

Novel treatment strategies for the protection of the preterm brain

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Valorization

Preterm birth is the leading cause of perinatal morbidity and mortality in developed countries. In the Netherlands 7.7% of all neonates are born preterm (less than 37 weeks gestational age) of which 20% are born extremely preterm (less than 32 weeks gestational age). Although survival after preterm birth has increased in the last decades, still a large proportion of preterm infants suffer from long term morbidity and disability, which have a tremendous impact on patients and their families.

Brain injury is the biggest contributor to mortality and morbidity in preterm infants. 25-50% of preterm infants suffer from cognitive, socialization, attentional and/or behavioral disorders, whereas, 5-10% suffer from spastic motoric deficits (e.g. cerebral palsy). Current treatment strategies are focused on limiting secondary injury after hypoxia-ischemia by hypothermia treatment in term babies or treating symptoms and stabilizing vital signs in preterm babies. Infections are treated with anti-microbial agents who are aimed at the microorganisms but not at the injured brain.

Improvement of neurodevelopmental outcomes of this vulnerable group of patients would markedly decrease the burden for this vulnerable patient group. Therefore, the aim of the current thesis was to investigate therapeutic strategies for the prevention of injury of the preterm brain caused by infectious (intra-uterine *C. albicans* infection) and sterile inflammatory triggers (global hypoxia-ischemia). For this purpose we used a translational ovine model of preterm brain injury caused by either global hypoxia-ischemia or intra-uterine infection. We used ovine fetuses since ovine fetal development and physiology are similar to the human situation. Furthermore, the size of the fetus allows for chronic instrumentations and the long gestational period enables us to study specific developmental processes in detail allowing us to apply our therapies at specific time-points.

In this thesis we demonstrated that systemic administration of Multipotent Adult Progenitor Cells (MAPCs) protected the fetal brain following global hypoxia-ischemia. Especially preterm infants suffering from global HI might benefit from MAPC treatment, since early interventions (within 6 hours following global HI) are mandated. Autologous transplantation of sufficient numbers of bone marrow stromal cells is time-consuming and exceeds the therapeutic window of opportunity. MAPC treatment has already been translated into a FDA-approved clinical-grade product, named MultiStem[®] (Athersys Inc.), and is therefore already available “off-the-shelf”.

Currently, MultiStem[®] is tested extensively in phase 2 clinical trials of ischemic stroke, and preclinical trials traumatic brain injury, multiple sclerosis, and spinal cord injury. Besides neurological indications, other diseases such as cardiovascular, and inflammatory and immune conditions benefit from MAPC treatment. The results in this thesis contribute to the addition of new group of patient, namely preterm infants.

The therapeutic effects of MAPC are mostly attributed to anti-inflammatory properties. The origin of many complications of preterm birth can be narrowed down to

inflammation, both *in utero* and postnatally. Therefore, the application of MAPC might reach further than neuroprotection. Future studies will investigate the therapeutic efficacy of MAPC treatment in other neonatal diseases such as bronchopulmonary dysplasia and necrotizing enterocolitis.

Besides anti-inflammatory properties, regenerative properties are also assigned to the therapeutic potential of MAPC cells. This paves the way for MAPC treatment as a secondary treatment in case of injury caused with an infectious origin, as proposed in chapter 4 in which we demonstrated that anti-microbial treatment reduced systemic inflammation, but could not protect against injury of the preterm brain.

As the therapeutic potential of stem cells is mostly achieved by secretion of paracrine factors, packed in extracellular vesicles, we assessed the therapeutic potential of systemic administration of these vesicles, rather than administration of the complete cell product. Administration of EVs poses advantages over administration of cells as (1) the risk of malignant transformation is greatly reduced because EVs are non-self-replicating (51). (2) Lacking an own metabolism, the EVs activity can hardly be influenced by the *in vivo* environment in patients, thus allowing for a much better characterization of their functional properties. (3) Owing to their small size EVs are less likely to generate emboli upon intravenous administration, as may be the case with MSCs. (4) In addition, EVs can be sterilized by filtration. Thus, from a regulatory point of view, the production and especially the quality control of EV fractions for clinical treatment application is less complicated than for a cellular therapeutic of *in vitro* expanded cells (52). (5) Last but not least, EVs can be developed independently of the original donor as they are derived from MSCs from unrelated donors and thus offer the opportunity to be turned into an “off-the-shelf” product. MAPC cells are ideal candidates for an off-the-shelf cell-based product and might provide the source for a superior cell-free product as well.

The ability of cells to adjust their secretome *in vivo* in reaction to their microenvironment appears to be crucial for the efficacy of cell-based therapy. Due to a lack of a metabolism, EV therapy is static, and the therapeutic properties of the EVs are determined by the *in vitro* conditions of the donor cell. Since *in vitro* conditions can be altered, creation of EVs with specific therapeutic properties is possible. Moreover, with the genetic stability of MAPCs, uniform cellular responses upon *in vitro* stimulation might be expected and reflected in the generation of EVs with specific therapeutic capacities (i.e. immune-modulatory, regenerative), that can be administered off-the-shelf at specific time-points. Determination of these time-points requires specific biomarkers, for which our translational ovine model is well-suited.

In conclusion, the therapies tested in this thesis show great promise and form the basis of additional research and clinical trials, leading to improved neurodevelopmental outcomes, and, ultimately, increased disability-free lives.