

Intrauterine Stem Cell Therapy

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Stem cells are found in embryonic, fetal and most adult tissues, and are characterised by two properties: the ability to self renew and to differentiate into multiple discrete tissue types. They offer great promise for tissue regeneration, cell replacement and gene therapy against a range of human diseases, but, with the exception of haemopoietic stem cells, clinical application at present remains largely speculative. Recent success with stem cell transplantation in adults and children, together with advances in stem cell biology and gene manipulation now render intrauterine correction of genetic defects using stem cells a realistic prospect.

Rationale

Genetic diseases with intrauterine onset can lead to intrauterine death or permanent neurological or musculoskeletal damage in early life. Examples include skeletal dysplasias, mucopolysaccharidoses, cerebral gangliosidoses, the leukodystrophies, a thalassaemia and muscular dystrophies. To prevent this, stem cells have been proposed as vehicles for fetal gene or protein delivery, because of their ability to expand and give rise to large numbers of multipotent cells. Transplantation in early intrauterine life has a number of advantages over postnatal transplantation, raising the possibility that intrauterine therapy may eventually be advantageous for genetic diseases of postnatal onset. Firstly, *in utero* therapy may be delivered in time to prevent irreversible disease manifestation (as in a thalassaemia or congenital myotonic dystrophy). Second, it obviates the severe treatment-associated morbidity associated with postnatal bone marrow transplantation. Third, the fetal environment is highly proliferative with exponential expansion of stem cell populations, and large-scale migrations of stem cells occur naturally only in fetal life. Next, there is a huge stoichiometric advantage in that the fetus is only about 75 g in size at 13 weeks gestation, allowing transplantation of many more cells than can be given postnatally. There is also some evidence that fetal cells have a competitive advantage over adult cells.¹ Finally, the immunological naiveté of the early gestational fetus produces tolerance to foreign antigens if presented before a critical window, around 12 to 14 weeks gestation.

The alternative approach is *in utero* gene therapy. For *in vivo* gene therapy, a vector containing the therapeutic gene is transferred directly to the fetus and transduces stem and other

cells *in situ*. The risk of unintentional transduction of germ cell or maternal cells to date has precluded work on human fetuses. Direct injection of gene vectors *in utero* has until recently proved disappointing in animal models, because of poor uptake of vectors in differentiated tissues, immune responses and transient transgene expression.² In contrast, *ex vivo* gene therapy uses autologous stem cells that are first obtained from the fetus, transduced *in vitro* and then transplanted back to the fetus, advantages being the selective transduction of target cells and minimisation of the immune response. Results from postnatal gene therapy trials validate the clinical effectiveness of the *ex vivo* approach.

Haemopoietic Stem Cell Transplantation

To date, intrauterine transplantation has mainly been evaluated using haemopoietic stem cells because of their well-established proliferative and multi-lineage potential and years of clinical experience in adult transplantation. Most attempts at haemopoietic stem cell transplantation *in utero* have failed, because the mid trimester fetus is immunocompetent and rejects allogeneic cells.³ Nevertheless, there are several well documented reports of successful engraftment and immune reconstitution following intrauterine transplantation of haploidentical paternal bone marrow in severe combined immunodeficiency.^{4,5} This disease, however, can instead be treated postnatally, either by allogeneic haemopoietic stem cell transplantation or by *ex vivo* gene therapy using autologous haemopoietic stem cells. Animal studies of intrauterine gene delivery using *ex vivo* transduced autologous haemopoietic stem cells have found limited transduction and sub-therapeutic expression with retroviral vectors, due to quiescence of haemopoietic stem cells and their low levels of the amphotropic receptor required for viral entry.^{3,6}

Mesenchymal Stem Cell Transplantation

Mesenchymal stem cells represent an attractive alternative for fetal therapy. Mesenchymal stem cells from adult bone marrow^{7,8} have been well characterised and are under intensive investigation for therapeutic applications in co-transplantation, muscular dystrophy, myocardial and neural injury, and tissue engineering. They divide rapidly and have high amphotropic receptor levels and are readily transducible with integrating vectors, maintaining transgene expression *in vitro* and *in vivo* without affecting multi-potentiality.^{9,10} Use of mesenchymal

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stem cells should obviate the rejection associated with intrauterine haemopoietic stem cell transplantation. Although immune recognition (MLC, T cell alloreactivity, high NK cells) seems established in humans by the late first trimester, mesenchymal stem cells downregulate certain immune responses,¹¹ which may be advantageous even for autologous use to obviate reactions against transgene products. Adult mesenchymal stem cells suppress T lymphocyte proliferation,^{12,13} and allogeneic/xenogeneic mesenchymal stem cells engraft in a wide range of organs in immunocompetent animal models.^{14,15}

The recent identification of mesenchymal stem cells in the human fetus raises the possibility not only of using a primitive mesenchymal stem cell population, but one which could be harvested for autologous use. Our group has characterised in human fetal blood a population of adherent cells with an immunophenotype comparable to adult mesenchymal stem cells, which self renew over 20 to 40 passages, and differentiate in permissive media along multiple mesenchymal and other lineages including muscle and neural tissue.¹⁶ These circulate only in the first trimester, support haemopoiesis in co-culture, and are similar to comparable populations in fetal liver and bone marrow, suggesting they are stromal cells migrating to sites of definitive haemopoiesis.¹⁶ Consistent with this, they express multiple adhesion molecules,¹⁷ and unlike other fetal cell types are found rarely in maternal blood in pregnancy, leading to the speculation that they engraft maternal organs and may be the cell type responsible for fetomaternal microchimerism.¹⁸ Transplantation of fetal mesenchymal stem cells in a xenogeneic fetal sheep model¹⁴ resulted in low level engraftment in multiple organs, with site specific tissue differentiation.¹⁹ Like adult mesenchymal stem cells, their fetal counterparts are HLA Class 2 negative and suppress mixed lymphocyte reactions.²⁰

Use of fetal mesenchymal stem cells for downstream therapeutic applications obviates some of the ethical and safety concerns (i.e malignant teratoma formation) associated with pluripotent embryonic stem cells on the one hand, while on the other reduces the disadvantage with adult mesenchymal stem cells of limited stem cell potential. Although there remain residual ethical issues with allogeneic use of fetal mesenchymal stem cells from discarded fetal tissue, these should be obviated by collection of autologous fetal blood or liver mesenchymal stem cells in continuing affected pregnancies. Given their ready transducibility (>95% using retroviral and lentiviral vectors),²¹ fetal mesenchymal stem cells may be accessible vehicles for autologous *ex vivo* fetal gene therapy. Fetal interventional techniques under high-resolution ultrasound have already developed to the extent that fetal blood sampling can be performed in ongoing pregnancies as early as 12 weeks with <5% loss rate,²² and advances in imaging and thin gauge fetoscopic cannulation should further render mesenchymal stem cell harvest and reinfusion feasible in the late first/early second trimester.^{23,24} However reports of leukaemia developing following postnatal gene therapy, presumably due to insertional

mutagenesis, currently limit the *ex vivo* gene therapy approach, although this may be able to be overcome by clonal expansion of transduced stem cells after insertional site analysis.

Co-transplantation of adult mesenchymal stem cells has been shown to enhance engraftment of haemopoietic stem cells *in utero* in animal models,²⁵ suggesting that co-transplantation of fetal mesenchymal stem cells may also result in accelerated haemopoietic engraftment following intrauterine transplantation.²⁶

Candidate Diseases

Conditions which may be appropriate for development of intrauterine fetal mesenchymal stem cell therapy include mesenchymal deficiency conditions which improve after postnatal bone marrow transplantation (which contains mesenchymal as well as haemopoietic stem cells) and for which there is a survival advantage for wild type cells. Such approaches first require testing in murine models of genetic disease to demonstrate not only engraftment and tissue-specific differentiation, but also long-term correction of the underlying histological, phenotypic and functional defect.

Duchenne muscular dystrophy is a progressive X-linked myopathy where the absence of dystrophin in muscle fibres leads to damage and regeneration cycles, eventually exhausting the muscle's innate ability to regenerate, resulting in fibrosis. Muscle wasting is apparent by 3 to 5 years with affected individuals wheelchair-bound by 12 years and dying in the third decade from respiratory failure or cardiomyopathy. There is no effective treatment and myoblast transplants have proved disappointing. Skeletal muscle repair after infusion of bone marrow-derived stem cells results in engraftment in up to 10% of muscle fibres,^{27,28} while infusion of adult synovial mesenchymal stem cells in a murine muscular dystrophy model induced synthesis of human dystrophin and decreased abnormal muscle fibres.²⁹ Long-term persistence of donor nuclei in Duchenne muscle has been documented after bone marrow transplantation.³⁰ The utility of a fetal-to-fetal approach is supported by mid-gestation intrauterine transplants in a mouse model of muscular dystrophy with unpurified fetal liver or adult bone marrow, which resulted in widespread albeit low level engraftment and myogenic differentiation.³¹

Another disease for which stem cell-based therapy has been proposed is osteogenesis imperfecta, a disorder of bone fragility and skeletal deformity characterised by defective type I collagen, the major structural protein in bone, which affects 1/10,000 births and for which there is no cure. Already, a few children with osteogenesis imperfecta have been transplanted with allogeneic bone marrow, which engrafted in the genetically defective bone, improving bone mineral content and growth velocity over 3 years follow-up.³² Subsequent therapy using adult mesenchymal stem cells without myeloablation boosted responses further, without apparent immune response in the host.³³ Anecdotally, human fetal liver mesenchymal stem cell transplants have been attempted in a couple of fetuses with

intermediate severity osteogenesis imperfecta, with low level engraftment on preliminary analysis. The development of *ex vivo* gene therapy for osteogenesis imperfecta is more problematic, as it is a dominant negative disorder with therapy needing to suppress the mutant allele in addition to ensuring expression of the wild type allele.

Following animal studies suggesting improvement in the characteristic skeletal and neurological deficit, infusion of bone marrow-derived mesenchymal stem cells in Hurler syndrome is similarly being investigated in affected children and might also eventually be applied *in utero*.³⁴ Niemann Pick disease, a lysosomal storage disorder, is another condition for which mesenchymal stem cell transplants have resulted in histological, biochemical and clinical improvement in murine models.³⁵

Conclusion

Just as gene therapy 10 years ago, stem cell therapy promises widespread application in many branches of medicine including fetal medicine. Theoretical ontological advantages of intrauterine transplantation include increased engraftment and avoidance of immune rejection, as well as treatment before disease morbidity. Human fetal mesenchymal stem cells may have therapeutic application *in utero*, either allogeneic, or as autologous donors of therapeutic genes in inherited deficiency diseases, such as muscular dystrophy, skeletal dysplasias, and inborn errors of metabolism. Understanding stem cell fate after intrauterine transplantation is a necessary prerequisite to further development of this approach in mouse models of debilitating human disease.

Acknowledgement

Dr Chan is supported by the Institute of Obstetrics and Gynaecology Trust. We acknowledge grant funding from Wellbeing, Action Research, and the Hammersmith Hospitals Special Trustees.

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