Abstract

Duchenne muscular dystrophy (DMD) is a lethal muscle disease for which an effective treatment is urgently needed. The use of stem cells to produce normal muscle cells to replace the missing dystrophin protein has attracted much attention. Claims of success using stem cell treatment in animal models of human muscle diseases require careful evaluation and are not necessarily easily extrapolated to the clinical situation. Recent studies in the dystrophic dog model have been claimed to show that injected mesangioblasts, stem cells derived from blood vessels, reduce the severity of the disease. However, the authors’ interpretation of the results did not consider that benefits might arise from the concomitant use of immunosuppressive drugs alone.

Keywords: Muscular dystrophy; Stem cell therapy; Mesangioblasts; Skeletal muscle; Immunosuppressive drugs

1. DMD and diverse approaches to therapy

The severe X-linked disease Duchenne muscular dystrophy (DMD) is manifested in young boys and results in progressive muscle weakness with loss of ambulation by 10–12 years of age and ultimate death from respiratory or cardiac failure usually around 20 to 30 years of age. DMD results from defects in the gene coding for the cytoskeletal protein dystrophin, a subsarcolemmal protein that normally stabilises the transmembrane dystrophin–glycoprotein complex and is required for muscle membrane integrity in response to exercise-induced muscle contraction [1,2]. Severely defective dystrophin protein leads to sarcolemmal fragility that results in necrosis of the muscle fibres. In boys with DMD, repeated cycles of membrane damage and muscle necrosis ultimately lead to replacement of myofibres by fibrous connective tissue and fat. This also occurs in the dystrophic golden retriever dog model of DMD, whereas the widely used mdx mouse model of DMD (that shares a similar gene defect) has a far less severe clinical phenotype and pathology. Many different strategies have been employed to replace the defective dystrophin gene/protein such as genetic correction of the defect by viral delivery of replacement mini-dystrophin genes [2]. Other promising approaches are to skip the mutation, to upregulate other proteins that may compensate for the defective dystrophin, or to use drugs or nutritional interventions to modulate the severity of the disease [1–3].

Alternative strategies focus on replacing the dystrophic muscle nuclei with normal nuclei derived from either myoblasts or stem cells. Myoblast Transfer Therapy (MTT) takes advantage of the fact that skeletal muscle is multinucleated and is formed by fusion of mononucleated precursor cells (myoblasts) and MTT relies on the delivery of normal nuclei into the dystrophic muscle fibres by biological fusion as routinely occurs during muscle repair. Quantitation of survival of the cultured donor myoblasts after intramuscular injection in mdx mice by several laboratories shows extensive and rapid cell death, with often 80% of donor myoblasts dying within days [4]. Incorporation of
surviving donor myoblasts into mdx myofibres can be increased by exercise or other agents that further damage the host mdx myofibres [5]. Extrapolation of studies in (small) mice to clinical treatment of (large) humans can be very difficult. Human trials of MTT started in the 1990s and initially had little success although perseverance has produced slight improvements based, in part, on conditions developed from experiments in monkeys [6]. These combined studies identify the key issues to address as: finding a superior source of donor myoblasts with a strong capacity to replicate, increasing the survival of donor cells after intramuscular or systemic delivery, enhancing dispersal and expansion of these normal donor cells and effective fusion with the dystrophic muscle cells. For all of these reasons, over the last 7 years attention has turned to stem cells as an alternative promising source of normal donor myoblasts for more effective MTT.

2. Stem cells to replace dystrophin

The two critical properties of a stem cell are self-renewal combined with the capacity to turn into various lineages of cells. Thus huge numbers of cells can be generated from the original cell and these progeny can be converted into the desired lineage. In the case of DMD the stem cell should produce large numbers of skeletal muscle precursor cells (myoblasts). There has been a tremendous interest in stem cells, extracted from many different sources ranging from embryonic to adult tissues, as potential therapeutic tools for many diseases, including neuromuscular disorders. Different types of stem cells have been explored for the treatment of DMD and the ideal scenario of a bone-marrow derived stem cell that circulates to reach all muscles has unfortunately proved disappointing and clinically irrelevant to date [7]. Claims of therapeutic breakthroughs that distort the realistic clinical application of scientific advances cause much concern to the scientific and wider community and have been the subject of several editorials [8,9]: this is especially disconcerting to parent groups and families of affected DMD boys who have such a vital interest in the outcome of scientific experiments and potential treatment.

Blood vessels have recently been identified as a promising source of novel stem cells called mesangioblasts. These cells were initially extracted from embryonic dorsal aorta of mouse embryos aged E9.5 days and claimed to have a striking capacity to form muscle and repopulate diseased skeletal muscles in mice lacking β-sarcoglycan (part of the dystrophin–glycoprotein membrane complex) [10]. These experiments were recently extended to dystrophic golden retriever dogs that more closely resemble the severe condition of DMD [11]. In this case, the mesangioblasts were instead extracted from vessels in skeletal muscles of young dogs and the cells injected systemically into the femoral artery of one leg of dystrophic dogs aged 75–159 days. In some cases autologous cells were genetically corrected before injection back into the same dog. In other cases normal heterologous cells were injected into dystrophic dogs that were immunosuppressed (with either cyclosporine, rapamycin or rapamycin with IL-10) to prevent immune rejection of the foreign donor cells. Dogs were also treated with steroids although the dosage and regime are not stated and the duration is unclear. It is noted that in comparison with the more extensively studied mesangioblasts from mice, the dog [11] and human [12] mesangioblasts have a finite life span.

It was concluded that injected mesangioblasts, especially heterologous stem cells, improved the health of the dystrophic dogs. These important observations have generated much expectation about the possibilities of these new stem cells. However several issues require clarification as discussed in detail elsewhere [13]. In particular, in two of the four cases analysed in detail by Cossu and colleagues there was a poor correlation between the extent of dystrophin restoration and the health of individual dogs [11]. This is evident for one of the healthiest dogs (Varus, injected with heterologous wild type cells) yet with no striking difference in numbers of dystrophin-positive fibres between injected and un.injected contralateral muscles, whereas another dog (Vampire, injected with autologous cells) had a major decline in health but more dystrophin-positive myofibres in all three injected muscles especially the gastrocnemius. It is recognized that the wide biological variation that occurs between individual dogs (although all carry the same gene mutation), presents difficulties and complicates experimental analysis. Thus detailed dystrophin analysis of muscles from the remaining five out of nine test dogs and controls would be very helpful to further evaluate the key proposal that dystrophin replacement (by mesangioblast engraftment) improves the health of treated dogs.

3. Benefits of immunosuppressive drugs

Some basic controls were unfortunately absent from this study. Treatment of control dogs with the same immunosuppressive drug combinations but without stem cell injections (or ideally with injection of a control cell) would test for any effects of drug treatments per se. This is critically important since it is well documented that cyclosporine and other anti-inflammatory drugs can reduce the severity of muscular dystrophy (discussed in [13,3]). The benefits of cyclosporine are exemplified by a clinical MTT trial which concluded that cyclosporine alone accounted for the significant long-term improvement in DMD boys [14], plus strong evidence from experiments in mdx mice [15] and are further endorsed by recent clinical trials being conducted in DMD boys.
with combined steroid and cyclosporine treatment [16]. The authors do not mention any of this literature and merely state that 'the differential treatment with cyclosporine should not matter because the drug itself had no effect on dogs transplanted with wild-type bone marrow' [11]. The possibility that the combination of immunosuppressive drugs and steroids has superior benefits to steroids only on dystrophopathy and that such drug treatment alone might present an alternative to the complications of mesangioblast transplantation into dystrophic muscle plus immunosuppression and steroids [11], requires rigorous evaluation.

The lack of reference by the authors [11] to the well-documented benefits of a range of anti-inflammatory drugs on muscular dystrophy [3] is especially surprising considering a recent paper published by the same group that specifically tested a non-steroidal anti-inflammatory drug (HC 1026), or prednisolone, in combination with mesangioblast stem cells [17]. Here they show that long-term drug treatment with HC 1026 or prednisolone significantly reduced the severity of the dystrophopathy in two mouse models of muscular dystrophy, they claim that HC 1026 improves engraftment of mesangioblasts to the muscles of α-sarcoglycan null mice (prednisolone was not tested in this context), and they state that 'these results open a window for an effective cure for muscular dystrophy'. Unfortunately, HC 1026 treatment alone was not compared directly with this drug + mesangioblasts in respect to overall effects on muscle physiology, serum creatine kinase levels or exercise performance. While data for HC 1026 or prednisolone alone are presented and suggest some equivalent benefits to those seen for the HC 1026 + stem cell treatment, a direct comparison is difficult due to differences in the duration of treatments and ages of the mice between the two sets of data [17]. Thus what remains unclear is the extent to which mesangioblasts per se have a significant impact on dystrophic muscle function, compared with drug treatment alone.

The possibility of using stem cells to treat neuromuscular diseases such as DMD has generated intense interest. Mesangioblasts offer a novel source of stem cells that can be delivered through blood, with the potential to generate many myoblasts to replace the defective genes in DMD and other myopathies. Before clinical trials are seriously considered, however, there is an urgent need for carefully conducted clinical trials in the dystrophic dog and mouse models, with adequate controls.

References