Blood-Brain Barrier Alterations Following Intracerebral Grafting: Solid and Cell Suspensions of Fetal Central Nervous System Tissue

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Abstract : The time course of angiogenesis and the status of the BBB within solid and cell suspension grafts of fetal CNS tissue following intracerebral transplantation is studied. Adult rats (n=40) received fetal forebrain tissue as either solid or cell suspension grafts to their corpus callosun bilaterally. The presence and the viability of the grafts was examined in fifty micron coronal sections treated by cresyl violet (Nissl) and acetylcholinesterase (AChE) histochemistry. BBB permeability within the grafts were determined by horseradish peroxide (HRP) leakege given systemically on the first, third, seventh and tenth post-operative days, to four separate groups of rats (n:10). HRP leakage was detected in both solid and cell suspension grafts on the first and third days following transplantation indicating a disrupted BBB. By the seventh postoperative day, no HRP reaction was detected. These results suggest that the BBB permeability of the vessels within intracerebral solid grafts depends on the transplanted tissue itself while the host CNS tissue provokes the formation of an intact BBB for cell suspension grafts.

Key Words : Angiogenesis, Blood-brain barrier, Horseradish peroxide, Neural transplantion.

INTRODUCTION

Neural transplantation is a significant tool to study the aspects of development and plasticity in the central nervous system (CNS), as well as to demonstrate the ability of grafted neural tissue to restore certain physiological dysfunction (1,8,10,29). Experimental studies with the extension to human subjects have shown that the host and the graft but anatomical evidence for such connections is limited. On the other hand, the status of the blood-brain barrier (BBB) following graft placement could have as yet undetermined consequences in terms of graft survival and amelioration of the functional disturbance. The barrier changes after transplantation may serve to promote graft survival by permitting transplanted cells to be exposed to blood trophic substances. Moreover, a disrupted BBB helps the host tissue to be exposed to the macromolecules and neurotransmitters within the graft which in fact, is the aim of the transplantation procedure. Therefore the status of BBB following transplantation is of crucial importance and manipulating the permeability of the vessels within the grafted tissue may provide a new therapeutic insight for human applications. On the other hand, exposure of the graft to host blood antibodies through a compromised BBB may provoke an immune response to the grafted tissue resulting with graft rejection. There is evidence that the duration of BBB permeability correlates closely with the sequence of immunological rejection of the graft (23,35). In the present study the time course of angiogenesis and the status of the BBB within solid and cell suspension grafts of fetal CNS tissue following intracerebral transplantation, are examined.

METHODS

Fetal tissue of CNS origin in either solid or cell suspension form were transplanted into the cerebral parenchyma of adult male Sprague-Dawley rats weighing approximately 250 grams. Fetal CNS tissue

was obtained from timed pregnat (15 and 16 days postcoitus) females. The donor rats were anesthetized with an intramuscular overdose of ketamine hydrochloride (50 mg/ml) / xylazine hydrochloride (%2.5) solution and the fetuses were removed individually under aseptic conditions. The basal forebrain tissue was dissected from the fetuses and pooled in Hank's Buffered Saline Solution (HBSS). Pieces of tissue approximately 1.0 mm3 in size were used as solid grafts. For the preparation of cell suspension, the remaining pieces were gently transferred to a microvial and 0.1% trypsin (Type II: crude, Sigma) in phosphate buffered saline (PBS) was added (300 microliters per dissected brain). The suspension was incubated at 37 C0 for 20 minutes. The trypsin was removed and washed off three times with PBS. Finally 10 microliters of the solution per tissue were added and the pieces that settled were dissociated into a suspension by repeated pipetting through a Pasteur pipette. The resulting milky fluid was kept at room temperature until the end of the experiment. Ten microliters were injected stereotaxically with a Hamilton syringe (26 no.needle gauge) into the host corpus callosum of adult, male rats of the same strain, at coordinates 2.5 mm. anterior to the bregma, 2.0 mm. off midline, 3.0 mm. deep from the cortical surface. The contralateral side received a solid graft of fetal basal forebrain approximately 1x1x1 mm. in size at the same coordinates, through a modified 20 gauge spinal needle. The basal forebrain region was specifically chosen because it is rich in cholinergic neurons that cen be easily detected by acetyl cholinesterase (AChE) histochemistry. Thirty animals received both cell suspension and solid grafts of fetal CNS tissue. Recipients were kept at room temperature with water and food ad libidum.

The rats, divided into four groups (n:10 for each) were sacrified on the first, third, seventh and tenth days after transplantation, respectively. Prior to sacrifice, each animal received diisopropylfuorophosphate (DBF), 1.0 mg/kg/rat intramuscularly, to suppress neurophil staining. Six hours following DBF injections, the animals were anesthetized and horseradish peroxide (HRP) Type VI 0.75 mg/5 gm body weight was given through the femoral vein. HRP was allowed to circulate for one bour and the rats were perfused with 0.1 M PBS for 5 minutes followed by 1.25% glutaraldehyde / 1.0% paraformaldehyde solution in PBS. Ten minutes later the perfusate was changed to PBS again to clear the fixative. The brains were removed and stored overnight in the same fixative at 4 C0. Coronal sections of 50 microns through the forebrain were obtained by an Oxford vibrotome, and submitted for cresyl violet stain, HRP and AChE histochemistry.

HRP histology was performed using the tetramethylbenzidine (TMB) method (20). HRP sections were counterstained with neutral red prior to examination. AChE histochemistry was performed according to the method of Naik (21). Serial sections were examined by AChE and cresyl violet staining for the presence and location of the graft and BBB leakage was determined according to the presence of HRP reaction product in the graft and the surrounding parenchyma.

RESULTS

Cresyl violet (nissl) stained sections demonstrated the presence of both solid and cell suspension grafts of CNS tissue in the host corpus callosum at the predetermined coordinates (Fig. 1a and c). In corresponding sections, the presence of AChE-positive neurons within the fetal grafts was detected by AChE histochemistry in the host rostral corpus callosum, where host cholinergic neurons are usually absent (Fig. 1b and d). By this method, both presence of the fetal tissue within the host parenchyma and localisation of the surviving recipient neurons were assessed. Detection of HRP reaction product both in the graft and the surrounding tissue was done under dark field microscopy in the corresponding sections. Furthermore, the extent of neovascularization was observed within the graft tissue and the surrounding brain outlined by the reaction product in the vessels.

One to three days post-transplantation :

Sections taken on the first postoperative day demonstrated that the solid tissue grafts were loosely adherent to the adjancent surfaces to some extent, with hemorrhagic debris present. The cell suspension grafts on the corresponding sides showed considerably less tauma tothe parenchyma compared with the solid grafts. By the third day, cells within the solid grafts stained well with cresyl violet, and demarcation with the surrounding brain was less obvious. In corresponding sections which were treated with AChE histochemistry, AChE positive neurons were present only at periphery of the solid grafts indicating neuron damage at the central parts. Cell

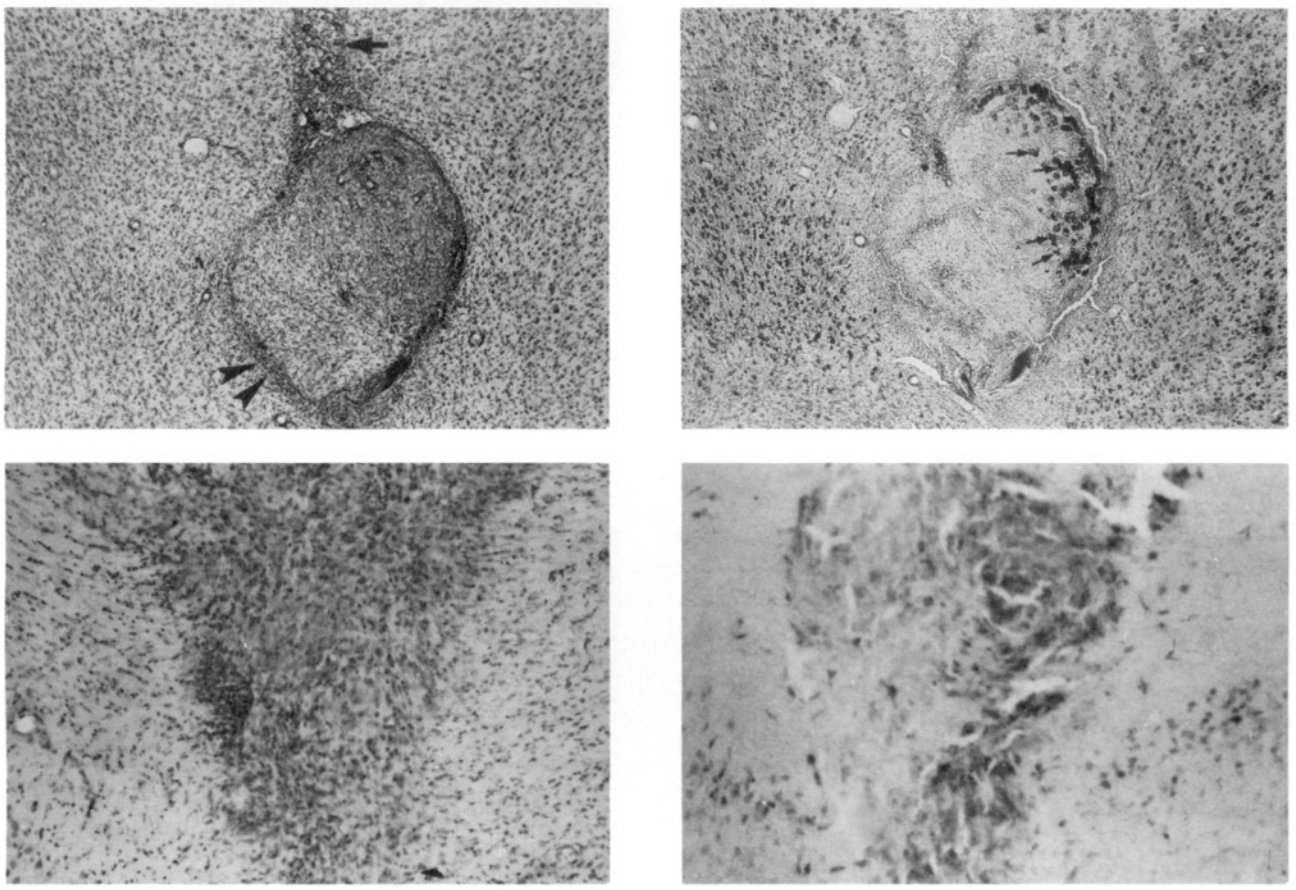

Fig. 1 : 50u thick coronal sections through the transplantation site showing intraparenchymal grafts of fetal CNS tissue in solid from within the host corpus callosum on the third postoperative day. a) Nissl stained sections where reactive glial accumulation is apparent at the graft-host parenchyma interface (arrow heads). Same infiltration is also present along the cannula tract (arrow). b) Corresponding section treated with AChE histochemistry. ACh positive neurons within the graft appear dark on the right corner (arrows). Remaining parts of the graft contain nly "ghosts" of neurons indicating cellular breakdown. c) Nissl stained section of the contralateral side of the host corpus callosum that received fetal CNS tissue in cell suspension from. Gliotic reaction is less apparent compared to solid grafts. d) AChE histochemistry for the same section demonstrates ACh positive cells distributed evenly throughout the graft indicating a better survival rate compared to the solid counterpart.

suspension grafts, on the other hand, showed uniform distribution of AChE positive neurons indicating a rapid reperfusion. Dark field microscopy exhibited a massive HRP reaction throughout the first to third days, along the needle tract and the grafts, including the adjacent host tissue (Fig. 2a and b). The leakage was more pronounced on the solid graft side, indicating the greater surgical trauma exerted by solid grafting techniques. Host blood vessels were outlined with HRP reaction product. No HRP labelled blood vessels could be detected in either solid or cell suspension grafts. There was no remarkable change in the first and third days of both solid and cell suspension tissue with respect to HRP leakage. On the third day, less macrophage and glial response was present in cresyl violet stained sections.

Seven to ten days post-transplantation :

By the seventh day the macrophage response was decreasing in the surrounding host tissue. HRP reaction product was absent within the parenchyma of the solid and cell suspension grafts (Fig. 3a and b). The host-graft interface showed some slight presence of reaction product in the solid grafts as well as the needle tracts for both sides. Grafts surviving to ten days showed results similar to those of seven days. Viable cells were demonstrated by cresyl violet staining and AChE histochemistry in corresponding sections for both solid and cell suspension grafts. At this time, reaction product was no longer present at the host-graft interface with HRP histochemistry. Vessels filled with reaction product could be traced to penetrate the cell suspension grafts. At this time,

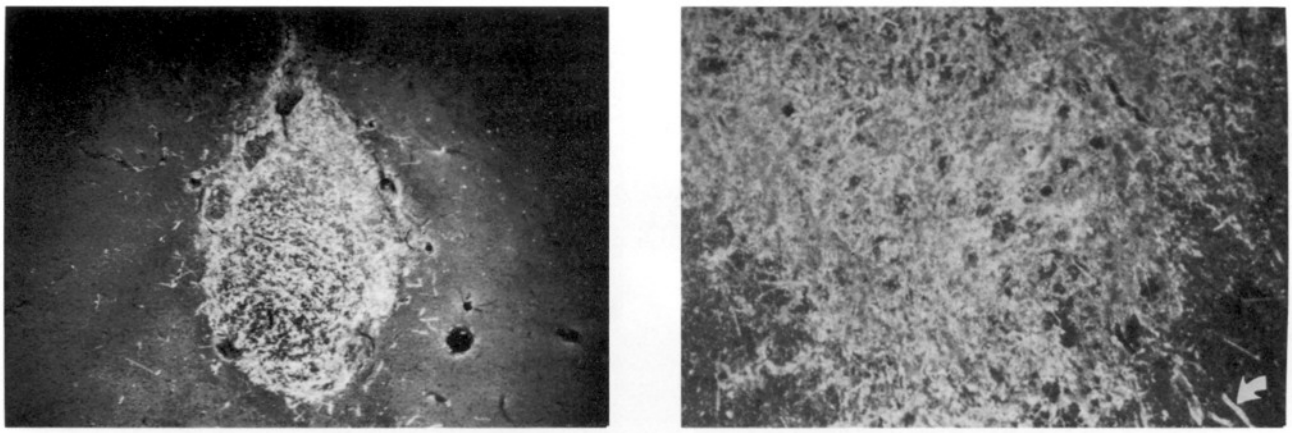

Fig. 2 : HRP histochemistry of, a) solid, and b) cell suspension grafts in coronal sections under dark field microscopy, three days post-grafting. Both grafts and the neighbouring host tissue are filled with HRP reaction product indicating a leaky BBB. Vasculature with normal BBB properties is outlined by HRP reaction within the host parenchyma (arrows).

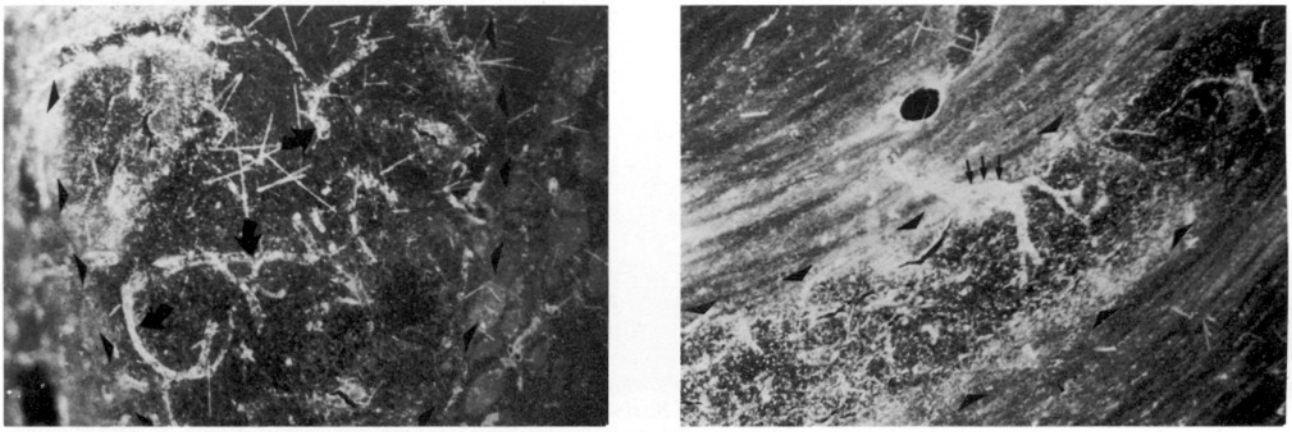

Fig. 3 : HRP histochemistry on the seventh day, a) solid, b) cell suspension grafts on contralateral sides of the corpus callosum in the same host. No HRP leakage is detected in the graft tissue (arrow heads) or the neigbouring host parenchyma. Non-leaky vessels within the solid and cell suspension grafts are delineated with the reaction product (arrows). Note the presence of a large caliber vessel originating from the host tissue and penetrating the graft (small arrows).

reaction product was no longer present at the host-graft interface with HRP histochemistry. Vessels filled with reaction product could be traced to penetrate the cell suspension grafts. Although the solid grafts also harboured fine caliber vessels throughout the tissue, alliance with the host tissue was less conspicuous.

DISCUSSION

Survival and functional effectiveness of intracerebrally transplanted nervous tissue depend on its rapid reperfusion through the host parenchyma (6,8,9,11,17,18,22). Few reports addressing the time course of angiogenesis within intracerebral solid grafts are available (1,2,8,9,17,18,28,29). According to the studies with solid grafts, reperfusion of the grafted tissue starts within hours and revascularization occurs within 24-72 hours (8,9,17,18). In our study, similar results were obtained with the solid fetal CNS grafts, which were found to be filled with HRP reaction product within 24 hours following transplantation. When a tissue receives its blood supply solely by the ingrowth of new vessels as in transplanted neoplasms or in traumatized brain, neovascularization does not begin before 3 days (4,6,11,22,28). Therefore, it is speculated that existing vessels in the solid implants are reperfused rather than replaced by ingrowing host vessels (1,8,17,22,24,28).

Our study was designed to examine the time course of angiogenesis in solid versus cell suspension grafts under similar conditions, i.e., the same host received both types of fetal tissue at corresponding coordinates. Our findings suggested that, there was

no difference on the time course of revascularization of solid and cell suspension grafts, both were found to be filled with blood-borne HRP within 24 hours. Since cell suspension grafts did not contain any vascular elements, the vasculature of the grafts must have been derived from the host. It is obscure whether solid grafts are solely reperfused by the ingrowing vessels from the host or whether some kind of anastomosis takes place with the host and graft vasculature. One finding in this study to support new growth in solid grafts is that only neurons at the host-graft interface were found to survive as shown by AChE histochemistry. On the other hand, surviving neurons within cell suspensions were uniformly distributed which is a staightforward advantage of this technique over solid tissue implantation, as cited elsewhere (8,9,12,13). Nevertheless, the overall healing and neovascularization process was completed by seven to ten days in both solid and cell suspension grafts in our study. At the seventh day following transplantation, vessels within the grafted tissue were clearly outlined with the HRP reaction product.

Another crucial aspect for a functioning intracerebral neural graft is the status of the BBB. Endothelial cells lining the capillaries of the CNS have structural and functional characteristics that constitute a regulatory barrier between the blood and the brain (25-27,36). This barrier selectively isolates circulating compounds in the blood from those produced by the brain providing a homeostatic environment in both the extracellular and cerebrospinal fluid compartments (31,36). The integrity of the BBB is altered by neoplasm growth, trauma or stroke, leading to functional and structural disturbances in the brain tissue (15,34,35). Similar changes apply to intracerebral neural transplantation. The mechanism of BBB disruption following neural grafting can result from both mechanical trauma and angiogenesis provoked by the graft (2,5,6,11,18,19,30). Mechanical trauma induces a chain of events within the brain, including the formation of new blood vessels. New host capillaries regress to an early developmental stage of increased permeability (17,35). An altered BBB accelerates graft rejection by exposing the graft's antigenic structure to the host's blood-borne immune defensive mechanism. There is an ongoing discussion on the status of BBB following intracerebral transplantation of neural tissue (7,8,10,15,28,-30,32). While most studies with solid tissue have demonstrated reformation of the BBB following transplantation, challenging results are also reported (17,18,28-30). In our study, solid as well as cell suspension grafts examined at the first and third days after grafting showed a diffuse peroxidase reaction in the graft and the surrounding tissue. At sevne and ten days, no HRP reaction product from the grafts was found, indicating that the healing and neovascularization processes had stabilized in both types of graft in the same time sequence. The probable reason for the controversy with studies showing a continuous BBB leakage after transplantation is the graft type and the site being used. Studies suggest that the grafted tissue maintains its vascular phenotype despite the change in environment (1,8-10, 13,33). Therefore when a CNS tissue which originally has impermeable vessels is transplanted in solid from, it is most likely that those properties would withstand in the new location. When non-CNS solid tissue such as adrenal medulla or superior cervical ganglion is used, originally leaky vessels within the graft are not expected to change their vascular phenotype in the host parenchyma resulting in continuous leakage at the graft site. On the other land, perfusion after grafting cell suspensions totally depends on neovascularization, because the enzymatic treatment disrupts the vasculature so the donor tissue has no inherent vascular structures. The ingrowing vascular network should maintain its barrier properties depending the local environment (3,14,16). This was confirmed in our study: the neovasculature of the cell suspension grafts of CNS tissue established a non-leaky barrier in the same time as its solid counterpart. On the contrary, when grafts are placed in CNS areas close to previntricular organs or the cortical surface with incomplete BBB, the graft tissue will have leaky vessels due to the local environment (7). In fact, in studies (17,18,28-30) indicating a permanent BBB breakdown following transplantation, either non-CNS tissue were used as donors or the grafts were placed in leaky areas of the CNS.

In conclusion, CNS tissue in either solid or cell suspension from is rapidly perfused within 24 hours following intracerebral transplantation. The vascular network with BBB properties is not established for seven days. These results confirm previous studies that a solid tissue with BBB properties will reestablish those properties, indicating blood vessels within the grafted tissue are preserved and maintain the morphological and permeability characteristics they manifest initially (2,3,6). Moreover, suspension grafts

of the same origin show the same properties under similar conditions. According to our data, it can be speculated that the vascular properties of intracerebrally grafted tissue are dictated by the graft itself and the local environment depending on the transplantation technique used.

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