Antistreptokinase antibodies influence Adenosine diphosphate-inducible platelet aggregation

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Abstract: The objective of the study was to investigate the effect of streptokinase/antistreptokinase antibodies on Adenosine 5'-diphosphate (ADP)-inducible platelet aggregation under steptokinase treatment.
ADP-inducible platelet aggregation was determined using light transmission aggregometry (LTA). Baseline rabbits (7) and subjects after intravenous infusion of streptokinase (7) were observed. The presence of antistreptokinase antibodies and plasminogen activators inhibitor type 1 (PAI-1) were determined using ELISA. 7 days after intravenous infusion of streptokinase (Sk) ADP-inducible platelet aggregation in platelet rich plasma (PRP) did not increase baseline level, and was inhibited by Sk in a dose-dependent manner. Sk in a concentration of 16 mkg/ml was associated with significantly reduced ADP-inducible platelet aggregation. Concentration of PAI-1 was significantly elevated in platelet fraction. ADP-inducible platelet aggregation peaked at 7 weeks after intravenous infusion of Sk; Sk-inducible inhibition was not observed. 7 days after administration of Sk ADP-inducible platelet aggregation was normal, although PAI-1 level was elevated in platelet fraction. Platelet aggregation in response to ADP was significantly increased at 7 weeks after Sk administration when compared to baseline level. Inhibition effect of streptokinase on ADP-inducible platelet aggregation was not observed.

Key words: haemostatic system, streptokinase, platelets, ADP-inducible platelet aggregation

INTRODUCTION

Streptokinase is a bacteria-derived protein and one of the plasminogen activators currently available for therapeutic use. While effective in thrombolysis, the antigenic profile and propensity of Sk to cause hemorrhagic complications led to the search for the best treatment option for a given disorder [1-3]. Low titers of antistreptokinase antibody are ubiquitous in human subjects due to past streptococcal exposure or Sk administration. In the presence of a high titer of antibody, the usual dose of Sk may be ineffective, and fail to activate the fibrinolytic system. Furthermore, exposure to Sk induces synthesis of specific antibodies that may initiate platelet aggregation and paradoxical clot propagation during treatment [4, 5]. Whereas atherothrombotic events are associated with platelet reactivity, there is insufficient data available on this aspect [6-8]. We investigated the effect of Sk on ADP-inducible platelet aggregation and PAI-1 concentration in platelet fraction as an indicator of platelet reactivity.

MATERIALS AND METHODS

Animals. The study population comprised 7 rabbits aged no older than 2 years. To mimic a clinical situation all subjects received streptokinase intravenously. Sk at 22,000 IU/kg of body weight was administered [9, 10], after the baseline

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blood samples had been drawn. Further samples were taken at 7 days and 7 weeks after Sk infusion.

Blood sampling. Blood was drawn by clean venipuncture from an ear vein using a 20-gauge butterfly needle (0.9 × 26 mm; Medicare, UK) into a Corning Centrifuge Tube containing 3.8% sodium citrate (9 parts whole blood, 1 part sodium citrate). All samples were mixed by gently inverting the tubes. Time interval between blood sampling and testing did not exceed 3 h.

Immuno Soluble Assay. In order to exclude factors affecting antibodies formation in response to Sk infusion, we determined titers of antistreptokinase antibody prior to administration of the thrombolytic agent. 7 days after Sk infusion, antistreptokinase antibody titers were not observed in the whole population. 7 weeks after thrombolytic agent administration, streptokinsse-specific antibody titers approached 1:100. PAI-1 concentration in platelet fraction was determined using ELISA.

Light transmission aggregometry. PRP was obtained according to [11, 12] by centrifugation of citrate-anticoagulated whole blood at 150 × g for 20 min at room temperature. Platelet-poor plasma (PPP) was obtained from the remaining specimen by re-centrifugation at 2,000 × g for 10 min. The baseline optical density was set with PPP. The maximal aggregation response was registered and used to differentiate between baseline group and group after Sk administration. ADP-inducible platelet aggregation was examined using aggregometer AP2110 c.c. "Solar" Belarus. Streptokinase was purchased from Belfarm, Belarus. Platelet aggregation was induced by 2.5 mkM ADP (Renam; Russia). Platelet count

was adjusted to 200,000 per 1 ml with PPP for each separate sample.

Statistical analysis. Data are expressed as means ± standard deviation (SD). Data were analyzed by SPSS for Windows package (version 16.0, SPSS Inc.) For comparison of ADP-inducible platelet aggregation under Sk treatment the Mann-Whitney U-test was used since the distribution of the data were not normal. For the comparison of ADP-inducible platelet aggregation before and 7 days and 7 weeks after Sk infusion, one-way ANOVA followed by the *post-hoc* Bonferroni's test was used. PAI-1 concentrations in platelet fractions before and 7 days after intravenous infusion of Sk were compared via an independent t-test. For all statistical assessments a value of p < 0.05 was accepted to be statistically significant. Histograms of ADP- inducible platelet aggregation are typical for the series of the repeated experiments (at least 3 in every series).

RESULTS

Titers of antistreptokinase antibody were not detected in the study population 7 days after Sk administration. ADP-inducible platelet aggregation in PRP of baseline group (A) and subjects after thrombolytic agent administration (B) were used as controls. We showed that inhibition effect of Sk was dose-dependent. The most significant inhibition of ADP-inducible platelet aggregation was observed at 16 mkg/ml of thrombolytic agent (Figure 1). ADP-inducible platelet aggregation was much
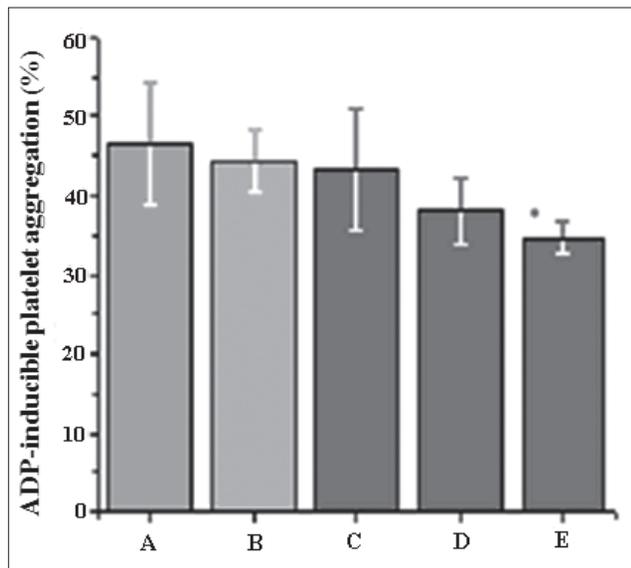
Figure 1 ADP-inducible platelet aggregation in PRP of baseline rabbits (before Sk administration) (A); in PRP taken at 7 days after intravenous infusion of Sk (B); in PRP taken at 7 days after intravenous infusion of Sk and stimulated by Sk in concentration of 12 mkg/ml (C), 14 mkg/ml (D), 16 mkg/ml (E).
Levene statistic: F=4.999, df1=3, df2=24, p<0.008;
Kruskal-Wallis: df=3, p<0.002;
*Mann-Whitney U test: p<0.001 relative to (B).

less affected by lower doses of Sk. Higher concentrations of thrombolytic agent showed the same statistically insignificant results (data not shown). ADP-inducible platelet aggregation did not differ significantly between baseline group (44.3±3.6%) and group after Sk administration (46.6±7.6%).

To estimate the reactivity of the platelets 7 days after Sk administration, we determined PAI-1 concentration in platelet fraction after 1 hour incubation at 37°C (Figure 2). It was shown that Sk administration increased PAI-1 level in platelet fraction from 41±5 ng/ml (baseline) to 95.8±7.4 ng/ml (p≤0.002).
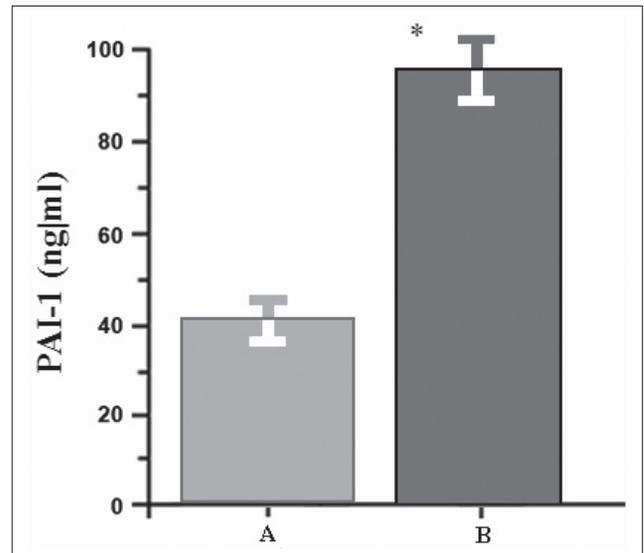
Figure 2 PAI-1 concentration in platelet fraction before (A) and 7 days after intravenous infusion of Sk (B)
* p < 0.001 relative to (A).

There was no significant difference in Sk-inducible platelet aggregation between baseline animals and those after thrombolytic agent administration. In neither group did we observe significant platelet aggregation in response to Sk (Figure 3).

The further PRP samples were taken at 7 weeks after thrombolytic agent administration modeling. ADP-inducible platelet aggregation approached 81±5%, considerably
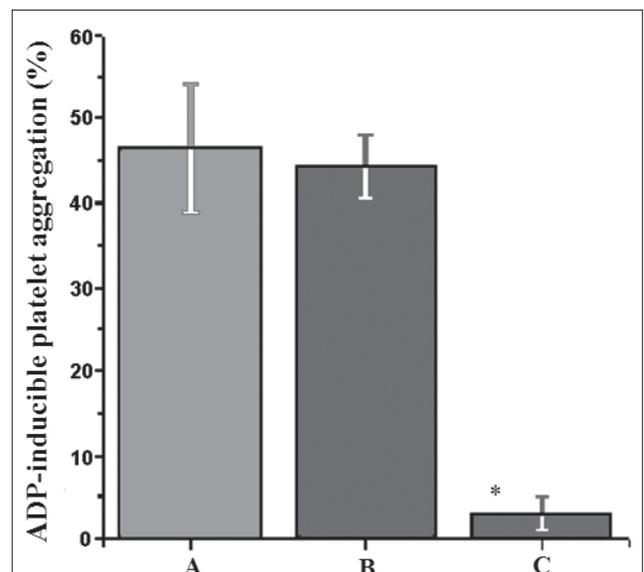
Figure 3 ADP-inducible platelet aggregation in PRP of baseline rabbits (before Sk administration) (A); in PRP taken at 7 days after intravenous infusion of Sk under treatment with ADP (B) and Sk (C)
Levene statistic: F=5.640, df1=2, df2=18, p<0.013;
Kruskal-Wallis: df=2, p<0.001;
*Mann-Whitney U test: p<0.001 relative to (B).

exceeding the control value (46±7%). The titers of antistreptokinase antibody were 1:100 according to the data of ELISA assay. Inhibition effect of Sk on ADP-dependent platelet aggregation was not observed. Sk in a concentration of 16 mkg/ml and 32 mkg/ml caused an insignificant increment in the aggregation level (Figure 4).
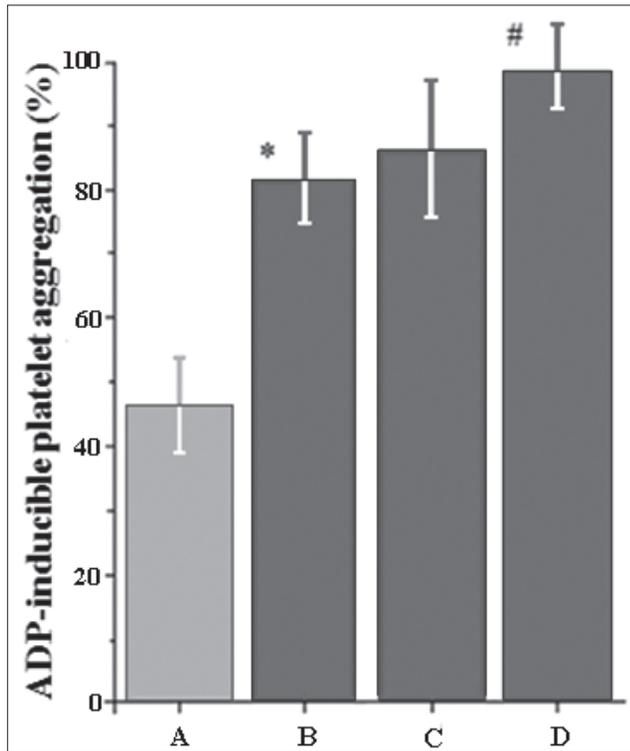

Figure 4 ADP-inducible platelet aggregation in PRP of baseline rabbits (before streptokinase administration) (A); in PRP taken at 7 weeks after intravenous infusion of Sk (B) in PRP taken at 7 weeks after intravenous infusion of Sk and stimulated by Sk in concentration of 16 mkg/ml (C) and 32 mkg/ml (D).
Levene statistic: F=1.871, df1=2, df2=18, p<0.183;
ANOVA: F=86.019, p < 0.001;
*Bonferroni: p < 0,001 relative to A.
Levene statistic: F=9.365, df1=2, df2=18, p<0.002
Kruskal-Wallis df=2, p<0.001
#Mann-Whitney U test: p<0.001 relative to (B)

DISCUSSION

LTA and ELISA assay was used to determine platelet reactivity. LTA is considered the standard method for the assessment of platelet aggregation ability; its limitations, however, comprise operator dependency and lack of standardization. In consideration of this, we also assessed platelet reactivity by PAI-1 secretion using ELISA. Several studies have shown that PAI-1 levels are elevated in patients with coronary heart disease, and may play an important role in the development of atherothrombosis by decreasing fibrin degradation [13, 14]. Increased expression of PAI-1 is found in atherosclerotic lesions. Thus, PAI-1 may be associated with the development of atherothrombotic cardiovascular disease, not only systemically, but also locally [15]. The concentration of PAI-1 in platelets accounts for more than 90% of the blood PAI-1. These data suggest that platelet PAI-1 might be released in large amounts into the plasma, resulting in an increase in thrombus formation [16].

Only baseline animals without titers of antistreptokinase antibody were included to the study [17-21]. We assessed

ADP-inducible platelet aggregation at 7 days after administration of Sk, when high titers of antistreptokinase antibody should not have occurred. 7 weeks after the infusion of Sk an enhancement of the titers was expected. It was noticed that there was no correlation between the amount of streptokinase (Belfarm; Belarus) and its activity. For *in vitro* experiments we used the amount values (mkg/ml) to standardize effects caused by Sk at 7 days and 7 weeks after administration of the thrombolytic agent.

7 days after streptokinase infusion, ADP-inducible platelet aggregation did not differ from the baseline level. A clear dose-dependent manner of platelet aggregation inhibition by streptokinase was observed. The agent in a concentration of 16 mkg/ml caused the most significant reduction of ADP-inducible platelet aggregation. Our data are related to several previous reports [22-24]. However, 1 hour incubation at 37°C of platelet fraction revealed increased PAI-1 level when compared to baseline. This may be attributable to the increased platelet reactivity. Sk-inducible platelet aggregation in PRP samples was not observed. It should be noted that Sk was used in a concentration of 32 mkg/ml, which exceeded the generally accepted therapy dose.

ADP-inducible platelet aggregation was significantly increased in the presence of a high titer of antistreptokinase antibody (7 weeks after Sk administration), when compared to the initial group.

In previous reports we showed that Sk in the presence of a high titer of antibody affected platelet reactivity in PRP without plasminogen [25]. This correlates with current data and may be attributable to the fact that Sk effects are not limited by its presence in the circulation. As platelet reactivity to ADP is associated with acute coronary syndrome [26, 27], a constellation of factors associated with platelet reactivity and streptokinase effects may contribute to a new clot formation.

The current study has some limitations: the relatively small number of animals and the lack of human ADP-inducible platelet aggregation data under the same conditions. Despite these limitations, the following recommendations may be given:
– the titer of antistreptokinase antibody should be assessed before Sk administration;
– Sk with high activity should be used to avoid excessive protein concentration in circulation and excessive antibodies formation.

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