

ZONULA OCCLUDENS TOXIN INDUCES A DECREASED EXPRESSION OF THE TIGHT JUNCTION PROTEIN OCCLUDIN

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Background: *Vibrio cholerae* elaborates Zonula occludens toxin (Zot), a 45 KDa protein that increases the intestinal permeability by opening intercellular tight junctions (tj). Zot localizes in the bacterial outer membrane with subsequent cleavage and secretion of a carboxyl-terminal fragment in the host intestinal milieu. Zot structure-function analysis confirmed that this fragment is the active domain responsible for the tj permeating effect.

Aim: To establish the relationship between Zot-mediated tj disassembly and expression of the tj protein occludin, using both rat intestinal epithelial cells monolayers and ex vivo rat intestine.

Methods: Rat intestine mounted on microsnapwell system was incubated with either Zot or its C-terminal active domain (Delta G). The Transepithelial Electrical resistance (TEER) was monitored and total RNA was extracted at increasing time intervals. Occludin expression was determined by Real Time PCR using the TaqMan probes technique. The occludin expression was also monitored in IEC6 monolayers. Results: Incubation of intestinal rat tissue with either Zot or Delta G led to a decrease in occludin expression and an increase in intestinal permeability starting as soon as 15 min (Table). The effects of Zot on both TEER and occludin expression returned to baseline following removal of the molecule from the bathing media (120min, Table). No significant changes were observed when the tissue was exposed to bovine serum albumin (BSA) used as negative control. Zot also induced a reduction in occludin expression in IEC6 cells (Table).

Conclusions: Our results suggest that Zot-induced tj disassembly involves down-regulation of occludin expression.

Effects of Zot and its active domain Delta G on TEER and occludin expression

	Rat jejunum*						IEC6 cells*	
	BSA		Zot		Delta G		occludin (%)	
Time (min)	TEER (%)	occludin (%)	TEER (%)	occludin (%)	TEER (%)	occludin (%)	BSA	Zot
0	0	0	0	0	0	0	0	0
15	1	5	19	7	2	27	0.7	19.5
30	7	5	15	8	17	30	7.1	22.1
60	8	0	51	11	29	25	3.2	4.2
90	8	-3	41	12	30	29	2	12.8
120(0)**	24	1	60	1	42	36	6.1	6.5
60**			42	ND				
90**			18	ND				
120**			15	0				

*Data are expressed as percentage decrement as compared to baseline values **Time points following Zot withdrawal from the upper chamber of the microsnapwell.