TRANSMUCOSAL POTENTIAL DIFFERENCE IN EXPERIMENTAL COLITIS IN RATS

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Abstract—Colon transmucosal potential difference (TPD), macro- and microscopic lesions, myeloperoxidase activity, and leukotriene levels were studied after the induction of experimental colitis in the rat. Forty-three male Wistar rats were subjected to the instillation of 200 mg/ml 2,4,6-trinitrobenzenesulfonic acid (TNB) solution through a rectal cannula. TPD measurements were made at different distances from the anus before and 24 h and one, two, three, and four weeks after lesion induction. Leukotriene B₄ levels were assayed by intracolonic dialysis 24 h and one, two, three, and four weeks after lesion induction. Macro- and microscopic evaluations were made of the bowel lesions, and myeloperoxidase activity was assayed. The mean basal TPD was −46.06 mV at 1 cm from the anus, and +10.86 mV in the proximal colon. Twenty-four hours after lesion induction the values proved markedly positive. This was correlated with an abrupt increase in LTB₄ levels and myeloperoxidase activity. After one week the TPD values exhibited a greater electronegativity, returning to basal values by the fourth week after lesion induction. This coincided with an improved macroscopic lesion index, LTB₄ levels, and myeloperoxidase activity. In conclusion, TPD is a useful indicator of acute colonic lesions and correlates well with LTB₄ and myeloperoxidase assays. Moreover, the parameter is able to delimit lesion evolution, reflecting possible ad integrum restoration of the bowel mucosa.

INTRODUCTION

Although the etiology of inflammatory bowel disease is uncertain, there is general agreement that the immune system is responsible for the tissue damage observed (1). A number of mediators are involved in immune and inflammatory cell response, including a variety of cytokines, eicosanoids, and reactive oxygen.
species that may damage the bowel (2). The difficulties encountered in attempting to determine these mediators in the mucosa of patients with ulcerative colitis has led to the development of experimental models to investigate the inflammatory mechanisms involved and to evaluate the effects of different therapeutic agents. Recently, Morris and coworkers (3–5) developed a model of chronic inflammatory bowel disease in the rat through the intraluminal instillation of the hapten 2,4,6-trinitrobenzenesulfonic acid (TNB). When coupled to high-molecular-weight substances such as tissue proteins, TNB induces an immunologic response (6, 7). In turn, the combination of TNB and ethanol produces severe transmural granulomatous inflammation (8, 9), with ulcers exhibiting the histopathological features of Crohn's disease (10, 11).

The integrity of the gastrointestinal mucosa may be assessed by measuring the transmucosal potential difference (TPD), which reflects ion flux between the apical pole and basal membrane of the digestive tract cells. Studies have shown TPD to be similar in both man and experimental animals in vitro (12).

TPD determinations have been used mainly to study the lesions effects of certain drugs on the stomach (13, 14) and to investigate gastric cytoprotective mechanisms. However, to date no experimental studies have evaluated TPD as an indicator not only of acute damage but also of the evolution of experimentally induced colic lesions. The aim of the present study was to investigate TPD as an indicator of colon mucosa integrity and to study its response to TNB administration. In addition, the resulting macro- and microscopic alterations of the colon mucosa were studied, along with the variations in lesive substances released by bowel injury.

MATERIALS AND METHODS

Lesion Induction. Male Wistar rats (140–180 g) were fasted for 24 h prior to the experiment, with tap water provided ad libitum. Colon lesions were induced according to the method described previously (4, 15), with slight modifications. Following sodium thiobarbital anesthesia (0.04 mg/100 g intraperitoneally), 0.25 ml of a solution containing 200 mg/ml TNB (Carlo Erba, Italy) in 10% ethanol was slowly injected using a 1-ml syringe equipped with a rubber cannula and Luer lock. The solution was instilled in the rectum to a distance of 8 cm. The same cannula was then used to inject 0.5 ml of air to completely clear the cannula of TNB solution. Finally, the cannula was removed and the animals were kept for 30 sec in a supine Trendelenburg position.

The rats were divided into five groups according to the day of sacrifice after lesion induction: group 1 (N = 6) (killed after 24 hours, group 2 (N = 9) day 7, group 3 (N = 8) day 14, group 4 (N = 10) day 21, and group 5 (N = 10) day 28. The animals were weighed three times a week, and mortality was recorded.

Measurement of TPD. Transmucosal potential difference was determined according to the method described previously (13) for the gastric mucosa, with slight modifications. Thus, we employed a standard calomel electrode, pH/mV saturated in 3 M KCl (Inglo, Urdorf, Switzerland). A second, metal-tip electrode was used as the reference electrode. Leads from both electrodes were