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Original Research

Relationship Between Plasma and Peritoneal Fluid Concentrations of D-dimer and Transforming Growth Factor Beta 1 in Horses With Colic

David Argüelles [a,b], María A. Delgado [a], Lara Armengou [a], Jorge U. Carmona [c], Marta Prades [a,*]

[a] Departament de Medicina i Cirurgia Animals, Facultat de Veterinària, Universitat Autonòma de Barcelona, Cerdanyola del Vallès, Spain
[b] Department of Equine and Small Animal Medicine, Faculty of Veterinary Medicine, University of Helsinki, Helsinki, Finland
[c] Grupo de Investigación Terapia Regenerativa, Departamento de Salud Animal, Universidad de Caldas, Manizales, Colombia

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ABSTRACT

The aim of the study was to analyze the correlation between D-dimer and transforming growth factor beta 1 (TGF-β_1) in plasma and peritoneal fluid of horses with different types of colic. The study included 124 horses with colic and 12 control horses. The horses with colic were grouped according to diagnosis (obstructions, enteritis, ischemic problems, peritonitis, and other mixed problems), results of the peritoneal fluid analysis (transudate, modified transudate, and exudate), and outcome (survivors and nonsurvivors). There were significant weak correlations between the evaluated proteins and the diagnosis, the outcome, and the type of peritoneal fluid. However, when specific colic groups were evaluated significant ($P = .0001$), moderate correlations were observed between plasma D-dimer and peritoneal TGF-β_1 concentrations in the enteritis (0.67) and mixed (0.82) colic groups. The results suggest a concomitant activation of the fibrinolysis system and the peritoneal local compensatory anti-inflammatory response mediated by TGF-β_1 in horses with colic due to enteritis and other problems excluding obstructions, ischemic problems, and peritonitis.

1. Introduction

The procoagulant activity of mesothelial cells deposits fibrin as part of the repair process to peritoneal injury. It is aided mainly by the release of mesothelial plasminogen activator inhibitor type 1, a fibrinolysis inhibitor that helps fibrin formation. Fibrinolytic activity is also activated by the secretion of a tissue-plasminogen activator, which is essential to destroy the fibrin formed after various gastrointestinal (GI) injuries and to decrease subsequent risk of adhesion formation [1,2]. Thus, activation of peritoneal fibrinolysis activity produces an increase in the peritoneal D-dimer concentration, which is a degradation fragment released exclusively by the plasmin-mediated lysis of cross-linked fibrin [3].

Peritoneal D-dimer is a specific marker of peritoneal fibrinolysis activity and, subsequently, of peritoneal fibrinogenesis. The test for measuring plasma and peritoneal D-dimer concentration has been used for assessing plasma hypercoagulation and hyperfibrinolysis in horses [4,5]. Plasma D-dimer concentration is a sensitive marker in assessing fibrinolytic activity and thus coagulation activity. Any increase in D-dimer concentration is related to an increase in fibrin destruction (hyperfibrinolysis), subsequently to an increase in fibrin formation (hypercoagulation or hyperfibrinogenesis) [3].

* Corresponding author at: Marta Prades, Departament de Medicina i Cirurgia Animals, Facultat de Veterinària, Universitat Autonòma de Barcelona, Edifici V, 08193, Cerdanyola del Vallès, Spain
E-mail address: marta.prades@uab.cat (M. Prades).

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Transforming growth factor beta 1 (TGF-β_1) is a pleiotropic protein involved in cellular proliferation induction, connective tissue deposition, and cellular death processes [6]. However, the most important function of that protein is controlling several immune-tolerance mechanisms by modulating the proliferation, differentiation, and survival of several types of lymphocytes [7]. Thus, in conjunction with other proteins such as interleukin 10, TGF-β_1 is considered to be one of the most powerful anti-inflammatory proteins in mammals. Both proteins are upregulated when systemic inflammatory response syndrome is unchained in horses with diseases that are severe or which have a poor prognosis, such as extensive trauma, sepsis, or cancer, among others [8].

Recently, a study showed that horses with colic presenting severe GI disorders also had a significant increase in the peritoneal TGF-β_1 concentration [9]. In addition, other studies demonstrated that the peritoneal D-dimer concentration was significantly greater in horses with altered peritoneal fluid when compared with horses with a normal peritoneal fluid analysis. These results confirmed that those horses with inflammatory and ischemic problems, as well as those with peritonitis, had greater peritoneal fibrinolytic activity (hyperfibrinolysis) due to greater peritoneal fibrin formation and deposition (hyperfibrinogenesis) [3,4].

To our knowledge, there are no studies of horses with colic that showed a relationship between the peritoneal fibrinolysis activity (using D-dimer measurements) and the peritoneal TGF-β_1 concentration. Knowing the biological relationship between the fibrinolysis system and the compensatory anti-inflammatory response mediated by TGF-β_1 could be useful for understanding the adaptive mechanisms during the development of GI disease in horses.

The aim of this study was to investigate the possible relationship between peritoneal TGF-β_1 and D-dimer concentrations with the outcome (survivors and nonsurvivors) and with the type of peritoneal fluid (transudate, modified transudate, and exudate) in horses with colic.

2. Material and Methods

This study was approved by the Ethical Committee for Animal Experimentation of the Universitat Autonòma de Barcelona. The clinically healthy horses were used as controls after obtaining the owner's consent. This research was performed after two former studies evaluating in an independent fashion of the peritoneal and plasma concentrations of D-dimer [3] and TGF-β_1 [9]. However, the horses enrolled in this study were different from the former studies.

2.1. Study Design

In this prospective clinical study, horses (at least 1 year of age or older) with colic admitted between September 2008 and September 2010, from which peritoneal fluid samples were collected at admission for diagnostic purposes with the owner's permission, were included. In addition samples from healthy horses, without GI disorders, were collected using the same techniques and were considered to be controls.

According to the diagnosis, horses with colic were grouped into one of the following five groups: obstructive group, including noninflammatory, nonstrangulating disorders such as impactions and large colon displacements without signs of intestinal devitalization that resolved with medical therapy; enteritis group, including horses with duodenojejunitis and typhlocolitis; ischemic group, including horses with surgical disorders, such as volvulus, torsion, inguinal hernias, and epiploic foramen entrapment; peritonitis group, including horses with gastric or intestinal ruptures, as well as those with septic peritonitis caused by bowel devitalization without rupture; mixed or other processes group, including horses with two or more intestinal disorders (diagnosed clinically or over the necropsy) and horses with abdominal malignancy (diagnosed clinically and complemented with necropsy findings). The diagnoses were made based on clinical history, a complete physical examination, and the results of complementary diagnostic tests (cell blood count, plasma biochemistry, blood gas analysis, abdominal ultrasonography, and peritoneal fluid analysis). Findings of abdominal radiology, laparoscopy, or postmortem examination were used for this classification whenever they were performed.

To assess the prognostic value of D-dimer and TGF-β_1 peritoneal concentrations of horses on admission, horses were grouped according to the outcome: survivors (horses that were discharged from the hospital) and nonsurvivors (horses that died during the hospitalization). An additional economic constraint group was also considered as an outcome because some horses were euthanized due to economic reasons and not due to their pathologies.

Horses were also grouped according to peritoneal fluid analysis, namely into transudate (nucleated cell count [NCC] $\leq$5,000 cells/μL and total protein $\leq$2.5 g/dL), modified transudate (NCC $\leq$5,000 cells/μL and total protein $>$2.5 g/dL or NCC $>$5,000 cells/μL and total protein $\leq$2.5 g/dL, with a normal peritoneal fluid cytology), and exudate (NCC $>$5,000 cells/μL and total protein $>$2.5 g/dL with inflammatory peritoneal fluid cytology) [3].

2.2. Sample Collection

Blood was collected in 4.5-mL tubes containing 3.8% sodium citrate (wt/vol) (BD Vacutainer, Franklin Lakes, NJ, USA). Peritoneal fluid was collected aseptically, using a sterile, blunt teat cannula, 2 cm to the right of the middle of the most dependent area of the ventral abdomen, according to the standard technique. Peritoneal fluid was collected in tubes containing sodium citrate for D-dimer and TGF-β_1 analysis and in 1-mL tubes containing K_3EDTA (Greiner Vacuette Minicollect K3 EDTA tube; Greiner Bio-one GmbH, Kremsmünster, Austria) for total NCCs, total peritoneal protein concentration (TP) measurements, and cytologic evaluation. Blood and peritoneal citrated samples were immediately centrifuged at 1,000*g* for 15 minutes, separated from the sediment and frozen at −84°C until assayed.

2.3. Peritoneal Fluid Analysis

A routine analysis was performed on EDTA samples by a specialized laboratory within 12 hours after collection. An