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Oral pretreatment with recombinant human lactoferrin limits trauma-hemorrhagic shock–induced gut injury and the biological activity of mesenteric lymph

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ABSTRACT

Background: Lactoferrin (LF) is a pleiotropic glycoprotein that is found in bodily secretions and is postulated to enhance the gastrointestinal barrier and promote mucosal immunity. Thus, the ability of talactoferrin, an oral recombinant form of human LF, to limit gut injury and the production of biologically active gut-derived products was tested using a rat model of trauma–hemorrhagic shock (T/HS).

Methods: Male rats were orally dosed with vehicle or talactoferrin (1000 mg/kg, every day) for 5 d before being subjected to T/HS or trauma–sham shock (T/SS). Subsequently, rats were subjected to a laparotomy (trauma) and hemorrhagic shock (mean arterial pressure, 30–35 mm Hg × 90 min) or to T/SS, followed by resuscitation with their shed blood. Before inducing shock, the mesenteric lymphatic duct was catheterized for collection of mesenteric lymph. Four hours after the end of the shock or sham-shock period, rats were sacrificed, a segment of the distal ileum was collected for morphologic analysis, and lymph samples were processed and frozen. Subsequently, lymph samples were tested in several pharmacodynamic assays, including endothelial cell permeability, neutrophil respiratory burst activity, and red blood cell (RBC) deformability. Total white blood cell counts in lymph samples were also quantified.

Results: Pretreatment with talactoferrin reduced the incidence of T/HS-induced morphologic injury of ileum to T/SS levels. Post-T/HS lymph from vehicle-treated rats increased endothelial monolayer permeability and neutrophil priming for an augmented respiratory burst, and induced loss of RBC deformability, compared with T/SS groups. Talactoferrin pretreatment significantly reduced the biological activity of T/HS lymph on respiratory burst activity and RBC deformability, but had no effect on the lymph cell count or endothelial cell permeability.

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Conclusions: These results provide a proof of principle that prophylactic dosing of oral talactoferrin can potentially protect the gut in a T/HS model and limit the production of biologically active factors in rat gastrointestinal tissue subjected to ischemia-reperfusion –type injuries.

1. Introduction

Given its pleiotropic nature, lactoferrin (LF), an iron-binding protein, has many properties that could be beneficial in infectious, inflammatory, or shock states. For example, it has been described as a soluble mediator that bridges the innate and adaptive immune systems to regulate many immune cell responses, and studies have demonstrated a role for LF in the control of proinflammatory cytokines during acute inflammation [1,2]. In addition, LF has also been shown to have gut-protective effects and talactoferrin, which is an oral recombinant form of human LF, has been shown to reduce bacterial translocation from the gut in a rat model of necrotizing enterocolitis [3], whereas bovine LF (bLF) can protect mice from mortality induced by lipopolysaccharide injection [4]. Several antimicrobial and anti-inflammatory mechanisms have been proposed by which LF may prevent bacterial translocation [5]. Furthermore, LF receptors have been identified on the surface of various cells, including cell lines that are characteristic of intestinal enterocytes, for example, HT29 and Caco-2 cells [6], and it has been postulated that LF has a barrier protective function in infants and may act to tighten the junctions between gut epithelial cells after birth [7].

Recently, the role of LF in gut-related injury was investigated in two independent studies. Doursout *et al.* [8] demonstrated that rats pretreated with oral LF 1 h before endotoxin injection were protected from endotoxin-induced hypotension; they also had significantly diminished serum tumor necrosis factor α (TNF-α) and interleukin 6 (IL-6). A protective effect on mean arterial pressure (MAP) was also apparent when LF was administered 18 h before lipopolysaccharide (LPS) challenge. Pretreatment with LF at either 1 or 18 h conferred significant protection from anatomical injury to the gut wall, based on histologic examination. More recently, using a superior mesenteric artery occlusion model, Zhang *et al.* [9] found that prophylactic bLF (daily administration of 200 mg/kg for 14 d) protected against gut damage. Together, these studies suggested that LF may protect the gut from intestinal injury by mitigating oxidative injury, acute inflammation, or an excessive apoptotic response.

Given the potential for LF to be used as an oral therapeutic agent [10], we investigated the role of talactoferrin in abrogating gut injury and the pathologic activity of mesenteric lymphatic fluid in a rat trauma–hemorrhagic shock (T/HS) model [11]. Mesenteric lymph bioactivity was chosen as a marker against which to test talactoferrin, because previous studies have documented that gut-induced distant organ failure after trauma–hemorrhage is mediated by factors exiting the stressed gut *via* the intestinal lymphatics [12]. Talactoferrin has a major advantage over LF, in that talactoferrin is produced through the recombinant technology in *Aspergillus niger*, whereas LF must be obtained through isolation from biological samples. Our T/HS results with talactoferrin extend the observations made with LF, because pretreatment with talactoferrin abrogated T/HS-induced gut injury and significantly reduced the biological activity of T/HS mesenteric lymph.

2. Materials and methods

2.1. Animals

Specific pathogen-free male Sprague-Dawley rats (Charles River, Wilmington, MA) weighing 350–450 g were housed under barrier-sustained conditions and kept at 25°C with 12-h light–dark cycles. The animals had free access to water and food. All animals were maintained in accordance with the recommendations of the "Guide for the Care and Use of Laboratory Animals," and the experiments were approved by the New Jersey Medical School Animal Care.

2.2. Talactoferrin

Clinical study grade human recombinant LF (talactoferrin), expressed in *A niger* var *awamori*, was provided by Agennix, Inc (Houston, TX) as a sterile stock solution of 100 mg/mL in 14 mmol/L of sodium phosphate and 45 mmol/L of sodium chloride at pH 7.0. Talactoferrin solution was <20% iron saturated and contained 58–62 EU/mL (5.8–6.2 ng/mL) of endotoxin (per Limulus amebocyte lysate testing; Associates of Cape Cod, East Falmouth, MA).

2.3. Experimental design

The goal of this study was to investigate the ability of pretreatment with oral talactoferrin to limit T/HS-induced gut injury and the generation of biologically active intestinal lymph. The rationale for this study is based on two notions. First, a previous study documented that talactoferrin limits systemic injury in a rat model of necrotizing enterocolitis [3]. Secondly, because gut injury and the production of gut-derived proinflammatory products could be contributing to the adverse systemic effects observed in these disease conditions, it is possible that talactoferrin could be exerting its protective effects, at least in part by limiting gut injury and the production of biologically active intestinal lymph. Thus, in a proof-of-principle study, rats were subjected to T/HS or trauma–sham shock (T/SS) and mesenteric lymph duct cannulation after receiving oral pretreatment with talactoferrin (1000 mg/kg; every day) or vehicle for 4 d before the surgery and just after the placement of the mesenteric lymph duct catheters and just before the induction of T/HS (90 min and MAP 30–35 mm Hg) or T/SS. Eight animals per group were studied. T/SS and T/HS rats