P.fr.162

Fenspiride interferes with neutrophil migration into the peritoneal cavity induced by endotoxin and activated zymosan

Cunha, F.Q., Boukili, M.A., Mota, J.I.B., Vargaftig, B.B. and Ferreira, S.H.

Dept. Pharmacology, Faculty of Medicine of Ribeirão Preto, Brazil and Unité de Pharmacologie cellulaire, Unité Associée Institut Pasteur-INSERM 285, 25 rue du Dr. Roux, Paris 75015, France

Since fenspiride inhibits exudation and neutrophil migration after zymosan (Z) injection into the rat pleural cavity (Lima et al., Rhinology suppl. 4, 87-95, 1988) we hypothesized that it might inhibit the release and/or the effects of chemotactic factors. Macrophages trigger neutrophil recruitment via Macrophage Neutrophil Chemotactic Factor (MNCF) (Cunha and Ferreira, Europ. J. Pharmacol. 129, 65-76,1986; Souza et al., Agents Actions, 24, 378-380, 1988), which differs from other macrophage-born cytokines by inducing migration in corticosteroid-treated animals. We now studied the ability of fenspiride to interfere with the migration of neutrophils into the peritoneal cavity of Wistar rats (180-200 g) injected i.p. with MNCF, Z-activated plasma (ZAP), carrageenin (C, 300 mg), lipopolysaccharide (LPS, E. coli, 200 ng) and Z (1 mg). Air pouches were produced (Edwards et al., J. Path., 134, 147-153, 1981), 20 ml of sterile air being injected s.c..into the backs of the rats. 3 days later air (10 ml) was re-injected and after 3 days, C (300 mg/ml) or LPS (200 ng/ml) were injected into the pouch. 4 hours later, the animals the peritoneal or pouch contents were collected with PBS. Cell counts were performed, the results being reported as the number of cells per ml. The increase in vascular permeability was evaluated by the determination of the concentration of extravasated Evans blue (25 mg/kg, injected i.v. 10 min after the i.p. injection of 3 ml of PBS, of C (300 mg/3 ml) or LPS (200 ng/3 ml). Fenspiride (Pneumorel® Biopharma, Neuilly sur Seine, France) was administered per os (60-200 mg/kg) one hour before the injection of the inflammatory agents, control animals receiving 3 ml of PBS. To study if fenspiride interferes with cell migration by inhibiting synthesis of MNCF, macrophages were harvested from peritoneal cavities stimulated with thioglycollate (10 ml of 3% thioglycollate in water i.p.,) 4 days earlier and were incubated in tissue culture dishes for 1 h at 37°C in an air-5% CO2 atmosphere. The adherent monolayers (95% macrophages) were washed and after 30 min at 37°C with LPS (5 mg/ml), the supernatants were discarded and the cells washed three times. This was followed by an incubation with 4 ml of LPS-free medium for 1 hour at 37°C. Fenspiride (100 mM) was added to the cell suspension 1 hour before LPS. The cell-free incubation fluids were sterilized and injected i.p. to dexamethasone-treated rats (0.5 mg/kg; 1 hour before) at 3 ml/cavity, which is equivalent to the material released by 3×10^6 macrophages. After 4 h, neutrophil migration was evaluated. Finally, to study migration by MNCF and ZAP, rats were treated with dexamethasone (0.5 mg/kg, s.c.), fenspiride (100-200 mg/kg, p.o.) or dexamethasone plus fenspiride, 1 hour before the i.p. challenge with 3 ml of MNCF or of ZAP. After 4 h, neutrophil migration was evaluated. ZAP was prepared by incubating guinea-pig plasma for 45 min with backers yeast (20 mg/ml).

LPs-induced neutrophil migration into the peritoneal cavity was inhibited by fenspiride at 100 and 200 mg/kg (56 and 58% inhibition, respectively), the dose of 60 mg/kg being inactive. Migration by Z was inhibited by 52% by 100 mg/kg of fenspiride, but migration by C was unaffected. Fenspiride also failed to prevent migration into C-injected pouches, but was effective against LPS (57% inhibition). In agreement, fenspiride reduced the increased vascular permeability by LPS (60% inhibition), but not by C. Fenspiride failed to block MNCF generation by LPS-stimulated macrophages. Rat treatment with dexamethasone, fenspiride alone or associated did not inhibit neutrophil migration into peritoneal cavities induced by MNCF, whereas migration by ZAP was blocked by 62%. Since, in contrast to MNCF, the recruitment of neutrophils in vivo by Z and LPS is partially dependent on the release of complement, it is possible that fenspiride modulates neutrophil migration by interfering with complement.