The effect of a compound prescription on the intestinal microflora of hamsters with colitis

Lin-xiang LIU,* Nian-xiang XIE, Hong-yue SONG and Hui-zhen NIU

Institute of Chinese Materia Medica, Academy of Traditional Chinese Medicine

(Received January 8, 1992. Accepted April 16, 1992.)

Abstract

The changes of the intestinal microflora in hamsters with colitis before and after treatment with a compound prescription of traditional Chinese medicines were studied. The hamster colitis model was established by injecting lincomycin (i.p.) into the animals. Microflora analysis was carried out by two methods : direct microscopic examination and the isolation of bacteria by using various selective media. The results showed that drastic changes were observed in the model animals, whereas in the animals treated with the compound prescription, the microflora was kept at a level similar to that in normal ones.

Key words intestinal microflora, microflora analysis, colitis animal model, compound prescription.

Introduction

In our previous studies,[1,2] we screened and constituted an effective compound prescription on hamster colitis from several classical prescriptions known to have the functions of "reinforcing spleen" and "eliminating dampness" in the sense of traditional Chinese medicine. The prescription was composed of the following herbal medicines : coptis root (*Coptis chinensis*), scutellaria root (*Scutellaria baicalensis*), dangshen (*Codonopsis pilosula*), white atractylodes rhizome (*Atractylodes macrocepha*), poria (*Poria cocos*), Alismatis rhizome (*Alisma orientalis*), aucklandia root (*Aucklandia lappa*), and licorice root (*Glycyrrhiza uralensis*). According to the theory of Chinese materia medica, the prescription possesses the functions of "clearing heat, drying dampness and invigorating spleen". An analysis based on several classics of traditional Chinese medicine revealed that colitis is mainly caused by "spleen deficiency" and "damp heat". In the previous experiment[2] using the hamster model, the prescription was proved to have the effects of decreasing animal mortality, the diarrhea rate, positive toxin detection rate, and the isolation rate of the pathogenic agent, *Clostridium difficile*.

The aim of the present study is to observe the variations of the intestinal microflora of the hamster colitis model under the influence of the prescription, to find out if the prescription can restore the disturbed microflora, so as to elucidate one of the mechanisms of its curative efficacy.

Materials and Methods

Hamster colitis model : The establishment of the model and the administration of the prescription were performed using previously described methods.[1,3] In brief, male Chinese golden hamsters, weighing 70-110 g, were injected intraperitoneally with 5-7 mg of lincomycin per animal. Three days after the injection, the animals suffered from diarrhea, and succumbed successively to the colitis, if not treated.

Administration of the drugs : The hamsters were grouped randomly into four. Two days before the injection of lincomycin, the hamsters of the first group were administered orally with a

*中華人民共和国北京 100700
中医研究院 中薬研究所 劉 林祥
Beijing 100700, China

Journal of Medical and Pharmaceutical Society for WAKAN-YAKU **9**, 47－51, 1992

decoction of the compound prescription (abbreviated as COMP) twice a day for another seven days after the injection. The proportion (%) of the ingredients in the COMP are as follows : dangshen 15, poria 15, coptis root 15, scutellaria root 15, and the other four are 10 each. The dosage of the decoction contents was 5 times that of the adult patients calculated by the body surface formula.[4] The members of the second group were administered with demethyl-Vancomycin (DMV) solution for seven days after lincomycin injection. The DMV is a new product of the Huabei Pharmaceutical Works (Hebei Province, China). Its minimum inhibitory concentration (MIC) against *C. difficile* is identical to that of vancomycin (Eli Lilly Co.) (unpublished data). A dosage of 10 mg of DMV per hamster a day was used as a positive drug control. The third group members were administered distilled water as control (LINCO). The fourth group serves as normal control (NORM) without lincomycin injection but were administered water the same as the treatment of the third group.

Direct microscopic examination: On day 10, all animals were sacrificed, and the cecal contents were removed aceptically, weighed, and added to 0.05 % yeast extract water to make a 10 % (w/v) suspension, and then further diluted 10 times with the same diluent. On a microscopic slide, divisions of one square centimeter each were made by a glass pencil. Every 10 μl of the diluted cecal suspension was dispersed evenly in one division on the slide, allowed to air dry, Gram stained, and examined under light microscope. The bacteria observed were classified by cell morphology into four categories : (A) Gram positive large rods (*C. difficile* included), (B) Gram positive cocci (coccobacilli included), (C) Gram positive short rod, and (D) Gram negative rods. During examination, the slide was moved at a definite pattern. The bacterial numbers of ten fields were calculated. Bacterial numbers of each category within one test group were totaled and the mean and S. D. values were calculated.

Microflora analysis : Selective and differential media used to isolate aerobic and anaerobic bacteria were as follows : EMB (for the isolation of *E. coli*), EC (for *Streptococcus faecalis*), NBGT (for *Bacteroides spp*), PS (for anaerobic cocci), FS (for *Fusobacterium spp*), BS (for *Bifidobacterium spp*), and LGA (for *C. difficile*). All of the media used were prepared with laboratory chemicals based on the formulas from Mitsuoka[5] except LGA.[6] 0.1 ml of cecal suspension of each dilution was inoculated, spread onto the agar surface in triplicate, and incubated in a conventional incubator or in an anaerobic glove box depending on the nature of the isolated bacteria. The bacteria were identified according to their colony characteristics, cell morphology, Gram stain reaction and biochemical reactions (anaerobes by API 20 A). The bacterial counts were expressed as mean log CFU/g wet feces.

Results

Direct microscopic examination

In the four categories, the most significant difference was observed in the number of the Gram positive large rods among the test groups. In normal feces, very few Gram positive large rods were observed. They significantly increased after the animals were injected with lincomycin (Fig. 1). *C. difficile* are included in this category. Morphological examination showed us that the increased bacteria are mainly *C. difficile*. In the feces of the animals which received DMV or COMP, the number of the Gram positive large rods decreased drastically. The changes in the bacterial numbers of the Gram positive rods and that of the Gram positive cocci showed similar patterns: among the animals injected with lincomycin, DMV treatment significantly decreased the bacterial numbers, whereas that of the COMP treated group were not so intense though the bacterial numbers decreased as well. The situation of the Gram negative rods was somewhat different. After the animals were injected with lincomycin, the numbers of these bacteria decreased, whereas DMV and COMP maintained the normal level or improved their growth (Fig. 1). It is well known that the Gram negative rods, especially *Bacteroides*, are important members of the intestinal flora.[7,8]

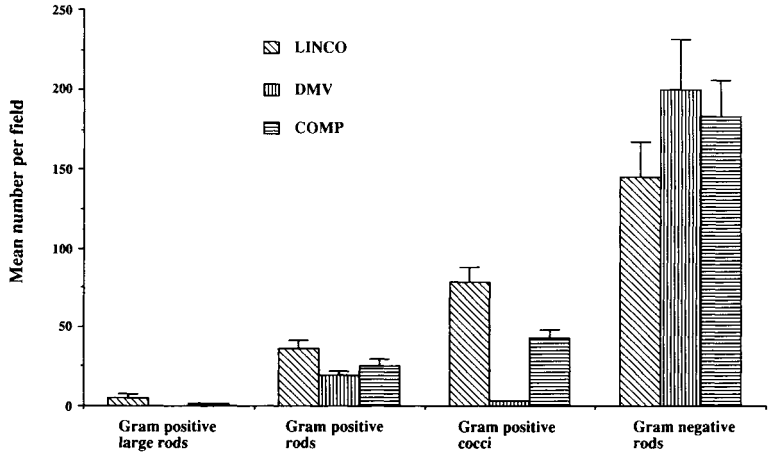

Fig. 1 Changes in numbers of various bacteria under direct microscopic examination.

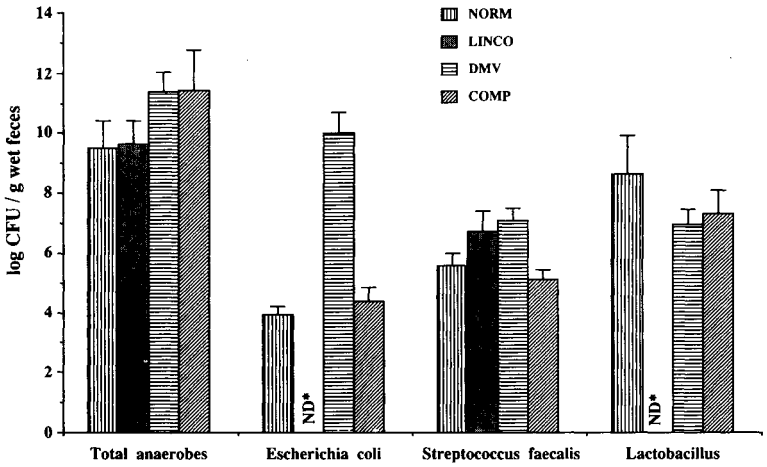

Fig. 2 Changes in numbers of various bacteria by isolation.
*ND : not detected

Microflora analysis by the isolation method

The total counts of the anaerobic bacteria were increased in the DMV and COMP treated groups. The fact is important because maintaining the stability of the anaerobic bacterial flora is essential to its colonization resistance against exogenous pathogens. The most significant change in the facultative anaerobes is that the lincomycin has exclusively inhibited the growth of *E. coli*. As most of the Gram positive bacteria are sensitive to vancomycin,[9] the inhibition of their growth will cause the count of *E. coli* to increase. This was seen in the DMV treated group. The COMP can maintain the amount of *E. coli* by preventing the decrease caused by lincomycin to its normal level (Fig. 2). Lincomycin also inhibited the growth of *Lactobacillus* and *Bifidobacterium* distinctly (Fig. 2, 3), whereas the COMP and DMV prevented the decrease of the numbers of the two bacteria. As to the Gram positive anaer-

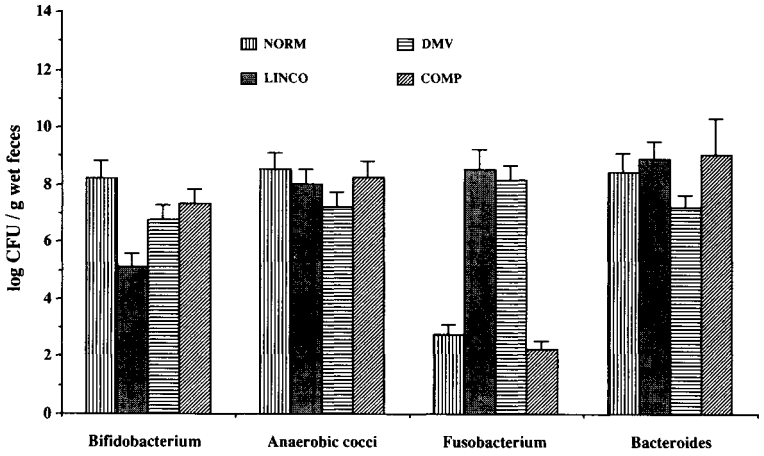

Fig. 3 Changes in numbers of various bacteria by isolation.

obic cocci, lincomycin and the COMP had no significant influence on them, but DMV had a decreasing effect (Fig. 1). The number of Fusobacteria in the antibiotic treated groups increased significantly than that of normal animals, but COMP maintained the number at a normal level. Thus the COMP is able to keep the amount of the anaerobic rods at a normal level (Fig. 3) even after the lincomycin injection.

Discussion

There are numerous bacteria living in the digestive tract of human beings. Undergoing a long period of development and innumerable generations of their multiplication, an ecological relationship and adaptation have been formed among them and between the flora and the host. The maintenance of the intestinal flora in the normal state is highly important to the nutrition, health and immunity of a human.

In the present study, direct microscopic observation revealed that the Gram positive large rods were presented in vast amounts in the lincomycin treated animal feces, but they were rare if the animals were treated with DMV or COMP. The numbers of the Gram negative rods were significantly larger in the latter two groups of animals.

Microflora analysis showed the same pattern, *i.e.*, the amount of the Gram negative rods was maintained at normal levels. In the lower digestive tract of man and animals, the Gram negative as well as Gram positive rods have numerical superiority. If their amount decreased sharply, the intestinal immunity must be injured. The multiplication of *Lactobacillus* and *Bifidobacterium* were significantly inhibited in the lincomycin treated animals, but they were normal on the whole in the two drug treated groups. The situation of *E. coli* was similar to that above. The DMV possesses stronger inhibitory activities against the Gram positive bacteria, whereas the decoction of the COMP of Chinese materia medica did not possess inhibitory activity against these bacteria *in vitro* (unpublished data). Therefore, the curative effects of the prescription are mild and ecologically effective.

和文抄録

本研究の目的は漢方方剤の投与より大腸炎モデル（ハムスター）の腸内菌変化に関する検討であった。使用した方剤は実験性ハムスター大腸炎に有効であると証明された。実験方法として糞便懸濁液直接塗抹標本法と選択培地分離培養法を用いた結果，lincomycinの前処理により腸内菌菌叢が著しく変化した。demethyl vancomycinの投与で病原菌の増殖が抑制されると共に，正常腸内菌の抑制，あるいは

異常な増殖も認められた。一方，漢方方剤の投与では病原菌の増殖は抑制されたが，正常菌叢は維持された。

Refernces

1）劉林祥，謝念祥，李志軍，宋紅月，牛恵珍：中薬複方治療抗生素性腹瀉及難辨梭菌結腸炎的研究，I. 利用地鼠結腸炎模型研究経典中薬複方的作用．中西医結合雑誌 **10**（特集），152-154, 1990.

2）劉林祥，謝念祥，李志軍，郝近大，宋紅月，牛恵珍：中薬複方治療抗生素性腹瀉及難辨梭菌結腸炎的研究，II. 利用地鼠結腸炎模型研究自組複方的作用．中西医結合雑誌 **10**（特集），155-157, 1990.

3）劉林祥，張相勇，李志軍，宋紅月，牛恵珍：地鼠結腸炎模型的研究．微生物学報 **25**, 60-65, 1985.

4）上海薬物所：人體臨床試用新抗癌薬物剤量的預測方法．薬物研究 **10**, 20-22, 1975.

5）光岡知足：腸内細菌学，朝倉書店出版，東京，pp. 473-480, 1990.

6）劉林祥，謝念祥，李志軍，牛恵珍，宋紅月：用潔霉素及慶大霉素製備難辨梭菌的分離培養基．微生物学通報 **14**, 19-23, 1987.

7）Rotimi, V.O., and Duerden, B.I.: Bacteroides species in the normal neonatal faecal flora. *J. Hyg. Camb.* **87**, 299-304, 1981.

8）Drasar, B.S., and Hill, M.J.: Human Intestinal Flora, London Academic Press, 1974.

9）Barker, B.M., and Prescott, B.F.: Antimicrobial Agents in Medicine, Blackwell Scientific Publications, London, 1973.