COMPARISON OF IN VITRO EFFECTS OF FLUNIXIN AND TOLFENAMIC ACID ON HUMAN LEUKOCYTE AND PLATELET FUNCTIONS

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Abstract—A study was made to compare the effects of two nonsteroidal antiinflammatory drugs (NSAIDs), flunixin and tolfenamic acid, on the leukotriene B₄ (LTB₄) production and migration of human polymorphonuclear leukocytes (PMNs) as well as on platelet aggregation and thromboxane B₂ (TxB₂) production during clotting. Tolfenamic acid inhibited LTB₄ production in PMNs as well as FMLP- and LTB₄-induced PMN migration (IC₅₀ values 23 ± 3, 39 ± 5, and 68 ± 13 μM, respectively), whereas flunixin inhibited these cell functions only with the highest concentration tested (100 μM). On the other hand, flunixin was clearly a more potent inhibitor of TxB₂ production and adrenaline-induced platelet aggregation than tolfenamic acid, the IC₅₀ values in TxB₂ production being 0.28 ± 0.02 μM and 2.6 ± 0.3 μM for flunixin and tolfenamic acid, respectively. We suggest that inhibition of PMN functions may be an additional mechanism in the antiinflammatory action of tolfenamic acid. At least in human PMNs and platelets, flunixin seems to be only an inhibitor of cyclooxygenase.

INTRODUCTION

Nonsteroidal antiinflammatory drugs (NSAIDs) exhibit differences in their ability to suppress polymorphonuclear leukocyte (PMN) functions, which is a contributing factor in their antiinflammatory activity besides their inhibitory action on prostanoid synthesis (for reviews see 1, 2). PMN functions that can be

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modified by drugs include adhesion, aggregation, degranulation, directed migration, mediator synthesis, and superoxide production. NSAIDs, although having different chemical constitutions, are known to inhibit the synthesis of cyclooxygenase products of arachidonate metabolism (for reviews see 3, 4). Inhibition of PMN functions is not yet accepted as a general mechanism of action of NSAIDs, and chemically different NSAIDs seem to have varying stimulus- and cell-specific mechanisms (1, 2).

We have shown earlier that in contrast to most other NSAIDs, tolfenamic acid \([N-(2-methyl-3-chlorophenyl)anthranilic acid]\) inhibits leukotriene B\(_4\) (LTB\(_4\)) synthesis and migration of human PMNs in concentrations comparable to those detected in vivo during drug treatment (5–7). Flunixin [2-(2-methyl-3-trifluoromethylanilino)nicotinic acid] is an antiinflammatory compound widely used for veterinary purposes. Chemically it resembles fenamates, but has a pyridinecarboxylic acid instead of benzoic acid (Figure 1). It has been reported to inhibit prostanoid production (8, 9) and, in higher concentrations, to suppress functions of PMNs from some animal species (10–12).

The aim of the present study was to compare the effects of flunixin and tolfenamic acid by measuring variables important in inflammation or thrombosis, i.e., LTB\(_4\) synthesis in isolated human PMNs, migration of human PMNs towards LTB\(_4\) and FMLP, and thromboxane B\(_2\) (TxB\(_2\)) formation in human platelets during clotting and aggregation of human platelets in vitro.

**MATERIALS AND METHODS**

*Isolation of Human PMNs.* Blood was collected by venipuncture from healthy volunteers who had abstained from any drugs for at least one week before sampling. A buffy-coat preparation of citrated blood was layered on Ficoll-Paque (Pharmacia Fine Chemicals AB, Uppsala, Sweden) and centrifuged according to Bøyum (13). Red cells were removed by dextran sedimentation followed by lysis of the remaining erythrocytes with Tris-buffered 0.15 M NH\(_4\)Cl. PMNs were washed twice with Dulbecco’s phosphate-buffered saline (DPBS). After the isolation procedure, the viability