Pathophysiological significance of a reaction in mouse gastrointestinal tract associated with delayed-type hypersensitivity

Wan-Gui Yu, Ping Lin, Hui Pan, Lan Xiao, En-Cong Gong, Lin Mei

Wan-Gui Yu, Department of Physiology, Medical College of Yangtze University, Jingzhou 434000, Hubei Province, China
Ping Lin, Department of Physiology, Medical School of Hubei Institute for Nationalities, Enshi, 445000, Hubei Province, China
Hui Pan, Lan Xiao, Lin Mei, Department of Physiology and Pathophysiology, Peking University Health Science Center, Beijing, 100083, China
En-Cong Gong, Department of Pathology, Peking University Health Science Center, Beijing, 100083, China
Correspondence to: Dr. Lin Mei, Department of Physiology and Pathophysiology, Peking University Health Science Center, Beijing, 100083, China
Supported by the National Natural Science Foundation of China, No. 30170549

Abstract

AIM: To explore the pathophysiological significance of delayed type hypersensitivity (DTH) reaction in mouse gastrointestinal tract induced by an allergen 2,4-dinitrochlorobenzene (DNCB).

METHODS: BALB/c mice were randomly divided into control and DTH, groups. After sensitized by DNCB smeared on the abdominal skin, the mice were challenged with DNCB by gavage or enema. The weight, stool viscosity and hematochezia were observed and accumulated as a reaction in mouse gastrointestinal tract associated with delayed-type hypersensitivity (DTH), thereby resulting in ulcerative colitis, colitis induced by DNCB in rabbit, guinea pig and rat has been reported, but that in mice has not been reported. In this study, DNCB-induced DTH in mice GI tract were found, and its pathophysiological significance was discussed.

RESULTS: Active charcoal propulsion rates of small intestine in the DNCB gavages groups were significantly higher than that in the control group (P<0.01). The DAI scores and pathological score in DNCB enema groups were also higher than that in the control group (P<0.05), and there were significant rises in LMIF activity in DNCB enema groups as compared with control groups (P<0.05).

CONCLUSION: Mouse gastrointestinal DTH reaction could be induced by DNCB, which might facilitate the mechanism underlying the ulcerative colitis.

Yu WG, Lin P, Pan H, Xiao L, Gong EC, Mei L. Pathophysiological significance of a reaction in mouse gastrointestinal tract associated with delayed-type hypersensitivity. World J Gastroenterol 2004; 10(15): 2254-2258
http://www.wjgnet.com/1007-9327/10/2254.asp

INTRODUCTION

The gastrointestinal tract (GI) is not only an important organ in digestion and endocrine, but also the largest peripheral immunological organ in the body. The secretory immunoglobulin A (s-IgA) and T cell are responsible for developing mucosa vaccines and producing oral tolerance. Furthermore, the manifestation of T cell mediated reaction in GI tract was most often in the way of delayed-type hypersensitivity (DTH), thereby resulting in ulcerative colitis, 2,4-dinitrochlorobenzene (DNCB), a chemical compound of low molecular weight, could combine with tissue protein to function as a full antigen in activating T cell mediated DTH reaction, such as skin DTH and colon inflammation/ulcer. Gastrointestinal DTH (or colitis) induced by DNCB in rabbit, guinea pig and rat has been reported, but that in mice has not been reported. In this study, DNCB-induced DTH in mice GI tract were found, and its pathophysiological significance was discussed.

MATERIALS AND METHODS

Animals

Healthy male BALB/c mice weighing 18-21 g (supplied by the Department of Experimental Animals, Peking University Health Science Center) were used. Animals were fed with a standard diet and allowed free access to water.

Preparation of major chemicals

DNCB solution For DNCB sensitization, 330 mg DNCB (Beijing Chemical Reagent Company, Beijing, P.R. China) was dissolved in 10 mL of acetone-olive oil (1:1) vehicle. For DNCB gavage, 300 mg DNCB was first mixed with a minimum volume of polysorbate 80, and a minimum volume of ethanol was added until the mixture was completely dissolved, followed by the addition of olive oil to achieve a final DNCB concentration of 6.6 g/L. The ratios of polysorbate 80-ethanol-olive oil in this vehicle were 6.6%, 8.8% and 84.6%, respectively. Then the 6.6% solution was diluted to 1.3 g/L and 0.3 g/L DNCB solution. For DNCB enema, DNCB was dissolved in 600 mL/L ethanol to achieve 4 g/L, 2 g/L and 1 g/L DNCB solution, separately. All these DNCB solutions were stored at 4 °C.

Activated charcoal suspension The suspension was made according to Qi et al. with a little modification. A total of 6 g activated charcoal (Tianjin 6th Chemical Reagent Company, Tianjin, China) and 2 g astragalin gum (Beijing Chemical Reagent Company, China) were dissolved in 50 mL of normal saline (NS) just before intragastric use.

RPMI 1640 One milliliter of RPMI 1640 culture medium contained 100 IU penicillin and 100 μg streptomycin, and then the pH was adjusted to 6.8-7.2.

Phytohemagglutinin (PHA) A 100 mg PHA was dissolved in 10 mL incomplete RPMI1640, and then sterilized and preserved at -20 °C.

Ammonium chloride solution (erythrocyte lytic fluid) A total of 1.03 g Tris and 3.735 g NH₄Cl were dissolved in 500 mL distilled water.

Induction of DTH response in GI tract

Grouping Ninety-six BALB/c mice were randomly divided into 10 groups, including DTH, DTH negative (DTH) group and control group.
Sensitization On the first day of experiment, mouse fur on abdomen (2.5 cm diameter) was shaved. A total of 50 µL of 33g/L DNCB was smeared on the shaved skin once a day for 1 or 4 d. Mice in the DTH−, and control groups were smeared with acetone-olive oil vehicle.

Gavage All the mice were fasted for 6 h before gavage. On the second day of experiment the mice in DTH−, DTH1 and DTH2 groups were gavaged with 0.8 mL of 0.3 g/L, 1.3/L and 6.6 g/L DNCB, respectively. The DTH− group was gavaged 1.3 g/L DNCB and the control group was gavaged polysorbate 80-ethanol-oil vehicle. On the third day, 0.8 mL of activated charcoal was gavaged to each mouse. Twenty minutes later, the mice were killed by decapitation for determination of gastrointestinal motility.

Enema On the 5th day, a silica-gel tube with 10 mm diameter was inserted into the colons of the mice, its tip being 3-3.5 cm far from the anus. The mice in DTH−, DTH1 and DTH2 groups were intracolonically administered 1 g/L, 2 g/L and 4 g/L DNCB (2 µL/g) once a day for 4 d. DTH− group was administered 2 g/L DNCB and the control group was administered 600 mL/L ethanol vehicle.

Evaluation of the animals Gastrointestinal motility After each mouse was killed, the whole small intestine was taken out, rinsed with NS quickly, and the intestinal wall was cut open longitudinally, laid and unfolded on a flat plate for estimation of the active charcoal migration distance (cm). The gastrointestinal motility was expressed by charcoal propulsion rate by using the formula: charcoal propulsion rate=migration distance of active charcoal / the distance from pylorus-duodenum junction to ileocecum×100%.

Body mass and stool By using disease activity index (DAI) score[10], the body mass and stool were scored as follows: Body mass: score 0-normal; score 1-1.5% lower than normal; score 2-6-10% lower than normal; score 3-11-15% lower than normal; and score 4-above 15% lower than normal. Stool viscosity: score 0-normal; score 2-fluffy; and score 4-diarrhea. Stool hemorrhage: score 0-normal; and score 2-apparent hemorrhage.

Pathological score On the 9th day, all the mice received enema were killed by cervical dislocation. The colon was cut open longitudinally along the attachments of mesenteries and was first macroscopically observed. Then specimens were taken from inflammatory/ulcerative colon and were fixed in 40 g/L formaldehyde. Paraffin-embedded 5-µm thick section was made for HE staining. The macroscopological score and microscopy score were obtained by adopting Dr. Murano’s method[10] with slight modification as follows: Macropathological score: score 0-no cementation; score 1-severe cementation or local congestion; score 2-severe cementation; score 3-atrophic change with ulcer and serious infiltration of chronic inflammatory cells in colon mucosa; score 2-mucosa hyperemia, infiltration of chronic inflammatory cells and decrease of the number of goblet cell in colon mucosa; score 1-mucosa hyperemia, infiltration of chronic inflammatory cells and far from the anus. The mice in DTH1, DTH2 and DTH3 groups was inserted into the colons of the mice, its tip being 3-3.5 cm far from the anus. The mice in DTH−, DTH1 and DTH2 groups were intracolonically administered 1 g/L, 2 g/L and 4 g/L DNCB (2 µL/g) once a day for 4 d. DTH− group was administered 2 g/L DNCB and the control group was administered 600 mL/L ethanol vehicle.

Measurement of leukocyte migration inhibitory factor (LIMIF) activity LMIF preparation By following our previous method[11], the spleen lymphocytes (2×106/mL) of mice received enema were cultured in PHA (60 µg/mL) for 72 h. Then the supernatant containing LMIF component was collected by centrifugation (1 500 r/min, 15 min), followed by lyophilization. The lyophilized powder was stored at -20 °C. Working solution (LMIF solution) was prepared by dissolving the lyophilized powder in RPMI-1640 to one third of its original volume before using.

Preparation of peripheral leukocytes A suspension of indicator cells (migrated leukocytes) was prepared from the anti-coagulated whole blood of guinea pigs. Three percent gelatin of 1/3 volume of the blood was added to the blood before culturing the sample at 37 °C for 30 min. The upper layer rich in leukocytes was taken out and centrifuged (1 000 r/min) for 10 min. Then the sediment was washed twice with Hank’s solution free of Mg2+ and Ca2+, and again centrifuged (1 000 r/min) for 10 min. Finally, the sediment was used as the indicator cell and was adjusted to a concentration of (1.6-1.8)x107 cells/mL RPMI 1640.

Detection of leukocyte absorbance By following our previous work[11] with a little improvement, the leukocyte was injected into a capillary tube of 80 µL volume. After heat-sealing one end of the tube and spinning at 1 000 r/min for 10 min, the tubes were cut at the liquid-cell interface. The portion containing the cells was placed inside each chamber of a 4-well plate filled with 210 µL LMIF solution for culture. Twelve hours later, 200 µL solution was taken out from each chamber and was put in an well of a 96-well plate, followed by an addition of 20 µL methylthiazoletetrazolium, MTT) was added into each well before the plate was incubated at 37 °C for 4 h. Then the supernatant was removed. 200 µL of DMSO was added into each well and the plate was shaken for 10 min. The absorbance (A value) of the migration cells was determined in Micro plate Reader at a wavelength of 570 nm. The average of A values of 3 wells was regarded as a mean A value. The above processes were repeated for 9 times.

Statistical analysis Data were expressed as mean±SE. Analysis of variance (ANOVA) and student’s t test were used for comparison among the groups and between paired data. P<0.05 was considered to be statistically significant.

RESULTS Gastrointestinal motility Active charcoal propulsion rates of small intestine in the DTH1 (0.3 g/L DNCB) and DTH− (1.3 g/L DNCB) groups were significantly higher than that in the control group (P<0.01) and DTH2 groups (P<0.05 or P>0.01), whereas there was no significant difference between the control group and DTH1 group (Figure 1). All the mice in DTH− group died after the 0.66% DNCB gavage.

Figure 1 Effect of DNCB gavage on gastrointestinal motility in sensitized mice. a<0.01 vs control, b<0.05 vs DTH−, c<0.01 vs DTH1, and DTH2 n=8 in control; n=7 in DTH−, DTH1 and DTH2 groups.
Figure 2 Effect of DNCB enema on DAI score in sensitized mice. \( P < 0.05 \) vs control. \( n=16 \) in control group and DTH4 group; \( n=11 \) in DTH5 group; \( n=17 \) in DTH6 group.

**DAI score and pathological score**

Diarrhea was first found in the mice 24 h after DNCB enema, and weight loss was found 3 d later. Serious weight loss and obvious diarrhea were seen in the 4 g/L DNCB group, 24% of whom died. DAI scores are shown in Figure 2.

Pathologically, the control and DTH(-) groups had normal histological structures and glands, no ulcer was found except for occasional slight mucosa congestion (Figure 3A). In DTH1 (1 g/L DNCB) group, there was slight colic edema, mucosa congestion, infiltration of lymphocytes and a decrease in the number of glands and goblet cells (Figure 3B). In DTH2 (2 g/L DNCB) group, intestinal adhesion and flatulence were found. A more disturbing array of glands, local erosion, dramatic decrease in goblet cells and diffuse inflammatory cellular infiltration were found (Figure 3C). In DTH3 (4 g/L DNCB) group, more extensive colic cementation, expansion of the proximal intestinal cavity, some white exudates, mucosa congestion, necrosis and multiple ulcers were found. Under the microscope, mucosa atrophy, decrease of glands and disturbance of tissue structure were observed (Figure 3D), moreover, erosion, hemorrhage, necrosis as well as deeper/extensive ulcers were easily seen (Figure 3E). The pathological score in each group is shown in Figure 4.

**The LMIF activities and its relationship with colitis**

With the increased DNCB doses in enema, which exacerbated colonic tissue damage, we could see an increase of the LMIF activity (A value decreased). The LMIF activity was significantly increased in the DTH5 and DTH6 groups as compared with the control group (\( P<0.01 \), student’s \( t \)-test) (Table 1). There were also significant differences in LMIF activity (\( P<0.01 \)) among DTH4, DTH5 and DTH6 groups (ANOVA).

![Figure 3](image-url) The pathological change of colon by DNCB enema in sensitized mice. A1: control 600 mL/L ethanol, normal histological structures. A2: DTH(-), only slight mucosa congestion. B: 1 g/L DNCB, hyperemia, infiltration of chronic inflammatory cells, decrease of the number of goblet cell in colon mucosa. C: 2 g/L DNCB, superficial erosion, chronic inflammation of colon mucosa. D: 4 g/L DNCB, atrophic changes of colon mucosa with chronic inflammation. E: 4 g/L DNCB, ulcer and hemorrhage in colon mucosa (HE, ×200).
we proved, using the specific DTH index of LMIF [14,15], that tests[12] inferred the possibility of GI being influenced by DTH cellular immune and GI disorder, but they either indirectly researchers explored the relations between DNCB-activated which he thought as being a cellular immune induced by DNCB and ulcer could be induced by DNCB enema in guinea pigs[4], reaction? Early in the 1960’s, Bicks What is the pathophysiological significance of GI DTH response, LMIF activity and UC. Secondly, we think that the distinctive (unique) significance of LMIF in UC and its pivotal role in connecting DTH with UC have been shown by Bartnik and Murakami.

To sum up, we have enough reasons to conclude that the GI DTH in mouse may provide not only experimental models of human UC, but also insight into pathogenic mechanisms of the UC, an inflammatory bowel disease (IBD) of unknown etiology.

REFERENCES
1. Takahashi I, Kiyono H. Gut as the largest immunologic tissue. J Parenter Enteral Nutr 1999; 23(Suppl): S7-12
2. Czerkinsky C, Anjuer F, McGhee JR, George-Chandy A, Holmgren J, Kienny MP, Fujishiba K, Meštecký J, Pierrefite-Carle V, Rask C, Sun J. Mucosal immunity and tolerance: relevance to vaccine development. Immunol Rev 1999; 170: 197-222
3. Gallaüerade V, Desvignes C, Peyron E, Kaeiüer D. Oral tolerance to hapten: intestinal epithelial cells from 2,4- dinitrochlorobenzene-fed mice inhibit haptenspecific T cell activation in vitro. Eur J Immunol 1995; 25: 1385-1390
4. Bicks RO, Rosenberg EW. A chronic delayed hypersensitivity reaction in the guinea pig colon. Gastroenterology 1964; 46: 543-549
5. Rabin BS, Rogers SJ. A cell-mediated immune model of inflammatory bowel disease in the rabbit. Gastroenterology 1978; 75: 29-33
6. Mei L, Li LQ, Li YF, Deng YL, Sun CW, Ding GF, Fan SG. Conditioned immunosuppressive effect of cyclophosphamide on delayed-type hypersensitivity response and a preliminary analysis of its mechanism. Neuroimmunomodulation 2000; 8: 45-50
7. Glick ME, Falchuk ZM. Dinitrochlorobenzene-induced colitis in the guinea-pig: studies of colonic lamina propria lymphocytes. Gut 1981; 22: 120-125
8. Zhang YB, Zou YH, Lian ZC, Chen WQ. Experimental model of ulcerative colitis in rat and its abnormality of colonic electricity. Laboratory Animal Science Administration 2002; 19: 5-7
9. Qi HB, Luo JY, Liu X. Effect of enterokinetic prucalopride on intestinal motility in fast rats. World J Gastroenterol 2003; 9: 2065-2067
10. Murano M, Maemura K, Hirata I, Toshina K, Nishikawa T, Hamamoto N, Sasaki S, Saito O, Katsu K. Therapeutic effect of intracolonically administered nucleic acid factor kappa B (p65) antisense oligonucleotide on mouse dextran sulphate sodium

Table 1 Effect of DNCB enema on LMIF activity in sensitized mice

| Group       | Skin smearing (50 μL/ mouse) | Enema (2 μL/ g bm) | A value (mean±SE) |
|-------------|------------------------------|-------------------|------------------|
| Control     | Acetone-olive oil            | 600 mL/L ethanol  | 0.426±0.009      |
| DTH1        | Acetone-olive oil            | 2 g/ L DNCB       | 0.401±0.007      |
| DTH2        | 33 g/ L DNCB                | 1 g/ L DNCB       | 0.363±0.038      |
| DTH3        | 33 g/ L DNCB                | 2 g/ L DNCB       | 0.220±0.012      |
| DTH4        | 33 g/ L DNCB                | 4 g/ L DNCB       | 0.139±0.019      |

\*p<0.01 when compared with the control and DTH1 (student's t-test). \*p<0.01 between DTH1, DTH2, and DTH3 groups (ANOVA); n=9 in each group. A, the absorbance of migration leukocytes. The skin smearing and enema were completed daily for 4 d.
11 Mei L, Li LQ, Fan SG, Ding GF. An assay of leukocyte migration inhibitory factor (LMIF) and the conditioned suppression effect on LMIF. Chin J Microbiol Immunol 1998; 18: 474-478

12 Shell-Duncan B, Wood JW. The evaluation of delayed-type hypersensitivity responsiveness and nutritional status as predictors of gastro-intestinal and acute respiratory infection: a prospective field study among traditional nomadic Kenyan children. J Trop Pediatr 1997; 43: 25-32

13 Gold D. Delayed-type hypersensitivity to Entamoeba histolytica in mice and hamsters: a comparison. Parasitol Res 1989; 75: 335-342

14 Bloom BR, Bennett B. Mechanism of a reaction in vitro associated with delayed-type hypersensitivity. Science 1966; 153: 80-82

15 Wolberg WH, Goelzer ML. In vitro assay of cell mediated immunity in human cancer: Definition of leukocyte migration inhibitory factor. Nature 1971; 229: 632-634

16 Matsui Y, Oshima S. Migration inhibition and stimulation factors produced from peripheral blood lymphocyte cultures of sensitized guinea pigs. Asian Pac J Allergy Immunol 1985; 3: 151-155

17 Malorny U, Goebeler M, Gutwald J, Roth J, Sorg C. Difference in migration inhibitory factor production by C57Bl/6 and BALB/c mice in allergic and irritant contact dermatitis. Int Arch Allergy Appl Immun 1990; 92: 356-360

18 Soffer EE. Diarrhea and malabsorption In: Stoller JK, Ahmad M, Longworth DL, eds. The cleveland clinic intensive review of internal medicine. New York Lippincott Williams Wilkins 2000: 730-732

19 Stenson WF. Inflammatory bowel disease In: Yamada T, Apers DH, Laine L, Owyang C, Powell DW, eds. Textbook of gastroenterology. New York Lippincott Williams Wilkins 1999: 1782-1783

20 Bartnik W, ReMine SG, Shorter RG. Leukocyte migration inhibitory factor (LMIF) release by human colonic lymphocytes. Arch Immunol Ther Exp 1981; 29: 397-405

21 Murakami H, Akbar SM, Matsui H, Horiike N, Onji M. Macrophage migration inhibitory factor activates antigen-presenting dendritic cells and induces inflammatory cytokines in ulcerative colitis. Clin Exp Immunol 2002; 128: 504-510

22 Zhou T, Lin P, Pan H, Mei L. Ulcerative colitis: a review in its pathogenesis and immune mechanisms. Shijie Huaren Xiaohua Zazhi 2003; 11: 1782-1786

Edited by kumar M and Xu FM