduced the CoQ_{10} distribution in the liver from the PC emulsion. Differences in disappearance rates from the plasma are thus considered to be due to the extent of CoQ_{10} distribution in the liver. CoQ_{10} concentration in the heart, a target organ, was greatest with the PC emulsion. Its distribution was related to lipoprotein lipase (LPL) activity in this organ. The effects caused by HCO-60, however, could not be explained by LPL activity alone. CoQ_{10} distribution in the adrenal gland and kidney can be explained partly by LPL activity but in the presence of HCO-60, the distribution mechanism apparently involves other factors.

We have previously reported on the tissue distribution of the highly lipophilic coenzyme Q_{10} (CoQ_{10}), which is used for treatment of congestive heart failure and angina pectoris, intravenously administered as a soya-bean oil fat emulsion (Yamaguchi et al 1984). The distribution of CoQ_{10} was found to depend on the concentration of egg yolk phospholipid (PL) used as emulsifying agent and to increase, particularly in the heart, a target organ of this drug, when PL was present at 1·2 to 24% (w/v).

A 1·2% PL emulsion used as a vehicle for CoQ_{10} by Yamaguchi et al (1984) had a metabolic fate similar to that of chylomicrons (Robinson & Quarfordt 1979) and was a substrate for lipoprotein lipase (LPL) which has a high activity in the heart (Borensztajn et al 1972; Odonkor & Rogers 1984). The effect of this lipase on the emulsion was considered to be closely related to the distribution of CoQ_{10} from emulsion in the heart. We therefore investigated the effects of emulsifying agents on CoQ_{10} tissue distribution in rats and also how LPL activity in the heart, adrenal gland and kidney is related to CoQ_{10} distribution in these tissues.

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MATERIALS AND METHODS

Materials

Soya bean oil (Wako Pure Chemical Industries, Ltd, Tokyo), a polyethylene derivative of hydrogenated castor oil (HCO-60, a gift from Nikko Chemical Co., Tokyo), egg yolk phosphatidyl choline (PC; 99% purity, provided by QP Co., Tokyo) and egg yolk sphingomyelin (SPM; 99% purity, Sigma Chemical Co., St Louis) were used without further purification. All-*trans* [3′-^{14}C]CoQ_{10} ([^{14}C]CoQ_{10}; 99% purity, 7·95 μCi mg^{-1}) was kindly supplied by Eisai Co., Tokyo. Bovine serum albumin (Fraction V) was obtained from Sigma Chemical Co. All other chemicals and reagents were of analytical grade or better.

Preparation of emulsions

Three emulsions containing [^{14}C]CoQ_{10} were prepared, using PC, SPM or a combination of PC and HCO-60 (PC + HCO-60) as emulsifying agents. In the PC emulsion, 5 mg of [^{14}C]CoQ_{10} was dissolved in 0·5 g of soya bean oil followed by the addition of chloroform solution (0·8 ml) containing 60 mg of PC. The system thus obtained was thoroughly mixed.

The chloroform in the mixture was evaporated under N_2 stream at room temperature (20 °C) and 125 mg of glycerol and 4 ml of purified water was added to the residual mixture. The mixture was emulsified three times, 1 min each, in an ice-cold bath with a sonicator (100W: 5202 PZT, Ohtake Co., Tokyo) and the volume adjusted to 5 ml with purified water. The SPM emulsion was prepared with 60 mg of SPM and emulsification was at 50 °C, while for the PC + HCO-60 emulsion, 60 mg of PC and 35 mg of HCO-60 were used, sonication conditions being the same as for the PC emulsion. The final concentrations of soya bean oil and glycerol in all the emulsions were 10 and 2·5% (w/v), respectively. The phospholipid concentration was 1·2% (w/v) and that of HCO-60, 0·7% (w/v).

Emulsion particle size

Five ml of the PC and PC + HCO-60 emulsions were centrifuged for 30 min at 3000*g* at room temperature; the lower phase was similarly centrifuged again and the concentration of CoQ_{10} in the lower phase was adjusted to 0·2 mg ml^{-1} with 2·5% (w/v) glycerol. The SPM emulsion was centrifuged at 1000*g* for 30 min. Emulsion particle size, as observed by an electron microscope (JEM 100CX, JEOL Ltd, Tokyo), was less than 0·5 μm in all emulsions.

Blood and tissue collection

Wistar male rats, 230–280 g were used. The methods of intravenous emulsion administration and blood and tissue collection were decribed by Yamaguchi et al (1984). All tissues were removed for assay at 6 or 12 h after administration of the emulsion to rats.

Preparation of lipoprotein lipase (LPL)

LPL from the tissues was extracted according to Korn (1955a, b). Heart, adrenal gland and kidney were removed after being perfused with 0·9% ice-cold NaCl through the portal vein. Each tissue was homogenized (Physcotron, Nichion Irikaseisakusho, Tokyo) with twenty times the tissue volume of cold acetone (−20 °C). The homogenate was filtered under reduced pressure, washed with acetone and ether, and dried to a powder at room temperature. After adding 1 ml of 0·025 M ammonia per 50 mg of the powder, the mixture was stirred for 60 min at 0 °C and centrifuged for 30 min at 25 000*g* at 4 °C. The supernatant was adjusted to pH 8·0 with 1 M acetic acid and used as an LPL solution.

Determination of LPL activity

LPL activity was determined by a modification of Korn's method (1955a, b). The following reaction composition was used: 0·05 ml of 0·3 M Tris-HCl buffer (pH 8·5), 0·05 ml of 20% BSA (Tris-HCl buffer solution, pH 8·5), 0·05 ml of a mixture of each emulsion and rat serum (1 : 1 v/v) prewarmed at 37 °C for 30 min, and 0·35 ml of the LPL solution. The solution was mixed, preincubated for 5 min at 37 °C and then incubated for 60 min. Free fatty acids released from the emulsion were determined.

Assay

The concentration of [^{14}C]CoQ_{10} in the plasma and tissues was assayed as described by Yamaguchi et al (1984). The amount of the metabolites of CoQ_{10} is negligibly small in blood until 12 h after oral administration in rats (Fujita et al 1972), but CoQ_{10} in plasma and tissue was measured as CoQ_{10} equivalents since the assay could not differentiate between unchanged CoQ_{10} and its metabolites. The sensitivity limit was 0·005 CoQ_{10} equivalents μg g^{-1} tissue. Free fatty acids released in the assay medium were determined as palmitic acid equivalents by the colorimetric method of Duncombe (1964) and Itaya & Ui (1965) with a slight modification. To 0·5 ml of the sample was added 0·5 ml of the copper reagent (Duncombe 1964; Itaya & Ui 1965) and 2 ml of the mixed solvent, n-heptane–chloroform (30 : 70 w/w). This mixture was shaken vigorously for 20 s and centrifuged for 10 min at 3000 rev min^{-1}. An amount, 91·2 ml, of the supernatant was mixed with 1·2 ml of 0·1% bathocuproine (2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline) as a colouring reagent and the system was allowed to stand for 30 min at room temperature. The absorbance of the mixed solution was read at 480 nm.

RESULTS

Fig. 1 shows the plasma concentration vs time for CoQ_{10} from the three emulsions administered intravenously to rats. The PC emulsion was essentially the same as the PL 1·2% emulsion of Yamaguchi et al (1984), but its particle size of less than 0·5 μm delayed the disappearance of CoQ_{10} from the plasma more than that of the PL 1·2% emulsion. Although there was no significant difference in the disappearance of CoQ_{10} from the plasma between the SPM and the PC emulsions, the SPM preparation tended to be the slower. The addition of HCO-60 to the PC emulsion caused a clear delay in the disappearance of CoQ_{10} from the plasma.

CoQ_{10} concentrations in various rat tissues at 6 and 12 h following intravenous administration are listed in Table 1. Tissue concentrations at 12 h were largely maintained without significant change from

Table 1. Coenzyme Q_{10} (CoQ_{10}) concentrations (CoQ_{10} μg g^{-1} tissue) in various rat tissues at 6 and 12 h following the intravenous administration of 0·6 mg kg^{-1} of [^{14}C]CoQ_{10} in emulsion prepared with either PC[a], PC + HCO-60[b], or SPM[c]. Values at 6 h represent the mean (s.e.) of three to six rats. Values at 12 h represent the mean of two rats; deviation from the mean was less than 20% in all cases.

Dosage forms	Time (h)	Plasma	Brain	Lung	Spleen	Liver	Heart	Adrenal gland	Kidney	Muscle
PC emulsion	6	0·470 (0·059)	0·030 (0·005)	0·498 (0·015)	4·63 (0·55)	10·2 (0·36)	4·65 (0 25)	6·40 (0·82)	0·139 (0·007)	0·067 (0·018)
	12	0·358	0·016	0·649	6·55	10·3	4·83	5·79	0·108	0·042
PC + HCO-60 emulsion	6	5·13[d] (0·44)	0·054[e] (0·004)	0·949[d] (0·133)	23·8[d] (1·3)	6·55[d] (0·87)	0·469[d] (0·042)	10·1[e] (1·1)	0·330[d] (0·032)	0·089 (0·01)
	12	2·67	0·033	1·23	32·7	6·89	0·375	54·3	0·254	0·078
SPM emulsion	6	0·407[f] (0·061)	0·006[e,f] (0·002)	0·329[d] (0·061)	63·4[d] (20·1)	9·29 (2·14)	1·18[d] (0·68)	2·20[e,f] (0·64)	0·073[e,f] (0·019)	0·017[f] (0·007)
	12	0·091	0·009	0·690	165	3·88	0·561	1·18	0·113	0·023

[a] Egg yolk phosphatidyl choline. [b] Polyoxyethylene derivative of hydrogenated castor oil. [c] Egg yolk sphingomyelin. [d] Significantly different from the PC emulsion ($P < 0·01$). [e] Significantly different from the PC emulsion ($0·01 < P < 0·05$). [f] Significantly different from the PC + HCO-60 emulsion ($P < 0·01$).

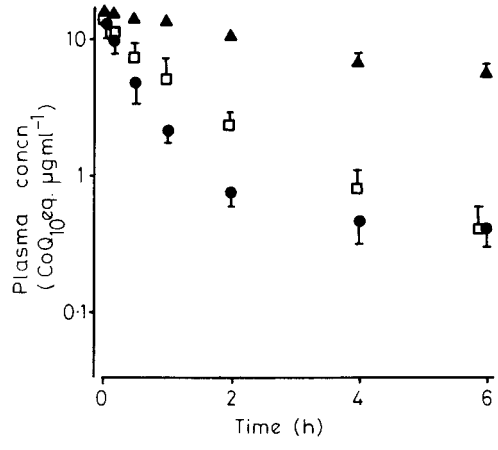

FIG. 1. Plasma concentration time courses in rats of coenzyme Q_{10} (CoQ_{10}) after intravenous administration of 0·6 mg kg^{-1} of [^{14}C]CoQ_{10} in emulsion prepared with egg yolk phosphatidyl choline (PC), PC and a polyoxethylene derivative of hydrogenated castor oil (HCO-60), or egg yolk sphingomyelin (SPM). Each value represents the mean ± s.e. of three to six rats. ● PC emulsion; ▲ PC + HCO-60 emulsion; □ SPM emulsion.

those at 6 h. Regardless of the small particle size, CoQ_{10} distribution to the reticuloendothelial system (RES) varied considerably. In the spleen, the rank order for CoQ_{10} concentration was SPM emulsion > PC + HCO-60 emulsion > PC emulsion. In the liver, the CoQ_{10} concentration was least from the PC + HCO-60 emulsion while that in the lung was small for all emulsions, but greatest for the PC + HCO-60 emulsion. In the heart, a target organ, CoQ_{10} concentration was in the rank order PC emulsion > SPM emulsion > PC + HCO-60 emulsion.

Table 2. Comparison of lipoprotein lipase activity in the heart, adrenal gland and kidney for the PC, PC + HCO-60 and SPM emulsions. Each value, the mean (s.e.) of three to nine experiments, represents the amount of free fatty acid as palmitic acid equivalents (μequiv. ml^{-1}) released in the assay medium.

Dosage forms	Heart	Adrenal gland	Kidney
PC emulsion	15·4 (1·47)	5·20[e] (2·01)	3·09[e] (0·44)
PC + HCO-60 emulsion	7·73[a] (0·088)	8·38 (0 28)	3·22[e,f] (0·71)
SPM emulsion	1·72[a,c] (0·22)	2·92[d] (1·17)	1·82[b] (0·32)

[a] $P < 0·01$ vs the PC emulsion.
[b] $0·01 < P < 0·05$ vs the PC emulsion.
[c] $P < 0·01$ vs the PC + HCO-60 emulsion.
[d] $0·01 < P < 0·05$ vs the PC + HCO-60 emulsion.
[e] $P < 0·01$ vs the heart.
[f] $P < 0·01$ vs the adrenal gland.

The results for LPL activity in the heart, adrenal gland and kidney are shown in Table 2. The adrenal gland and kidney were tissues which, respectively, had a higher and lower CoQ_{10} concentration than the heart (Table 1). LPL from the heart hydrolysed triglyceride in the PC emulsion thereby releasing the largest amount of free fatty acid, while free fatty acid release from the PC + HCO-60 emulsion was significantly greater than that from the SPM emulsion. In contrast to LPL in the heart, LPL from the adrenal gland and kidney tended to release the larger amount of free fatty acid from the PC + HCO-60 emulsion, while free fatty acid release from the SPM emulsion was least in these tissues. For the PC emulsion, the rank order of LPL activity in the

tissues was heart > adrenal gland > kidney; for the PC + HCO-60 emulsion, it was heart = adrenal gland > kidney. No differences in LPL activity for SPM emulsion could be detected in these tissues.

DISCUSSION

The disappearance of CoQ_{10} from the plasma, following its intravenous administration in fat emulsions of particle size less than 0·5 μm and emulsification with three different emulsifiers, is consistent with the results presented by Yamaguchi et al (1984). They found that CoQ_{10} disappeared following its administration in a HCO-60 solution from the plasma, more slowly than when it was given in a PL emulsion (Fig. 1). In Table 1, the importance of the type of emulsifier on tissue distribution of CoQ_{10} in the reticuloendothelial system is evident. The relatively slower rate of disappearance of CoQ_{10} from the plasma following its administration in the PC + HCO-60 emulsion corresponds to the lower concentration of CoQ_{10} in the liver (Fig. 1, Table 1).

The CoQ_{10} concentration in the heart was greatest from the PC emulsion (Table 1). The high LPL activity in the heart (Borensztajn et al 1972; Odonkor & Rogers 1984), in addition to the high solubility of CoQ_{10} in soya bean oil, suggests that the release of CoQ_{10} from soya bean oil particles by LPL is an important factor controlling CoQ_{10} distribution in this tissue. The extent of hydrolysis by heart LPL of each emulsion was therefore examined (Table 2) and found to be dependent on the particular emulsifier used, hydrolysis being greatest with the PC emulsion. In the heart, where the release of free fatty acid from the PC emulsion by LPL was highest, CoQ_{10} distribution from the emulsion was also at its greatest. Similarly, in the adrenal gland and kidney, the higher the LPL activity, the greater was the CoQ_{10} distribution (Tables 1 and 2).

However, there remain some points which cannot be resolved on the basis of the relationship between tissue distribution and LPL activity. The following are examples: (1) in the heart, CoQ_{10} distribution from the PC + HCO-60 emulsion, though not significant, was less than that from the SPM emulsion. However, the ability of heart LPL to hydrolyse the emulsion was significantly greater with the PC + HCO-60 emulsion than with the SPM emulsion; (2) with the PC emulsion, CoQ_{10} concentrations were not significantly different in the heart and adrenal gland, but the heart LPL activity was significantly greater than adrenal gland LPL. For the PC + HCO-60 emulsion, the CoQ_{10} concentration was greater in the adrenal gland than in the heart while their LPL activities did not differ significantly. A fat emulsion and chylomicron undergo similar metabolic fates to form low density lipoprotein (Robinson & Quarfordt 1979). CoQ_{10} in the plasma distributes into low density lipoprotein (Nakamura et al 1982) so the distribution of CoQ_{10} in the adrenal gland may possibly be due to the uptake of lipoprotein containing CoQ_{10} mediated by lipoprotein receptors in the adrenal gland, as reported by Kovanien et al (1979); (3) CoQ_{10} concentration in the kidney was greater for the PC + HCO-60 emulsion than for the PC emulsion, while kidney LPL did not show a significant difference between these emulsions. This is consistent with the data of Yamaguchi et al (1984), which indicate the CoQ_{10} concentration in the kidney to be higher with the HCO-60 than the PL emulsions.

In summary, CoQ_{10} tissue distribution following its intravenous administration in fat emulsions is influenced by the emulsifying agent used. For the PC emulsion, CoQ_{10} distribution in the heart was related to LPL activity. However, the presence of HCO-60 as an emulsifying agent brought about CoQ_{10} distribution which could not be explained by LPL activity alone. For distribution to the adrenal gland and kidney, a mechanism based on LPL activity, with other mechanisms based on the properties of a fat emulsion or emulsifying agent, is possible.

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