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A review of Duchenne muscular dystrophy focusing on cardiac involvement

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Abstract

Duchenne muscular dystrophy (DMD) is a lethal x-linked recessive disorder, characterised by progressive skeletal muscle weakness, accompanied by cardiac and smooth muscle dysfunction. Although the main cause of death in DMD used to be respiratory problems caused by deterioration of the diaphragm, developments in respiratory care means that cardiac failure is now the major cause of death, despite the introduction of cardio-protective treatment such as angiotensin-converting-enzyme inhibitors and beta-blockers. Once affected, the heart progressively deteriorates over time, with the main problem being due to the death of cardiomyocytes. A loss of dystrophin makes the sarcolemma more susceptible to damage, leading to an influx of calcium ions into the cell, activating proteases and ultimately resulting in cardiomyocyte death. The dead cells are replaced by fibrotic tissue which causes dilated cardiomyopathy. There is at present no cure for DMD, however there is much on-going research producing positive results based around the pathophysiology in DMD patients.

Key words: Cardiomyopathy, Duchenne muscular dystrophy, Dystrophin

Introduction

Muscular dystrophy is a group of inherited disorders that involve muscle weakness and the loss of muscle tissue through cycles of degeneration/regeneration, fibrosis and replacement of muscle with fatty tissue. Duchenne muscular dystrophy (DMD) and its milder variant Becker muscular dystrophy (BMD) result from mutations in the gene that encodes the 427-kDa cytoskeletal protein dystrophin (Johnson, 2002). Dystrophin is a rod-shaped protein responsible for connecting the extracellular matrix to the cytoskeleton of each muscle fibre via the dystrophin-associated complex (DGC), a protein complex at the muscle cell membrane (sarcolemma). Absence of dystrophin is associated with the loss of the DGC and structural weakness of the sarcolemma during contractile activity (Blake et al, 2002). DMD is a progressive and severe X-linked recessive muscle-wasting disease, with an incidence of 1 in every 3500 male births (Beaudet et al, 2008). DMD is the most common muscular dystrophy in children, presenting in early childhood and characterised by proximal muscle weakness and calf hypertrophy in affected boys. Patients are usually confined to a wheelchair by the age of 12, with death occurring in their second or third decade. Most DMD mutations result in a complete lack of dystrophin, whereas mutations resulting in BMD tend to produce a functional, but truncated version of the dystrophin protein (Bushby et al, 2010). The incidence of BMD is less than DMD being 1 in 180 000 (Betancur et al, 2011). BMD is a milder form of the disease and has a later onset; patients can usually walk and have a much longer life span, although cardiac complications are very common in later life.

Dystrophin

The dystrophin gene is the largest described, spanning 2.5 megabases of the genomic sequence, accounting for 0.1% of the total human genome and is composed of 79 exons (Blake et al, 2002). Dystrophin is a rod-shaped protein, responsible for connecting the extracellular matrix to the cytoskeleton of each muscle fibre via a large protein complex containing many subunits (Johnson, 2002). Mutations in the dystrophin gene lead to a reduction in the stability of the cell membrane. Dystrophin is a cytosolic adapter protein, binding actin filaments to dystroglycan, an adhesion receptor (Berk et al, 2008). It is a member of the β-spectrin/α-actinin protein family, characterised by an NH_2-terminal actin binding domain followed by a variable number of repeating units. Dystrophin can be organised into four separate regions based on sequence homologies and protein binding capabilities. These are the actin-binding domain at the NH_2 terminus, the central rod domain, the cysteine-rich domain and the COOH-terminal domain (Rando, 2001). The role of dystrophin is to link the cytoskeleton to the sarcolemma (Campbell, 1995). This link extends to the extracellular matrix via beta-dystroglycan interacting with alpha-dystroglycan, which binds extracellular matrix proteins including laminin alpha2, and proteoglycans: agrin and perlecan that possess laminin globular (LG) domains. The absence of dystrophin disrupts this link leading to compromised muscle sarcolemmal integrity (Bansal et al, 2011). The transmembrane segment of the dystroglycan β subunit associates with a complex of integral membrane proteins; its cytosolic domain binds dystrophin and other adapter proteins, as well as various intracellular signalling proteins. The resulting large, hererometric assemblage, the dystrophin glycoprotein complex (DGC) links the extracellular matrix to the cytoskeleton and signalling pathways within muscle and other types of cell (Campbell et al, 2000). Mutations in dystrophin can disrupt the

DGC-mediated link between the exterior and the interior of muscle cells, leading to muscular dystrophy.

Cardiac involvement

Cardiac failure is the major cause of death in DMD despite the introduction of cardio-protective treatment such as angiotensin-converting-enzymes (ACE) inhibitors and beta-blockers (Ameen and Robson, 2010). DMD patients develop hypertrophic and dilated cardiomyopathy, which ultimately results in cardiac death in an increasing number of cases. Although present treatment options and frequent monitoring have led to vast improvements in the quality of life of DMD patients, their longer lifespan indicates that the cardiomyopathy that develops needs to be addressed. Signs of cardiac involvement have been found in 90% of DMD patients via the use of echo and electrocardiogram (ECG), with ECG abnormalities being detectable from as early as six years of age (Annane et al, 2010). Clinically apparent cardiomyopathy is first evident after 10 years of age, affects one-third of patients by the age of 14 years and is present in all patients over 18 years of age (Kornberg and Yiu, 2008). Preclinical cardiac involvement is seen in 25% of patients less than six years of age (Bain et al, 1990). Atrial and ventricular arrhythmias occur, including premature ventricular beats and more complex or sustained ventricular ectopy, which increases with age and ventricular dysfunction. Despite the high frequency of cardiac involvement, most patients are fairly asymptomatic due to physical inactivity (Cox and Kenkel, 1990). The occurrence of BMD patients in developing heart problems due to dilated cardiomyopathy is 70%, with heart failure being the primary cause of their death (Allen et al, 2009a; Finsterer and Stollberger, 2008). ECG and echo changes have also been found in up to 90% of DMD and BMD carriers, with 7-11% developing dilated cardiomyopathy. This represents a major group of dystrophic patients for whom the heart is the major site of pathology.

As with skeletal muscle, the absence of dystrophin results in the sarcolemma of cardiomyocytes becoming more susceptible to damage from muscle contractions. Allen and Williams (2007) found that stretch-activated ion channels do not open appropriately in dystrophin deficient cardiomyocytes when they are stretched during ventricular filling; this results in an increase in the influx of calcium into the cell. The tears in the membrane allow the entry of extracellular calcium, raising intracellular calcium levels. This leads to the activation of calcium-activated proteases, such as calcium induced Calpains, which destroy the plasma membranes, allowing even more calcium into the cell. Eventually the chronic calcium overload will result in the death of the cardiomyocyte.

Cardiomyocyte death usually occurs in very discrete small areas of the heart known as microinfarcts (Lannaccone et al, 2003). Within these areas, cardiomyocyte death initiates an inflammatory cascade. Macrophages migrate into the heart to remove the damaged and dead cells. Fibroblasts subsequently invade the area and form fibrocollagenous scar tissue resulting in the deposition of fibrotic tissue in the walls of the heart, replacing the contractile cardiomyocyte (Allen et al, 2009b). As the fibrosis spreads, the ventricle thins and enlarges resulting in dilated cardiomyopathy in DMD and BMD patients.

Present treatment for DMD cardiomyopathy

The standard therapy for DMD includes the use of glucocorticoids from the age of five or six. In addition present treatments using β adrenergic blockage and ACE inhibitors have proven to be effective at remodelling the heart (Bourke et al, 2003). ACE inhibitors block the conversion of angiotensin I to angiotensin II, which prevents the deposition of fibrotic material in the walls of the heart (Lawer et al, 1997). An uncontrolled retrospective study indicated that echocardiographic parameters, including fractional shortening, left ventricular ejection fraction and sphericity index in DMD and BMD patients improved three years after administration of ACE inhibitors alone and in combination with β-blockers (Belmont et al, 2005). A double-blind trial was conducted at the same time to assess the effect of preventative afterload reduction in muscular dystrophy patients. DMD patients between the ages of 9.5 and 13 years with normal ventricular function were randomly chosen to receive three years of ACE inhibitor perindopril or a placebo. Following the three years each participant received perindopril for two years. A lower left ventricular fraction was found in the patients who did not receive perindopril for the first years (Bécane et al, 2005). A later study by Coursey et al (2006) tested another ACE inhibitor called enalapril. Results showed that normalisation of fractional shortening occurred in 43% of patients taking enalapril; this improvement was maintained for up to four years. These two studies suggest that early pharmacological interventions are required and should be encouraged before cardiac symptoms are apparent in order to reserve cardiac function.

New treatment options for muscular dystrophies and the heart

There is at present no cure for either DMD or BMD; however there is a lot of on-going research into these diseases. In the 1960s and 1970s there were few survivors of DMD beyond teenage years, but with developments in standards of patient care and disease management, lifespan has gradually extended (Baudouin et al, 2002). Pharmacological intervention has begun to change the natural history of DMD, with further advances and more effective treatment to target the underlying pathology of DMD.

Corticosteroids

The corticosteroids, deflazacort and prednisone, are currently the only evidence-based effective treatments for DMD (Ashwal et al, 2005; Kuntzer et al, 2008). The exact cellular mechanisms responsible for corticosteroids' beneficial effect are still unknown (Berket et al, 2010; Brooke et al, 2005). Despite this lack of knowledge of their action corticosteroids have been shown to slow the rate of disease progression and also extend functional abilities for two years or longer (Altman et al, 2012; Armaroli et al, 2012; Ashwal et al, 2005; Kuntzer et al, 2008). Improved pulmonary function, time to rise from supine to standing, time to climb four stairs and time to walk nine metres are all outcomes documenting the beneficial action of corticosteroids. Brooke et al (2001) found that glucocorticoid corticosteroids improve muscle strength and function in children with DMD treated from six months to two years as shown in randomized controlled clinical trials. Currently the most effective doses appear to be prednisone 0.75 mg/kg/day and deflazacort 0.9mg/Kg which appear to be equally effective in improving muscle strength and function in the short term (Ashwal et al, 2005). In nonrandomised trials, boys who were treated longer than two years with either prednisone or deflazacort showed significant benefits in

Table 1: Summary of current therapies for DMD

Intervention	Action/Effect
Stem cell therapy	Introduce dystrophin producing cells (Deutekom and Ommen, 2003).
Myoblast transplantation	Introduce dystrophin producing cells (Deutekom and Ommen, 2003).
Corticosteroids	Slows progression of muscle weakness, exact mechanism in DMD is unknown (Moxley et al, 2012).
Myostatin inhibition	Inhibit myostatin; a growth differentiation factor, that inhibits muscle differentiation and growth (Moxley et al, 2012).
Utrophin upregulation	Replacement of dystrophin (Deutekom and Ommen, 2003).
Phosophodiesterase inhibitors	Inhibit the inactivation of nitric oxide from the cGMP pathway to prevent contraction coupling damage (Moxley et al, 2012).
Stop codon reading through	Ribosomal read through of stop codons in mRNA to produce a full length protein. Ataluren and Gentamicin are examples (Lee et al, 2012).
Exon Skipping	Removes specific exons from pre-mRNA to give a restored reading frame to produce a less severe phenotype, similar to BMD (Anthony et al, 2012).
Gene therapy	Introduce mini-dystrophin into cells via a vector (adenoviral vectors) to replace depleted dystrophin (Duan and Zhang, 2012)
Nutritional	E.g. the addition of gelatine, creatine and monohydrate may increase muscle protein synthesis by increasing ATP levels (Moxley et al, 2012).

ambulation, delayed onset of scoliosis and pulmonary dysfunction, preserved cardiac function, survival and quality of life compared to untreated counterparts (Balaban et al, 2005; Altman et al, 2006; Hoyle et al, 2006). No clinically severe side effects have been observed in the short term studies performed with the use of corticosteroids, however, boys treated with corticosteroids for a mean time of 5.5 years were significantly shorter and had delayed puberty. Patients' major concern with early corticosteroid treatment is with the effect on growth rate; however most feel that the benefits of the drug outweigh the risks (Altman et al, 2012; Armaroli et al, 2012). Side effects involving increased frequency of long bone fractures and vertebral fractures should be considered, although there is a lack of knowledge on how likely and severe these side effects are and what interventions are best to prevent or manage them (Armaroli et al, 2012). Despite the progress described above, further randomised trials are required to decide the optimal dose of steroids, the age to start taking corticosteroids and the prime dosing schedule to improve function with least side effects. In addition, long-term control studies will also be necessary to determine the long-term effects of corticosteroids on ambulation, bone, cardiac and respiratory function, behavioural problems and on patients' quality of life. Uncontrolled studies of cohorts of DMD patients receiving prednisone or deflazacort for five years and longer have provided some encouraging results. Furthermore, Robert (2012) is performing

a large multicentre clinical trial to address the question of the optimal corticosteroid regimen for DMD and is recruiting patients this year.

Stop codon read-through: gentamicin and ataluren

Up to 20% of DMD patients have a premature stop codon (Ahn and Kunkel, 1993; Moxley et al, 2012). Aminoglycosides interfere with stop codons by introducing a nucleotide sequence at the aminoacyl transfer RNA acceptor site, allowing translation of mRNA into a protein of full length (Anderson et al, 1965; Davies et al, 1964). Barton-Davis et al (1999) demonstrated that daily injections of gentamicin resulted in the production of a full-length dystrophin protein and a decrease in serum creatine levels. However trials in 2001 and 2003 showed less positive, varying results with no dystrophin increase in the first trial and very little in the second (Burstein et al, 2001; Comi et al, 2003). Ataluren (PTC-124) is an oral nonaminoglyoside nonsense mutation suppressor, currently being used in clinical trials for both DMD and cystic fibrosis (Lee et al, 2012). In vitro, Ataluren is thought to be superior to gentamicin in terms of bioavailability (Almstead et al, 2007). Phase I and phase II trials have demonstrated an encouraging safety profile. However, the primary endpoint; a six minute walk test showed improvement, but not of statistical significance, so the study was completed early (Atkinson, 2011). Refinements have been made in the design of the study and current studies are recruiting 110 patients for a nine month trial, involving three daily doses (10, 10 and 20 mg/kg) for up to 36 weeks (Barth, 2011), to establish Ataluren's long-term safety.

Exon skipping

Antisense oligonucleotides (AOs) targeted to splicing elements within DMD pre-mRNA can induce the skipping of targeted exons, restoring the open reading frame and the consequent production of a shorter but functional dystrophin protein. This results in a phenotype similar to BMD (Moxley et al, 2012). This treatment could be potentially beneficial to approximately 72% of DMD patients (Aartsma-Rus et al, 2003). AOs are 20-30 nucleotides long and are complimentary in sequence to regions of the pre-mRNA transcript; they bind to specific sites on pre-mRNA to promote specific exon exclusion from the mature-mRNA. They can be delivered systemically or directly injected into muscle. Two AOs (Drisapersen and Eteplirsen) currently show promise for targeting exon 51. Furthermore multiple intravenous AO trials in preclinical studies using canines resulted in functional improvement (Hoffman et al, 2009). In 2007, four patients received an intramuscular dose of Drisapersen (Aartsma-Rus et al, 2007). Their muscle biopsies showed increased sarcolemma dystrophin expression, however functional improvement was not observed. Phase I or phase IIa studies were then carried out in which twelve patients received five weeks of subcutaneously injections. Results showed dystrophin expression in all twelve of the patients and ten out of the twelve patients demonstrated functional improvement in the 6 minute walk test. Although no serious adverse effects were documented, all treated patients developed proteinuria (Aartsma-Rus et al, 2011). A current phase III randomised, double blind and placebo-controlled clinical study aims to assess the safety and efficacy of Drisapersen in DMD patients (GlaxoSmithKline, 2011). Eteplirsen, also an AO that targets exon 5, is showing promising results. A study involving seven DMD patients receiving injections of Eteplirsen into the exterior digitorum brevis muscle (two patients received a dose of 0.09 mg, the other five 0.9 mg) produced a 44-79% increase in dystrophin positive fibres in the patients biopsies who received a dose of

0.9 mg (Anthony et al, 2012). A Phase II, open label, dose escalation study with systemic Eteplirsen showed that 7 of 19 patients had slight improvement with positive staining of sarcolemmal dystrophin, ranging from 8.9 to 16.4% (Abbs et al, 2011). There was a dose-response effect and no side effects related to the drug.

Gene therapy: Viral vectors

The dystrophin gene consists of over 2.4M base pairs and is the largest human gene (Bertelson et al, 1987). Due to its large size, it is not possible to fit the entire gene into an adeno-associated virus, which is currently the best vehicle for gene transfer. The capacity for packing of the adeno-associated virus is 4.7 kb (Bourque et al, 2002). Smaller versions of dystrophin (minidystrophin) have been developed to solve the problem of fitting the gene into the virus (Bourque et al, 2002; Bowles et al, 2010; Duan and Zhang, 2012). Studies have demonstrated that following their transfer there is protection of the plasma membrane of myofibers in adult *mdx* muscle (Li et al, 2000). The first clinical gene therapy trial in a study of six boys with frame-shift mutations was reported by Bowles et al (2010). A mini-dystrophin gene was delivered in an adeno-associated virus into one of the biceps muscle while the opposite bicep received saline. Muscle biopsy specimens were assessed on day 42, for four patients and day 90, for two patients. Vector DNA was detected in all patients, however functional protein was not visualised. Future studies are ongoing which include plans for systemic and regional intra-arterial delivery of a mini-dystrophin gene contained within a vector. Immune reaction to viral vector transfer however still remains an important problem that needs addressing.

P2X7 Receptor

The lack of functional dystrophin in muscular dystrophy patients leads to raised levels of intracellular free calcium in the sub-sarcolemmal region of dystrophic muscle. Arkle et al (2006) have shown that dystrophin appears to have a function in controlling purinergic responses. In dystrophic (*mdx*) mouse myoblasts, a purinergic phenotype arises in which exposure to extracellular ATP triggers an increase in cytosolic Ca^{2+} which is primarily caused by activation of P2X7 receptors. P2X7 receptors are a family of ionotrophic ATP-gated ion channels which are permeable to small cations including Ca^{2+}. ATP and other inflammatory mediators have been shown to up-regulate the expression and function of P2X7 receptors and they are now considered as a ‘danger’ sensor, detecting ATP where tissue damage occurs (Khakh and North, 2006). At an intracellular level the ATP content of muscles is high and ATP is released in small amounts in response to physiological muscle activity, acting through purinergic receptors to modulate skeletal muscle plasticity (Almarza et al, 2009). In muscular dystrophy patients, the fragility of myofibres results in large amounts of ATP being released into the extracellular space. It is possible that these high concentrations of ATP acting on the P2X7 receptor could contribute to DMD and BMD pathology by chronically increasing intracellular Ca^{2+} levels. Arkle et al (2012) have shown that P2X7 receptor expression and function are significantly altered in mouse dystrophic myoblasts and myotubes *in vitro* and *ex vivo* and also in *mdx* muscle *in vivo*. Pharmacological inhibition of this receptor in *mdx* mice *in vivo* resulted in a significantly lower number of relevant fibres in skeletal muscle, where such suppression indicates a reduced number of degeneration-regeneration cycles in treated animals (Davies et al, 2006). This suggests that treatment with P2X7 antagonists may hinder the dystrophic process.

P2X7 Receptor antagonists

P2X7 receptor antagonists that have been identified in recent years include Brilliant Blue G (BBG), KN-62, PPADS and oATP (Donnell-Roberts and Jarvis, 2007). A feature shared between these antagonists is their differential affinity for human versus rodent P2X7 receptors (Chambers et al, 2007). KN-62 for example, potently blocks human P2X7 receptors, but is ineffective at the rat receptor (Humpreys et al, 1998). Other antagonists such as oATP and PPADS show only weak affinity for blocking P2X7 receptors and have been found to be non-selective (Donnelly-Roberts and Jarvis, 2007). The inability of these antagonists to block rodent P2X7 receptors presents problems when determining the role of P2X7 modulation in experimental models. More recent, structurally distinct and competitive P2X7 receptor antagonists (A-740003 and A-438079) have been described, that show less species differences between humans and rats (Carroll et al, 2003). An even more recent study investigated a structurally novel, competitive P2X7 receptor antagonist, A-804598 (Carroll et al, 2009).

Conclusion

In conclusion present treatment options and frequent monitoring have led to vast improvements in the quality of life of DMD patients. The longer lifespan however, has left DMD patients exposed to increased risk of cardiac problems. The cardiomyopathy that develops is a major health issue that needs to be addressed. The traditional view that medication should be started only when symptoms have become evident is being challenged. Treatments are now being initiated before symptoms are seen to help protect from heart damage. As the number of boys with DMD are surviving longer and developing cardiomyopathy, new treatment regimens are being examined to help treat cardiomyopathy and cure DMD altogether.

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