Commentary

COMMENTARY ON THE PRESENT STATE OF KNOWLEDGE FOR MYOBLAST TRANSFER THERAPY

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HISTORY OF THE CLINICAL TRIALS

Five major clinical trials of Myoblast Transfer Therapy (MTT) have recently been carried out in North America and Italy as a potential therapy for boys with the lethal X-linked myopathy, Duchenne Muscular Dystrophy (DMD). This cell therapy is based on the knowledge that (i) DMD is caused by a gene defect resulting in a lack of the subsarcolemmal protein, dystrophin and that (ii) skeletal muscles are multinucleated cells which are formed by the fusion of mononucleated precursor cells (myoblasts). MTT aims to replace the missing protein dystrophin by the introduction of normal myoblasts into dystrophic muscle (1). To date the approach has been to increase the number of normal cells by growing them in tissue culture before injection into the dystrophic host muscles.

The decision by clinicians to proceed with clinical trials arose from a meeting on Myoblast Transfer Therapy organised by the Muscular Dystrophy Association of America in New York in June 1989 (2). Most of the research scientists present at this meeting strongly opposed the decision as they felt that human trials were premature since so few animal studies had been carried out.

The clinical trials generated much controversy but the consistent outcome was that they were unsuccessful in terms of both muscle strength and effective replacement of dystrophin (3–8). The controversy unfortunately continues with testimonials from the Cell Therapy Research Foundation in Memphis claiming improved muscle function resulting from MTT despite the lack of any scientific evidence to substantiate this. This apparent conflict produces a great dilemma for the parents of affected boys. The formal clinical trials represented a very high cost in terms of the finding resources and time which were diverted away from basic research, and in emotional terms for the families involved. Although there was essentially no success with these MTT trials, no deleterious effects on the affected boys were reported; however, it should be noted that “bystander” damage to host muscles has been reported after MTT in animals (9). One central problem with these clinical trials is that little information is available regarding the fate of the injected donor cells as muscle biopsies sample only a portion of the entire muscle and it is neither ethical nor feasible to more closely monitor such cells in the severely compromised muscles of the dystrophic boys.

ANIMAL STUDIES

The fate of similarly injected donor cells can, fortunately, be readily monitored in animal models of DMD. There is a mouse and a dog model which are both genetically and biochemically homologous to DMD, as the same gene is defective and no dystrophin is present in all cases (10). The most widely used model is the mdx mouse although it lacks the severe pathology of DMD as it seems to have an excellent capacity to regenerate after repeated cycles of necrosis. The dog model has a much closer pathology to DMD. It would seem appropriate to first confirm the success of MTT in both of these animal models before extending this treatment to the clinical situation but, unfortunately, this was never done.

Most of the animal experiments of MTT, to date, have been carried out in mouse (or rat) models. An assessment of the literature clearly shows that MTT has been demonstrated successfully in these animal models largely under conditions which are quite unlike those used in the clinical trials of MTT. Examination of over 30 research papers on this subject, reveals that the vast majority of animal studies do not show survival and fusion of cultured donor myoblasts with muscles of “untreated”
dystrophic host mice. Instead, a range of experimental variations were employed which have no equivalence to the conditions used in the clinical trials of MTT. In 13 of these studies, myoblasts were injected into “nude” or SCID host mice, yet these animals are immunocompromised (i.e. their immune system does not reject “foreign” tissues) unlike “normal” mice or the human condition. Indeed, the seminal 1989 Nature paper of Partridge et al. (11), clearly demonstrated superior MTT in nude mdx host mice as compared with “normal” mdx hosts (which had been made tolerant to donor cells at birth). In 9 papers, the host muscles were pre-irradiated to impair proliferation of host myoblasts and thereby enhance the contribution of donor myoblasts to new muscle formation; a side effect is that myoblast proliferation appears to be greatly enhanced in irradiated muscle and myoblast movement may also be increased. In 11 papers, myogenic cell lines such as C2C12 cells were injected instead of primary myoblasts. Since these transformed myogenic cells readily move throughout the host muscles and have been shown to form tumors in vivo, in marked contrasts with primary myoblasts, there are clearly important differences between these two types of donor cells. While these interesting papers show what is possible under certain “optimal” experimental conditions, it is essential to recognise that they do not address why the clinical trials failed. Only when MTT is studied under conditions which genuinely approach the clinical situation can the problems be accurately analysed and strategies developed to enhance the success of such potential therapy.

Only about half a dozen studies, most published very recently, have used experimental conditions which are equivalent to the clinical situation. The importance of using appropriate conditions for the MTT studies has been recognised by the laboratory of Jacques Tremblay and his group of Quebec. They have demonstrated successful MTT where immunosuppressive drugs have been given to the host animals (12). This procedure does have parallels with the clinical situation where such intervention is possible and, indeed, immunosuppression was used in some of the unsuccessful clinical trials, including the case where 10% of donor dystrophin was detected in muscle biopsied from one boy (6).

STRATEGIES TO ENHANCE MTT

Available information from the animal experiments indicates that the cultured donor myoblasts perish from about 48 hours after injection and few remain by one week. The reasons for this massive and rapid cell death are not known. Some role of the host immune system is implied by the finding that donor cell survival is significantly improved in nude or SCID host mice. Having identified this major problem, strategies are clearly required to dramatically enhance the survival of the injected cultured myoblasts in “normal” dystrophic hosts. One approach may be the application of growth factors or extracellular matrix components to the cultured cells, as a 4 fold increase in survival after injection has been demonstrated with myoblasts grown in the presence of basic fibroblast growth factor (13). In marked contrast to the poor survival of isolated cultured myoblasts after injection, excellent long-term survival of donor myoblasts (up to a year) has been demonstrated where intact or sliced donor muscle grafts were implanted into “untreated” dystrophic muscles (14). Extensive movement of these donor myoblasts and fusion with the host muscles was also seen with grafts of sliced muscle. This alternative grafting strategy appears to readily overcome the problem of donor cell survival and provides a potential long-term “sink” of normal donor myoblasts within the dystrophic muscles. The challenge in this situation is to optimise the conditions which will enhance the movement and proliferation of the donor myoblasts, their fusion with the dystrophic host muscles and the long-term survival of such mosaic myofibres.

The band-wagon appears to have passed by Myoblast Transfer Therapy as a potential treatment for myopathies such as DMD due largely to the premature nature and apparent failure of the clinical trials. Yet, this strategy for cell therapy for skeletal myopathies still has great appeal. The appropriate animal experiments have only recently been undertaken and it would seem that such cell therapy for skeletal muscle may only now start to be seriously addressed. The identification of where the major problems lie in MTT provides the opportunity for numerous new approaches to such therapy and raises many fundamental and challenging questions about the biology of skeletal muscle cells. It is hoped that these new developments will rekindle interest and stimulate much research on MTT.

REFERENCES


