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PARASITOLOGY

Relevant aspects of golden retriever muscular dystrophy for the study of Duchenne muscular dystrophy in humans

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ABSTRACT: *Golden Retriever muscular dystrophy (GRMD) is the most representative model for studying Duchenne muscular dystrophy (DMD) in humans, owing its phenotypic expression. DMD is a recessive disorder linked to the X chromosome in which the loss of dystrophin induces progressive weakness and degeneration of the skeletal and cardiac muscles, which lead to replacement by connective and adipose tissues. Onset of clinical signs occurs between 2 and 5 years of age, and many patients die from heart or respiratory failure. The main studies concerning dystrophic Golden Retrievers (DGR) sought to elucidate the pathophysiology of the disease and its clinical implications to develop therapies and alternative treatments to improve the quality of life and increase longevity of DMD patients. This review presents an overview of relevant contributions of the DGR model for elucidating DMD in humans.*
Key words: *Duchenne muscular dystrophy, animal model, dystrophic Golden Retriever.*

Aspectos relevantes da distrofia muscular do Golden Retriever para o estudo da distrofia muscular de Duchenne em humanos

RESUMO: *A distrofia muscular do Golden Retriever (DMGR) é o modelo mais representativo para o estudo da distrofia muscular de Duchenne (DMD) em humanos devido a sua expressão fenotípica. A DMD é uma desordem genética recessiva ligada ao cromossomo X onde a perda da distrofina induz fraqueza progressiva e degeneração do músculo esquelético e cardíaco conduzindo a substituição do músculo por tecido conjuntivo e adiposo. O início da doença ocorre entre 2 e 5 anos de idade e muitos pacientes morrem por insuficiência cardíaca ou respiratória. Os principais estudos realizados no Golden Retriever distrófico (GRD) buscam elucidar a fisiopatogenia da doença e suas implicações clínicas na tentativa de testar terapias e tratamentos alternativos para melhoria da qualidade de vida do paciente distrófico e aumentar sua longevidade. Esta revisão apresenta uma visão geral sobre relevante contribuição do modelo GRD para elucidar a DMD em humanos.*
Palavras-chave: *Distrofia muscular de Duchenne, modelo animal, Golden Retriever distrófico.*

INTRODUCTION

Duchenne muscular dystrophy in humans is a recessive disorder linked to the X chromosome that is characterized by a mutation of the dystrophin gene. It manifests as progressive degeneration and necrosis of skeletal and cardiac muscles, with subsequent replacement by connective and adipose tissue, leading to generalized muscle weakness (VALENTINE et al., 1989; COLLINS & MORGAN, 2003; BANKS & CHAMBERLAIN, 2008; KASPAR et al., 2009; NAKAMURA & TAKEDA 2011).

Sex-linked muscular dystrophy associated with dystrophin deficiency has also been reported in some breeds of dogs, and is best seen in the Golden Retriever breed, as a condition known as golden retriever muscular dystrophy (GRMD) (BERGMAN et al., 2002). This is the most common form of muscular dystrophy in dogs (SHELTON et al., 2001). In the case of a dystrophic golden retriever dog (DGR), the mating of heterozygous female dogs with affected male dogs produces normal male dogs, heterozygous female dogs, and affected male and female dogs (VALENTINE et al., 1988). GRMD colonies were established in the United States, Australia, Italy, France and Brazil (NGUYEN et al., 2002). Several experimental models of DMD have been developed in animals. Animal models genetically homologous to DMD-such as the mouse

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with a mutation on the X chromosome (mdx), and the cat with hypertrophic feline muscular dystrophy-present moderate clinical characteristics and little or no endomysial fibrosis (BULFIELD et al., 1984). However, GRMD is the most relevant animal model for the study of DMD, particularly considering its potential therapeutic approach (MIYAZATO et al., 2011b).

GRMD is similar to DMD in humans due to clinical and pathological characteristics, in which young adult dogs often die from respiratory or cardiac failure (VALENTINE et al., 1988). A high mortality rate is typically observed in the first two weeks of life (VALENTINE et al., 1988; VALENTINE & COOPER, 1991; HOWELL et al., 1994).

Despite the considerable understanding of the pathophysiology, molecular basis, and therapeutic approaches of DMD, no effective treatment is available yet to halt the progression of the disease (ALLAMAND & CAMPBELL, 2000; DECONINCK & DAN, 2007; BANKS & CHAMBERLAIN, 2008). In this sense, the use of an animal model, mainly a canine model, in DMD research continues to provide valuable clues in the elucidation of the pathogenesis and the development of new therapies (ALLAMAND & CAMPBELL, 2000; COLLINS & MORGAN, 2003; BANKS & CHAMBERLAIN, 2008; NAKAMURA & TAKEDA, 2011; MIYAZATO et al., 2011c; KORNEGAY et al., 2012). Different canine models of DMD have been reportedly used for studying the disease (COOPER et al., 1988; SHARP et al., 1992; SCHATZBERG et al., 1999; SHIMATSU et al., 2003; WALMSLEY et al., 2010). Golden retriever dog with muscular dystrophy is the main lineage studied and is the best characterized. This review presents an overview of DMD and the pathological, immunological and molecular studies that have involved the GRMD model, highlighting its contribution to a better understanding pathophysiology and morphofunctional expression of the disease.

Current overview of Duchenne muscular dystrophy

Duchenne muscular dystrophy (DMD), also called progressive muscular dystrophy, is a recessive X-linked hereditary disorder. It results from changes in the short arm of the X chromosome in the *DMD* gene of the xp21 region. This gene has 79 exons and encodes a protein called dystrophin (ZATZ, 2004). Thirty-eight relevant human mutation sites have been described (EMERY et al., 2015), and genetic mutations of any order (deletion, duplication, insertion or of point) (McGREEVY et al., 2015) can lead to the absence of dystrophin in the sarcoplasmic membrane of the muscle cells and cause its rupture, calcium influx and subsequent activation of endogenous protease with activation of inflammatory cascade, and consequent necrosis and replacement of muscle tissue by adipose and fibrous tissue (DILAYLA & ABREU, 2015).

DMD is the most common muscular disorder in children, with phenotypic manifestation in male patients only. The incidence varies from 1:3,000 to 1:5,000 live births (McGREEVY et al., 2015). It manifests itself in early childhood, between 3 and 5 years of age, and is characterized by proximal muscle weakness and calf hypertrophy. Affected boys demonstrate delays in motor development; are easily fatigued; and have difficulty of running, jumping or standing up from the ground. Indeed, many of them have frequent falls or tip-toe walking. Typical associated posture is of lumbar lordosis and anserine gait (ZATZ, 2004). Less frequently, DMD causes a delay in language or overall development, or incidental findings of an increase in the serum levels of creatine kinase or transaminases when these investigations are performed for other reasons (JONES et al., 2003).

The course of the disease may lead to boys becoming wheelchair-bound between the ages of 8 and 12, the same age at which there is cardiac and respiratory compromise (JONES et al., 2003). At this time, the development of hypertrophic cardiomyopathy secondary to cardiac fibrosis also occurs. These patients often demonstrate persistent tachycardia, ventricular and atrial arrhythmias, premature ventricular complexes or sustained ventricular ectopic beats. Many patients are asymptomatic regarding cardiac dysfunction; however, because they are physically inactive (NIGRO et al., 1990). Respiratory dysfunction begins around the age of 10 years, with chronic respiratory failure presenting due to restrictive lung disease. Vital capacity reduces approximately 8% to 12% per year, and when it reaches 1 liter, the risk of death in the next one to two years is very high (PHILLIPS et al., 2001). Average life expectancy is considered to be 20 years, when the patient approaches death due to cardiorespiratory complications (JONES et al., 2003).

Several symptomatic therapies have been proposed, and the association of some of them may be necessary, depending on the condition of each patient. They extend from physiotherapy to the use of corticosteroids, and aim at delaying the progression of the disease and increasing the length and quality of patients life. However, new therapeutic strategies have been studied in the search for correction of the underlying cause and cure. These therapies are: (1) gene therapies, or the use of viral vectors, plasmid vectors, exon skipping and gene correction; (2) cell therapies

(stem cells), or the use of embryonic stem cells and adult stem cells; (3) pharmacological therapies, or the use of stop codon read-through, absence of dystrophin (utrophin) compensation and muscle growth and regeneration stimulators (GUEDES, 2012); and, more recently, the especially promising technique of (4) genome editing, such as CRISPR (clustered regularly interspaced short palindromic repeats) and TALEN (transcription activator-like effector nucleases) (McGREEVY et al., 2015; KAISER, 2016).

Dog as model of muscular dystrophy

Canine model is one of the best phenocopies of DMD in humans, when compared with other study models (BOGDANOVICH et al., 2003; COLLINS & MORGAN, 2003). Spontaneous mutations in the dystrophin-encoding gene, resulting in muscular dystrophy linked to the X chromosome, have been reported in several dog breeds (COLLINS & MORGAN, 2003; SHELTON & ENGVALL, 2005; NAKAMURA & TAKEDA, 2011). Genetic mutations in the Golden Retriever, Rottweiler, German Short-haired Pointer, Cavalier King Charles Spaniels, Beagle and Weimaraner breeds were described (COOPER et al., 1988; SHARP et al., 1992; SCHATZBERG et al., 1999; SHIMATSU et al., 2003; BALTZER et al., 2007; WALMSLEY et al., 2010). All canine models of DMD, type of mutation, and site of each dystrophin gene mutation are described in table 1.

Canine muscular dystrophy linked to the X chromosome is recognized and well-characterized in golden retriever dogs, where it occurs spontaneously (SHIMATSO et al., 2003). In Brazil, studies on genetics, clinic, pathology, molecular biology, and immunocytochemistry performed in the canine model have been useful in the evaluation of Duchenne muscular dystrophy in humans.

GRDM dogs have been extensively studied and have led to a better understanding of the condition for research on DMD in humans, especially in the experimental evaluation of new treatments in preclinical trials (COLLINS & MORGAN, 2003; SHELTON & ENGVALL, 2005; BANKS & CHAMBERLAIN, 2008; SILVA et al., 2009; NAKAMURA & TAKEDA, 2011). Both DMD and DGR present progressive clinical signs and severe myopathy with initial fiber necrosis and regeneration associated with connective tissue proliferation in the endomysium and perimysium (VALENTINE et al., 1990,; MIJAZATO et al., 2011b; KORNEGAY et al., 2012). Also, adult dogs have a body mass compatible with that of patients with DMD. Thus, DGR is indicated as an excellent model for studying the disease in humans (COOPER et al., 1988). Table 2 summarizes all the common characteristics of DMD and GRDM that show the benefits of using GRDM as an experimental model of the disease in humans.

Course of the disease in the DGR dog

GRMD is characterized by progressive muscle weakness and atrophy. These characteristics are similar to those of human DMD (NAKAMURA & TAKEDA, 2011). Extremely high levels of creatine kinase (CK) are identified in the first days of life, with a peak at six to eight weeks after birth, even before clinical signs appear, suggesting that the onset of

Table 1 - Comparison table of the site and types of genetic mutations in dog models of DMD according to breed.

Breed	Type of Mutation	Site of the Mutation
Cavalier King Charles Spaniel (CKCS-MD)	Point mutation	Intron 50
Golden Retriever (GRMD)		Intron 6
Rottweiler		Exon 52
Mixed Breed Golden Retriever- Beagle (CXMD)	Deletion mutation	Intron 6
Cocker Spaniel		Exon 65
Tibetan Terrier		Exons 8-29
German Short-haired Pointer (GSHP)		All the gene
Mixed Breed Pembroke Welsh- Labrador Retriever (CKCS-MD)		Exons 45-53
Pembroke Welsh Corgi	Insert mutation	Intron 13
Labrador Retriever		Intron 19
Japanese Spitz	Chromosome inversion	
Mixed Breed Labrador and Poodle	Not known	
Springer		

(McGREEVY et al. 2015; DUAN et al., 2015).

Table 2 - Summary of characteristics that show the benefits of using GRDM as an experimental model of DMD.

Features common to GRDM and DMD	
Histopathological characteristics	Spontaneous occurrence
	Few myofibrils containing central nucleation
	Severe myopathy with early necrosis of muscle fibers
	Muscle regeneration associated with connective tissue proliferation in endomysium and perimysium
Phenotypic characteristics	Ventricular dilation and thinning of the myocardium due to replacement by fibrous tissue
	Equivalent body mass as adults
	Lumbar kyphosis and lordosis
Clinical manifestations	Progressive muscle weakness
	Muscle atrophy
	Joint Contractures
	Hypersalivation and dysphagia
	Life expectancy approximately 25% of normal
	Development of muscular contractures with development of lumbar kyphosis and lordosis
	Abnormalities in gait diagnosed early
	Contraction injuries
	Cardiac arrhythmias
	Respiratory function impairment
	Death due to cardiorespiratory insufficiency at approximately 3 years
GRDM - Early diagnosis	
Increased CK in the first days of life (peak 6-8 weeks)	
Development of secondary lesions at 2 months	
Changes in gait and posture occur between 6-10 weeks	
Early signs of arrhythmias at 6 months	
Severe cardiomyopathy with signs of HF at 2 years	
CK Creatine kinase; HF Heart failure.	

lesions could be in the uterus, which could allow for earlier diagnosis of the disease (VALENTINE et al., 1988; NGUYEN et al., 2002; COLLINS & MORGAN, 2003). However, the high mortality rate observed in GRMD during the first 2 weeks of life coinciding with a high CK concentration suggested that DGR puppies could develop massive muscular necrosis mainly of the respiratory muscles (NGUYEN et al., 2002; COLLINS & MORGAN, 2003). CK levels remain elevated during the advanced stage of the disease, indicating persistent muscle fiber necrosis, but a progressive decline in these levels is observed in dogs aged 3-and-a-half to 6 years, similar to that seen in patients with DMD (VALENTINE et al., al., 1988). Dogs that survive during the neonatal period will present intermittent cycles of muscular degeneration and necrosis with sufficient initial regeneration, to compensate for fibers degeneration (Valentine et al., 1990; NGUYEN et al., 2002~; BANKS & CHAMBERLAIN, 2008).

Pathogenesis of muscular dystrophy can be divided into two phases: phase I involves the direct effects of dystrophin deficiency, and phase II is related to endomysial fibrosis and muscular atrophy (HOFFMAN & GORSOSPE, 1991; NGUYEN et al., 2002). Phase I lesions are reported in all species lacking dystrophin (humans, mice, dogs and cats) and are characterized by hyalinization, hypertrophy, intracellular accumulation of calcium salts and necrosis with subsequent regeneration of myofibers to compensate degeneration, in addition to the expressive inflammatory response (NGUYEN et al., 2002; MIYAZATO et al., 2011a; MIYAZATO et al., 2011b). Phase II lesions can be observed in DGR dogs at 2 months of age, and consist of increased endomysial connective tissue (endomysial fibrosis) and replacement of muscle fibers by fibrous tissue and adipose cells. In addition, marked muscle atrophy, complete mineralization of the foci of myofibers, and heterogeneity of fiber diameter are also observed in muscles in phase II of the disease. Only children with DMD, DGR dogs and diaphragms of mdx mice present this stage of the disease. Moreover, the DGR dog is the only animal model that shows (early at 2 months) the widespread development of secondary lesions due to dystrophin deficiency in a manner

compatible with human boys with DMD (NGUYEN et al., 2002; BANKS & CHAMBERLAIN, 2008; LESSA et al., 2014). The major ultrastructural findings in DGR dogs included dilatation of the sarcoplasmic reticulum, hypercontracted fibers, necrosis, degeneration of subsarcolemmal areas, and cytoplasmic disorganization (VALENTINE et al., 1990).

A study involving 16 male golden retriever dogs deficient in dystrophin aged five to 51 months showed, in the histopathological analysis, the presence of hyalinization and necrosis of muscle fibers as well as an increase of inflammatory cells, particularly mononuclear cells. In addition, variations in fiber size, dystrophic calcification, regeneration of fibers of smaller diameter and infiltration of adipose cells were observed in the interfascicular areas. There was also a significant increase in the number of positive fibers for calcium deposition in the skeletal muscles of these dogs, suggesting that the disruption of calcium homeostasis is associated with the severe degenerative lesions observed in these animals. Furthermore, histological evaluation of the skeletal muscle fibers of DGR dogs evidenced irregular shape and loss of the mosaic pattern among the different types of muscle fibers. In this study, cardiac involvement with pronounced lesions was also observed in 87.5% of the DGR analyzed. Analysis of the cardiac muscle of these dystrophic dogs revealed increased connective tissue deposition, presence of inflammatory cells, necrosis and some fibers with dystrophic calcification (MIYAZATO et al., 2011a; MIYAZATO et al., 2011b). The DGR animals aged 10 to 20 months of age presented deposition of fibrous and adipose tissue in extensive areas of the left and right ventricles, which compromises correct functioning of heart, progressing to heart failure (MALVESTIO et al., 2015).

Recently, in a histopathological, biochemical and immunological study carried out on the gastrocnemius muscle and myotendinous junction of DGR animals, similar alterations to those observed in humans affected by muscular dystrophy were observed, such as fibrosis, hyalinization, hypertrophy, necrosis, calcification and regeneration. However, lesions morphology in the myotendinous junctions was more pronounced than those of the gastrocnemius muscle, possibly due to the increase in Type II fibers, decreased MHCI complex expression, and decreased cytotoxic activity of CD8 cells. Such results strongly suggested that the immune system acts on the inflammatory process in DMGR (BERETTA et al., 2014).

Clinical aspects of the disease in the DGR dog

Clinical manifestations in DGR dogs are progressive, with a gradual loss of muscle mass and development of contractures that often lead to skeletal deformities (COOPER et al., 1988; SHELTON & ENGVALL, 2005; NAKAMURA & TAKEDA, 2011). Extensive muscle degeneration and generalized necrosis are identified soon after birth (VALENTINE et al., 1988; NAKAMURA & TAKEDA, 2011). Clinical weakness and gait abnormalities are the first manifestations in puppies affected by DMD at 6 to 10 weeks old, similar to that observed in DMD children who exhibit their first clinical signs at 2 to 4 years old (VALENTINE et al., 1988). A notable feature in DGR dogs is the enlargement of the base of the tongue due to hypertrophy and muscle pseudohypertrophy, which leads to pharyngeal and esophageal dysfunction, resulting in dysphagia, salivation, regurgitation, and finally, aspiration pneumonia (VALENTINE et al., 1988; NAKAMURA & TAKEDA, 2011; KORNEGAY et al., 2012). Progressive gait impairment is evidenced in DGR dogs by the sixth week of age as a result of atrophy of trunk and temporalis muscles, plantar posture due to overextension at the carpus, overflexion at the tarsus and abduction of the paws, as well as progressive lumbar kyphosis and consequent lordosis (VALENTINE et al., 1988; SHELTON & ENGVALL, 2005; NAKAMURA & TAKEDA, 2011; KORNEGAY et al., 2012). In addition, the affected dogs present muscular atrophy, weakness and greater susceptibility to injury induced by contraction (NGUYEN et al., 2002; BANKS & CHAMBERLAIN, 2008). Cardiomyopathy is often observed in young DGR dogs. In the ECG evaluation, these animals present ventricular arrhythmias and increase in Q/R space at six months of age, which progress with age (MOISE et al., 1991). Moreover, increased echogenicity corresponding to the areas of mineralization at necropsy are observed on the echocardiogram at 6-and-a-half months of age. Ventricular dilatation and myocardial thinning due to fibrous tissue replacement, with decreased myocardial contractile function, are frequent findings in DGR dogs at 2 years of age (MOISE et al., 1991). As in patients with DMD, impairment of respiratory function is also demonstrated in DGR dogs and, together with cardiomyopathy, is the main cause of death in these animals (VALENTINE et al., 1989; COLLINS & MORGAN, 2003; BANKS & CHAMBERLAIN, 2008; NAKAMURA & TAKEDA, 2011). It was demonstrated that homozygous (carrier) females and small dogs affected could have milder clinical signs and only some histological lesions seen in male DGR (VALENTINE et al., 1988. KORNEGAY et al., 2012). A previous

study documented that mixed breed DGR-labrador retriever dogs could have a less severe histopathological phenotype of the disease, as evidenced by the degree of necrosis and differentiation of muscle fibers, supporting the idea that cross-breeding could reduce disease severity (MIYAZATO et al., 2011c).

Advances in DMD therapy using GRMD

The therapeutic strategies used for DMD can be categorized as pharmacological, molecular, cellular or a combination of them. Table 3 summarizes the therapeutic techniques already used in dogs. Pharmacological therapies aim to control pathogenic mechanisms, seeking to ameliorate the dystrophic phenotype of the disease. The two most common molecular approaches are gene therapy, in which the dystrophin gene is introduced into the muscle by local injection, or through the vasculature using plasmids or viral vectors, and genetic correction, involving the introduction of oligonucleotides to restore the correct nucleotide sequence. Regarding such cell-based therapies, the approach could be through transplantation of normal cells such as myoblasts or stem cells into the diseased muscle (KORNEGAY et al., 2012).

Genome editing therapy is based on an RNA strands that guide a nuclease enzyme to cut the DNA at a precise point in the genome, which is the site of the defective exon. Cells, then, repair the vacant space by assembling the broken strands, or by using a DNA template provided to create a new sequence (KAISER, 2016). Several studies have demonstrated the effectiveness of TALENs (Transcription Activator-Like Effector Nuclease) and CRISPR (Clustered Regularly Interspaced Short Palindromic Repeat) in studies of gene repair in induced pluripotent stem cells (iPSCs). The CRISPR technique is an extremely accurate genome-editing technique with very promising results. CRISPR-Cas9 technology has already been used in the correction of the mutant dystrophin gene in mice, avoiding the development of muscular dystrophy, and also in the repair of cystic fibrosis transmembrane receptor locus by homologous recombination in cultured intestinal stem cells from human patients with this disease (WU et al., 2013). LI et al. (2015) performed the genetic correction of the gene that causes muscular dystrophy in humans and obtained promising results with three different techniques, demonstrating that the CRISPR technique was

Table 3 - Summary of muscular dystrophy therapies used in dogs.

Therapeutic technique employed		Breed	Reference
Pharmaceutical Therapy	Daily oral administration of prednisone for 4 months	DGR	LIU et al., 2004
	Chronic infusion of P188		TOWNSEND et al., 2010
	Daily oral administration of losartan potassium		SILVA et al., 2009
	Proteasome Inhibitor (Bortezomib)		ARAUJO et al., 2013
	Association of Prednisone and Cyclosporine A		BARTHÉLÉMY et al., 2012
	Myostatin	Healthy Dogs	BRADLEY et al., 2008
Cell Therapy	Transplantation of Myoblasts	DGR	KORNEGAT et al., 2012
	Transplantation of Mesangioblasts		SAMPAOLESI et al., 2006
	Intravenous and intramuscular transplantation of human immature dental pulp stem cells (hIDPSC)		KERKIS et al., 2008
	Canine umbilical cord mesenchymal stem cells		ZUCCONI et al., 2011
	Transplantation of adipose cells derived from human stroma		VIEIRA et al., 2012
Gene Therapy	Intravenous injection of AAV9 with minigene	DGR	KORNEGAY et al., 2010
	Intramuscular injection of AAV-8 with minigene		HAKIM et al., 2014
	Direct injection of chimeric oligonucleotide		BARTLETT et al., 2000
	Intravenous injection of AAV9 with recombinant		SHIN et al., 2011
	Direct injection of plasmids containing the complete genetic code of dystrophin		BRAUN, 2004
	AAV-based Exon Skipping – Intravenous injection		VULIN et al., 2012
	AAV-based Exon Skipping – Intracardiac Injection		BARBASH et al., 2013
	Injection of microgene Δ3990 (ΔR3-19 / 20-21/C)	Healthy Dogs	KORNEGAY et al., 2010
	Exon Skipping	$CXMD_J$	YOKOTA et al., 2009

equally effective in the TALEN technique and that both obtained minimal mutagenic effects outside the initial target when directed to a single sequence region. The method was considered accurate and efficient with very promising prospects.

Subsequently, MAGGIO et al. (2016) obtained promising results using viral vectors to correct mutations in the dystrophin gene with CRISPR-Cas9 associated or not with TALENs in cultures of progenitor muscle cells derived from mdx mice. The authors were able to correct the mutation in the dystrophin gene in up to 37% of the cell population evaluated. Likewise, BENGTSSON et al. (2017) reported a significant increase in the expression of dystrophin with an increase in the functional capacity of the muscle fibers of mdx^{4cv} mice.

Many studies in DGR dogs have been conducted to evaluate the role of pharmacological therapies for the treatment of DMD. Functional and histopathological changes were evaluated in DGR dogs submitted to a daily oral administration of prednisone for four months. Results showed hypertrophy of the cranial sartorius muscle, an improvement of the tibiotarsal joint angulation, increased calcification of myofibers, and decreased fetal myosin expression. These findings show the benefits, but also deleterious histopathological alterations, in these animals after treatment with prednisone (LIU et al., 2004). Another study demonstrated that chronic infusion of a membrane-sealing poloxamer, P188, reduced myocardial fibrosis of DGR dogs, prevented serum increase of cardiac troponin I (cTnI) and atrial natriuretic peptide (ANP), and prevented left ventricular remodeling. These findings have important clinical relevance because P188 could act as a therapeutic strategy for dystrophic cardiomyopathy, which is the second most common cause of death in patients with DMD (TOWNSEND et al., 2010). In DMD patients, losartan potassium is used as an anti-fibrosis treatment because of its inhibition of TGF-beta due to the fact that it is an angiotensin inhibitor, and DMD patients may often present with increased levels of potassium, urea and creatinine. However, the use of losartan potassium in DGR administered orally at a dose of 50mg for nine weeks did not influence renal function, serum potassium level or blood pressure (SILVA et al., 2009).

Another study showed that DGR dogs treated with bortezomib, a proteasome inhibitor, presented greater uniformity of fiber diameter and decreased lymphocyte invasion, suggesting attenuation of the inflammatory process and a reduction of t connective tissue deposit in the endomysium and perimysium of the muscle fibers. Also, increased α- and β-dystroglican expression was also reported, which indicates an improvement in the histopathological phenotype of the disease. These results suggested that bortezomib could block the activation of phospho-NFκB, which could be important in the pathogenesis of DMD and possibly represent a line of research in DMD therapy (ARAUJO et al., 2013).

More recently, the role of the combination of cyclosporin A and prednisolone at immunosuppressive levels in the course of the disease of DGR dogs has been verified. Results showed an intense effect on the DGR phenotype after treatment, leading to exacerbation of calcification lesions, atrophy, decreased muscle strength and increased fatigue. However, there was a remarkable improvement in disease progression at the systemic level and in animals' locomotion, as well as a decrease in CK values. This fact emphasizes the interest of a multiparametric evaluation in the canine model of DMD, which could reproduce the complexity of the human disease (BARTHÉLÉMY et al., 2012). Another study suggested that for effective immunosuppressive treatment in dogs, the dosage of ciclosporin A requires adjustments in the course of therapy, guided by serum values and the animals age (MORINI et al., 2008).

Three four-day-old DGR dogs submitted to intravenous injection with an adenovirus-associated vector (AAV9) carrying a human codon with dystrophin minigene under the control of the cytomegalovirus (CMV), presented generalized muscle transgene expression after 16 weeks of treatment (KORNEGAY et al., 2010). In DGR dogs, the point of the mutation is within intron 6, and this leads to the deletion of exon 7 in the dystrophin mRNA, with a resulting frameshift caused by the early termination of the translation. Direct injection of the chimeric oligonucleotide into the cranial tibial muscle of a 6-week-old affected male dog resulted in repair of the dystrophin mutation site after 48 weeks of treatment (BARTLETT et al., 2000). Also, affected dogs treated by transendocardial route with an adenoviral vector carrying a recombinant to correct the mutation site in exons 6 and 8 and thus reestablish the dystrophin protein sequence in the hearts of affected dogs, presented restoration of cardiac dystrophin expression at 13 months as confirmed by reverse transcriptase-PCR (RT-PCR). This finding was accompanied by an improvement in cardiac function and a reduction of fibrosis, both verified by magnetic resonance imaging (MRI) (BISH et al., 2012).

Regarding cell-based therapies, it is known that various cell types such as adult, fetal and embryonic stem cells can contribute to the regeneration of diseased muscle. However, it was

reported that canine myoblast transplantation proved to be incapable of achieving significant implantation in DGR dogs (KORNEGAY et al., 2012). Conversely, transplantation of mesangioblasts (stem cells associated with vessels) in DGR dogs resulted in a great recovery of dystrophin expression and in the reestablishment of muscular morphology and function (confirmed by the measurement of the contraction force of the individual fibers), which allowed for a remarkable clinical improvement and preservation of motor activity (SAMPAOLESI et al., 2006). Another study evaluating the human immaturedental pulp stem cells (hIDPSC) transplanted by arterial or muscular injection in four DGR dogs showed minimal expression of dystrophin in treated animals. However, improvement in clinical status was observed in animals who received monthly arterial injections, suggesting that multiple systemic deliveries could be more efficient than local injections (KERKIS et al., 2008). In this context, mesenchymal stem cells derived from the canine umbilical cord were identified in the dystrophic muscle after its systemic administration. However, the expression of dystrophin was not detected in the animals after transplantation. Results showed that umbilical cord mesenchymal stem cells could be able to reach the animal's musculature, but would not be able to reach full differentiation in skeletal muscle cells (ZUCCONI et al., 2011). More recently, it has been reported that adipose cells derived from human stroma, when injected systemically into the dog's cephalic vein, could have the ability to penetrate, implant and express the dystrophin in the dystrophic muscle of the DGR up to 6 months after transplantation. Thus, introduction of large numbers of human mesenchymal cells into a large animal model without immunosuppression is a safe procedure that may have significant implications for future therapy in patients with DMD (VIEIRA et al., 2012).

CONCLUSION

Despite progress made in understanding the genetics and pathogenesis of DMD, the cure for the disease, which has a 100% mortality rate, has not yet been established. Possibly, this failure could be explained by the fact that most of even the current research is still being performed on murine models that do not reliably reproduce the pathogenesis of the disease. The canine GRMD model is undoubtedly the closest to the human disease, presenting severe cardiac and skeletal alterations similar to those observed in those affected by DMD. Therefore, GRMD animals are extremely useful for a better understanding of pathological mechanisms and for pre-clinical studies recently developed using pharmacological, genetic and cell-based therapies to seek cures or to increase life expectancy of those affected by DMD.

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