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EUROPEAN TOAD GLANDULAR SECRETIONS COMPONENTS INDUCE PLATELET ADHESION AND AGGREGATION

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Amphibian skin secretions are enriched with complex cocktails of bioactive molecules spanning a wide spectrum of biological activities. Although recently there has been a spate of interest concerning the potential therapeutic effects of the biologically active compounds derived from amphibians' dermal secretions, most of these substances are not yet widely implemented in medical and pharmaceutical industry, as the mechanism of their action and possible side effects remain insufficiently studied. Since our previous studies demonstrated that the European toad parotoid glands secretions induced platelet aggregation in platelet rich plasma, this work was designed to study the *in vitro* effects of the active fraction of this toad secretions upon pure platelets function.

Adult specimens of the European toad (*Bufo bufo*) (n=50) were collected during spring spawning (males and females) on the lake Didorivka in Holosiivskyi district of Kyiv, Ukraine. Skin secretions were collected as follows: toad was put into a petri dish and, after mechanically stimulated the parotoid glands zone with fingers for 1-2 min, washed with ultra-pure water. Water suspension of skin secretions was centrifuged at 2,500 g for 15 min; the supernatant was lyophilized (Telstar LyoQuest) and kept at 4 °C till use. Before the experiment lyophilized skin secretions (30 mg) were dissolved in 1 mL of 0.05 M Tris-HCl buffer (pH 7.4) and centrifuged at 10,000 g for 5 min. Protein concentration in the supernatant was assayed by Bradford method with BSA as a standard. Chromatographic separation of general skin secretions was carried out using Superdex G75 SEC (size exclusion chromatography) column (flow rate 0.75 ml/min) equilibrated with 0.05 M Tris-HCl buffer (pH 7.4), containing 0.13 M NaCl. Rabbit platelet-rich plasma ($2x10^5$ cells/µL) and platelet-poor plasma were obtained following the standard protocol. Platelets were purified by column chromatography on Sepharose 4B. Platelet aggregation was measured using aggregometer AT-02 (Medtech, Russia). Platelets adhesion onto collagen-, fibrinogen-, and albumin-coated surfaces was determined by measuring the activity of acid phosphatase.

European toad skin secretions were fractionated and the flow-through fractions were studied on the ability to induce platelet aggregation. One fraction dose-dependently induced aggregation of isolated platelets: the pro-aggregatory effect of this fraction was the same as the effect of $5x10^{-6}$ M ADP. This active fraction was used in further experiments. The studied fraction was shown to induce platelet adhesion onto fibrinogen-coated surface. The number of platelets that adhered to this surface upon the active fraction treatment was $109x10^3$ cells. Despite, it was 50 % and 63 % lower than after thrombin (0,6 U/mL) and ADP ($5x10^{-6}$ M) stimulation, respectively, it was rather higher than the same to the collagen- and albumin-coated surfaces.

Our results showed that the components of European toad parotoid gland secretions may be a promising source of natural compounds which can modulate platelet functions and might be used for the treatment of diseases that involve aberrant platelet activity. These findings might serve as a scientific platform to further explore platelets as they involve fundamental cellular regulatory mechanisms, and, moreover, they emerged as important markers for various types of diseases. In addition, the detailed studying of platelets might help scientists to search for new pharmacological approaches to detect different blood diseases.