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EFFECTS OF CCl_4 POISONING ON METABOLISM OF DOLICHOL IN RAT LIVER MICROSOMES AND GOLGI APPARATUS

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Carbon tetrachloride (CCl_4) poisoning affects glycoprotein processing and maturation at the level of rat liver microsomes and Golgi apparatus. HPLC analysis showed that within 5–60 min after CCl_4 administration the levels of total dolichol, free dolichol and dolichyl-phosphate strongly decreased both in total microsomes and in Golgi apparatus. The most marked and early reduction of total dolichol was observed in the secretory membranes of Golgi area already 15 min after CCl_4 poisoning.

The incubation of CCl_4-pretreated isolated hepatocytes with [3H]-mevalonate showed a significant slowing down of the label incorporation into both free-dolichol and dolichyl-phosphate.

Moreover, lipid peroxidation might cause alterations in the molecular structure of both free-dolichol and dolichyl-phosphate. A notable prevention of dolichol decrease was observed in animals pretreated with vitamin E.

The results suggest that the prooxidant activity of CCl_4 is able to affect the metabolism of dolichol either by increasing the oxidative degradation or impairing the biosynthetic pathway.

KEY WORDS: CCl_4, glycosylation, dolichol, liver, microsome, golgi apparatus.

INTRODUCTION

The rat poisoning with carbon tetrachloride (CCl_4) leads to a precocious impairment of the hepatocellular system which provides for terminal glycosylation and maturation of glyco- and lipoproteins at the level of the Golgi apparatus. In fact, a few min after acute treatment with CCl_4 a structural derangement of liver Golgi apparatus takes place,[1] and the earliest functional change is a block of lipoprotein transit through the Golgi cisternae and vesicles both in the isolated hepatocyte model and in the whole animal.[2,3] Furthermore, at 15 min of rat intoxication CCl_4 strongly inactivates galactosyltransferase activity in Golgi secretory and formative membranes.[1–4]

Lipid peroxidation appears to be the major mechanism involved in the inactivation of glycosylation reactions;[3] this is suggested both by the susceptibility of Golgi galactosyl- and sialyltransferase activities to the toxic action of aldehydic products of lipid peroxidation[5] and by the marked protection of these activities afforded by rat supplementation with vitamin E.[3,4]

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The glycosylation of proteins is a multistage process which starts in the endoplasmic reticulum where the synthesis of N-linked oligosaccharide occurs and comes to its end in the Golgi apparatus where a specific glycosyltransferase system provides for the terminal glycosylation reactions.[6]

Both in the membranes of endoplasmic reticulum and Golgi apparatus several long-chain polyisoprenoid alcohols, named dolichol, are present either as free dolichol or dolichyl-phosphate (dolichyl-P). Recent observations indicate that the biosynthetic pathways of free-dolichol and dolichyl-P may be distinct and that these forms are not freely interconvertible.[7] The latter form is mostly present in the endoplasmic reticulum and acts as glycosyl carrier across these membranes in the initial stages of the membrane-directed biosynthesis of the N-linked oligosaccharide chains of glycoproteins.[8,9]

The highest concentration of free dolichol occurs in Golgi apparatus[10] with a possible role either in terminal glycoprotein processing or in movements through the Golgi membranes towards the secretory region.

Since the levels of both free dolichol and dolichyl-P seem to be essential for the entire steps of glycosylation pathway, this investigation has been undertaken with the aim of clarifying whether the free radical activation of CCl_4 might impair these reactions by affecting the metabolism of dolichol in rat liver microsomes and Golgi apparatus.

MATERIALS AND METHODS

"In Vivo" Experiments

Male Sprague Dawley rats (CD-COBS Charles River, Calco, Italy), weighing 200 $\pm$ 25 g, were fed on a standard synthetic diet, devoid of antioxidants. Groups of 6 animals, fasted for 16 h, received by oral intubation a single dose of CCl_4 (250 μl/100 g b.w. as a solution 50% v/v in mineral oil), or mineral oil alone. The animals were sacrificed at different times within 5–60 min after intubation. In the experimental groups in which the protective effect of vitamin E was tested, 10 mg/100 g b.w. of alpha-tocopherol, dissolved in 1 vol of ethanol followed by the addition of 9 vol of 16% Tween 80 in 0.9% NaCl, were injected intraperitoneally 15 h before the animals were killed.

Preparations of Golgi apparatus subfractions (F1 and F2 as secretory region and F3 as formative region) were obtained from total microsomes of pooled livers by discontinuous sucrose density gradient ultracentrifugation according to the method previously described.[11] Morphological and chemical characterization of Golgi membranes were carried out as reported.[3]

"In Vitro" Experiments

After hepatocyte isolation, as described by Poli *et al.*,[12] the cells were pre-incubated with CCl_4 (129 μM) for different times within 15–60 min and then with 5-(3H)mevalonolactone (38.8 Ci/mmol) for 10 min as described by Ekström *et al.*[7]

After incubation the isolated liver cells were sonicated and the microsomal fraction was isolated.

Extraction of Total Dolichol

The liver homogenate and the subcellular fractions were digested with 0.5 N ethanolic KOH at 60°C for 1 h. Total dolichol (as free-dolichol, dolichol-fatty acid, dolichyl-P and other polyisoprenoids compounds) was extracted three times with 7 volumes of ethanol/hexane (2:5 v/v).[13]

Extraction of Free Dolichol and Dolichyl-P

The microsomes were supplemented with 4 ml 0.25% pyrogallol in methanol and 2 ml 60% KOH. Alkaline hydrolysis, which converts fatty acid dolichol to free dolichol, was performed at 85°C for 45 min. The mixtures were adjusted to a chloroform/methanol/water (C/M/W) ratio 3/2/1 and the chloroform phase was washed with upper phase four times and evaporated to dryness.[14] The samples dissolved in CM (2:1), were applied to a 5 cm DEAE cellulose column equilibrated in CM (2:1). The column was washed with 10 ml of CM (2:1) and 10 ml of CM (3:1). Dolichyl-P was eluted with 200 mM ammonium acetate in CM 3:1.[15]

Analysis of Dolichol

After evaporation, the samples, containing either total dolichol, or free dolichol or dolichyl-P, dissolved in methanol, were analyzed by high performance liquid chromatography (HPLC) in a μBondapak C18 reversed phase column (Waters 3.9 mm × 30 cm). The mobile phase was methanol/isopropanol containing 10 mM phosphoric acid (solvent B). The gradient was isocratic at 2% B for 5 min, increased linearly in 3 min to 50% B, and maintained for 5 min; the flow rate was 2.0 ml/min. The effluent was monitored at 210 nm.

In the labelling experiments the effluent was collected in a fraction collector and radioactivity measured in a beta-counter Tri-carb 4000, Packard.

Stimulation of Microsomal Lipid Peroxidation

Incubation of microsomes isolated from control and vitamin E pretreated rats was carried out in presence of ascorbate (500 μM) + $FeSO_4$ (20 μM) to initiate non-enzymic lipid peroxidation.[16] After 60 min of incubation at 37°C, lipid peroxidation was measured by determining thiobarbiturate-reactive substances.[17] Free dolichol and dolichyl-P were extracted and analyzed as described.

Generation of Oxygen-Derived Free Radicals

The reaction of xanthine with xanthine-oxidase was used.[18] The reaction mixture contained 2 mM xanthine, 100 μM EDTA in 50 mM phosphate buffer at pH 7.4, 0.5 units xanthine-oxidase and 50 μg free dolichol or dolichyl-P standards. After 30 min incubation at 37°C, the residual amount of dolichol was assayed by HPLC.

Protein Analysis

Protein was determined by the method of Hartree[19] with bovine serum albumin as standard.

Statistical Evaluation

The results of 4 to 8 sets experiments have been averaged and expressed as means ± standard deviation (SD). The differences were calculated by Student's t-test and were considered significant when the t-values corresponded to $P < 0.01$.

Chemicals

All chemicals were of reagent grade and were obtained from Merck AG, Darmstadt, FRG, and BDH Italia, Milan, Italy. Dolichol standards were purchased from Sigma Chemical Co. St. Louis USA. Internal standards (C125-free dolichol and C120-dolichyl-P) were kindly supplied from Prof. T. Chojnacki (Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warszawa, Poland). 5-(3H)-mevalonolactone (specific activity 38.8 Ci/mmol) was obtained from New England Nuclear, GmbH, Dreieich, FRG.

RESULTS

The levels and distribution of total dolichol in liver homogenate and in subcellular fractions obtained both from control and CCl_4-treated rats 60′ min after intoxication are reported in Table 1. These results show that the subcellular fractions normally contain dolichol which is differently concentrated in each fraction. CCl_4 affects the levels of total dolichol both in whole homogenate and in subcellular fractions: the percentage of decrease is 39%, 32% and 54% in homogenate, cytosol and microsomes, respectively.

The time course of the total dolichol content in liver microsomes of CCl_4-poisoned rats is shown in Table 2. The levels of microsomal dolichol decreased gradually after CCl_4 administration by the 27%, 36% and 54% at 15, 30, 60 min, respectively. The normal levels of dolichyl-P represent about 45% of the total dolichol present in microsomes; these results are in agreement with other data reporting that rough and smooth endoplasmic reticulum are enriched in dolichyl-P which represents the 54% and the 36% of total dolichol.[20] The quantitative determination of both free-dolichol and dolichyl-P purified from microsomal extracts showed a significant rate of reduction 60 min after CCl_4 intoxication (48% and 46%, respectively) (Table 3).

TABLE 1
Levels of total dolichol in liver homogenate and subcellular fractions from untreated and CCl_4-treated rats 60 min before sacrifice

Fraction	Total dolichol		
	Control	CCl_4	Decrease %
Homogenate	304 ± 45	185 ± 27*	39
Cytosol	37 ± 4	25 ± 3*	32
Microsomes	125 ± 16	58 ± 9*	54

The values are expressed as ng total dolichol/mg protein and represent the mean ± S.D. of 4 experiments.

$P < 0.01$ in comparison with control rats.

TABLE 2
Total dolichol content of rat liver microsomes at different times after CCl_4 administration to untreated rats or pretreated with vitamin E 15 h before sacrifice

Time	CCl_4	Decrease %	Vit E	Vit E + CCl_4	Decrease %
Control	125 ± 16	-	126 ± 18	122 ± 18	-
5′	108 ± 14	14	121 ± 13	115 ± 15	3
10′	98 ± 10	22	119 ± 15	110 ± 13	10
15′	91 ± 6*	27	123 ± 21	107 ± 15**	12
30′	80 ± 12*	36	127 ± 16	105 ± 13**	14
60′	58 ± 9*	54	124 ± 20	101 ± 15**	17

The data are expressed as ng total dolichol/mg protein and represent the mean ± SD of 4–8 sets of experiments.
* $P < 0.01$ in comparison with control rats.
** $P < 0.01$ in comparison with CCl_4-intoxicated rats at the same time.

While only 3% of dolichyl-P is present in Golgi apparatus, this compartment has the highest content in total dolichol which is approximately 16-fold enriched with respect to microsomes (Table 4).

CCl_4 affects the levels of total dolichol in Golgi area early with respect to microsomes. In fact, the secretory fractions F1 and F2 showed a significant decrease of the levels of total dolichol already at 5 min after poisoning. The most marked decrease was present in both secretory (F1, F2) and formative fractions at 15 min (56%, 47%, 28% in F1, F2, F3, respectively) (Table 5).

The decrease of dolichol levels in whole homogenate and in each investigated subcellular compartment suggests that the disappearance of these molecules is not dependent on an altered cellular distribution.

The CCl_4-induced effects on dolichol metabolism might be due to an impairment of the polyprenol synthetic pathway. To check whether the dolichol synthetized through the mevalonate pathway was affected by CCl_4, isolated rat hepatocytes were incubated with [3H] mevalonate at different times after exposure to CCl_4. The incorporation of the label in free dolichol and dolichyl-P extracted from the hepatocyte microsomes was significantly lower than that observed in untreated cells within 15 and 60 min after CCl_4 treatment, and the most marked decrease was observed at 60 min both in free-dolichol (75%) and dolichyl-P (70%) (Figure 1). In order to verify the labelling pattern of each polyprenol unit, the position of the individual dolichols

TABLE 3
Free-dolichol and dolichyl-P content of rat liver microsomes 60 min after CCl_4 administration to untreated rats or pretreated with vitamin E 15 h before sacrifice

Treatment	Free dolichol	Decrease %	Dolichyl-P	Decrease %
Control	67 ± 8	-	56 ± 7	-
Vit E	60 ± 10	11	54 ± 9	3
CCl_4	35 ± 5*	48	30 ± 3*	46
CCl_4 + Vit E	58 ± 6**	13	50 ± 5**	11

The data are expressed as ng dolichol/mg protein and represent the mean ± SD of 4–8 sets of experiments.
* $P < 0.01$ in comparison with control rats.
** $P < 0.01$ in comparison with CCl_4-intoxicated rats.

TABLE 4
Enrichment of total dolichol levels in normal rat liver Golgi apparatus (GA) subfractions in comparison with microsomes

	Total dolichol (ng/mg prot)	Enrichment
Microsomes	125 ± 15	1
GA F1	7565 ± 376	63
GA F2	1405 ± 168	12
GA F3	430 ± 45	3

The data are expressed as ng total dolichol/mg protein and represent the mean ± SD of 4–8 sets of experiments.

after CCl_4 intoxication and the radioactivity measured in the collected fractions have been compared (Figure 2).

Evidence has been reported that through the stimulation of lipid peroxidation, CCl_4 strongly inactivates the galactosyl-transferase activity in the entire Golgi area and that the pre-treatment of rats with alpha-tocopherol prevents this CCl_4-induced enzymatic change.[3] Therefore, groups of rats have been pretreated with vitamin E 15 h before poisoning. The increased antioxidant potential of liver membranes which follows the alpha-tocopherol treatment resulted in a good prevention of the CCl_4-induced alterations of the levels of dolichol both in microsomes and in each Golgi fraction (Tables 2, 3, 5).

Since vitamin E is able to prevent the prooxidant effect of CCl_4 without affecting the covalent binding of its metabolites to cell protein and lipid,[21,22] this finding indicates that lipid peroxidation could be the mechanism mainly responsible for the degradation of dolichol on the membranes of liver submicrosomal compartments.

TABLE 5
Total dolichol content of liver Golgi apparatus subfractions at different times after CCl_4 administration to untreated rats or pretreated with vitamin E 15 h before sacrifice

Time	CCl_4	Decrease %	Vit E	Vit E + CCl_4	Decrease %
F1					
Control	7565 ± 37	-	7538 ± 538	7590 ± 415	-
5′	6643 ± 31*	12	7590 ± 415	7384 ± 412**	3
10′	5354 ± 60*	29	7602 ± 540	6891 ± 442**	9
15′	3351 ± 30*	56	7530 ± 427	5769 ± 486**	24
F2					
Control	1405 ± 168	-	1427 ± 285	1421 ± 137	-
5′	1191 ± 145	16	1421 ± 137	1345 ± 139	4
10′	985 ± 87*	30	1485 ± 195	1211 ± 105**	14
15′	748 ± 47*	47	1431 ± 156	1107 ± 85**	21
F3					
Control	430 ± 45	-	441 ± 43	435 ± 38	-
5′	396 ± 82	8	420 ± 39	405 ± 52	4
10′	361 ± 60	17	435 ± 38	386 ± 25	8
15′	312 ± 31*	28	430 ± 51	360 ± 30**	14

The data are expressed as ng total dolichol/mg protein and represent the mean ± SD of 4–8 sets of experiments.

* $P < 0.01$ in comparison with control rats.

** $P < 0.01$ in comparison with CCl_4-intoxicated rats at the same time.

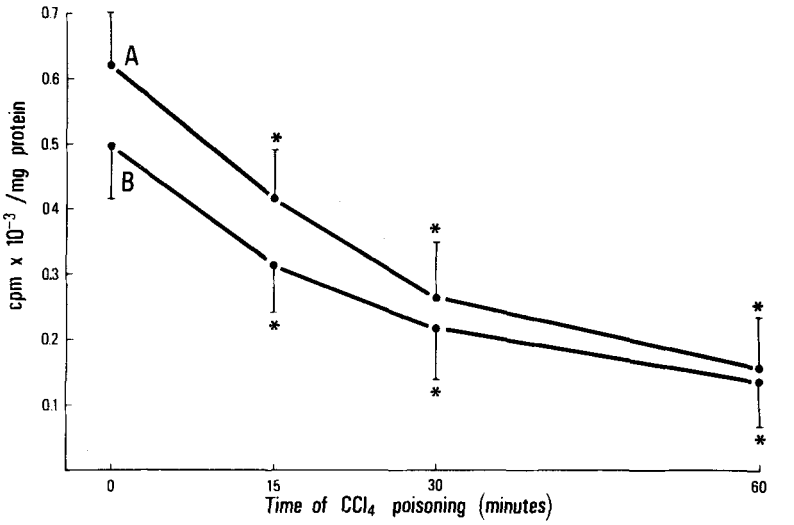

FIGURE 1 Distribution of radioactivity into free dolichol (A) and dolichyl-P (B) after [3H] mevalonate labeling of CCl_4-treated isolated hepatocytes.

The cells were incubated for 10 minutes with the label. The individual fractions were taken from the hepatocyte microsomes after isolation in reversed phase HPLC.

The values are the mean of 4 experiments ± SD and are expressed as cpm/mg protein.

* $P < 0.01$ in comparison with control.

In order to verify this possibility two different experimental models have been performed "*in vitro*". Lipid peroxidation was initiated in the liver microsomes by the addition of ascorbate/Fe++ and the amount of dolichol and dolichyl-P measured along with that of malondialdehyde formed. As shown in Table 6 the prooxidant effect of iron decreased by respectively 52% and 44% the recovery of both free-dolichol and dolichyl-P. On the contrary, in the microsomes prepared from rats pretreated with Vitamin E, where no lipid peroxidation was detectable, the disappearance of both the two forms of dolichol was greatly diminished.

The susceptibility of the dolichol molecule to undergo radical attack is confirmed by experiments where 50 μg of pure free-dolichol or dolichyl-P were exposed to a free radical generating system consisting of xanthine and xanthine-oxidase. Following 30 min incubation the recovery from the system was decreased by about 60% for free-dolichol and 57% for dolichyl-P.

DISCUSSION

The results presented in this paper indicate that CCl_4 affects the liver metabolism of dolichol; this event may be involved, at least in part, in the impairment of glycosylation mechanisms both in endoplasmic reticulum and in Golgi apparatus, as previously reported.[1-5] In fact, the levels of dolichol, particularly dolichyl-P, are considered rate-limiting for the synthesis of glycoproteins;[23,24] this explains why microsomes, with respect to whole liver, are enriched almost 10 fold in dolichyl-P[25]

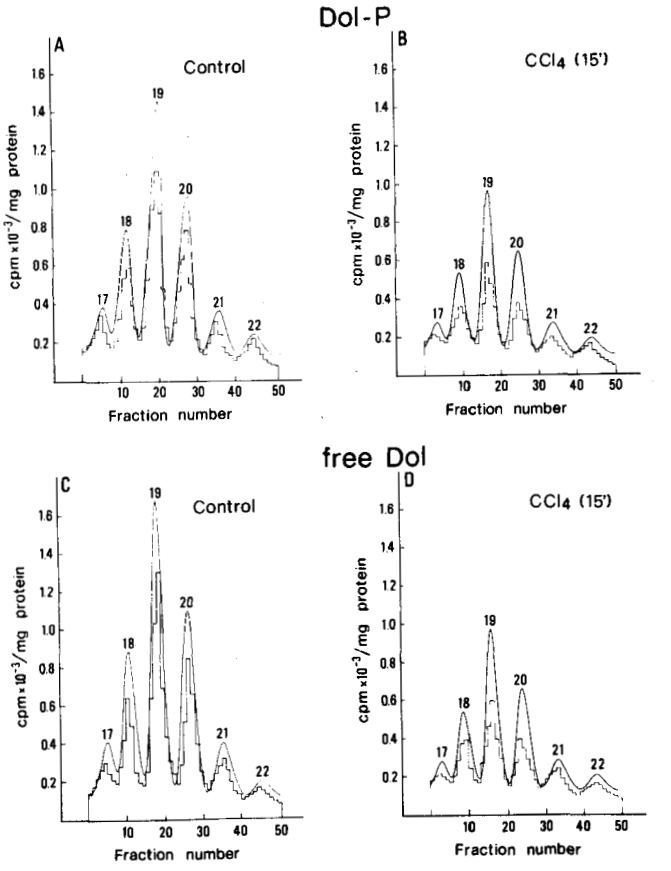

FIGURE 2 [3H] mevalonate labelling of free dolichol and dolichyl-P in microsomes of isolated hepatocytes.

The experiments were performed as described in Figure 1.

The continuous curves give the position of the individual dolichol as obtained by 210 nm recording of eluent and the staples denote the radioactivity measured in the collected fractions. The number above the peaks denote the numbers of isoprenes in the individual polyprenols.

A = dolichyl-P in untreated cells (dol-P).

B = dolichyl-P in hepatocytes 15 min after CCl_4 administration.

C = free-dolichol in untreated cells (free-dol).

D = free-dolichol in hepatocytes 15 min after CCl_4 administration.

which provides both for the formation of the precursor oligosaccharide and for the sugar transport from the cytosol to the membranes of endoplasmic reticulum.[9,26,27]

Furthermore, the high enrichment of free dolichol in Golgi apparatus suggests a role also for these membranes in the metabolism and functions of polyisoprenoids: several data indicate that Golgi membranes contain a specific dolichyl-P phosphatase which is able to modulate the levels of microsomal dolichyl-P.[10,28] On the other hand, a potential role has been postulated for free dolichol in controlling the fluidity and the permeability properties of membranes;[29–32] such effects may explain the involvement

TABLE 6
Effect of ascorbate/Fe++ on free dolichol and dolichyl-P levels of liver microsomes isolated from untreated and vit E pretreated rats

1	Free-dolichol	Dolichyl-P	MDA
Vitamin E untreated rats			
Control	70.1 ± 6.5	57.3 ± 7.3	1.8 ± 0.2
Ascorbate/Fe++	36.4 ± 5.1*	25.1 ± 4.3*	17.7 ± 3
Vitamin E pretreated rats			
Control	69.8 ± 6.0	58.2 ± 7.2	2.1 ± 0.3
Ascorbate/Fe++	58.3 ± 6.1**	44.5 ± 5.2**	2.5 ± 0.4

Lipid peroxidation was measured after 60 min incubation by determining the amount of malondialdehyde (MDA) formed.

The data are expressed as ng dolichol/mg protein and nmol aldehyde/mg protein and represent the mean ± S.D. of 4 experiments.

* $P < 0.01$ in comparison with control microsomes.

** $P < 0.01$ in comparison with ascorbate/Fe++ incubated microsomes.

of these molecules in membrane movements between the Golgi region and the plasma membrane and in the glycosylation reactions.[6,33]

The CCl_4-induced changes in dolichol levels could be dependent on alterations either of the dolichol synthetic pathway or of their breakdown. In this report we have investigated the involvement of both the mechanisms. Mevalonate is a precursor of all isoprenoid compounds such as cholesterol and ubiquinone and it has been demonstrated that the biosynthesis of cholesterol and dolichol is regulated by independent mechanisms.[34–36] After hepatocyte incubation with CCl_4 the incorporation of the labelled precursor into both free-dolichol and dolichyl-P markedly decreased as the time of poisoning increased. However, the measurements of half lives of dolichol in liver cells generally give values between 80 and 118 h for free dolichol and 17 h for dolichyl-P;[37] therefore the impairment of the synthetic process of isoprenoid compounds should result in a decrease of dolichol levels only after these times. Since a marked decrease of dolichol appeared in our experiments in a very short time after CCl_4 poisoning, it must be related mostly to a precocious oxidative degradation of these molecules.

As mentioned before, the prevention of changes in dolichol levels afforded by the rat pretreatment with alpha-tocopherol indicates that CCl_4 acts through its prooxidant activity also within few min after poisoning. This is in agreement with other data showing that already 5 min after CCl_4-intoxication evidence of lipid peroxidation is given by the appearance of the diene conjugation band.[38–40] In our previous experiments the lipids of the three liver Golgi fractions obtained from CCl_4-poisoned rats showed early the appearance of a diene conjugation band which disappeared, particularly in the secretory Golgi membranes, after rat pretreatment with alpha- tocopherol.[3]

Moreover, the involvement of lipid peroxidation in the mechanism of microsomal dolichol degradation was also supported by the significant decrease of both free dolichol and dolichyl-P in liver microsomes incubated with the prooxidant system ascorbate-Fe++. It is therefore possible that highly reactive peroxyl and alkoxyl radicals might react within the cellular membranes with dolichol causing alterations which did not allow measurement by HPLC. Consistently with the hypothesis that free radical attack produces a decrease in the measurable amount of dolichol is the finding that incubation of free dolichol and dolichyl-P with a free radical generating

system similarly causes a 60% loss of both the molecules. According to Chojnacki and Dallner[37] the rates of dolichol turnover suggest the existence of breakdown pathways involved in the catabolism of these molecules; our results could support this possibility.

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References

1. U.M. Marinari, M.A. Pronzato, D. Cottalasso, A. Zicca-Cadoni, G. Nanni, G. Poli, E. Chiarpotto, E. Albano, F. Biasi and M.U. Dianzani (1985) CCl_4-induced early functional impairment of rat liver Golgi apparatus. In Free Radical in Liver Injury, edited by G. Poli, K.H. Cheeseman, M.U. Dianzani and T.F. Slater, pp. 179–183, IRL Press Limited, Oxford, England.
2. G. Poli, E. Chiarpotto, E. Albano, D. Cottalasso, G. Nanni, U.M. Marinari, A.M. Bassi and M.U. Dianzani (1984) Carbon tetrachloride-induced inhibition of hepatocyte lipoprotein secretion: functional impairment of Golgi apparatus in the early phases of such injury, *Life Science*, **36,** 533–539.
3. G. Poli, D. Cottalasso, M.A. Pronzato, E. Chiarpotto, F. Biasi, F.P. Corongiu, U.M. Marinari, G. Nanni and M.U. Dianzani (1990) Lipid peroxidation and covalent binding in the early functional impairment of liver Golgi apparatus by carbon tetrachloride. *Cell Biochemistry and Function*, **8,** 1–10.
4. D. Cottalasso, M.A. Pronzato, C. Rolla, U.M. Marinari, G. Nanni, E. Chiarpotto, F. Biasi, E. Albano, G. Poli and M.U. Dianzani (1984) Effect of vitamin E pretreatment on CCl_4-induced impairment in vivo of glycosylation and lipoprotein secretion by rat liver Golgi apparatus. *IRCS Medical Science*, **12,** 904–905.
5. U.M. Marinari, M.A. Pronzato, D. Cottalasso, C. Rolla, F. Biasi, G. Poli, G. Nanni and M.U. Dianzani (1987) Inhibition of liver Golgi glycosylation activities by carbonyl products of lipid peroxidation. *Free Radical Research Communications*, **3,** 319–324.
6. G.B. Hirschberg and M.D. Snider (1987) Topography of glycosylation in the rough endoplasmic reticulum and Golgi apparatus. *Annual Review of Biochemistry*, **56,** 63–87.
7. T.J. Ekström, T. Chojnacki and G. Dallner (1984) Metabolic labelling of dolichol and dolichyl phosphate in isolate hepatocytes. *Journal of Biological Chemistry*, **259,** 10460–10468.
8. D.K. Struck and W.J. Lennarz (1980) The function of saccharide-lipids in synthesis of glycoproteins. In the Biochemistry of Glycoproteins and proteoglycans, edited by W.J. Lennarz, pp. 35–73. New York: Plenum Press.
9. R. Kornfeld and S. Kornfeld (1985) Assembly of asparagine-linked oligosaccharides. *Annual Review of Biochemistry*, **54,** 631–644.
10. J.W. Rip, N. Chaudhary and K.K. Carroll (1983) Distribution and metabolism of dolichol and dolichyl phosphate in rat liver. *Canadian Journal of Biochemistry and Cell Biology*, **61,** 1025–1031.
11. G. Nanni, D. Cottalasso, G. Cetta, M.A. Pronzato and U.M. Marinari (1986) Ethanol-induced impairment of glycoprotein metabolism in liver Golgi apparatus. *Front. Gastrointestinal Research*, **9,** 50–69.
12. G. Poli, E. Gravela, E. Albano and M.U. Dianzani (1979) Studies on fatty liver with isolated hepatocytes. *Experimental Molecular Pathology*, **30,** 116.
13. W.L. Adair and R.K. Keller (1985) Isolation and assay of dolichol and dolichyl phosphate. *Methods in Enzymology*, **111,** 201–215.
14. C. Edlund, J. Ericsson and G. Dallner (1987) Changes in hepatic dolichol and dolichyl monophosphate caused by treatment of rats with inducers of the endoplasmic reticulum and peroxisomes and during ontogeny. *Chemical Biological Interactions*, **62,** 191–208.
15. P.K. Keller, M.S. Fuller, G.D. Rottler and L.W. Connelly (1985) Extraction of dolichyl phosphate and its quantitation by straight-phase high-performance liquid chromatography. *Analytical Biochemistry*, **147,** 166–173.

16. G. Poli and E. Gravela (1982) Lipid peroxidation in isolated hepatocytes. In: Free radicals, lipid peroxidation and cancer, Edited by D.C.H. McBrien and T.F. Slater, Academic Press, 215–241.
17. G. Poli, E. Gravela, E. Albano and M.U. Dianzani (1979) Studies on fatty liver with isolated hepatocytes. *Experimental Molecular Pathology*, **30,** 116–127.
18. I. Fridovich (1985) Xanthine oxidase. In: CRC Handbook of methods for oxygen radical research, Edited by R.A. Greenwald, CRC Press, Florida, 51–53.
19. E.F. Hartree (1972) Determination of protein: a modification of the Lowry method that gives a linear photometric response. *Analytical Biochemistry*, **48,** 422–427.
20. J.W. Rip, C.A. Rupar, K. Ravi and K.K. Carroll (1985) Distribution metabolism and function of dolichol and polyprenols, *Progress in Lipid Research*, **24,** 269–309.
21. M.U. Dianzani, U.M. Marinari and G. Poli (1984) The contribution of covalent binding and lipid peroxidation in the pathogenesis of fatty liver by CCl_4. In Proc. Iuphar 9th Int. Congr. Pharmacol., edited by W. Paton, J. Hitchell and P. Turner, vol. 2, pp. 259–264, The Macmillan Ltd: London.
22. M.U. Dianzani and G. Poli (1985) Lipid peroxidation and haloalkylation in CCl_4-induced liver injury. In Free Radicals in liver injury, edited by G. Poli, K.H. Cheeseman, M.U. Dianzani and T.F. Slater, pp. 149–158, IRL Press: Oxford.
23. C.D. Carson and W.J. Lennarz (1979) Inhibition of polyisoprenoids and glycoprotein biosynthesis caused abnormal embryonic development. *Proceedings of the National Academy of Sciences U.S.A.*, **76,** 5709–5713.
24. J.E.R. Potter, M.J. James and A.A. Kandutsch (1981) Sequential cycles of cholesterol and dolichol synthesis in mouse spleen during phenylaydrazine-induced erytropoiesis. *Journal of Biological Chemistry*, **256,** 2371–2376.
25. N. Chaudhary, D.J. Freeman, J.W. Rip and K.K. Carroll (1982) Direct estimation of dolichyl phosphate in rat liver by high pressure liquid chromatography. *Lipids*, **17,** 558–560.
26. J.A. Hanover and W.J. Lennarz (1980) N-linked glycoprotein assembly evidences that oligosaccharide attachment occurs within the lumen of the endoplasmic reticulum. *Journal of Biological Chemistry*, **255,** 3600–3604.
27. J.A. Hanover and W.J. Lennarz (1982) Transmembrane assembly of N-linked glycoproteins. Studies on the topology of saccharide lipid synthesis. *Journal of Biological Chemistry*, **257,** 2787–2794.
28. C.A. Rupar, J.W. Rip, N. Chaudhary and K.K. Carroll (1982) The subcellular localization of enzymes of dolichol metabolism in rat liver. *Journal of Biological Chemistry*, **257,** 3090–3094.
29. C.S. Lai and J.S. Schutzbach (1984) Dolichol induced membrane leakage of liposomes composed of phosphatidylethanolamine and phosphatidylcholine. *FEBS Letters*, **169,** 279–282.
30. J.A. Monti, S.T. Christian and J.S. Schutzbach (1987) Effects of dolichol on membrane permeability. *Biochimica Biophysica Acta*, **905,** 133–142.
31. C. Valtersson, G. Van Duijn, A.J. Verkleij, T. Chojnacki, B. de Kruijff and G. Dallner (1985) The influence of dolichol esters, and dolichyl phosphate on phospholipid polymorphism and fluidity in model membranes. *Journal of Biological Chemistry*, **260,** 2742–2751.
32. G. Van Duijn, C. Valtersson, T. Chojnacki, A.J. Verkleij, G. Dallner and B. de Kruijff (1986) Dolichyl phosphate induced non bilayer structures, vesicles fusion and transbilayer movement of lipids: a model membrane study. *Biochimica Biophysica Acta*, **861,** 211–213.
33. J.A. Hanover and W.J. Lennarz (1981) Transmembrane assembly of membrane and secretory glycoproteins. *Archives of Biochemistry and Biophysics*, **211,** 1–19.
34. R.K. Keller, W.L. Adair and G.C. Ness (1979) Studies on the regulation of glycoprotein biosynthesis. *Journal of Biological Chemistry*, **254,** 9966–9969.
35. J.E. Potter and A.A. Kandutsch (1982) Increased synthesis and concentration of dolichyl phosphate in mouse spleen during phenylhydrazine-induced erythropoiesis. *Biochemical and Biophysical Research Communications*, **106,** 691–696.
36. M.J. James and A.A. Kandutsch (1980) Inter-relationships between dolichol and sterol synthesis in mammalian cell cultures. *Journal of Biological Chemistry*, **254,** 8442–8446.
37. T. Chojnacki and G. Dallner (1988) The biological role of dolichol. *Biochemical Journal*, **251,** 1–9.
38. F.P. Corongiu, M. Lai and A. Milia (1983) Carbon tetrachloride, bromotrichloromethane and ethanol acute intoxication. New chemical evidence for lipid peroxidation in rat tissue microsomes. *Biochemical Journal*, **212,** 625–631.
39. K.S. Rao and R.O. Recknagel (1969) Early incorporation of carbon-labelled carbon tetrachloride into rat particulate lipids and proteins. *Experimental Molecular Pathology*, **10,** 219–228.
40. F.P. Corongiu, M.A. Dessi, S. Vargiolu, G. Poli, K.H. Cheeseman, M.U. Dianzani and T.F. Slater (1985) Antioxidant activity of alpha-tocopherol against lipid peroxidation in rat liver microsomes. In Free Radical in Liver Injury. Edited by G. Poli, K.H. Cheeseman, M.U. Dianzani and T.F. Slater, pp. 81–86, IRL Press: Oxford.

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