Mechanisms involved in the crotoxin effect on experimental acute intestinal inflammation induced by TNBS in mice

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Introduction: The breaking of tolerance to enteric bacteria followed by infiltration and activation of immune cells are characteristics of gastrointestinal disorders. As known, the intestinal epithelial cells are involved in the cellular influx and consequent establishment of the local immune reaction. However, the mechanisms involved in this exacerbated inflammatory process are not completely elucidated. Crotoxin (CTX) is the main component of the *Crotalus durissus terrificus* (*C. d. terrificus*) rattlesnake venom and, among its biological activities includes the immunomodulatory effect. It was previously shown that the CTX is able to ameliorate the acute intestinal inflammatory response induced by the intrarectal instillation of trinitrobenzene 2,4,6 sulfonic acid (TNBS) in mice. **Objectives:** Evaluate the mechanisms involved in the modulatory effect of CTX on the establishment of the acute intestinal inflammation induced by TNBS in mice focusing on neutrophils and macrophages migration, as well as the effect of the CTX on intestinal epithelial cell activation *in vitro*. **Methods and Results:** CTX was isolated from *C.d.terrificus* venom by ion exchange chromatography. The purity and MW of the purified CTX were confirmed by SDS-PAGE. The Caco-2 cell line (human colorectal adenocarcinoma) were cultured for 17-21 days and stimulated with IFN-γ in the presence or not of CTX for 18 h. After this, the expression of ICAM-1 was evaluated by flow cytometry and IL-8 release in supernatants by ELISA. Besides, we analyzed the action of CTX on the migration of human neutrophils induced by the Caco-2 cell line in *transwell* system. The CTX was able to reduce ICAM-1 expression, IL-8 production and decreased neutrophil influx on Caco-2 cultures stimulated with IFN-γ. To address the modulatory effect of CTX on the intestinal inflammatory process, groups of mice received the intrarectal instillation of TNBS and after 12 hour, the toxin was injected intraperitoneal route. After 24 and 48 h of the TNBS-instillation, there was observed a higher percentage of weight loss and clinical score in the TNBS-group compared with the control group. In addition, necrotic area was also verified in the colon of the TNBS-group, confirming the establishment of the experimental colitis in the mice. However, the CTX administration resulted in decreased weight loss, clinical score and necrotic area in TNBS mice-group. Furthermore, this toxin was able to interfere with the migration of neutrophils and macrophages in TNBS-mice group. Therefore, these data allow us to continue with the main objective of to elucidate the modulatory effect exerted by CTX on the immune system, such as the intestinal inflammatory reaction.

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