Effect of Sodium Citrate on the Rate of Cholesterol Esterification and on the Formation of Primary Products of Lipid Peroxidation in Human Blood. Are These Processes Related?

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In vitro experiments show that sodium citrate in a final concentration of 130 mM induces a 4- to 5-fold increase in the activity of lecithin-cholesterol acyltransferase. A parallel determination of the blood content of primary products of lipid peroxidation reveals a 20-30% decrease in diene and triene conjugates and lipid hydroperoxides.

Key Words: *cholesterol esterification; lecithin-cholesterol acyltransferase; high density lipoproteins; lipid peroxidation*

High density lipoproteins (HDL), especially HDL_3, protect low density lipoproteins (LDL) from lipid peroxidation (LPO) [1,9,10,12]. In an attempt to elucidate the mechanism responsible for the antioxidant activity of HDL_3 [1] it was found that partially purified lecithin-cholesterol acyltransferase (LCAT) from human plasma, an enzyme that catalyzes cholesterol esterification in human and animal plasma and is associated with HDL_3, hinders the formation of malonic dialdehyde during autoxidation of LDL. The addition of an enzyme inhibitor (parahydroxymercurobenzoate) or heat inactivation abolishes the antioxidant effect of the enzyme.

It has recently been reported that sodium citrate stimulates cholesterol esterification [2]. Thus, the question arises: as to whether the sodium citrate-induced acceleration of the LCAT reaction coincides with a decrease in the blood content of LPO products. If this is found to be the case, it will be additional evidence that LCAT is involved in the antioxidant activity of HDL.

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This study was performed *in vitro*. Our objective was to examine the effect of sodium citrate on the rate of the LCAT reaction in human plasma and on the formation of LPO products (diene and triene conjugates and lipid hydroxyperoxides) in the blood.

MATERIALS AND METHODS

Freshly prepared plasma from healthy donors' blood was used. The rate of cholesterol esterification was assessed from the LCAT activity.The incubation mixture contained 1 ml donor plasma and 0.05 ml sodium citrate, which was dissolved in 0.01 M Tris-HCl, pH 7.4, to a final concentration of 130 μmol/ml; it was taken into account that the plasma itself contained 16 μmol/ml citrate added as an anticoagulant. Incubation was carried out for 45 min at 37°C with constant shaking. LCAT activity was assayed in 0.01-ml aliquots; the contents of diene and triene conjugates and lipid hydroperoxides were measured in 0.2-ml aliquots. LCAT activity was determined by a previously described method [7] with modifications [15] and expressed in μmol/liter 2-^{14}C-cholesterol esterified in the plasma during a 1-h incubation.

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Diene and triene conjugates were determined in methanol-hexane extracts (5:1 v/v) spectrophotometrically at wavelengths 233 and 277 nm [8], and lipid hydroperoxides were determined as described elsewhere [4]. Statistical analysis was performed by a nonparametric method (comparison of two variables).

RESULTS

The rate of cholesterol esterification reported to be the same in citrate plasma and blood serum of the same donors [7]. This indicates that the concentration of sodium citrate generally used to prevent blood coagulation is insufficient for the activation of cholesterol esterification. Therefore, in order to stimulate the LCAT reaction we added sodium citrate to citrated donor plasma to provide a high concentration of this agent (130 μmol/ml).

This led to a 4- to 5-fold increase in LCAT activity (Table 1). The stimulatory effect of sodium citrate on enzyme activity has been demonstrated by others [7,13].

It should be stressed that in this study we determined the enzyme activity, but not the ability of plasma to esterify cholesterol [2], which depends on the enzyme activity and concentration, the concentration and properties of the substrate (HDL), cofactors (apoproteins), the presence of LCAT inhibitors, and so on. These factors probably account for the difference in the intensity of the effect of sodium citrate on LCAT activity and cholesterol esterification: 4-5-fold in our study and 33% in [2].

The results obtained allowed us to employ sodium citrate as a tool to study the activities of LCAT and lysolecithin acyltransferase and the cholesterol-acceptor properties of HDL.

Sodium citrate is known to form complexes with transition metals which activate LPO in the blood. Therefore, it was important to examine the effect of sodium citrate in the concentration activating LCAT on the LPO content in human blood.

A parallel determination of the content of LPO products revealed a decrease (by 20-30%) in the formation of diene and triene conjugates and lipid hydroperoxides (Fig. 1). Changes in the content of LPO products occurring during stimulation of the enzyme activity by sodium citrate were less pronounced, probably due to the potent antioxidant system operating in the plasma.

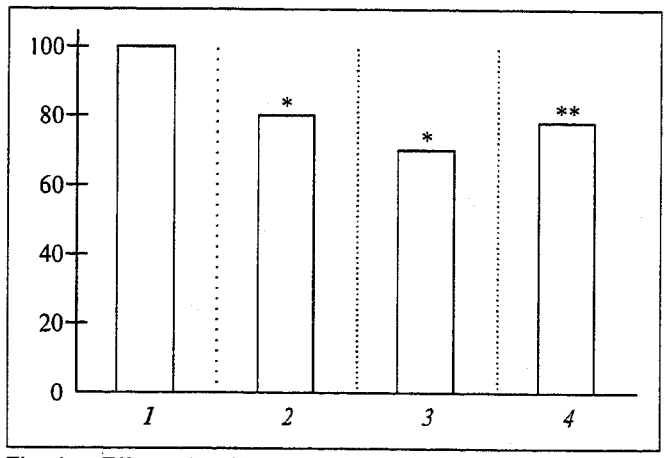

Fig. 1. Effect of sodium citrate on the content of LPO products in human plasma. *1*) control; *2*) diene conjugates; *3*) triene conjugates; *4*) lipid hydroperoxides. Ordinate: plasma content of lipid peroxides (%); *1*) the plasma lipid peroxide content without the addition of sodium citrate is taken as 100%; *2*) 125.2 μmol/liter; *3*) 49.7 μmol/liter; *4*) 49.9 μmol/liter. $^{*}p=0.02$, $^{**}p<0.01$ compared with the control.

Thus, our results indicate that high concentrations of sodium citrate stimulate LCAT activity and simultaneously suppress the formation of primary LPO products. This points to an inverse dependence between the rate of the LCAT reaction and the content of LPO products in human blood.

Recently it was found that after peroxide modification (in the presence of Cu^{2+}) HDL lose the ability to activate LCAT [11]; this effect was observed at a Cu concentration of 0.1 μM, which is two orders of magnitude lower than the physiological level, i.e., under conditions where other physicochemical properties of HDL (electrophoretic mobility in polyacrylamide gel, formation of malonic dialdehyde, apoA-I polymerization, etc.) are not altered. From these and previous [1] findings and from the published data [11] it follows that sodium citrate activation of the LCAT reaction in the plasma is probably coupled to the complexation of transition metals with sodium citrate, which abolishes the

TABLE 1. Effect of Sodium Citrate on LCAT Activity ($n=3$)

Number of experiment	Without citrate		With citrate	
	μmol esterified cholesterol/liters/h	%	μmol esterified cholesterol/liters/h	%
1	80	100	325	406
2	130	100	548	422
3	178	100	900	506

Note. Percentage changes are statistically significant at $p<0.005$.

inhibitory effect of these metals on LCAT or stimulates the ability of HDL to activate the enzyme.

The possibility that these two processes (cholesterol esterification and the formation of LPO products) in the plasma are interrelated [1] cannot be ruled out. It is known that during the LCAT reaction the readily oxidizing unsaturated fatty acid of lecithin, which is located on the surface of a lipoprotein particle in the plasma, is transferred to cholesterol with ester formation; owing to its hydrophobicity, the ester is transported to the center of the particle. Thus, the LCAT reaction facilitates the removal of a highly reactive fatty acid from the surface of the lipoprotein particle to its center, where it becomes included in a less reactive cholesterol ester [3,5,14]. Thus, the oxidation cascade on the surface of the lipoprotein particle is delayed or interrupted.

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