

RESEARCH NOTE

The Effect of Cholera Toxin and its Toxoid on Ileal Loops of the Rat

Ibrahim Felipe Heneine⁺,
Orivaldo A Rocha, Geovanni D Cassali

Departamento de Patologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Caixa Postal 486, 31270-901 Belo Horizonte, MG, Brasil

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In a previous paper (IF Heneine et al. 1992 *Brazilian J Med Biol Res* 25: 913-917) we described a cholera toxoid prepared by a simple and inexpensive procedure that proved unable to provoke subcutaneous tissue lesions, but retained the capacity to induce, when injected parenterally, antibodies against the virulent toxin. The toxoid was stable and did not show reversion to toxicity. In this paper we are reporting the comparative ability between the toxin and the toxoid to provoke intestinal tissue lesions, using the rat ileal loop (KMS Aziz et al. 1968 *Nature* 220: 814-815).

For the experiments, 40 days old Wistar rats from the animal house of the Departamento de Patologia Geral were kept fasting for 72 hr with plenty of water and a 5% sucrose solution. A group of eight animals was anesthetized with ether and two ileal segments of 8 to 10 cm each, near the cecum, were ligated with least damage

to the peritoneum. Each segment was injected intraluminal with toxin (10 µg/0.2 ml of phosphate buffer 0.05 M, pH 7.4) or toxoid (same dose). In some loops buffer only was injected. The animals were left for 6 hr with water and sucrose solution, when they were killed by an ether overdose.

The macroscopic findings showed that the virulent toxin caused a watery dilatation of the ileal segment, whereas the toxoid and the controls did not. The extravasated fluid was turbid, and in some loops, sanguinolent. The weight in grams of the loops was: Toxin, 2.35 ± 0.17 (mean \pm SEM, $P < 0.05$ in relation to toxoid); toxoid, 0.76 ± 0.06 ; control, 0.71 ± 0.06 ($n = 5$ for all experiments).

The microscopic aspect of loops treated with toxin, varied from a mild edema in the mucosal corium, to extensive lesions. Necrosis, with an almost complete disappearance of the mucosa and submucosa, and presence of an amorphous proteinaceous eosinophilic mass was seen. The muscularis externa appear distended and in some places ruptured by the volume increase. On the other hand, the toxoid did not induce morphological alterations, giving an aspect similar to controls.

Preliminary results indicated that the toxoid, two doses of 10 µg/ml with 15 days interval, administered by gavage in a 5% sodium bicarbonate solution, confer protection to ileal loop challenge with 20 µg of virulent toxin.

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⁺Corresponding author

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