BIOCHEMICAL PARAMETERS OF EVALUATION OF RENAL AND HEPATIC FUNCTION OF COLIC HORSES SUBMITTED TO LAPAROTOMY THAT HAVE SURVIVED OR NOT

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ABSTRACT

We examined 46 adult horses - six healthy, as control, (group 1), and 40 horses with colic submitted to treatment by laparotomy. Twenty animals had no postoperative complication (group 2), and twenty died or were euthanized from seven to ten days after the surgery (group 3). There was an increase in serum urea and creatinine concentration and AST, FA and GGT activity of animals from group 1 and group 2, indicative of renal and hepatic injury. The changes were associated with dehydration and endotoxins. Depending on the severity of the colic, animals may develop kidney and liver failure.

KEYWORDS: acute abdomen; clinical pathology; horse.

PARÂMETROS BIOQUÍMICOS DE AVALIAÇÃO DA FUNÇÃO RENAL E HEPÁTICA DE EQUINOS COM CÓLICA SUBMETIDOS A LAPAROTOMIA, SOBREVIVENTES OU NÃO

RESUMO

Foram examinados 46 equinos adultos, seis animais hígidos (G1) e 40 com cólica, submetidos à laparotomia. Desses, vinte recuperaram-se sem intercorrência pós-operatória (G2) e 20 foram a óbito ou foram sacrificados sete a dez dias após a cirurgia (G3). Houve aumento na concentração sérica de ureia e de creatinina e na atividade da aspartato aminotransferase, fosfatase alcalina e gama glutamiltransferase nos animais do G2 e do G3, indicativo de lesão renal e hepática. As alterações foram associadas à desidratação e às endotoxinas. Animais que apresentam distúrbio gastrointestinal mais severo são mais propensos a desenvolverem insuficiência renal e hepática, as quais contribuem negativamente para o prognóstico.

PALAVRAS-CHAVE: abdômen agudo; cavalo; patologia clínica.

INTRODUCTION

Renal and liver diseases secondary to severe gastrointestinal disorders are relatively common in horses and, although the progression to kidney and liver failure is rare (DIVERS, 2005), severe changes in these organs relate negatively with the recovery of horses with colic (DAVIS et al., 2003). The alterations are due to the action of endotoxins, persistent dehydration, use of nephrotoxic and non-steroidal anti-inflammatory drugs, disseminated intravascular coagulation, metabolic and electrolyte

abnormalities (GROOVER et al., 2003; GEOR, 2007).

Biochemical tests are essential for the differential diagnosis of kidney and liver disease and/or failure, and, when evaluated over time, may assist in prognosis. Specific liver enzymes include sorbitol dehydrogenase (SDH) and gamma-glutamyl transferase (GGT), which reflect hepatocellular and biliary injury, respectively. Aspartate aminotransferase (AST) and alkaline phosphatase (AP) also reflect hepatocellular and biliary injury, respectively, but they are not specific to the liver (DIVERS, 2005). Typically, the evaluation of serum concentrations of urea and creatinine is recommended for the diagnosis of renal diseases (AROSALO et al., 2007).

The aim of this study was to investigate possible changes in the enzymatic activity of GGT, AST, AP and serum urea and creatinine of horses with colic, submitted to laparotomy, which survived or not.

MATERIAL AND METHODS

We used 46 adult horses of different breeds and sex, which constituted three experimental groups. The control group (G1) consisted of six healthy horses, two females, three castrated males and one non-castrated male, submitted to exploratory laparotomy and experimental intestinal manipulation. The age of the animals ranged from 2 to 15 years, body condition score from three to four, and body weight of 295.9 kg.

Group 2 consisted of horses with colic, which were submitted to laparotomy for correction of nephrosplenic incarceration of large colon (six animals), large colon torsion (two animals), small colon impaction (two animals), ileum impaction (four animals), large colon impaction (four animals) and inguinal hernia (two animals). Animals in Group 2 underwent an uneventful postoperative period. Group 3 was composed of twenty horses with colic, which were submitted to laparotomy for correction of large colon torsion (four animals), inguinal hernia (six animals), epiploic foramen incarceration (two animals), ileocecal intussusception (one animal) and volvulus of the small intestine (seven animals). Animals in Group 3 died or were euthanized seven to ten days after surgery. In animals of G2 and G3, the colic symptoms appeared 12 to 19 hours before the examination.

Before anesthesia, all horses with colic underwent fluid replacement therapy with Ringer's solution with lactate, calculating the volume of replacement in function to the estimated volume loss (hematocrit, %) x body weight (kg). For pain control, the animals received either flunixin meglumine (0.5 mg kg^{-1}, IV), xylazine (0.2 mg kg^{-1}, IV) or butorphanol (0.2 mg kg^{-1}, IV), and all underwent gastric emptying through a nasogastric tube.

For sedation, animals received xylazine (0.5 mg kg^{-1}, IV). Then we proceeded to pressure infusion of guaiacol glyceryl ether 10% (100 mg kg^{-1}, IV) and midazolam (0.05 mg kg^{-1}, IV), followed by ketamine (2 mg kg^{-1}, IV). After orotracheal intubation, sedation was maintained with halothane volatilized with 15 ml/kg of oxygen in a semi-closed-circuit anesthesia with spontaneous ventilation. During surgery, we administered 5 to 10 mL/kg/min (IV) of Ringer's solution with lactate to help to maintain the mean arterial pressure between 70 and 100 mmHg.

During the postoperative period, we performed antimicrobial therapy with benzathine penicillin, at a dose of 30,000UI kg^{-1}, intramuscularly (IM), every 48 hours, completing three applications, and gentamicin at a dose of 4mg kg^{-1} (IV), every 24 hours, completing five applications. We administered flunixin meglumine at a dose of 0.5 mg kg^{-1}, IV, every 12 hours, for three days, as analgesic and anti-inflammatory. We performed electrolyte replacements with Ringer's solution with lactate and saline solution, calculated in terms of maintenance rates (60 mL kg^{-1} day), and hydration of the animals, as described previously. The drugs selected and the duration of administration varied according to the severity of disease and response to the treatment.

We obtained blood samples by jugular puncture with 25x8 needles in plastic disposable syringes, immediately before anesthesia and 24, 48, 72 hours, seven and ten days after surgery. We used blood samples collected in tubes without anticoagulant for the evaluation of biochemical parameters, serum urea by UV kinetics (Diacetyl monoxiona method), creatinine colorimetric (Lustosa-Basques´ method), aspartate aminotransferase (UV kinetics method), alkaline phosphatase PNP kinetics (Roy modified method), and gamma-glutamyltransferase (Szasz modified method). Subsequently we centrifuged the samples at 2000 rpm for five minutes, and after syneresis, the serum obtained was placed in *Eppendorf* tubes, identified, and stored at -20°C until the moment of evaluations. After that, we analyzed the samples with the aid of a set of diagnostic reagents for[1] subsequent spectrophotometric readings[2]. The hematocrit was obtained in microhematocrit tubes centrifuged at 14.000G during five minutes for posterior reading

[1] Labtest - Labtest Diagnóstica S.A., Lagoa Santa - MG
[2] Labquest - CELM, modelo E-225-D

with a special scale. The trial was approved by the Ethics and Animal Welfare Committee, protocols 023232-05 and 013332-07.

We used a completely randomized design with three groups and six replications. We applied Tukey test ($P < 0.05$) for comparison of means using the SAS[3] statistical software when we observed significance between groups and moments.

RESULTS AND DISCUSSION

We verified increase in serum urea in horses of G2 and G3, before laparotomy (time 0), and maintenance in the moments 24 hours, 48 hours, 72 hours, seven and ten days in animals of G3 (Table 1). We observed similar results for the variable creatinine. The increases were associated with dehydration that can be due to the hematocrit values (Table 2). For AROSALO et al. (2007) and GEOR (2007), the activity of endotoxins, nephrotoxic drugs, such as gentamicin, the non-steroidal anti-inflammatories and the anesthetics drugs are also responsible for the onset of renal disorders.

The hypovolemic or septic shock reduces renal blood flow affecting both the perfusion pressure and vascular resistance. In such conditions, hypovolemia stimulates baroreceptors, causing compensatory peripheral vasoconstriction, also involving the renal vasculature. The increase in sympathetic activity on the kidneys primarily affects the renal afferent arterioles, and also alters the intrarenal flow distribution. The reduction in renal blood flow decreases glomerular filtration with the consequent increase in tubular reabsorption and decrease in urine flow, also resulting in increased blood urea and blood (DIVERS et al., 1987).

AROSALO et al. (2007) also pointed out dehydration as being responsible for the increase in serum urea and creatinine in equine with colic, and for the increase in toxicity of nephrotoxic drugs. Such statement could partially explain differences in serum urea and creatinine presented by animals in groups G2 and G3, which received similar drugs, yet only animals of G3 were permanently dehydrated. Additionally, the severity of gastrointestinal disorder should be considered since, in these cases, endotoxins can lead to septic shock, as previously explained.

GUNSON & SOMA (1983) observed, in a clinical trial, that horses undergoing water deprivation and concomitant administration of non-steroidal anti-inflammatories developed acute necrosis of renal papilla, whereas animals that received the same anti-inflammatory therapy, but were not water deprived, or vice versa, did not develop renal failure.

Only animals in G3 showed persistent dehydration (0 to 10 days) (Table 2), which, according to SEANOR et al. (1984) e SOUTHWOOD (2006), can lead to pre-renal azotemia and result in renal ischemia or renal failure if not corrected in time. As mentioned earlier, the horses with colic were constantly submitted to volemic therapy for replacement and hydro-electrolyte maintenance. However, it is known that endotoxins induce hypotension, which is persistent in spite of adequate administration of fluids and electrolytes (KATZ et al., 2003). Abnormal serum creatinine and urea in animals under constant hydro-electrolytic correction indicate that approximately 75% of nephrons are already damaged. However, depending on the cause and extent of damage, the kidney can still recover (AROSALO et al., 2007).

DIVERS et al. (1987) verified similar laboratory findings, besides observing, by microscopic examination, varying degrees of tubular necrosis and cortical bone in three of four horses with colic which developed acute renal failure, related to hypotension, sepsis and disseminated intravascular coagulation.

SCHULZE et al. (2004) also aimed at assessing the damage caused by the deficit of intravascular volume and / or endotoxemia in kidney structures and evaluated, by means of optical and electron microscopy, the kidneys of nine horses, of which six had shown intestinal strangulation obstruction and three, simple obstructions. Distension of the proximal tubule, increased number of apical vacuoles, increase in size, loss of brush border of epithelial cells of the proximal tubule, areas of disseminated intravascular thrombosis and interstitial edema were observed by light microscopy. Apical bubbles and luminal changes were apparent in scanning electron microscopy. Cellular necrosis and isolated epithelial cells in the tubular lumen were detected by the study of transmission electron microscopy. The findings allowed the authors to correlate the morphological changes observed in the proximal tubule with the elevations in creatinine concentration. The proximal tubule is the primary site of reabsorption of water and electrolytes, particularly sodium. Therefore, the destruction of the proximal tubule epithelium reduces one of the primary functions of the kidney.

[3] Statistical Analysis of System - version 8.

Table 1. Mean and standard error of serum urea and creatinine in healthy horses (G1), in horses with colic submitted to laparotomy, without intercurrences in the postoperative (G2), and horses with colic which died seven to ten days after surgery (G3)

Parameter	Before laparotomy	Time after undergoing laparotomy				
		24 hours	48 hours	72 hours	7 days	10 days
Urea (mg/dL)						
G1	30.4±1.8Cb	33.3±2.6Bb	35.2 ±2.4Bab	36.3 ±1.8Bb	39.9 ±2.6Ba	37.3±2.4Bb
G2	48.0±4.4Ba	34.6±3.0Bb	33.6±3.0Bb	40.8±5.6Bb	42.7±5.6Bab	40.1±4.8Bb
G3	68.5±8.3Aa	40.4±5.2Ab	39.8±8.3Ab	56.1±11.4Aab	67.4±12.9Aa	49.7±3.8Aab
Creatinine (mg/dL)						
G1	1.10±0.05Cb	1.50±0.04Ba	1.56±0.05Ba	1.60±0.05Ba	1.52±0.04Ba	1.60±0.03Ba
G2	2.07±0.14Ba	1.63±0.15Bb	1,63±0,15Bb	1.41±0.06Bb	1.38±0.06Bb	1.61±0.10Bb
G3	2.88±0.27Aa	1.91±0.10Aa	2.57±0.04Aa	2.85±0.76Aa	2.80±0.60Aa	2.53±0.06Aa

Different capital letters in the columns indicate significant differences among groups (P <0.05).
Different lowercase letters in the lines indicate significant differences between time moments (P <0.05).

Table 2. Mean and standard error of hematocrit in healthy horses (G1), in horses with colic submitted to laparotomy, without intercurrences in the postoperative (G2), and horses with colic which died seven to ten days after surgery (G3)

Parameter	Before laparotomy	Time after undergoing laparotomy				
		24 hours	48 hours	72 hours	7 days	10 days
Hematocrit (%)						
G1	29.06±0.69Ca	30.00±0.59Ba	30.60±0.69Ba	32.80±0.59Ba	32.05±0.50Ba	32.65±0.69Ba
G2	40.74±1.63Ba	33.50±2.21Bb	33.66±1.25Bb	34.84±1.10Bb	32.78±1.16Bb	34.04±1.13Bb
G3	60.72±1.93Aa	58.20±2.29Aa	55.11±1.61Aa	42.10±1.85Ab	44.58±1.36Ab	44.33±1.55A

Different capital letters in the columns indicate significant differences among groups (P <0.05).
Different lowercase letters in the lines indicate significant differences between time moments (P <0.05).

Treatment of acute kidney disease is, according to SEANOR et al. (1984), DIVERS et al. (1987) and GEOR (2007), quite simple and favorable, including the correction of hydro-electrolytic deficits and metabolic disorders. For DIVERS et al. (1987), furosemide, mannitol and dopamine should be only used in horses with oliguria. The use of low-dose dopamine was effective in restoring diuresis in one horse with changes of hemodynamic and renal functions associated with proximal duodenum-jejunitis (LEMOS et al., 2007). GEOR (2007), on the other hand, advises against the use of drugs promoters of renal blood flow and urine production. However, all researchers agree that removing the cause is paramount to the success of the treatment, and the prognosis is more favorable in non-oliguric horses, in which azotemia is resolved within 72 hours of treatment (GROOVER et al., 2006).

In this essay, corrective therapies specifically targeting the restoration of renal function and / or correction of the cause were not implemented because, as it is a casuistic essay and not an experimental one, the biochemical measurements were not performed in real time. All animals underwent hydro-electrolytic, primary and fundamental for the restoration of normal renal function, but only animals in G2 responded favorably to therapy.

The means and standard errors for the variables aspartate aminotransferase, gamma-glutamyl transferase, and alkaline phosphatase are shown in Table 3.

Table 3. Mean and standard error of aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP) in healthy horses (G1), in horses with colic submitted to laparotomy, without intercurrences in the postoperative (G2), and horses with colic which died seven to ten days after surgery (G3)

Parameter	Before laparotomy	Time after undergoing laparotomy				
		24 hours	48 hours	72 hours	7 days	10 days
AST (U/L)						
G1	172.1±18.1Ba	160.5±8.9Ba	152.1±7.0Bb	172.1±18.1Ba	160.5±8.9Ba	152.1±7.0Bb
G2	286.3±25.2Ab	376.0±34.6Ab	386.0±34.6Ab	421.0±36.2Aa	453.0±45.0Aa	407.9±42.9Aa
G3	296.9±23.9Ab	267.9±44.4Ab	377.7±57.5Ab	365.3±37.8Ab	353.9±43.9Ab	415.3±42.3Aa
GGT (U/L)						
G1	21.2±1.1Ba	18.0±1.8Bb	19.0±1.8Ab	23.3±1.8Ba	18.0±1.8Ab	21.2±1.9Ba
G2	22.0±1.6Ba	17.4±1.3Bb	18.4±1.3Ab	19.8±1.6Ba	19.4±1.3Aa	20.2±1.6Ba
G3	29.0±2.9Aa	27.6±1.9Aa	18.2±1.5Aab	28.2±1.1Aa	19.1±1.3Aab	27.5±2.9Aa
AP (U/L)						
G1	165.0±12.7Ba	166.1±14.7Ba	174.1±14.5Ba	165.0±12.7Ca	166.0±14.7Ca	174.1±14.5Ba
G2	178.2±14.9Bb	208.4±16.6Bb	200.4±16.6Bb	234.7±16.9Ba	209.9±21.0Ba	246.1±21.6Ba
G3	233.4±30.6Ab	236.6±29.2Ab	298.8±28.0Aa	391.7±48.3Aa	307.1±35.5Aa	372.4±36.8Aa

Different capital letters in the columns indicate significant differences among groups ($P < 0.05$).
Different lowercase letters in the lines indicate significant differences between time moments ($P < 0.05$).

Liver diseases are also common in horses with gastrointestinal disorders such as colic, diarrhea and / or endotoxemias, but progression to liver failure is rare. The diagnosis of liver disorders is generally based on the evaluation of serum enzymes, whereas the liver biopsy is specific for assessing liver function (BERGERO, 2008). In this essay, we observed, for the variable AST, that animals in G2 and G3 presented similar values and both groups were similar to GI at all evaluation moments. We verified increase in AP in animals in G3 at all evaluation moments. We also observed increased values in animals in G2 and G3 at the moments 72 hours and seven days. Only animals in G3 showed an increase in serum GGT activity at the moments 0, 24, 72 hours and 10 days.

Changes in serum AST activity, because it is not an hepato-specific enzyme, may also be related to lesions in the skeletal muscles, which, in horses with colic, are due to dehydration, intramuscular injections, anesthetic drugs, muscle injuries, the intestinal change itself and factors intrinsic to exploratory laparotomy (TRALL, 2007). Regarding muscle damage in the animals tested, there was a significant increase ($P < 0.05$) in creatine kinase (CK) in the animals and moments studied.

The alkaline phosphatase, although present in many tissues besides the liver, is useful for the diagnosis of liver disease, particularly cholestatic disease (FERNANDEZ et al., 2008). However, the mucosa of the small intestine in many animal species including horses, is extremely rich in AP, while the mucosa of the large intestine presents small amounts of this enzyme (DAVIES et al., 1984), which would explain the increased in AP only in the animals in G2, but four animals had localized lesions in the small intestine, besides presenting persistent dehydration, as previously mentioned.

GGT is an induction enzyme that may have its serum activity immediately increased due to acute liver injury, possibly because of the release of membrane fragments containing GGT. In the case of cholestasis, there is an increase in production, release and consequently in its activity (DURHAM et al., 2003; TRALL, 2007; BERGERO, 2008). Increased serum GGT activity was also observed by DAVIS et al. (2003) in horses with colic; however, these authors found that animals with proximal enteritis are more likely to develop liver disease than horses with small bowel strangulation obstructions. The mechanism may involve ascending infection of the organ through the bile duct, absorption of toxins and inflammatory mediators through the portal circulation, blockage of the bile duct or liver hypoxia associated with systemic inflammatory response syndrome (SIRS) and with the shock (DURHAM et al., 2003; BERGERO, 2008).

CONCLUSIONS

Horses with colic present changes in serum urea and creatinine and in the activity of aspartate

aminotransferase, alkaline phosphatase and gamma-glutamyl transferase, indicative of renal and liver diseases, respectively. Animals showing more severe gastrointestinal disorder are more likely to develop kidney and liver failure, which contribute negatively on prognosis.

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