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Low-dose heparin: a novel approach in immunosuppression

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Abstract Very low subcutaneous doses of standard and low molecular weight heparin inhibited the traffic of sensitized lymphocytes to a graft site, reduced in situ mononuclear cell infiltration and prolonged skin allograft survival in mice. Similar effects were caused by low doses of oral heparin, while higher oral doses prolonged graft survival. Our results suggest that oral heparin may have immunosuppressive properties applicable in clinical transplantation.

Key words Heparin · Skin grafts Immunosuppression

Introduction

Recent results emphasize the possibly important role of heparin and other glycosaminoglycans in the regulation of a variety of immunobiological processes. These effects, apparently unrelated to the well-known anticoagulant action of heparin, may also be relevant in transplantation leading to enhanced graft survival [2, 7]. Heparin has been shown to preserve the integrity of vessel walls, inhibiting smooth muscle cell proliferation and migration, impairing intimal thickening and preventing endothelial injury [1, 5]. Heparin can bind growth factors and cytokines [8]. Its ability to bind TNF may be of particular interest in view of the postulated role of TNF in graft destruction [6]. The fact that extracellular matrix proteins (ECM) have heparin-binding regions suggests that heparinoids play a role in the regulation of leucocyte ECM interactions which in turn may influence tissue infiltration including at the site of an allograft [8].

Very low non-anticoagulant doses of heparin (VLDH) inhibit lymphocyte traffic to mouse skin allografts and prolong their survival [2]. VLDH have also been shown by us to be effective in controlling chronic renal allograft rejection [4]. In this report we confirm and extend those results by showing that low molecular weight (LMW) heparin and oral heparin are also effective.

Materials and methods

Skin grafting

Grafts were exchanged between C_3H (H-2^k, donors) and Balb/c (H-2^d, recipients) as described previously [7].

Homing of lymphocytes to an allograft

This phenomenon was studied using ^{51}Cr-labelled sensitized (by prior skin allografting) spleen lymphocytes (or nylon-purified T cells) on consecutive days post-transplant [2]. The radioactivity of removed skin transplants was determined in a gamma counter 4 h after administration of the cells (total radioactivity 6×10^5).

Histopathological examination of skin allografts

Allografts were removed on consecutive days post-transplant and examined using standard technique. The degree of mononuclear cell infiltration was ranked from + to + + + +.

Heparins

Standard heparin (Polfa Pharmaceuticals, Warsaw) as well as LMW heparin (Lovenox, Rhone-Poulenc) were used. Heparin was given either subcutaneously (s.c.) 5–50 μg/mouse daily or in the drinking water. To avoid contamination with heparin-degrading bacteria, drinking bottles were sterilized and changed every 2 or 3 days.

Statistical analysis

The Mann-Whitney-Wilcoxon test using Statgraphics software were used for the analysis.

Results

In the first part of this work, we extended our previous findings by showing that LMW heparin, as well as its standard form, is immunosuppressive when given via the s.c. route and causes a significant prolongation of skin allograft survival. The lowest dose causing an immunosuppressive effect was 5 μg/mouse (Fig. 1), which is identical to the effective dose of standard heparin. This dose of heparin was effective when given every day and every second day, but not when given every third day post-transplant (not shown).

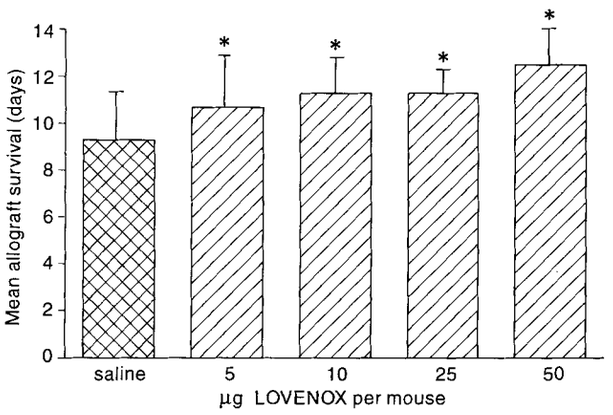

Fig. 1 Effect of different s.c. doses of LMW heparin (Lovenox) on skin allograft survival in mice. Mean values of three experiments ± SD. Each experimental group included 10–12 animals (* $P < 0.05$)

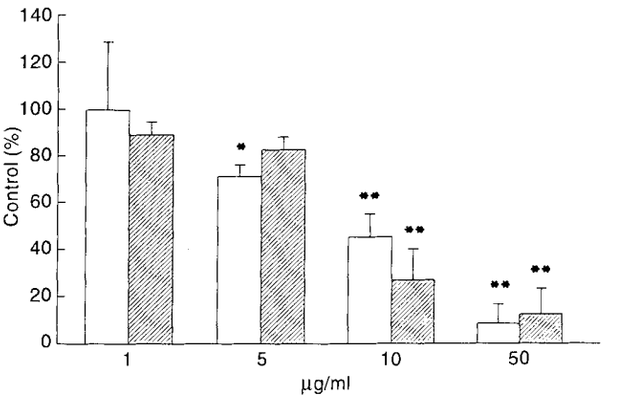

Fig. 3 Both standard and LMW heparin are active in inhibiting sensitized lymphocyte migration to an allograft when given orally. Control: mice receiving water alone. Mean values of three experiments ± SD (□ LMW heparin, ▫ standard heparin; * $P < 0.02$, ** $P < 0.001$)

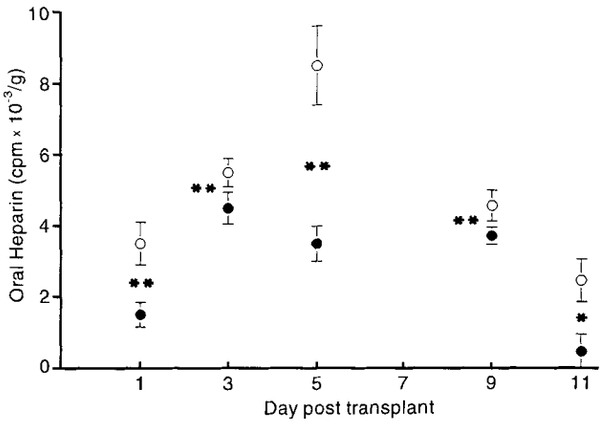

Fig. 2 Inhibitory action of oral heparin on sensitized lymphocyte traffic to an allograft site. Data expressed as cpm per gram of graft tissue. Mean values of four experiments ± SD (● heparin, ○ water; * $P < 0.05$, ** $P < 0.005$)

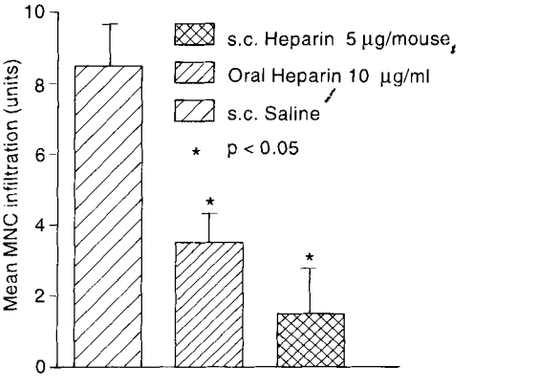

Fig. 4 Subcutaneous and oral heparin inhibit mononuclear cell infiltration of the allograft site. The data are depicted as the mean sum of infiltration ranks (on consecutive days post-transplant) in each group of mice. Mean values of four experiments ± SD (▩ s.c. heparin 5 μg/mouse, ▨ oral heparin 10 μg/ml, ▫ s.c. saline; * $P < 0.05$)

Oral heparin significantly inhibits the traffic of sensitized cells to an allograft, and this effect was most evident on day 5 post-transplant (Fig. 2). Both standard and LMW heparin were active in inhibiting T-cell migration to the allograft when given orally (Fig. 3).

A heparin-dependent decrease in lymphoid cell migration to the allograft was paralelled by a reduction in mononuclear cell infiltration of allografts from mice receiving s.c. or oral heparin (Fig. 4). While low doses of oral heparins can significantly decrease lymphoid cell traffic to skin transplants, the doses causing prolongation of skin transplant survival were much higher and corresponded to at least 2 mg/ml (3–4 mg/day) in drinking water, in contrast to the effective s.c. dose of 5 μg/mouse.

Discussion

Our results confirm our earlier studies indicating that very low s.c. doses of heparin inhibit lymphoid cell migration to skin transplants and infiltration of allografts [2, 7]. We showed further that LMW heparin is also active. More importantly, low doses of oral heparin decreased cell traffic to skin transplants and higher doses prolonged allograft survival. Thus, it appears that oral administration of standard and LMW heparin could be used as an efficacious and safe adjunct therapy in clinical immunosuppression. Our recent preliminary results in patients with rheumatoid arthritis fully support this view [3].

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