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Phosphorylase re-expression, increase in the force of contraction and decreased fatigue following notexin-induced muscle damage and regeneration in the ovine model of McArdle disease

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Abstract

McArdle disease is caused by a deficiency of myophosphorylase and currently a satisfactory treatment is not available. The injection of notexin into, or the layering of notexin onto, the muscles of affected sheep resulted in necrosis followed by regeneration of muscle fibres with the expression of both non-muscle isoforms of phosphorylase within the fibres and a reduction of the amount of glycogen in the muscle with an increase in the strength of contraction and a decrease in fatiguability in the muscle fibres. The sustained re-expression of both the brain and liver isoforms of phosphorylase within the muscle fibres provides further emphasis that strategies to enhance the re-expression of these isoforms should be investigated as a possible treatment for McArdle disease.

Keywords: Myophosphorylase; Glycogen phosphorylase; McArdle disease; Ovine model of McArdle disease; Notexin; Phosphorylase isoforms.

1. Introduction

McArdle disease is transmitted as an autosomal recessive metabolic myopathy caused by a deficiency of muscle glycogen phosphorylase. As a consequence glycogen metabolism is blocked inhibiting aerobic and anaerobic glycolysis [1–3]. Patients with this condition experience exercise intolerance with premature fatigue, myalgia and stiffness leading to contracture and rhadomyolosis. Muscle weakness predominately affecting the upper limbs and paraspinal muscles may occur as a late consequence in some patients. Acute rhabdomyolysis can be severe and results in collapse and acute renal failure. Severe generalised weakness at, or soon after birth, with respiratory insufficiency and death in infancy has been reported [4,5]. Other than aerobic conditioning which can improve exercise tolerance [6], there is no satisfactory treatment to reverse McArdle disease. More than 100 different mutations of the myophosphorylase gene (PYGM) have been reported in human patients with McArdle disease: ($n = 133$ PYGM mutations – Human Gene Mutation Database (HGMD http://www.hgmd.org) 9.3.12). There are three glycogen phosphorylase isoenzymes (brain (pygb) liver (pygl) and muscle (pygm)) each encoded by a different gene. In mature skeletal muscle, the muscle isoform of glycogen phosphorylase is predominantly expressed while in

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regenerating muscle fibres the non-muscle isoforms of glycogen phosphorylase pygb and pygl are expressed [6,7].

Since 1997, a unique flock of Merino sheep carrying a mutation in the PYGM has been maintained at the Murdoch University Veterinary School farm in Western Australia and has been available for study. In affected animals (double recessive) as determined by genotyping, there is an absence of glycogen phosphorylase activity in muscle fibres and the sheep exhibit similar clinical effects and morphological changes to those seen in humans. Expression of the brain and liver isoforms of glycogen phosphorylase has been shown to be present in developing and regenerating muscle fibres but are not expressed in mature muscle fibres [8]. The mutation in the sheep model is a mutation of the acceptor splice site in intron 19 of PYGM [9]. The splice site mutation leads to the activation of a cryptic acceptor splice site which in turn causes an eight-base deletion from the mRNA at the 5′ end of exon 20 resulting in a frameshift and a downstream premature stop codon removing the last 31 amino acid residues from the myophosphorylase protein.

McArdle disease has also been described in Charolais cattle [10] but the ovine model is the first animal model of the condition in a species with a similar body mass throughout life to that of humans. Affected sheep provide a useful animal model for the preclinical testing of putative therapy regimes and for the evaluation of the risks and benefits of such therapies. The ovine model has been used for pre-clinical gene therapy trials [8], and phosphorylase activity has been detected at the sites of injection of myophosphorylase adeno and adeno-associated viral vectors carrying myophosphorylase cDNA or Lac Z cDNA [11]. The phosphorylase activity was a mixture of expression of the human muscle glycogen phosphorylase resulting from the presence of human myophosphorylase cDNA and re-expression of the ovine brain and liver isoforms in regenerating muscle fibres.

The results reported in the present paper examine the re-expression of the ovine brain and liver isoforms of phosphorylase in skeletal muscle of sheep with McArdle disease following the injection of notexin, a myotoxic phospholipase derived from the venom of the Australian Tiger Snake *Notechis scutatus scutatus*. It has previously been reported that necrosis and hyalinization of muscle fibres with neutrophil infiltration begins soon after injection of notexin; regeneration commences by 3 days post injection; myotube formation is visible by 5 days and by 21 days fully differentiated muscle fibres are found [12,13]. However, the basal lamina is left intact and the vascular supply is not impeded by notexin [14]. In the experiments reported here, notexin was administered to sheep with McArdle disease in one of two ways, either as a single injection into the muscle or as an application layered onto the surface of the muscle. The changes in the glycogen content and muscle function were observed in biopsies taken at 10, 21, 30 or 31, 60, 90 and 120 days after notexin administration (Table 1), to ascertain whether muscle function can be improved by stimulation of phosphorylase in muscles by notexin.

2. Materials and methods

2.1. Approvals for surgical and experimental procedures

All trials and procedures involving animals were carried out with the approval of the Murdoch University Animal Ethics Committee, the University of Western Australia or IMVS Institutional Biosafety Committee. All injections or applications of notexin and biopsies were performed under general anaesthesia. In young lambs up to 3 months old, isoflurane was given by mask and in older animals Alfaxan-CD-RTU (Jurox Pty. Ltd.) was used intravenously for induction, followed by isofluorane via intubation or mask.

2.2. Preparation of notexin

The notexin was obtained as freeze-dried powder from Venom Supplies Pty Ltd, Tanunda, South Australia. Prior to injection, the notexin was diluted to a concentration of 5 μg/ml in sterile saline.

2.3. Biopsies

In order to observe the changes in the effects produced by the administration of notexin at various times, the injections or layering were made in or onto several muscles (Table 1) all of which were long muscles and it was possible to leave an area of undamaged muscle around each site and therefore when biopsies were taken, the risk of changes being present as a result of scarring or regeneration from adjacent sites was avoided.

Incisions were made in the direction in which the fibres were running such that the biopsies were 2.0 to 3.0 cm long and 0.5 cm in diameter. They were transversely cut at approximately 0.5 cm intervals into a series of blocks and each of the blocks was immediately frozen in liquid nitrogen-cooled isopentane. The blocks of muscle remained frozen at −80 °C until they were required for sectioning or for glycogen analysis. Sections for histochemistry were cut on a cryostat (Tissue-Tek (Beyer) or Leica CM 1510) and stained.

Biopsies of semitendinosus were also taken for measurements of the force of contraction and the fatiguability of treated and untreated muscle 21 days after the injection of notexin. The measurements from notexin treated muscles from sheep with McArdle disease were compared with those from untreated muscle and with muscle from untreated normal sheep.

2.3.1. Control uninjected or unlayered sites (Table 1)

During 4 trials (2, 3, 5 and 8) a total of 42 biopsies were taken from 20 affected animals from sites in the muscles where notexin had neither been injected nor layered and