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Investigating sodium valproate as a treatment for McArdle disease in sheep

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Abstract

McArdle disease is due to an absence of the enzyme muscle glycogen phosphorylase and results in significant physical impairment in humans. We hypothesised that sodium valproate, an HDAC inhibitor, might have the ability to up-regulate the enzyme. We treated McArdle sheep with sodium valproate given enterically at 20–60 mg/kg body wt. Compared with untreated control animals, there was increased expression of phosphorylase in muscle fibres. The response was dose dependent and reached a maximum 2 hours after the application and increased with repeated applications. Improvement in mobility could not be demonstrated. These findings suggest that sodium valproate is a potential therapeutic treatment for McArdle disease.

Keywords: McArdle disease; Sheep; Glycogen storage disease type V; Glycogen phosphorylase; Sodium valproate; HDCAI

1. Introduction

McArdle disease (glycogen storage disease V), is an autosomal recessive disorder affecting approximately 1 in 100,000 persons and it is the most common of the muscle glycogenoses. The condition results from complete (or almost complete) absence of the enzyme muscle glycogen phosphorylase (pygm) and is due to mutations in this enzyme [1–3]. Clinical signs relate to the inability to perform strenuous and isometric activities and include muscle fatigue, contracture and rhabdomyolysis, which, when severe, results in myoglobinuria and acute renal failure. At present there is no satisfactory treatment [4]. There are two additional glycogen phosphorylase isoenzymes (brain (pygb) and liver (pygl)), which are encoded by different genes. In mature skeletal muscle, the muscle isoform of glycogen phosphorylase is predominantly expressed, and in developing and regenerating muscle fibres the brain and liver isoforms are also expressed [1,5–7].

Since 1997 a unique flock of Merino sheep carrying a mutation for McArdle disease has been maintained at the Murdoch University Veterinary School farm in Western Australia and has been used for studies of the disease. In the affected animals there is an absence of skeletal muscle glycogen phosphorylase activity and the sheep exhibit similar clinical effects and morphological changes to those seen in human patients. As with humans, expression of the brain and liver isoforms of glycogen phosphorylase has been shown to be present in developing but not in mature muscle fibres of normal and McArdle disease sheep [2]. McArdle disease has also been described in Charolais cattle [8] and more recently a 'knock-in' mouse has been bred [9] but the ovine model is the first animal model of the condition in a species which has, throughout life, a similar body mass to that of humans. The disease in sheep provides a unique and authentic animal model for the preclinical testing of putative therapy regimes and for the evaluation of the risks and benefits of such therapies. The injection of vectors carrying myophosphorylase cDNA or Lac Z cDNA phosphorylase activity initiated phosphorylase activity with a mixture of expression of the human muscle glycogen phosphorylase and re-expression of the ovine brain and liver isoforms of phosphorylase in regenerating muscle fibres [10]. The intramuscular injection into, or the layering of notexin onto, the surface of the muscle of sheep with McArdle disease also resulted in the expression of the brain and liver isoforms of phosphorylase and an increase in strength in regenerating muscle fibres at these sites [11,12].

In the present paper the effect of sodium valproate on the expression of phosphorylase in skeletal muscles of sheep with McArdle disease was examined. Sodium valproate is

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commonly used to treat epilepsy. It belongs to a family of drugs known as Histone Deacetylase Inhibitors (HDACIs) that can affect gene expression by acetylating lysine residues, which in turn have a direct effect on chromatin [13]. These results indicate that sodium valproate could be a potential therapeutic target for McArdle disease.

2. Materials and methods

2.1. 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 surgical procedures and injections of sodium valproate 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 isoflurane via intubation or mask.

2.1.1. Trial 1. Administration of sodium valproate by intramuscular injection

Five ml of sodium valproate (0.5 g/30 ml) or sterile normal saline was injected into 8 muscles of three, 5 week old, affected lambs. The valproate injections were made into the peroneus tertius and gluteobiceps muscles of the right pelvic limb and into the extensor carpi radialis and ulnaris lateralis of the right thoracic limb. The same muscles in the left limbs were injected with 5 ml sterile saline. In 2 of the lambs the injections of valproate were made on 3 consecutive days; in the third lamb the injections of saline (left) and valproate (right) were made on the third day only. Each injection was given over a 1 minute period.

4 days after the first of the multiple injections and 2 days after the single injection, biopsies were taken, under general anaesthetic, from the gluteobiceps and extensor carpi radialis sites of both limbs in all 3 sheep. 9 days later, biopsies were taken from the remaining 4 sites. The biopsies were 2.5–3 cms long and 0.5 cms in diameter. Each biopsy was cut into 4–6 blocks and frozen in liquid nitrogen-cooled isopentane and kept at −80 °C. Seven sections were cut from each of the frozen blocks. Sections were stained with H&E and histochemically for glycogen phosphorylase, and developmental myosin heavy chain (to look for evidence of regenerating fibres).

2.1.2. Trial 2. Enteric administration of sodium valproate solution

Damage to muscle fibres by the injection in trial 1 may have resulted in expression of phosphorylase in regenerating fibres. Therefore an enteric route of administration was chosen for trials 2 and 3. As sheep are ruminants, it was decided to bypass the rumen, reticulum and omasum by administering the sodium valproate directly into the abomasum, which is the equivalent of the stomach in non-ruminants. In trial 2 the doses of sodium valproate were given twice daily in solution via a mushroom headed catheter which was implanted under anaesthesia into the abomasum 10 days prior to the administration of the first dose. For two days following surgery the lambs were injected with oxytetracycline and temgesic to prevent infection and provide pain relief. They were checked twice daily and temperatures were taken once a day.

The silicone mushroom headed catheters were implanted into the abomasum of 3 lambs at 12 days old (Group 1) and 5 lambs 12–18 days after birth (Group 2). A pre-treatment biopsy was taken from the abdominal muscle of each lamb at the implantation site during surgery (Table 1). Ten days later, blood was taken to determine haematological and biochemical parameters. This was followed on the same day by the first dose of sodium valproate with Epilim liquid (Sanofi-Synthelabo Australia Pty Ltd) at a dose of 2.5 mg/kg body weight twice daily for both groups. The dose for group 1 was increased by 2.5 mg per day until 7 days later at the start of week 2, when a dose of 10 mg sodium valproate per kg was given twice per day (i.e. 20 mg/kg per day). This dose regime was continued until the start of week 14 when the dosage was increased at a rate of 2 mg/kg a day until the dose of 10 mg/kg twice per day was reached at the end of week 15. The dose of 20 mg/kg twice per day was continued until the animals were euthanased at the start of week 20. The lambs in group 2 were given 10 mg/kg twice daily until the start of week 10 when the dosage was increased at a rate of 2 mg/kg daily until a dose of 15 mg/kg twice daily was reached at the end of week 11. The concentration of valproate in the blood was measured 10 times in group 1 and 5 times in group 2 at varying times in the trial (Table 1).

In order to identify any toxic effects, blood was taken for biochemical and haematological parameters. Blood samples were analysed for creatine kinase (CK), gamma glutamate transferase, aspartate transaminase (AST), bilirubin, albumin, calcium, creatinine, glucose, protein, phosphate, urea and magnesium prior to and during dosing. Haematological analysis was carried out for blood cell counts (WBC, RBC, neutrophil, lymphocyte, monocyte, eosinophil and basophil) as were platelet counts and haemoglobin estimations.

In both groups of animals, muscle biopsies were taken throughout the trial and at post-mortem examination to test histochemically for the presence of phosphorylase positive muscle fibres (Table 1). On the day of cannulation, the abdominal wall muscle was taken; in week 5 for group 2 and week 6 for group 1, right extensor carpi radialis and left peroneus tertius; in week 9 group 2 and week 10 group 1 right semitendinosus, and left ulnaris lateralis, and in week 13 group 2 and week 17 group 1 right peroneus tertius. At euthanasia, the extensor digitorum lateralis, triceps, supraspinatus, gastrocnemius, gluteus medius, sternocephalicus, diaphragm were taken.

2.1.3. Trial 3. Enteric administration of sodium valproate tablets

2.1.3.1. Treatment group. Six affected ewes that had not been used in previous treatment trials were dosed with sodium valproate in an enteric coated tablet form (Epilim EC200 or Epilim EC500 Sanofi-Synthelabo Pty Ltd). As lambs, 2 years earlier, small muscle samples had been taken from 2 of the animals. The age of these ewes at the start of the trial was between 2½ and 5 years.