Best Practice & Research Clinical Obstetrics and Gynaecology
Vol. 18, No. 6, pp. 977–994, 2004
doi:10.1016/j.bpobgyn.2004.06.008
available online at http://www.sciencedirect.com

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Perinatal applications of neural stem cells

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The brain, unlike many tissues, has a limited capacity for self-repair and so there has been great interest in the possibility of transplanting neural cells to replace those lost through injury or disease. Encouraging research in humans is already underway examining the possibility of neural cell replacement in adult neurodegenerative conditions such as Parkinson's disease and Huntington disease. In addition, experiments exploring neural stem cell replacement in rodent models of acute stroke, demyelination and spinal cord injury have demonstrated functional improvements in treated animals. When considering perinatal neural stem cell therapy, it should not be overlooked that the immature, developing brain might provide a more favourable environment for stem cell integration. However, considerable advances need to be made both in understanding the basic biology of neural stem cells, including the instructive signals that determine their proliferation and differentiation, and in characterising their responses when transplanted in a damaged or diseased area of the brain.

Key words: brain; neural; perinatal; stem cell therapy.

WHAT ARE NEURAL STEM CELLS?

Neural stem cells (NSCs) can be defined as lineage-committed cells found within the central nervous system (CNS). These cells have the ability to self-renew and can be maintained in culture with mitogens such as epidermal growth factor (EGF) and fibroblast growth factor 2 (FGF2). They can be propagated in aggregates (known as neurospheres)[1–3] (see Figure 5, Chapter 3) or as cell monolayers.[4] NSCs are more restricted in their differentiation capacity than embryonic stem cells, giving rise predominantly to the three major cell types of the CNS: neurons, astrocytes and oligodendrocytes. NSCs have been isolated from many areas of the developing human

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brain[5] as well as two well-defined areas of the adult central CNS—the subventricular zone and the hippocampus.[6,7] Although their discovery and isolation is promising for the understanding of both brain development and repair, the basic biology of NSCs is still not well understood. Indeed, the unequivocal identification of true NSCs remains difficult, as there are no specific markers for this cell type. Nestin, for example, is a widely used marker and is highly expressed in the developing neuroepithelium and NSC[8,9], but is also found on other cell types such as endothelial cells, developing myoblasts and reactive astrocytes.[10] Other markers used to define NSCs include Musashi and notch-1[11,12] but none of these is absolutely specific. NSCs are thus defined largely on their culture properties, their proliferation and their differentiation capacity both in vitro and in vivo. For the purposes of this article, NSCs have been defined as neural cells with the potential to self-renew and to generate all the different cell types of the nervous system following differentiation.

CAN THE BRAIN REPAIR ITSELF WITHOUT THE NEED FOR NSC TRANSPLANTATION?

Much research is now focused on understanding the brain's intrinsic potential for repair in degenerative diseases and following cerebral injury. This research both investigates the potential for endogenous repair and also aims to learn more about the permissive and restrictive cues in the damaged brain that will be crucially important if cell replacement therapy is seriously considered in the future. It is now clear that injury to the CNS does indeed result in the proliferation of endogenous neural precursors, although the numbers are insufficient to enable functional recovery. A number of studies using BrdU labelling, to label proliferating cells, in conjunction with neuron-specific markers have demonstrated the expansion and subsequent differentiation of endogenous neural precursors following experimental stroke.[13] Similarly, NSC proliferation has been found to increase ten-fold in the subgranular zone of the dentate gyrus after global ischaemia in the gerbil.[14] In a separate model, employing chemically induced seizures in the rodent, a pronounced increase in the generation of new neuronal precursors in the subventricular zone (SVZ) and their subsequent migration and integration towards the olfactory bulb was reported.[15] Although it has been proposed that ischaemia-induced neurogenesis might contribute to the specific recovery of memory function lost following injury, a high proportion of the dividing cells are lost over the weeks following injury. It remains to be demonstrated whether such responses are specific or represent a generic global response that occurs in areas that already have ongoing adult neurogenesis. However, the demonstration of the continued production, and survival of neural cell types following injury, has led to renewed interest in mechanisms of the endogenous cell response and whether this could be exploited further to instruct repair following injury. At present there is no prospect of the endogenous NSC response to injury being sufficient to replace neural cell loss completely.

REPAIRING THE INJURED NERVOUS SYSTEM WITH CELL-BASED THERAPY

As outlined above, there is good evidence that endogenous neural cell proliferation and differentiation occurs following cerebral injury. Neural cells, however, have a limited

capacity to regenerate and the small population of endogenous NSCs seems unable fully to reconstitute and restore function after damage. This has led several groups to examine the potential of cell replacement therapy after cerebral injury.[16,17] The main sources of cells for potential therapeutic replacement are: neural precursor cells from fetuses; NSCs from fetal brain; NSCs from adult brain; and neural cells derived from embryonic stem cells. In addition, recent research suggests that functional neural cells can be derived from non-neural precursors (see below).

Although the focus of this review is the potential of NSCs for treatment, the use of fetal tissue grafts will be discussed briefly because it is already in clinical use for neurodegenerative conditions. In animal models, fetal neural cell transplantation has been used with success in several adult brain injury models. Fetal cortical grafts survive in the infarct area following focal forebrain ischaemic injury in adult rats and appear to receive connections from the surrounding brain with a resulting improvement in motor function[18], spatial learning and memory.[19] Experience already exists of fetal neural cell transfer in humans suffering neurodegenerative disorders. Over 300 patients with Parkinson's disease have now received grafts of fetal mesencephalic precursors into the striatum. These grafts are spontaneously active and can restore dopamine release to near-normal levels with symptomatic improvement. There is a downside however; in a clinical trail from Denver and New York, 15% of grafted patients developed unacceptable dyskinesias.[20] Furthermore, the supply of fetal neural tissue is limited and consequently only small numbers of neurons are available. This could partially overcome by in vitro expansion, but fetal tissue contains a heterogenous population of cells, many of which are post-mitotic. A further barrier is the poor survival of grafted neural cells—it has been estimated that as many as 95% of transplanted neural precursors die by apoptosis.[21] The limitation of poor tissue supply might be overcome by generating dopaminergic neurons from neural stem cells (or embryonic stem cells). Indeed, rat embryonic day-12 NSCs propagated in culture retain the capacity to differentiate into dopaminergic neurons and to improve outcome in a rat model of Parkinson's disease.[22]

These studies suggest that NSCs might provide a highly proliferative, homogenous pool of cells with advantages over fetal tissue grafts. NSC transplantation has been used with some success in other adult injury models. Cortical neurons undergoing injury-induced apoptosis can be replaced by transformed neural progenitor cells. Significantly, these cells demonstrated appropriate differentiation in situ into region-specific neuronal and glial subtypes determined by the site of injection.[23] With relevance to perinatal therapy, stable clones of NSCs can contribute to normal brain development when injected into the germinal zones of newborn mice.[24] Another proposed advantage of stem cells is that they can be modified using cloning technology to express the patient's own genotype, or to express a transgene. This technology could potentially be used to provide a source of immune-compatible cells for transplantation or even to transfer a gene product. Table 1 summarises useful properties of NSCs that might be important for perinatal cell therapy.

POTENTIAL SOURCES OF NEURAL STEM CELLS FOR THERAPY

Neural stem cells derived from the central nervous system—fetal and adult

NSCs have been identified in many areas of the developing mammalian brain, and also in specific regions of the adult CNS. Such cells are easy to expand and have been

Table 1. Properties of neural stem cells (NSC) advantageous for perinatal cell therapy.

Property	Potential benefits
Highly proliferative	Allowing large-scale production and storage. In addition, NSCs might proliferate in vivo and potentially generate a new stem cell pool after transplantation
Plasticity	Ability to differentiate into neuronal and glial subtypes
Migration	Wide dissemination after intraventricular injection in the developing brain Allows easy gene and global cell replacement
Targeting to injured tissue	NSCs migrate to areas of injury allowing treatment of focal injury
Integration	Potential of NSCs to form functional connections with endogenous brain
Few side effects	NSC transplants might not need immunosuppression Unlike embryonic stem cells, NSC are not tumourogenic,
Genetic manipulation	Stem cells are readily transduced. This enables the delivery of beneficial genes and their products to areas of damaged brain

demonstrated to differentiate into all three neural cell types—neurons, oligodendrocytes and astrocytes—by clonal analysis[25,26], labelling[27] and in transplantation experiments. When considering NSCs for replacement therapies, it is important to recognise that cells from different sites are not identical, displaying different growth characteristics, trophic factor requirements and restricted patterns of differentiation.[28–30] In addition, these multipotent progenitors clearly differ in their potential according to the developmental stage at which they were isolated and the specific brain region from which they were isolated.[28,31]

Although the bulk of experimental data has been obtained using rodent NSCs, similar multipotent cells have been identified in the human.[5,32] There are clearly similarities between the in vivo and culture properties of NSCs from rodents and from man but there are notable differences too. These differences are discussed at length in Ginis and Rao's recent review.[33] One of the most evident is that differentiation of rat neurospheres generates large quantities of oligodendrocytes, whereas similar experiments in human cells generate relatively few. The reason for this is not clear and more work is needed to determine what signals influence the cell phenotype and, indeed, to identify the specific cues for terminal differentiation.

Uchida et al have described the isolation of NSCs and the subsequent neurosphere culture from fresh human fetal tissue.[34] NSCs were obtained from dissociated brain and spinal cord, and enriched by fluorescence-activated cell sorting. Subsequently, it was demonstrated that single cells, with the phenotype CD133+, CD34−, CD45−, CD24−/lo, 5E12+, could generate neurospheres, self-renew and differentiate into neurons and glia. When these cells were injected into the lateral ventricles of immunodeficient newborn NOD/SCID mice they showed engraftment, migration and region-specific neuronal differentiation on examination up to 7 months later.[34]

Although ethically controversial, human fetal CNS tissue represents a potential source of human NSC for future research and therapy. An attractive property of fetal NSCs is their proliferative capacity. In one study, human neural progenitors isolated from embryonic forebrain were expanded for up to a year in culture using EGF, FGF and leukaemia inhibitory factor (LIF). Prolonged culture did not profoundly affect the potential of these cells, and indeed injection of these cell lines into the developing rat brain showed extensive migration and integration.[35] Although these results are encouraging, it is clear that challenges remain in determining the best source and

developmental age to acquire NSCs from fetal tissue, and to define the optimal ex vivo propagation conditions for each therapeutic application.

Another potential source of human NSCs is from the adult brain, and indeed, such cells have now been cultured from human cadavers up to 5 days after death.[36] At present, NSCs derived from adults are difficult to isolate, found in smaller numbers, have a more limited proliferative capacity and seem to have a more restricted differentiation potential than their fetal counterparts. In the light of current knowledge, it would appear that fetal NSCs will be more useful for cell therapy. However, the highly controversial nature of this source of neural tissue has provided an impetus for understanding adult NSCs in more detail. Whether fetal or adult NSCs are used in cerebral transplants, little is known about the cell-intrinsic and cell-external factors that influence proliferative capacity and fate choice. By careful examination of the effects of defined neurotrophic factors and accurate definition of the factors in the transplant microenvironment, it might well be possible to improve the potential of NSC therapy in the future.

Neural stem cells derived from embryonic stem cells

Embryonic stem (ES) cells from the inner cell mass of the blastocyst can give rise to all tissues in the body, including those of the nervous system. ES cells thus provide another promising alternative source of tissue for therapeutic applications. ES cells are multipotent, can be propagated in vitro and can be engineered to express therapeutic genes. The first demonstration that mouse ES cells can be induced to express multiple neural phenotypes in culture was reported by Bain and colleagues[37], who used retinoic acid as the trigger for differentiation. The newly formed neurons not only expressed lineage-specific markers but also were also capable of generating action potentials. ES cells can be maintained in long-term culture as floating aggregates (embryoid bodies) and retain their ability to differentiate into cell types of all three germ layers. Several groups have now proposed culture conditions that enrich neural progenitors from murine ES cells.[38–40] Okabe et al developed elaborate sequential culture conditions that differentiate the ES cells towards a nestin-positive neural precursor phenotype in medium supplemented with insulin, transferrin, selenium and fibronectin. These more committed progenitors can then be propagated with the mitogen FGF2 and subsequently differentiated into neuronal and glial phenotypes following growth factor withdrawal.[38] Other groups have successfully purified NSCs from ES cultures by selecting cells expressing the cell surface markers A2B5 or PSA-NCAM[39] or the transcription factors Sox 1 and Sox 2.[41] It should be pointed out that not all neural markers are conserved between species and so the identification of neural cells derived from human ES cultures will require careful analysis. Moreover, many of the early lineage markers are either expressed transiently or are confined to restricted subregions of the brain. For example, in embryogenesis, Sox 1 is confined to the neuroepithelium of the neural plate, whereas Sox 2 is found in the early neural crest. Some of these markers are also confined to neural subtypes; thus, Mash 1, Mash 4A, Pax 3 and Pax 6 are known to be important in dorsoventral neural tube patterning. This knowledge, if exploited carefully, could facilitate the isolation of different populations of ES-derived neural precursors. Each clone could then be analysed for both neurogenic potential and survival following engraftment so as to select the best cell type for therapeutic use.

Indeed, with the increased availability of human ES cells[42], it has been demonstrated that they can be propagated as efficiently as murine ES cells and can also differentiate

along neural lineages. For example, Carpenter et al have demonstrated neural differentiation of human ES cells using combinations of growth factors and retinoic acid.[43] As with murine ES cells, the more committed neural progenitors could be identified by antibody selection using the cell surface markers A2B5 and PSA-NCAM. Other groups have confirmed these findings and have shown that enriched neural precursors from human ES cells can incorporate into brain tissue and differentiate in vivo.[44] Su-Chan Zhang transplanted enriched neural precursors into the lateral ventricles of newborn mice and observed migration to multiple brain regions, followed by differentiation into cells with mature neuronal and astrocytic phenotypes. Interestingly no mature oligodendrocytes were identified.[45] Such in vivo studies are important in two respects: first, they suggest that transplanted cells do have the potential to populate the brain and, second, they highlight the fact that more manipulation might be necessary before the required neural cell types are efficiently generated.[24,46] Nevertheless, early success in neural differentiation of cell grafts in vivo has led to further work in injury models to see whether transplanted cells can integrate and functionally improve outcome following CNS injury involving loss of multiple cell types.[47,48] Although some success has been reported, it is clear that there is still a significant gap in our knowledge of how to direct appropriate differentiation of ES cells in vivo.

Neural stem cells and neural cells from non-neural tissues

A growing number of studies suggest that non-neural cell types (e.g. bone marrow cells) can be propagated and differentiated into neural lineages. Much of this work is controversial but if useful neural cells could be obtained from this source, it would circumvent the ethical problems of using embryonic stem cells and fetal tissue, and the practical issues of deriving cells from post-mortem brains. The early in vivo experimental data was based on bone marrow transplantation (BMT) studies in lethally irradiated mice. The transplanted cells were followed by genetic differences (e.g. mouse strain, sex, expression of green fluorescent protein) between the injected cells and the host. In one case of female mice rescued by male BMT, up to 2.4% of neuronal cells were found to be male as defined by localisation of the Y chromosome.[49] Similar studies have demonstrated the presence of donor markers in neurons of the olfactory bulb.[50] Similar work has also been undertaken in humans. Post-mortem studies of females who had BMT from male donors revealed a small proportion (0.1%) of Purkinje cells that carried the Y chromosome and were presumed to be of donor origin.[51] It has been claimed, however, that the few apparent donor-derived cells do not represent differentiation ('transdifferentiation') of donor cells but are the result of cell fusion between the endogenous Purkinje cell and a donor cell forming stable heterokaryons[52] that express markers of both recipient and donor tissue. This possibility has been confirmed in murine models and it is thought that the fusion event reprogrammes the donor cell. Therefore fusion, although unable to increase the number of cells in a damaged CNS, might contribute to replacing damaged cells. If the mechanisms of reprogramming could be understood in detail and the frequency of fusion increased, this approach could still be useful for therapy, although the possible genomic instability of heterokaryotic cells would have to be taken into consideration.

Fusion of donor cells with host brain cells might explain the apparent transdifferentiation of blood to brain in vivo but it cannot explain all of the in vitro data. There are now several published protocols for directing bone marrow stromal

cells to neural lineages.[53–57] The approaches used are different and range from using chemical demethylating or reducing agents, to retinoids to more physiological growth factors. Although controversial, much of the transdifferentiation data is tantalising and is not easily explained by cell fusion. In these experiments, the generation of neural cells from blood could either be due to the presence of a minute subpopulation of highly pluripotent cells in the marrow, or explained by the reprogramming (trans- or de-differentiation) of an already committed blood progenitor. Verfaillie's group has described the 'multipotent adult progenitor cell' (MAPC) as a bone marrow-derived cell that has multi-tissue potential[56], including neural lineages. When transplanted, these cells have been shown to ameliorate neurological deficits in a rat model of cerebral ischaemia.[58]

Blood or bone marrow would be an ethically acceptable and easily accessible source of cells for neural replacement, although research is at an early stage and the findings controversial. However, reproductive cloning has already demonstrated as 'proof of principle' the phenomenon of nuclear reprogramming. Future research must focus on the mechanisms involved.

PERINATAL NEURAL STEM CELL THERAPY FOR BRAIN DISEASE

Perinatal applications of stem cell therapies are thought by some to be limited to a few rare disorders. Until recently, the application of cell therapies was considered only for focal brain diseases or insults. This was based on the fact that it would not be practicable to deliver cells (with multiple injections) to multiple sites in the brain. However, the majority of neurological diseases that manifest in childhood are global and affect widespread areas of the CNS and multiple cell types. These include genetic causes such as inborn errors of metabolism, lysosomal storage diseases and leukodystrophies, as well as the widespread abnormalities that can follow acute brain injury after asphyxia or the more subtle white matter abnormality that occurs in the majority of extremely preterm infants. We would argue that the global nature of these diseases might not preclude the use of perinatal cellular therapy for two reasons: first, NSCs have been shown to migrate extensively in the developing brain, and more readily than in the mature brain; and second, in other injury models, NSCs seem to migrate and even home to widespread sites of injury.

Considerations when using perinatal neural stem cell therapy

One of the major obstacles to using human NSC perinatal therapy is that, at present, for the majority of brain diseases the pathogenesis is not well understood at the molecular/cellular level. It is likely that a detailed knowledge of the timing of pathological events and the associated environmental signals would aid successful treatment design. Indeed, it is intuitive that cell replacement might be more successful in cases where a specific population of cells is lost in an environment where other cells are competent; however, this is rarely the case. There is frequently more global and ongoing cell loss with a distorted and abnormal microenvironment and cytoarchitecture. Although undifferentiated NSCs offer the potential to replace numerous cell types, this is unlikely to occur effectively in the absence of appropriate signals. NSCs can undoubtedly respond appropriately to developmental cues but much work needs to be done to examine the transplant environment and how this can be manipulated in

disease and injury. A further problem when considering many of the neurodegenerative conditions of childhood (e.g. inborn errors of metabolism) is that the process is ongoing and the environment inherently toxic. Unless the transplanted cells, or the local environment, are manipulated, the graft would itself be vulnerable and ultimately lost. Indeed, even when the disease process is not ongoing, graft loss by apoptosis is a problem and hampers long-term success; this will be discussed later. Although NSC therapy in humans is some way off, examples of potential therapeutic targets (metabolic brain disease, white matter disease and hypoxic ischaemic encephalopathy) are discussed below.

Neural cell therapy for metabolic brain diseases

One of the major advantages of considering perinatal cell therapy for inborn errors of metabolism is that, if the diagnosis is known, treatment could be commenced early to prevent or minimise ongoing brain damage or deterioration. It is important to recognise, however, that the majority of infants with metabolic diseases (frequently autosomal recessive) do not have affected parents and so treatment before the onset of symptoms or manifestations, especially in utero, would not generally be possible. There are other important issues to take into account. First, it could be argued that metabolic diseases are multiorgan diseases and should therefore be treated with global cell therapy such as BMT, although this approach might have little impact on neurological deterioration. One example of this is the treatment of metachromatic leukodystrophy (arylsulfatase deficiency) with BMT: only in the kidney and liver of transplanted animals was lipid storage improved; in the brain, neuronal damage was as severe as in the untreated animals.[59] Second, in a given metabolic disorder where the majority of cells are likely to be affected, it would be unlikely that a cell replacement strategy would be helpful. In these cases NSCs could be used as vehicles to deliver a missing or aberrant gene/protein, or simply to generate trophic support for endogenous cells to slow their degeneration. Third, cell therapy would have to be designed in such a way that grafted cells would escape the pathological processes affecting host cells. Already several researchers have examined NSC therapy in models with inborn errors of metabolism with mixed results. Meng et al investigated the possibility of using NSCs in the treatment of metabolic brain disease in a murine model of mucopolysaccharidosis type VII.[60] This condition arises from a defect in the β-glucuronidase gene and results in lysosomal accumulation of glycosaminoglycans in the brain, with subsequent neurodegeneration. In this study NSCs were modified to overexpress the missing enzyme (β-glucuronidase) and transplanted into the cerebral ventricles of newborn affected mice. These NSCs migrated widely, produced large quantities of β-glucuronidase and resulted in a dramatic clearance of the lysosomal accumulation in host cells to near normal levels. Such experiments prove the principle that NSCs can be used as gene delivery vehicles in genetic deficiency disorders. One downside in this experiment was that graft survival was limited by cell death by apoptosis and so the benefits would not be long lasting—improving graft survival is an important concept that will be discussed later.

Neural cell therapy for white matter disease

Although in the majority of CNS diseases a number of different cell types are affected, cell replacement therapy has been most successfully used in models in which a single cell type is damaged or missing. One example is white matter disease and there are several rodent models with abnormalities in oligodendrocytes—the myelin-forming

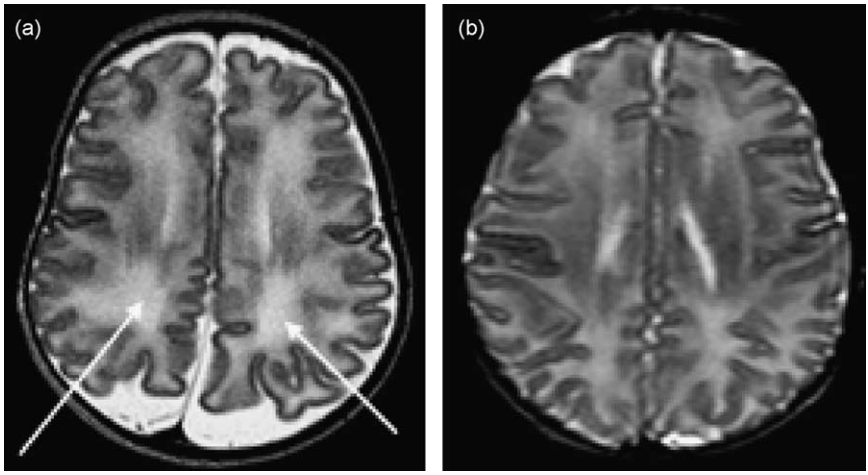

Figure 1. White matter abnormality in extremely preterm infants. T2 weighted transverse MR images. (a) An image of a preterm infant at term-corrected age demonstrating patchy high signal intensity in the white matter (arrows). Overt white matter cystic change (periventricular leukomalacia) is now very rare but more subtle white matter signal change on MR imaging occurs in the majority of infants <28 weeks. This pattern represents abnormality[62] and is not present in normal term-born infants. (b). This MR feature probably represents loss or maldevelopment of oligodendrocytes and their precursors.

cells of the CNS. As well as demonstrating proof of principle, these models will provide useful prototypes for perinatal therapy. For example, there is accumulating evidence that although periventricular leukomalacia is becoming rare, brain injury or abnormality found in the majority of survivors of extremely preterm birth remains predominantly white matter (Figure 1) and involves oligodendrocyte precursor loss. Magnetic resonance imaging studies have confirmed involvement of the white matter[61,62] and in vitro data also suggest that oligodendrocyte precursors, abundant in the preterm brain, are very much more vulnerable to a variety of stressors than mature oligodendrocytes.[63] So oligodendrocyte death or maldevelopment seems to be a primary event in preterm brain injury. If cell-based therapy is to be considered seriously for these infants, we will need better tools to estimate long-term neurodevelopmental outcome in the perinatal period in order to optimise patient selection, and a better understanding of the pathogenesis of the condition. Neither of these obstacles is close to solution at present.

Despite the distance between the theory and clinical practice of NSC transplants, laboratory work examining oligodendrocyte replacement has provided some encouraging results. For example, NSCs transplanted into the cerebral ventricles of shiverer mice (a strain that has dysfunctional oligodendrocytes and lacks myelin basic protein) showed widespread engraftment and resulted in effective myelination.[64]

A further aspect of cell therapy is the developmental stage at which cells are transplanted. More committed oligodendrocyte progenitor cells (OPC) from 21–23 week human fetal brains have also been isolated, purified and cultured. These cells were xenografted into shiverer brains, where they developed into oligodendrocytes that myelinated host axons.[65] Interestingly, in this study OPC from adults generated oligodendrocytes more efficiently than fetal OPC. Although these studies highlight that both NSCs and more committed progenitors can replace a single cell type, other

studies in different models are less encouraging. Thus, NSC transplantation into twitcher mice (a model of Krabbe's disease in which an absence of galactocerebroside results in the accumulation of the toxic lipid psychosine)[66] resulted in no improvement in disease symptoms or survival despite extensive differentiation and myelination. It is possible that the transplanted cells could not sufficiently influence the toxic environment of the endogenous cells.

Neural stem cell replacement in hypoxic ischaemic encephalopathy

Hypoxic ischaemic encephalopathy (HIE or newborn encephalopathy) is another important cause of newborn brain injury (Figure 2). After such an insult there is immediate necrotic cell death followed by further delayed cell death by apoptosis. Indeed, various neuroprotective strategies are currently being evaluated in animal models in an attempt to reduce the apoptotic cell death and therefore improve neurodevelopmental outcome. Following a successful pilot study[67], a large-scale human clinical trial is now underway of whole-body hypothermia as a neuroprotective treatment. Anti-apoptotic therapies might help minimise later cell death but will not impact on immediate necrotic cell death and so cell replacement might be considered in the future. In HIE the pattern of brain injury depends on the type and severity of insult, but in severe cases involves both grey and white matter. NSC transplantation in animal models of HIE are encouraging in that NSCs survive, migrate into the infarct areas and differentiate into what seems appropriate neuronal and glial subtypes. However, there are considerable problems extending successful animal studies to humans. Animal models use a clearly defined injury, often with carotid artery ligation with hypoxia. By contrast, in the clinical arena HIE is the endpoint of a variety of pathways, and usually not an isolated and clearly defined acute insult. This makes evaluation and study difficult.

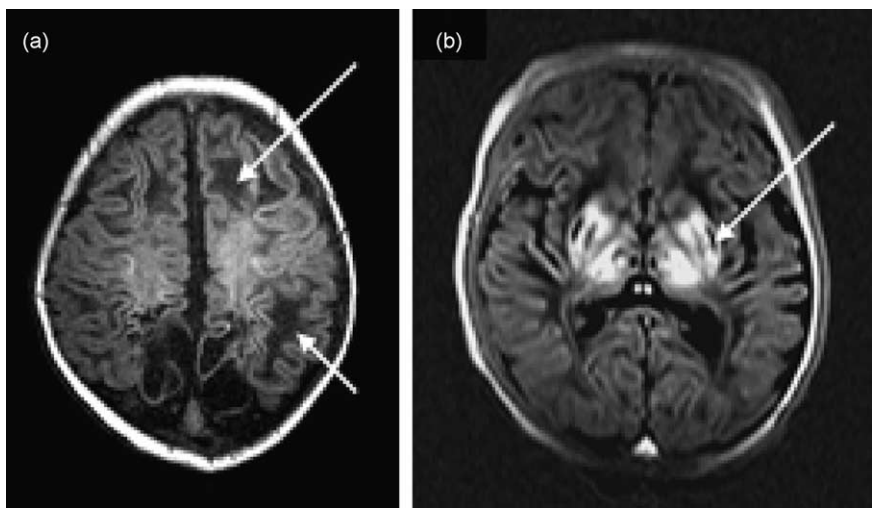

Figure 2. Hypoxic ischaemic encephalopathy in newborn infants leads to grey and white matter loss with subsequent neurodisability. T1 transverse MR images of two infants with severe brain injury following hypoxic ischaemic encephalopathy. (a) A transverse image acquired in an infant at 18 days age demonstrating large areas of low signal intensity in the white matter (arrows), which later atrophy. (b) An image at the level of the basal ganglia of an infant at 20 days age showing loss of grey and white matter with cystic change in the basal ganglia (arrow).

Indeed, even with advanced imaging methods it remains a challenge to give an early estimate of long-term prognosis in moderately affected patients, and thus difficult to define a population for study to be confident of outcome differences.

FACTORS AFFECTING TRANSPLANT SUCCESS

A number of factors can influence the success of cell-based therapies. Environmental factors, the tissue source of stem cells, their developmental stage, programmed cell death (by apoptosis) and the receptiveness of the host environment will all contribute to the ultimate survival of grafted cells. For example, it appears that neural precursors cultured from different areas of the brain have different properties. In a recent study, Svendsen's laboratory found that cells isolated from the developing rat brain, propagated as neurospheres, have different growth properties and give rise to cells with distinct phenotypes depending on their site of origin.[31] The stage of differentiation of the transplanted cells might also be a factor. For demyelinating diseases, multipotential neural precursors rather than more restricted oligodendrocyte precursors might be more useful.[64,68,69] Whereas cells committed to a defined lineage before injection might generate a larger proportion of a given cell type, these will not have the advantage of cell plasticity and might display reduced proliferative potential. The developmental age of the NSCs used for transplantation might also be a significant factor in determining the successful outcome. In conclusion, many factors influence success of cell therapy and very much more research is needed to understand them more fully.

Survival of grafts—death by apoptosis

Areas of adult neurogenesis have been shown to contain high numbers of apoptotic cells.[70] Presumably, during normal adult neurogenesis, programmed cell death plays an important regulatory function by eliminating excess or unhealthy cells from neurogenic regions similar to that observed in the embryo.[71] Apoptosis of transplanted NSCs might also be a major factor in determining the successful outcome of cell therapy, and some experimental data in adult models supports this. Anti-apoptotic therapies, such as inhibition of caspases, can significantly increase the survival of nigral transplants in a model of Parkinson's disease.[21] Survival of dopaminergic grafts into the striatum of Parkinsonian rats is also significantly increased if the transplants are spiked with a small population of fibroblasts expressing FGF, which both acts as a survival signal and enhances neuronal differentiation.[72] In the generation of dopaminergic neurons derived from cultures of pluripotent mouse ES cells, increased numbers of tyrosine hydroxylase-positive(TH, the first and rate-limiting enzyme in dopamine synthesis) and dopamine-producing neurons were obtained in the presence of neurotrophins in combination with defined survival promoting factors, including IL-1β and GDNF.[73] In parallel experiments, the mRNA level of the anti-apoptotic gene bcl-2 was also increased in these cultures. However, the role of Bcl-2 in graft survival is not straightforward. Whereas Bcl-2 protects against apoptosis in culture, NSCs isolated from transgenic mice overexpressing this anti-apoptotic gene do not show increased survival on grafting, although they do, rather intriguingly, display improved fibre outgrowth.[74] One should also consider that engineering NSCs to express pro-survival

genes could increase the risk of tumourogenesis. Clearly, more work is needed to determine the role of apoptosis in the survival of stem cell transplants.

Cell environment

Cell—cell interactions are be important in determining the correct terminal differentiation of NSCs. The importance of external cues from the environment has been demonstrated both in vitro and in vivo. In culture, neural precursor cells from embryonic striatum or the adult subependymal zone in the presence of FGF yielded only small numbers of neurons producing TH, whereas the inclusion of conditioned medium from glial cell cultures increased the yield of TH-positive neurons by more than 17-fold. Embryonic striatal precursors were significantly more responsive to the differentiation environment, further indicating that stem cells from earlier developmental sources might provide more successful transplants.[75] The importance of the host environment has also been demonstrated by transplantation into the cerebral ventricles of embryonic hosts in utero. Not only do donor cells differentiate but they acquire the specific phenotype of the surrounding cells. Thus, McKay and co-workers found that cells that had incorporated into the host hippocampus assumed morphologies resembling granule and pyramidal neurons, whereas those that integrated into the inferior colliculus resembled tectal neurons that reside in this region.[76] Although there are encouraging data suggesting that pluripotent cells can respond appropriately to developmental cues from the brain, little is known about the extrinsic signals and molecular events that direct this process.

Despite these somewhat daunting hurdles, stem cell therapy has been successfully employed in animal models of CNS lesions where there is significant cell loss, for example to repair focal infarctions resulting from stroke. It seems that the brain can detect and respond to even small changes in cell number or subtle perturbations in normal function by providing the appropriate cues for stem cells to differentiate and repair the damage.[23] At the other extreme, what would be the outcome of grafts in a situation where cell loss was so extensive that tissue structure was significantly disrupted? In an important new development, transplantation of a polymer scaffold seeded with NSCs was associated with significant improvement in motor function in a severe traumatic spinal cord injury model in rats.[77] Clearly, appropriate differentiation and integration is the goal of cell therapy. Intriguingly, there are now a variety of transplantation studies in adult models of injury (e.g. stroke) where a functional improvement has been seen in lesioned animals after stem cell therapy even though the graft does not appear to have integrated. It has been speculated that the clinical improvement might be due to trophic signals from injected cells promoting survival and repair of endogenous tissue. Such observations indicate that the environment into which the cells are transplanted might instruct their fate, but also that the donor cells themselves might have the ability to modify the host environment and potentially enhance endogenous repair.

Other variables include the site of NSC injection, because this can also influence the fate of transplanted cells. By marking transplanted cells with GFP, human NSCs have been shown to differentiate into post-mitotic neurons throughout the brain, whereas differentiation into glial cell types occurred predominantly at the sight of injection.[35] Such outcomes can be overridden by employing genetically modified donor cells that offer the advantage of combining cell replacement with gene therapy.[78] For instance, transplants into a refractory environment (e.g. where activated microglia are present)

would have an increased chance of success if the cells transferred contained a gene to counteract this hostile environment. For example, it has been suggested that ectopic expression of the neural cell adhesion molecule, L1, in astrocytes can increase the speed and efficiency of innervation of branching axons, thus improving the transplant success of grafted NSCs.[79]

SUMMARY

Many issues that remain to be clarified about NSC transplantation into injured brains. Although imaging of the developing brain has become more sophisticated and can now even be undertaken in utero (Figure 3), the pathogenesis of many conditions is not well understood. This precludes the directed use of NSCs for perinatal therapy at present. Also, there is ongoing debate as to which cells are best suited for cell therapy.[80] In an ideal world, one would be able to stimulate the proliferation and appropriate differentiation of endogenous stem cells. In fact, a number of vector-driven growth-factor-based therapies might work, at least in part, through this mechanism. Early experiments in stem cell transplantation suggested that embryonic tissue was significantly more plastic than that derived from the adult. Although subsequent research has indicated that adult NSCs possess a broader developmental potential than was first thought, they have a more limited lifespan than ES or fetal-derived cells.

Any research that relies on embryonic or fetal tissues (especially when derived as a result of therapeutic cloning) will be ethically controversial. Consequently, efforts should also focus on adult sources of stem cells for neural cell replacement. Whether the starting material is fetal or adult-derived, cell replacement strategies must also contend with the influence of environmental signals. In several models of adult brain repair, transplants are prone to apoptosis for prolonged periods after transfer and so clinical improvement might only be temporary.[47] Considerable work is therefore needed to identify the triggers for specific neural cell survival and integration, and further to determine how the environment of the injured brain might be manipulated to become more permissive for effective repair.

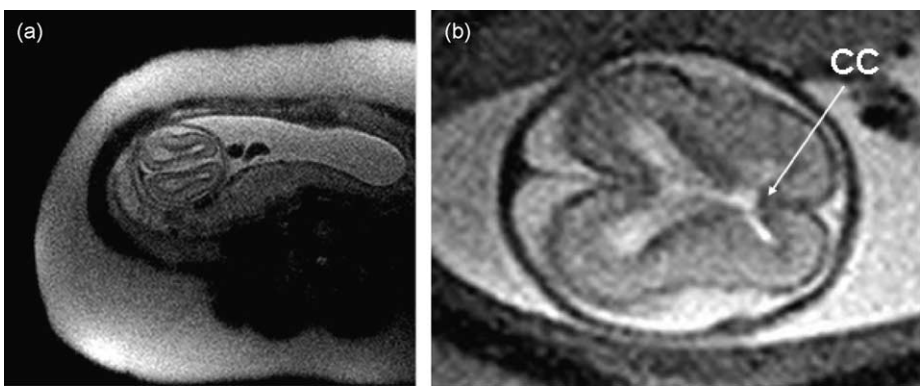

Figure 3. Fetal MR imaging can allow earlier diagnosis of structural brain abnormalities. (a) Fetal MR imaging allows details of brain structure and development in utero (arrow). This technique allows earlier diagnosis of structural brain disease. This example is of an infant with an absent corpus callosum. For comparison a normal fetal MR at the same level, (b) shows the normal corpus callosum (CC).

Practice points

- NSCs can be obtained from fetal and adult brain for study and therapy
- embryonic stem cells and bone-marrow-derived stem cells can differentiate into neural cells
- NSC therapy is successful in several animal models of acute brain injury, metabolic disease and neurodegeneration but research is at an early stage
- perinatal therapy offers the potential advantage of treating brain disease before it is too advanced/irreversible
- the pathogenesis of rare metabolic brain diseases or other neurodegeneration is poorly understood

Research agenda

- the understanding of the molecular and cellular basis of perinatal brain diseases
- research into the brain environment in disease and the factors that could enhance endogenous brain repair, and optimise NSC graft survival, differentiation and integration
- determining the 'optimum' source of stem cell for brain repair in any given condition
- researching the benefits of perinatal versus later cell therapy

ACKNOWLEDGEMENTS

The authors would like to thank Professor M. Rutherford and colleagues in the Robert Steiner MR Unit, Imperial College London, for providing the MR images used in this article.

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