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## Enzymatic Manipulation of the Site of Spinal Cord Injury Allows Better Survival and Adhesion of Allogeneic Homotopic Fetal Transplants in Adult Rats

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Spinal cord (SC) contusion in rats yields an experimental model of SC trauma in humans. This model has often been criticized for its lack of reproducibility. Both histological observations and functional recovery cannot be reproduced consistently. The recent demonstration that homotopic fetal transplants in newborn and adult SC can improve locomotion, discloses the ability of fetal neural tissue to partially restore SC function (Kunkel-Badgen, 1990; Stokes and Reier, 1992).

A necrotic area at the site of the lesion is formed in the first days after SC contusion. In the later post-traumatic stages this area evolves into a cavitation. Host-graft integration of tissues transplanted into the lesioned area at any stage post-lesion will be diminished or prevented in acute stages after trauma by necrotic tissue and in subacute and chronic stages post-injury by cellular and scarring tissue (Reier, 1988).

We have hypothesized that enzymatic manipulation of the lesioned area could allow a better host-graft integration. To test this hypothesis 20 anesthetized adult Long-Evans rats weighing 240-270 g were subjected to one-level laminectomy (T9). The SC injury was produced by the weight-drop method (15 g/10 cm). Nine days after SC injury all rats were transplanted with pieces of fetal thoracic SC (E-15). The surgical site was reopened and the contused area was identified. Dura and SC were sagittally opened. Before transplantation, in 10 rats (control group) the lesioned area was freed of necrotic and scar tissues with light aspiration (2-3 cm Hg), taking care to maintain the integrity of preserved SC. In the other 10 rats (experimental group) the area of the lesion was also prepared using light aspiration, followed by the application of a mixture of collagenase (0.25%) and

hialuronidase (0.1%) to the area, which was changed frequently for 20 minutes. Finally, EDTA (0.1 M) was applied for 30 seconds. Methylprednisolone was administered i.p. at the start of the surgery for transplantation and at 2 and 4 hours (30 mg/kg/dose) thereafter; the drug was re-administered i.m. 8 and 24 hours after surgery (60 mg/kg/dose). For conventional histologic analysis rats were killed 9 days after transplantation. Sagittal sections of SC were obtained and stained with H&E and Gallego stains. From these sections the best slide of each case was chosen for morphometric analyses. A 10x micrograph was obtained including the whole live graft, and the transplant was drawn on millimetric paper indicating the host-graft contact area. A stereological method was used to evaluate the amount of surviving tissue. The total number of crosses in the area of live transplants was registered in each case. To evaluate the host-graft adhesion a similar stereological test was used, but in this case the total squares following the line of host-graft interface were registered. To evaluate statistical differences a t-test analysis was used.

The proportion of rats showing any amount of surviving grafts was 100% for the experimental group, and 90% for the control group. The proportion of rats showing any amount of host-graft adhesion was 70% in the experimental group and 50% in the control group. In the experimental group the total amount of surviving grafts measured a total of 2,286 crosses (mean 228.6; SD 83.9); in the control group the total was 1,604 crosses (mean 160.4; SD 138.2). The difference between the two measurements was not significant. The host-graft adhesion in the experimental group measured 359 squares (mean 35.9; SD 34.4) and 133 squares in the

control group (mean 13.3; SD 16.6). The difference was statistically significant ( $p < 0.05$ ). The greater amount of host-graft adhesion in the experimental group could be attributed to an enhanced host-graft contact after the removal of the necrotic and scar tissues by enzymatic manipulation. This is a gentle procedure that eliminates the barrier for transplant integration and preserves the lesioned SC.

#### CONCLUSION

The enzymatic manipulation of the lesioned area 9 days after SC contusion allows a better host-graft adhesion than that seen in the control group.

The proportion of rats showing any amount of surviving grafts was high in both experimental and control groups. There were no differences in the amount of surviving grafts between the two groups. This enzymatic manipulation should now be evaluated in later stages of SC injury, in order to determine whether it is possible to remove the scarring from the lesioned site and maintain the integrity of the preserved SC.