Cholinesterase in the atherosclerotic intima and in fibroblast cultures

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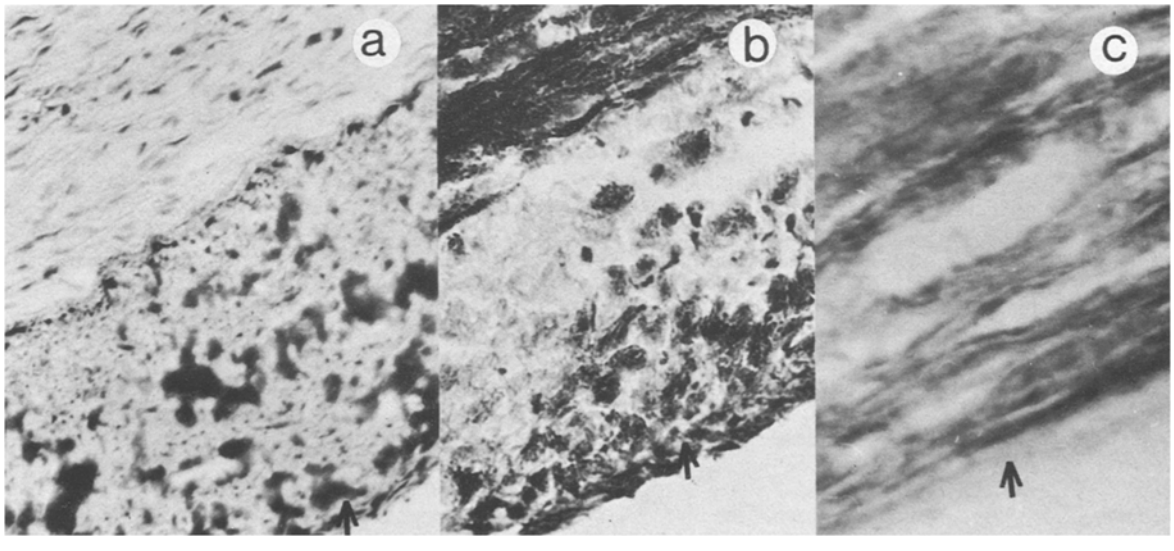
Summary. Cholinesterase activity was present in the atheromatous plaque of the rabbit's atherosclerotic aorta. Cholinesterase activity was significantly increased in rat fibroblast cultures grown in the presence of hypercholesterolemic serum. Cholesterol ester synthesis in these cultures was inhibited by neostigmine, a cholinesterase inhibitor.

Enzymes associated with the synthesis of cholesterol ester[2] and fatty acids[3] have been found to be increased in the atherosclerotic intima. Increased cholesterol ester and fatty acid synthesis have also been observed[4] in fibroblast and aortic smooth muscle cell cultures in the presence of hypercholesterolemic serum. Cholinesterase (ChE) is one other enzyme which has been implicated in the control of fatty acid metabolism[5]. In order to test this hypothesis, we examined the atherosclerotic aorta of rabbits and fibroblast grown in the presence of hypercholesterolemic serum for the changes in ChE activity.

Materials and methods. Atherosclerosis was induced in 12 rabbits by feeding them 1 g of cholesterol daily for 3 months. Another 12 rabbits were fed with only rabbit chow and used as controls. Serum cholesterol values in the cholesterol fed rabbits ranged from 800 mg to 1200 mg% with a mean of 1050 mg%. The serum cholesterol in the control rabbits varied from 50 to 80 mg% with a mean of 70 mg%. Frozen sections of aorta from the test rabbits and control rabbits were stained for lipids with oil-red-O and for ChE activity using acetylthiocholine[6] as substrate with a 6-h incubation period. Addition of eserine (10^{-4} M) completely inhibited the ChE activity. Fibroblast cultures were grown from rats (Sprague-Dawley) skeletal muscles obtained under aseptic conditions[7]. After 6 passages, the cultured cells were incubated for 48 h in media containing 10% normal serum, 10% hypercholesterolemic serum (whole serum) and 10% hypercholesterolemic serum (whole serum) + neostigmine (10^{-4} M). In the incubation studies whole sera from the normal and hypercholesterolemic rabbits were used. To each incubation flask we added 2 µCi of 2-^{14}C acetate (New England Nuclear). The cells were then washed 3 times in phosphate buffered saline (Ca^{++} and Mg^{++} free) and harvested as described previously[4]. The washed cell pellets were treated with distilled water containing 1% Triton X-100, frozen and thawed twice, and centrifuged at 3000 rpm for 20 min. The supernatant was used for further studies. ChE activity was measured using propionylthiocholine as a substrate[8]. Lipids were extracted with chloroform:methanol (2:1)[9] and separated by TLC[10]. Cholesterol ester spots were removed directly into vials containing 10 ml of the liquid scintillation counting fluid[11] and counted using Beckman LS-330 liquid scintillation system. The proteins were measured by Lowry's Method[12].

Results and discussion. Spots of ChE activity were clearly visible in atherosclerotic plaques of rabbits (figure). The sites of ChE activity corresponded with the sites of (lipid staining) of oil-red-O stained adjacent serial sections. ChE activity in normal aorta was seen in the media as shown before[13]. We also observed increased ChE activity in 2 human atherosclerotic aortas (unpublished).

In the fibroblast cultures, ChE activity was significantly increased only when cells were grown in the presence of hypercholesterolemic serum. Under the same conditions, cholesterol ester synthesis was markedly increased compared to controls grown in normal rabbit serum. The addition of neostigmine, an anti-ChE compound, to the hypercholesterolemic serum resulted in a significant decrease in fibroblast ChE activity and in cholesterol ester synthesis (table). In a separate experiment, we investigated the effect of neostigmine (10^{-4} M) on cholesterol ester synthesis in the presence of rabbit aortic homogenates using 9, 10^{-3} H (N) oleic acid (New England Nuclear)[14] incorporation into cholesterol esters. Under these experimental conditions, there was no inhibition of cholesterol ester synthetase by neostigmine.



a Lipid stained spots in the atherosclerotic intima indicated by the arrow. *b* Cholinesterase activity in the atherosclerotic intima (arrow). Marked cholinesterase activity is also seen in the media in the same section. *c* Cholinesterase activity is seen only in the media of the control aorta. Intima (arrow) shows no cholinesterase activity. All sections from arch of aorta. × 250.

The enhanced enzyme specific activity suggests that ChE is synthesized by the fibroblasts in the presence of hypercholesterolemic serum. The increased enzyme activity does not appear to be derived from the incubation media since addition of horse serum ChE (1 mg/ml) to this medium did not result in increased enzyme activity in the cultured cells. The presence of ChE in the atheroslcerotic intima could also be due to synthesis of this enzyme by intimal cells stimulated by increased cholesterol ester and fatty acid synthesis. It has been suggested[5] that the principal biological function of ChE in the liver is to remove toxic butyrylcholine formed from butyryl CoA, an intermediate in fatty acid metabolism in mammals. A similar role for ChE in the reticuloendothelial system has also been proposed[15]. This proposed function of ChE is further supported by our findings of decreased cholesterol ester synthesis by neostigmine in the fibroblasts. It has also been suggested[16] that ChE acts as a tissue growth factor by maintaining ideal conditions for fatty acid metabolism. In the atherosclerotic intima, ChE may play a similar role in relation to lipid metabolism and smooth muscle cell proliferation.

Cholinesterase activity and 1-^{14}C acetate incorporation into the cholesterol ester in fibroblast cultures in presence of normal serum (NS); NS + cholinesterase (ChE); Hypercholesterolemic serum (HCS) and HCS + neostigmine (N)

Type and No. of experiments	Cholesterol ester (cpm/mg protein)	Cholinesterase units*/ mg protein/h
A NS (3)	1150± 230 (SEM)	370±98
B NS+HChE[1] (3)	1310± 150	498±30
C HCS (4)	12150± 950	792±77
D HCS+N (3)	7830±1370	454±56

* ChE units. nmoles of thiocholine formed/mg protein/h. [1]HChE-horse serum cholinesterase. Statistical analysis by Student's t-test. Cholesterol ester C vs A and B, $p < 0.001$; C vs D, $p < 0.01$. Cholinesterase - C vs A, B and D, $p < 0.001$.

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Induction of lymphomas by urethane in combination with diethylstilboestrol in CFLP mice[1]

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Summary. The combination of urethane and the non-steroidal oestrogen diethylstilboestrol induced lymphomas in CFLP mice.

It is known that synergism can be demonstrated in the leukemogenic effect of oestradiol and urethane repeatedly administered to C57BL mice[2]. In the present paper it is reported that urethane in combination with the non-steroidal oestrogen diethylstilboestrol (DES) induces lymphomas in CFLP mice of closed bred colony.

Materials and methods. 6–8 weeks old male CFLP, as well as newborn CFLP, BALB/c, C3H/He-mg, CBA/Ca and AKR mice, obtained from the Laboratory Animals Breeding Centre of Hungary (Gödöllö) were used. The adult CFLP mice were treated with a single dose of 1000 mg/kg urethane (Reanal, Budapest) i.p. and of 50 mg/kg DES (Gedeon Richter Ltd., Budapest) s.c. at 14 days interval or simultaneously. The autopsy of those animals which seemed to be ill was performed immediately, whereas the survivors were killed within 300 days. To transplant the lymphomas induced by combined treatment, the thymomas and the pathologic spleens were carefully homogenized with Potter-Elvehjem homogenizer in M 199 medium at 37 °C and filtrated through a 10-layered gauze. In all cases, 5×10^6 cells were transplanted i.p. into 10/10 newborn mice. For the light microscopy formalin fixation, paraffin embedding and hemalaun-eosin staining, whereas for electron microscopy glutaraldehyde and OsO_4 fixation, araldite embedding and uranyl acetate and lead citrate staining were applied. The ultrathin sections were studied with JEOL 100 B electron microscope.

Results and discussion. The autopsy of mice treated with urethane and DES revealed 2 tumour types. On the one hand, the combination of urethane + DES induced malignant lymphomas in some of the mice. Results are shown in the table. The difference of the frequency of lymphomas in experimental groups against the controls proved to be significant when DES was administered simultaneously with or after urethane treatment (χ^2-test, $p < 0.01$ and $p < 0.001$, respectively). However, between the effects of the sequential combinations, no significant difference was found in respect to frequency and average latency period of lymphomas. On the other hand, multiple lung tumours developed in all animals treated with urethane, irrespective of the administration of DES. The development of lung tumours was expected according to our previous results