

Serum Enzymatic Changes in Halothane Anesthetized Athletic Horses

Alterações sérico enzimáticas em cavalos atletas anestesiados com halotano

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Abstract

Six horses (5 male, 1 female), 2 to 5 years of age, and weighing 430 to 510 kg, from the Brazilian Jockey Club (Rio de Janeiro, Brazil) were anaesthetised to perform elective arthroscopic surgery during 1:30 hr. All the animals were examined before the anaesthetic procedure. Food but not water was withheld from these horses for 12 hr. Xylazine (0.75 mg/kg IV bwt) was administered as a preanaesthetic medication. Ketamine (2.2 mg/kg IV bwt) followed by rapid IV infusion of a 5% solution of guaiphenesin (100 mg/kg bwt) were used to induced anesthesia. The horse's trachea was intubated and the animals were positioned in right lateral recumbency on a padded cart. The endotracheal tube was connected to a semi-closed rebreathing circuit system that delivered a halothane-oxygen mixture. The facial artery was cannulated percutaneously for direct blood pressure measurement and connected to anaeroid manometer. Blood was collected from the jugular vein for biochemical analysis before anaesthesia (hour 0, baseline) and 1, 2, 8, 24, and 48 hr post anaesthetic induction. The enzymes creatine kinase (CK) and isoenzymes (CK-MB, CK-MM), aspartate transaminase (AST) and lactate dehydrogenase (LDH) were analyzed. No signs of post-anaesthetic complications were observed in the immediate post-operative period. In conclusion our data support that in short procedures, there are enzymatic changes even without clinical signs of myopathy.

Key words: horses; enzymes; isoenzymes; myopathy

Introduction

Mortality and morbidity are in close relationship to patient preoperative condition before prolonged anaesthesia and surgery. Mortality/morbidity rates in equine anaesthesia are higher than in other domestic

species (Tevick, 1983; Young & Taylor, 1993). Two major complications account for the greater anaesthetic risk. First, intraoperative profound cardiovascular depression and pulmonary dysfunction which may occur even in healthy animals, and second, a prolonged intra and postoperative recumbency (Dismore & Hall, 1993).

Post-anaesthetic myopathy is only observed in the recovery period and attempts to detect if any signs during anaesthesia have been disappointing. Often the first signs are unsuccessful attempts of the animal to stand on its own, and soon become apparent that some muscle groups are abnormally functioning, usually those that were on the underside of the recumbent horse during anaesthesia. Muscles are swollen, firm, painful, and non-functional and the horse behaves with considerable distress.

Nineteen factors, which contribute to post anaesthetic lameness in horses, have been addressed (Richey et al., 1990). These factors were divided as (1) intrinsic: breed, weight, age, sex, degree of fitness, history of exercise-induced myopathy; (2) extrinsic: season of the year, drug administration before induction, intra-operative drug administration, induction technique, anaesthetic maintenance, rate of fluid administration, duration of anaesthesia, type of procedure performed, recumbent position; and (3) physiological: hypotension, hypoxemia, hypercarbia, and acidemia. Myopathy causes uncomfortable situation for the animal, resulting hyperventilation, tachycardia, sweating, and dehydration. When the muscle cells are damaged, its contents are liberated. Consequently, in acute situations the serum enzymes creatine kinase (CK), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) are markedly elevated.

The purpose of the present study was to determine enzymatic changes (CK-MB, CK-MM), AST and LDH in response to short periods of normotensive (> 65 mmHg) anaesthesia, similar to that observed in the clinical setting.

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Material and Methods

Six horses (5 male, 1 female), 2 to 5 years, and weighing 430 to 510 kg, from Brazilian Jockey Club (Rio de Janeiro, Brazil) were anaesthetised to perform elective arthroscopic surgery during 1:30 hr. All the animals were examined before the anaesthetic procedure. Food but not water was withheld from these horses for 12 hr. Xylazine - Bayer (0.75 mg/kg IV bwt) was administered as a preanaesthetic medication. Ketamine - Parke-Davis (2.2 mg/kg IV bwt) followed by rapid IV infusion of a 5% solution of guaiphenesin - Henrifarma (100 mg/kg bwt) was used to induced anesthesia. The horse's trachea was intubated and the animals were positioned in right lateral recumbency on a padded cart. The left (non-dependent) limbs were placed on holders and the right forelimb (dependent) was pulled forward.

The endotracheal tube was connected to a semi-closed rebreathing circuit system that delivered a halothane-oxygen mixture. The facial artery was cannulated percutaneously for direct blood pressure measurement and connected to anaeroid manometer. Mean arterial blood pressure was maintained above 65 mm Hg and Lactated Ringer solution was administered IV at a rate of 10 ml/kg/hr during anaesthesia. After anaesthetic recovery, the animal was submitted to clinical examination in order to detect signs of post-anaesthetic myopathy.

Blood was collected from the jugular vein for biochemical analysis before anaesthesia (hour 0, baseline) and 1, 2, 8, 24, and 48 hr post anaesthetic induction. The enzymes CK and isoenzymes (CK-MB, CK-MM), AST and LDH were analyzed. The venous blood samples were centrifuged and analyzed at the same day they were collected. The samples were analyzed by spectrophotometer ISP #, using Merck laboratorial kits*. # Viatron ISP - Vital Scientific; * GRANUTEST@ 2.5, 1.12134.0001 ck; GRANUTEST@ 2.5 PLUS, 1.12946.0001 CK-MB DS; 1.03349.0001 LDH (optimized UV test), Merck-1- test@; Monotest bAST/Teste UVN@ 736 791 to 80 ml.

Statistical analysis - All data were expressed as mean \pm standard deviation. Enzymatic data were submitted to

Bonferroni "t" test for multiple comparisons (SAS 6.0, 1995). A $p < 0.01$ was used to indicate statistical differences.

Results

No signs of post-anaesthetic complications were observed in the immediate post-operative period. All the animals had a smooth and uneventful recovery, without signs of muscle weakness, swelling or lameness. The animals could walk normally quite after and on the following days of anaesthetic exposure.

There was a significant ($p < 0.01$) increase in CK and its isoenzymes values obtained 8 and 24 hr post-induction. During the anaesthetic procedure there was a slight, but non-significant increase in both AST and LDH (Table).

Discussion

It has hypothesised that halothane might be a triggering factor in post-anaesthetic myopathy. Some animals showing post-anaesthetic myopathy also had an abnormal halothane-caffeine contracture test a sign of malignant hyperthermia susceptibility. However maintained six standardbred horses on isoflurane vaporised in oxygen anaesthesia for 3 hr twice. During normotensive (> 80 mm Hg) and hypotensive anaesthesia (50-65 mmHg) blood gases were kept within the normal limits with the aid of controlled ventilation when indicated. Three animals showed severe signs of post-anaesthetic myopathy during recovery in hypotensive anaesthesia and were euthanatised. Therefore post-anaesthetic myopathy is closely linked to inadequate blood pressure during anaesthesia rather than to a reaction to a specific agent, and rigorous care must be taken to maintain normotension.

Most authors agree that arterial blood pressure is one of the major causes of post-anaesthetic myopathy (Grandy et al., 1987; Lindsay et al., 1989; Muir, 1991; Dobromyllskij, 1993; Young, 1993) An anaesthetised horse positioned on lateral recumbency, and correctly positioned on a thick padded cart, has a mean intracompartimental pressure between 30 and 40 mmHg. The difference between blood

Table — Mean \pm SD values of serum creatine kinase (CK) and isoenzymes muscle and brain-CK (CK-MB) and muscle-CK (CK-MM), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) from six isoflurane anaesthetised horses basely (0), and 1, 2, 8, 24, 48 and 72 hr post-induction

Time (hr)	CK	CK - MB	CK - MM	AST	LDH
Normal values	100 - 300	$\pm 14\%$ CK	CK - CKMB	150 - 400	> 250
0	318 ± 112^a	29 ± 16^a	289 ± 102^a	87 ± 15^a	250 ± 79^a
1	309 ± 98^a	24 ± 10^a	284 ± 89^a	88 ± 18^a	447 ± 191^a
2	329 ± 48^a	25 ± 7^a	304 ± 47^a	87 ± 25^a	480 ± 303^a
8	2670 ± 1165^b	264 ± 105^b	2406 ± 1071^b	82 ± 17^a	428 ± 153^a
24	1574 ± 1114^b	181 ± 122^b	1392 ± 995^b	103 ± 24^a	393 ± 190^a
48	481 ± 131^a	45 ± 11^a	436 ± 121^a	154 ± 92^a	300 ± 92^a

Different letter in the same column indicates statistical significant values ($P < 0.01$). $CK_{total} = CK-MB + CK-MM$

arterial pressure and intracompartmental pressure has to be higher than 30 mmHg to yield adequate perfusion (Whithesides et al., 1975). Mean arterial blood pressure obtained from the animals in this study was 67 mmHg, therefore sufficiently to allow muscle perfusion (White & Suarez, 1986).

Creatine kinase was increased (1.235 ± 813 IU/l) 6 hr after halothane normotensive (80-90 mmHg) anaesthesia for a 4 hr period and the magnitude of increase was greater (9.337 ± 2.348 IU/l) and long-lasting (48 hr) after hypotensive anaesthesia (50-55 mmHg) (Lindsay et al., 1989). Steffey et al. (1990) observed a greater CK value (57.150 IU/l) in 5 hr halothane anaesthesia spontaneously breathed horses, positioned in sternal recumbency.

Small, non-significant, increases in CK values 30 min after a 3:30 hr period of halothane induced hypotensive anaesthesia (55-65 mm Hg) have been reported (Muir, 1991). A large CK increase (3.383 ± 4.350) was observed after the horses stood and may reflect postural changes and improved tissue perfusion to previously damaged areas and/or further muscle destruction during the anaesthetic recovery. Stover et al. (1988) studied five horses anaesthetised (1 hr/day) on three successive days and non-significant biochemical parameter increase was detected, probably because short anaesthetic period.

In this study enzyme values for CK and isoenzymes (CK-MB) and (CK-MM) were increased at 8 (2670 ± 1165 IU/l) and 24 (1574 ± 1114 IU/l) hr post-induction of anaesthesia. The animals stood smoothly, without signs of post anaesthetic lameness. This data agree with previous work where asymptomatic horses often have high CK values, and disastrous cases with large muscle damage can show CK values > 50000 IU/l (Young & Taylor, 1993).

We measured two isoenzymes of CK. A rise in the serum levels of certain isoenzymes (CK-MM) is more valuable than the total CK value for diagnosing muscle damage. Isoenzyme determination is a more expensive and time-consuming process than assaying total CK. The values of CK-MB in normal situations are $\pm 14\%$ of the total CK. CK-MB values lower than 6% indicate an unbalanced CK-MB/CK-MM ratio and consequently muscle damage (Cardinet, 1989). In this study CK-MB and CK-MM increased in the same proportion, consequently muscle damage was insufficient to change relation between the two isoenzymes.

The enzyme AST is commonly used to indicate hepatocellular damage. Apart from liver, it is also present in heart and skeletal muscles, and kidney and thus this enzyme serum activity will be increased in muscle and liver damage (Pearson & Craig, 1980). AST is markedly elevated in acute severe muscle damage (Muir, 1991). Routine training may turn cell membrane less susceptible to damage and/or the extra cellular changes noxious to it and so reduce normal wear and tear processes (Mullen et al., 1979; Cribb, 1988). The activities of AST recorded in

this study support this hypothesis, and become lower than those obtained in well trained thoroughbreds.

Increases in mean AST values (309 ± 54 IU/l) during hypotension anaesthesia (605 ± 280 IU/l) 1 hr after anaesthesia have been reported (Muir, 1991). In this study AST did not change significantly during the first 24 hr post-anaesthesia, where a slight non-significant increase at this point 24 ($103,4 \pm 24,6$ IU/l) and 72 hr ($154,8 \pm 92,1$ IU/l) was observed. Dobromyllskij (1993) and Cardinet (1989) obtained similar results during normotension (85-95 mmHg) at 24-48 hr after anaesthesia.

Lactate dehydrogenase is present in many tissues and catalyses the reduction of pyruvate to lactate. Muir (1991), Dobromyllskij (1993) and Whithesides et al. (1975), reported that this enzyme is increased in muscle damage. An increase in LDH was observed after the horses were turned over, indicating that values increased only after reperfusion to dependent muscles (Muir, 1991). We observed a smooth non-significant increase (1 to 24 hr post-induction) in LDH, as reported by Cardinet (1989).

Conclusions

We concluded that an increase in CK values could be observed after short halothane based anaesthetic procedures without clinical signs of myopathy. The increase in CK-MM values was not sufficient to cause misbalance between the two CK isoenzymes studied.

Resumo

Alterações sérico enzimáticas em cavalos atletas anestesiados com halotano

Seis cavalos (5 machos, 1 fêmea) de 2 a 5 anos, pesando entre 430 e 510 kg, provenientes do Jockey Clube Brasileiro, no Rio de Janeiro, foram anestesiados e submetidos a cirurgia artroscópica eletiva, com duração média de 1 h 30 min. Todos os animais foram examinados antes do procedimento anestesiológico e submetidos a jejum alimentar por 12 h. Como medicação pré-anestésica foi utilizada xilazina (0,75mg/kg.IV) e, para indução anestésica, foram utilizadas a ketamina (2,2mg/kg.IV) seguida por rápida infusão venosa de uma solução de éter gliceril guaiacol a 5% (100mg/kg). Os animais foram intubados, posicionados em uma mesa em decúbito lateral direito e conectados a um circuito valvular, semi-fechado com absorção de CO₂ e mantidos com uma mescla de halotano/oxigênio. A artéria facial foi canulada percutaneamente e conectada a um manômetro anaeróide para a mensuração direta da pressão arterial. Foram coletadas amostras de sangue para análises bioquímicas antes da anestesia (tempo zero) e nos tempos 1, 2, 8, 24 e 48 h após a indução anestésica. Foram mensuradas as enzimas creatina kinase (CK) e suas isoenzimas (CK-MB, CK-MM), aspartato transaminase (AST) e lactato

desidrogenase (LDH). Durante o período pós-operatório imediato, nenhum sinal clínico de complicações pós-anestésicas foi observado. Podemos concluir que, mesmo em procedimentos anestesiológicos de curta duração, existem alterações enzimáticas sem presença de sinais clínicos de miopatia.

Palavras chave: cavalo; enzimas; isoenzimas; miopatia

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