Glycoconjugate Journal 15, 523–526 (1998)

EGC2

Heparin potentiates *in vivo* neutrophil migration induced by IL-8

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Chemokine IL-8 attracts neutrophils by a haptotactic gradient, made possible by its interaction with proteoglycans of the extracellular matrix. Heparan sulfate, but not heparin, potentiates the attraction exerted *in vitro* by IL-8. In the present study we first confirmed this *in vitro* phenomenon, observing that IL-8 activity was potentiated 100% by heparan sulfate, but not by heparin. Then, we evaluated the interference of heparan sulfate or heparin on *in vivo* neutrophil migration induced by IL-8. The activity of rat IL-8 (3.5 μg/animal) preincubated with heparan sulfate (50 μg/animal) or heparin (77 μg/animal) was assayed on the rat dorsal air pouch. Contrary to *in vitro* experiments, heparin, but not heparan sulfate, potentiated the *in vivo* IL-8 activity two-fold. We investigated the relationship between this observation and that reported by others, that IL-8-induced migration depends on the presence of mast cells, which contain heparin-rich granules. We studied the neutrophil migration induced by IL-8 (3.5 μg/animal) into the rat peritoneal cavity depleted of mast cells. Neutrophil migration was reduced by 32% when compared to that observed in normal animals. The response of depleted rats was reconstituted by preincubation of IL-8 with heparin (77 μg/animal). These data suggest that heparin released from cytoplasmic granules may be the contribution of mast cells to IL-8-induced neutrophil migration.

***Keywords*: IL-8, heparin, heparan sulfate, mast cell, neutrophil migration**

Introduction

Interleukin 8 (IL-8) is a neutrophil attractant produced *in vitro* by lipopolysaccharide (LPS) stimulated monocytes [1]. The attractant activity of IL-8 seems to depend more on the formation of a haptotactic (insoluble) gradient than on the formation of a chemotactic (soluble) gradient [2–4]. Since IL-8 binds to immobilized heparin [5] and since another proteoglycan, heparan sulfate, potentiates the chemotactic ability of this interleukin *in vitro* [6], it was assumed that, *in vivo*, the interaction of IL-8 with these proteoglycans is important for the formation of the haptotactic gradient [6–8]. Other research indicate that IL-8 may have a direct chemoattractant activity on neutrophils: IL-8 is active *in vitro* [9] and its *in vivo* activity does not require DNA-dependent RNA synthesis [10, 11]. On the other hand, it has been suggested that the chemoattractant action of IL-8 may depend on resident cells. Mice of a mast cell-deficient strain do not respond to IL-8 [3] and the effect of IL-8 in rats depends on the presence of resident mast cells [12]. According to Perretti and coworkers [13], the influence of these cells on IL-8 activity may be associated with the secretion of endogenous histamine, which is a potent inducer of the expression of endothelial selectins. The objective of the present study was to determine the effect of heparin on IL-8-induced neutrophil migration *in vivo* and to investigate the role of mast cells, as cells that synthesize and store heparin, in the neutrophil migration induced by IL-8.

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Materials and methods

IL-8 preparation

IL-8 was obtained from the supernatant of a culture of LPS-stimulated rat macrophages, as described by Yoshimura and coworkers [1] and purified by the method of Van Damme and coworkers [5].

In vivo neutrophil migration assays

Neutrophil migration *in vivo* was measured using either the dorsal air pouch [14] or the peritoneal cavity of rats [15] as

models. In the first model, PBS, heparin and heparan sulfate were used as the negative controls whereas in the second model, PBS and heparin were used as the negative controls. Neutrophil migration was assessed 6 or 4 h after sample injection into the dorsal air pouch or peritoneal cavity, respectively.

Peritoneal mast cell depletion

Rats were depleted of their peritoneal mast cells by intraperitoneal (*ip*) injection of 20 ml distilled water as described by Mendonça and coworkers [16].

In vitro neutrophil migration assay

Human neutrophil migration *in vitro* was assayed using a 48-well Boyden-modified microchamber [17].

Results

Effect of heparin or heparan sulfate on the neutrophil attractant activity of IL-8

In vivo

IL-8 (3.5 μg per animal), when injected into the dorsal air pouch of rats, caused migration of $2.5 \pm 0.29 \times 10^6$ neutrophils/ml of cavity wash. This neutrophil migration was increased two-fold ($5.15 \pm 0.60 \times 10^6$ neutrophils/ml) when IL-8 was preincubated with heparin (77 μg per animal, data not shown). Lower doses of heparin, 38.5 or 58 μg per animal, had no effect on IL-8 activity and higher doses could not be tested because of the hemorragic effect of heparin. The ability of heparin to interfere with IL-8-induced neutrophil migration was confirmed in a second experiment (Figure 1A), in which the effect was doubled. When heparin was replaced with an equivalent dose of heparan sulfate, no significant effect was observed. Heparin and heparan sulfate administered separately did not induce migration to the air pouch. In experiments carried out on the peritoneal cavity, heparin had a small potentiating effect (about 14%) on the chemotactic activity of IL-8, as shown in Figure 2.

In vitro

In contrast to the *in vivo* assays, the *in vitro* chemoattractant activity of IL-8 (35 ng/well) on human neutrophils was not affected by preincubation with heparin (3 μg/well) but was potentiated 100% by incubation with heparan sulfate (3 μg/well). Heparin or heparan sulfate alone had no chemotactic activity on human neutrophils (Figure 1B).

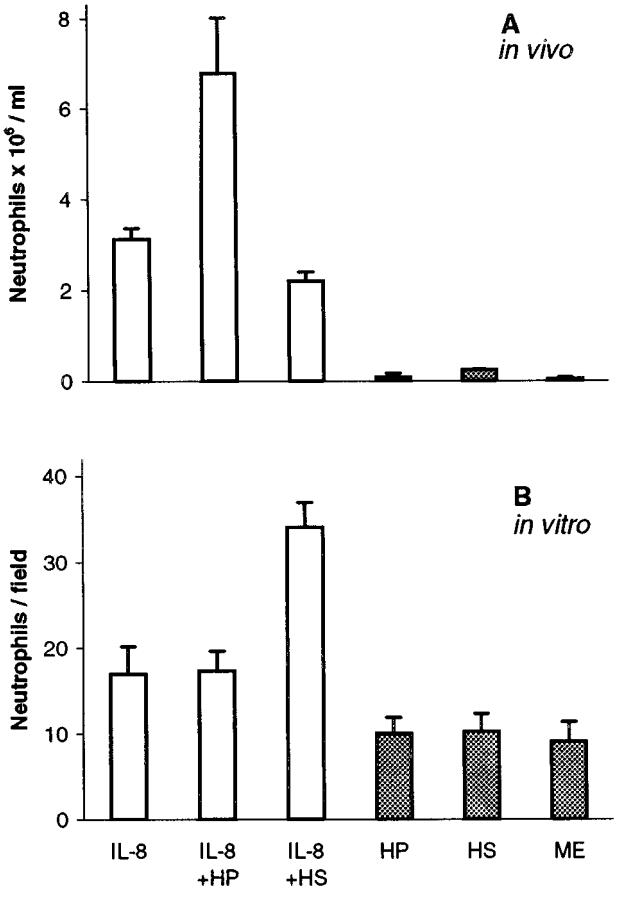

Figure 1. Effect of heparin (HP) or heparan sulfate (HS) on neutrophil migration induced by IL-8.

A. *In vivo* neutrophil migration to the air pouch of rats induced by IL-8 (3.5 μg/animal, open bars) preincubated or not with heparin (77 μg/animal) or heparan sulfate (50 μg/animal). The effects of medium (ME), heparin or heparan sulfate alone were assessed (closed bars). Neutrophil migration was assessed in five animals. The results are reported as mean ± SEM number of neutrophils $\times 10^6$/ml.

B. *In vitro* neutrophil migration in a microchamber induced by a suboptimal concentration of IL-8 (35 ng/well, open bars), preincubated or not with heparin or heparan sulfate (3.0 μg/well). The effects of medium (ME), heparin or heparan sulfate alone were assessed (closed bars). Neutrophils migrating through the polycarbonate filter pores during the 1 h incubation at 37°C in 5% CO_2 were counted (five fields per assay, each sample assayed in triplicate). Data are reported as mean ± SEM neutrophil number per field.

Effect of mast cells on the chemoattractant activity of IL-8

The demonstration that heparin potentiates the neutrophil chemoattractant activity of IL-8 *in vivo* (Figure 1A), taken together with evidence that cytoplasmic granules of mast cells are rich in heparin [18], stimulated us to evaluate the effect of mast cells on the neutrophil migration induced by IL-8. A model of resident mast cell depletion was first standardized by intraperitoneal injection of distilled water as described by Mendonça and coworkers [16]. In order to characterize the model of mast cell depletion we carried out counts of the mast cells present in the peritoneal wash fluid 3, 4, 5 or 6 days after the injection of distilled water. Absolute depletion of the mast cell population occurred on the fifth day. This interval was used in subsequent experiments concerning mast cell depletion. We also showed that mast cell depletion did not modify the neutrophil migration

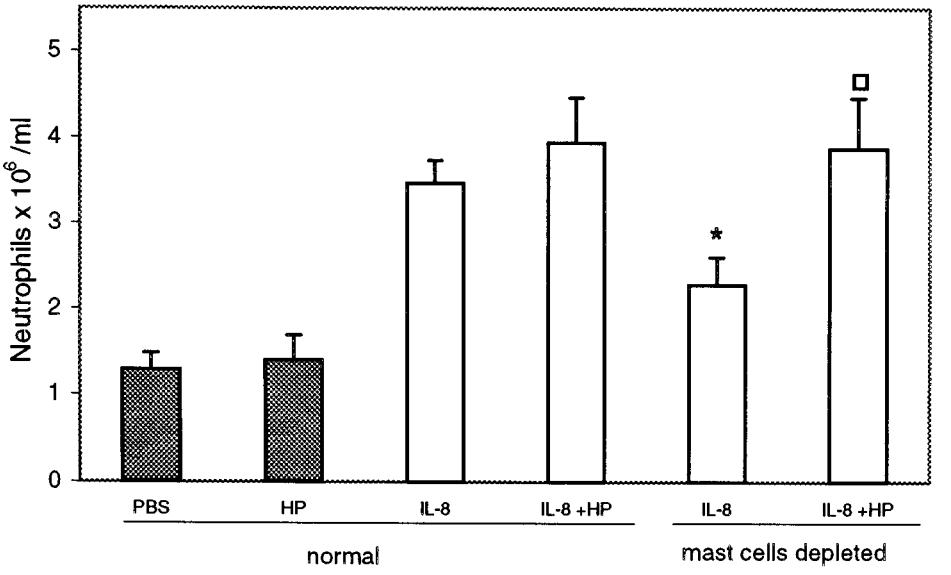

Figure 2. Effect of heparin (HP) on neutrophil migration induced by IL-8 on rats submitted or not to mast cell depletion by *ip* injection of distilled water. IL-8 (3.5 μg/animal, open bars) combined or not with heparin (77 μg/animal) was injected *ip* in normal rats or in mast cell-depleted rats. The effects of medium (PBS) and heparin alone were assessed (closed bars). The results are reported as mean ± SEM number of neutrophils $\times 10^5$/ml ($n = 5$ normal animals; $n = 10$ mast cell-depleted animals). *$p < 0.001$ compared to IL-8 in normal rats, □ $p < 0.001$ compared to IL-8 alone in mast cell depleted rats.

induced by thioglycollate administered *ip* five days after the *ip* water injection. The number of neutrophils in the cavity wash ($7.14 \times 10^6 \pm 0.19$ neutrophils/ml) was similar to that observed in the cavity of control animals ($6.15 \pm 0.35 \times 10^6$ neutrophils/ml). The neutrophil attractant effect of an *ip* injection of IL-8 was then compared in animals submitted or not to mast cell depletion (Figure 2). Neutrophil migration to the depleted cavity was 32% lower. Figure 2 shows that incubation of IL-8 with heparin had no significant effect on neutrophil migration in normal animals, but in mast cell-depleted animals heparin increased neutrophil migration by 45%, reaching values close to those induced by IL-8 in non-depleted animals.

Discussion

Heparin potentiated the *in vivo* attractant activity of IL-8 by more than 100% in rats. This potentiating effect occurred after incubation of 3.5 μg IL-8 with 77 μg of heparin per animal as demonstrated in Figure 1A. This effect was not observed when heparin was replaced by heparan sulfate in the same procedure (Figure 1A). In order to account for the apparent discrepancy between the present data and those reported by Webb and coworkers [6], who described a potentiating effect of heparan sulfate on the chemoattraction induced by IL-8 *in vitro* and no effect of heparin, we carried out an *in vitro* assay. This assay (Figure 1B) confirmed the observations of Webb [6], suggesting the hypothesis that heparan sulfate, as an IL-8 ligand [7], may correspond to the final potentiator of IL-8 and that the potentiating effect of heparin *in vivo* could be mediated by its ability to induce the expression of endothelial heparan sulfate as previously reported [19]. The absence of an *in vivo* effect of heparan sulfate on IL-8 activity may be attributed to the fact that the compound was offered in the soluble form which may not permit the formation of a chemokine haptotactic gradient. However, this gradient may be formed when the expression of heparan sulfate is induced *in vivo* by heparin, or when heparan sulfate binds to the surface of the pores of the polycarbonate filter of the migration microchamber *in vitro*. We then determined if there was a relationship between the effect of heparin of IL-8-induced neutrophil migration and the dependence on the presence of mast cells for IL-8 to exert its attractant effect [3,12]. We evaluated whether in mast cell-depleted animals, the administration of heparin would compensate for the absence of this cell type with respect to IL-8-induced neutrophil migration. Neutrophil migration induced by IL-8 *ip* injection in mast cell-depleted rats was 32% less than in non-depleted animals stimulated with the same dose of IL-8 (Figure 2). This confirms the report that the attractant effect of IL-8 on neutrophils is influenced by the presence of mast cells [12]. Administration of heparin in combination with IL-8 to mast cell-depleted animals quantitatively reestablished the attractant effect of IL-8, as shown by the fact that the number of migrating neutrophils was similar to that observed in non-depleted animals after stimulation with the same dose of IL-8 (Figure 2). These observations, although preliminary, favor the view that heparin potentiates the attractant action of IL-8 and that this phenomenon may be responsible for

the effect of the presence of mast cells on IL-8's attractant activity on neutrophils *in vivo*.

Acknowledgments

This work was funded by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Conselho Nacional de Pesquisa e Desenvolvimento (CNPq), and Financiadora de Estudos e Projetos (FINEP), Brazil. The authors thank Dr Lewis Joel Greene for his critical review of the manuscript.

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Received 9 September 1996, revised 24 June 1997, accepted 29 July 1997