Rationale for Regenerative Treatment in Neonatology

Argumente für eine regenerative Therapie in der Neonatologie

R. Haase[1], **D. Körholz**[2], **E. Herting**[3], **R. V. Sorg**[4], **L. P. Müller**[5], **U. Göbel**[6]

Affiliation addresses are listed at the end of the article

In this issue of the "Klinische Pädiatrie" Gortner et al. [8] report on regenerative treatment modalities in neonatology to overcome long term sequelae of prematurity and asphyxia, since both are associated with a high risk of adverse outcome leading to lifelong severe disability. According to the review by Gortner et al. [8] severe bronchopulmonary dysplasia (BPD) and hypoxic-ischaemic encephalopathia (HIE) might be preferable fields for regenerative treatment in neonatology using cord blood stem cells (CBSC), endothelial progenitor cells (EPC), mesenchymal stem cells (MSC) or MSC derived exosomes.

During the past 3 decades clinical results in neonatology have improved dramatically. Over the last years the survival of preterm infants has significantly increased and exceeds now 80% for children born at 24–26 weeks gestational age [16,31–33]. Despite progress in neonatology in preterm infants short and long term outcome is mainly influenced by respiratory diseases and neurological damages. For the survivors the risk for the development of chronic pulmonary disorders and sensoric, neurologic, cognitive and behavioral deficits [4,15,24,25,34] remains almost unchanged. For term infants asphyxia is further a main cause for neonatal death and hypoxic brain damage [3]. About 30% of the surviving newborns will suffer from cerebral palsy or other neurodevelopmental disabilities in later life [29]. New approaches such as hypothermia for treatment of perinatal asphyxia have substantially increased the proportion of surviving infants without neurological damage [25,27,28]. However, infants with less severe encephalopathy have the most benefit from hypothermia. Although ongoing or planned studies hopefully will clarify the potential of new approaches for the treatment of preterm infants and newborns with perinatal asphyxia negative long term effects of perinatal problems such as neurodevelopmental delay and bronchopulmonary dysplasia will remain a major challenge for the caring pediatricians.

Regenerative medicine is not new for pediatricians. Allogenic or autologous hematopoietic stem cells from bone marrow, peripheral blood or umbilical cord blood have been used in pediatric oncology since 2 decades for hematopoietic reconstitution after myeloablative therapies or treatment of bone marrow failure syndromes [30]. Stem cell transplantations were performed even before birth in children affected by diseases with known molecular basis and assured diagnosis [17]. MSCs are considered and used as a treatment alternative in severe graft vs. host disease [18,20,23]. MSCs were also used for treatment of inherited diseases such as osteogenesis imperfecta more than 10 years ago [11]. In contrast, the use of regenerative medicine in specific diseases of neonates is still very limited and most experiences refer to preclinical studies with MSCs. There are only 2 clinical studies dealing with MSCs for treatment of BPD (ClinicalTrials.gov identifier NCT01297205 and NCT01207869) and one for treatment of HIE (ClinicalTrials.gov identifier: NCT00593242) listed in the NIH registry. The preclinical studies uniformly demonstrate positive effects of MSC in the treatment of BPD and HIE. Regarding neonatal brain damage MSCs were effective in reducing the volume of the lesion and in improving functional outcome [40]. Of interest, in animal experiments methods of regenerative medicine were more successful in neonatal than in adult models, maybe due to higher plasticity and inherited increased tolerance of neonatal compared to adult neuronal cells [40,41]. However, also in adult animals with hypoxic brain lesions, MSCs were mostly associated with improved outcome [10]. Animal studies with rodents after induction of experimental BPD consistently showed that treatment with MSCs leads to preservation of alveolar structure and lung vascularization, decreased inflammation and improved pulmonary outcome and survival [1,36,39]. An apparent problem of most animal models for BPD is its monocausal genesis by using high concentrations of oxygen to induce experimental BPD. However, in patients, BPD is a multifactorial disease and hyperoxygenation may be only one important factor in its pathogenesis. Beside gestational age other factors like prenatal or postnatal infection, premature rupture of membranes, prenatal corticosteroids, the presence or absence of a patent ductus arteriosus, fluid management and strategies of breathing support or mechanical ventilation may also influence the occurrence of BPD.

Regenerative medicine might also be important for neonatal diseases affecting other organs than lungs and brain. So, the use of MSCs was described in a neonatal rat model of necrotizing enterocolitis (NEC) [35]. 4 days after intraperitoneal injection in the NEC + MSC group the animals had more weight gain and a better histopathological outcome. NEC is a multifactorial disease with bacterial translocation as an important contributing factor. Therefore one might speculate that the known immunosuppressive activity of MSCs might promote bacterial overgrowth and increase transmural bacterial translocation. However, in this study bacterial translocation was reduced due to accelerated intestinal barrier recon-

Bibliography
DOI http://dx.doi.org/10.1055/s-0032-1316289
Klin Padiatr 2012; 224: 230–232
© Georg Thieme Verlag KG Stuttgart · New York
ISSN 0300-8630

Correspondence
Prof. Dr. Ulrich Göbel
ESPED-Geschäftsstelle
Koordiierungszentrum für Klinische Studien
Universitätsklinikum Heinrich-Heine-Universität Düsseldorf
Moorenstraße 5
40225 Düsseldorf
Tel.: +49/211/81-16263
Fax: +49/211/81-16262
goebelu@arcor.de

This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited.

stitution. These results suggest that MSCs might be a therapeutic approach to avoid the devastating long term consequences of severe NEC, but further studies are necessary to examine long term (study period included 4 days after injection of MSCs) effects and risks.

Since there is a lot of experiences using stem cells from pediatric patients with immune deficiency syndromes or acute leukemia [9,13], the different hazards of the treatment strategy have not to be overlooked. Therefore, MSCs for the treatment of BPD or neonatal brain damage as suggested from preclinical studies might be initially limited to patients with severe complications which significantly increase the risk of mortality in the individual patient or which are associated with a high risk of lung or brain damage leading to a lifelong major reduction of quality of life in the respective patient. Individual treatment approaches [21] should be abandoned because they will not lead to a significant increase of knowledge for these therapies and may lead to publications showing positive effects while negative results may be suppressed. Thus, regenerative therapies should only be performed in well-designed multicentre clinical trials which are registered at the NIH.

However, these preclinical studies raise important questions which have to be answered in a clinical setting:

When is the appropriate time for initiation of these treatments – acute intervention during occurrence of the damage or later on when the initial inflammatory reaction has already ceased? MSCs influence the regional microenvironment by paracrine secretion of cytokines and chemokines. Several studies have shown that MSCs homing occurs predominantly in tissues with the presence of injury or inflammation, but the underlying mechanism is not fully understood. In contrast, it was also demonstrated that MSCs are trapped in the lungs after intravenous application [19]. While this may be beneficial for treating lung damage it raises the question for the appropriate mode of application in other diseases [14]. Furthermore, the number of engrafted cells is usually too low to explain the full therapeutic benefit [39]. MSCs do not only have a regenerative potential by simply replacing damaged resident cells, but also anti-inflammatory, anti-apoptotic and growth promoting effects and might support tissue regeneration from host cell progenitors [7,23,42,43]. Thus it may be preferable to start regenerative treatment rather early so that cell damage of resident cells induced by local inflammation may be limited [5]. On the other hand prematurity and HIE might be caused from prenatal processes that might decrease the positive properties of autologous MSCs. Therefore – which source of MSCs should we use? MSCs can be harvested from bone marrow and adipose tissue, but early postnatally also from cord blood, Wharton's jelly or the placenta [40]. For neonatologists the umbilical cord, cord blood and the placenta are of special interest. Another question is directed towards the optimal cell dose – amount of cells, single doses versus repetitive doses, as it has been used for treatment of autoimmune diseases [26]. New data suggest, that MSCs may exert therapeutic effects through highly different mechanisms, such as cytokine release mediating effects over "long distance" [19] as well as direct mitochondrial transfer [12]. Finally, the question on what severe side effects might occur along with these treatment modalities may help to decide which of these strategies might be used or which cells might be preferably be used in near future. From animal and human studies acute toxicity appears similarly negligible but less is known about long term effects [5,38]. Due to their low immunogenic potential allogeneic MSC do not to induce rejection and they do also not lead to graft vs. host disease. Regarding MSCs and cancer – data from the literature are conflicting but results of some animal studies still suggest a tumor induction and growth promoting role of MSCs [6,37]. Thus, the long-term safety of MSCs has to be assessed.

As for cellular therapy in general [22], there remains the question on how to finance these treatment modalities for our small patients. Based on the experiences of paediatric oncology it might also be true for neonatology that there is no relevant market for industrial companies sponsoring these trials. Therefore the necessary studies should be supported by public funding through institutions such as "Bundesministerium für Forschung und Technologie" or a foundation for neonatal care which might act similar as the "Deutsche Kinderkrebshilfe". Reimbursement for approved treatment has to be taken over by the public health insurance companies based on approval and agreement by the regulatory agencies ("Gemeinsamer Bundesausschuss") will allow the necessary measures to achieve a standardized assurance for this treatment modality. This advancement would offer all high risk term and preterm infants the opportunity to be treated in quality controlled clinical trials, as shown earlier in Pediatric Oncology [2].

Finally, this concept may be most successful if the Society of Neonatology will invite all researchers who fulfil the essential requirements for participation to take part in those trials. The success of German Neonatal Network (GNN) has shown the great interest of neonatologists in the implementation of cooperative studies [32]. The contribution of all eligible patients will allow to answer as soon as possible the most important questions in this very promising field of regenerative medicine.

Affiliations

[1] Neonatologie, Universitätsklinik und Poliklinik für Kinder- und Jugendmedizin, Martin-Luther-Universität Halle-Wittenberg, Halle(Saale)
[2] Universitätsklinik und Poliklinik für Kinder- und Jugendmedizin, Martin-Luther-Universität Halle-Wittenberg, Halle(Saale)
[3] Klinik für Kinder- und Jugendmedizin, Universitätskinderklinikum Schleswig-Holstein, Campus Lübeck
[4] Institut für Transplantationsdiagnostik und Zelltherapeutika Düsseldorf
[5] Universitätsklinik für Innere Medizin IV, Martin-Luther-Universität Halle-Wittenberg, Halle(Saale)
[6] ESPED-Geschäftsstelle, Koordinierungszentrum für Klinische Studien, Universitätsklinikum, Heinrich-Heine-Universität Düsseldorf

References

1 *Alphonse RS*, *Thebaud B*. Growth factors, stem cells and bronchopulmonary dysplasia. Neonatology 2011; 99: 326–337
2 *Berthold F*, *Bode G*, *Bocker A et al.* Measures of quality assurance for in-patient pediatric oncology units. Klin Padiatr 2006; 218: 293–295
3 *Born M*, *Scheef L*, *Boecker H et al.* Cerebral MRI of preterm neonates at term equivalent age at 3 Tesla: hints at white matter damage on T2- and diffusion weighted images. Klin Padiatr 2010; 222: 443–448
4 *Dalous J*, *Larghero J*, *Baud O*. Transplantation of umbilical cord-derived mesenchymal stem cells as a novel strategy to protect the central nervous system: technical aspects, preclinical studies, and clinical perspectives. Pediatr Res 2012; 71: 482–490
5 *Dazzi F*, *van Laar JM*, *Cope A et al.* Cell therapy for autoimmune diseases. Arthritis Res Ther 2007; 9: 206
6 *De Boeck A*, *Pauwels P*, *Hensen K et al.* Bone marrow-derived mesenchymal stem cells promote colorectal cancer progression through paracrine neuregulin 1/HER3 signalling. Gut 2012 [Epub ahead of print]. doi: 10.1136/gutjnl-2011-301393.
7 *De Miguel MP*, *Fuentes-Julian S*, *Blazquez-Martinez A et al.* Immunosuppressive properties of mesenchymal stem cells: advances and applications. Curr Mol Med 2012 [Epub ahead of print]. doi: 10.2174/156652412800619950.

This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited.

8 *Gortner L*, *Felderhoff-Müser U*, *Monz D et al.* Regenerative Therapies in Neonatology: State of the Art and Perspectives Klin Padiatr. 2012; 224
9 *Honig M*, *Schulz A*, *Friedrich W*. Hematopoietic stem cell transplantation for severe combined immunodeficiency. Klin Padiatr 2011; 223: 320–325
10 *Honmou O*, *Onodera R*, *Sasaki M et al.* Mesenchymal stem cells: therapeutic outlook for stroke. Trends Mol Med 2012; 18: 292–297
11 *Horwitz EM*, *Gordon PL*, *Koo WK et al.* Isolated allogeneic bone marrow-derived mesenchymal cells engraft and stimulate growth in children with osteogenesis imperfecta: Implications for cell therapy of bone. Proc Natl Acad Sci USA 2002; 99: 8932–8937
12 *Islam MN*, *Das SR*, *Emin MT et al.* Mitochondrial transfer from bone-marrow-derived stromal cells to pulmonary alveoli protects against acute lung injury. Nat Med 2012; 18: 759–765
13 *Jude V*, *Chan KW*. Recent advances in hematopoietic stem cell transplantation for childhood acute lymphoblastic leukemia. Curr Hematol Malig Rep 2010; 5: 129–134
14 *Karp JM*, *Leng Teo GS*. Mesenchymal stem cell homing: the devil is in the details. Cell Stem Cell 2009; 4: 206–216
15 *Kribs A*, *Hartel C*, *Kattner E et al.* Surfactant without intubation in preterm infants with respiratory distress: first multi-center data. Klin Padiatr 2010; 222: 13–17
16 *Kyser KL*, *Morriss FH Jr*, *Bell EF et al.* Improving survival of extremely preterm infants born between 22 and 25 weeks of gestation. Obstet Gynecol 2012; 119: 795–800
17 *Lanfranchi A*, *Porta F*, *Chirico G*. Stem cells and the frontiers of neonatology. Early Hum Dev 2009; 85: S15–S18
18 *Le Blanc K*, *Frassoni F*, *Ball L et al.* Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. Lancet 2008; 371: 1579–1586
19 *Lee RH*, *Pulin AA*, *Seo MJ et al.* Intravenous hMSCs improve myocardial infarction in mice because cells embolized in lung are activated to secrete the anti-inflammatory protein TSG-6. Cell Stem Cell 2009; 5: 54–63
20 *Lin Y*, *Hogan WJ*. Clinical Application of Mesenchymal Stem Cells in the Treatment and Prevention of Graft-versus-Host Disease. Adv Hematol 2011; 2011: 427863
21 *Luan Z*, *Liu WP*, *Qu SQ et al.* Treatment of newborns with severe injured brain with transplantation of human neural precursor cells. Zhonghua Er Ke Za Zhi 2011; 49: 445–449
22 *Maziarz RT*, *Driscoll D*. Hematopoietic stem cell transplantation and implications for cell therapy reimbursement. Cell Stem Cell 2011; 8: 609–612
23 *Meisel R*, *Brockers S*, *Heseler K et al.* Human but not murine multipotent mesenchymal stromal cells exhibit broad-spectrum antimicrobial effector function mediated by indoleamine 2,3-dioxygenase. Leukemia 2011; 25: 648–654
24 *Moll M*, *Schoning M*, *Golz R et al.* 2-year follow-up examinations (Bayley II) in infants born at <32 weeks in a German perinatal center. Klin Padiatr 2011; 223: 251–254
25 *Poets CF*. What are the main research findings during the last 5 years that have changed my approach to clinical practice? Arch Dis Child Fetal Neonatal Ed. 2011 [Epub ahead of print]. doi: 10.1136/archdischild-2011-300641.
26 *Ra JC*, *Kang SK*, *Shin IS et al.* Stem cell treatment for patients with autoimmune disease by systemic infusion of culture-expanded autologous adipose tissue derived mesenchymal stem cells. J Transl Med 2011; 9: 181
27 *Roehr CC*, *Hansmann G*, *Hoehn T et al.* The 2010 Guidelines on Neonatal Resuscitation (AHA, ERC, ILCOR): similarities and differences – what progress has been made since 2005? Klin Padiatr 2011; 223: 299–307
28 *Roka A*, *Azzopardi D*. Therapeutic hypothermia for neonatal hypoxic ischaemic encephalopathy. Early Hum Dev 2010; 86: 361–367
29 *Saugstad OD*. Reducing global neonatal mortality is possible. Neonatology 2011; 99: 250–257
30 *Schonberger S*, *Niehues T*, *Meisel R et al.* Transplantation of haematopoietic stem cells derived from cord blood, bone marrow or peripheral blood: a single centre matched-pair analysis in a heterogeneous risk population. Klin Padiatr 2004; 216: 356–363
31 *Seaton SE*, *King S*, *Manktelow BN et al.* Babies born at the threshold of viability: changes in survival and workload over 20 years. Arch Dis Child Fetal Neonatal Ed. 2012 [Epub ahead of print]. doi: 10.1136/fetalneonatal-2011-301572.
32 *Stichtenoth G*, *Demmert M*, *Bohnhorst B et al.* Major contributors to hospital mortality in very-low-birth-weight infants: data of the birth year 2010 cohort of the German Neonatal Network. Klin Padiatr 2012; 224: 276–281
33 *Stoll BJ*, *Hansen NI*, *Bell EF et al.* Neonatal outcomes of extremely preterm infants from the NICHD Neonatal Research Network. Pediatrics 2010; 126: 443–456
34 *Strassburg HM*. Neuropediatric diagnosis and rehabilitation of very and extremely premature infants. Klin Padiatr 2010; 222: 425–426
35 *Tayman C*, *Uckan D*, *Kilic E et al.* Mesenchymal stem cell therapy in necrotizing enterocolitis: a rat study. Pediatr Res 2011; 70: 489–494
36 *Tropea KA*, *Leder E*, *Aslam M et al.* Bronchioalveolar stem cells increase after mesenchymal stromal cell treatment in a mouse model of bronchopulmonary dysplasia. Am J Physiol 2012; 302: L829–L837
37 *Tsukamoto S*, *Honoki K*, *Fujii H et al.* Mesenchymal stem cells promote tumor engraftment and metastatic colonization in rat osteosarcoma model. Int J Oncol 2012; 40: 163–169
38 *Tyndall A*. Application of autologous stem cell transplantation in various adult and pediatric rheumatic diseases. Pediatr Res 2012; 71: 433–438
39 *van Haaften T*, *Byrne R*, *Bonnet S et al.* Airway delivery of mesenchymal stem cells prevents arrested alveolar growth in neonatal lung injury in rats. Am J Respir Crit Care Med 2009; 180: 1131–1142
40 *van Velthoven CT*, *Kavelaars A*, *Heijnen CJ*. Mesenchymal stem cells as a treatment for neonatal ischemic brain damage. Pediatr Res 2012; 71: 474–481
41 *van Velthoven CT*, *Kavelaars A*, *van Bel F et al.* Nasal administration of stem cells: a promising novel route to treat neonatal ischemic brain damage. Pediatr Res 2010; 68: 419–422
42 *Winter M*, *Wang XN*, *Daubener W et al.* Suppression of cellular immunity by cord blood-derived unrestricted somatic stem cells is cytokine-dependent. J Cell Mol Med 2009; 13: 2465–2475
43 *Yi T*, *Song SU*. Immunomodulatory properties of mesenchymal stem cells and their therapeutic applications. Arch Pharm Res 2012; 35: 213–221

This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited.