HUMAN RECOMBINANT PLATELET PHOSPHOLIPASE A$_2$ EXACERBATES POLY-L-ARGININE INDUCED RAT PAW EDEMA

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Abstract—In this study by using the human recombinant non-pancreatic-secreted platelet PLA$_2$ (r-hnps-PLA$_2$) and rabbit polyclonal antibodies directed against either the human (group II) or the porcine enzyme (group I), we have shown a possible involvement of platelet PLA$_2$ in poly-l-arginine (25 kDa)-induced rat paw edema. Local treatment of rats with the anti-platelet-PLA$_2$ antibody (anti-hnps-PLA$_2$) but not with anti-porcine-PLA$_2$ antibody (anti-porc-PLA$_2$) significantly reduced the edema induced by a maximal dose of poly-l-arginine (1 mg/paw). Furthermore when r-hnps-PLA$_2$ (1-10 µg) was injected together with a subliminal dose of poly-l-arginine (50 µg/paw), a dose-dependent increase in both edema and protein leakage was observed. This effect was selectively inhibited by the anti-hnps-PLA$_2$ (10-100 µg/paw) but not anti-porc-PLA$_2$ (10-100 µg/paw). Thus, platelets seem to be involved in both vascular and cellular components of the inflammatory response by contributing, most likely in the early phase, to the edema formation through secretion of PLA$_2$.

INTRODUCTION

Phospholipases A$_2$ enzymes exist in two forms: intracellular (1, 2) and extracellular (3–5). Recently, on the basis of their amino acidic sequences, extracellular PLA$_2$s have been divided in three groups (6). The platelet is a source of extracellular group II phospholipase A$_2$ (5). A group II human phospholipase A$_2$ (r-hnps-PLA$_2$) secreted from platelets has been recently cloned and the recombinant protein produced (5, 7). Recombinant hnps-PLA$_2$ is a basic enzyme (pI > 10.5) having a cluster of amino acids near its amino terminus (5), which have been proposed to play an important role in the interaction of PLA$_2$ enzymes with specific biological targets (8, 9). The recombinant human enzyme has been
already shown to be proinflammatory when injected into the joints of rabbits, approximately at the same concentrations of those found in the joints of arthritic patients (7). A similar inflammatory pattern was observed when the native form of the enzyme was injected intrarticularly in rat joints (10). Injection of r-hnps-PLA₂ in the rat hind paw did not cause edema but r-hnps-PLA₂ has been shown to potentiate rat adjuvant arthritis (11). The role of platelets in inflammation is not very clear yet. Activated platelets and leukocytes have been shown to release polycationic substances that are able to increase vascular permeability (12, 13). Moreover, cationic substances such as poly-L-arginine have been shown to activate leukocytes and to stimulate the respiratory burst (14, 15). A model of polycation-induced rat paw edema recently has been described that depends on both cationic charge and molecular weight of the protein (16). Because r-hnps-PLA₂ is positively charged and it has been shown to be released in vitro upon platelet stimulation with thrombin (3, 5) or in vivo in the rat by intravenous administration of ADP (17), we have designed these experiments to investigate a possible role of the platelet-secreted enzyme and consequently of the platelets themselves in the poly-L-arginine rat paw edema model.

MATERIALS AND METHODS

**Drugs.** Human recombinant non-pancreatic-secreted phospholipase A₂ (Dr. Browning, Biogen) was produced from a stable transfected mammalian cell line (7) and was supplied in 0.02 M sterile HEPES buffer, pH 7.4, NaCl 0.15 M. The purity was estimated to be greater than 95% (SDS gel analysis, Schagger and Jagow gel system). Endotoxin presence as estimated as 1–2 ng/100 µg of hnsp-PLA₂ (colorimetric assay). Polyclonal antibodies to the human and to the pancreatic PLA₂ enzymes were prepared by immunizing New Zealand white male rabbits with 20 µg of enzyme in complete Freund’s adjuvant by injection directly into the lymphonodi. The animals had intramuscular booster injections with 50 µg/rabbit in incomplete Freund’s adjuvant multiple times. Protein A or protein G Sepharose was used to purify rabbit IgG using conventional methods. Both antibodies were supplied lyophilized and reconstituted with distilled water when they were needed. All the successive dilution were performed using sterile saline. Poly-L-arginine, 24 kDa, and Evans blue were purchased from Sigma (St. Louis, Missouri). Formamide and all salts were obtained from Carlo Erba (Milan, Italy).

**Measurement of Rat Paw Edema.** Male wistar rats (Charles River, 120–150 g) were used throughout the experiments. Edema was induced into the left hind paw by a single subplantar injection of different doses of polyarginine, 24 kDa, dissolved in saline. Paw volume was measured immediately before the injection and each hour for 6 h thereafter by a hydroplethysmometer (model 7150, Ugo Basile, Italy). The final volume injected into the paw was always 0.1 ml. A dose–response curve was generated (data not shown) and the dose of 50 µg/paw was shown to be able to elicit a subliminal edema and used to carry out the entire study. Results are expressed as increase in the paw volume (milliliters) calculated by subtracting the basal volume. In some cases edema was evaluated as the area under the curve using the Simpson’s rule (18).

**Assessment of Vascular Response with Evans Blue.** Evans blue (25 mg/kg as a 2.5% solution in 0.45% NaCl) was injected intravenously immediately before subplantar injection of poly-L-