Effect of Serum from Cardiovascular Patients on Catalytic Activity of Secretory Phospholipase A_2 (IIA)

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Incubation of patients' serum catalytically active by type IIA secretory phospholipase A_2 (SP-IIA) with serum containing the enzyme in a high concentration but exhibiting no catalytic activity in 1:1 volume ratio led to a significant inhibition of SP-IIA catalytic activity. Donor and patient sera with low levels of SP-IIA had no effect on the serum with SP-IIA activity under these conditions. However, the increase in the content of patients' serum with a low level of SP-IIA in the incubation mixture to 1:2 (v/v) and of donor serum to 1:3 (v/v) also led to blockade of SP-IIA catalytic activity. These results indicate that human serum contains an SP-IIA inhibitor and its concentration decreases significantly in sera with SP-IIA activity.

Key Words: *type IIA secretory phospholipase A_2; inhibitor; serum; cardiovascular diseases*

The development of cardiovascular diseases (CVD) is associated with acute and chronic inflammatory processes. Serum level of type IIA secretory phospholipase A_2 (SP-IIA; a proinflammatory enzyme with normally negligible concentration) increases significantly during inflammation [4]. SP-IIA is an important inflammation mediator [5]. High level of this enzyme persisting for a long time in the serum is considered to be an independent risk factor and CVD predictor in coronary patients [2,3].

SP-IIA is secreted in catalytically active form by blood and liver cells [6]. The mechanisms of CP-IIA inactivation in human serum are unknown. We previously showed that in some cardiovascular patients the serum with high levels of CP-IIA exhibited no CP-IIA activity. These data suggest the presence of SP-IIA inhibitor in patients' blood.

We measured serum SP-IIA levels in cardiovascular patients and evaluated the effects of these sera on catalytic activity of SP-IIA.

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MATERIALS AND METHODS

The study was carried out in 5 donors and 15 patients with stable angina pectoris, functional classes II-III. Venous blood specimens were collected from the ulnar vein. The blood was centrifugated for 20 min at 3000 rpm. The sera were stored at -70°C until the study.

Serum SP-IIA was measured by enzyme immunoassay using human SP-IIA EIA kit (Cayman Chemical Company). Serum specimens were diluted with buffer from the kit in 1:20 proportion, after which 100-μl diluted samples were tested in two repetitions. EIA kit is specific for SP-IIA and does not react with phospholipase A_2 types I, IV, and V or with any of inflammation mediators (TNF, IL-1, erythrocyte activation factor). The minimum concentration detectable by EIA is 15.6 pg/ml.

In order to evaluate SP-IIA catalytic activity, water emulsion of L-3-phosphatidyl-N-methyl-^{14}C-choline-1,2-dipalmitoyl was used as the substrate (57 mCi/mol; Amersham). The studied mixture (250 μl) contained 5 nmol radioactive substrate, 20 μl serum, and 100 nM Tris-HCl (pH 8.0)

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containing 2 mM $CaCl_2$. The mixture was incubated for 30 min at 37°C and constant shaking. The reaction was stopped by adding 1.5 ml chloroform:methanol (2:1) mixture. Extracted lipids were separated by thin layer chromatography on silica gel plates (DC, Merck) in chloroform:methanol:28% NH_4OH (60:30:8) system. Lipid spots were developed by iodine vapor. Fractions corresponding to lysophosphatidylcholine were scraped off and put into flasks with 7 ml Unisolve 100 scintillation fluid (Koeh-Light Ltd.). Radioactivity was measured on a Rackbeta 125 β-scintillation counter (LKB). Catalytic activity of SP-IIA was estimated in units per liter of serum [6]. Inhibition of SP-IIA activity was evaluated by adding catalytically inert sera (10, 20, 40, 60 μl) into incubation mixture.

The results were processed using Student's test. The differences were considered significant at $p<0.05$.

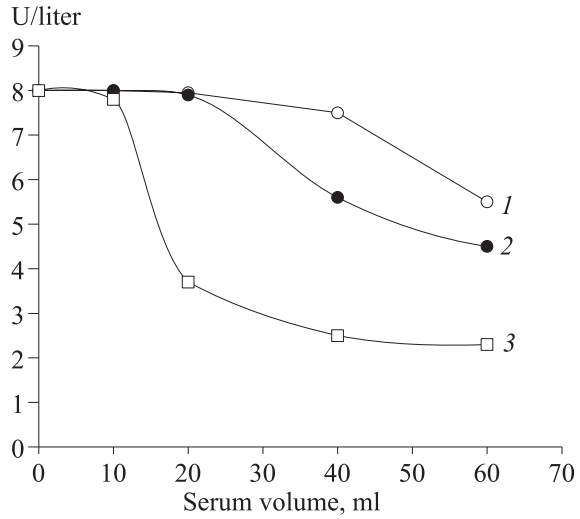

Fig. 1. Relationship between inhibition of SP-IIA catalytic activity and serum volume. *1*) donors; *2*) group 2 patients; *3*) group 2 patients.

RESULTS

The type of SP-IIA present in the serum was evaluated using manoalide (specific and irreversible SP-IIA inhibitor). Manoalide in a concentration of 0.1 μM completely blocked SP-IIA activity, preventing accumulation of radiolabeled product lysophosphatidylcholine in the reaction mixture (data are not presented), this indicating the presence of SP-IIA in patient sera.

In 5 patients whose sera showed SP-IIA activity the enzyme concentration was 4.5±1.1 μg/liter, which was significantly higher than in donors (1.40±0.25 μg/liter; $n=5$). Patients whose sera showed no SP-IIA activity formed two groups. Group 1 consisted of 5 patients with low SP-IIA concentration (2.40± 0.13 μg/liter), comparable with the enzyme concentration in the sera of donors. Group 2 consisted of 5 patients with high concentration of SP-IIA (22.56± 5.25 μg/liter).

Incubation of serum with SP-IIA activity with catalytically inert serum from patients with high enzyme concentration in 1:1 volume ratio (20:20 μl) led to significant inhibition of SP-IIA activity (Fig. 1). Normal donor serum or serum from patients with low concentration of SP-IIA had no effect on the serum with SP-IIA activity under these conditions. However, increasing the percent of serum from group 1 patients in the incubation mixture (to 1:2; 20:40 μl) and of normal donor serum (to 1:3; 20:60 μl) also led to SP-IIA blockade

These results indicate the presence of SP-IIA inhibitor in human serum, the concentration of this inhibitor decreasing significantly in the sera with SP-IIA activity.

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