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Paper Poster Session
Clostridium difficile: epidemiology and risk factors

Albumin prevents Clostridium difficile-related cytotoxicity through toxin B binding

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Background: *Clostridium difficile* is an anaerobic Gram positive, spore-forming, bacillus causing illness in animals and humans ranging from mild diarrhea to fulminant life-treathening colitis. This bacterium causes 60% of antibiotic-associated diarrhea cases and exerts its pathogenetic properties through the production of two potent exotoxins, *i.e.* toxin A (TcdA) and toxin B (TcdB). *C.difficile* infection (CDI) has emerged as a major enteric pathogen with worldwide distribution representing the most frequently reported nosocomial pathogen in the United States. Notably, toxemia is significantly associated with the clinical severity of CDI. Low albumin levels represent a risk factor for both acquiring and developing severe CDI, and subsequent studies showed that hypoalbuminemia predisposes to CDI. However, the mechanism by which hypoalbuminemia predisposes to the disease is not yet understood. Plasma albumin is the most abundant protein, acts as transporter of numerous endogenous and exogenous compounds, affects the pharmacokinetics of many drugs, regulates chemical modifications of some ligands, and inactivates some toxic compounds. The aim of this work was to evaluate *in vitro* the protective role of albumin towards *C.difficile* toxins-dependent citotoxicity.

Material/methods: The ability of human serum albumin to exert a protective role towards TcdA and TcdB toxicity has been evaluated using human epithelial colorectal adenocarcinoma cells (CaCo-2) treated with either TcdA or TcdB or both toxins, and then analysing their metabolic activity in the absence and presence of human albumin. We also analysed, by immunoblot experiments, the capability of human albumin to inhibit toxins entry into CaCo-2 infected cells. Furthermore, the thermodynamic parameters of albumin binding to TcdA and TcdB have been evaluated by HPLC chromatography and fluorimetry approaches.

Results: Results obtained showed that TcdA exerts no significant effects on CaCo-2 metabolic activity both in the absence and in the presence of HSA. On the contrary albumin exerts a protective effect on CaCo-2 cells from the cytotoxic effect of TcdB, and a partially protective effect towards TcdA and Tcdb co-infection of CaCo-2 cells. Noteworthy, by immunoblot experiments we observed that albumin inhibits TcdB entry into CaCo-2 cells, demonstrating that the albumin-dependent protective effect is determined by TcdB sequestration outside the intestinal cells. Finally, the thermodynamic parameters of TcdB binding to albumin were determined (K_d = 1nM). On the contrary, no binding of TcdA to albumin has been observed.

Conclusions: We demonstrated that the administration of albumin protects enterocytes from *C. difficile* induced death. This finding is consistent with a protective role of albumin in CDI rather than being a proxy for underlying disease. A possible explanation is the ability of albumin to scavenge TcdB thus reducing the systemic manifestations of CDI. This discovery could help to understand the clinical significance of the known association between albumin levels and survival in patients with CDI.