INTRAVASCULAR PLUG FORMATION INDUCED BY POLY-APS IS THE PRINCIPAL MECHANISM OF THE TOXIN'S LETHALITY IN RATS/RAT TISSUES

MATIJAŽ BUNC¹, LUCIJA ŠARC², JANEZ ROZMAN¹, TOM TURK³, KRISTINA SEPČIĆ³ and DUŠAN ŠUPUT¹

¹Institute of Pathophysiology, Medical Faculty, Ljubljana, ²Department of Toxicology, Clinical Center, Ljubljana, ³Department of Biology, Biotechnical Faculty, Ljubljana, Slovenia.

Abstract: Toxic water soluble polymeric 3-alkylpyridinium salts isolated from the sponge Raniera sarai strongly inhibited AChE in vitro. In vivo, experimental animals died due to plugs formed in microcirculation. The mechanism of this plug formation is unknown. In vitro, the toxin did not affect the coagulation rate, but the rate of platelet aggregation was accelerated in a dose-dependent manner. The hemolytic activity of poly-APS was diminished by the addition of serum proteins in a dose-dependent manner. These results support the conclusion that non-specific binding to proteins is the underlying mechanism of the lethality of poly APS.

Key Words: Poly APS, Coagulation, Inhibition Of Hemolysis, Serum Proteins.

INTRODUCTION

Water soluble poly-APS with MW 18900 and 5520 Da were isolated from the marine sponge Raniera sarai [1]. In vitro, they strongly inhibited acetylcholinesterase (AChE) [2]. They bind to lipids and aggregate lipid vesicles [3]. After an i.v. application of lethal doses of the toxin to experimental animals, their ECG readings showed signs of hypoxia [4]. Arterial blood pressure decreased, and breathing stopped after a few breaths. 2.7 mg/kg was the estimated lethal dose for rats. Autopsies of the experimental animals killed by the toxin revealed that the lumen of medium and small sized blood vessels in the heart and lungs were filled with granular brownish material - plugs - a mixture of fibrin, red blood cells, and platelets. Therefore, it was reasonable to test the effects of the toxin on the coagulation pathway, platelet aggregation, serum protein precipitation, and hemolytic activity.

MATERIALS AND METHODS

The coagulation pathway was tested using standard laboratory methods. The precipitation of serum proteins with poly-APS was measured using SDS-polyacrylamide gel electrophoresis. The inhibition of hemolysis by serum proteins was determined spectrophotometrically. Hemolytic activity was
monitored using the turbidimetric method. The inhibition of hemolysis by the serum was determined by measuring the time it took for absorbance to decrease by 50% in (t50) in the presence of the serum, and comparing this time with the t50 of 1 hemolytic unit (HU, 0.8 μg/ml of poly-APS) without the serum. Blood serum with 20 mg/l of proteins was diluted with erythrocyte buffer in dilutions from 1:0 to 1:4092. A quantitative assessment of the effects of poly-APS on platelets was performed using optical aggregometry as described in Medical Laboratory Hematology [5]. Washed rat platelets were incubated with the following final concentrations of poly-APS (mg/ml): 1.08, 0.1, 0.01, 0.001, 0.0001; and measured by means of optical aggregometry.

RESULTS

In vitro, the toxin had no direct effects on coagulation. The effects of poly-APS on rat platelets were dose dependent. 1.08 mg/ml poly-APS caused 100% aggregation of platelets, whereas at 0.0001 mg/ml the aggregation was less than 2% (1.4%±0.3). SDS-PAGE analysis of the sediment and supernatant, obtained after incubation of the serum with poly-APS followed by centrifuging, revealed that serum proteins are present in both the sediment and in the supernatant. Serum proteins inhibited hemolysis by poly-APS when added to the erythrocyte suspension. The inhibition was 100% with dilutions lower than 1:16, and partial inhibition (3%±2) persisted even at very high dilutions (1:128) of the blood serum.

DISCUSSION

Previous data [4] suggested that plug formation might be an important factor in the lethal effects of the toxin in rats. This study has shown that in vitro, the toxin accelerates the rate of platelet aggregation in a dose-dependent manner. Inhibition of the hemolytic activity of poly-APS by serum proteins could explain the fact that hemolytic activity was hardly ever found in vivo. Our results support the view that non-specific binding of poly-APS to plasma proteins, and possibly also to membrane-bound proteins on platelets, is the underlying mechanism of the lethality of poly-APS.

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REFERENCES


