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INHIBITION OF LIVER GOLGI GLYCOSYLATION ACTIVITIES BY CARBONYL PRODUCTS OF LIPID PEROXIDATION

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The present report deals with the investigation of the effect of 4-hydroxy-*trans* 2,3-nonenal (HNE), hexanal (HEX) and malondialdehyde (MDA), the major products of lipid peroxidation, on the glycosylation pathway of rat liver Golgi apparatus. Defined concentrations of the aldehydes were added to isolated fractions of formative (F_3) and secretory ($F_1 + F_2$) Golgi compartments, then incubated at 37°C for 10 min. At the end of the incubation the activity of galactosyl-(GT) and sialyl-(ST)transferases, the main enzymes of the terminal protein and lipoprotein glycosylation, was evalued. A significant impairment of both these activities was observed with HNE and HEX but not with MDA.

These data suggest that aldehydes generated during peroxidation reactions are able to impair the protein and lipoprotein maturation mechanism which is normally achieved through a complete glycosylation.

KEY WORDS: Lipid peroxidation, aldehydes, liver golgi, glycosyltransferases.

INTRODUCTION

Recent reports produced evidence that carbon tetrachloride (CCl_4) poisoning induces a very early increase in the lipid content of liver Golgi apparatus either in the isolated hepatocyte model and in the whole animal.[1,2] In both experimental conditions lipid accumulation in the Golgi apparatus occurred soon after CCl_4 treatment and in any case before being evident also in the cytosol. These findings indicated that the secretory damage at the Golgi level precedes the CCl_4-induced impairment of the lipoprotein transport by microtubules.[3]

The only slight prevention afforded by vitamin E rat pretreatment[2,4] suggested that the derangement of lipoprotein release from the Golgi apparatus due to CCl_4 is mainly dependent on mechanisms other than lipid peroxidation, i.e. the covalent binding of free radical metabolites of CCl_4 to cell structures.

It is well-known that liver Golgi apparatus is an important cell site not only for assembly and transport of lipoprotein micelles but also for their complete maturation.[5–7] The latter event includes apolipoprotein terminal glycosylation by a specific multiglycosyltransferase system.[8,9] After CCl_4 poisoning galactosyltransferase activity showed an early reduction which was significantly lower than normal at 15 min of rat

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intoxication both in the secretory and formative membranes of the Golgi apparatus.[2] The animal supplementation with vitamin E, which is able to prevent the CCl_4 pro-oxidant effect,[10] led to a complete protection against the CCl_4-induced impairment of glycosylation reactions.[4] So, the damage appears to be dependent upon lipid peroxidation and possibly involving aldehydic compounds. In fact, it is now well established that among the toxic compounds generated from the peroxidative breakdown of membrane polyunsaturated fatty acids, aldehydes are likely the most diffusible ones, able then to reach cellular targets far from their site of origin by this way amplifying the damage.[11–14] Many of these carbonyl compounds (identified as alkanals, 2-alkenals, ketones and 4-hydroxyalkenals) have been found to exert several toxic effects on enzymatic activities and metabolic cell functions.[15–17] Particular attention has been drawn on 4-hydroxy-*trans*-2,3-nonenal (HNE)[18] and hexanal (HEX). These carbonyls are produced in relatively high amounts in peroxidized liver microsomes,[19] intact hepatocytes[13] and whole liver.[20]

The present investigation deals with the demonstration that HNE and HEX, contrary to malondialdehyde (MDA), can inhibit the liver Golgi glycosylation processes, possibly through the covalent binding to galactosyl- and sialyl-transferase enzymes.

MATERIALS AND METHODS

Female Sprague Dawley rats (CD-COBS, Charles River, Calco, Italy) weighing 200 ± 25, were fed a standard synthetic diet free of antioxidants.

Groups of 8–10 animals having fasted for 16 h were sacrificed; the livers were removed, pooled, washed in ice-cold 0.25 M sucrose, forced through a tissue press and then homogenized in 0.5 M sucrose with eight strokes in a Potter Elvehjem homogenizer to give a 20% (w/v) homogenate.

The isolation of Golgi apparatus was carried out according to the method of Ehrenreich *et al.*[21] with minor modifications.[22] Three fractions of purified Golgi membranes were obtained from the total microsomal fraction by discontinuous sucrose density gradient ultracentrifugation. The pooled fractions ($F_1 + F_2$), which represent the secretory membranes, and the fraction (F_3), which represent the formative membranes of Golgi apparatus were used as a source of glycosylation enzymes. The purification of the Golgi membranes was checked by electron microscopyas previously described.[2]

The biochemical characterization was carried out by measuring the enzymatic markers of Golgi membranes and of other subcellular organelles.[1]

The effects of HNE, HEX and MDA on the glycosylation activities were assayed by incubating different concentrations of the aldehydes with the Golgi secretory ($F_1 + F_2$) or formative (F_3) fractions for 10 min at 37°C.

At the end of incubation, aliquots of each Golgi fraction were processed to measure the galactosyl-transferase and sialyl-transferase specific activities according to the methods described by Fleischer.[23,24]

UDP-galactose: N-acetylglucosamine galactosyl-transferase activity (GT) was expressed as nmol galactose transferred to N-acetyl glucosamine/h/mg protein.

CMP-sialic acid (NANA): lactose sialyl-transferase activity (ST) was expressed as nmol sialic acid transferred to lactose/h/mg protein.

Protein was determined by the method of Hartree[25] with bovine serum albumin as a standard.

All measurements were expressed as means of 6 to 8 sets of experiments ± standard deviation (SD). The differences were calculated by Student's t-test and were considered significant when the t-values corresponded to $P < 0.01$.

All products were of analytical grade and were purchased from the following sources: standard oligosaccharides from Calbiochem, San Diego, USA; unlabelled UDP galactose and CMP-NANA from Sigma Chemical Co, St Louis, USA. Dowex resins from Fluka AG, Buchs, Switzerland; UDP(^{14}C)-galactose, specific activity 337 mCi/mmol and CMP(^{14}C)-NANA, specific activity 1.8 mCi/mmol, from The Radiochemical Centre, Amersham, UK. MDA was freshly prepared from 1,1,3,3-tetramethoxypropane by incubation in 0.1 N HCl for about 16 h at room temperature and subsequent neutralization. Synthetic HEX and HNE were kindly supplied by Prof. H. Esterbauer (Institute of Biochemistry, University of Graz, Austria) as chloroform stock solutions and stored at −20°C.

The stability and the purity of the stored aldehyde solutions were well preserved, as confirmed by u.v., mass spectrometry and t.l.c. analyses (H. Esterbauer, unpublished data). Aqueous solutions of the aldehydes were freshly prepared from the stock solutions after chloroform evaporation under nitrogen flush. The concentration of each compound was measured as previously described.[13,19] In aqueous solution, at pH 6 to 8, neither condensation nor decomposition of aldehydic compounds take place at least for 6 h, as checked by u.v. and t.l.c. analyses (H. Esterbauer, personal communication). Other chemicals were obtained from BDH Italia, Milano, Italy or from Merck AG, Darmstadt, FRG.

RESULTS

The treatment of rat liver Golgi with HNE or HEX induced changes of galactosyl- and sialyl-transferase activity which are dependent on the aldehyde concentration. Table I shows that the aldehyde able to induce a significant inhibition of the glycosyla-

TABLE I

Effect of 4-hydroxy-*trans*-2,3-nonenal (HNE) on galactosyl- and sialyl-transferase activities of rat liver Golgi apparatus[a]

	HNE final concentrations (mM)						
	Control	0.025	0.05	0.1	0.2	0.3	0.5
Galactosyltransferase[b]							
$F_1 + F_2$	162 ± 18	160 ± 25 (1%)	107 ± 18[c] (34%)	84 ± 17[c] (48%)	67 ± 10[c] (59%)	38 ± 6[c] (77%)	3 ± 0.6[c] (98%)
F_3	85 ± 9	90 ± 10	81 ± 21 (19%)	50 ± 10[c] (41%)	23 ± 6[c] (73%)	18 ± 5[c] (79%)	2 ± 0.5[c] (99%)
Sialyltransferase[b]							
$F_1 \pm F_2$	56 ± 12	55 ± 14 (2%)	48 ± 11 (15%)	24 ± 3[c] (57%)	18 ± 4[c] (68%)	6 ± 2[c] (89%)	2 ± 0.6[c] (98%)
F_3	68 ± 13	67 ± 11 (2%)	51 ± 10 (25%)	28 ± 6[c] (59%)	20 ± 5[c] (71%)	15 ± 3[c] (78%)	1 ± 0.4[c] (98%)

[a] Isolated Golgi membranes have been incubated for 10 min at 37°C with or without HNE.
[b] The enzyme specific activities (see Methods) are expressed as mean values ± SD of 6 to 8 experiments. Figures in parentheses are percent inhibition as to the control (HNE untreated samples) taken as 100%.
[c] Significant as to the corresponding controls: $P < 0.01$.

TABLE II

Effect of Hexanal (HEX) on galactosyl- and sialyl-transferase activities of rat liver liver Golgi apparatus[a]

	HEX final concentrations (mM)			
	Control	0.25	0.5	1
Galactosyltransferase[b]				
$F_1 + F_2$	162 ± 18	175 ± 13	146 ± 11 (10%)	70 ± 10[c] (57%)
F_3	85 ± 9	83 ± 10 (2%)	31 ± 6[c] (74%)	18 ± 4[c] (79%)
Sialyltransferase[b]				
$F_1 + F_2$	56 ± 12	53 ± 6 (5%)	48 ± 7 (15%)	17 ± 6[c] (70%)
F_3	68 ± 13	64 ± 13 (6%)	45 ± 10[c] (34%)	25 ± 6[c] (64%)

[a] Isolated Golgi membranes have been incubated for 10 min at 37°C with or without HEX.
[b] The enzyme specific activities (see Methods) are expressed as mean values ± SD of 6 to 8 experiments. Figures in parentheses are percent inhibition as to the control (HEX untreated samples) taken as 100%.
[c] Significant as to the corresponding controls: $P < 0.01$.

tion enzymes at the lowest concentration is HNE. In fact, in the secretory fractions ($F_1 + F_2$) GT activity shows a reduction of 34%, 48%, 59% and 77% respectively at 0.05, 0.1, 0.2 and 0.3 mM HNE. In the formative fraction (F_3) the GT inactivation appears significant at 0.1 mM (41%), reaching values of 73% and 79% at 0.2 and 0.3 mM, respectively.

In a similar way HNE is also inhibiting ST activity. Moreover the aldehyde produces a total destruction of both the enzymatic activities at 0 .5 mM.

When HEX instead of HNE is used, much higher concentrations of aldehyde are needed to obtain the same rate of enzyme inactivation (Table II). In fact 1 mM HEX gives about the same inhibition than 0.2 mM HNE of GT and ST activities from both formative and secretory membranes. Only as regards (F_3) membranes the effect of the alkanal is already significant at 0.5 mM concentration.

On the other hand, MDA does not significantly decrease the examined enzyme activities when used at concentrations up to 1 mM, so confirming even in this case its lower toxicity if compared to the other aldehydes (Table III).

DISCUSSION

The results presented in this paper suggest that the CCl_4-induced impairment of glycosylation mechanisms can be, at least in part, mediated by aldehydic products of lipid peroxidation. Carbonyls other than MDA are more likely involved, probably in connection with their higher lipophilicity. In fact both GT and ST of rat liver Golgi are membrane-bound enzymes[23,24] and it has been demonstrated that the membrane concentrations of aldehydes like hydroxyalkenals or hexanal are much greater than that of MDA.[19]

These findings are supported by previous studies that stressed the high biological reactivity of HNE with membrane-bound enzymes such as glucose-6-phosphatase, cytochrome P-450, adenylate cyclase, aminopyrine dimethylase, NADPH cytochrome c reductase.[16,17,26]

TABLE III

Effect of Malondialdehyde (MDA) on galactosyl- and sialyl-transferase activities of rat liver Golgi apparatus[a]

	MDA final concentrations (mM)		
	Control	0.5	1
Galactosyltransferase[b]			
$F_1 + F_2$	162 ± 18	170 ± 15	130 ± 20[c] (20%)
F_3	85 ± 9	82 ± 11[c] (4%)	77 ± 10[c] (10%)
Sialyltransferase[b]			
$F_1 + F_2$	56 ± 12	60 ± 10	48 ± 8[c] (15%)
F_3	68 ± 13	63 ± 11[c] (7%)	59 ± 13[c] (14%)

[a] Isolated Golgi membranes have been incubated for 10 min at 37°C with or without MDA.
[b] The enzyme specific activities (see Methods) are expressed as mean values ± SD of 6 to 8 experiments. Figures in parentheses are percent inhibition as to the control (MDA untreated samples) taken as 100%.
[c] Not significant as to the corresponding controls: $P > 0.05$

Furthermore, the aldehydic products of lipid peroxidation are able to exert their cytotoxic effects at the level of both formative and secretory sides of liver Golgi membranes. There is general agreement that the glycosylation of proteins and lipoproteins starts with the addition of core sugars in the membranes of endoplasmic reticulum. The newly formed glycolipoproteins are then transferred to the Golgi apparatus, where during the progress of these molecules, glycosylation is completed by galactosyl- and sialyl-transferases which occur throughout the formative and secretory Golgi apparatus.[9] It has been shown that both transferases are mainly oriented towards the luminal side of Golgi membranes and this finding fits well with the main biological role of these enzymes that is the maturation of secreted glycolipoproteins.[24] Therefore GT and ST seem to be easily accessible to the toxic action of diffusible products of lipid peroxidation. In particular the enzyme inhibition due to HNE was observed already at concentrations of the aldehyde likely occurring in oxidative stress.[13] In conclusion, the derangement of glycosylation processes could result "*in vivo*" in the production of unglycosylated or abnormally glycosylated proteins and lipoproteins and, consequently, in changes of their functions, interactions with other molecules and secretion into the blood stream.[22] In relation to the mechanism(s) by which carbonyl compounds could inactivate Golgi glycosylation, it seems of primary interest the demonstration that aldehydes are able to covalently bind to proteins of different cell types with the formation of protein-carbonyl adducts.[27]

Whether these adducts are really produced also with GT and ST and, in the positive case, in which percent they are stable or unstable is still unknown and represents the target of our present studies.

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