

Prevention of GI Absorption of Bacterial Toxins: an *In Vitro* Evaluation of the Potential for prophylactic use of a Novel Oral Adsorbent (AST-120, Spherical Carbon Adsorbent)

Keith Anderson PhD, Laurent Fischer MD

Background:

- AST-120 is currently in clinical trials for fistulizing Crohn's disease, Pouchitis, IBS, PPI-resistant GERD and Hepatic Encephalopathy.
- AST-120 is an orally-administered, non-absorbed, carbonaceous microsphere with very high specific surface area (>1600 m²/g).
- AST-120 selectively adsorbs a broad range of small molecular weight organic compounds putatively involved in inflammation of the digestive tract.
- Gut barrier failure and bacterial translocation/endotoxemia have been implicated in exacerbating and perpetuating inflammation of the gut and liver^{1,2,3}.

Methods:

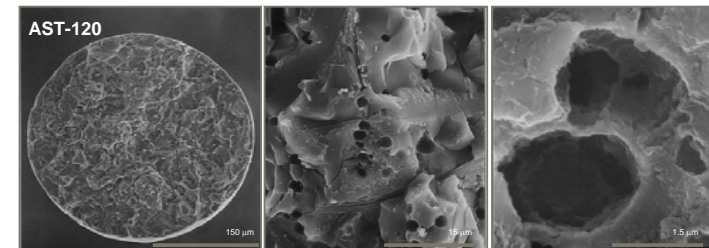
- *E. coli*, *S. dysenteriae*, *V. cholerae* and *C. difficile* were obtained from ATCC (Manassas, VA).
- Bacterial cultures were incubated in Tryptic Soy Broth (TSB) overnight at 37°C.
- Cells were lysed via centrifugation at 900g for 20-30 min at 4°C and supernatants were resuspended in phosphate buffered saline (pH 7.4).
- 0.25g of AST-120 was added to 25 mL of PBS/toxin combinations representing low, medium and high toxin concentrations.
- Flasks were incubated over a 6h period at 37°C while shaking at 78 rpm.
- Residual toxin concentrations were quantitated by standardized test kits applying immunoassay technology for the *E. coli* STa toxin or reverse passive latex agglutination technology for Shiga toxin (STx), Cholera toxin (CT) and *C. difficile* Toxin (Toxin A).

References

- ¹ Probiotics and antibodies to TNF inhibit inflammatory activity and improve nonalcoholic fatty liver disease. Li. et al. *Hepatology* (2003); 37: 343-350
- ² Multifactorial gut barrier failure in cirrhosis and bacterial translocation: working out the role of probiotics and antioxidants. Albillos et al. *J Hepatology* (2002); 37: 523-526
- ³ The intestine and its microflora are partners for the protection of the host: report on the Danone Symposium "The Intelligent Intestine". Bourlioux et al. *Am J Clin Nutr* (2003); 78: 675-683

Aim:

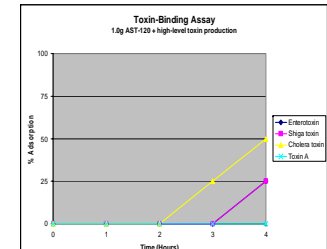
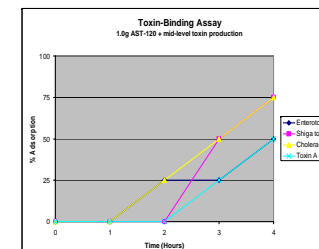
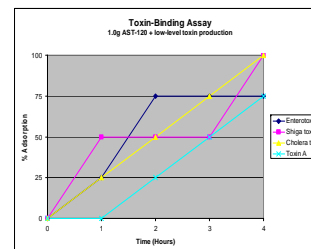
This investigation is intended to evaluate the binding potential of AST-120 for GI enterotoxins implicated in exacerbation of inflammatory bowel disease.



Corresponding O.D. readings and toxin levels expressed in ng/mL

Toxin	Low-level O.D.= Conc.	Mid-level O.D.=Conc.	High-level O.D.=Conc.
STa	5.0=10ng/mL	6.5=13ng/mL	8.0=16ng/mL
STx	0.45=1-2ng/mL	1.5=3-6ng/mL	5.0=11-22ng/mL
CT	0.9=1-2ng/mL	3.0=3-6ng/mL	5.0=6-11ng/mL
Toxin A	2.0=2ng/mL	3.0=3ng/mL	4.0=4ng/mL

Toxin	Specific Binding Capacity
<i>E. coli</i> heat stable ET (STa)	925 ng / g
<i>S. dysenteriae</i> Type1 Shiga Toxin (STx)	413 ng / g
<i>V. cholerae</i> Cholera toxin (CT)	425 ng / g
<i>C. difficile</i> Toxin A	150 ng / g



Discussion:

AST-120 has demonstrated binding affinity for known potent bacterial exotoxins. Although the features of AST-120 are not optimized for adsorption of proteins, it has been confirmed that binding capacity for these exotoxins are retained. It is postulated that the ability of AST-120 to improve symptomology in inflammatory disease processes is, in part, conferred by the ability to irreversibly adsorb bacterial exotoxins thus preventing end organ damage. AST-120 is a broad range scavenger of inflammatory mediators and/or stimulants present in the GI tract and has been designed for chronic treatment of inflammatory GI and liver diseases. Further investigation for use of AST-120 in prevention of clinical complications linked to bacterial toxins is warranted.