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A Platelet-Activating Factor Antagonist Inhibits Interleukin 1-Induced Inflammation

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Treatment with a platelet-activating factor receptor antagonist, SRI 63-441, inhibited interleukin 1-induced increases in vascular permeability and leukocyte infiltration in the rabbit eye following the intravitreal injection of human interleukin 1-alpha. Treatment with the prostaglandin-synthetase inhibitor, flurbiprofen, or the corticosteroid, prednisolone, resulted in comparable attenuation of the increase in vascular permeability. In contrast to the effect of flurbiprofen, SRI 63-441 did not reduce interleukin 1-induced increases in prostaglandin E_2 levels. Combined treatment with the platelet-activating factor antagonist and inhibitors of prostaglandin synthesis nearly prevented interleukin 1-induced increases in vascular permeability or cellular infiltration. These findings suggest a role for platelet-activating factor in interleukin 1-induced inflammation. Platelet-activating factor and prostaglandins may act synergistically as mediators of interleukin 1-induced vascular permeability.

Interleukin 1 is a polypeptide cytokine implicated in immunologic and inflammatory processes (1-10). Release of IL1-like activity occurs in inflamed tissues including arthritic joints, ultraviolet-irradiated skin, gingiva and the central nervous system (11-19). Furthermore, IL1-like peptide is synthesized by ocular cells and is found in fluids from sites of ocular inflammation and injury (20-23).

When exogenously administered in the skin and joint, IL1 promotes inflammatory increases in vascular permeability and cellular infiltration (24-26). We and others have recently demonstrated that locally injected IL1 in low doses results in acute ocular inflammation (27,28).

The potential for platelet-activating factor to contribute to IL1-induced inflammation is suggested by the finding that IL1 promotes PAF synthesis in endothelial cells in vitro (29). PAF is an extremely potent and rapid acting inducer of vascular permeability as well as a chemoattractant for polymorphonuclear leukocytes (30-34). PAF is 1000 to 10000 times as active on

Abbreviations: IL1, interleukin 1; PAF, platelet-activating factor; rIL1α, recombinant human interleukin 1-alpha.

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a molar basis compared to histamine or bradykinin in inducing cutaneous vascular permeability in rabbits, guinea pigs or rats (30,31).

In order to elucidate the role of PAF in mediating IL1-induced inflammation, we have studied the ability of a PAF receptor antagonist, SRI 63-441, to inhibit vascular permeability, cellular infiltration and increases in prostaglandin E_2 resulting from the intravitreal injection of interleukin 1. Furthermore, we compared the actions of inhibitors of prostaglandin synthesis to those of the PAF receptor antagonist in attenuating IL1-induced inflammation. Finally, to understand the interaction of eicosanoid metabolites with PAF in causing IL1-induced vascular permeability and cellular emigration, we tested whether a non-steroidal anti-inflammatory agent or corticosteroid might have additive or synergistic effects with a PAF receptor antagonist.

Materials and Methods

Animals

Female New Zealand white rabbits weighing 2.0 to 2.7 kilograms were purchased commercially and housed at the animal care facility at the Oregon Health Sciences University. The animals were fed standard laboratory chow.

Interleukin 1 Administration

The intravitreal injection of interleukin 1 produces an acute inflammatory response characterized by a cellular infiltrate in the anterior chamber, iris vessel dilatation, and protein extravasation (28). Pathologically, the iris demonstrates edema, cellular infiltration and hemorrhage. These changes become evident within 6 hours and are maximal 24 hours after the injection (28).

Recombinant human interleukin 1-alpha obtained from Hoffmann-LaRoche Inc. (Nutley, N.J) contained less than 20 ng of endotoxin per 10^6 units by the Limulus assay as performed by the supplier. The activity of the rIL1α was determined by a thymocyte proliferation assay with 1 unit equivalent to an amount inducing 50% of maximal stimulation (35).

rIL1α was inactivated by heating to 70° C for 30 minutes. Injections of active and heat-inactivated IL1 were made into the central vitreous with a 28 gauge needle. Care was taken to avoid the lens using direct visualization. The eye was topically anesthetized with proparacaine 0.5% prior to injection. Active or heat-inactivated rIL1α was diluted to desired concentrations in pyrogen free saline with a final volume of 50 microliters for injection. All injections were performed with non-inflammatory amounts of a protein carrier, either 2% fetal calf serum or 0.25% human serum albumin.

Two different lots of rIL1α were used. Lot 14493-50/93 was used for studies involving measurements of ocular vascular permeability and cellular infiltration. In these studies, 200 units of rIL1α (2 ng protein) were injected into the vitreous of the right eye. A second lot, lot IL-1 1/87, was used for studies involving measurement of aqueous humor prostaglandin E_2 levels. 400 units of rIL1α (1 ng protein) were injected into the vitreous of the right eye in these experiments.

Aqueous Humor Protein, Leukocyte Counts and Prostaglandin E_2 Levels

6 hours following rIL1α injection, rabbits were sacrificed by barbiturate overdose. Vascular permeability and cellular infiltration were determined by measurement of accumulated protein and leukocytes in the aqueous humor aspirated immediately at the time of death. Direct quantitation of protein in aqueous humor aspirates was performed by binding of brilliant blue (Biorad,

Richmond CA) and measurement of absorbance at 595 nM (36). Cell counts were performed in a hemocytometer. When adequate sample was available, Wright-stained slides were prepared by cytocentrifugation for differential cell counts. Prostaglandin E_2 levels in the aqueous humor were measured by a standard radioimmunoassay kit (NEN Dupont, Boston, MA).

Treatments

i) Platelet-Activating Factor Antagonist

The platelet-activating factor receptor antagonist, SRI 63-441, was obtained from Sandoz Research Institute (East Hanover, NJ) (37). The drug was given at doses of 20 mg/kg intravenously in pyrogen-free saline 2 minutes before rILlα injection and 2 and 4 hours following rILlα injection.

ii) Flurbiprofen, Prednisolone

Fifty microliters of flurbiprofen sodium 0.03% (Allergan, Puerto Rico) or prednisolone acetate 1% (Allergan, Irvine, CA) was given topically every 30 or 60 minutes respectively starting 2 hours before rILlα injection until the time of sample collection.

Statistical Analysis

Each result is presented as a mean ± standard error. Statistical comparisons between untreated and treated groups were performed using Student's *t* test.

Results

Interleukin 1-Induced Ocular Vascular Permeability (Figure 1)

6 hours after the intravitreal injection of 200 units of IL1 alone, there was a rise of protein in the aqueous humor to 14.9 ± 3.7 mg/ml (n=7). In comparison, protein levels in the non-injected contralateral eye were 0.3 ±

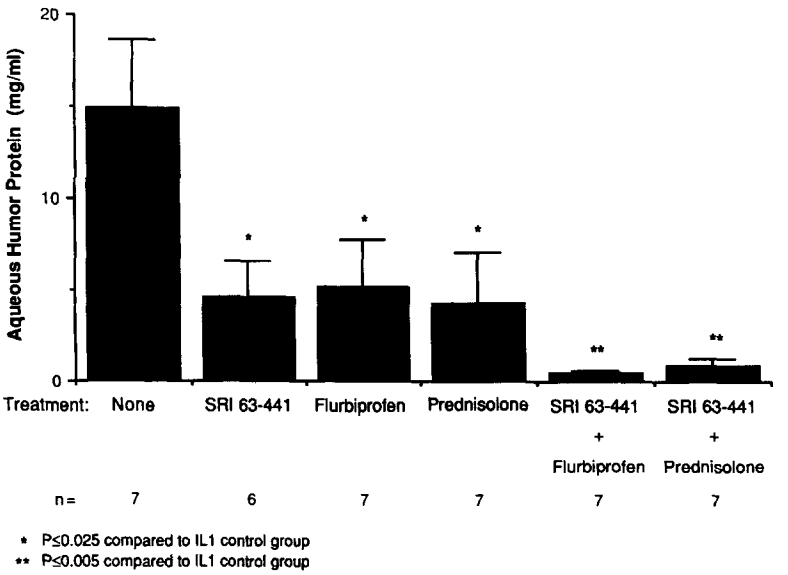

Fig. 1) Intravenous SRI 63-441, topical flurbiprofen or topical prednisolone comparably reduce the increase in ocular vascular permeability induced by intravitreal IL1 injection. Combined treatment of the PAF receptor antagonist with either the prostaglandin synthesis inhibitor, flurbiprofen, or the corticosteroid, prednisolone, nearly prevented IL1-induced breakdown of the blood-aqueous barrier.

0.1 mg/ml (n=7). Heat inactivation of IL1 completely prevented the increase in vascular permeability seen with active IL1 resulting in protein levels of 0.2 ± 0.1 mg/ml (n=3). Heat treatment does not alter ocular inflammatory effects induced by endotoxin (28). When SRI 63-441 was administered intravenously, the rise in aqueous humor protein was attenuated to 4.6 ± 2.0 mg/ml (n=6), ($P \leq 0.025$). Topical treatment with the prostaglandin-synthetase inhibitor, flurbiprofen or the corticosteroid, prednisolone, resulted in comparable attenuation of the increase in aqueous humor protein levels to 5.2 ± 2.5 mg/ml (n=7) ($P \leq 0.025$) or 4.3 ± 2.8 mg/ml (n=7) ($P \leq 0.025$), respectively. The combination of the PAF antagonist treatment with either flurbiprofen or prednisolone treatment resulted in further lowering of protein levels to 0.5 ± 0.1 mg/ml (n=7) ($P \leq 0.005$) or 0.9 ± 0.4 mg/ml (n=7) ($P \leq 0.005$) respectively.

Interleukin 1-Induced Anterior Chamber Cellular Infiltration (Figure 2)

The intravitreal injection of 200 units of IL1 usually induced an accumulation of leukocytes into the anterior chamber within 6 hours. In all samples tested, whether from drug-treated (n=5) or non-treated (n=3) rabbits, between 96% and 100% of the leukocytes present were polymorphonuclear leukocytes. In untreated rabbits receiving interleukin 1 alone, 398 ± 206 leukocytes/μl (n=7) were present in the aqueous humor 6 hours after injection. In comparison, non-injected eyes or eyes receiving IL1 that was heat-inactivated did not have a cellular response. SRI 63-441 and prednisolone

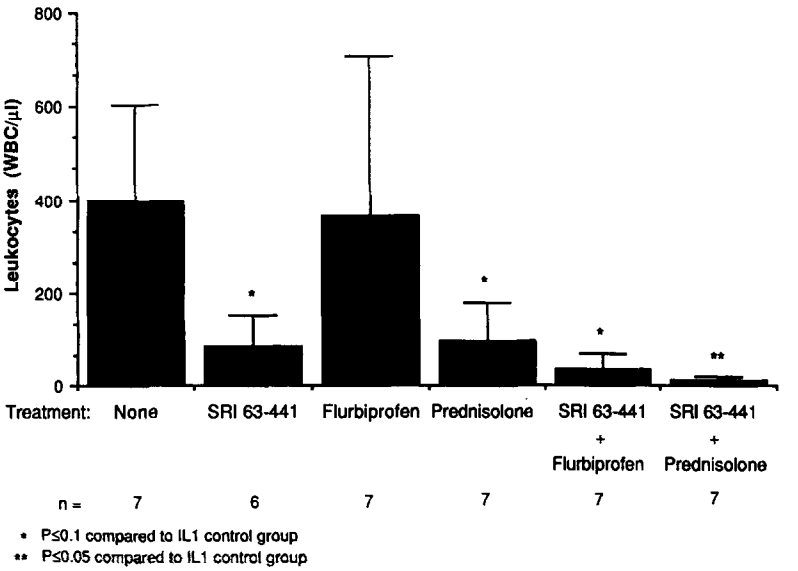

Fig. 2) Intravenous SRI 63-441 or topical prednisolone decreased the number of cells entering the anterior chamber following intravitreal IL1 injection. In contrast, treatment with flurbiprofen alone did not prevent the accumulation of leukocytes. Combined treatment with flurbiprofen or prednisolone and the PAF antagonist further reduced IL1-induced increases in leukocytes.

each reduced the accumulation of cells in the anterior chamber following IL1 to 84 ± 67 leukocytes/μl (n=6) ($P \leq 0.1$) or 99 ± 82 leukocytes/μl (n=7) ($P \leq 0.1$) respectively. In contrast, treatment with flurbiprofen did not prevent the accumulation of leukocytes into the anterior chamber following intravitreal IL1 injection. Aqueous humor from IL1 injected rabbits treated with flurbiprofen had 365 ± 341 leukocytes/μl (n=7). Adding flurbiprofen treatment to SRI 63-441 treatment resulted in a reduction in leukocyte infiltration to 37 ± 32 leukocytes/μl (n=7) ($P \leq 0.1$). Combined therapy of the PAF antagonist with prednisolone further decreased the number of cells entering the anterior chamber to 11 ± 8 leukocytes/μl (n=7) ($P \leq 0.05$).

Interleukin 1-Induced Aqueous Humor Prostaglandin E_2 Levels (Figure 3)

Interleukin 1 (400 Units) caused an elevation in aqueous humor prostaglandin E_2 levels to 500.6 ± 79.2 pg/ml (n=6) compared to 8.0 ± 1.7 pg/ml (n=6) in contralateral non-injected eyes . Flurbiprofen lowered the rise in prostaglandin E_2 levels induced by IL1 to 46.9 ± 28.1 pg/ml (n=6) ($P \leq 0.005$). On the other hand, SRI 63-441 did not decrease the rise in aqueous humor prostaglandin E_2 levels. SRI 63-441 treated rabbits had aqueous humor prostaglandin E_2 levels of 1404.7 ± 531.0 pg/ml (n=6). In comparison to normal aqueous humor, the intravenous injection of SRI 63-441 alone did not increase aqueous humor prostaglandin E_2 levels in eyes not injected with interleukin 1 (11.1 ± 1.9 pg/ml, n=6). Furthermore, SRI 63-441 did not directly interfere with the prostaglandin E_2 radioimmunoassay. Although flurbiprofen and SRI 63-441 give comparable reductions in the IL1-induced rise

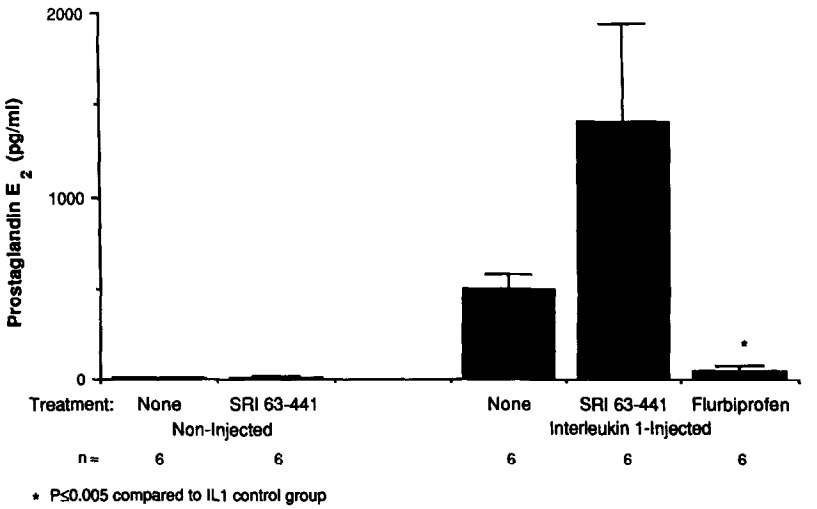

Fig. 3) Intravitreal IL1 caused an elevation in aqueous humor prostaglandin E_2 levels. Flurbiprofen treatment lowered the rise in prostaglandin E_2 levels. In contrast, SRI 63-441 did not reduce increases in prostaglandin E_2. SRI 63-441 alone did not increase aqueous humor prostaglandin E_2 levels in eyes not injected with interleukin 1.

of aqueous humor protein, there is a significant difference in the ability of these agents to reduce aqueous humor prostaglandin E_2 levels following these treatments.

Discussion

Interleukin 1 is suggested to be an important mediator of acute and chronic inflammation (1,2). It has been shown to promote vascular permeability, inflammatory cell infiltration and other inflammatory changes in many models of inflammation (24-28). However, the mechanisms by which IL1 contributes to inflammation are not well understood.

Platelet-activating factor is an excellent candidate to mediate many of the inflammatory actions of IL1. PAF may be synthesized by endothelial cells in response to IL1 (29). Moreover, major activities of PAF mimic important biologic functions of IL1. For example, IL1 and PAF both promote increases in vascular permeability, induce chemotaxis of granulocytes and monocytes, and activate neutrophils to marginate, aggregate, and degranulate (4-10,24-28,30-34). Both are potential mediators of gram-negative sepsis (37,38).

We present in this paper, the first evidence to our knowledge that PAF may mediate IL1-induced inflammation in vivo. This evidence is based on our finding that a specific PAF-receptor antagonist, SRI 63-441, significantly blocked IL1-induced increases in vascular permeability and tended to reduce cellular infiltration in the rabbit eye. The failure of the PAF antagonist to inhibit the rise in prostaglandin E_2 suggests that the PAF antagonist is not exerting these anti-inflammatory effects by inhibiting eicosanoid synthesis.

Vascular alteration in inflammatory reactions may depend on several mediators acting in synergy (39-41). Several studies have shown synergism between prostaglandins and PAF in models of cutaneous edema (42-45). We found that the combination of an antagonist of prostaglandin synthesis, flurbiprofen, or a corticosteroid, prednisolone, together with the PAF receptor antagonist, SRI 63-441, nearly abolished the IL1-induced breakdown in the blood-aqueous permeability barrier. These results suggest that PAF may act together with prostaglandins to promote IL1-induced increases in vascular permeability.

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