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Original Research

Mucosal mRNA Cytokines' Profile of Gastric Wall in Neonatal Foals: Comparison with Endoscopy and Histology

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ABSTRACT

Gastritis and gastric ulcerations occur frequently in neonatal foals. The relationship between cytokines expressed by gastric mucosa and gastric histopathology in healthy or sick foals has never been investigated. The aim of this study was to compare the histological diagnosis and endoscopic view with cytokine expression (TNF-α, IL-1β, IL-4, IL-8, IL-13, and IFN-γ) of gastric mucosa. Twenty-two foals were definitively enrolled in the study: 19 were critically ill, and 3 were healthy foals. Gastric biopsy specimens were collected for histological examination and for cytokine mRNA qualitative real-time PCR analysis. This study shows that there is a substantial agreement between histology and endoscopy and that foals with evidence of gastritis and gastric ulcerations have higher probability of expressing TNF-α. Moreover, the overall profile of cytokines expression, with a low percentage of IFN-γ, a high percentage of IL-4, and the absence of IL-13, suggests a down-regulation of the Th1 cell-mediated immune response and an impaired Th2 response in the gastric wall in the neonatal period.

1. Introduction

Gastric ulcer disease is common in foals and horses, and the term equine gastric ulcer syndrome (EGUS) is used to describe this disease. Unlike the typical squamous mucosal ulceration seen in mature horses, lesions in the foals often also affect the glandular mucosa and/or the duodenum. For this reason, this disease is also called gastroduodenal ulcerative disease (GDUD) [1].

Erosions and ulcers are seen in 50% of foals less than 40 days old [2,3]. Two different pathogeneses are recognized for the ulcer in the squamous and glandular mucosa. Gastric ulceration in the squamous mucosa, along the greater curvature adjacent to the margo plicatus, is directly related to the degree and severity of gastric acid exposure. In neonatal foals, although the alkalinizing effect of milk ingestion, intragastric pH is lowest during periods of sleep. Moreover, infrequent or interrupted feeding and recumbency lead to lower gastric fluid pH [4].

Glandular gastric ulcers occur most frequently in foals. It is probably due to decreased blood flow, mucus and bicarbonate secretion. Decreased blood flow in the gastrointestinal tract due to systemic hypotension or hypovolemia is a common feature in critically ill foals affected by sepsis or perinatal asphyxia syndrome (PAS). Gastrointestinal tract ischemia and subsequent reperfusion injury could result in myriad effects, including decreased motility and alterations in mucosal protection, mucosal blood flow, and acid production [4,5]. Mucosal blood flow, which is enhanced by prostaglandins of the E series, is likely the most important contributor to overall gastric mucosal health [1,4,5]. The stress of birth may also lead to an excess release of endogenous corticosteroid, which can inhibit prostaglandin synthesis [1].

Endoscopy is the primary method used for the identification of gastric ulceration in the adult horse, and it can

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be useful for assessing the extent of disease and monitoring the response to treatment. Two studies compared endoscopy and histology of equine gastric ulcers; the first study compared endoscopy with histopathology of gastric ulcers at necropsy, and stated that endoscopy alone did not accurately assess the depth or severity of squamous gastric ulceration and underestimates the number of gastric glandular ulcerations [6]. The second study compared endoscopy with histopathology of biopsy specimens, and it emphasized the absence of any correlation between the endoscopic and histological findings [7].

Cytokines play a central role in the regulation of the mucosal immune system and are extremely important in mucosal defense. Their role in the pathogenesis of gastric ulcers has been studied in humans, adults [8] and children [9], in dogs [10], in cats [11], and in rats [12]. To better understand the pathogenesis of gastric ulcers, the presence of cytokines in the biopsy specimens of stomach of horses with naturally occurring lesions has been investigated only in one recent study [7]. However, there are no studies in critically ill foals.

The first aim of this study was to test the hypothesis that the endoscopic view can be compared to the histological diagnosis of gastritis and gastric ulceration (G/GU), considered the "gold standard." The second aim was to evaluate the cytokines expression (TNF-α, IL-1β, IL-4, IL-8, IL-13, IFN-γ) of gastric mucosa in healthy and critically ill foals less than 30 days of age. The third aim was to test the hypothesis that cytokines patterns are related to the evidence of G/GU, to the main diagnoses, to the administration of medication to treat or prevent gastric ulcers, to the administration of nonsteroidal anti-inflammatory drugs (NSAIDs) to the presence of gastroenteric symptoms, and to the type of nutrition.

2. Materials and Methods

Twenty-four foals were initially enrolled in the study. Twenty-one foals were critically ill at less than 30 days of age, admitted to the Equine Perinatology Unit (EPU) of the University of Bologna, and 3 were healthy foals born at the same unit during the 2009 foaling season. Healthy foals were born from mares referred to the EPU for attended delivery and had an Apgar score of $\geq$9 [13] and a normal clinical evaluation. The inclusion criteria for critically ill foals was a systemic illness requiring level 2 or 3 intensive care [14]. As histopathology is considered the "gold standard" for the diagnosis of G/GU, 2 foals were excluded from the study and were not evaluated further because the samples taken for histopathology were not suitable for analysis. Data from these foals are not included in the descriptive statistics or any analyses.

At admission and twice for every day of hospitalization, all sick foals receive a complete clinical examination. The foals had a variety of clinical diagnoses: 4 had prematurity/dismaturity, 7 had sepsis, 2 had perinatal asphyxia syndrome, 1 had meconium retention, 1 had trauma, 1 had hemorrhagic shock, and 3 foals were not classified. Because foals often suffer from multiple problems and have more than one clinical diagnosis, they are classified depending on the most life-threatening condition at admission.

If they were not able to suckle from the mare's udder, the type of enteral or parenteral nutrition was recorded. Assisted enteral nutrition included the use of a small nasogastric tube or the use of a bowl and mare's milk, or milk replacement was administered every 2 hours for the first 2 weeks of life and than every 3 hours. Foals with severe gastrointestinal conditions or severe neurologic dysfunctions (depression, recumbence, seizures) were placed on enteric rest and received parenteral nutrition (PN). For each foal, the administration of proton pump inhibitor (omeprazole paste), histamine type 2 receptor antagonist (ranitidine), and mucosal protectant (sucralfate) was recorded, as well as the administration of NSAIDs (flunixin meglumine or phenylbutazone).

Foals that were recumbent and foals that were placed on enteric rest were separated from the mares with a removable partition. All critically ill foals received broad spectrum antibiotics and fluid therapy.

The presence of the clinical signs of G/GU such as anorexia, ptyalism, bruxism, diarrhea, abdominal distension, gastric reflux, and dorsal recumbency was recorded.

All clinical data were collected by the two admitting clinicians (C.C. and J.M.). All procedures performed on the foals were approved by the Ethic-Scientific Committee for Experiments on Animals of the University of Bologna, in accordance with DL 116/92, and approved by the Ministry of Health. Oral informed consent for foal participation was given by the owners.

The foals underwent gastroscopy (Pentax EG-290P; insertion tube, 9.8 mm; instrument channel, 9.8 mm; working length, 1050 mm) without fasting and sedation. In the critically ill foals, the endoscopic evaluation was performed within 7 days from admission or during the administration of the treatment mentioned, while in the healthy foals, endoscopy was conducted within 60 hours after birth. Endoscopy was performed by placing the foal in left lateral recumbency and proceeding from nostril to esophagus to the lower esophageal sphincter. After moderate wall distension by insufflations of air, all mucosal surface was examined, eventually changing the body position if the content hid the wall, and maneuvering the endoscope to obtain a retroflexed view of the cardial region. Normal gastric mucosa was defined as an intact epithelium with no appearance of hyperemia or hyperkeratosis. Lesions were defined as superficial when only the mucosa was involved and the interior of the lesion had a typically pink appearance. The lesions were defined as deep when they included structures under the mucosa, with raised edges and a pink granulation tissue-like appearance crater. The lesions were defined as active in the presence of a hyperemic or darkened necrotic lesion crater. The most severe lesions had an active hemorrhage or adherent blood clots. All endoscopic examinations were performed by the same operator (M.P.), with over 20 years' experience in endoscopy, blinded to the clinical condition of the foals. The two pathologists (G.B., M.M.) were blinded both to the clinical condition (C.C., J.M.) of the foals and to the endoscopic view. Also the investigators evaluating PCR analysis (A.P., C.S.) were blinded to the clinical condition of the foals and to the endoscopic and histologic view.

For each foal, five gastric biopsy specimens were taken in the same area; 2 of 5 were snap frozen in liquid nitrogen for cytokine mRNA qualitative RT-PCR analysis, and 3 of 5 were used for histology.