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Morphological abnormalities and apoptosis in lamellar tissue of equines after intestinal obstruction and treatment with hydrocortisone

[*Alterações morfológicas e apoptose em tecido laminar de equinos após obstrução intestinal e tratamento com hidrocortisona*]

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ABSTRACT

Four experimental groups of equines were used in order to study morphological abnormalities and apoptosis in lamellar tissue. Group Cg (control) was composed of animals without any surgical procedure; group Ig (instrumented), animals that underwent enterotomy; group Tg (treated), animals that were subjected to intestinal obstruction and were treated with hydrocortisone; and group Ug (untreated), animals that were subjected to intestinal obstruction without treatment. The lamellar tissue was analyzed regarding the presence of tissue abnormalities and apoptosis. No morphological abnormalities were observed in animals of surgical groups, and no difference in apoptosis was observed between groups. It was concluded that intestinal obstruction allowed laminitis to develop, probably by systemic activation, and that the maneuvers performed in the enterotomy aggravated the process. Hydrocortisone did not aggravate the lesions of the lamellar tissue.

Keywords: equine, laminitis, apoptosis, intestinal obstruction, morphological abnormalities

RESUMO

Foram utilizados quatro grupos de equinos para estudar alterações morfológicas e apoptose no tecido lamelar. O grupo CG (controle) foi composto por animais sem o procedimento cirúrgico; o grupo Ig (instrumentado), por animais submetidos à enterotomia; o grupo Tg (tratado), por animais submetidos à obstrução intestinal e tratados com hidrocortisona; e o grupo Ug (não tratado), por animais submetidos à obstrução intestinal, sem tratamento. O tecido laminar foi analisado quanto à presença de alterações morfológicas e de apoptose. Foram observadas alterações morfológicas nos equinos submetidos a procedimento cirúrgico, mas não houve diferença entre os grupos com relação às células apoptóticas. Conclui-se que a obstrução intestinal permite o desenvolvimento da laminite provavelmente por ativação sistêmica e que as manobras realizadas na enterotomia podem ser consideradas como agravantes no processo. A hidrocortisona não agravou as lesões do tecido laminar.

*Palavras-chave**:** equino, laminite, apoptose, obstrução intestinal, alterações morfológicas*

INTRODUCTION

Laminitis occurs secondarily to other diseases, such as those of the gastrointestinal tract that follow a course involving sepsis (Dabareiner et al., 1998; Eades et al., 2002). It is known that metalloproteinases (MMP) 2 and 9 are involved in the process of laminitis for excessive degradation of collagens types IV and V and laminin, which are crucial structural elements of the laminae. These enzymes are responsive for physiological control of hoof growth, and can be activated by means of inflammatory mediators, like tumor necrose factor alfa (TNF-α) and cytokines (Han et al., 2000; Belknap and Black, 2005; Black et al., 2006) in inflammatory conditions.

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There is also evidence that apoptosis is involved in lamellar lesions. Faleiros et al. (2004) observed that the number of apoptotic cells is low in the equine hoof during the normal physiologic state and higher in animals showing acute and chronic laminitis. The apoptosis occur due to activation of caspases in cell cytosol, causing cell death that differs from necrosis, without inducing an evident inflammatory response (Hathaway and Kuechle, 2002; Creagh et al., 2003).

Several studies show that laminitis in prodromal phase is associated with inflammatory response, for elevation in inflammatory mediators, like TNF- α, cytokines, and reactive oxygen species (Loftus et al., 2007). Since laminitis is associated with systemic inflammation, administration of anti-inflammatory agents can prevent it from appearing for reducing the systemic inflammatory response (Campbell et al., 2007). However, this approach is questionable when it is observed that laminitis has developed after glucocorticoids administration (Pollitt, 1999; Johnson et al., 2002).

The objective of this study was to analyze the morphological and apoptotic abnormalities in hoof lamellar tissue after intestinal obstruction and to evaluate the effect of administering hydrocortisone.

MATERIAL AND METHODS

Twenty, males or females, mixed breed, healthy equines without presenting locomotor abnormalities were used. The control group (Cg, n = 7) was composed of normal animals obtained from a commercial slaughterhouse (Pomar Slaughterhouse and Cold Store – Araguari, Brazil). The lamellar tissue material of the other groups was obtained from another study ("Experimental obstruction of the jejunum of equines with or without hydrocortisone treatment: clinical, hematological, biochemical, and anatomopathological characteristics", approved by the Animal Wellbeing and Ethics Committee, FCAV-UNESP, Jaboticabal, SP – protocol number 013598-06), in which the animals were randomly distributed into three groups and underwent a surgical procedure: instrumented group (Ig, n = 5), in which the surgical procedure involved maneuvers carried out by means of enterotomy, with manipulation of the loops and incision of the jejunum in order to emplace a balloon (without inflation); treated group (Tg, n = 4), with jejunal obstruction induced by the inflation of the balloon and intravenously administration of hydrocortisone (Solu-Cortef, Rhodia Farma), at a dose of 4mg/kg; and untreated group (Ug, n = 4), with jejunal distension caused by balloon inflation, without receiving treatment.

The animals had previously been subjected to fasting and water deprivation and were then sedated with 10% xylazine hydrochloride (Sedazine, Fort Dodge) and butorphanol (Torbugesic, Fort Dodge). Local anesthesia was achieved by means of the inverted "L" technique, using 2% lidocaine hydrochloride (Xilestesin, Cristália). The middle third of the jejunum was exposed through the left paracostal fossa, in a quadrupedal position, and was subjected to intramural compression of the intestinal wall by implanting an empty latex balloon in the lumen, by means of enterotomy (Faleiros et al., 2002). The balloon was then inflated with air until reaching a pressure of 12mmHg. After remaining inflated for four hours, the balloon was removed. Then, after 18 hours of circulatory reperfusion, the animal was sacrificed by means of an overdose of anesthetic (Thiopentax, Cristália).

Metacarpophalangeal disarticulation was carried out on both thoracic limbs. The hoof was sectioned in the medial plane (Pollitt, 1996) to remove lamellar fragments. These were then fixed in a 10% formol solution for 24 hours. Subsequently, they were transferred to a 70% alcohol solution, processed and embedded in paraffin. Sections of 5µm thickness were cut, and these were stained by means of the hematoxylin-eosin (HE) and periodic acid-Schiff (PAS) histochemical methods. The lesions were classified in accordance with the methodology of Pollitt (1996), and they are presented in Table 1.

Table 1. Descriptions of the histological lesions observed, corresponding to the scores established for evaluating fragments of lamellar tissue[1]

Score	Histological characteristics
0	SEL with rounded and delimited extremity, interfingering with SDL; rectilinear PELc with rounded extremity; oval and apical nuclei of BC; and BM with well-defined outline.
0.5[2]	PEL and SEL with normal architecture, with the presence of rounded BC nuclei; but still with apical location.
1.0	Thin SEL that are difficult to identify individually; rounded BC nuclei close to the BM; and PEL with less thin and undulating extremities.
1.5[2]	Thin and elongated SEL, adhering to each other; rounded BC nuclei close to the BM; PEL with thin and undulating extremities; but the BM is at a normal distance from the PEL. BM with irregular outline.
2.0	All the aforecited abnormalities, with significantly greater distance between the keratinized axes of the PEL and the BM. The latter is serrated and fragmented in PAS staining.
3.0	Severe destruction of lamellar architecture. Fragmentation and absence of BM, with SEL extremities significantly thinned and elongated, forming an amorphous mass of tissue between the PEL and PD.

[1]Pollit (1996).
[2]Intermediate values used in this study for classifying of lesions.
SEL: secondary epidermal lamina; SDL: secondary dermal lamina; PEL: primary epidermal lamina; BC: basal cell; BM: basal membrane; PAS: periodic acid-Schiff staining; PDL: primary dermal lamina.

The presence of apoptosis in the lamellar tissue was evaluated by means of immunohistochemistry using lamellar fragments embedded in paraffin and sectioned in slices of 3µm. The sections were deparaffinized in a heated chamber at 60ºC, followed by washes in xylol and alcohol at decreasing concentrations. For antigen recovery, a citrate buffer (pH 6.0) was used. Blocking of endogenous peroxidase was carried out using a commercial blocker (TA-060-HP, Hydrogen Peroxidase Block, Lab Vision). The sections were incubated with primary anti-rabbit anti-caspase 3 antibodies (ASP 175, Cell Signaling) for 15 hours, at a dilution of 1:700. Next, they were incubated with the biotin-streptavidin complex (LSAB, K0690, Dako). The chromogen used was diaminobenzidine (K3468, Dako) and counterstaining was performed with Harris hematoxylin. The positive control for caspase 3 was a section through an equine lymph node, and the negative control consisted of omitting the primary antibody of the tissue, and thus only incubating the antibody diluent (S3022, Dako). The cells immunolabeled by caspase 3 were counted in relation to the area of the histological section (cells/mm^2) in hoof epidermal tissue.

The results regarding morphological abnormalities and apoptotic cells were taken to be the means from observations on each right and left limbs. The statistical method was the Kruskal-Wallis test, and differences of groups were investigated using Dunn's multiple comparison test ($P<0.05$).

RESULTS

No locomotion abnormalities were observed after the surgical procedure. The morphological abnormalities that were observed using optical microscopy consisted of spherical nuclei of basal cells, thinning of the secondary epidermal lamina, undulation of the primary epidermal lamina, and irregular outline of the basal membrane, for staining with periodic acid-Shiff (PAS), among the animals in the Ig, Tg, and Ug groups (Figure 1). These alterations were classified with level of lesions in 0, 0.5, 1.0, and 1.5, that were saw in lamellar tissue (Table 1). Some animals in the control group (Cg) presented slight abnormalities, to those score 0.5 was attributed. In relation to level of alterations, there was no difference between the animals in the Ig, Tg, and Ug groups. However, the animals in the groups with intestinal obstruction (Tg and Ug) presented a difference in relation to Cg. The animals in Ig did not differ from those in the other groups (Figure 2).

The immunolabeling for caspase 3 was nuclear in the basal cells of secondary epidermal lamellae, and there was no labeling in the negative controls (Figure 3). Immunolabeled cells were observed in all groups, with no differences among them (Figure 4).

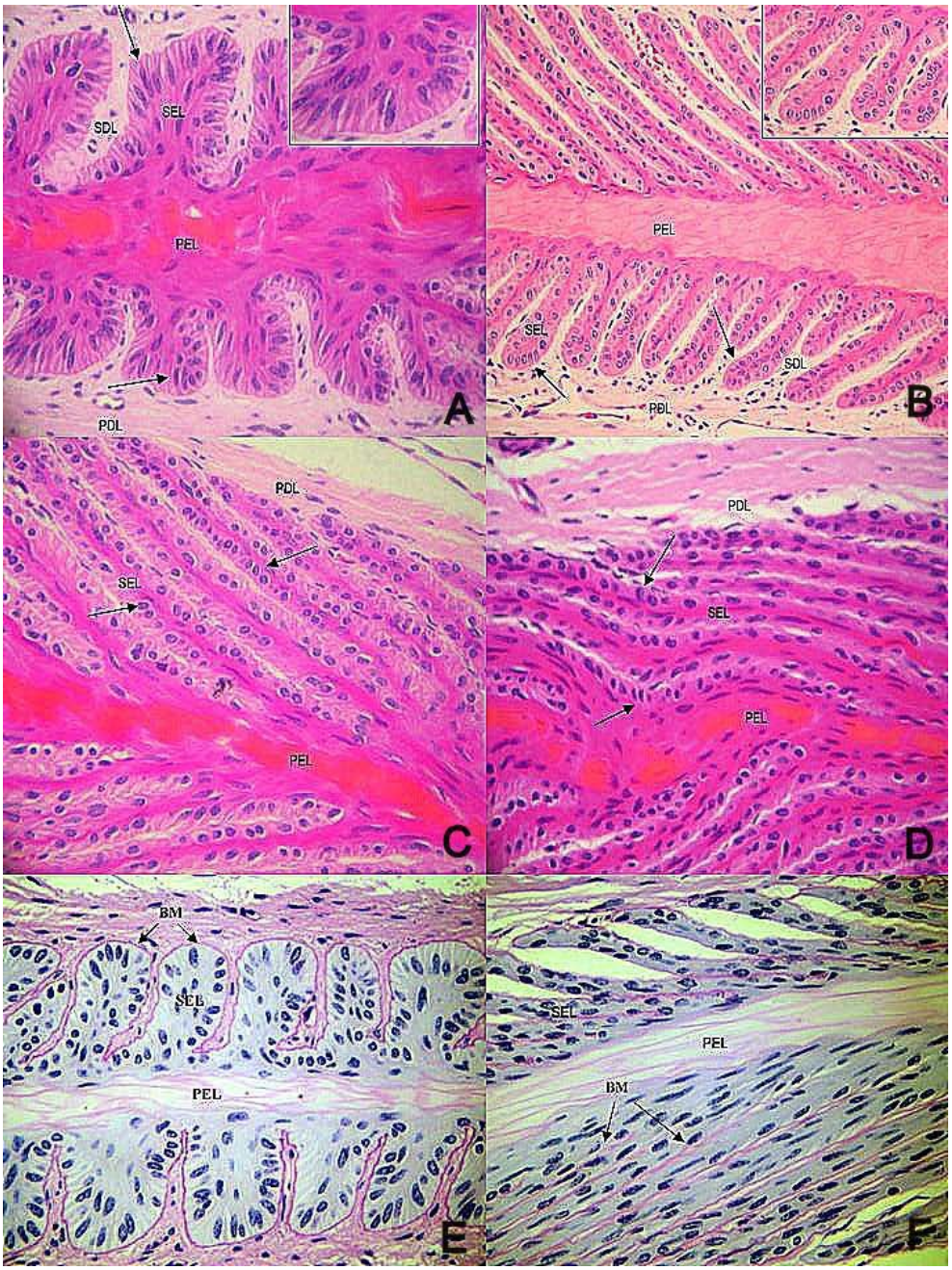

Figure 1. Photomicrographs of laminas from equine hoofs in the experimental groups**. (A)** Presence of basal cell (BC) nuclei that are oval and apical (arrows) and secondary epidermal laminas (SEL) with rounded extremities (Cg, score 0, HE, obj.40x). **(B)** Thinning of some SELs (dotted black arrows), SELs with rounded extremity (dotted white arrows), presence of spherical BC nuclei (continuous black arrows) and oval nuclei (continuous white arrows) (Ig, score 0.5. HE, obj.20x). **(C)** Spherical BC nuclei close to BM (black arrows), thinning of the SEL extremities and reduction of the space between them (white arrow) (Tg, score 1, HE, obj.40x). **(D)** Spherical BC nuclei (arrows) and pronounced thinning of the SEL extremities (Ug, score 1.5, obj.40x). **(E)** Whole basal membrane (BM) (arrows) (Cg, score 0, PAS, approximated to obj.40x). **(F)** BM with irregular outline and smudged (arrows) in some segments of the SEL (Tg, score 1, PAS, obj.40x). (B) Tg, score 1, demonstrating absence of BM (arrows, insert) in some segments of SEL (PAS, obj.40x).

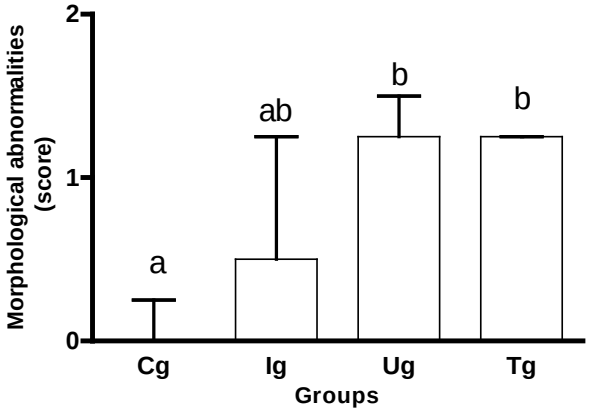

Figure 2. Medians and interquartile deviations of the scores for morphological abnormalities of the laminar tissue observed in the control and experimental groups (Ig, Tg and Ug). Scores in columns marked with the same letter do not differ (P>0.05).

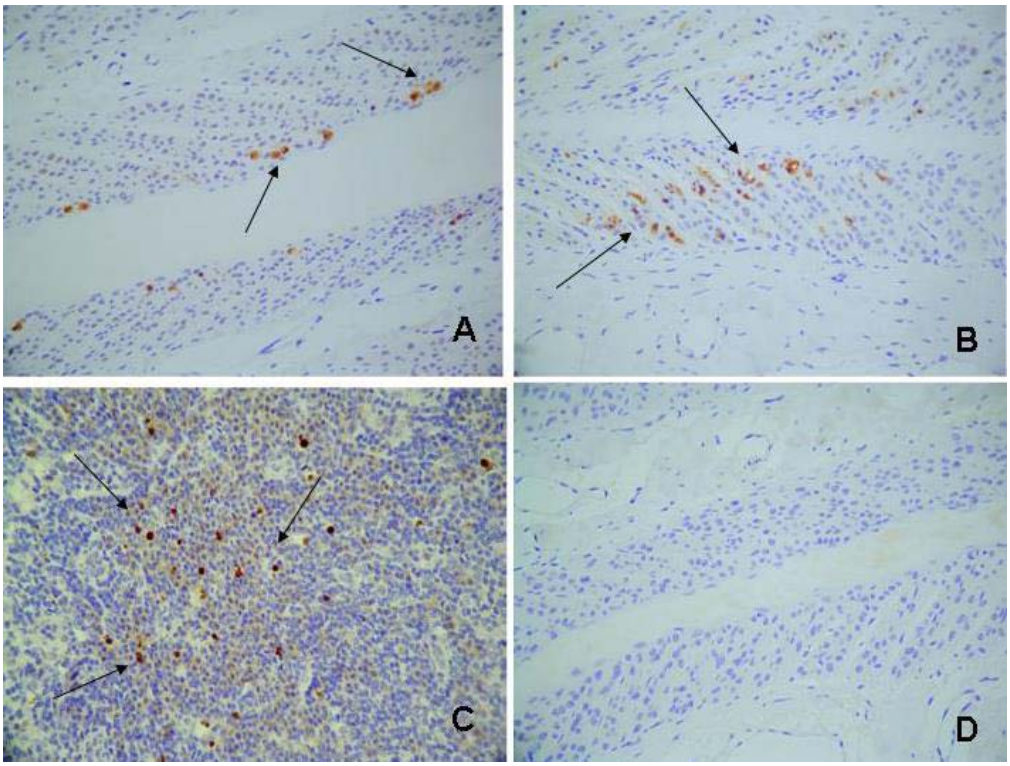

Figure 3. Immunohistochemistry for caspases 3 and 14. Photomicrographs of the equine lamellar tissue (A, C, D, F); photomicrograph of equine lymph node (B) and photomicrograph of equine skin (E). **(A)** Immunolabeling for caspase 3 in the basal cell (BC) nuclei (arrows, Ug). **(B)** Positive control for caspase 3 (arrows). **(C)** Negative control for the immunohistochemical reaction for caspase 3 (Tg). **(D)** Negative immunolabeling for caspase 14 (Tg). **(E)** Positive control for caspase 14 (arrow). **(F)** Negative control for the immunohistochemical reaction for caspase 14 (Tg). Streptavidin/biotin immunohistochemical method (LSAB kit, chromogen DAB, obj. 40x).

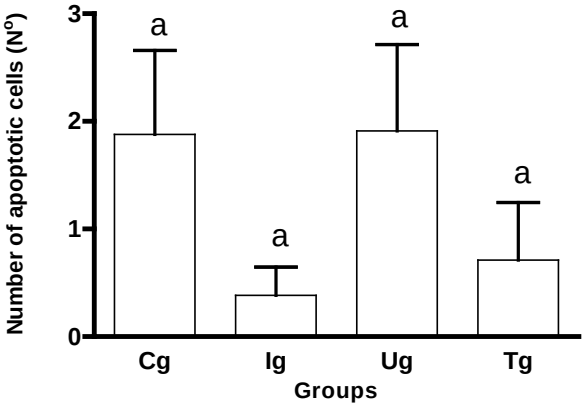

Figure 4. Means and standard deviations for the number of apoptotic cells in the control and experimental groups (Ig, Tg, and Ug). Scores in columns marked with the same letter do not differ (P>0.05).

DISCUSSION

The main morphological abnormalities that were observed in the lamellar tissue and classified with grades 1 and 1.5, as described in the results, characterize the initial events of laminitis, according to Croser and Pollitt (2006). These abnormalities were higher among the animals in Ig, Tg, and Ug, and some histological sections of this horses demonstrated basal membrane with a smudged appearance, with an undefined outline in some segments. This characteristic can be related to degradation of the basal membrane during the initial phase, probably by MMP, that are collagenotic enzymes (Croser and Pollitt, 2006). This process leads to failure of the union between the dermal and epidermal laminas (Pollitt, 1996; Pollitt and Daradka, 1998; Croser and Pollitt, 2006).

All the animals in the groups subjected to the surgical procedures presented morphological abnormalities, in varied grades. The difference in the severity of the abnormalities observed between the obstructed groups (Tg and Ug) and Cg indicates that the damage caused by intestinal ischemia can give rise to a predisposition towards laminitis.

Experimental findings have demonstrated that there is a systemic inflammatory response that contributes towards the initial phase of laminitis (Black et al., 2006; Hurley et al., 2006; Loftus et al., 2007; Noschka et al., 2009). In the same way as in organ failure among humans, this can cause lamellar abnormalities such as distant lesions in others organs (Belknap et al., 2009). The intestinal injury model has been used in other species as a means of inducing sepsis, which is intrinsically related to systemic inflammation (Benjamin, 2001). This may also occur because of the lesions from intestinal ischemia/reperfusion (Bianco-Blatlles et al., 2008), and this probably occurred during the obstruction of the lumen in the animals of the present experiment. Faleiros et al. (2002) reported, under similar conditions, that distension of the small colon induced pulmonary accumulation of neutrophils.

The tissue lesions that were found among these equines were more severe than those reported by Rio Tinto et al. (2004), who analyzed horse lamellar tissue after intestinal lesion. This may have occurred because those authors used intestinal reperfusion of 12 hours, i.e. less than the 18 hours used here. These data indicate that the severity of the lamellar lesions is related to the duration of the intestinal injury.

The absence of difference between the lesions found among the horses without obstruction (Ig) and those in the obstructed groups (Tg and Ug) demonstrates that enterotomy can reveal abnormalities in lamellar tissue even if they are less severe, since there were no differences in the lesions between Ig and Cg. Enterotomy, with manipulation of the intestinal loops, causes the release of prostacyclins (Gottlieb et al., 1989) and predisposition towards translocation of intestinal bacteria (Reddy et al., 2006). This would be related to the development of the

systemic inflammatory response, and would explain the lamellar abnormalities found among the animals that underwent enterotomy. Hiki et al. (2006) demonstrated that there are significantly higher inflammatory cytokine counts and systemic endotoxin concentrations in pigs subjected to traditional gastrectomy, with manipulation of the intestinal loops, than when laparoscopy is used. Moreover, it can be suggested that, according to the ischemia theory, these abnormalities were caused by vasoconstriction mediated by the prostacyclin, as described by Menzies-Gow et al. (2008).

According to Pollitt (1996), morphological abnormalities classified with scores greater than 1 induce clinical signs of laminitis, and such abnormalities were observed among the animals of this study (score 1.5). Considering that laminitis secondary to a condition of acute abdomen evolves 24 to 48 hours after the onset of this condition (White, 1990), these animals could have developed the postural and locomotor abnormalities, characteristic of this disease, if they had not been sacrificed after 18 hours of reperfusion. However, it is impossible to affirm that the condition would be one of laminitis with clinical symptoms. Such alterations might have been subclinical, given that it has been found that only 25% of animals with gastrointestinal disorders develop clinical signs of laminitis (Cohen et al., 1994).

The subtle abnormalities (score 0.5) observed among some animals in the Cg (15%) represent the possibility of occurrences among normal animals, induced by systemic inflammatory processes of low severity.

Immunolabeling for caspase 3 was observed in the histological sections from the animals of this study, but the number of immunolabeled cells did not vary between the groups. This demonstrates that, during the initial phase of the lamellar lesion, there is no excessive activation of caspases, considering that the data on the groups subjected to surgical procedures did not differ from the data on the Cg group. Apoptosis is physiologically present in lamellar tissue, as observed in the present study and also by Faleiros et al. (2004) and Souza (2007), thereby promoting cell renovation. Faleiros et al. (2004) reported an increased apoptosis rate among horses with naturally acquired laminitis with up to one week of evolution, compared with animals at the prodromal stage or with chronic laminitis. Souza (2007) observed increased numbers of apoptotic cells among equines 48 hours after inducing laminitis. Thus, with results of this study and the former ones, maybe occurrences of apoptosis should be related to the severity of the lesions of laminitis that are found after the disease onset.

Although there is no hard evidence to support the aforecited hypothesis, it can be envisaged that activation of the apoptotic cascade is related to tissue regeneration and renovation in laminitis. This would constitute an attempt to reestablish the structural integrity of the lamellar tissue, considering that the tissue lesions were triggered without their participation and that these events occur in normal tissue, probably with the same function.

Administration of glucocorticoids in high doses and for prolonged periods leads to the development of laminitis (Johnson et al., 2002), probably due to the induction of insulin resistance (Tiley et al., 2007) or reduction of blood perfusion in the hoof (Cornelisse et al., 2006). So, it can must state that hydrocortisone aggravated the morphological abnormalities in the animals subjected to surgical procedures (Tg), because there was no difference with the untreated group. This corroborates the findings of Rio Tinto et al. (2004).

CONCLUSIONS

The model of intestinal injury due to jejunal obstruction was efficient for reproducing the conditions present in the gastrointestinal disorders that lead to lamellar tissue lesions. The results also suggest that the tissue damage promoted by the maneuvers performed during the enterotomy also favor occurrences of this disease. Administration of hydrocortisone did not aggravate the laminar abnormalities, thus suggesting that its therapeutic use does not present a risk of developing laminitis.

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REFERENCES

BELKNAP J.K.; BLACK S.J. 2005. Review of the pathophysiology of the developmental stages of equine laminitis. In: ANNUAL CONVENTION OF THE AMERICAN ASSOCIATION OF EQUINE PRACTITIONERS, 51., 2005, Seattle. Proceedings… Seattle, USA. Disponível em: http://www.ivis.org/proceedings/aaep/2005/belknap/chapter.asp?LA=1. Acessado em: 31 out. 2007.

BENJAMIN, C.F. Atualização sobre mediadores e modelos experimentais de sepse. *Medicina*, v.34, p.18-26, 2001.

BELKNAP, J.; MOORE, J.N.; CROUSER, E.C. Sepsis - from human organ failure to laminar failure. *Vet. Immunol. Immunopathol.*, v.129, p.155-157, 2009.

BIANCO-BLATLLES, M.D.; SOSUNOV, A.; POLIN, R.A. et al. Systemic inflammation following hind-limb ischemia - reperfusion affects brain in neonatal mice. *Develop. Neurosci.*, v.30, p.367-373, 2008.

BLACK, S.J.; LUNN, D.P.; YIN, C. et al. Leukocyte emigration in the early stages of laminitis. *Vet. Immunol. Immunophatol*., v.109, p.161-166, 2006.

CAMPEBELL, R.C.; PEIRÓ, J.R.; ROSA, P.C.S. et al. Endotoxemia por lipopolissacarídeo de *Escherichia coli*, em equinos: efeitos de anti-inflamatórios nas concentrações sérica e peritonial do fator de necrose tumoral alfa (TNF-α). *Arq. Bras. Med. Vet. Zootec.*, v.59, p.837-843, 2007.

COHEN, N.D.; PARSON, E.M.; SEAHORN, T.L. et al. Prevalence and factors associated with development of laminitis in horses with duodenitis/proximal jejunitis: 33 cases. *J.Am.Vet. Med. Assoc.*, v.204, p.250-254, 1994.

CORNELISSE, C.J.; ROBINSON, N.E.; BERNEY, C.A. et al. Thermographic study of *in vivo* modulation of vascular responses to phenylephrine and endothelin-1 by dexamethasone in the horse. *Equine. Vet. J.*, v.38, p.119-126, 2006.

CREAGH, E.M.; CONROY, H.; MARTIN S.J. Caspase-activation pathways in apoptosis and immunity. *Immunolology Reviews.*, v.193, p.10-21, 2003.

CROSER, E.L.; POLLITT, C.C. Acute laminitis: descriptive evaluation of serial hoof biopsies. In: ANNUAL CONVENTION OF THE AMERICAN ASSOCIATION OF EQUINE PRACTITIONERS, 52., 2006, San Antonio, TX. *Proceedings*… San Antonio, TX, USA., Disponível em: <http://www.ivis.org/Proceedings/aaep/2006/croser/chapter.asp?LA>. Acessado em: 10 jan. 2009.

DABAREINER, R.M.; WHITE, N.A.; DONALDSON, L. Evaluation of Carolina rinse as a treatment for ischemiareperfusion of the equine jejunum. *Vet. Surg*., v.27, p.521, 1998.

EADES, S.C.; ASHLEY, M.S.; HOLM, D.V.M. et al. A review of the pathophysiology and treatment of acute laminitis: phatophysiologic and therapeutic implications of endothelin-1. *AAEP Proc.*, v.48, p.353-361, 2002.

FALEIROS, R.R.; MACORIS, D.G.; ALESSI A.C. et al. Effect of intraluminal distention on microvascular perfusion in the equine small colon. *Am. J. Vet. Res.*, v.63, p.1292-1297, 2002.

FALEIROS, R.R.; STOKES, A.M.; EADES, S.C. et al. Assessment of apoptosis in epidermal lamellar cells in clinically normal horses and those with laminitis. *Am. J. Vet. Res.*, v.65, p.578-585, 2004.

GOTTLIEB, A.; SKRINSKA, V.A.; O'HARA, P. et al. The role of prostacyclin the mesenteric traction syndrome during anesthesia for abdominal aortic reconstructive surgery. *Ann. Surg.*, v.209, p.363-367, 1989.

HAN, Y.; TUAN, T.L.; WU, H. et al. TNF-α stimulates activation of pro-MMP-2 in human skin through NF-κB mediated induction of MT1-MMP. *J. Cell Sci*., v.114, p.131-139, 2000.

HATHAWAY, T.R.; KUECHLE, M.K. Apoptosis and cutaneous biology. *Adv. Dermatol.*, v.18, p.287-311, 2002.

HIKI, N.; SHIMIZU, N.; YAMAGUCHI, H. et al. Manipulation of the small intestine as a cause of the increased inflammatory response after open compared with laparoscopic surgery. *Br. J. Surg.*, v.96, p.195-204, 2006.

HURLEY, D.J.; PARKS, R.J.; REBER, A.J. et al. Dynamic changes in circulating leukocytes during the induction of equine laminitis with black walnut extract. *Vet. Immunol. Immunophatol.*, v.110, p.195-206, 2006.

JOHNSON, P.J.; SLIGHT, S.H.; GANJAM, V.K. et al. Glucocorticoids and laminitis in the horse. *Vet. Clin. N. Am.* :*Equine Pract.*, v.18, p.219-236, 2002.

LOFTUS, J.P; BLACK, S.J.; PETTIGREW, A. et al. Early laminar events involving endothelial activation in horses with black walnut-induced laminitis. *Am. J. Vet. Res*., v.68, p.1205-1211, 2007.

MENZIES-GOW, N.J.; BAILEY, S.R.; BERHANE, Y. et al. Evaluation of the induction of vasoactive mediators from equine digital vein endothelial cells by endotoxin. *Am. J. Vet. Res.*, v.69, p.349-355, 2008.

NOSCHKA, E.; VANDENPLAS, M.L.; HURLEY, D.J. et al. Temporal aspects of laminar gene expression during the developmental stages of equine laminitis. *Vet. Immunol. Immunopathol.*, v.129, p.242-253, 2009.

POLLITT, C.C. Basement membrane pathology: a feature of acute equine laminitis. *Equine Vet. J.*, v.28, p.38-46, 1996.

POLLITT, C.C. Equine laminitis: a revised pathophysiology. *AAEP Proc.*, v.45, p.188-192, 1999.

POLLITT, C.C.; DARADKA, M. Equine laminitis basement membrane pathology: loss of type IV collagen, type VII collagen and laminin immunostaining. *Equine Vet. J.*, v.26, suppl, p.139-144, 1998.

REDDY, B.S.; GATT, M.; SOWDI, R. et al. Surgical manipulation of the large intestine increases bacterial translocation in patients undergoing elective colorectal surgery. *Colorectal Dis.*, v.8, p.596-600, 2006.

RIO TINTO, J.J.M.; ALVES, G.E.S.; FALEIROS, R.R. et al. Utilização de hidrocortisona em eqüinos submetidos a isquemia e reperfusão no jejuno e suas consequências sob o cório lamelar. *Arq. Bras. Med. Vet. Zootec*., v.56, p.292-299, 2004.

SOUZA, A.H. Aspectos clínico, fisiopatológico e terapêutico na laminite experimental em equinos. 2007. 219f. Tese (Doutorado) - Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, Jaboticabal.

TILEY, H.A.; GEOR, R.J.; McCUTCHEON, L.J. Effects of dexamethasone on glucose dynamics and insulin sensitivity in healthy horses. *Am. J. Vet. Res.*, v.68, p.753-759, 2007.

WHITE, N.A. Intensive care, monitoring, and complications of acute abdominal disease. In: WHITE, N.A. *The equine acute abdomen*. Philadelphia: Lea & Febiger, p.309-335, 1990.