S10-4

Method for Treating, Preventing, or Inhibiting Enterotoxigenic *Escherichia coli* Infections with Bovine Erythrocyte Preparations

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Diarrhea caused by enterotoxigenic *Escherichia coli* (ETEC), commonly referred to as travelers' diarrhea, is a common health problem among travelers visiting less developed or tropical countries (Peltola, H., *et al.* 1991. Lancet 338:1285-1289; Ericsson, C. D. *et al.* 1993. Clin. Infect. Dis. 16:616-626). Diarrhea caused by ETEC and other ETEC infections are important concerns for military personnel when deployed to less developed countries (Wolf, M. K., *et al.* 1993. Clin. Microbiol. 31:851-856 ; Bourgeois, A. L., *et al.* 1993. Am. J. Trop. Med. Hyg. 48:243-248). ETEC may be transmitted by food or water contaminated with animal or human feces. ETEC produces two toxins, a heat-stable toxin (ST) and a heat-labile toxin (LT). ETEC infections may cause profuse watery diarrhea, abdominal cramping, fever, nausea, vomiting, chills, loss of appetite, headache, muscle aches, and bloating.

The current therapy for travelers' diarrhea is to initiate treatment with agents such as bismuth subsalicylate (Pepto-Bismol®), antidiarrheals such as diphenoxylate with atropine (Lomotil®), loperamide HCl (Immodium®), attapulgite (Kaopectate®) and the like, rehydration therapy, and combinations thereof. The majority of the treatments involve the non-specific removal of the offending agents (*i.e.* toxins) from the intestinal tract. Only in moderate to severe cases of diarrhea where distressing or incapacitating symptoms are reported is antimicrobial therapy recommended. ETEC is frequently resistant to common antibiotics such as trimethoprim-sulfamethoxazole and ampicillin. Fluoroquinolones such as ciprofloxacin have shown some efficacy. Antibiotics are not usually effective at reducing clinical symptoms of the disease and problems associated with antibiotic resistance can occur. Prophylactic use of antibiotics is not recommended. Thus, therapies that specifically remove ETEC from the intestine are needed to provide more effective treatments for ETEC diarrhea.

In order to initiate the infectious process of diarrhea, ETEC must adhere to the host intestinal epithelial cells via the binding between bacterial adhesins and host receptors (Beachey, E. H. 1981. J. Infect. Dis. 143:325-345; Satterwhite, T. K., *et al.* 1978; Lancet. 2:181-184; Warner, L. and Y. S. Kim. 1989 in "Intestinal Receptors for Microbial Attachment", Eds. M. J. G. Farthing, and G. T. Kensch). This binding is commonly referred to as adhesion-receptor interaction. One treatment method that is based on this adhesin-receptor interaction involves an oligosaccharide covalently

attached to a solid support, wherein the oligosaccharide binds *E. coli* heat-labile toxin. However, it does not prevent or treat ETEC infections as the methods merely immobilize or neutralize the heat-labile toxin (LT). Thus, the enterotoxigenic *E. coli* are still capable of adhering to the intestinal epithelial cells, colonizing and producing more LT and a need still exists for a method for treating, preventing, or inhibiting ETEC infections, diseases, or disorders such as travelers' diarrhea.

Bovine erythrocytes (RBC) are known to agglutinate ETEC strains (Cassels, F. J., and M. W. Wolf, 1995. J. Ind. Microbiol. 15:214-226; De Graaf, F. K., and F. R. Mooi, 1986. Adv. Microb. Physiol. 28:65-143; Evans, D., Jr., *et al.* 1979. Infect. Immun. 23:336-346; Khalil, S. B., *et al.* 1999. Infect. Immun. 67:4019-4026; Ryu, H., *et al.* 2001. Infect. Immun. 69:640-649). Therefore, adhesion of ETEC to host epithelial cells may be prevented, inhibited, or reduced by binding to RBC preparations. Specifically, prevention, inhibition, or reduction of ETEC adhesion may be achieved by competitive binding to bRBC preparations as the binding affinity between ETEC and bovine erythrocytes is greater than the binding affinity between ETEC and intestinal epithelial cells.

The RBC preparations may be used in combination with or as a substitution for treatments of ETEC infections and related conditions. For example, the RBC preparations may also be used alone or in combination with at least one supplementary active compound such as bismuth subsalicylate, diphenoxylate with atropine, loperamide HCl, attapulgite, ciprofloxacin, and the like to treat, prevent or inhibit ETEC infections. Additionally, the RBC preparations may be used alone or in combination with compositions which bind *E. coli* heat-labile toxin (LT). The formulation for treating, preventing, or inhibiting an enterotoxigenic *E. coli* (ETEC) infection, such as travelers' diarrhea or infant diarrhea, comprising a therapeutically effective amount of at least one RBC preparation and a carrier suitable for oral administration. The formulation may further comprise at least one supplementary active compound such as an anti-diarrheal or an antibiotic. Preferred supplementary active compounds include bismuth subsalicylate, diphenoxylate with atropine, loperamide HCl, attapulgite, and ciprofloxacin. The formulation may further include a composition that binds LT. In some preferred embodiments, the formulation comprises a carrier such as an ingestible carrier. The carrier may be a pharmaceutical carrier or a foodstuff.

In this study, we prepared whole erythrocytes, erythrocyte ghosts, erythrocyte fractions, erythrocyte extracts and glycolipid receptors from bovine erythrocytes. The ability of the RBC preparations to prevent, inhibit, or reduce adhesion of ETEC to the intestinal wall of a subject were measured by both *in vitro* and *in vivo* assays. Bovine RBC preparations were found to agglutinate many ETEC strains expressing colonization factors (CF, the adhesion protein) such as CFA/I, CFA/III, CS1, CS2, CS4, CS5, CS7, CS15, CS17, CS19, PCF0159 and PCF0166. By high performance thin layer chromatography (HPTLC, Magnani, J. *et al.* 1982. JBC 257 :14365), the RBC ghost was found to contain several glycolipids and phospholipids, such as asialo-GM2, asialo-GM1, GM1, lactosylceramide and phosphyatidylethanolamine, that are well-known receptors for many human ETEC strains. Also several ETEC strains (E9034A, 60R315, C91f, and the like)

were found to bind the lipid extract of bRBC by the HPTLC overlay procedure. By the test of the absorptive capacity of bRBC, mixing intact RBC, 0.1% and 0.01% total volume, with a CFA/I bearing H10407 suspension that the bacterial concentration was reduced by 59% and 57% after 2 minutes incubation. For the competition assay using rabbit brush borders (RBB), the RBC ghosts attached to ETEC that were bound to the brush borders, and appeared to be competing off RBB-bound bacteria away from the RBB, when examined under a phase contrast microscope.