Distribution, metabolism and excretion of [1-¹⁴C]dolichol injected intravenously into rats

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[1-¹⁴C]Dolichol mixed *in vitro* with rat serum and injected intravenously into rats was rapidly cleared from the circulation in a manner consistent with a two-compartment model. About 80% of the radioactivity recovered from animals killed after 1 day was in the liver, with smaller amounts being found in lung, carcass (internal organs removed), gastrointestinal tract and contents, and spleen. The kidneys, testes and heart contained little radioactivity, and the brain did not appear to take up any [1-¹⁴C]dolichol. The half-life for the turnover of radioactivity from [1-¹⁴C]dolichol in tissues varied considerably, being 2 days for the lung, 17 for liver and about 50 days for the carcass. After 1 day, and also after 4 and 21 days, most of the radioactivity in all tissues was as [1-¹⁴C]dolichol and as [1-¹⁴C]dolichyl fatty acyl ester, although a small amount of incorporation of [1-¹⁴C]dolichol radioactivity into phospholipids was also observed. Faeces collected over the first 4 days after injection contained 13% of the [1-¹⁴C]dolichol dose, but urine and expired air contained only small amounts of radioactivity. Radioactivity in faeces was nearly all as unchanged [1-¹⁴C]dolichol and as [1-¹⁴C]dolichyl fatty acyl ester. The [1-¹⁴C]dolichol remaining in liver after 21 days appeared to be in a pool (possibly lysosomes) where most of it was not subject to excretion.

The role of phosphorylated dolichol intermediates in the assembly of oligosaccharide chains that are subsequently transferred to protein is quite well understood (Hubbard & Ivatt, 1981). The formation of these *N*-linked secretory and membrane-bound glycoproteins takes place in the rough-endoplasmic-reticulum component of the microsomal fraction. The subcellular distribution of dolichol (alcohol and fatty acyl ester) and dolichyl phosphate in liver (Rip *et al.*, 1981, 1983a) is such that only a very small portion of the total dolichol in liver appears likely to be involved in the assembly of glycoproteins. This is because the sum of all dolichol intermediates that participate directly in synthesis (i.e. all phosphorylated and pyrophosphorylated forms, including those that are glycosylated) account for only 7–8% of the total dolichol in normal rat liver (Rip *et al.*, 1983a). The remainder (over 90%) is found as dolichol (alcohol) and as fatty acyl ester, and only about 5% of this material is in the microsomal fraction.

Alcohol and ester forms in the microsomal fraction can presumably act as precursors to dolichyl phosphate, since the necessary enzymes, dolichol kinase and dolichyl ester hydrolase, are known to

exist. The remaining 95% of the combined alcohol and acyl ester forms appears in subcellular fractions that do not carry out *N*-glycosylation reactions. Although the lysosomes in particular accumulate substantial amounts of dolichol, they do not appear to be able to metabolize it effectively.

The availability of [1-¹⁴C]dolichol (Takigawa *et al.*, 1984) has provided us with a means of studying the metabolism of dolichol, and has allowed us to obtain information concerning the clearance, tissue distribution and excretion of dolichol injected intravenously.

Experimental

Chemicals

[1-¹⁴C]Dolichol (50 mCi/mmol) was a gift from the Kuraray Co., Okayama, Japan. H.p.l.c. revealed a series of homologues from 15 to 24 isoprene units, in which dolichols of 18 and 19 isoprene residues predominate. The radiochemical purity of the product was over 99.5%. All other chemicals used in this study were a minimum of reagent grade.

Animals

Male Wistar rats [CrI:(WI)BR strain; Canadian Breeding Farms and Laboratories, LaPrairie, Que., Canada] weighing 300–350 g were individually housed and given free access to water and Purina chow.

Injection of [$1\text{-}^{14}\text{C}$]dolichol

[$1\text{-}^{14}\text{C}$]Dolichol was freed of carrier solvent under a stream of N_2 . Fresh rat serum was then added to it to yield a [$1\text{-}^{14}\text{C}$]dolichol/serum mixture, which was shaken gently for 4 h. After this time all radioactivity had become associated with serum components, and a 0.4 ml portion (9×10^6 d.p.m.) of the mixture was injected into the tail vein.

Clearance of radioactivity from circulation

Small amounts of blood (12.5 μl) were removed from the tail vein at various times after injection, with calibrated heparinized capillary tubes. The radioactivity in blood was counted directly in Scintiverse (Fisher Scientific, Toronto, Ont., Canada) by using scintillation counting.

Excretion of radioactivity

Radioactivity excreted in urine and in faeces and expired as $^{14}\text{CO}_2$ in air was measured. Animals were placed in metabolic chambers (Delmar Glass Co., Maywood, IL, U.S.A.), and 24 h collections of faeces and urine were obtained. Amounts of $^{14}\text{CO}_2$ in expired air were measured by placing individual animals in small Plexiglass metabolic cages with an air flow of 600–700 ml/min. Air coming from the chamber was bubbled through a solution of ethanol/ethanolamine (2:1, v/v) to trap respiratory CO_2 (Kim *et al.*, 1976). For a 15 min collection period, 10 ml of solution was used and the radioactivity present in a 2 ml portion was determined by scintillation counting.

Tissue distribution of radioactivity

Animals were killed by decapitation and tissues were removed, washed free of blood and frozen. For some experiments serum was collected in order to determine the amount of radioactivity present in lipoproteins.

Tissues, urine, faeces and lipoproteins were saponified (Burgos *et al.*, 1963), and non-saponifiable lipids were extracted into diethyl ether. Ether extracts were then washed with water and dried under N_2 . Dried lipids were dissolved in 5–10 ml of chloroform/methanol (2:1, v/v), and a portion was counted for radioactivity. In some cases liver and faecal total lipids were extracted with 20 vol. of chloroform/methanol (2:1, v/v). These extracts

were washed with water, dried under N_2 and taken up in a small volume of chloroform/methanol (2:1, v/v).

Identification of radioactivity

Radioactive compounds present in non-saponifiable or total lipid extracts were separated and identified by spotting samples along with authentic [$1\text{-}^{14}\text{C}$]dolichol on plates of silica gel G-25 and developing in hexane/diethyl ether/acetic acid (140:60:3, by vol.). Radioactive bands were located by autoradiography of the developed t.l.c. plates (Kodak X Omat AR film). Autoradiograms were used to locate precisely ^{14}C -labelled compounds, which were then scraped into scintillation vials and their radioactivities counted.

Isolation of lipoproteins

Lipoproteins were separated by ultracentrifugation in an SW 40 rotor (35000 rev./min for 24 h), with the use of a discontinuous gradient (Redgrave *et al.*, 1975). The individual lipoproteins were located by using the lipoprotein-staining procedure previously described (Terpstra *et al.*, 1981), aspirated from tubes, pooled and frozen.

H.p.l.c.

In addition to autoradiography, h.p.l.c. was used to investigate the amounts of [$1\text{-}^{14}\text{C}$]dolichol and [$1\text{-}^{14}\text{C}$]dolichyl ester present in total lipid extracts from liver and from faeces. Chromatography was carried out in a reverse-phase C_{18} column in a Hewlett-Packard 1084-B chromatograph, with elution by a methanol/propan-2-ol gradient system (Rip *et al.*, 1981).

Results

Distribution of [$1\text{-}^{14}\text{C}$]dolichol radioactivity in serum

After mixing of serum and [$1\text{-}^{14}\text{C}$]dolichol *in vitro* some 70% of the radioactivity appeared in very-low-density lipoproteins, with lesser amounts being present in low-density and high-density lipoproteins (Fig. 1). Autoradiography of t.l.c. plates on which chloroform/methanol (2:1, v/v) extracts of serum fractions had been run in hexane/diethyl ether/acetic acid (140:60:3, by vol.) indicated that nearly all the radioactivity remained as [$1\text{-}^{14}\text{C}$]dolichol. A small amount (2% of total radioactivity) of a second component corresponding in mobility to dolichyl fatty acyl ester was also observed, but the actual nature of the component was not identified.

Clearance of circulating radioactivity

Radioactivity injected intravenously was rapidly cleared from the blood. The clearance curve (Fig. 2) consistent with a two-compartment model was obtained by using the method of residuals (Gibaldi

Fig. 1. Radioactivity in serum lipoproteins after mixing of [$1\text{-}^{14}\text{C}$]dolichol and rat serum in vitro

Lipoproteins were saponified and radioactivity was determined as indicated in the Experimental section. Abbreviations: VLDL, LDL and HDL, very-low-density, low-density and high-density lipoproteins respectively.

Fig. 2. Blood concentrations of radioactivity at various times after intravenous injection of [$1\text{-}^{14}\text{C}$]dolichol

Blood was collected and radioactivity was determined as indicated in the Experimental section. The ○ symbols represent the rapidly cleared component, and the straight line joining ● symbols represents the slowly cleared component.

& Perrier, 1975). Apparent elimination constants (0.0139min^{-1} and 0.0004min^{-1}) and half-lives (0.83h and 26.4h) were obtained for the early rapid phase and the later slow phase respectively. The rapid phase probably represents uptake of

Fig. 3. Radioactivity in lipoproteins isolated from serum 4 days after intravenous injection of [$1\text{-}^{14}\text{C}$]dolichol. Individual lipoproteins were saponified and radioactivity was determined as indicated in the Experimental section. Abbreviations: as defined in Fig. 1 legend.

[$1\text{-}^{14}\text{C}$]dolichol by the liver, since this tissue contains 82% of the radioactivity recovered from animals killed after 24h. The second, slower, phase may represent redistribution of radioactivity initially taken up by tissues such as the lung, which take up considerable radioactivity but do not retain it for any length of time.

Serum was collected from rats killed 4 days after injection to determine if most of the radioactivity in serum was still associated with very-low-density lipoproteins. However, at this time only 5% of the radioactivity in serum was found in very-low-density lipoproteins, whereas 45% was associated with high-density lipoproteins (Fig. 3). Previous studies (Rip *et al.*, 1983b; Keenan *et al.*, 1977a) have suggested that dolichol is carried by high-density lipoproteins *in vivo*.

In addition to liver, the lungs, the gastrointestinal tract and contents, the carcass and the spleen took up significant quantities of radioactivity (Table 1), whereas little of the radioisotope appeared in kidneys, testes, heart and especially in the brain. On a weight basis (Table 1) the liver, lung and spleen had much greater amounts of radioactivity than other tissues. Only about 3% of the injected dose of [$1\text{-}^{14}\text{C}$]dolichol remained associated with blood after 1 day. T.l.c. of non-saponifiable lipids indicated that [$1\text{-}^{14}\text{C}$]dolichol accounted for at least 98% of radioactivity present in all organs and tissues of animals killed after 24h.

Analysis, by autoradiography and h.p.l.c., of total lipid extracts from livers removed from animals at various times after injection, indicated that 40–80% of [$1\text{-}^{14}\text{C}$]dolichol was esterified.

Clearance of radioactivity from rat organs

Animals injected with [$1\text{-}^{14}\text{C}$]dolichol were killed after 1, 4 and 21 days. At 21 days about half

Table 1. *Tissue distribution of radioactivity from [1-¹⁴C]dolichol 24 h after intravenous injection*
 For experimental details see the text. Values indicated represent the averages $\pm$ S.E.M. for determinations on tissues from three animals. Total blood radioactivity is based on a blood volume (ml/kg) of 60.

Tissue	$10^{-3} \times$ Radioactivity (d.p.m./g)	$10^{-3} \times$ Total radioactivity (d.p.m.)	Total radioactivity (% of dose)
Liver	382.2 ± 28.0	5709 ± 32	63.4
Lung	198.2 ± 60.8	531 ± 160	5.9
Carcass	0.93 ± 0.07	315 ± 10	3.5
Gastrointestinal tract + contents	10.2 ± 1.8	309 ± 23	3.4
Blood	15.5 ± 1.6	279 ± 20	3.1
Spleen	159.7 ± 9.4	100 ± 9	1.1
Kidney	3.0 ± 0.4	9 ± 1.1	0.10
Testes	1.6 ± 0.1	5.1 ± 0.4	0.056
Heart	1.0 ± 0.6	1.4 ± 1.0	0.015
Brain	0.28 ± 0.01	0.5 ± 0.02	0.006

Fig. 4. *Clearance of radioactivity from rat tissues after administration of [1-¹⁴C]dolichol*

Animals were injected and radioactivity was monitored as indicated in the Experimental section. Individual points represent the averages $\pm$ S.E.M. for three determinations.

of the injected radioactivity had been lost from the whole animal and from the liver (Fig. 4). Radioactivity in the lungs and in the gastrointestinal tract decreased over this period to 4.9 and 12.5% of the amount present after 1 day. Little change occurred in the relatively small amounts of radio-

activity present in testes, kidneys, heart and brain, and the spleen actually accumulated radioactivity over the duration of the experiment. [1-¹⁴C]Dolichol accounted for nearly all radioactivity present in lung, spleen, kidney, testes, heart and brain after 1, 4 or 21 days.

Our earliest experiments suggested significant metabolism of [1-¹⁴C]dolichol to more polar metabolites in liver, in the gastrointestinal tract and in the carcass. This was based on the appearance on autoradiograms of bands in addition to dolichol (one band common to liver, carcass and the gastrointestinal tract, and a second unique to the carcass). These bands appeared to account for about 10% of the radioactivity in liver, and as much as 50–75% of total radioactivity in the gastrointestinal tract and carcass, on the basis of the relative intensities of the bands present. However when these 'metabolite' bands were scraped off and their radioactivities counted, they contained little or no radioactivity in the case of the carcass and the gastrointestinal tract, and less radioactivity than expected in the liver. The additional bands observed in these tissues could be reproduced by using non-saponifiable lipids from tissues of animals that had not received [1-¹⁴C]dolichol. This suggests that a chemical interaction of the lipid components on the plate with the X-ray film was responsible for the presence of artefactually darkened areas on the autoradiograms described above. The band observed in liver accounted for only 2.5 and 4.0% of radioactivity present in liver after 4 and 21 days respectively, on the basis of scintillation counting.

Radioactivity in expired air, faeces and urine

At 24 h after injection ¹⁴CO₂ was being evolved from [1-¹⁴C]dolichol at a very low rate (420–480 d.p.m./h per animal). This suggested that respiration was not a major route for the elimination of radioactivity, since animals killed after 4

Table 2. Excretion of radioactivity after intravenous injection of [1-¹⁴C]dolichol

For experimental details see the text. Except where indicated otherwise, values indicated are the averages $\pm$ S.E.M. for determinations on urine and faeces from four animals.

Day	$10^{-3} \times$ Radioactivity excreted (d.p.m./day per rat)	
	Faeces	Urine
1	65 $\pm$ 8	26.3 $\pm$ 3.2
2	408 $\pm$ 83	9.4 $\pm$ 2.0
3	406 $\pm$ 72	9.2 $\pm$ 0.7
4	318 $\pm$ 54	10.1 $\pm$ 0.8
9	62 $\pm$ 5	—
15	—	4.5–5.5*
20	8 $\pm$ 2	—

* Range determined with a total of four animals housed in pairs during collection.

days contained some 1.2×10^6 d.p.m. less than those killed 3 days earlier.

Radioactivity lost in urine (Table 2) accounted for only 5% of the total eliminated over the first 4 days, and was excreted at the rate of 4000–5000 d.p.m./day per rat on day 15. Radioactivity in urine was not extractable into diethyl ether, either directly or after acid and alkaline hydrolysis, indicating that the radioactivity is associated with a molecule more polar than dolichol.

Faecal excretion accounted for nearly all radioactivity lost from rats between day 1 and day 4. A small amount of the radioisotope was excreted within the first day (Table 2), followed by larger and nearly equal amounts on each of the next 3 days. Collections for day 9 and day 20 produced smaller but still significant amounts of radioactivity. During the first 4 days 1197000 ± 125000 d.p.m. (13% of the dose) was excreted in this manner. Nearly equal amounts of [1-¹⁴C]dolichol and [1-¹⁴C]dolichyl ester were present in total lipid extracts of faeces collected over the first 4 days after injection. The faecal dolichyl ester appeared to consist of two types, a minor component identical with the acyl ester in liver, and a second, more abundant, form intermediate in polarity between dolichol and dolichyl acyl ester from liver. This second acyl ester form had retention time on h.p.l.c. identical with that of dolichyl acetate.

Discussion

When [1-¹⁴C]dolichol was mixed with rat serum *in vitro* most of the radioactivity was associated with very-low-density and low-density lipo-

proteins, and only about 5% was in high-density lipoproteins (Fig. 1). However, in animals killed 4 days after injection, high-density lipoproteins contained 45% of the serum radioactivity. This difference may be due to more rapid clearance of very-low-density-lipoprotein and low-density lipoprotein dolichol from the serum. Earlier studies have shown that most of the dolichol normally present in serum is associated with high-density lipoproteins (Keenan *et al.*, 1977a; Rip *et al.*, 1983b).

[1-¹⁴C]Dolichol was rapidly cleared from the bloodstream, and 63% of the dose appeared in the liver within 24 h of injection. The lungs, carcass, gastrointestinal tract and contents, blood and the spleen accounted for 5.9, 3.5, 3.4, 3.1 and 1.1% of this dose respectively. The radioactivity in each of the other tissues after 1 day represented no more than 0.1% of the dose given.

Animals were allowed to bleed after decapitation, and tissues were minced and blotted to remove as much blood as possible before extraction. Less than 0.1 ml of residual blood could still account for all of the radioactivity found in heart and brain after 24 h, but in most other tissues only a small proportion of the [1-¹⁴C]dolichol present can be attributed to blood. The persistence of radioactivity in the tissues over a 21-day period (Fig. 4), long after radioactivity has been cleared from the circulation, provides further evidence that the radioactivity is largely associated with the tissues themselves rather than with entrapped blood.

The half-lives for the turnover of [1-¹⁴C]dolichol in the whole rat and in the liver were 16.2 and 16.6 days respectively. The lung and the gastrointestinal tract and contents lost radioactivity rapidly (half-lives of 1 and 7 days respectively), but radioactivity incorporated into the carcass was lost much more slowly. The faecal excretion patterns observed (Table 2) suggest that radioactivity is associated mainly with the contents of the gastrointestinal tract and not in the tract itself. After 24 h nearly all the radioactivity in any particular tissue after saponification was present as [1-¹⁴C]dolichol, and this pattern did not change significantly after 4 and after 21 days.

Phosphorylated forms of dolichol appear not to be efficiently extracted from saponification mixtures into diethyl ether, and are therefore not included in the sum of dolichol and dolichyl ester obtained. The amount of radioactivity from [1-¹⁴C]dolichol incorporated into phospholipid was estimated by fractionating a total lipid extract into neutral lipids and phospholipids by silicic acid chromatography (Rip *et al.*, 1983a). Liver phospholipids accounted for 4–5% of total lipid radioactivity on days 1 and 4, and a somewhat higher proportion on day 21.

The major route for the elimination of [1-¹⁴C]dolichol was in faeces, apparently via the bile. Highest concentrations of radioactivity appeared in faeces collected on the first few days after injection, and by day 5 13% of the dose had been eliminated. This value agrees with the data of Keenan *et al.* (1977b), who observed elimination of 16% of a dose of [1-³H]dolichol over a 90 h period. It is noteworthy that, although large amounts of [1-¹⁴C]dolichol were excreted on each of days 2, 3 and 4 after injection, the amounts cleared at later times such as day 20 are very small, even though livers still contain some 2.5×10^6 d.p.m. of [1-¹⁴C]dolichol. This indicates that liver may have at least two pools into which [1-¹⁴C]dolichol from serum is able to enter. Radioactivity from one pool is lost quickly and appears in faeces over a period of 2–3 weeks, whereas the second pool is nearly completely retained, since at 20 days only about 8000 d.p.m./day per rat is excreted. This suggests that [1-¹⁴C]dolichol that remains in liver beyond 3 weeks has been incorporated into a relatively stable environment, perhaps a membrane system, where it is not available for biliary excretion and where it is not acted on metabolically. It is possible that lysosomes, which are known to contain high dolichol concentrations (Rip *et al.*, 1983a,b; Wong *et al.*, 1982), offer such an environment.

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