Prostaglandin production by human polymorphonuclear leucocytes during phagocytosis in vitro

G. Tolone, L. Bonasera, M. Brai and C. Tolone[1]

Istituto di Patologia Generale dell'Università, Corso Tukory 211, I-90134 Palermo (Italy), 7 December 1976

Summary. Human polymorphonuclear leukocytes were found to be able to synthetize and release substantial amounts of PGE_2, when stimulated by a phagocytic stimulus such as zymosan particles coated with complement. Hydrocortisone, at a concentration of 10^{-5} M, which proved to be effective in other biological systems, failed to inhibit phagocytosis and PG release.

Several lines of evidence exist implicating polymorphonuclear (PMN) leukocytes as the main source of E-type prostaglandins (PGs) in the inflammatory response. In the phlogistic exudate produced by carrageenin injection in the rat, release of histamine and bradykinin occurs in the early phase while that of PGE_2 is delayed from 2 to 3 h[2], and its concentration increases steadily and parallels the time-course of migration of PMN leukocytes into the site of injury[3]. Moreover, production of PGE_1 and PGE_2 by several inflammatory cell populations has been reported[4,5]. In guinea-pigs, macrophage appears as the major cell-type responsible for PG release[6], while in rabbits PMN leukocytes seem to be mainly involved[4]. Differences in species probably account for such a discrepancy. In this paper we present evidence of a substantial production of PGE_2 by human PMN leukocytes during phagocytosis in vitro.

Material and methods. Polymorphonuclear leukocytes were purified according to Van Furth and Van Zwet[7]. Blood samples from human volunteers were collected in sterile tubes containing 30 µl heparin ml^{-1} blood. The erythrocytes were sedimented with a 5% (w/v) solution of dextran (2×10^5 mol.wt) in buffered saline (3 ml solution to 10 ml blood) for 30 min at 37 °C. The leukocyte-rich supernatant fluid was then removed and centrifuged for 10 min at $110 \times g$. The sedimented leukocytes were washed twice with heparin-saline, concentrated by centrifugation (10 min at $110 \times g$) and resuspended in gelatine-Hanks' salt solution at $1-2 \times 10^7$ cells ml^{-1}. Differentials were: PMN (94.1%); eosinophils (1.5%); mononuclear cells (4.4%). Cell viability was checked at the beginning and end of each experiment by dye exclusion of trypan blue[7].

Zymosan. Zymosan particles (Schuchardt, München, BRD) were treated with normal human serum (0.5 ml serum per mg dry substance), that is, coated with complement factors, then washed extensively with buffered saline and resuspended in gelatine-Hanks' salt solution at a final concentration of 4 mg ml^{-1}.

Phagocytosis. Zymosa particles (2.0 mg) were incubated (37 °C) with 5×10^6 PMN leukocytes in a final volume of 1 ml. In some tubes, hydrocortisone sodium succinate at a final concentration of 10^{-5} M steroid base was added. After 90 min, mixtures were centrifuged ($1200 \times g$) and supernatants assayed for prostaglandin and β-glucuronidase (βG) activities.

PG activity was tested by radioimmunoassay using a Calbiochem RIA set for PGs. The antiserum employed (sheep anti-PGE_2-bovine serum albumin) cross reacted with PGE_1 and, to a lesser extent, with $PGF_{2\alpha}$ and PGA_2. For platelet aggregation the procedure described by Born[8] was followed.

βG activity was measured according to Czarnetzki et al.[9]. Reaction mixtures consisting of 0.1 ml test samples, 0.1 ml phenolphthalein glucuronic acid 0.01 M and 0.5 ml acetate buffer 0.1 M, pH 5.0, were incubated at 37 °C for 12 h and read at 540 nm. Enzyme activity was expressed as increase in absorbance per ml (A_{540} ml^{-1}).

Release of prostaglandins and of lysosomal enzyme marker β-glucuronidase by human polymorphonuclear leukocytes phagocyting zymosan particles

	PMN leukocyte responses	
	βG (A_{540} ml^{-1})	PG (ng/10^6 cells)
Buffer control	0.22	0.1 ± 0.03*
Zymosan particles	1.52	10.8 ± 1.60
Zymosan particles and Hydrocortisone	1.38	11.2 ± 2.22

* Mean ± SEM. Supernatants of zymosan alone did not show prostaglandin activity.

Results and discussion. As the table shows, phagocytosis of zymosan particles by human PMN leukocytes led to a substantial production of PGs (10.8 ± 1.6 ng per 10^6 cells) which paralleled release of lysosomal enzyme marker β-glucuronidase. Cell viability was maintained during incubation as checked by dye exclusion. Prostaglandin activity was mainly E-type, as detected by radioimmunoassay, and resembled PGE_2 rather than PGE_1 due to the enhancing effect on ADP-induced platelet aggregation[10]. Hydrocortisone, at a concentration of 10^{-5} M, failed to inhibit PGs release from phagocyting leukocytes.

Present data strengthen the opinion that E-type PGs are involved in the inflammatory response in humans and support the hypothesis that these ubiquitous compounds may serve to provide a negative feedback mechanism for regulating extent and duration of inflammatory reactions. PGE_1 and PGE_2, in fact, are both effective inhibitors of histamine release by human basophils in type I hypersensitivity[11] and of lymphokine secretion by activated lymphocytes in type IV hypersensitivity[6]. In type III allergic reactions, although PGE_1 can inhibit the

1 Present address: Clinica Pediatrica Università Iª Napoli, Italy.

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release of lysosomal enzymes from PMN leukocytes[12], the effect has been considered of no physiological relevance, since these cells were thought to be poor PGs productors. Our data demonstrate that the statement is not correct and that PMN leukocytes are able to synthetize substantial amounts of PGE_2 when subjected to phagocytic stimuli. In our model, hydrocortisone, at a concentration proved to be effective in other biological systems[13-16], failed to inhibt release of prostaglandins and lysosomal enzyme β-glucuronidase. This finding is consistent with other reports[17-20] and casts doubts on whether corticosteroids may act as anti-inflammatory agents by interfering with PG-synthetase activity in cells involved in the inflammatory response.

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Evidence that superoxide radicals are involved in the hemolytic mechanism of phenylhydrazine[1]

A. Valenzuela, H. Ríos[2] and G. Neiman

Laboratorio de Bioquímica, Instituto de Nutrición y Tecnología de los Alimentos, Universidad de Chile, Casilla 15138, Santiago 11 (Chile), 8 December 1976

Summary. Phenylhydrazine produces in the red blood cell the same effect as the enzymic system xanthine oxidase-xanthine, a superoxide radical generator system. Both effects are inhibited by the enzyme superoxide dismutase.

Molecular oxygen is fundamental for life of aerobic organisms; however when it acts through the formation of highly active free radicals, it can turn toxic[3-6] and even mortal[7]. One of these free radicals is the superoxide radical (SR), which is generated by the reduction of molecular oxygen when it interacts with a bivalent metal[8]. Much evidence exists relating SR with the initiation of unsaturated fatty acids oxidation[9-11].

There is scarce information as regards the hemolytic mechanism of phenylhydrazine (PH), although it is well known that many of its effects are oxygen dependent. It was demonstrated[12] that PH in presence of hemoglobin produces hydrogen peroxide and therefore SR, since the former is an end product of the dismutation of this free radical. These SR could initiate the peroxidation of the lipidic components of the erythrocyte membrane producing its destruction and liberation of hemoglobin.

Using hemolytic and peroxidative measurements as a damage criterion, we found that the system xanthine oxidase (XO)-xanthine (X), which generates SR, produces a high cellular lysis level which is narrowly related to a high peroxidation grade. Both phenomena are inhibited by the enzyme superoxide dismutase (SOD). In addition, PH produces a similar hemolytic and peroxidative effect as the XO-X system, these processes being also inhibited by SOD. From this, we postulated that the hemolytic action of PH is closely related with that of SR.

Materials and methods. Chemicals: PH-HCl and Folin Ciocalteu's phenol reagent were obtained from E. Merck A. G., Darmstadt, Federal Republic of Germany. Sephadex G-100, epinephrine, xanthine, xanthine oxidase and 2-thiobarbituric acid were obtained from Sigma Chemical Co., St. Louis, U.S.A. All other chemicals used were of analytical grade.

Methods: SOD was prepared from bovine erythrocytes according to the method of McCord and Fridovich[13] with the following modification: the final extract was purified in a Sephadex G-100 (100 cm × 3 cm) column equilibrated and eluted with NaCl 0.15 M. The enzymic activity of SOD was assayed according to the method of Misra and Fridovich[14], obtaining an extract with an activity of 3500 units/mg protein. The denatured enzyme was obteined by heating at 100 °C for 20 min.

The hemolytic and peroxidation asays were carried out in rat's blood using oxalate as anticoagulant. The red blood cells were washed and suspended in an equal volume of NaCl 0.15 M and the assays were performed in several test tubes which contained: 1 ml of the cell suspension; 0.1 ml of 2×10^{-3} M PH; 0.1 ml of 2×10^{-3} M xanthine; enzymic extracts of SOD and xanthine oxidase. The final incubation volume was always 1.4 ml. These test tubes batteries were incubated at 37 °C with gentle agitation. At intervals of 30 min 1.5 ml of NaCl 0.15 M was added to the corresponding test tube, then after shaking, an aliquot of 1.5 ml was extracted for the hemolysis test and another of 0.8 ml for the peroxidation assay.

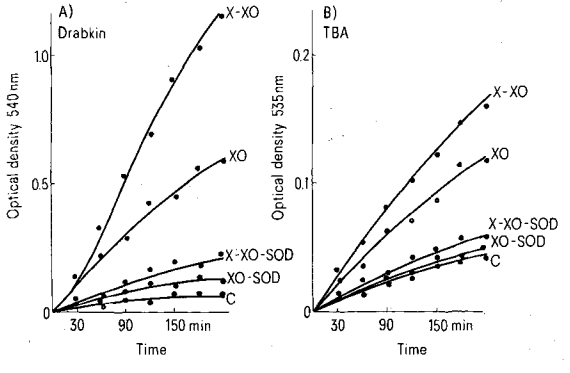

Fig. 1. Effect of the xanthine oxidase (XO)-xanthine (X) system on the hemolysis (A) and peroxidation (B) of red blood cells and its inhibition by SOD. The experimental conditions are described in the text. XO: 0.6 units (Sigma units); SOD: 1.2 units; C: control.