Effect of Plant Extracts on *trans*-Sialidase Activity in Human Blood Plasma

D. V. Aksenov*,, V. V. Kaplun*, V. V. Tertov*, I. A. Sobenin*,**, and A. N. Orekhov**,*****

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We studied the effects of plant extracts and products of their biological treatment on *trans*-sialidase activity in human blood plasma. Extracts of kelp, fucus, yarrow, Saint John's wort, onion, and honey *in vitro* decreased *trans*-sialidase activity. Extracts of pollen (beebread) and garlic powder produced the maximum inhibitory effect. *trans*-Sialidase activity of blood plasma *ex vivo* decreased 2-fold after peroral administration of pollen and garlic powder. A correlation was found between the decrease in *trans*-sialidase activity in blood plasma and ability of blood plasma to induce cholesterol accumulation in cultured cells from the intact human aortic intima.

Key Words: *trans-sialidase; low-density lipoproteins; atherosclerosis*

Accumulation of lipids (*e.g.*, cholesterol esters) in cells of the vascular wall is a key event in the pathogenesis of atherosclerosis. Circulating multiple-modified low-density lipoproteins (cmLDL) play an important role in the accumulation of intracellular lipids [2,3]. These LDL differ from native particles by their physicochemical properties. For example, cmLDL are characterized by lower content of sialic acid, cholesterol esters, and phospholipids, higher content of lysophospholipids and diglycerides, increased lipid oxidizability and negative surface charge, and small size and increased density of particles. cmLDL demonstrate increased association capacity and high atherogenicity, *i.e.* they induce cholesterol accumulation in cultured cells. cmLDL are probably a product of enzymatic modification of the lipoprotein particle. *trans*-Sialidase of human blood plasma is one of the modifying enzymes.

*Laboratory for Mechanisms of Atherogenesis, Institute of Experimental Cardiology, Russian Cardiology Research-and-Production Complex, Russian Ministry of Health; **Laboratory of Intercellular Interactions, Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences; ***Institute of Atherosclerosis, Russian Academy of Natural Sciences, Moscow. ***Address for correspondence:*** denaks@mail.ru D. V. Aksenov

Homogenous *trans*-sialidase was isolated from human blood plasma by chromatography on polysialic acid covalently bound to agarose [13]. LDL, glycoproteins, blood plasma gangliosides, and blood cell glycoconjugates serve as donors of sialic acid for *trans*-sialidase. Glycoconjugates of plasma components and blood cells can act as acceptors of sialic acid [9].

Treatment of native LDL with *trans*-sialidase results in their desialation and ability to induce cholesterol accumulation in cells of human aortic intima [9]. It can be hypothesized that the inhibition of *trans*-sialidase can decrease the rate of LDL modification and prevent cholesterol accumulation in vascular cells. Our previous studies showed that heparin and cytosine derivatives decrease *trans*-sialidase activity [8]. Much recent attention is paid to the use of natural products for the prevention and therapy of atherosclerosis. Here we studied the effect of plant preparations on *trans*-sialidase activity in human blood plasma.

MATERIALS AND METHODS

Ethanol (70%) extracts were obtained from plants, algae, and bee products. Plant products (Krasno-

gorskleksredstva) and long-acting preparations from pollen (Pollinat) and garlic powder (Allicor, INAT-Farma) were used.

Venous blood was taken from fasting donors and placed in tubes with 1 mg/ml EDTA. The plasma was separated from cells by 15-min centrifugation at 300 rpm (TJ-6 centrifuge, Beckman Instruments). LDL were isolated from blood plasma by 2-step ultracentrifugation as described previously [9]. LDL samples were dialyzed overnight against a 4000-fold volume of 50 mM Tris-HCl (pH 7.0) at 4°C and sterilized by filtration (0.45 μ). Native LDL were separated from cmLDL by affinity lectin-chromatography on a column packed with *Ricinus communis* agglutinin-agarose (RCA_{120}, Boehringer Mannheim GmbH) [13]. Sialylated LDL glycoconjugates were tritiated in the C-8-position as described previously [14].

For isolation of LDL-depleted plasma, 6 ml NaBr solution with a density of 1.065 g/ml was layered onto 4 ml plasma brought to 1.39 g/ml with dry NaBr. Centrifugation was performed at 40,000 rpm for 15 h (50Ti rotor, Beckman Instruments). Floating LDL were removed and LDL-depleted plasma was dialyzed overnight against a 4000-fold volume of 50 mM Tris-HCl (pH 7.0) at 4°C.

Plasma *trans*-sialidase was isolated by the method of affinity chromatography. LDL-depleted plasma (700 μl) was placed on a column packed with 2 ml Sepharose with covalently bound polysialic acid (Syntesome GmbH). The column was washed with 20 ml 50 mM Tris-HCl (pH 7.0). The bound enzyme was eluted with 5 ml 5 mM sialic acid in 50 mM Tris-HCl (pH 7.0). The enzyme solution was dialyzed against 10 mM Tris-HCl for 24 h. The buffer was replaced 3 times. The enzyme was concentrated by means membrane ultrafiltration (Amicon systems) and stored at -70°C. Protein concentration in the enzyme solution was measured by the method of Lowry.

[^{3}H]-Labeled LDL or glycoconjugates covalently bound to Sepharose served as donors of sialic acid. The reaction mixture contained 10-20 μl Sepharose-bound LDL, 50 μg asialofetuin, 1 mM dithiothreitol, 2 mM $CaCl_2$, and 10 μg enzyme. The volume of the reaction mixture was brought to 0.2 ml with 50 mM Tris-HCl (pH 7.0). Incubation was performed for 4 h in darkness at 37°C and constant agitation. After incubation, 300 μl water was added and the reaction mixture was centrifuged at 4500 rpm for 10 min. The supernatant (200 μl) was sampled. Radioactivity of the supernatant was measured on a 1215 Rack-Beta liquid scintillation counter (LKB).

Subendothelial smooth muscle cells were isolated from intact human aortic intima by collagenase treatment. The cells were cultured in a CO_2 incubator (Forma Scientific) at 37°C in a humid atmosphere (95% air and 5% CO_2) [4]. Cell lipids were extracted with a hexane-isopropanol mixture [1]. Intracellular cholesterol content was measured with a test system for measurement of total cholesterol concentration (Boehringer Mannheim).

Volunteers perorally received specified doses of plant preparations. The blood was taken after 2, 4, 6, and 12 h and *trans*-sialidase activity was measured.

The significance of differences was estimated by Student's *t* test (BMDP software).

RESULTS

Table 1 presents the data on the effect of extracts from plants, algae, and bee products in various concentrations on *trans*-sialidase activity in human blood plasma. Extracts of tormentil cinquefoil roots (tormentil, *Potentilla erecta L.*) and calendula flowers (*Calendula officinalis L.*) had no effect on enzyme activity. Extracts of Saint John's wort (*Hypericum perforatum L.*) and yarrow (*Achillea millefolium L.*) in concentrations of 100-1000 μg/ml decreased *trans*-sialidase activity by 45-71%.

The extract of *Rhodiola rosea* L. root in concentrations of 100-1000 μg/ml decreased *trans*-sialidase activity by 36-44%. Plants of the *Aralia* family, Chinese ginseng (*Panax ginseng C.A. Mey*) and spiny *Eleutherococcus* (*Eleutherococcus senticosus Maxim.*), were ineffective.

Among extracts of bee products, only extracts of honey and pollen inhibited *trans*-sialidase. The ethanol extract of pollen in concentrations of 1-1000 μg/ml decreased *trans*-sialidase activity by 51-86%. The aqueous extract had a similar effect on enzyme activity.

Brown algae extracts from kelp (*Laminaria saccharina*) and fucus (*Fucus vesiculosus*) in the highest concentrations decreased *trans*-sialidase activity by 24 and 36%, respectively.

Extracts from plants of the *Lily* family decreased *trans*-sialidase activity. The ethanol extract of garlic powder (*Allium sativum L.*) in a concentration of 1000 μg/ml decreased enzyme activity by 92%. The water and chloroform extracts of garlic powder had a similar effect.

Among the test preparations, extracts of pollen and garlic powder were most potent in decreasing *trans*-sialidase activity. Hence, we studied the *ex vivo* effect of these preparations on *trans*-sialidase activity.

trans-Sialidase activity in human blood plasma decreased most significantly 4 h after pollen ad-

TABLE 1. *In Vitro* Effect of Extracts from Plants, Algae, and Bee Products on *trans*-Sialidase Activity (% of control)

Component	Concentration, μg/ml				
	0	1	10	100	1000
Extract of tormentil cinquefoil roots (*Potentilla erecta L.*)	100±7	97±6	95±6	102±5	89±6
Extract of calendula flowers (*Calendula officinalis L.*)	100±7	103±3	105±8	92±6	88±4
Extract of Saint John's wort (*Hypericum perforatum L.*)	100±7	98±5	95±7	72±5*	57±4*
Extract of yarrow (*Achillea millefolium L.*)	100±7	92±8	78±4*	54±4*	27±3*
Extract of Chinese ginseng (*Panax ginseng C.A. Mey*)	100±7	98±6	99±3	95±5	88±6
Extract of spiny *Eleutherococcus* roots (*Eleutherococcus senticosus Maxim.*)	100±7	100±7	101±8	95±6	85±8
Extract of *Rhodiola rosea L.* root	100±7	98±5	89±5	72±4*	58±5*
Extract of propolis	100±7	108±10	103±7	98±3	88±5
Extract of honey	100±7	92±5	88±4	85±8	60±4*
Extract of pollen	100±7	49±4*	43±2*	21±3*	14±2*
Extract of brown algae, sugar kelp (*Laminaria saccharina*)	100±7	89±5	85±4	81±5*	74±3*
Extract of fucus (*Fucus vesiculosus*)	100±7	90±4	81±3*	75±4*	65±2*
Extract of bulb anion (*Allium cepa L.*)	100±7	95±7	92±6	82±2*	73±6*
Extract of garlic (*Allium sativum L.*)	100±7	81±6*	63±4*	32±3*	10±4*

Note. The effects of extracts in concentrations of 0.01 and 0.1 μg/ml are not shown, since they do not modulate *trans*-sialidase activity. *Significant decrease in enzyme activity, $p<0.005$.

ministration (Fig. 1, *a*). Pollen in a dose of 250-500 mg 2-fold decreased *trans*-sialidase activity in blood plasma (Fig. 1, *b*). Apart from pollen in capsule form, we used long-acting Pollinat tablets. These tablets contain 250 mg pollen in a polymeric matrix and produce a prolonged inhibitory effect persisting for up to 12 h (Fig. 2).

We studied the effect of Pollinat on *trans*-sialidase activity in blood plasma (Fig. 2, *a*). The decrease in *trans*-sialidase activity was accompanied

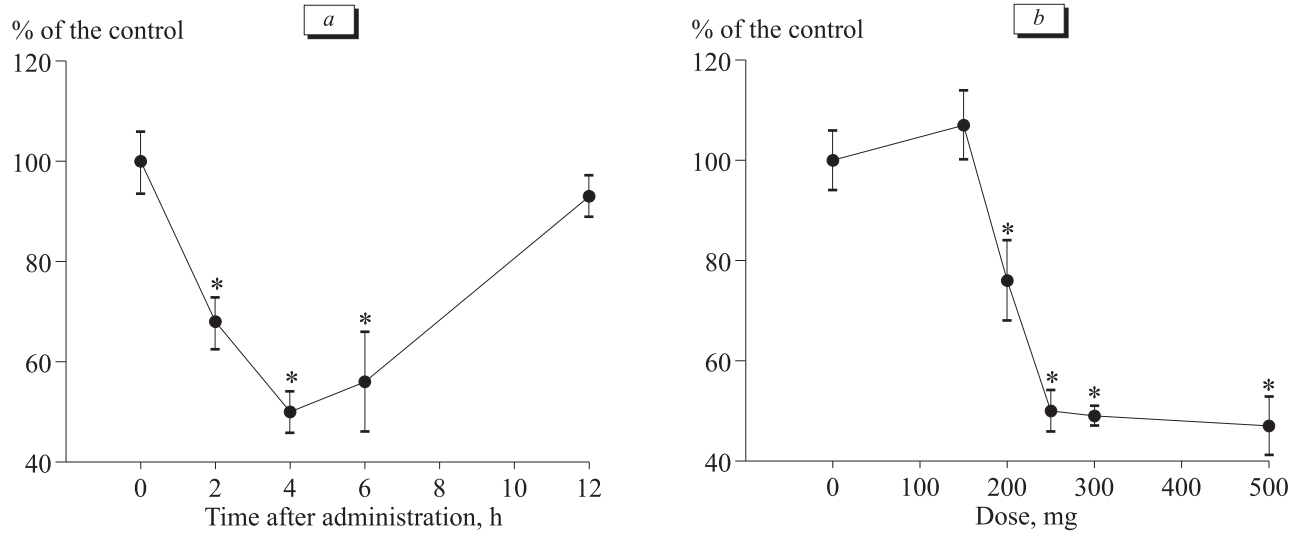

Fig. 1. *trans*-Sialidase activity in blood plasma *ex vivo* in dependence on the time after administration (*a*) and concentration of pollen (*b*). Here and in Fig. 3: *$p<0.05$ compared to the control.

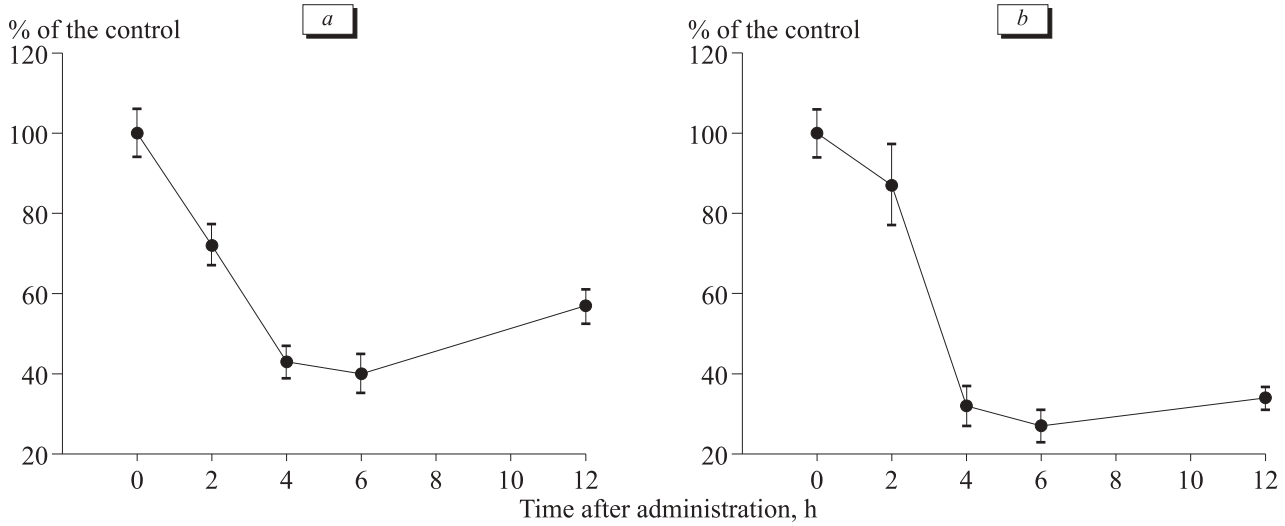

Fig. 2. Effect of the preparation Pollinat on *trans*-sialidase activity in blood plasma (*a*) and its atherogenicity (*b*).

by the reduction of plasma atherogenicity (Fig. 2, *b*). The correlation coefficient was 0.80 ($p<0.05$).

Figure 3 illustrates the dependence of *trans*-sialidase activity in blood plasma on the time after peroral administration of Allicor with 150 mg garlic powder. The inhibitory effect of the test preparation was manifested 4 h after treatment and persisted over the first 12 h.

A positive correlation was revealed between the garlic powder-induced decrease in plasma *trans*-sialidase activity and reduction of intracellular cholesterol accumulation under the influence of blood plasma from patients ($r=0.76$, $p<0.05$).

trans-Sialidase activity was measured in patients receiving the preparation of garlic powder in the morning and evening for 1 year. *trans*-Sialidase activity was high in 18 of 25 patients (group 1) and low in 8 patients (group 2). Administration of Allicor for 1 year was followed by a 32% decrease in *trans*-sialidase activity in patients with high basal enzyme activity. No significant changes in enzyme activity were found in group 2 patients.

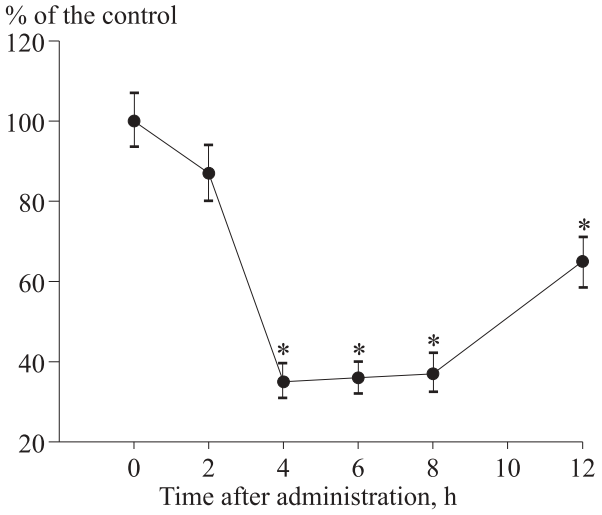

Fig. 3. Effect of the preparation Allicor on *trans*-sialidase activity in human blood plasma.

Treatment with plasma samples from group 1 patients 2.1-fold increased cholesterol accumulation in culture of intact human aortic intima cells

TABLE 2. Effect of Long-Term Treatment with Allicor on *trans*-Sialidase Activity in Human Blood Plasma (*S*±*SEM*)

Group of patients	*trans*-Sialidase activity in blood plasma, fmol/ml/min		Blood plasma atherogenicity, % of the control	
	before the start of treatment (basal level)	after 1 year	before the start of treatment (basal level)	after 1 year
With high basal activity (>75, *n*=14)	90±6	62±6*	205±17	134±15*
With low basal activity (<75, *n*=8)	40±3	40±6	115±7	108±6

Note. *$p<0.05$ compared to the basal level.

(Table 2). Plasma samples from group 2 patients exhibited no atherogenic activity. Atherogenicity of the plasma from group 1 (but not group 2) patients decreased after administration of Allicor for 1 year. The coefficient of correlation between the decrease in *trans*-sialidase activity and plasma atherogenicity was 0.75 ($p<0.05$).

Ex vivo experiments showed that the decrease in *trans*-sialidase activity correlates with reduction of plasma atherogenicity in patients receiving the preparation from pollen and garlic powder. It can be hypothesized that inhibition of *trans*-sialidase reduces atherogenicity of blood plasma. Our results should be taken into account during the development of new approaches to prevention and therapy of vascular atherosclerosis in humans.

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