Retrospective Study

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Evaluation of low-molecular-weight heparin for the prevention of equine laminitis after colic surgery

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Abstract

Objectives – The aim of this study is to describe the prevalence of postoperative laminitis in colic cases and to determine if low-molecular-weight heparin (LMWH) is effective in preventing this complication.
Design – Retrospective clinical study.
Animals – Client-owned horses.
Interventions – SC administration of enoxaparin during the postoperative period.
Measurements and Main Results – Medical records of 360 horses undergoing surgery for colic and surviving at least 3 days were evaluated. Fifty-six horses admitted before 1995 did not receive LMWH (control group) and 304 admitted after 1995 received LMWH as a prophylaxis for laminitis (treatment group). Three grades of severity were defined for laminitis. Prevalence and severity of laminitis were compared between the 2 groups. Several parameters recorded on admission (sex, age, breed, site and nature of the disease, heart rate, PCV, gravity score, and shock score) and the administration of LMWH were tested as risk factors in the development of laminitis in a logistic regression procedure. Prevalence and grade of laminitis were significantly lower in the treatment group. Only the absence of LMWH was recognized as a significant risk factor in the logistic regression model.
Conclusions – The administration of LMWH appears to be effective in the prophylaxis of laminitis following colic surgery and may be useful in the postoperative management of these horses.

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Keywords: colic, enoxaparin, fractionated heparin, horses, postoperative intensive care

Introduction

Laminitis is a painful systemic disease of complex etiology characterized by an aseptic diffuse pododermatitis. It is a well-recognized secondary disease occurring especially in horses suffering from acute gastrointestinal tract diseases such as strangulating obstruction, inflammatory bowel disease (anterior enteritis and enterocolitis), and grain overload.[1–3] According to Pollitt,[4] laminitis can be defined as a failure of the attachment between the distal phalanx and the inner hoof wall leading to pain and characteristic lameness. In a study evaluating prevalence and factors associated with the development of laminitis in horses with proximal enteritis by using physical examination data, clinicopathological data, and the initial treatment recorded on admission to the clinic, laminitis was only significantly correlated with weight and hemorrhagic reflux (1985–1991).[5] In another study on risk factors in the development of laminitis during hospitalization, patient characteristics (age, breed, and sex), results of laboratory testing, and comorbid disease states were evaluated, and using multivariate analysis laminitis was associated to endotoxemia.[3] Nevertheless, it is interesting to note in this last study that abdominal surgery for colic was significantly related to laminitis in the univariate model.[3]

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Modifications in the circulating white cell counts and leukocyte infiltration were reported in the prodromal stage of induced laminitis.[6,7] Concentrations of various inflammatory markers in the laminar tissues such as myeloperoxidase (MPO),[8] mRNA of interleukin-1β,[9] cyclooxygenase-2[10] and a cytokine-associated nuclear protein[11] were also higher at the same stage of development of laminitis. The presence of these inflammatory mediators is consistent with the inflammatory nature of the laminitis and its close relation to endotoxemia.

Increased concentrations of endothelin-1, a potent vasoconstrictor, released from endothelial cells during inflammatory response, were found in the laminar tissues of horses with laminitis.[1] Likewise, platelet activation and development of coagulation are known to promote endothelial activation and leukocyte margination into tissues in human sepsis. These elements of the hemostasis system probably play a similar role in the early stage of laminitis.[12] Laminitis may thus be linked to local ischemia due to hemodynamic alterations[13] and microthrombi that were found in the laminar tissues in the early stage of the disease.[14–16] Disseminated intravascular coagulation was suspected in the development phase of laminitis but evidence of systemic activation of the coagulation system was not apparent in several studies.[16–18] Because of its anticoagulant properties, heparin was used to prevent laminitis, but its beneficial effects in laminitis remained controversial. Evidence supporting the use of unfractionated heparin (UFH) for laminitis prevention has been variable in the literature.[5,19] Low-molecular-weight heparins (LMWH) are widely used in humans for their preventive and curative effects on venous thrombosis and thromboembolism after general, orthopedic, or neurosurgery.[20] The anti-inflammatory properties of UFH and LMWH are supported by clinical evidence in burns, asthma, rheumatoid arthritis, and inflammatory bowel diseases.[21,22] The effects of heparins on inflammatory cells have also been reported, in particular their inhibition of superoxide anion production by neutrophils, reduction of granulocyte activity, and attenuation of neutrophil migration.[21,22]

Although Hunt et al[23] indicated that laminitis was responsible for 2.4% of the postoperative fatalities in colic cases, little data are available concerning the prevalence of laminitis following laparotomy for colic. The treatment of laminitis remains difficult, the outcome uncertain, and the prophylaxis of questionable effectiveness. Despite our encouraging subjective clinical impressions, no objective analysis of the use of fractionated heparins has been made. In this report, we describe the results of a retrospective study performed to evaluate the clinical effects of fractionated heparin in preventing laminitis during the postoperative period in horses admitted for colic surgery.

Material and Methods

Animal selection

The medical records of horses referred to our clinic (Equine Clinic, Large Animal Surgery, University of Liège) between 1991 and the onset of 2007, were evaluated for inclusion in the study. Only the records of horses with intestinal disorders requiring surgical intervention and surviving at least 72 hours after surgery were selected for the retrospective study. The horses selected were divided into 2 groups. The first group included animals admitted to the clinic before 1995, which did not receive LMWH as prophylactic treatment against postoperative laminitis (control group). The second group consisted of horses admitted from 1995 until 2007, which all received LMWH as preventive treatment (treatment group). Rate and dosage of LMWH consisted of single daily SC administration of 0.35 mg/kg of enoxaparin.[a] The first injection was administered after the recovery from anesthesia and injections were continued for at least 3 days. Over the test period, all the surgical interventions and postoperative management were supervised by the same senior veterinarians. Other postoperative medical treatment included administration of antibiotics (ampicillin or penicillin-gentamycin), nonsteroidal anti-inflammatory drugs (flunixin meglumine and phenylbutazone), polyionic infusions (lactated Ringer's solution), analgesic medication (α_2-agonist), and nasogastric decompression. Over time, specific antibiotics, nonsteroidal anti-inflammatory drugs, α_2-agonist administration changed following the availability of new drugs, but no other drug classes or supportive care were introduced. Frog supports were systematically applied on forelimbs after surgery. In addition, acepromazine[b] (0.033 mg/kg, IM, q 8 h), dimethyl sulfoxide[c] (1 g/kg in 10% solution, IV, q 24 h for 3 d), or both were administered to horses that developed laminitis. Horses showing symptoms of laminitis at the time of admission were excluded from the study.

Laminitis grade determination

Horses developing laminitis during hospitalization were classified using the following grading system. They were classified as grade 1 when they showed bounding digital pulse and hot feet without lameness, and a further progression of the clinical signs that resolved within a few hours or days without development of laminitis; the clinical observations were thus interpreted as indicators for potential onset of laminitis. Grade 2 was defined as horses suffering from clinical

laminitis that were not consequently euthanized. Diagnoses were established by senior clinicians on the basis of the presence of clinical signs of active laminitis during the postoperative period: strong digital pulse, pain, lameness, toe-relieving, and heel-loading with or without localized depression of the coronary bands or other signs of pedal bone displacement, including radiographic evidence. The key indicators of grade 2 were the presence of pain and lameness. All horses from grade 2 successfully recovered with treatment. The grade 3 laminitis group included horses that were euthanized following development of severe and refractory laminitis with uncontrollable pain, breakthrough of the pedal bone, or both.

Shock status, gravity score, and disease classifications

On the basis of the data from the medical records, animals were evaluated for gravity (GS) and shock scores (SS) as described by Grulke et al.[24] The GS assessed the severity of intestinal obstruction and was based on the evaluation of 4 parameters (rectal palpation, borborygmi, abdominal distension, and pain). To each parameter, a grading value from 1 to 3 was attributed. The highest value of any 1 of the 4 parameters determined the final GS. The SS was based on 6 cardiovascular parameters (heart rate, respiratory rate, PCV, systolic arterial pressure, blood lactate concentration, and blood urea), to which a value ranging from 1 to 4 was attributed. As above, the highest value attributed to any 1 of the 6 parameters determined the final SS. The survival rate was shown to vary significantly according to attributed GS as well as SS in a study of 200 horses suffering from a wide range of intestinal diseases (small, large, strangulated, and non-strangulated diseases).[24]

Each horse was also classified according to the location of the disease (small versus large intestine) and the nature of the pathology (strangulated versus non-strangulated). Pathology was considered strangulated as soon as the obstruction blocked the vascularization. Horses with concomitant small and large intestine pathology were classified according to the site of the most important injury. However, for the strangulating status, horses with both conditions were classified in the strangulated disease group.

Determination of prevalence, severity, and risk factors for laminitis

The period prevalence was calculated for all horses, those from the control group and those from the treatment group, either by using all horses with laminitis or only animals with laminitis grades 2 and 3. The difference of prevalence was assessed by comparison of the proportion of laminitis within each group (control versus treatment group) using a χ^2 test[d] (2×2 contingency table). The severity of postoperative laminitis was also compared between the 2 groups using a Mann-Witney test.[d]

The relationship between the development of postoperative laminitis (all grades; horses with versus horses without laminitis) and the independent continuous variables (age, weight, heart rate, and PCV) were examined with a Student *t*-test.[e] The relationship between the development of laminitis and the independent categorical variables such as the sex (mares, geldings, stallions), the breed (Belgian Warmblood or ponies versus others), the location of the main injury (small versus large intestine), the nature of the disease (strangulated versus non-strangulated disease), the GS, the SS, and the preventive administration of enoxaparin (enoxaparin or not) were assessed using a χ^2 test.[e]

Variables with $P < 0.2$ were introduced in a stepwise multiple logistic regression model to obtain the effects of variables associated with laminitis, corrected for the effects of the other variables.[e] For all statistical testing, a $P < 0.05$ was considered significant.

Results

Three hundred and sixty horses met the conditions of the study and were selected. Fifty-six were in the control group (no LMWH) and 304 in the treatment group (LMWH administration). The number of horses for each group, the data registered from the characteristics, the condition (laminitis or not) and the grade of postoperative laminitis are summarized in Table 1. Information concerning the location, the nature, and the gravity of the intestinal disease on admission are shown in Table 2. The total prevalence of laminitis for both control and treatment group was 4.44% (95% confidence interval [CI], 2.65–7.26%). The prevalence of horses developing laminitis in the treatment group (3.29%; 95% CI, 1.68–6.15%) was significantly lower than in the control group (10.71%; 95% CI, 4.43–22.55%) ($P = 0.049$) (Figure 1). Furthermore, when considering only animals suffering from clinically significant laminitis (grades 2 and 3), the prevalence was unchanged in the control group and reduced to 0.32% (95% CI, 0.01–0.02%) in the treatment group (Figure 1). Overall, the laminitis grade was 1.68 ± 0.87. Laminitis grade was significantly less severe in the treatment group (1.1 ± 0.31) than in the control group (2.66 ± 0.51) ($P = 0.002$) (Figure 2). Mortality due to laminitis in horses suffering from this disease was 66.7% (4/6) in the control group. No fatal laminitis was observed in the LMWH treatment group. Among the horses in the treatment group only 1 developed clinically established laminitis (grade 2) with a rapid resolution. All the other

Table 1: Patient characteristics and laminitis grade of the control and treated groupsobjectsource>

	Number of horses	Breed*		Age (year) (mean ± SD)	Weight (kg) (mean ± SD)	Sex*			Postoperative Grade of laminitis*		
		Belgian Warmblood	Others			Mare	Gelding	Stallion	Grade 1	Grade 2	Grade 3
No LMWH Group (control)											
Laminitis	6	4	2	10.6 ± 5.86	530 ± 54.77	2	3	1	0	2	4
No Laminitis	50	36	14	8.44 ± 5.68	523 ± 121.55	26	13	11			
All Horses	**56**	**40**	**16**	**8.64 ± 5.67**	**524.64 ± 114.8**	**28**	**16**	**12**			
LMWH Group (treatment)											
Laminitis	10	7	3	11.7 ± 5.91	490 ± 119.04	6	0	4	9	1	0
No Laminitis	294	197	97	9.38 ± 5.37	508 ± 112.55	119	128	47			
All Horses	**304**	**204**	**100**	**9.46 ± 5.39**	**507.53 ± 112.57**	**125**	**128**	**51**			
All Groups	**360**	**244**	**116**	**9.34 ± 5.44**	**509.84 ± 112.86**	**153**	**144**	**63**	**9**	**3**	**4**

*Number of horses. LMWH, low-molecular-weight heparin.

Table 2: Location, nature, and gravity of the intestinal disease at admission according to the preventive LMWH administration and the development of a postoperative laminitis

	Number of horses	Location of the main injury*		Nature of the Injury*		Gravity Score*			Shock Score*		
		Small Intestine	Large Intestine	Strangulated	No Strangulated	1	2	3	1	2	3
No LMWH Group (control)											
Laminitis	6	3	3	4	2	0	5	1	2	2	2
No Laminitis	50	21	29	24	26	3	35	12	26	21	3
All Horses	**56**	**24**	**32**	**28**	**28**	**3**	**40**	**13**	**28**	**23**	**5**
LMWH Group (treatment)											
Laminitis	10	6	4	8	2	0	5	5	9	1	0
No Laminitis	294	110	184	158	136	29	171	94	209	79	6
All Horses	**304**	**116**	**188**	**166**	**138**	**29**	**176**	**99**	**218**	**80**	**6**
All Groups	**360**	**140**	**220**	**194**	**166**	**32**	**216**	**112**	**246**	**103**	**11**

*Number of horses. LMWH, low-molecular-weight heparin.

animals were classified as grade 1, which only reflects a suspicion of laminitis development.

The univariate analyses showed that variables collected on admission were not relevant in the development of postoperative laminitis. The only factor that significantly influenced the prevalence of postoperative laminitis was the preventive administration of enoxaparin immediately following surgery. The final logistic regression confirmed that the absence of the administration of enoxaparin was a risk factor in the development of postoperative laminitis ($P = 0.02$; odds ratio, 0.27; 95% CI, 0.092–0.77).

Discussion

In this study, we used a historical case-control group consisting of horses admitted before 1995. At that time we initiated LMWH administration following surgery for colic. Taking into account the severe and painful character and the consequences of laminitis on the outcome for the horses, the creation of a new control group by omission of the administration of LMWH in recent years, was not ethically defensible. Furthermore, the study was based on naturally occurring disease in client-owned horses, so that the best treatment available was mandatory. The perioperative management of horses was similar during the entire period of the study. We did not consider the minor modifications reported above as the cause of the sudden change in the prevalence of postoperative laminitis.

As expected from our clinical impression, the statistical analysis of this study confirmed that LMWH administration appeared to reduce the prevalence and the severity of postoperative laminitis in our hospital. Interestingly, no horse in the control group was classified as grade 1 laminitis. These observations are noteworthy,

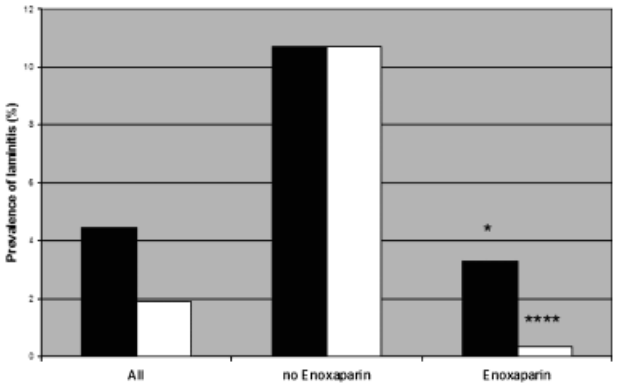

Figure 1: Prevalence of laminitis after colic surgery either in all horses (All), horses which received enoxaparin as preventive treatment against laminitis (Enoxaparin) and horses that did not receive enoxaparin as preventive treatment (no Enoxaparin). Results are presented for the 3 grades of laminitis (■) and for the grades 2 and 3 only (□). *$P < 0.05$; ****$P < 0.001$ between groups.

especially when laminitis was the cause of death in 2.4% of the postoperative colic cases.[23] The authors suggested that heparin should be included in the preventive treatment of laminitis when endotoxemia or hypoperfusion is suspected.[23] UFH was reported as being effective in preventing laminitis during proximal enteritis, but researchers have raised questions about this result and underlined the necessity of further prospective investigation.[5] Reduction of the prevalence of laminitis was also described in horses with small intestinal disorders requiring surgical correction but the reduction did not attain statistical significance.[19] As the effectiveness of UFH could be based on its anticoagulant properties, the variability in response to this treatment may be linked to reduced antithrombin activity in horses with intestinal strangulation. The variation in the timing of administration and dosage could also explain the differences observed between studies.[19,25] The use of LMWH might explain the discrepancies observed with the use of UFH. To the authors' knowledge, our study reports for the first time the capacity of a specific type of heparin to reduce the occurrence of postoperative laminitis following intestinal disease requiring surgical correction.

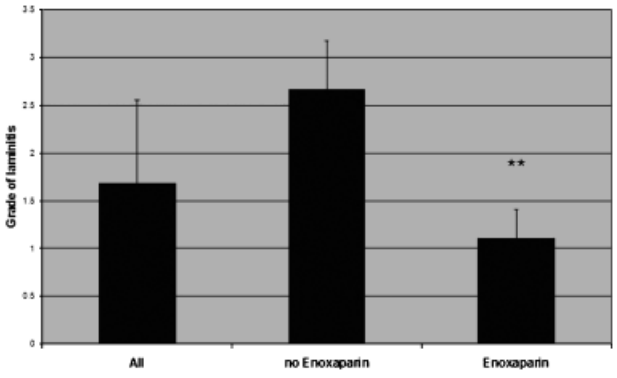

Figure 2: Mean grade (± SD) of laminitis after colic surgery either in total horses (All), horses that received enoxaparin as preventive treatment against laminitis (Enoxaparin) and horses that did not receive enoxaparin (no Enoxaparin). **$P < 0.01$ between groups.

Surprisingly, we did not observe significant effects arising from the location, type, SS and GS on the development of laminitis. However, development of laminitis was marginally associated (univariate analysis) with small intestine diseases, strangulated diseases, and the SS. In surgical colic cases, patients with small intestinal disease generally present as a strangulated obstruction[24] and intestinal ischemia leads to the shock following endotoxemia.[26] Thus, these data agree with previous results in which endotoxemia was correlated with the development of laminitis.[3] The results of this study are limited by the small sample size of the laminitis group and the study design (retrospective case control) and need to be verified by future prospective controlled studies of this important issue.

LMWH is widely used in human medicine and does not present important adverse effects. The preventive dose for deep venous thrombosis after general surgery in humans is 20–40 mg enoxaparin (SQ, q 24 h), according to the thromboembolic risk level of the patient.[27] These doses correspond to a range of 0.28–0.57 mg/kg for an average 70 kg human. Therefore, the dose of enoxaparin we used (0.35 mg/kg) in horses was similar to that usually adopted in human medicine.

The pharmacokinetics of UFH and LMWH are different. The binding to circulating and cellular proteins is less for LMWH than for UFH. Consequently, the clearance of UFH is faster than that of LMWH,[20] the bioavailability and the plasma half-life are greater for LMWH than for UFH at low dose levels, and the anticoagulant response is more predictable for LMWH.[28–32] These properties allow the clinical use of LMWH SC without monitoring of the anticoagulant effects,[20] whereas UFH necessitates either a continuous IV infusion or SC injections with an initial dose sufficient to counteract its restricted bioavailability,[29,33] and monitoring of the coagulation status (based on the activated partial thromboplastin time).[20] Moreover, to prevent venous thrombosis in general surgical patients, low doses of LMWH administered SC once daily proved to be as effective and safe as UFH administered in the same way 2 or 3 times daily, with a similar small risk of bleeding.[20] A study in horses concluded that LMWH may be used more safely and conveniently than UFH.

The SC administration of UFH twice a day or LMWH once daily allowed for the attainment of an adequate concentration in plasma for the purpose of prophylaxis (venous thrombosis) but LMWH administration achieved sufficient concentrations after the first injection without altering clotting and bleeding time during the treatment.[34] Heparin-induced thrombocytopenia is an adverse effect of heparin treatment in humans, which can be complicated by thrombotic events.[35] In horses, thrombocytopenia and erythrocyte agglutination were also associated with heparin and could impair microcirculation.[25,34] Both in humans and horses the use of LMWH reduced the risk of development of these complications as compared with UFH.[34,35] Thus, pharmacokinetic advantages of LMWH could explain the differences between the 2 types of heparins in the prophylaxis of laminitis and could play a role in our results.

Recently, a study performed by Riggs et al[8] showed neutrophil activation and degranulation with release and infiltration of MPO in the laminar tissues during the prodromal stage of induced-laminitis. Likewise, we demonstrated the capacity of the venous and arterial endothelial cells from the distal limb to take up active MPO and the inhibitory effects of UFH and LMWH on the MPO activity and on the MPO capture by these endothelial cells.[36] However, the MPO could intervene in several factors implicated in the pathophysiology of laminitis such as on the nitric oxide (NO) availability or on the matrix metalloproteinases (MMP).

In laminitis, NO depletion could be one of the mechanisms leading to local vasoconstriction and subsequent ischemia. Baldus et al[37] reported that MPO enhances the catabolism of NO during myocardial ischemia and reperfusion. Cell-bound MPO could thus lead to the lamellar hypoperfusion occuring during laminitis, either by vasomotor dysfunction by the way of NO depletion or by the formation of microthrombi enhanced by oxidative injuries on endothelial cells. Moreover, the importance of NO as an inhibitor of neutrophil-endothelial cell interactions has been demonstrated.[38] These findings are interesting when we consider the capacity of heparins to restore vasodilator function by increasing bioavailability of NO.[39] Additionally, LMWHs have shown protective effects on inflammatory response by inhibiting neutrophil activation (notably based on MPO assay) and release of proinflammatory cytokines (tumor necrosis factor, interleukin-12) in experimentally induced colitis or hepatic ischemia-reperfusion injuries in rats.[40,41]

The enzymatic theory of laminitis suggests that laminitis triggering factors induce the production and activation of the MMP-2 and -9 from keratinocytes and neutrophils, in the laminar tissues.[42] Induction of the MMP-2 and -9 production is mediated by proinflammatory cytokines such as the interleukin-1β, the tumor necrosis factor-α and by the transforming growth factor.[43,44] The activation of MMP depends on several serine proteases such as trypsin, plasmin, neutrophil elastase, and cathepsin G, but also on reactive oxygen species.[43,45] MPO is responsible for the production of hypochlorous acid, one of the most potent oxidant agent, and could therefore be responsible for the activation of the pro-MMP in laminar tissues. As mentioned above, LMWH inhibits MPO activity and its uptake by endothelial cells.[36] Thus, LMWH would have the power to inhibit the MMP activation pathway by acting on the enzymes that transform the pro-MMP into active MMP. These properties could explain that heparins have beneficial effects on laminitis through properties other than the anticoagulant ones.

Conclusions

The literature reports interesting properties of LMWHs in diverse clinical applications in humans. However, to the authors' knowledge, the present study is the first one that reports the successful use of LMWH in the prevention of equine laminitis. This study opens the field to prospective evaluation and necessitates further investigation to confirm our results. Additionally, more fundamental investigation into the mechanisms of the action of LMWH in the prevention of laminitis and generally in vascular inflammatory diseases should be conducted.

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Footnotes

[a] Clexane, Aventis Pharma NV/SA, Boulevard de la Plaine, Brussels, Belgium.
[b] Combistress, KELA Laboratoria N.V., Hoogstraaten, Belgium.
[c] Dimethylsulfoxide, V.M.D. NV/SA, Arendonk, Belgium.
[d] GraphPad Instat, GraphPad Software Inc, San Diego, CA.
[e] SAS, SAS Institute Inc, Cary, NC.

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