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Inhibition of Platelet Aggregation under the Action of Sodium Hypochlorite. Effect of Blood Plasma Components

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The proteins fibrinogen and serum albumin and the amino acid alanine modified by sodium hypochlorite are shown to inhibit thrombin-induced aggregation of isolated platelets. The hypochlorite sodium-treated proteins and amino acids acquire the capacity to counter platelet aggregation as a result of the formation of chloramine derivatives. The aggregating capacity of hypochlorite sodium-inactivated platelets can be restored by native plasma and fibrinogen.

Key Words: *platelets; aggregation; sodium hypochlorite; plasma; chloramine; amino acids*

It has been postulated that the elimination of certain thromboses necessitates generalized inactivation of platelets through inhibition of their function regardless of the nature of the stimulus and the mechanism of cellular signaling [5]. Clearly, the structure of the platelet plasma membrane as a whole needs to be transformed (e.g., by chemical modification of sulfhydryls and amino groups) if such inhibition is to be achieved.

In recent years, an intensive search has been underway for methods by which platelet concentrates intended for transfusion can be best stored. The storage life of platelets is limited, in particular, because of their activation under the influence of difficult-to-control factors [10]. Hence the need for platelet-inhibiting agents that would meet two requirements: inhibit platelet activity for a prolonged period and allow its rapid restitution at the time of transfusion.

One candidate for such an agent appears to be sodium hypochlorite (NaOCl). This compound forms in the body as a product of the myeloperoxidase reaction when neutrophils are activated [9]. NaOCl ions effectively react with sulfur-containing chemical groups and with amino groups [3,11,12]. Our earlier studies [2,3] showed that the NaOCl contained in a platelet-enriched plasma sample impedes platelet aggregation and that its presence there may elicit reversible interaction between the platelets and plasma components, suggesting a potential for reactivating these cells.

In the present study we explored the possibility of restituting the activity of NaOCl-inactivated platelets by plasma and its individual components and, conversely, of inhibiting platelet activity by plasma components modified by NaOCl ions.

MATERIALS AND METHODS

Human thrombin from the Chronolog reagent kit, bovine fibrinogen (Serva), human serum albumin (Serva), and alanine (Reanal) were used. NaOCl was obtained by electrolysis of a 0.9% NaCl solution.

Isolated platelets were obtained from platelet-enriched rabbit plasma [6]. Briefly, EDTA (1 mM)

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was added to the plasma and the cells were precipitated by centrifugation at 1850 g for 6 min, washed in the incubation medium supplemented with 0.5 mM EDTA, and finally suspended in the incubation medium. This was composed of 134 mM NaCl, 5 mM KCl, 0.4 mM Na_2HPO_4, 0.1 mM NaH_2PO_4, 1 mM $MgSO_4$, 10 mM HEPES, and 5 mM glucose (pH 7.4).

Platelet aggregation was measured by turbidimetry after Born [1,7] after adding 0.1 ml $CaCl_2$ (1 mM) and then thrombin (to a final concentration of 0.1 activity unit per ml) to the suspension, which contained platelets in a concentration close to that in the original platelet-enriched plasma. The quantitative index of platelet aggregability was the maximal change in the transmittance of the suspension recorded 5-7 min after the addition of thrombin. The data were presented as the percentage ratio $\Delta T/\Delta Tc$, where ΔTc is the change in the transmittance of the suspension of native (control) cells and ΔT is the change in that of the suspension of NaOCl-treated cells. In tests run to evaluate the effects of plasma and fibrinogen on platelets, the cells were washed free of these substances using the procedure described above for the isolation of cells from the platelet-enriched plasma.

RESULTS

We used the proteins serum albumin and fibrinogen and the amino acid alanine both in tests with platelet inhibition and in those with platelet reactivation. These three plasma components were selected for testing because albumin constitutes the major protein fraction of plasma, fibrinogen binds to the platelet membrane and can form intercellular bridges in platelet aggregates [8], while alanine is one of the amino acids that circulate in the blood in abundance.

The anti-aggregatory effect of each NaOCl-modified plasma component was measured using two samples. One of these was a mixture of platelets and of the compound in question at concentrations more or less equal to their concentrations in the original platelet-enriched plasma. To this mixture NaOCl was added in a concentration so selected as to markedly weaken platelet aggregation. In preparing the other sample, the plasma component in the form of a concentrated solution was first modified with NaOCl (the ratio of their concentrations was the same as in the first sample), after which the modified component was added to the platelet suspension in the same final concentration as in the first sample. As shown in Table 1, both proteins and alanine displayed pronounced anti-aggregating activity: the degree of aggregation decreased by 40-20% after NaOCl was added to the solution (sample 2) and by 70-50% after it was added to the mixture (sample 1). In the case of platelet-enriched plasma, that part of the anti-aggregatory effect mediated by the plasma [3] appears to be due to the modification of many proteins and amino acids.

One product of the reaction between NaOCl and alanine should be chloramine derivatives [4,11] in which the chloramine group can be reconverted into an amino group with the formation of chloride ion under the action of reduced glutathione [12]. It follows from the data in Table 1 that the anti-aggregating activity of the modified alanine will be drastically reduced by adding reduced glutathione to a NaOCl-containing alanine solution. This implies that the anti-aggregatory properties of NaOCl-modified alanine are determined by the chloramine group. The same is possibly true of proteins.

Next, we studied the action of alanine modified under conditions conducive to the formation mainly of alanine monochloramine (chloroalanine) [4]. For this, a concentrated alanine solution (120 mM) was rapidly introduced into the NaOCl solution (12 mM) in a ratio of 10:1.2 by volume. The degree of platelet aggregation was 100% without chloroalanine, 48±5% after the addition of 50 μM chloroalanine, and 1.6±1% after 100 μM chloroalanine was added. These results show that chloroalanine and NaOCl have comparable capacities for inhibiting the aggregation of isolated platelets.

In tests aimed to evaluate the restitution of aggregability in modified platelets, we first mixed 0.3 ml of platelet suspension and 0.3 ml of NaOCl and added 2.4 ml of plasma to the mixture 1 min later. For tests with plasma components, we mixed 0.8 ml of platelet suspension with 0.1 ml NaOCl and added 0.1 ml of the solution of the test compound 1 min later. The final NaOCl concentration was 30-40 μM. Since the procedure for measuring platelet aggregation included preliminary washing of these cells, an additional control sample was tested. This comprised platelets modified with NaOCl and then washed once in the EDTA-containing medium and finally suspended in the incubation medium described above. After a short-term (for 5 min) incubation of the modified platelets with native plasma, the degree of their aggregation increased from 52±2% to 109±6% and did not differ from that of native cells. The NaOCl-modified platelets washed in the EDTA-containing medium also showed increased aggregability, but the increase was much smaller

Table 1. Anti-Aggregating Activity of Plasma Components Modified by Sodium Hypochlorite

Compound	Final NaOCl concentration, μM	$\Delta T/\Delta Tc$, %	
		NaOCl added to mixture of cells and the compound	NaOCl added to solution of the compound
Albumin, 4%	300	31±4	68±6
Alanine, 1.2 mM	35	59±2	58±5 (100±10)
Fibrinogen, 0.35%	220	57±5	81±5

Note. Figures in parentheses indicate the degree of aggregation observed when reduced glutathione was added (25 μM) to the solution of NaOCl-modified alanine.

(66±5% *vs.* 52±3%). After albumin (4%) or alanine (1.2 mM) was added to the suspension of modified platelets in a concentration close to their levels in blood plasma, the degree of aggregation was 60±2% and 47±8%, respectively, *vs.* 63.2% and 52±2% observed for the original samples, i.e., the platelet aggregability slightly decreased. It is of interest that treatment of modified platelets with fibrinogen (0.35%) led to a considerable increase in their aggregability (from 52±3% to 85±5%), this increase being significantly greater than in the control tests with washing described above.

The present results indicate that native plasma is capable of effectively eliminating the modifications undergone by platelets under the direct action of sodium hypochlorite, and that one of the plasma components responsible for this effect is fibrinogen. This special property of fibrinogen, not shared by albumin, is possibly due to its capacity to be adsorbed onto the plasma membrane. However, since fibrinogen does not restore platelet aggregability completely, certain other plasma components must also possess aggregation-restituting capacity.

In summary, the proteins fibrinogen and serum albumin and the amino acid alanine are capable, when modified by NaOCl, of inhibiting thrombin-induced platelet aggregation, and this capacity of the modified plasma components is due in large measure to the formation of chloramine derivatives. The platelet aggregability suppressed by NaOCl can be restored by treatment with native plasma or fibrinogen.

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