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# A new chronic ulcerative colitis model produced by combined methods in rats

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## INTRODUCTION

The etiopathogenesis of ulcerative colitis (UC) remains obscure, due to lack of an ideal animal model[1-8]. With the improvement of theory and methodology in the last 30 years, people used to adopt chemicals (acetic acid, ethanol, carrageen, etc.), immunotechniques (humoral or cellular immunity, immune complex) and substance derived from UC patients to set up various kinds of UC animal model, which mimic the pathologic changes of human UC, so far these remain far from reality[6-10]. A possible exception is the spontaneous colitis developed in the cotton-top tamarin when captured, but this animal is rarely available and expensive preventing its usage[11]. Therefore, establishing an ideal animal model becomes the focus and key of the research study of UC. Nowadays, the models produced by 2,4-dinitrochlorobenzene (DNCB) and acetic acid (AA) came into use because of their simplicity and pathologic changes simulating those of human UC[12-21], however, the characteristics of short course of DNCB method and absence of immunoreactivity in AA method, these two models are not ideal either. In the present study, we established a new rat UC model produced by combination of DNCB and AA, and observed the changes of general condition, the disease course, the pathology, ultrastructure, apoptosis, immunoreaction and intracolonic pressure, in order to develop a more ideal UC animal model.

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## MATERIALS AND METHODS

### *Materials*

Eighty healthy male and female adult Wistar rats weighing 250g - 350g were used in this study. They were provided by the Department of Experimental Animal of our hospital, and were raised in the SPF environment (constant tempera ture, humidity and sterilized water, food and padding) and acclimatized to the surrounding for 7 days prior to the experiments.

DNCB (First Reagent Factory of Shanghai), AA (Dongtai Reagent Factory), $CD_4$, $CD_{29}$ and FITC or PE conjugated monoclonal antibodies (Immunotech, Marseilles, France), PC polygraf HR multichannel recording system (CTD-SYNECTICS Ltd), scanning electron microscope (EX1200, Japan), flow cytometry FACScan (Becton Dickenson Immunocytometry System).

### *Methods*

**Animal models** Eighty Wistar rats were divided randomly into 4g roups, 20 each. Group A (DNCB +AA): after the nape hair was depleted by 10% $Na_2S$, DNCB acetone solution (20g/L) was dropped to the nape of the rats (0.3mL for each) once daily for 14 days, on the 15th day, nylon catheter (3mm in diameter) was inserted into the colon at the site of 8cm from the anus, and 0.25mL 0.1% DNCB, 50 mL/L 0.04 mol/L solution alcohol were infused, on the 16th day, 2mL AA solution (80mL/L) were infused into the same site for 15s, then 5mL normal saline (0.9%) was used to washout AA. Group B (DNCB only): from the 1st day to the 14th day, the method was similar to that for Group A, from the 15th day to the 18th day, 0.25 mL 0.1% DNCB (50 mL/L) alcohol solution (0.04mol/L) were infused into the colon at 8cm depth by the same nylon catheter once a day for each. Group C (AA only): 2mL AA (80mL/L) solution were infused into the colon at 8 cm depth by intracolonic administration with nylon catheter (3mm in diameter) for 15s, then 5mL saline for washing the AA. Group D (saline control): equivalent volume of normal saline was given in the method similar to that for Group A.

**Pathological observation** After the model had been established, the rats were killed at wk1, 2, 4, 8 and 16, and the distal colon (7cm-9cm) were removed longitudinally and washed to remove the luminal contents, tissues were fixed in 10% neutral-buffered formalin, dehydrated according to the routine, embedded in paraffin wax and sectioned.

Finally, the sections were stained with HE and observed microscopically. Apoptotic cells were identified morphologically[22], for cell shrink age, chromatin condensation, formation of apoptotic bodies. Apoptosis was calculated randomly by counting the apoptotic bodies in the lamina propria for at least 200 cells[22].

**Electron microscopy** The tissues were cut into small pieces (0.5mm in diameter) and fixed first in 2.5% glutaraldehyde buffered in 0.1M PBS (pH 7.2) at 4℃ for at least 2h. The tissues were washed with the same buffer and then fixed in 1% osmium tetroxide in phosphate buffer at 4℃ for 2h, dehydrated in graded series of acetone and embedded in epoxy resin 812. The ultra-thin sections were observed under EX1200 electron microscope.

**Measurement of $CD_4^+CD_{29}^+$** Blood $CD_4^+CD_{29}^+$ were measured using flow cytometry according to our previous article[15,23].

**Colonic pressure and motility** Intracolonic pressure and motility were measured according to our previous report through pressure tranducer and recorded by PC Polygraf HR multichannel recording system[24] (Figure 1). During manometry, the catheter lumen was infused with 0.9% saline at 0.2mL/min using a miniature hydraulic infusion pump. The baseline resistance of intra colonic pressure was set at zero. The catheter (outer diameter 3mm with 4 side holes for 4 channels) was inserted into the colon at 9cm depth from the anus without laparotomy, and was withdrawn at 1mm-2mm increment, and measurement was not started until 5min after the tip of the catheter dropped out into the rectum.

**Statistical analysis** The data were expressed as $\bar{x}\pm s$, and analyzed, using the Student's *t* test $P<0.05$ was consid ered significant.

## RESULTS

**General condition and disease course** Anorexia, bloody diarrhea, mucus in stools were seen in all A, B and C groups after 1 to 2 weeks, which lasted for 16 weeks in group A with weight loss and 8 weeks in group C, while in group B, the bloody diarrhea and mucus in stools decreased gradually and recovered 2 weeks later. No symptoms were observed in group D.

**Pathological findings** Diffuse hemorrhage, edema, congestion, superficial ulceration in the mucosa with infiltration of lymphocytes, plasma cells and polymorphonuclear cells, cryptitis, crypt abscess could be observed in all A, B and C groups (Figure 2), and these characteristics lasted for 16 weeks in group A and 8 weeks in group C while only 2 weeks in group B. In group D, the bowel wall was normal by gross and microscopic examinations.

**Ultrastructural changes** There were decreased number of cells, shortened microvilli, swollen mitochondria with depleted ridge, maldevelopment of goblet cell and increased number of lysosomes during the acute phase (Figure 3), which recovered to normal gradually (Figure 4).

**Apoptosis** The apoptosis indexes, $9.9 \pm 3.8$ in group A, $8.6 \pm 3.5$ in group B, $8.1 \pm 2.9$ in group C, were significantly higher than those in group D ($4.0 \pm 2.1$, $P<0.05$). Under scanning electronmicroscope, shrinkage of cells, condensation and margination of chromatin could be seen (Figure 5).

**Immunoreactivity** The changes of $CD_4^+CD_{29}^+$ in ulcerative colitis models were shown in Table 1.

$CD_4^+CD_{29}^+$ increased significantly ($P < 0.01$) in group A and B but not in group C and D 1 week after set up of model ($P>0.05$).

**Table 1 Changes of $CD_4^+CD_{29}^+$ in ulcerative colitis models (%, $\bar{x}\pm s$)**

| Group | *n* | Prior to set up of model | 1 week after set up of model |
|---|---|---|---|
| A | 20 | 5.01±2.01 | 11.17±2.18[b] |
| B | 20 | 4.95±1.87 | 10.98±2.87[b] |
| C | 20 | 4.93±1.96 | 5.06±2.03 |
| D | 20 | 4.76±1.56 | 4.91±1.93 |

[b]$P<0.01$ *vs* prior to set up of model.

**Colonic pressure and motility** One week after set up of the model in group A, the basal intracolonic pressure was apparently lower than that of the pre-model (proximal pressure: 0.78 kPa ± 0.13 kPa *vs* 0.88 kPa ± 0.14 kPa, distal pressure 0.76 kPa ± 0.11kPa *vs* 0.89kPa±0.15kPa, $P<0.05$). The frequency of migrating motor complex waves *in vivo* were significantly faster 1 week after set up of the model as compared with that prior to (1.59/ min ± 0.27/ min *vs* 0.60/ min ± 0.12/ min, $P<0.05$) in 7 rats of group A, this belonged to the pathologic colon of high dysrhythmia. The amplitude of migrating motor complex waves decreased markedly in 10 rats of group A after 1 week of post-model than that of pre-model (proximal pressure: 0.64 kPa ± 0.24 kPa *vs* 1.98 kPa ± 0.38 kPa, distal pressure: 0.92 kPa ± 0.37 kPa *vs* 2.45kPa±0.63kPa, $P<0.01$), which belonged to asthenia colon. In the other 3 rats, while the proximal amplitude of migrating motor complex waves were lowered significantly (0.96 kPa ± 0.31kPa), the distal amplitude of migrating motor complex waves remained still higher (2.35kPa± 0.50kPa).

**Success rate** The ulceration pattern was present in all 20 rats of group A, the success rate therefore, could be considered 100%.

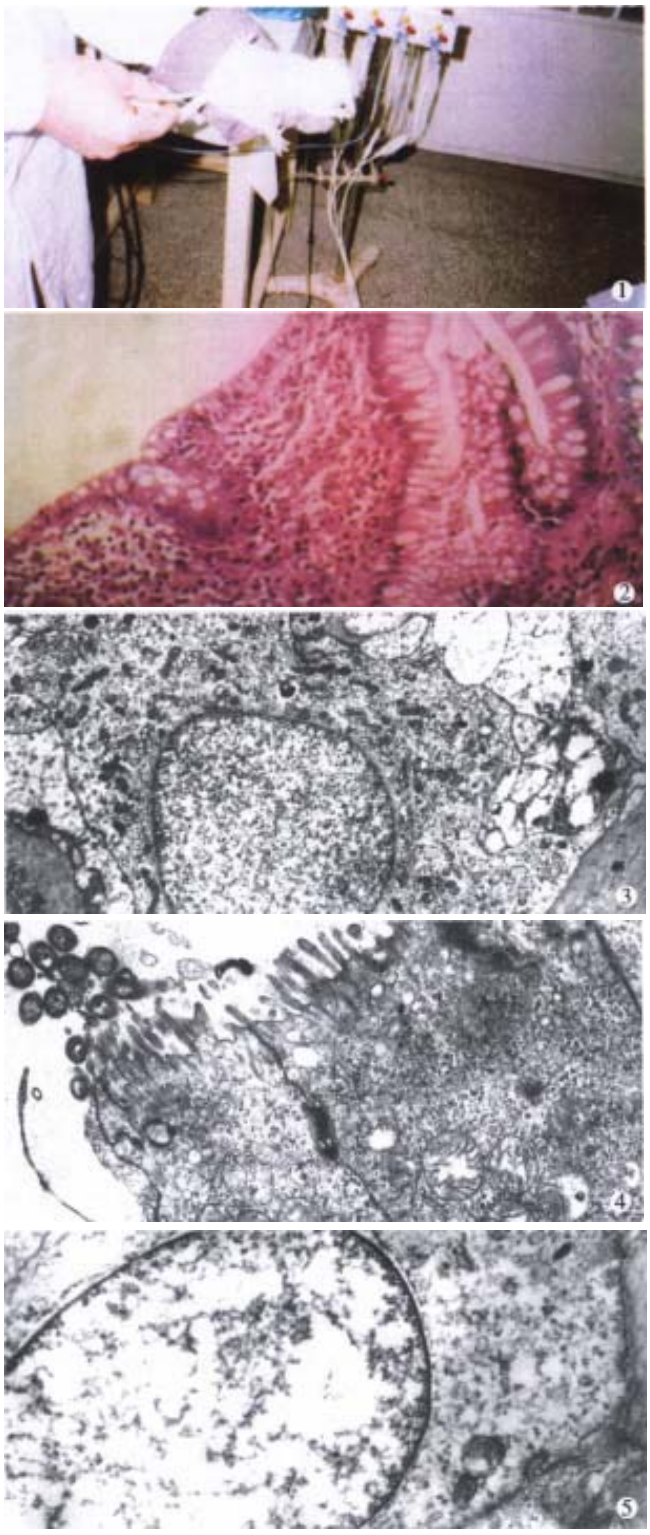

**Figure 1** Measurement of intracolonic pressure and motility in UC rats without laparotomy.
**Figure 2** Pathological features of ulcers and crypt abscess. HE×25
**Figure 3** Swelling of mitochondria with ridge depletion in goblet cell. ×4000
**Figure 4** In healing phase, the decreased number of the cell count and swelling of mitochondria recovered to normal gradually. ×10K
**Figure 5** Apoptosis of epithelia cell with condensa tion and margination of chromatin. ×12K

## DISCUSSION

So far, the precise etiopathogenesis of UC remained unelucidated[1,25-40], though experimental colitis had been produced by various methods mimiking human UC somehow, up to the present there is not one ideal animal model which conforms with human UC in pathogenesis, pathology and biologic behavior[6-21,41,42]. An ideal animal model should fullfil the following requirements[15,24]: ① it should reflect the histological characteristics of the diseases; ② it should be autoimmune in nature; ③ it should have the similar clinical manifestations as in human UC; ④ it should be simple and reproducible. In this study, the UC model produced by AA method was related to its chemical stimulation to the colonic mucosa[12-14], which led to increment of vascular permeability and activate inflammation mediators, resulting in bloody diarrhea, mucus in stools and histological features as diffuse edema, congestion and ulceration, crypt abscess and mucosal infiltration of inflammation cells. It can be used to study the inflammatory mechanism and anti-inflammatory drugs[43-45], but it lacked the immune response which is a dsawback.

DNCB is a hapten, when bound with tissue proteins will be able to elicit immunologic response and induce colitis[18-21]. The clinical symptoms and histological features, in particular $CD_4^+CD_{29}^+$ cells are similar to those of human UC, but the self-limited course of 2 weeks, is too short to be utilized as an ideal model.

In order to overcome these shortcomings we establish a new chronic UC model by using DNCB and AA in combination which has the following advantages[15-17]. ① Clinically it manifests mucus in stools, bloody diarrhea and weight loss, just like those occur in human UC. ② It can reflect the pathologic characteristics of UC, such as continuous superficial colonic inflammation. Microscopically there exist mucosal edema and congestion, infiltration of lymphocytes, plasma cells and polymorphonuclear cells, crypt abscresses and ulceration. ③ It is an immune response model, immunology is well studied in UC[1,46-49]. One of the important immunoregulatory abnormality in UC is related to the T cell response[23,50]. $CD_4$ interacts with HLA class II molecules, $CD_4$ positive T cells can be divided into Th1 and Th2 cells whreas $CD_{29}$ reacts with 130 KD integrin $\beta_1$ subunit which is expressed as a heterodimeric complex with one of six $\alpha$ subunits, forming the very late activation antigen (VLA) subfamily of adhesion receptors. The $\beta_1$ subunit has a broad distribution, and is expressed on lymphocytes, monocytes but weakly on granulocytes. These receptors are involved in a variety of cell-cell and cell- matrix interactions. Co-expression of $CD_4^+$ and $CD_{29}^+$ can be used as a marker to identify the Th1 cells subgroup, whose main function is to help B lymphocyte to induce

antibody production and cell-mediated dissolution[15,23]. The increase of $CD_4^+CD_{29}^+$ can lead to highly activated B lymphocyte and immunoregulatory abnormality. In this test, $CD_4^+$ $CD_{29}^+$ cells are significantly higher after the set up of models by combination of DNCB and AA, Which is in accord with the requirement of immune response similar to that in human UC. ④ It has a long disease course, if can last at least for 16 weeks with chronic damage of the bowel hence, eligible for the assessment of the drug effects. ⑤ The ultrastructural features are similar to those of human UC. The decreased number of epithelial cells and shortened microvilli, swelling of mitochondria with depletion of ridges can lead to impairment of water absorption of colon resulting in diarrhea. The maldevelopment of goblet cell, may lead to mucus in stools. ⑥ The above findings are not only consistent with the changes in human UC[51-59], but also concordant with other reports[60,61]. The model produced by combination of DNCB and AA is a more ideal animal model of UC. Apoptosis was first described by Kerr[62] and is referred to as programmed cell death, which is genetically controlled. In active UC, injury is common. Did apoptotic cells increase in colonic epithelia which led to ulceration This hypothesis had been studied since 1996[63-67]. Apoptosis of the normal colon was localized in the superficial epithelium, far less than one apoptotic body per crypt. In active UC, the loss of epithelial cells occurred mainly by apoptosis in crypts of involved and uninvolved adjacent areas, resulting in impairment of protective mucosal barrier. The mediators of apoptosis are partly related to the Fas/Fas-L interaction and/or changes in Bcl-2 expression. Our previous study demonstrated the number of apoptotic cells of colonic mucosal epithelial cells in human UC increased as shown by flow cytometry[68], This led to the damage of the barrier resulting in ulceration. Under scanning electron microscope, shrinkage of cells, condensation and margination of chromatin could all be seen.

The symptoms of diarrhea and abdominal pain are partly related with colonic motility disturbance[69-75]. In this study, we first used PC Polygraf HR multichannel recording system to measure the intracolonic pressure and motility in physiological and pathological conditions from multiple sites over prolonged periods. The resultant signals were digitized, analyzed and displayed in a readily interpretable manner, and could be easily subjected to a variety of statistical manipulations, as the colon was situated within the abdominal cavity, the temperature, humidity and pH were maintained by the rats themselves, and not interfered by operative maneuver. In this study, UC rats are characterized by decreased intracolonic basal pressure and disturbance of frequency and amplitude of migrating motor complex which may lead to the symptoms of urgency, diarrhea and abdominal pain. In conclusion, the new chronic UC rat model produced by combination of DNCB and AA is similar to human UC in clinical manifestations, histology, ultrastructural changes, immune response, apoptosis and colonic motility, and it is simple, inexpensive and reproducible with high successful rate.

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