CLOSTRIDIUM DIFFICILE TOXIN IN THE PIGLET

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Introduction. Swine dysentery is an important cause of morbidity and mortality in feeding units. Although several microbial agents may contribute to the disease, Treponema hyodysenteriae is generally considered to be the organism common to most cases. In spite of this common association of T. hyodysenteriae, its presence cannot be confirmed by microbiologic methods or histologic staining techniques in a significant proportion of swine dysentery cases at our laboratory. Because there are many cases of swine colitis which remain undiagnosed, laboratory investigations must continue for other pathogenic microorganisms.

Recently Clostridium difficile has been identified as an agent which, under certain conditions, produces an enterotoxin that can cause pseudomembranous colitis in man. Usually, a disruption of the normal intestinal flora by an antimicrobial agent has preceded the development of this type of colitis (Bartlett, et al, 1978). Inoculation of gnotobiotic pigs with C. difficile has been shown to produce colitis and the enterotoxin was recovered from the feces (Lysons, et al, 1980).

This study was designed to demonstrate any effect of C. difficile toxin on the colonic mucosa of conventional piglets.

Methods. Toxins were prepared from two (CTD-134 and -191) C. difficile isolates which had been recovered from human patients with pseudomembranous colitis. The isolates were incubated for 48 hours in a meat broth media. The toxin was collected as a crude extract via centrifugation. Specificity of the toxin was determined by intraperitoneal injection in mice and hamsters using C. sordelli antitoxin to neutralize the toxin and prevent death. Further assay and neutralization of the toxin was conducted on Crandell feline kidney cell culture, using C. sordelli antitoxin. The level of toxin was expressed as a titer based on the log of the dilution producing 50% cytotoxicity in cell culture. The T. hyodysenteriae isolate was recovered from a diseased animal. This isolate was suspended in sterile phosphate buffered saline and adjusted to a McFarland No.3 concentration for use as an inoculum.

Two groups of conventional piglets were used in the study. Group I included 10 piglets weighing 25 to 50 kg. Group II was comprised of 5 piglets ranging in weight from 44 to 60 kg. Following 24 hours of fasting each piglet was anesthetized with methoxyflurane and nitrous oxide. A mid-line abdominal approach was used to surgically produce 2 to 3 cm. long segments in the spiral colon by ligating the entire circumference of a colonic loop. A small (1cm. long) segment was created between each major segment to reduce the possibility of leakage between segments. The major segments were instilled with 5ml. of material (from anterior to posterior) as follows:

	Segment				
Group	1	2	3	4	5
I	Toxin A	Toxin B	Filtered meat broth	No Treat	*
II	Toxin C	Filtered meat broth	Toxin D	No Treat	No Loop

Note: Toxin A (titer = 4.5) and toxin B (titer = 3.1) were produced in separate incubations of isolate No. CTD-134. Toxic C (titer = 4.2) and Toxin D (titer = 3.5) were produced in separate incubations of isolate No. CTD-191. *T. hyodysenteriae inoculum.

The abdominal incisions were closed and the pigs maintained as a group with routine care and feed. Piglets were electrocuted and necropsied at 72 hours post-surgery. Contents of each colonic segement were cultured for enteric bacterial pathogens and assayed for C. difficile toxin. Tissue specimens from each segment were fixed in formalin and processed routinely to produce hematoxylin-eosin stained slides for histopathologic examination.

Results. Group I: The piglets ate normally for 36 to 48 hours post-surgery, after which they gradually deteriorated with mild dehydration, depression, and abdominal distension at time of euthanasia (72 hrs.).

Gross pathologic changes included marked distention of the small intestine and colon proximal to the ligations. With the exception of three piglets, each segment (No. 5) of the colon inoculated with T. hyodysenteriae was markedly distended with grayish mucoid fluid. One of the three non-affected segments had accidently had the T. hyodysenteriae inoculum injected into the mesentery instead of the colon lumen. The lumina of segments No. 1 through 4 were similar, being dry with desiccated mucus and feces. The mucosa appeared normal. Other than the T. hyodysenteriae isolated from the No. 5 segments, no pathogens were isolated on bacteriologic culture.

Microscopic examination of each colonic segment inoculated with T. hyodysenteriae revealed moderate to marked dilation of the glandular crypts with mucinous material. There were focal sites of epithelial erosion and a slight to moderate increase in cellularity of the lamina propria. Occasionally there was a surface exudate comprised of mucin and leukocytes. Although the segments which received the C. difficile toxin had mild changes which included slightly dilated crypts and a mild increase in lamina propria cellularity, these segments did not differ significantly from the segments which were blank (no inoculum).

Group II: Four of the 5 piglets survived the surgery without complications. Each segment receiving toxin had gross characteristics similar to the toxin inoculated segments in Group I piglets, i.e, desiccated mucus and feces. Bacterial cultures were negative for pathogenic organisms. Microscopically, the colonic mucosa of all segments was essentially the same as described in Group I.

Conclusions. Toxin from two isolates of C. difficile failed to produce pathologic changes when applied in vivo to the colonic mucosa of conventional piglets.

In view of the colitis developed in C. difficile inoculated gnotobiotic pigs (Lysons, et al, 1980), the failure of the direct application of toxin to produce lesions in this study may have been related to: a) inadequate toxin concentration in the inoculum, b) dilution of the toxin by contents of the colonic segment resulting in low levels of toxin at the tissue level, c) interference with toxin action by the bacterial flora or other material in the colon and/or d) non-susceptibility of the conventional piglet to C. difficile toxin.

Selected references: Bartlett, J. G, Moon, N., Chang, T. W., et al: Gastroenterology, 1978, 75:778; Bartlett, J. G., Chang, T. W., Moon, N. and Onderdonk, A. B.: Am. J. Vet. Res., 1978, 39:1525; Larson, H. E., Price, A. B., Honor, P. and Borriello, S. P: Lancet, 1978, 2:1063; Lysons, R. T., Hall, G. A., Lemcke, R. M., Bew, J. and Luther, P. D.: Proc. Congr. Int. Pig Vet. Soc. 1980:231.

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