Volume 228, number 1, 179–181 FEB 05560 February 1988

Phospholipid peroxidation as a factor in gallstone pathogenesis

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Received 3 December 1987

Phospholipid peroxidation markedly reduces the stability of mixed micellar systems composed of cholate, phosphatidylcholine and supersaturating levels of cholesterol. This suggests that lipid peroxidation is likely to play a significant role in the precipitation of cholesterol from gallbladder bile, thus in the pathogenesis of cholesterol gallstones. This conclusion is supported by studies of the nucleation time of cholesterol in gallbladder biles, which was significantly reduced by exposure to a stream of oxygen. This effect of phospholipid peroxidation on cholesterol solubility may occur in other biological fluids as well. In view of the increased lipid peroxidation in the elderly, it may explain the effect of age on the frequency of various diseases related to cholesterol precipitation.

Lipid peroxidation; Cholesterol precipitation; Gallstone

1. INTRODUCTION

Cholesterol gallbladder stones are a frequent disease in the western hemisphere [1]. It is only rarely fatal, yet it constitutes one of the major causes of surgery as its pharmacological treatment, even when successful, is often followed by the reappearance of cholesterol stones [2]. The detailed etiology of this disease is therefore of great importance and much work has been devoted over the last two decades towards the understanding of the role of various factors in the precipitation of cholesterol from bile. This research yielded detailed phase diagrams for model systems composed of cholesterol, phosphatidylcholine (PC) and bile salts, on the basis of which an index (CSI) was defined to describe the degree of cholesterol supersaturation [3,4]. This index, however, is of questionable significance since supersaturation of the bile is merely a necessary but not a sufficient condition for cholesterol precipitation [5]. The latter process may be induced by various heterogeneous nucleating agents in gallstone patients [6,7] and/or inhibited by antinucleating agents in normal subjects [8]. A variety of alleged promotors and inhibitors of nucleation were studied for their effect on cholesterol precipitation both in model systems and intact bile samples [9]. These studies indicate the complexity of the system in terms of the large number of factors which may affect cholesterol precipitation from it.

The present study is devoted to the possible effect of yet another factor, peroxidized lipids. Being aware of the general trend of an increase in the fraction of peroxidized lipids in the aged [10] and of the increasing frequency of gallbladder stone formation in the elderly [11], we found it of interest to study the effect of phospholipid peroxidation on the precipitation of cholesterol from its supersaturated dispersions.

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2. MATERIALS AND METHODS

Phosphatidylcholine from egg yolk was purified chromatographically according to Singleton et al. [12]. Its purity was confirmed by thin-layer chromatography and its concentration measured according to Stewart [13]. Sodium cholate (Sigma) was crystallized from ethanol. Oxidized PC was obtained by exposing a dispersion of PC (50 mM), in a solution containing

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00145793/88/$3.50

H_2O_2 (0.1 mM), $FeSO_4$ (0.2 mM), ascorbic acid (0.25 mM) and NaCl (150 mM), to ultrasonic irradiation for 30 min. The oxidized lipid was then extracted with chloroform (30 vols) and analysed for total phospholipid content [13] and for conjugated dienes (ϵ = 28000 A per mol at 233 mM, in ethanol [14]). These analyses showed that the above procedure resulted in peroxidation of 15% of the PC. Supersaturated (mixed micellar) systems containing PC, cholesterol and cholate were then made with a varying fraction of the PC substituted by the oxidized PC. This was done by mixing the appropriate volumes of solutions of PC, peroxidized PC and cholesterol in chloroform, evaporating the mixed solution to dryness, dispersing the residue in a saline solution, sonicating it to constant turbidity and then solubilizing the resultant small vesicles by cholate.

3. RESULTS AND DISCUSSION

Curve A of fig.1 describes the time dependency of the turbidity of a system made by mixing equal volumes of a sonicated dispersion of 40 mM PC and 16 mM cholesterol with a solution of 160 mM cholate in saline. As is obvious from this curve, the latter protocol initially resulted in solubilization of the vesicles, yielding essentially transparent mixed micellar solution. This system is obviously supersaturated with cholesterol (CSI = 1.3; [3]) and, as expected, it transformed spontaneously into a multiphase turbid system, similar to that observed by Kibe et al. [15] and by Halpern et al. [16] in their studies of supersaturated 'model biles'. Nonetheless, the rate of this process was very slow; the turbidity remained low for several hours, although 15 h after preparation, phase transformation did occur. The rate was markedly increased by lipid peroxidation, as is evident from curves B–F of fig.1. These curves describe the turbidity increase of systems made as in curve A, only that the solution of PC in chloroform was contaminated by increasing amounts of peroxidized phospholipid prior to being mixed with the solutions of cholesterol in chloroform. All the systems described in fig.1 were unstable. Fifteen hours after preparation they were all very turbid ($A \simeq$ 2.5). The time dependencies were all characterized by a phase of slow increase in turbidity which was then followed by a much faster turbidity increase. This happened after a period of time (τ), which can be used as an indicator for the instability of the mixed micellar supersaturated system.

The correlation between τ and the degree of lipid peroxidation, presented in the inset, suggests that lipid peroxidation destabilizes the supersaturated

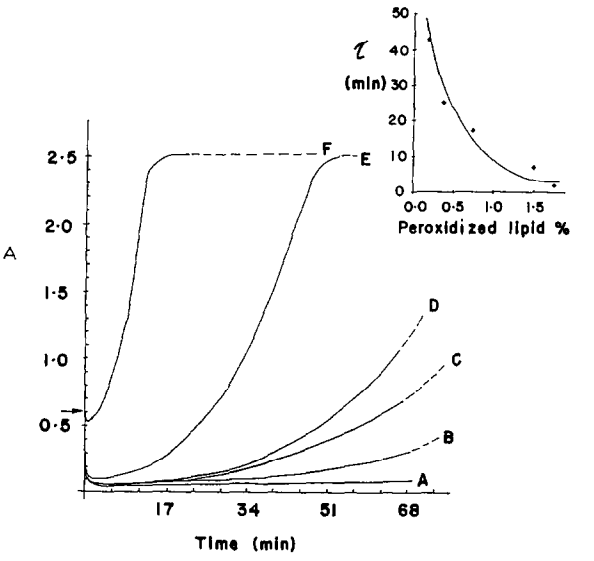

Fig.1. Time courses of the turbidity of systems containing cholate (80 mM), cholesterol (8 mM) and phosphatidylcholine (20 mM). All the systems were made by solubilizing vesicles made of PC and cholesterol by mixing them with equal volumes of cholate solutions. Prior to being solubilized, the vesicles had an A value (at 570 mm) of 0.60 ± 0.05 A, independent of phospholipid composition. The phospholipid used for the experiment of curve A was not contaminated by peroxidized PC. The phospholipid used for the other experiments was contaminated by peroxidized lipids, made as described in the text. The level of peroxidized lipid was 0.18% in B; 0.37% in C; 0.75% in D; 1.5% in E and 1.75% in F. The inset describes τ, defined as the time at which the line describing the initial increase in turbidity, following solubilization, intersects with the time describing the maximal increase in turbidity.

PC-cholesterol-cholate mixed micellar systems. To test the possibility of a causal relationship between PC peroxidation and cholesterol precipitation in the gallbladder, we have investigated the effect of exposure of native gallbladder bile samples to molecular oxygen on the nucleation time of cholesterol from them [17]. Bile samples of 8 patients were each divided into equal volumes. One part was exposed to a stream of oxygen and the other to a stream of nitrogen. Following 30 min of exposure to the gas, both samples were sealed and subsequently observed daily, until the appearance of cholesterol crystals. In the samples exposed to N_2, such crystals were observed (by light microscopy, [17]) 5.2 ± 3.0 days after sealing, as compared to 2.1 ± 1.0 days in samples exposed to O_2. Signed rank test of the difference between the

two series of bile samples resulted in a p value lower than 0.02. This of course does not prove that lipid peroxidation causes cholesterol precipitation in vivo, but it does suggest that lipid peroxidation may play a significant role in gallbladder stone formation. This has to be considered in the context of its possible contribution to the increased frequency of gallstones in the aged [11].

Furthermore, we find it very tempting to speculate that any carrier of cholesterol is likely to be destabilized by peroxidation of the lipids by which cholesterol (or cholesterol esters) is solubilized, as it brings cholesterol into a proximity with the polar hydroxy groups of the peroxidized lipids. This leads us to the working hypothesis, which should be thoroughly investigated, that peroxidation of triglyceride acyl chains of lipoproteins may play a role in the precipitation of cholesterol in blood vessels, as well.

REFERENCES

[1] Fisher, M.M. (1978) in: Gallstones (Fisher, M.M. et al. eds), p.1, Plenum, New York.
[2] Schoenfield, L.J. (1983) Harrison's Principles of Internal Medicine (Isselbacher, K.J. et al. eds) 16th edn, McGraw-Hill, New York.
[3] Carey, M.C. (1978) J. Lip. Res. 19, 945–955.
[4] Carey, M.C. and Small, D.M. (1978) Man. J. Clin. Invest. 61, 998–1026.
[5] Holzbach, R.T., March, M., Olszewszki, M. and Holan, K. (1978) J. Clin. Invest. 52, 1467–1479.
[6] Pattinson, M.R. (1985) FEBS Lett. 181, 339–342.
[7] Gollish, S.H., Burnstein, M.J., Ilson, R.G., Petrunka, C.N. and Strasberg, S.M. (1983) Gut 24, 836–844.
[8] Sewell, R.B., Mao, S.J.T., Kawamoto, T. and LaRusso, N.F. (1983) J. Lipid Res. 24, 391–401.
[9] Burnstein, J.M., Ilson, R.G., Petrunka, C.N., Taylor, R.D. and Strasberg, S.M. (1983) Gastroenterology 85, 801–807.
[10] Hirai, S., Okamoto, K. and Morimatsu, M. (1982) in: Lipid Peroxides in Biology and Medicine (Yogi, K. ed.) p.305–315, Academic Press, Orlando, FL.
[11] Einarsson, K., Nilsell, K., Leijd, B. and Angelin, B. (1985) New Engl. J. Med. 313, 277–282.
[12] Singleton, W.S., Gray, M.S., Brown, M.L. and White, J.L. (1965) J. Am. Oil Chem. Soc. 42, 53–56.
[13] Stewart, J.C.M. (1980) Anal. Biochem. 104, 10–14.
[14] Esterbauer, H. (1982) in: Free Radicals, Lipid Peroxidation and Cancer (McBrien, D.C.H. and Slater, T.R. eds) pp.101–128, Academic Press, New York.
[15] Kibe, A., Dudley, M.A., Halpern, Z., Lynn, M.P., Breuer, A.C. and Holzbach, R.T. (1985) J. Lip. Res. 26, 1102–1111.
[16] Halpern, Z., Dudley, A.M., Lynn, M.P., Nader, J.M., Breuer, A.C. and Holzbach, R.T. (1986) J. Lip. Res. 27, 295–306.
[17] Halpern, Z., Dudley, M.A., Kibe, A., Lynn, M.P. and Holzbach, R.T. (1986) Gastroenterology 90, 875–885.