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Effect of Local Administration of Levan on Skin Homograft Rejection in Mice (39500)

ILAN BLEIBERG, GIDEON STRASSMAN, JUDITH LEIBOVICI, AND MOSHE WOLMAN[1]

Departments of Cell Biology, Histology, and Pathology, Tel-Aviv University, Sackler School of Medicine, Ramat Aviv 61999, Israel, and the Chaim Sheba Medical Center, Tel-Hashomer, Israel

Various immunosuppressors have been used to prolong the survival of homografts and thereby delay the rejection of implanted organs and tissues. Levan, a high molecular weight polysaccharide, has been shown to inhibit the inflammatory response by preventing both transport of proteins, including immunoglobulins (1, 2), and diapedesis of cells across the endothelium (3). Repeated intraperitoneal injections of levan were found to delay homologous skin graft rejection in mice significantly (4). The present work describes the effects of local subcutaneous injections of levan on graft rejection.

Materials and methods. Balb/C male mice, 8–10 weeks old, were used as recipients and as donors of isologous skin. Homologous skin samples were taken from C3H mice.

Each animal was grafted with isologous skin on one side of the back and homologous skin on the other side. The isografts served as controls for the skin-grafting technique.

Skin transplantation was performed according to Billingham and Silvers (5), with the addition that residual hair was removed by BaS and the skin was cleansed with ethanol and ether after shaving. Grafts were obtained with a punch, 5 mm in outer diameter. Micropore surgical tape (Blenderm 3M Company, St. Paul, Minnesota) was placed over the transplant with a cellophane window to permit daily inspection. The cellophane was prevented from adhering to the graft by a plastic ring (10 mm across and 2 mm thick) placed between them.

Native levan was obtained from the Technical Unit, Department of Biological Chemistry of the Hebrew University in Jerusalem. Five percent levan solution in saline was prepared according to Shilo, Wolman, and Wolman (3).

Levan was administered either intraperitoneally (ip) or by subcutaneous (sc) injection:

One group of mice received ip injections of 10 mg of levan on Days 8, 6, and 4 before grafting and 25-mg daily sc injections beginning 1 day before grafting until homograft rejection.

Other groups of mice received daily sc injections of 5 or 10 mg close to (<1.5 cm) or far from (>4 cm) the graft site, beginning 1 day before grafting and continuing until rejection.

Results. The effects of ip and local sc injections of levan are summarized in Table I. The mean survival time (MST) of the grafts in the control untreated mice was 10.1 days. Daily sc injections of 5 mg of levan adjacent to the skin graft increased MST to 15.5 days, and 10 mg increased it to 17.2 days. A dose of 10 mg injected at a distince from the skin graft, in which any possibility of local damage to the graft was eliminated, yielded an MST of 17.8 days. When given ip, a dosage of 25 mg of levan was required to obtain a similar effect (MST of 16.6 days).

The distribution of survival times of animals treated by various routes and dosages of levan is illustrated in Fig. 1. In the untreated control mice, 87% of the homografts were rejected by Day 11, while none of the grafts in the levan-treated animals was rejected at that time. The rejection delay was dose dependent, as can be seen in animals given different amounts of levan at the same distance from the graft. One hundred percent graft rejection occurred on Day 17 with a 5-mg daily dose of levan, while with 10 mg this occurred on Day 22. No significant differences were noted between sc injections adjacent to or remote

[1] Established Investigator of the Chief Scientist's Bureau, Ministry of Health, Israel.

TABLE I. THE EFFECT OF VARIOUS ROUTES OF LEVAN ADMINISTRATION ON THE MEAN SURVIVAL TIME OF SKIN HOMOGRAFTS

Group	Dosage (mg)	Route of administration	Number of mice	Mean survival time (MST in days)	P
Control	–	–	15	10.1 ± 1.4	
Levan	25	ip	11	16.6 ± 2.7	<0.005
Levan	5	sc[a]	12	15.55 ± 1.1	<0.005
Levan	10	sc[a]	11	17.2 ± 2.9	<0.005
Levan	10	sc[b]	19	17.8 ± 2.0	<0.005

[a] <1.5 cm from the graft.
[b] >4 cm from the graft.

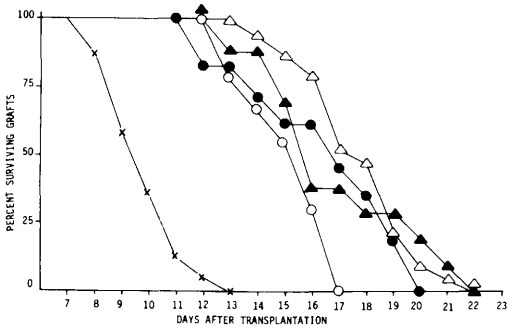

FIG. 1. Effect of levan on survival of skin grafts in Balb/C mice: ×, control; ●, 25 mg ip; ○, 5 mg local; ▲, 10 mg local; △, 10 mg >4 cm from the graft.

from the transplant. Intraperitoneal injections of 25 mg delayed 100% rejection longer than local injections of 5 mg, but less than local 10-mg injections (20, 17, and 22 days, respectively).

Discussion. The inhibitory effect of levan administered ip on graft rejection has been demonstrated in a previous work (4). In view of the possible damage that ip injections can cause to the abdominal organs, the sc route was considered as an alternative.

Subcutaneous administration of a dose as low as 10 mg had an inhibitory effect on graft rejection similar to 25 mg of levan injected ip ($0.4 > P > 0.3$). Even 5 mg of sc levan inhibited the graft rejection to nearly the same extent ($0.1 > P > 0.05$). These findings indicate that local sc injections of the polysaccharide are at least as effective as systemic administration. In other experiments in our laboratory (unpublished results), the level of levan in the blood was found to be much lower when injected sc than ip with the presently used doses and schedules. This indicates that the inhibitory effect of levan on graft rejection primarily depends on the local concentration of levan in the graft bed area.

In view of the possibility that repeated injections of levan administered to the neighborhood of the graft bed might mechanically affect graft survival, we also injected levan sc at a distance from the grafted area. In this experiment the delay in rejection time was similar to that of mice injected with levan near the graft ($0.3 > P > 0.2$). We concluded that local sc injection of levan causes no physical damage to the graft.

Summary. The influence of subcutaneous levan administration on the survival time of homografts was compared to that of intraperitoneal administration of levan. It was found that the inhibitory effects on rejection produced by a high dosage of levan administered intraperitoneally were similar to those produced by a much lower subcutaneous dose. Similar inhibitory effects were observed when levan was injected subcutaneously close to or far from the graft, and it was concluded that the inhibition was not a result of physical damage to the graft bed or mechanical interference with the rejection process.

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