Neuromuscular Disorders 18 (2008) 355–364

www.elsevier.com/locate/nmd

Acquired multiple Acyl-CoA dehydrogenase deficiency in 10 horses with atypical myopathy

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Received 28 September 2007; received in revised form 7 February 2008; accepted 18 February 2008

Abstract

The aim of the current study was to assess lipid metabolism in horses with atypical myopathy.

Urine samples from 10 cases were subjected to analysis of organic acids, glycine conjugates, and acylcarnitines revealing increased mean excretion of lactic acid, ethylmalonic acid, 2-methylsuccinic acid, butyrylglycine, (*iso*)valerylglycine, hexanoylglycine, free carnitine, C2-, C3-, C4-, C5-, C6-, C8-, C8:1-, C10:1-, and C10:2-carnitine as compared with 15 control horses (12 healthy and three with acute myopathy due to other causes). Analysis of plasma revealed similar results for these predominantly short-chain acylcarnitines. Furthermore, measurement of dehydrogenase activities in lateral vastus muscle from one horse with atypical myopathy indeed showed deficiencies of short-chain acyl-CoA dehydrogenase (0.66 as compared with 2.27 and 2.48 in two controls), medium-chain acyl-CoA dehydrogenase (0.36 as compared with 4.31 and 4.82 in two controls) and isovaleryl-CoA dehydrogenase (0.74 as compared with 1.43 and 1.61 nmol min^{-1} mg^{-1} in two controls).

A deficiency of several mitochondrial dehydrogenases that utilize flavin adenine dinucleotide as cofactor including the acyl-CoA dehydrogenases of fatty acid β-oxidation, and enzymes that degrade the CoA-esters of glutaric acid, isovaleric acid, 2-methylbutyric acid, isobutyric acid, and sarcosine was suspected in 10 out of 10 cases as the possible etiology for a highly fatal and prevalent toxic equine muscle disease similar to the combined metabolic derangements seen in human multiple acyl-CoA dehydrogenase deficiency also known as glutaric acidemia type II.

Keywords: Horse; Multiple Acyl-CoA dehydrogenase deficiency; Myopathy; Lipid metabolism; Acylcarnitine; Organic acid; Glycine conjugate

1. Introduction

So-called atypical myopathy is an acute myopathy that appears in grazing horses [1–3]. To the authors' knowledge, the first case reports of myopathy in grazing horses concerned outbreaks that occurred in the autumn of 1939 in the North of Wales, UK [4]. Since the recognition of the syndrome, outbreaks of atypical myopathy have been reported in several European countries and case reports prior to the syndrome's identification suggest that the condition has also been encountered in Australia, Canada and the United States of America [2,5]. For example, in the autumn of 1995, over one hundred horses died from this condition in Northern Germany [1,2]. In autumn 2000, Belgium recognised its first cases of atypical myopathy

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0960-8966/$ - see front matter © 2008 Published by Elsevier B.V.
doi:10.1016/j.nmd.2008.02.007

and large outbreaks were recorded during cold periods in autumn and spring of the years 2002, 2004, and 2006 in Belgium and France. From 2004, the syndrome was recognised in more than ten European countries thereby suggesting its emerging nature. The syndrome is associated with a mortality rate of about 90% and death usually occurs within 72 h [2,3].

To date, a number of potential aetiological and contributory factors were considered, but both the exact aetiology and the pathophysiology have remained unresolved. Whatever the cause, particular weather conditions seem to trigger the appearance of the syndrome [2,5].

The main feature of this syndrome is the sudden onset of clinical signs characterized by acute generalised progressive myopathy. Serum biochemical abnormalities usually include markedly elevated muscle enzyme activities indicating severe muscle damage and at *post mortem* widespread myodegeneration are found in both skeletal muscle and myocardium [1–3]. It has been shown previously that in equine atypical myopathy predominantly type 1 muscle fibers were degenerated associated with the accumulation of neutral lipids [1].

The aim of the current study was to perform metabolic screening of lipid metabolism in horses with a tentative diagnosis of atypical myopathy.

2. Materials and methods

2.1. Horses

Five horses sampled during the autumn 2006 outbreak in the Netherlands and five Belgian cases from outbreaks in 2003, 2004, and 2006 with a tentative diagnosis of atypical myopathy were used. In nine out of these 10 horses (except case 5 which survived) the disease turned out to be fatal. The description and clinical course of these 10 horses (indicated as 1–10) is given in Table 1. Peak blood values in these horses are shown in Table 2. Inclusion criteria included access to pasture, no previous anaesthesia, nonexertional and nonrecurrent acute progressive rhabdomyolysis, absence of nonmuscular pathology and plasma creatine kinase activity >2000 IU L^{-1}.

Twelve clinically healthy warmblood mares belonging to Utrecht University housed in boxes and accustomed to frequent handling were used as healthy controls. These horses were 3.7–20.5 years of age (mean age ± SD, 9.6 ± 5.0 years) and weighed 470–758 kg (mean weight, 596 ± 84.7 kg). Their diet consisted of grass silage supplemented with concentrate feed and met nutrient requirements for maintenance and performance. The total diet contained 10% ash, 14.5% crude protein, 1.3% crude fat, 20% crude fiber, and 56.2% other carbohydrates. Water was provided ad libitum.

In addition, three horses suffering from acute myopathy due to other causes (including one case of post-anaesthetic myopathy (horse 11) and two cases of recurrent exertional rhabdomyolysis (horses 12 and 13)) without a tentative diagnosis of atypical myopathy were used as diseased controls. The description and clinical course of these three horses (indicated as 11–13) is also given in Table 1. Peak blood values in these horses are shown in Table 2.

2.2. Muscle pathology

Nine out of 10 horses (except case 5 which survived) with a tentative diagnosis of atypical myopathy were submitted for pathology. For histologic examination, muscle specimens were fixed in 4% (w/v) phosphate-buffered paraformaldehyde, sectioned at 5 μm, and stained with H&E. In addition, NADH, cytochrome *c* oxidase, succinate dehydrogenase, acid phosphatase, periodic acid Schiff, ATP-ase (pH 4.3 and 9.4), Sudan black B, and oil red O stains were performed on various muscle tissues frozen in isopentane that was precooled in liquid nitrogen, and stored at −80 °C.

2.3. Analysis of organic acids and glycine conjugates

Urine samples from all 10 cases with a tentative diagnosis of atypical myopathy were subjected to analysis of organic acids and glycine conjugates and results

Table 1
Breed, age, sex, and clinical progression of 10 horses with a tentative diagnosis of atypical myopathy (1–10) and three controls (11–13)

Horse number	Breed	Age	Gender	Clinical course
1	Fjord	2 years	Mare	Death within 3 days
2	Belgian draft	0.5 years	Mare	Death within 1 day
3	Standardbred	1.5 years	Mare	Death within 3 hours
4	Ardennais	0.5 years	Mare	Death within 10 hours
5	Draft crossbreed	10 years	Mare	Survived
6	Draft crossbreed	11 years	Mare	Death within 1 day
7	Pony	1 year	Mare	Death within 3 days
8	Friesian	10 months	Mare	Death within 1 day
9	Arabian	2 years	Stallion	Death within 1 day
10	Warmblood	3.5 years	Mare	Death within 2 days
11	Tinker	14 years	Gelding	Death within 2 days
12	Thoroughbred	4 years	Mare	Survived
13	Warmblood	10 years	Mare	Survived