Structure and Oxidation Capacity of Amino Acid Chloramine Derivatives and Their Effects on Platelet Aggregation

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Comparison of antiaggregation capacity of N-chloramine acids with different position of the chloramine group in the molecule showed that in the most efficient compounds the distance between the chloramine and carboxyl groups was 3-5 carbon atoms. This feature of antiaggregation activity was not related to the difference in oxidation capacity of N-chloramine acids. It was hypothesized that the revealed structural dependence of antiaggregation activity of N-chloramine acids is determined by the structure of platelet membrane, in particular, the presence of a negatively charged group near the site of interaction between N-chloramine acids and platelet membrane.

Key Words: *chloramine derivatives of amino acids*; *platelets; aggregation; chemiluminescence; luminol*

In living organism hypochlorous acid (ionized form: hypochlorite anion) is produced by phagocytes in the reaction catalyzed by myeloperoxidase. The interaction of hypochlorite with amino groups of free amino acids or peptides yields chloramine derivatives of the corresponding compounds [7,9,13]. During activation of neutrophils hypochlorite and chloramine derivatives of biogenic compounds can be locally accumulated in very high concentrations (up to 0.1 mM) [12].

Both hypochlorite and N-chloramine acids modulate activity of blood cells [8,11]. The chloramine derivatives of amino acids react with sulfur-containing groups and modify platelet membrane, which results in generalized inhibition of functional activity of platelets (inhibition of its activation irrespective of agonist nature) [1-4,10]. Inhibition of aggregation of the platelets depends on their structure and physicochemical parameters of N-chloramine acids such as their molecular weight and van der Waals volume. In platelet-rich plasma the antiaggregation capacity markedly increases with decreasing the molecular weight in the following order: N-chloroserine, N-chloroalanine, and N-chloroglycine. Probably, this relationship results from facilitated interaction of these N-chloramine acids with active chemical groups in the narrow "pocket" of the platelet membrane. N-Chloramine acids with greater molecular weight ranging from N-chlorophenylalanine to N-chloroleucine (198.5-164.5 D; van der Waals volume 0.169-0.151 nm^3) produce less pronounced effect on platelets [1].

Our aim was to study the effect of N-chloramine acids of the same molecular weight and with different distances between the chloramine and carboxyl groups and characterized by different oxidation capacity on platelet aggregation.

MATERIALS AND METHODS

We used commercial chemicals ADP, luminol (Sigma), sodium hypochlorite (Aldrich), and amino acids (Reanal).

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N-Chloramine acids were produced by interaction of sodium hypochlorite with amino acid solution in molar concentration, which exceeded the concentration of hypochlorite by 10%. The synthesis of N-chloramine acids was evaluated spectrophotometrically by absorption peak at 252-255 nm. The concentrations of these acids were determined by iodometric titration [1].

Rabbit platelets were examined in platelet-rich plasma (PRP). To obtain PRP, the blood drawn from rabbit marginal ear vein was stabilized with 3.8% sodium citrate (9:1 v/v) and centrifuged at 460*g* for 20 min. The supernatant cell suspension was used as PRP.

Platelet aggregation was measured using the turbidimetric method according to Born on an aggregometer constructed on the basis of a KFK-2MP electrophotometer (λ=670 nm). Chloramine derivatives of amino acids were incubated with PRP for 5 min before addition of ADP (10 μM). The quantitative index of platelet aggregation capacity was the maximum change in light transmission (ΔT) of their suspension recorded 7 min after addition of ADP. The index of the inhibitory action of the examined agents was the degree of inhibition $(\Delta T_C-\Delta T)/\Delta T_C$, where ΔT_C and ΔT were the changes in light transmission in the control and experimental samples, respectively.

The oxidation capacity of chloramines was compared by analyzing the kinetic curves of luminal-dependent chemiluminescence induced by these agents [5,6].

Chemiluminescence was measured via chemiluminescence channel of a P.I.C.A lumiaggregometer (Chrono-Log Corp.). To this end, luminol (0.1 ml) was rapidly introduced into chloramine solution (0.4 ml), the mixture being persistently agitated with a magnetic stirrer.

RESULTS

Antiaggregation effect of chloramines decreased with increasing the molecular weight from N-chloroalanine to N-chloroleucine (Fig. 1), which agrees with published data [1]. However, sometimes chloramines of the same molecular weight produced different antiaggregation effect. For example, chloramine derivatives of serine and GABA have similar molecular weight, but N-chloroGABA was more efficient in inhibiting platelet aggregation than N-chloroserine (Fig. 1). These N-chloramine acids differ in the position of chloramine group. By contrast to N-chloroserine, the chloramine group of N-chloroGABA is distanced from the carboxyl group by three carbon atoms. In the following experiments, we used two pairs of N-chloramine acids (N-chloro-α-alanine/N-chloro-β-alanine and N-chloroleucine/N-chloramine-ε-caproic acid) with the same molecular weight, but different distance between the chloramine and carboxyl groups (by one and five carbon atoms, respectively). These pairs of chloramines with identical molecular weight produced different antiaggregation effects on platelets, which was greater in the molecule with larger distance between the chloramine and carboxyl groups (Fig. 1). The most potent antiaggregation effect was produced by N-chloramine acids, where the distance between the functional groups was 3-5 carbon atoms (Fig. 2).

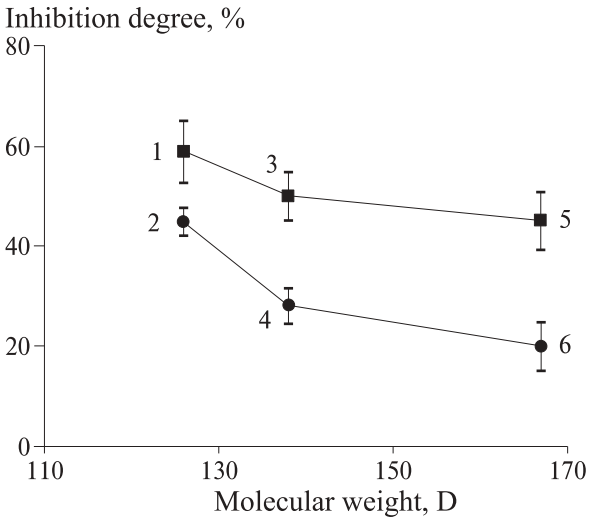

Fig. 1. Inhibitory action of N-chloramine acids with different position of chloramine group on aggregation of rabbit platelets in the platelet-rich plasma. The final concentration of N-chloramine acids was 0.5 mM. *1*) N-chloro-β-alanine, *2*) N-chloro-α-alanine, *3*) N-chloroGABA, *4*) N-chloroserine, *5*) N-chloramine-ε-caproic acid, *6*) N-chloroleucine.

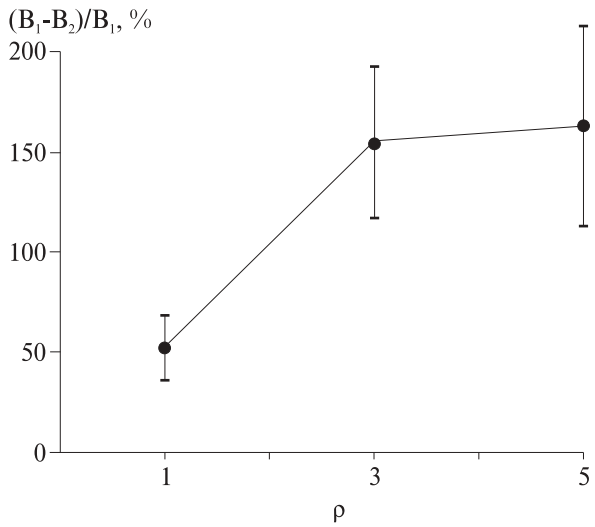

Fig. 2. Antiaggregation effect of N-chloramine acids with different distance between chloramine and carboxyl groups. ρ: number of carbon atoms between chloramine and carboxyl groups; B_1: degree of platelet aggregation inhibition induced by N-chloro-β-alanine, N-chloroGABA, or N-chloramine-ε-caproic acid; B_2: degree of platelet aggregation inhibition induced by N-chloro-α-alanine, N-chloroserine, or N-chloroleucine, respectively.

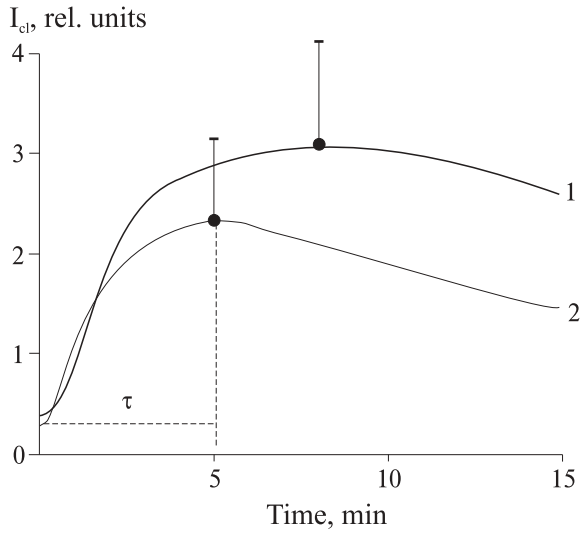

Fig. 3. Time dependence of luminol chemiluminescence (I_{cl}, 0.01 mM) induced by N-chloroGABA (*1*) or N-chloroserine (*2*) applied in concentration of 0.5 mM. τ is time to peak. The data were averaged from 5 independent experiments.

Theoretically, this structural phenomenon can be explained by strengthening of oxidation capacity of N-chloramine acids with increasing the distance between the chloramine and carboxyl groups. To test this hypothesis, we compared oxidation capacity of N-chloroGABA and N-chloroserine. It was previously established that oxidation capacity of N-chloramine acids could be assessed by chemiluminescence method [6]. Luminol chemiluminescence induced by N-chloramine acids lasted for tens minutes and had a maximum value (Fig. 3). The parameter inverse to the time of attaining the peak of luminol chemiluminescence induced by N-chloramine acids ($1/\tau$) reflects oxidation capacity of chloramine compounds [6]. For the analysis of oxidation capacity of N-chloramine acids, $1/\tau$ index should be measured for the same concentrations of the analyzed chemicals. In the luminol (0.01 mM)—chloramine (0.5 mM) system, $1/\tau$ were 0.20±0.04 and 0.125±0.025 min^{-1} for N-chloroserine and N-chloroGABA, respectively. This difference is significant according to the Mann—Whitney test ($p<0.05$), so the oxidation capacity of N-chloroserine is greater than that of N-chloroGABA. However, the latter agent exerted more potent antiaggregation effect on platelets (Fig. 1), so different antiaggregation potency of N-chloramine acids cannot be explained by their differences in oxidation capacity. Probably, the increase in antiaggregation potency with increasing the distance between the chloramine and carboxyl groups results from peculiarities in the structure of platelet membrane affecting exposure of the sulfur-containing target groups.

Thus, the degree of inhibition of platelet aggregation by N-chloramine acids depends not only on the molecular weight, but also on the distance between the chloramine and carboxyl groups. Probably, there is a negatively charged group in the sensitive site of the platelet membrane, which impedes interaction of chloramines with adjacent sulfur-containing groups.

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