Neural Tube Defects: Review of Experimental Evidence on Stem Cell Therapy and Newer Treatment Options

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Key Words

Neural tube defects · Myelomeningocele · Stem cells · Induced pluripotent stem cells · Combination therapies

Abstract

The failure of closure of the neural tube during development leads to malformations called neural tube defects (NTDs). The most common neural malformations in humans include anencephaly, encephalocele, exencephaly, craniorachischisis spina bifida with or without myelomeningocele, lipomyeloschisis, lipomyelomeningocele, meningocele and myelocystocele. Current preventive strategies are mainly based on pharmacologic/folic acid supplementation. However, stem cell-based and other combination approaches may emerge as potential treatment options for NTDs. This review provides an account of experimental evidence on stem cell therapy and newer treatment options for NTDs which have become available in recent years. Copyright © 2010 S. Karger AG, Basel

Introduction

Neural tube defects (NTDs) are the result of perturbation in the neurulation process. Neurulation, through a coordinated series of events, gives rise to the neural plate, neural folds and the neural tube which eventually dif-

ferentiates and develops into the future brain and spinal cord. Most NTDs arise in anterior and posterior neuropores as they close last. NTDs can be open or closed depending upon exposed or closed neural tissue. Open NTDs (ONTDs) are the result of primary neurulation and may involve any area of the central nervous system (CNS). Closed NTDs are due to defects in secondary neurulation and are mostly confined to the spine. Cranial manifestations of NTDs include myelomeningocele (MMC) (fig. 1, 2), meningoencephalocele (fig. 3, 4), anencephaly, encephalocele (meningocele or meningomyelocele), craniorachischisis totalis, and spinal presentations include spina bifida aperta, myeloschisis, congenital dermal sinus, lipomatous malformations (lipomyelomeningoceles), split-cord malformations, diastematomyelia, diplomyelia and caudal agenesis.

NTDs are complex multifactorial genetic defects involving both genetic and environmental factors. Geography, ethnicity, nutrition, maternal illness, maternal age and parity, previous abortions, multiple gestations, obesity, parental socioeconomic status and parental occupational exposure are important epidemiologic determinants of NTDs. Folate deficiency has been implicated as the major cause of NTDs after teratogens. Toxins and antiepileptic drugs such as valproate and carbamazepine can also induce NTDs. NTDs are often found associated with several other syndromes, chromosomal and single gene disorders, twinning, chromosomal aberrations as

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Fig. 1. Lumbosacral meningocele with hypertrichosis.

Fig. 2. Lumbosacral meningocele with exposed meninges.

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Fig. 3. Occipital meningocele with anencephaly.

Fig. 4. Nasal meningocele.

well as the CNS and non-neural malformations. The exact mechanism of NTDs is not known at present, but a range of mouse mutant models, especially the curly tail (ct) mutant which mimics human malformations in location, pathology and associated abnormalities and loop tail mutant which manifests into craniorachischisis, have

greatly enhanced our understanding of NTDs in humans [1, 2]. Several excellent reviews from time to time have elaborated the role of different genes in the neurulation process in general as well in folate metabolism pathway [3–8]. Extracellular matrix, cell surface glycoproteins, actin-containing microfilaments, growth factors and coor-

minated action of several genes have been implicated in the neurulation process. Until 2007, 190 naturally occurring or experimentally induced mouse mutants with NTDs had been described and on the basis of available literature on NTD producing loss of function mutations in mice, 28 genes are believed to be required for normal neurulation [9]. NTDs can also be produced surgically. Models based on mechanically induced NTDs due to disruption of normal neurulation through surgical intervention offers advantages to toxin-induced NTDs. The neurological evaluations demonstrated normal functioning in the large animals in which MMC could be mechanically created and surgically repaired [7, 8].

At present, oral folic acid intake remains the treatment of choice to prevent NTDs. Folic acid supplementation has been implicated in prevention of not only NTDs but also orofacial anomalies. A good deal of literature substantiates the role of folic acid in preventing preterm delivery, placental abruptions and infarctions. Periconceptional intake of multivitamins containing folic acid reduces the risk of having NTD in previously affected pregnancy. Recent literature suggests inositol as a possible adjunct to folic acid therapy [10, 11]. Complementary and alternative medicine therapy has been used in several diseases including spina bifida. *Cordyceps*, *Ocimum sanctum*, *Azadirachta indica* and other medicinal plants and their extracts which are reported to have immunomodulating, anti-cancer and multiple rejuvenating effects, may be investigated in NTD mutant mice in combination with stem cells [12].

At present the only option for prenatal therapy of spina bifida is fetal surgery, which remains under investigation in the Management of Myelomeningocele Study (MOMS) trial. Fichter et al. [13] have reviewed fetal spina bifida repair in great detail and have concluded that despite possible risks for fetus and mother, intrauterine MMC repair may prove to be beneficial in the fetuses before 26 weeks of gestational age, with small ventricles at the time of surgery, and lesion levels below L2. However, the chances for success of fetal surgery are probably very limited and it will remain a very aggressive option which is likely to be poorly accepted by most patients.

Stem Cell Therapy

Stem cells have emerged as a potential therapeutic modality for several diseases due to their reparative function. They are unspecialized cells which virtually can give rise to many or all of the different cell types in the

body. In recent years their therapeutic role has been investigated in treating a range of neurodegenerative diseases including stroke, cerebral palsy, spinal cord injury, post-polio syndrome, musculoskeletal disorders and congenital malformations.

Sim et al. [14] investigated the chronological changes of reclosure capacity in surgically induced spinal ONTDs of chick embryos. The chronological changes in reclosure capacity of ONTDs paralleled the proliferative activity of the neural tube, suggesting that manipulation of cell dynamics could enhance the reclosure process in embryonic spinal cord tissue. Extending these studies, Lee et al. [15] have reported enhancement of reclosure capacity by the intra-amniotic injection of human embryonic stem cells (hESCs) in surgically induced spinal ONTDs in chick embryos. The study reveals the possibility of use of hESCs in prenatal management of human NTDs. The experiment included longitudinal opening of neural tubes of chick embryos at the wing bud level for a length of six somites. hESCs were injected into the amniotic cavity with the aid of vehicle. Neural tube incision followed quick injection of 2 μ l of vehicle or hESC solution. The sizes of NTDs on postoperative reincubation days 3, 5 and 7 were compared between control (no injection) and vehicle (injection of glucose in PBS and hESC injection of hESCs in vehicle) groups. The postoperative day (POD) lengths were significantly smaller in the hESC cell group than in control and vehicle groups from POD 3 to POD 7, clearly demonstrating enhanced reclosure of the ONTDs with embryonic stem cells. The enhancement of reclosure capacity in this study could be due to the bridging effect required to fill the injury site and protect injured tissue to promote regeneration which in turn might have led to repair of structures and regain of function. To simulate the clinical situation of human NTDs, Lee et al. [24] further investigated reclosure of surgically induced spinal ONTDs by intra-amniotic injection of hESCs in chick embryos 24 h after lesion induction. The hESCs injected into the amniotic cavity at 24 h after ONTD induction enhanced reclosure ability.

Gupta et al. [16] performed a study with autologous stem cells in congenital anomalies on 17 patients with meningomyelocele. Stem cells were administered either during definitive surgery or postoperatively. CD34+ mononuclear cells separated from bone marrow were injected into the spinal cord and caudal space for meningomyelocele. The condition of the patient before surgery served as the control condition for that patient. Temperature charting and seeking evidence of infection or other complications were used as postoperative monitoring measures. In

meningomyelocele, antibiotic coverage was given for 7–10 days. Clinical status and muscle charting of meningo-myelocele patients were evaluated by two independent physiotherapists. Changes in power, sensation, and neurological status of bladder and bowel were examined. Patients in the study were of age group 0–1 month, 1–5 months, and 1–6 years in 6, 8 and 3 cases, respectively, and a history of rupture was present in 5 patients. Meningomyelocele repair had been performed in 4 patients in the past who presented persistent neurological deficiency. In 1 patient redo surgery for a tethered cord had been performed. In 13 patients, meningo-myelocele repair and stem cell injection were performed whereas in 3 patients only stem cell injection was given. Two patients had meningoceles. A smooth postoperative recovery in all subjects was recorded without complications. The initial results had been promising but the authors warrant long-term evaluation with randomized control trials to draw significant conclusions. Although the work is of significant value, being the first of its kind from India, experts in the field believe that this study is not without flaws because: (1) a traumatic paraplegia rat model cannot be a basis of meningo-myelocele experimentations in humans since neurons are normal until injury in traumatic paraplegia whereas in meningo-myelocele, neurons are congenital dysphasic; (2) the authors failed to demonstrate if the injected stem cells could really form functional neurons after transplantation; (3) the safety of the cells for human use was not ensured as they could become tumorigenic, and finally (4) because of the lack of proper assessment of recovery of function, especially in very small infants.

Fauza et al. [17] delivered neural stem cells (NSCs) to the spinal cord in lambs with experimental MMC as an adjuvant to fetal repair of spina bifida. The study included fetal lambs divided in three groups: group I animals had animals without repair; group II presented standard surgical MMC coverage, and group III had MMC coverage plus delivery of a murine NSC clone into the spinal cord defect. A 2 ml NSC suspension of 10^8 cells/ml was directly injected into the gray matter of the spinal cord at 4–6 equally spaced points. Animals in group II and III showed higher survival than group I. Animals in group I also presented inability to walk (major paraparesis) to a more significant extent than in groups II and III. A lower incidence of major paraparesis was recorded in group III compared to group II, but the results were not statistically significant. Group III, comprising animals without engraft, showed no indications of clinical worsening or impairment greater than animals in groups I and II. Group III presented increased power of muscular contractions and

tactile function in the affected areas compared to group II. In all animals in groups II and III, variable degrees of spinal cord tethering to the alloderm patch was recorded. Despite their xenologous origin and lack of host immunosuppression, all animals in group III showed NSC engraftment and survival in the spinal cord and no worsening from the existing condition was observed. Results showed noticeably higher NSC density within the most damaged portions of the spinal cord, suggesting selective NSC homing to abnormal areas. This effect was distinct in the periphery of the cord at the level of the defect where cord had been exposed to chemical insult from the amniotic fluid and possibly suffered mechanical damage. In hydro/syringomyelia-like lesions present within the cord of some of the animals in this group, some donor cells were found which mostly occur in MMC. This could be the consequence of needle-based cell injection in this model. There were very few non-peripheral areas with no morphologic evidence of injury with any engrafted cells. The authors did not find any clear relationship between the degree of NSC engraftment and clinical outcome in this initial series. No evidence of tumor formation was recorded at the engrafted sites in any of the animals. The study clearly pointed towards some role of NSCs in the regenerative process. The authors suggested validation of the effect with more experiments before NSC could be used as therapeutic modality.

Induced Pluripotent Stem Cells

First described by Takahashi and Yamanaka [18], these cells are basically reprogrammed somatic cells which eventually become pluripotent by introduction and ectopic expression of some specific transcription factors – *Oct4*, *Sox2*, *Klf4* and *Myc* being the most prominent. Induced pluripotent stem cells (iPSCs) share many similarities with hESCs and can differentiate into all major cell types representing three embryonic germ layers. This breakthrough technology on the one hand has circumvented the use of embryos and oocytes for research, on the other the use of animals can be spared significantly for drug discovery and toxicity testing. Generation of patient-specific iPSCs further bypass the problem of graft rejection thus proving to be an ideal choice for transplantation studies. In addition, iPSCs are capable of restoring organ function as well as reconstruction of the entire tissue in the event of injury or disease.

The discovery of iPSCs has initiated a flurry of research on finding treatment for a variety of CNS disor-

ders. Recently it has become possible to generate subject-specific iPSCs which can further differentiate into relevant cell types [19, 20]. Some recent examples include generation of spinal motor neurons and glia from a person suffering from amyotrophic lateral sclerosis, dopamine neurons derived from iPSCs derived from persons with Parkinson's disease, motor neurons derived from iPSCs derived from a 3-year-old child with spinal muscular atrophy and derivation of iPSCs from persons with familial dysautonomia – a rare genetic disease mainly affecting the peripheral nervous system [21–24].

Due to a direct role of cranial neural crest cells in neural tube closure, it is fairly reasonable to hypothesize that probably neural crest cells can be reprogrammed to derive specific NTD iPSCs which, if transplanted, could possibly adequately help in closure of neural tubes in young infants. For caution, such studies should first be demonstrated in animal models. To model the disease in vitro, neural crest stem cells can be isolated from adult palatum [25]. For rapid and successful replenishment of the diseased tissue with healthy cells, the migratory potential of such neural crest iPSCs need to be accessed and their dependency on extracellular matrix molecules, such as thrombospondin for migration, examined. Another candidate cell type for iPSCs therapy in NTD can be liver cells as these cells are a natural source of folic acid. This fact can be used in helping women with a family history of birth defects or with a previous child with a birth defect or multiple first-trimester abortions. Liver cells isolated from such a woman can be reprogrammed to endogenously secrete folic acid and administered right before the onset of pregnancy as a prophylactic measure to possibly prevent NTD in the newborn. But again, this idea is purely speculative and needs proper experimental validation, especially for their responsiveness to indigenous folate synthesis, absorption and transport as well as folate toxicity (in the event of excessive synthesis of folic acid).

iPSCs are unique in the sense that patient-specific cells can differentiate into specific cell lineages, thus providing a potential in vitro tool to study the complexity of the disease. The only concern with iPSCs at present is their safety for human use as misprogrammed and undifferentiated iPSCs may lead to tumors.

New Therapies

The marriage between chemical biology and molecular genetics has led to the emergence of a new discipline of chemical genomics aimed at discovering therapeutic

small molecules. A range of molecules including antisense oligonucleotides, peptides, small interfering (si) RNAs and microRNAs, CNS, dopaminergic, and natural product-based drug candidates. Zhou et al. [26] tested peptidergic agonists of activity-dependent neurotrophic factor on prenatal alcohol-induced NTDs and serotonin neuron loss in time pregnant B6 dams. The authors experimentally induced deficits in fetal 5-HT neurons in the caudal raphe through prenatal exposure to alcohol. Prenatal treatment with the neuroprotective peptides SAL or NAP could fully protect against reduction in 5-HT neurons in the caudal and rostral raphe, and NAP prevented delayed closing of the rhombencephalic ventral canal. A recent study by Zwerts et al. [27] has demonstrated the role of endothelial cell survivin in embryonic defects in angiogenesis, cardiogenesis, and neural tube closure. Survivin is an inhibitor of apoptosis protein which is a regulator of mitosis and is highly expressed in proliferating cells but low or absent in quiescent or terminally differentiated cells. It is a negative regulator of apoptosis and plays a critical role in maintaining prenatal vascular integrity. Their study showed that lack of endothelial surviving expression during embryonic development resulted in delayed neural tube closure along with in utero lethality and defective cardiac development. The offspring of diabetic mothers are more prone to congenital malformations. Wentzel et al. [28] have shown that the teratogenic effect of diabetic serum can be prevented by supplementation of superoxide dismutase and N-acetylcysteine in rat embryo culture. Small molecules such as monastrol or ispinesib, which can inhibit kinesin KIF11 and inhibitor of $\alpha 4$ integrin (CT301) which have shown to have neuroprotective function, may be investigated for their possible role in preventing NTDs. Gyuris et al. [29], using bromodomain-containing protein 2 (Brd2) null mice, have shown that Brd2 is required for neural tube closure and embryogenesis. The authors have nicely demonstrated that the disruption of Brd2 gene causes embryonic lethality and exhibits neural tube closure defects and most commonly the anencephaly of the hindbrain. The effect of transgenic Brd2 gene activity may be tested on neural tube closure to investigate if Brd2 gene overexpression could be used as a therapeutic target. Watson et al. [30] have shown that *Mrj*^{-/-} mouse embryos exhibit NTDs independent of extraembryonic defects. The study suggests using *Mrj*^{-/-} mutant embryos as an excellent model to study the relationship between stem cell self-renewal and neural tube closure. Neural stem cells with possible *Mrj* reprogramming for increased self-renewal can be a viable option for development of stem cell-based

therapy for NTDs. A recent revelation that synergistic inhibition of SMAD signaling by *Noggin* and SB431542 can cause efficient neural conversion of hESCs as well as direct differentiation of iPSCs into midbrain dopamine and spinal motor neurons, further envisage the possible scope of use of small molecules and stem cells for treating NTDs [31]. In a recent study, Camerer et al. [32] have demonstrated that local protease signaling contributes to neural tube closure in the mouse embryos. These signaling molecules can be exploited for their therapeutic suitability as target molecules in the treatment of neural tube and associated developmental defects. In addition, siRNAs and microRNAs, designed against a specific gene, that regulate developmental processes may be helpful in understanding the underlying cause of NTDs and in developing therapeutic strategies to prevent NTDs.

Conclusion

NTDs are preventable to some extent by proper consumption of folic acid. The newer treatment modalities with stem cells though demonstrate some improvement, but for safety, rigorous animal experimental studies are

advised before their use in human clinical trials. Before starting any such study, investigators should carefully design the experiment, keeping in mind all the considerations such as the use of proper controls, use of correct and appropriate experimental models, use of well-standardized and established procedures (especially when human subjects are involved) and proper tests to access the recovery of function. Assessment of migration and functional integration of stem cells should be thoroughly investigated in studies involving cell transplantation studies. The use of magnetic resonance imaging and nanotechnology procedures has been immensely helpful in tracing the fate of transplanted stem cells [33–36]. Alternative medicine in conjunction with stem cells and iPSCs may be useful in treating NTDs. Combination therapies using stem cells with plant extracts of known potential therapeutic value can be tested in animal models of NTD, to examine the therapeutic effect of such formulations.

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