Communication

Targeting of Coenzyme Q₁₀ Solubilized with Soy
Lecithin to Heart of Guinea Pigs

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The use of liposomes as carriers for the delivery of therapeutic and diagnostic agents to specific targets within the body has been investigated by many research groups. Mauk *et al.* (1) and Wu *et al.* (2) reported that liposomes carrying aminomannose derivatives showed early retention in the lung after intravenous injection. Takada *et al.* (3) also showed that liposomes coated with amylopectin derivatives were taken up into lung in considerable quantity after intravenous injection. The targeting to brain using liposomes carrying sulfatide has been reported by Naoi and Yagi (4). However, increased heart uptake of administrated agents, *i.e.* targeting to heart by means of liposomes or the other solubilized systems, has been not reported.

This paper deals with the targeting of coenzyme Q₁₀ to heart by means of a soy lecithin microemulsion acting as a solubilized system of coenzyme Q₁₀.

All-*trans*[3'-¹⁴C]coenzyme Q₁₀ was synthesized by Dr. K. Hamamura (Eisai Co., Ltd., Japan). Its radiochemical purity was over 99% as determined by thin layer chromatography (Kiesel gel 60 F-254, Merck) using benzene/chloroform (1:1, v/v) as a developing solvent. The specific radioactivity was 48 μ Ci per mg.

Polyoxyethylene(60)hydrogenated castor oil (HCO-60; Nikko Chem. Co., Japan) solution of [¹⁴C]coenzyme Q₁₀, micellar solution of [¹⁴C]coenzyme Q₁₀, was prepared as follows. Saline was slowly added to the mixture of [¹⁴C]coenzyme Q₁₀ and HCO-60 (1:4, w/w) at 40°C, resulting in a final concentration of HCO-60 of 0.24%. The diameter of individual micelles was estimated to be 10-14 nm by gel filtration using a Sepharose 6B column. Soy lecithin composed of phosphatidylcholine (over 87%), lysophosphatidylcholine (under 3%) and glycolipid (under 3%)

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was purchased from Ajinomoto Co., Ltd. (Japan). A soy lecithin microemulsion of [^{14}C]coenzyme Q_{10} was prepared as follows. [^{14}C]Coenzyme Q_{10} ($216\text{ }\mu\text{Ci}$), soy lecithin (3.9 mg) and D-sorbitol (90 mg) were suspended in distilled water, and then preliminary sonication was carried out using a Branson probe type sonifire operating at 40 W. Propylene glycol (90 mg) was added to the resulting suspension, and sonicated at 40 W to obtain a clear solution. The diameter of the emulsion particles was estimated at 15–50 nm by electron microscopic observation. [^{14}C]Coenzyme Q_{10} solubilized with HCO-60 or soy lecithin-system was administered intravenously into the femoral vein of male guinea pigs (weight 250–350 g) at a dose of 0.6 mg per kg.

Twenty or fifty μl of blood was taken from the ear vein at various intervals. Guinea pigs were killed by decapitation at 30 min and 24 h after the injection. Just before decapitation, the heart was perfused with ice-cold saline, and then the heart, liver and spleen were removed. Blood samples were solubilized with 0.75 ml of Soluene 350 (Packard)/isopropanol (1 : 1 by vol.), and several drops of 30% H_2O_2 were added for decolorization. In the case of the tissues, approx. 100 mg of each tissue was incubated with 0.5 ml of Soluene 350 at 50°C for 2 h. Five ml of scintillation fluid consisting of Instagel (Packard)/0.5 N HCl (9 : 1 by vol.) was added to the solubilized blood or tissue samples, and the radioactivity was measured with an Aloka LSC 900 liquid scintillation counter. In order to perform a whole-body autoradiographic study, [^{14}C]coenzyme Q_{10} solubilized with soy lecithin-system or HCO-60 were also administered intravenously to the guinea pigs at respective doses of 0.6 mg and 1.8 mg per kg. The animals were killed by immersion in solid CO_2 -hexane 10 min and 24 h after dosing. Sections were cut at $20\text{ }\mu\text{m}$ using a PMV Cryo-

Fig. 1. The clearance of radioactivity from blood after intravenous injection of [^{14}C]coenzyme Q_{10} solubilized with soy lecithin (microemulsion) and HCO-60 (micellar solution) into guinea pigs. Each value represents the means for three animals. [^{14}C]Coenzyme Q_{10} dosed at 0.6 mg/kg. ○, soy lecithin system; ●, HCO-60.

microtome and then exposed to X-ray film (Fuji, Type Kx) for 1 month.

For analysis of the redox level of [¹⁴C]coenzyme Q₁₀ administered to guinea pigs, the animals were sacrificed by decapitation at 30 min or 24 h after injection. The liver and heart were quickly removed, rinsed with ice-cold 0.15 M NaCl and then chilled in liquid N₂. The chilled sample was homogenized at -75°C (in dry ice/*n*-hexane) with 4 vol (v/w) of ethanol using a Polytron homogenizer (Hijiri Seiko, Japan). Two ml of homogenate was poured into a test tube containing 5 ml of *n*-hexane, and then 1 ml of H₂O was added. The tube was shaken to extract the quinone and quinol. The subsequent procedure for determination of Q₁₀ and Q₁₀H₂ by high performance liquid chromatography (HPLC) was described in our previous paper (5). Q₁₀ and Q₁₀H₂ separated by HPLC were fractionated, and then radioactivities were determined with an Aloka liquid scintillation spectrometer.

As shown in Fig. 1, the clearance of radioactivity from blood after injection of soy lecithin-[¹⁴C]coenzyme Q₁₀ was markedly faster in the initial phase compared with HCO-60-[¹⁴C]coenzyme Q₁₀. This result indicates that the [¹⁴C]coenzyme Q₁₀ solubilized with the soy lecithin-system was distributed rapidly into tissues.

Table 1 shows the levels of radioactivity in heart, liver and spleen after intravenous injection of [¹⁴C]coenzyme Q₁₀ solubilized with soy lecithin-system or HCO-60. The concentration in tissues 30 min after dosing with soy lecithin-[¹⁴C]coenzyme Q₁₀ was much higher than with HCO-60-[¹⁴C]coenzyme Q₁₀, as suggested in the results of blood concentration. After administration of soy lecithin-[¹⁴C]coenzyme Q₁₀, 3-4% of radioactivity dosed was found in the heart. These values were 165 (30 min) and 51 times (24 h) higher than those after dosing with HCO-60-[¹⁴C]coenzyme Q₁₀.

Figures 2 and 3 show the whole-body autoradiograms 10 min or 24 h after the

Table 1. Tissue distribution of radioactivity after intravenous injection of [¹⁴C]coenzyme Q₁₀ solubilized with soy lecithin and HCO-60 into guinea pigs. [¹⁴C]Coenzyme Q₁₀ dosed at 0.6 mg/kg.

Tissue	Time after injection (h)	Concentration (CoQ ₁₀ eq. µg/g)			
		HCO-60		Soy lecithin	
Heart	0.5	0.03	0.05 (0.02 ^a)	6.3 ± 3.5 ^b	(3.3)
	24	0.14	0.10 (0.07)	10.0 ± 4.0 ^c	(3.6)
Liver	0.5	1.1 ± 0.03 ^b	(6.9)	3.3 ± 0.8 ^b	(25.4)
	24	3.5 ± 0.3 ^b	(25.4)	6.6 ± 0.9 ^c	(31.2)
Spleen	0.5	0.93 ± 0.11 ^b	(0.2)	14.8 ± 3.3 ^b	(3.9)
	24	2.33 ± 0.31 ^b	(0.6)	6.4 ± 0.1 ^c	(1.6)

^a Value is the amount of radioactivity in tissue as % of the dose. ^b Value is the mean ± SE of 3 experiments. ^c Value is the mean ± SE of 6 experiments.

Fig. 2. Whole-body autoradiograms 10 min (a) and 24 h (b) after intravenous injections of $[^{14}\text{C}]$ coenzyme Q_{10} solubilized with soy lecithin into guinea pigs. $[^{14}\text{C}]$ Coenzyme Q_{10} dosed at 0.6 mg/kg.

injection of soy lecithin- and HCO-60- $[^{14}\text{C}]$ coenzyme Q_{10} into guinea pigs. As shown in Fig. 2, the radioactivity was found to be mainly localized in the heart muscle and brown adipose tissue 10 min after injection of soy lecithin- $[^{14}\text{C}]$ coenzyme Q_{10} . At 24 h after the injection, radioactivity was found mainly in the heart muscle, liver and spleen. On the other hand, no significant radioactivity was detected in the heart muscle 24 h after injection of HCO-60- $[^{14}\text{C}]$ coenzyme Q_{10} (Fig. 3). These results strongly suggest that the distribution and retention of $[^{14}\text{C}]$ coenzyme Q_{10} solubilized with soy lecithin-system in heart muscle acting as a targeting tissue of coenzyme Q_{10} , were markedly higher than with the HCO-60 solubilizing system.

Table 2 shows the redox levels of $[^{14}\text{C}]$ coenzyme Q_{10} in the heart and liver after intravenous injection of $[^{14}\text{C}]$ coenzyme Q_{10} solubilized with soy lecithin or HCO-60. The redox levels of $[^{14}\text{C}]$ coenzyme Q_{10} in the heart reached 22.5 and 58.1% at 15 min and 24 h respectively after injection of $[^{14}\text{C}]$ coenzyme Q_{10} solubilized with soy lecithin. In the liver, the redox levels were 26.5 and 67.6% at the same time points, respectively. On the other hand, the redox levels of $[^{14}\text{C}]$ coenzyme Q_{10} in the

Fig. 3. Whole-body autoradiogram 24 h after intravenous injection of [¹⁴C]coenzyme Q₁₀ solubilized with HCO-60. [¹⁴C]Coenzyme Q₁₀ dosed at 1.8 mg/kg.

Table 2. Redox levels of [¹⁴C]coenzyme Q₁₀ administered to guinea pigs.

Tissue	Heart		Liver	
	15 min	24 h	15 min	24 h
Soy lecithin system	22.5 ^a	58.1	26.5	67.6
HCO-60 system	—	trace	—	18.4

^a Each value represents the percentage of Q₁₀H₂ as compared to the sum of Q₁₀ and Q₁₀H₂, and is the average of two independent determinations.

heart and liver were trace and 18.4% at 24 h after injection of HCO-60-[¹⁴C]coenzyme Q₁₀, respectively. The redox levels of [¹⁴C]coenzyme Q₁₀ in the heart and liver after injection of soy lecithin-[¹⁴C]coenzyme Q₁₀ were much higher than those of HCO-60-[¹⁴C]coenzyme Q₁₀. This result indicates that [¹⁴C]coenzyme Q₁₀ solubilized with soy lecithin is more easily reduced than HCO-60-[¹⁴C]coenzyme Q₁₀. This fact is important since the reduced Q₁₀ may play a significant role as an anti-oxidant (6,7). Thus, the soy lecithin solubilizing system of coenzyme Q₁₀ acted as an excellent delivery system to the heart as a targeting tissue of coenzyme Q₁₀, and may prove to be highly effective in the therapy of heart disease such as myocardial ischemia and adriamycin toxicity.

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