EFFECTS OF NIFLUMIC ACID ON POLYPHOSPHOINOSITIDE AND OXIDATIVE METABOLISM IN POLYMORPHONUCLEAR LEUKOCYTES FROM HEALTHY AND THERMALLY INJURED RATS

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Abstract—Thermal injury in rats leads to an impairment of polymorphonuclear leukocyte (PMN) functions, particularly oxidative metabolism and phosphoinositide turnover. As prostaglandin E₂, which has immunosuppressive properties, is released in high levels after burn trauma, we investigated the in vitro and in vivo effects of a nonsteroidal antiinflammatory drug, niflumic acid, on oxidative and phosphoinositide metabolism in PMNs from healthy and burned rats. Given the role of fluoride ions on PMN, the influence of niflumic acid was compared with that of sodium fluoride (NaF) at equivalent doses of 10⁻³. In vitro, niflumic acid and sodium fluoride had no effect on oxidative metabolism in stimulated by formyl methionyl-leucyl-phenylalanine (FMLP) or opsonized zymosan (OZ) or nonstimulated PMNs from healthy and burned rats. Niflumic acid slightly increased the production of inositol phosphate by nonstimulated PMNs from healthy and burned rats. Niflumic acid and NaF partly restored the stimulating effect of FMLP on inositol phosphate production by PMNs from burned rats. In vivo treatment with niflumic acid and NaF increased the oxidative metabolism of PMNs from burned rats but not healthy rats. Niflumic acid, more than NaF, restored the activity of both stimulants on phosphoinositide metabolism in PMNs from burned rats. In conclusion, at non-antiinflammatory doses, while inhibiting cyclooxygenase activity, niflumic acid exerts a complex effect on the burn-induced depression of PMN functions. The fluoride anion induces similar but generally weaker effects and seem to be involved in the restoring effects of niflumic acid on PMN functions in burned rats.
INTRODUCTION

Severe thermal injury leads to alterations of various components of host defenses, including decreased humoral and cellular immunity (1) and inhibition of polymorphonuclear leukocyte (PMN) functions such as chemotaxis, phagocytosis, and bacterial killing (2). A temporal association between the depression of PMN bactericidal activity, complement consumption, and production of arachidonic acid metabolites via the cyclooxygenase pathway occurs in thermally injured animals (3). Elevated levels of E-series prostaglandins (PGE) are released from macrophages/monocytes and skin cells after burn injury (3). These PGEs inhibit various PMN functions including chemotaxis, aggregation, superoxide production, and lysosomal enzyme release (4). It has been suggested that defective PMN bactericidal activity caused by experimental thermal injury is related to the elevation of cAMP, and that PGE₁ plays an important role in this phenomenon (4). PGE also modulates humoral and cellular immune functions, acting as a feedback inhibitor for T-cell proliferation, lymphokine production, cytotoxicity, and other functions (5–7). Nonsteroidal antiinflammatory drugs (NSAIDs) were thus tested for their ability to counteract some detrimental effects of burn injury (8). Inhibition of prostaglandin (PG) production by NSAIDs generally results in enhanced immunological functions, as demonstrated in several in vivo and in vitro studies (7, 9). One such drug, niflumic acid, shows immunostimulating properties in mice (10).

We have previously shown, in a rat model of thermal injury, alterations of PMN responses to classical stimulants such as FMLP and OZ three days after injury. The oxidative burst was diminished (11), possibly due to alterations in signaling systems and particularly in phosphoinositide (PI) breakdown. The stimulating effects of FMLP and OZ on PI metabolism are reduced or abolished after burn injury (12).

In the present study, we investigated the effects of niflumic acid, a NSAID inhibitor of cyclooxygenase, on the oxidative burst and PI breakdown in PMNs from healthy and thermally injured rats both in vitro and in vivo. As the F⁻ ion may interfere with G-proteins involved in stimulus-coupling mechanisms (13, 14), we investigated its relative importance in the activity of niflumic acid by comparing the effects of NaF at equivalent doses.

MATERIALS AND METHODS

Reagents. Niflumic acid (UPSA Laboratories, Agen, France) was dissolved in phosphate buffer (Na₂HPO₄, 2H₂O 11.21 g, KH₂PO₄ 0.49 g, NaCl 3.00 g, H₂O qsp 1000 ml). Cytochrome c, FMLP, cytochalasin B, OZ, luminol, bovine serum albumin (BSA), and sodium fluoride (NaF) were obtained from Sigma (St. Louis, Missouri), Dowex AG1-X8 ion-exchange resin (200–400