Effect of Hyperbaric Oxygen Treatment on Fetal Spinal Cord Grafts: A Preliminary Experimental Study

Hiperbarik Oksijen Tedavisinin Fötal Spinal Kord Greftleri Üzerine Etkisi: Deneysel Bir Ön Çalışma

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Abstract: In fetal neural tissue transplantation, lack of oxygen supply to the graft in the acute stage is an important problem. This study evaluated the effects of hyperbaric oxygen treatment on fetal spinal cord grafts. Spinal cord tissues obtained from 16-day fetal rats were transplanted to the spinal cord of adult Wistar rats (n=30). The hosts were then divided into two groups. Group 1 were untreated controls, and Group 2 rats received 100% O_2 at 2.5 atmospheres pressure for 90 minutes twice a day for 7 days. All animals were sacrificed for histological examination. Degenerative changes, interface, host spinal cord edema, and vascularization of the graft were scored on three-grade scales, and the scores were statistically analyzed. The graft survival rates in Groups 1 and 2□ were 46.1% and 71.4%, respectively. Animals treated with HBO showed statistically less spinal cord edema than the untreated group ($P < 0.05$). The interface was also significantly better in the treated group ($P< 0.05$). These findings indicate that HBO therapy can increase the chances of graft survival. We believe that the edema-reducing effect of HBO prevented the graft from becoming displaced and, thus, contributed to the successful integration of graft and host.

Key words: Fetal graft; hyperbaric oxygen; neural transplantation; rat; spinal cord

Özet: Fötal nöral doku aktarımlarında, grefte erken dönemde oksijen desteğinin olmayışı önemli bir problemdir. Bu çalışmada, hiperbarik oksijen tedavisinin (HBOT) fötal spinal kord greftleri üzerine olan etkileri değerlendirilmiştir. 16 günlük fötal ratlardan sağlanan spinal kord dokuları erişkin Wistar ratların (n=30) spinal kordlarına nakledildi. Sonrasında iki guruba ayrıldılar. İkinci gruptaki denekler günde iki kez, 90 dakika olacak şekilde 7 gün, 2.5 atmosfer basınç altında % 100 oksijen tedavisi aldılar. Tüm denekler histolojik incelemeler için feda edildiler. Dejeneratif değişiklikler, greft-konakçı bütünlüğü, konakçı spinal kord ödemi ve greftin vaskülarizasyonu 3-dereceli ölçeklerde puanlandırıldı. Bu puanların istatiksel analizleri yapıldı. Greft yaşam oranları 1. Grup ve 2. Grupta sırasıyla % 46.1 ve % 71.4' dü. HBO tedavisi alan deneklerin spinal kordlarındaki ödem, tedavi yapılmayan deneklere göre istatistiksel olarak daha azdı ($p<0.05$). Konakçı-greft bütünlüğü yine bu grup deneklerde daha iyi ($p<0.05$) idi. Bu bulgular, HBOT'nin greftin yaşama şansını artırabileceğini göstermektedir. HBOT' nin ödem azaltıcı etkisinin, greftin kaviteden atılımını önlediğini, böylece konakçı-greft bütünleşmesine katkısının olduğunu düşünmekteyiz.

Anahtar kelimeler: Fötal greft, hiperbarik oksijen, nöral tranplantasyon, rat, spinal kord.

INTRODUCTION

Attempts to transplant fetal central nervous system (CNS) grafts into the spinal cord have shown that fetal cortical, spinal, and tegmental transplants can survive and grow in the adult rat spinal cord (5,6,14,15,18,34,66,72-74,85). However, experimental studies also indicate that the survival rate of implanted grafts is variable, and the results that have been presented by various authors are difficult to compare. The reported data are controversial. Transplants have been reported to produce improvement in some experimental models of spinal cord injury (7,14,49,73,82), but other experimental efforts to foster axonal regeneration in the transected spinal cord have yielded discouraging results (15,18,71). Although we currently know that transplants can survive at sites of complete spinal cord transection (28,35,71), the success rates for such procedures have been very low (18,20,28).

Today, the main question is not whether neural grafting to the spinal cord is possible, but how the survival of neural transplants can be enhanced, and what conditions lead to maximum graft survival and ultimate structural and functional restoration of the injured spinal cord. A number of recent studies have emphasized the importance of vascularization of fetal CNS grafts (13,47,48,52,53,81,91). In some investigations, pockets and lesions have been created to increase vascular supply to the transplant (45,46,66). In attempt to create an appropriate environment for the fetal CNS graft, some researchers have placed these grafts into resection lesions that were made 1-12 weeks earlier (8,34,45, 46,51,66,76). In addition, the use of various growth factors to modify the microenvironment after transplantation has been shown to improve graft survival and function (29,79,87). Other therapeutic agents or techniques have also been tried in efforts to promote long-term graft survival (1,28,31,43,53,70,86).

Hyperbaric oxygenation (HBO) is a widely recognized adjunctive therapy. Its beneficial effects as a therapeutic modality have led to a broad spectrum of uses, ranging from carbon monoxide poisoning to a variety neurological disorders (3,4,26,33,39,40,65,84). Lesions involving excessive tissue damage with acute oxygen deficiency, as in wounds that require skin transplants, radiation-induced necrosis, skin-muscle flaps, crush-syndrome, necrotic wound infections, compartment examined. syndrome, and burns are all further indications for HBO (21-23,56,58,80,83).

In contrast to other areas of hyperbaric medicine, little research has been done on fetal tissue transplantation (50). This study was designed to evaluate the possible beneficial effects of HBO therapy after transplantation of intact fetal rat spinal cord into a completely transected adult rat spinal cord. We histologically examined the viability, vascularization, and parenchymal integration of each graft. This is the first study to describe the experimental use of HBO for this purpose.

MATERIALS AND METHODS

Thirty adult male Wistar albino rats, each weighing 250 to 280 gm, were used as hosts. The donors were fetuses from time pregnant female Wistar rats at 16 days of gestation (E16). (Day 0 = day of insemination). All the male rats underwent embryonic homotopic transplantation to their spinal cord. The experimental protocol was approved by the Animal Care Committee at our institution, and was performed in accordance with the Principles of the Helsinki Declaration.

Donor tissue preparation:

Pregnant female rats at 16 days of gestation were anesthetized with intraperitoneal sodium pentobarbital (40 mg/kg), and fetuses were obtained by cesarean section. At the time of utilization, one uterine horn was placed in ice-cold lactated Ringer's solution and the fetuses were removed from the amniotic sacs. Each harvested fetus was checked for viability by observing color and movement. Harvested fetuses were anesthetized by hypothermia on a bed of crushed ice. Under magnification, the spinal cord was dissected free, and 1 to 2 mm long segments of the thoracic region were obtained using sharp microscissors. Exposure of the grafts to air was prevented by continuous irrigation with Ringer's solution.

Transplantation procedures:

The hosts were anesthetized with intraperitoneal sodium pentobarbital (40 mg/kg) and a laminectomy was performed at the mid-thoracic level. With the aid of an operating microscope, the dura mater was opened longitudinally with a 27-gauge needle and the spinal cord was transected subpially using a microaspiration technique (71). The procedure resulted in a cavity with a 1 to 2 mm gap between the stumps of the transected spinal cord. The cavity was then filled with Gelfoam to assist in hemostasis while the donor was prepared. Following preparation of the donor tissue, Gelfoam was

removed from the lesion site and the cavity was rinsed with cold sterile saline. Using jeweler's forceps, one or two fetal spinal cord fragments were immediately implanted into the cavity created by aspiration. Special care was taken to ensure that the fetal spinal cord transplant was oriented in the rostrocaudal direction. The lesion site was covered with a narrow strip of Gore Tex surgical membrane (W.L. Gore & Associates, Inc., Arizona) to limit fibroadhesive reactions. The muscle and skin wounds were then closed in layers, and the animals were maintained on heating pads until they recovered fully from the anesthetic.

Postoperative care:

Procedures were performed under sterile conditions and normothermia was maintained by means of warming pads. All operated animals received daily intramuscular injections of prophylactic antibiotics for 3 consecutive days after surgery. None of the rats were immunosuppressed. In the postoperative period, all the rats underwent manual bladder expressions twice daily, and this was usually continued to the end of the study.

HBO therapy:

After transplantation, the animals were divided into two groups. Those in Group 2 underwent HBO therapy, which was started 1 hour after transplantation. They were placed in a hyperbaric chamber designed for laboratory animals and received 100% oxygen at 2.5 atmospheres absolute (ATA) for 90 minutes, twice a day for 1 week. After each treatment period, all animals were returned to normobaria within 10 min, during which time they breathed compressed air. We used a regimen that has been found to be safe and effective (42,90). The rats tolerated HBO conditions well, and they displayed no signs of discomfort while receiving treatment. The pressure chamber was made of steel. A cylinder-shaped HBO room 70 cm long and 40 cm in diameter and resistant to 4 atmospheres of pressure was constructed for the experiment (Figure 1). It was connected to an oxygen tube by a three-valve system, and had a door and two observation windows. The inside pressure was monitored and adjusted using a manometer connected to the system.

Histological examination:

The rats were maintained on a 12 hr/12 hr light/dark cycle, with food and water available. After the last treatment, they were re-anesthetized with an overdose of intraperitoneal sodium pentobarbital and were perfused intracardially with 0.9% saline,

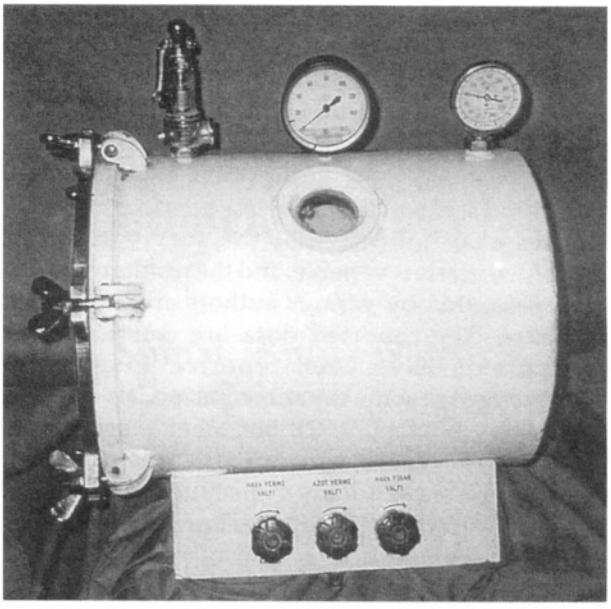

Figure 1: Photograph of the hyperbaric oxygen application chamber that was specifically designed for the experimental studies.

followed by 4% paraformaldehyde in 0.1 M phosphate buffer at pH 7.4. It was easy to re-expose the lesion site because of the use of the surgical membrane. The membrane minimized the problems associated with scarring and hypervascularity in terms of locating the graft site. After the lesion site was exposed, the cavity was examined visually for the presence of an obvious transplant. The spinal segments that included the transplants were immediately removed and drop-fixed in 10% buffered formalin. Following fixation in formalin, they were embedded in paraffin. Sections 5 µm-thick were cut in the longitudinal plane on a microtome, and were then stained with hematoxylin and eosin in order to examine the characteristics of the grafts and their relationship to host tissue. For each sample, seven sections were prepared and four fields were randomly selected. All slides were evaluated and graded by a blinded observer.

The slides were examined under light microscopy. Host spinal cord edema was evaluated using a three-point scale ranging from 1 for normal, to 3 for severe. Within the graft, degenerative changes including micro- and/or macrocyst formation, cavitation, necrosis, gliosis, and composition of neurons were assessed using a three-point scale of 1 for normal (none or mild degenerative changes), 2 for moderate, and 3 for severe. The degree of parenchymal integration (interface) was also scored on a three-point scale with the following specific

descriptives: 1 = an interface with large gaps, severe glial reaction (poorly integrated graft); 2 = an interface with small gaps, moderate glial reaction; and 3 = complete parenchymal integrity with no glial reaction and gaps. Vascularization of the graft was also scored semiquantitatively. Each branch was counted as a single vessel. A score of 1 to 3 was derived for vascularization in each selected field, with a score of 1 indicating an area containing 5 vessels; a score of 2 indicating an area containing 6-10 vessels; and a score of 3 indicating an area containing 11 vessels. The scores for these parameters were averaged and compared between the groups. In addition, the data were statistically analyzed using Chi-Square and Fisher's Exact Probability tests. Differences were considered to be statistically significant at $P < 0.05$.

RESULTS

The results of the histological examinations are presented in Table 1, and the mean scores are shown in Figure 2. None of the animals showed functional recovery.

Group 1:

Of 15 animals, 2 died from different causes at different times throughout the posttransplantation period. Two of the remaining 13 animals developed local postoperative infection at the operative site. Only 6 of 13 (46.1%) host animals had viable grafts at 7 days after transplantation, and the graft volumes in all 6 cases had decreased compared to their initial size. In the remaining seven cases, the graft had become displaced from the gap at the site of transection, presumably due to edema in the traumatized spinal cord. These displaced grafts had formed a dense degenerated fibroglial mass. The transplantation site was characterized by degenerating cellular debris, and contained aggregations of extravascular blood cells. In general, the embryonic spinal cord tissue in this group showed very limited growth and differentiation. The neurons were randomly dispersed or grouped in clusters, and most of them were undifferentiated (Figure 3). Although the transplants contained numerous areas of microvacuolation and degenerative neuronal changes, an area of intense necrosis cavitation and macrocyst formation was consistently seen in the central region of the graft. The mean score for the degenerative changes was 2.3.

Most of the grafts showed gliosis. Typical spinal cord cytoarchitecture was not observed. There was also more histological evidence of host spinal cord in this group compared than in the Group 2 animals. Most of the cases in Group 1 had moderate to severe edema, and the median score was 2.1 (Figure 4).

Table 1: Summary of histological findings.

a For degenerative changes, 1=none or mild, 3= severe
b For host spinal cord edema, 1=normal, 3=severe
c For interface, 1= poorly integrated graft, 3=complete integration,
d For vascularization, 1=an area containing 5 vessels, 3= an area containing > 11 vessels

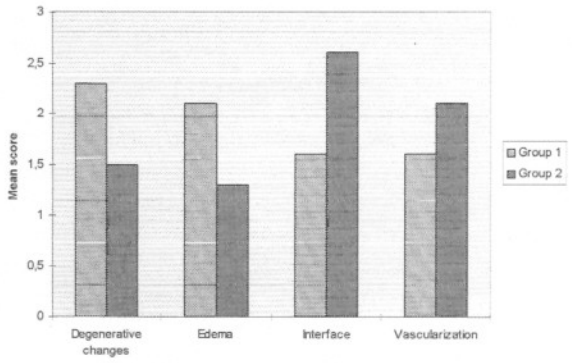

Figure 2: Bar graph depicting the mean scores for the control animals and those treated with HBO.

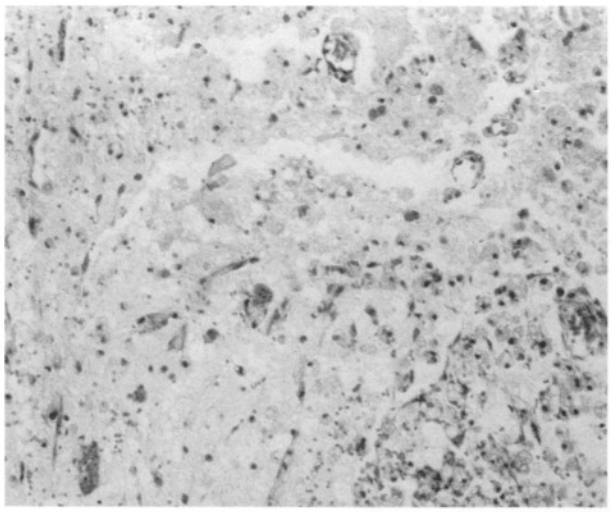

Figure 3: An example of a fetal spinal cord graft from a Group 1 animal. The tissue contains some small and many atrophied neurons. The internal structure of the graft is disorganized and there is obvious degenerating tissue (H&E x 200).

There were varying degrees of anatomical integration between transplant and host. Most of the transplants were only partially integrated with the parenchyma of the host spinal cord. Intense glial proliferation and cyst formation were commonly observed along the interface (Figure 5). The score for interface ranged from 1 to 3, and the mean score was 1.6.

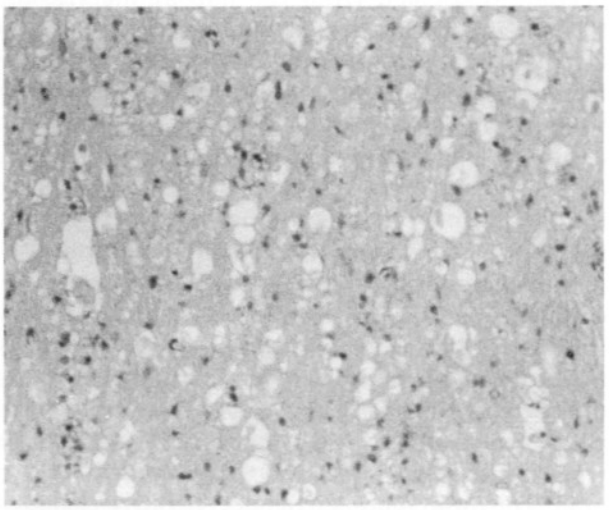

Figure 4: Section of a Group 1 host spinal cord showing Grade 2 edema formation (H&E x 200).

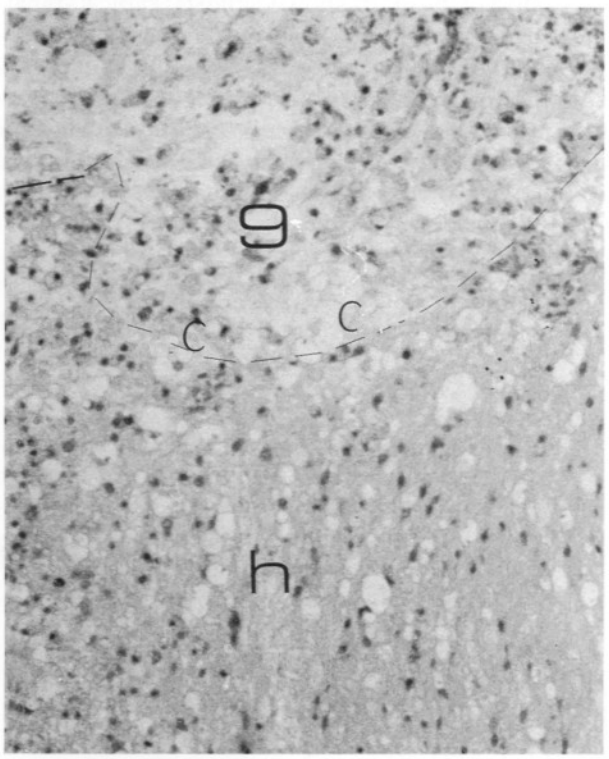

Figure 5: Section from a Group 1 rat showing the host-graft interface (broken line). Although the graft (g) is tightly juxtaposed to the host parenchyma (h), it is poorly integrated with it. There are many cysts (c) at the interface (H&E x 100).

Blood vessels of larger diameter were present in some sections of the grafts, but were few in number. A few vessels could also be identified at the host-graft interface. The mean score for vascularization was 1.6.

The graft site in this group also exhibited evidence of rejection. On histological examination, there was a dense accumulation of polymorphonuclear leukocytes and macrophages in the grafts (Figure 6), but no evidence of any local spread of infection into the host spinal cord.

Group2:

In this group, one animal died from unknown cause(s) in the early posttransplantation period. None developed infection. The transplant survival rate, excluding the case of postoperative death was 71.4%. Most of the viable grafts had not decreased in size, and had completely filled the original cavity. On macroscopic examination, 8 of 10 fetal grafts were grossly visible, and 3 grafts in this group had also expanded dorsally. The surface of the transplant had a whitish appearance that was clearly different from the adjacent spinal cord of the host.

Histologically, the transplants appeared to contain a higher density of neurons than the surrounding host spinal cord (Figure 7). There were

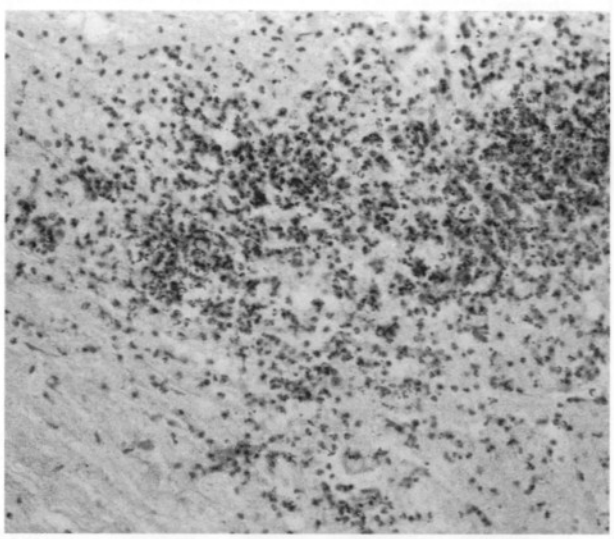

Figure 6: Light micrograph of a Group 1 animal showing the graft occupied by polymorphonuclear leukocytes, which contributed to the necrobiosis of many neurons (H&E x 100).

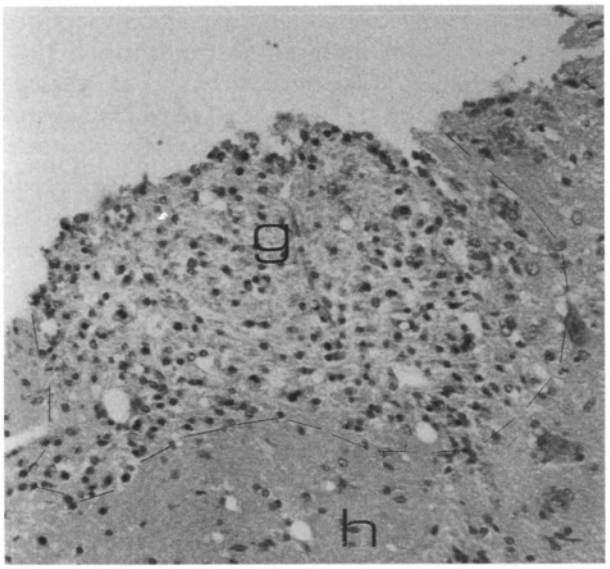

Figure 7: Photomicrograph showing the graft (g) with good parenchymal integration (broken line) in a Group 2 specimen. The graft is densely populated with cells. All the neurons appear healthy and normal, and there are no signs of obvious pathological reaction (H&E x 50).

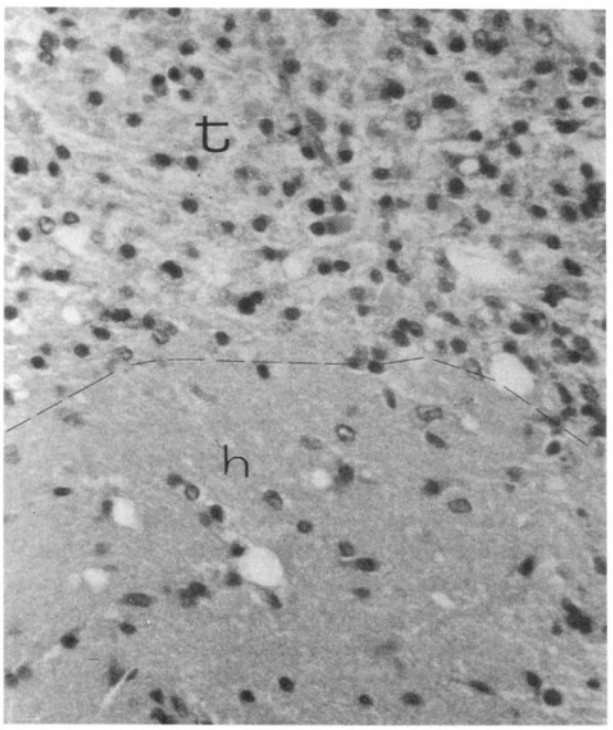

Figure 8: An example of an interface in a Group 2 animal. The broken line shows the boundary between the transplant and host tissue. The transplant (t) appears to be well integrated with the surrounding host neuropil (h) (H&E x 200).

many normal-looking, healthy, well-differentiated neurons, and they were homogeneous in composition compared to Group 1 grafts. Nevertheless, in a few cases, some areas of gliosis were present. Although regions of microcyst formation and micronecrosis were detected, in most cases there were no expansive areas of necrosis, hemorrhage, or macrocyst formation, all of which were routinely observed in the Group 1 rats. The mean score for degenerative changes was 1.5. No typical spinal cord architecture could be identified. Animals in this group showed statistically less spinal cord edema than the untreated group ($P< 0.05$). The mean score for edema was 1.3.

In general, the transplant fused well with the host spinal cord parenchyma. In the best cases, the regions of parenchymal integrity between graft and host (interface) occurred with no intervening connective tissue scar or cystic cavitation (Figure 8). The mean score for interface was 2.6, and there was a statistical difference between the two groups ($P< 0.05$). In addition, small blood vessels were observed along the host-transplant interface (Figure 9). Large, dilated, thin-walled vessels resembling dilated veins were often observed within the transplants. The grafts had good vascular supply (Figure 10). Although there was no statistical difference between the two groups, the mean score for graft vascularization in the second group was 2.1, which was higher than the score for the untreated grafts.

Microscopic examination revealed less inflammatory reaction in the HBO-treated group than in the controls. However, in some cases there were mononuclear cells surrounding the vessels in the grafts.

DISCUSSION

The results of this preliminary study indicate the following: 1. The survival of fetal (E16) homotopic rat spinal cord grafts transplanted to the completely transected spinal cord of adult rats appears to be enhanced by applying HBO; and 2. The parenchymal continuity between the graft and the host spinal cord, and the vascularization of the graft can be enhanced by administering HBO therapy. Although the achieved survival rate was not excellent, there were significant differences between the two groups. The

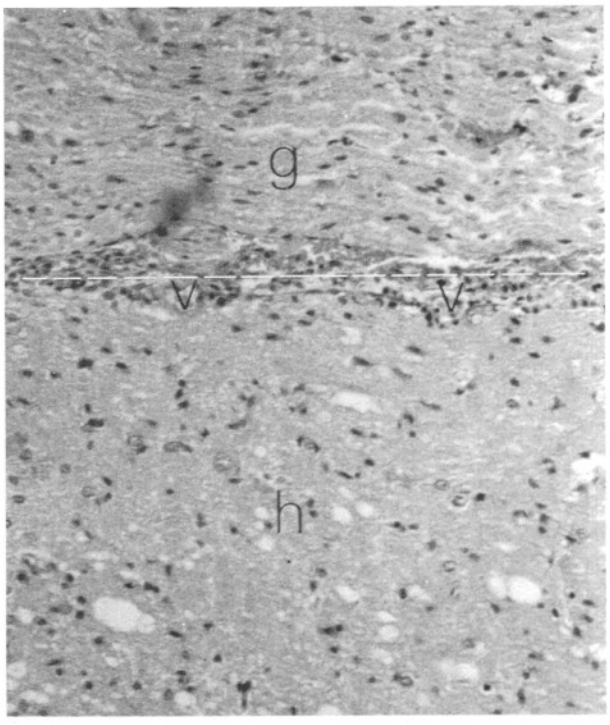

Figure 9: Section of a Group 2 specimen showing small blood vessels (v) along the host (h)-graft (g) interface (broken line) (H&E x 100).

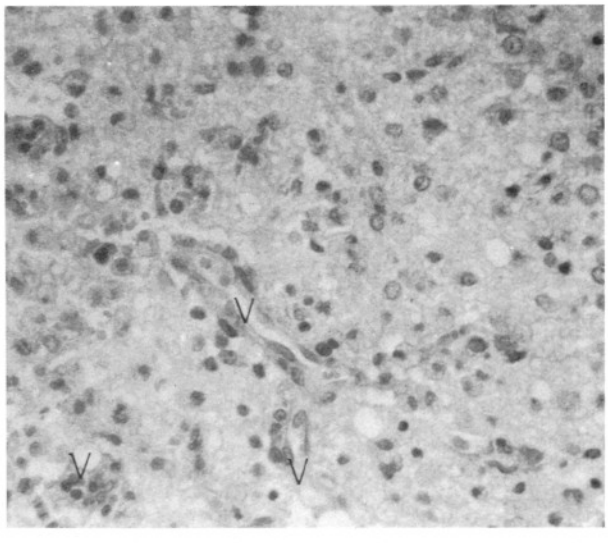

Figure 10: Many small blood vessels (v) were seen within the graft at 7 days after transplantation in the Group 2 animals (H&E x 100).

transplant survival rates in our groups, excluding postoperative deaths, were 46.1% and 71.4%, respectively. Note that the rate in the HBO-treated animals was approximately double the rate observed in the controls.

There are several possible explanations for this improved survival and graft-host integration: first, HBO counteracts tissue hypoxia by elevating tissue oxygen tension (PtO2) (25,44,57,60,63,64,77); second, HBO has the ability to reduce edema in traumatized tissues (25,61,77,84); and third, HBO has can facilitate the formation of new vessels (44,57,60,63).

Tissue Oxygenation:

It is well known that embryonic CNS tissues contain mitotically active precursors of all the neural and glial elements. These elements have remarkable growth potential, and are also capable of differentiation. Because they are resistant to trauma and hypoxia (54,81), they continue to develop and attain adult characteristics after transplantation. In addition, grafts of fetal CNS tissue have the capacity to maintain a relatively normal fetal microenvironment. It has been reported that they quickly establish an oxygen microenvironment after transplantation, and that oxygen transport is a regulated variable in the graft neuropil (81). The ability of a graft to develop and produce cells outside its normal environment has also been demonstrated in explant culture in vitro (78). Because neurogenesis is known to continue undisturbed for periods of 5 to 6 days in grafts obtained from rat fetuses (E16) (38), one would anticipate that the rate of microvessel proliferation known to occur in the normal early postnatal period would not match the increased metabolic demands of development. Due to these features, embryonic neural tissue can remain viable for some time, but this genetically preprogrammed capacity gradually diminishes as development proceeds. We termed this initial period the "no-flow critical phase" of transplantation. During this stage, it is important to give supportive treatment to prevent graft damage from infarction until blood flow becomes available. Oxygen is vital for the CNS, and oxygen deficiency, regardless of the cause, starts a vicious circle of pathological changes in CNS tissue.

In the present study, the object of therapy was to supply the graft tissue with adequate oxygen until normal reperfusion was established, and thus interrupt this vicious circle. Transplantation destroys a graft's blood supply, leaving it anoxic. This implies a rapid fall to zero of the oxygen tension of the graft, and leads to pathological changes in the graft tissue. The transplant may sustain a larger degree of cell death in this "no-flow" critical period. Processes of proliferation and differentiation may be delayed. This explains why grafts undergo an initial size reduction after transplantation, a phenomenon that has been

highlighted previously (29,68,69).

We believe that the amount of cell necrosis in the transplant plays an important role in determining the final volume of the developed graft. In the present study, most of the viable grafts treated with HBO showed no initial size decrease and grew more than those in the untreated animals. Moreover, some of the grafts even grew larger than their initial size at transplantation. This may be the result of better graft oxygenation.

We think that HBO helped enhance graft survivability and quality by preventing or limiting cell death. It has been shown that administration of HBO increases oxygen levels in the rat spinal cord (41,67). Measurement of PtO_2 levels within the injured spinal cords of adult rats after transplantation of fetal spinal cord tissue showed that even the more extensively developed transplants continued to show PtO_2 levels lower than those taken from normal spinal cord tissue (81). It was also shown that the low oxygen levels were significant particularly in the deeper samples from the graft (81). In our study, the grafts in Group 1 showed that intense degenerative changes had occurred in the central portions of the grafts. This can be explained by the difficulty of oxygenating these regions, even under conditions of 100% oxygen at ambient pressure (24). In contrast, with HBO at 2-2.5 ATA, diffusion of oxygen into the nonvascularized tissue is enhanced (26).

It has also been proven that at 3 ATA, there is a 15-fold increase in dissolved oxygen (9,83). This is sufficient to meet requirements for tissue oxygenation in the absence of vascularization. The HBO-treated group also showed microcyst formation and degenerative changes, but we did not observe severe pathological changes and macrocyst formation in the central portion of the grafts. Hyperbaric oxygenation involves applying oxygen under barometric pressure greater than that found on the Earth's surface at sea level. The improvement of graft oxygenation by HBO may concomitantly combat the ischemia and hypoxia associated with edema.

HBO therapy has been advocated as a method of improving tissue oxygen delivery, especially to areas of diminished flow (40). It counteracts tissue hypoxia by elevating tissue PtO_2 (10,22,25,44,57, 60,63,64,67,84), and also increases the amount of oxygen dissolved in the plasma, depending on the absolute pressure used (9,83,88). It has been proven that tissue oxygenation may be sustained by diffusion of oxygen from surrounding tissues. Under hyperbaric conditions, oxygen dissolved in plasma can spread into the extravascular spaces, and can nourish the tissues even in the absence of red blood cells (9,26,59). In this regard, even if there is no circulation HBO will be effective. In this study, lack of oxygen supply to the graft was our main reason for using this form of therapy. It is our belief that increasing the capillary PO_2 tension by HBO increases the amount of oxygen that reaches the developing graft.

Anti-edema effect:

A second important effect of HBO therapy is reduction of tissue edema. After transplantation, the graft may be in an unfavorable environment due to tissue edema and ischemia, which are not always immediately resolved (19,78,81). Even with the best technique, transplantation of neural tissues, being traumatic to both the transplant and the host spinal cord, produces some degree of pathological reaction in the transplant and the surrounding host cord. Edema can compress the capillaries and aggravate ischemia, starting another type of vicious circle. The edema that develops may either persist or worsen (10,25,80). Increased edema in the host spinal cord parenchyma may prevent initial parenchymal apposition between transplant and host tissue, which is essential for the eventual integration of the two (19,28,71). Edema can cause grafts to fold over or become deformed, and fetal grafts can be easily ejected from the spinal cord. It can also increase the diffusion distance from host to graft (25). HBO creates a favorable diffusion gradient through edema fluid and other barriers, such as blood, to the hypoxic cells (25).

In most of our Group 1 cases, the transplanted embryonic tissue did not stay between the stumps of the spinal cord, but went extraparenchymal and formed a dense degenerated mass. Similar observations have also been reported by other authors (19,28,71). Although it is not clear what caused the migration of the graft, there appears to be at least one possible explanation. Since the host spinal cord was traumatized by the aspiration procedure, it is possible that the migration of the graft may have been caused by edema in the adjacent host tissue. However, there was noticeably less edema in the HBO-treated group compared to the control animals, and most of the grafts in the treated group were adequately retained. This beneficial effect was thought to be due to the edema-reducing effect of HBO.

The HBO treatment improved host responsiveness by making the environment more favorable, and allowed the graft to make contact with the stumps of the host spinal cord. Accordingly, previous experimental and clinical studies have proven that HBO is a useful adjunct in the treatment of traumatic edema (10,32,61,77,80,83,84,90). It reduces edema through vasoconstriction and reduction of blood flow (25,26,83). The latter is compensated for by hyperoxia (25,61,84). It has also been shown that any vasoconstriction of the microarterioles that occurs during HBO exposure does not persist beyond the treatment period (90). HBO also reduces neuronal swelling by improving the cells' metabolism (16,26,33).

Vascularization:

A third determinant of graft survival is adequate vascularization of the graft. Under normobaric conditions, since tissue oxygenation is regulated by blood flow and metabolic need within very narrow constraints during development (30,81), interruption of blood flow means that the amount of oxygen available to the cells also falls to zero. It is clear from previous investigations that adequate vascularization of the graft is necessary not only to ensure the viability of its cells, but also to allow sufficient phenotypic expression of the graft and achieve a favorable effect in the host (47,48,52,53,87,91). Thus, rapid access to vascular supply is mandatory to ensure survival of any neural graft. Although, it has been reported that vascularization of the graft occurs within the first 24-72 hours after transplantation (13,47,48,52), some authors have emphasized that the vasculature of grafted neural tissue remains poorly defined for the first 7 days (2,11,91). In the revascularization process, whether the graft vessels are indigenous or host-derived is still controversial (2,47,48). In our opinion, the rapidity and the extent of vascularization, whatever its origin, is important. Although there was no statistical difference between the two groups in our study, the mean score for vascularization was higher in the HBO-treated grafts. In Group 2 animals, all the viable grafts became well-vascularized at the end of the first week, and small blood vessels were also identified at the graft-host interface, as observed in previous studies (11,12,53).

Because most of the grafts in Group 1 failed, it seems reasonable to assume that these tissues probably did not vascularize rapidly enough to ensure survival. Marx et al. (58) proved that HBO treatment produced eight- to ninefold greater vascular density than the vascularity observed in both normobaric oxygen and air-breathing controls. In accord with this, a number of studies have shown that HBO promotes capillary proliferation (22,44,57,60,63,64); however, the underlying mechanism of enhanced capillary angiogenesis is still uncertain. We believe that HBO supports hypoxic tissue, including primitive endothelial cells, by restoring abnormally low tissue oxygen tension levels. It can also be speculated that HBO and intermittent exposure to this oxygen pressure may have trophic effects on endothelial cells. The need for further studies on this subject is obvious.

Interface:

Likewise, the survival of fetal neural grafts is highly dependent on the establishment of parenchymal integration between the transplant and host (19,49,76). The region of parenchymal continuity between the two is known as the interface. The presence of a well-established interface indicates the existence of an ideal neural transplant, and represents histological evidence of graft survival. Grafts that are not anatomically integrated cannot be considered to have anatomical or functional significance for the host. They may survive for only a short time, and eventually degenerate (17,19,53,79). Thus, parenchymal integration of a fetal neural graft seems to be an indispensible requirement for the ultimate survival of the graft. A number of previous studies have indicated that considerable glial scarring at the interface prevents parenchymal integration of graft and host (19,28,29,34,71,74,81). The glial scar appears to form a mechanical barrier to graft-host neuronal connectivity (71,75). On the other hand, neural transplants that contain a number of surviving cells are able to attach themselves to the host parenchyma long before glial scar formation sets in (19), and most scarring develops after 7 days (53,89). Therefore, if the number of surviving cells within the graft can be increased in this critical period, it may be possible to produce much more healthy grafts.

In the present study, there were significant differences between the two groups with regard to parenchymal integration. Group 1 showed glial scarring and cyst formation at the host spinal cord-graft interface in each viable graft. In contrast, in the HBO-treated group, there were a few small cystic structures at the interface, and we observed a slight increase in gliosis, which did not affect the viability and anatomical integration of the transplant. Moreover, in the best cases, the interface was free of cystic formations and gliosis. In accord with previous studies, we suggest that absence of or limited gliosis is attributable to the presence of a healthy graft (6,19,34,76,85). The effectiveness of HBO in establishing parenchymal integration may be due to

enhanced survival of cells within the graft in the "no flow" critical phase, rather than to a direct effect on the interface.

None of the animals in the study received immunosuppressive therapy. The vast majority of grafts treated with HBO survived well, and there was little evidence of rejection. In Group 1 animals, a large amount of cell death might have been brought on by the invasion of polymorphonuclear leukocytes and macrophages, whose activity included clearing the graft. In contrast, stabilizing the graft by providing adequate oxygen could have limited the death of these cells in the acute stage, and thereby inhibited the subsequent inflammatory process.

The actions of HBO are not limited to reducing tissue edema, increasing tissue oxygen tension, and combating ischemia. Anaerobic metabolism results in acidosis and depletion of cellular energy. As the demands for energy production are no longer met, cells lose their ability to maintain normal ionic homeostasis. This abnormal cellular environment is extremely damaging to cell membranes (36,77). In addition, inadequate oxygen supply to the injured tissue results in the conversion of aerobic glucose metabolism to anaerobic metabolism (62). However, it has been shown that improving the oxygen supply to damaged cells results in normalization of the intracellular electrolyte balance (27,37). HBO has also a favorable effect on glucose metabolism (16,33). These facts support the theory that HBO therapy improves the chances for graft survival and integration with host spinal cord until blood flow becomes available, in party by allowing more aerobic glucose metabolism to occur and helping maintain normal ionic homeostasis during this phase.

Infection also adds significant risk to the transplantation procedure. It has been shown that hypoxia is a fundamental characteristic of wounds, and that oxygen availability is an important factor in wound repair and resistance to infection (24). HBO treatment is used as an adjunct to antibiotic medication in a variety of infectious diseases. It inhibits gram-positive as well as gram-negative organisms, and its positive impact in anaerobic infections is also well known (22,23,55,56). These effects may be an another important consideration in transplantation procedures. Although all the procedures in this study were performed under sterile conditions, two animals from Group 1 developed infections. In contrast, none of the HBO-treated rats had problems with infection.

Lastly, we want to emphasize that HBO cannot revitalize necrotic tissues, but by virtue of its effects can increase the chances that marginally viable cells will survive. Knowing this, there should be minimal delay between transplantation and the initiation of HBO treatment. We believe that prompt in treatment is important, but there are some specific issues that still need to be addressed, including optimal timing for initial treatment, duration and frequency of treatment, and others.

CONCLUSION

This is the first investigation that has evaluated the effects of HBO on grafted embryonic spinal cord tissue. Although the results of this preliminary experimental study are not dramatic, they demonstrate the beneficial effects of HBO therapy on fetal spinal grafts. HBO provides an appropriate milieu for graft growth, and can increase the likelihood of graft survival by restoring low tissue oxygen tension and reducing edema. Thus, it contributes to the integration of the graft with the host parenchyma. We do not view HBO as a solution to the many problems in this field, but believe that it can be used as an adjunct to other therapeutic agents or transplantation techniques used in fetal tissue transplantation. Although our results are encouraging, further investigation will more clearly delineate the role of HBO in embryonic neural tissue transplantation.

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