P.O.Box 2345, Beijing 100023,China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

World J Gastroenterol 2003;9(3):568-571
World Journal of Gastroenterology
 ISSN 1007-9327

• BASIC RESEARCH •

Combined therapy of allantoin, metronidazole, dexamethasone on the prevention of intra-abdominal adhesion in dogs and its quantitative analysis

Xiao-Chen Wang, Chang-Qing Gui, Qing-Shan Zheng

Xiao-Chen Wang, Qing-Shan Zheng, Anhui Provincial Center for Drug Clinical Evaluation & Yijishan Hospital of Wanan Medical Collage, Wuhu 241001, Anhui Province, China
Chang-Qing Gui, Department of Pharmacology, Wanan Medical Collage, Wuhu 241001, Anhui Province, China
Correspondence to: Dr. Qing-Shan Zheng, Anhui Provincial Center for Drug Clinical Evaluation & Yijishan Hospital of Wanan Medical Collage, 93 TuanJieDong Lu, Wuhu 241001, Anhui Province, China. editorys@mail.wh.ah.cn
Telephone: +86-553-573-8350 **Fax:** +86-553-573-8350
Received: 2002-10-30 **Accepted:** 2002-11-19

Abstract

AIM: To observe the preventive effects of combined therapy of AMD (allantoin, metronidazolem and dexamethasone in combination) on intra-abdominal adhesion in dogs.

METHODS: 20 dogs of both sexes were used in this study. After laparotomy under anesthesia, 2 cm section of cecal end was clamped and ligated, then 1 cm cecum section was cut and another 1 cm was kept. The cecum stump was closed with purse-string suture. Both parietal and visceral peritonea were stripped for an area of about 3×4 cm^2. Before the skin closure, the animals were divided into two groups randomly. The abdominal cavities in Group AMD (n=10) were rinsed by 200 ml of AMD solution, and with 50 ml left, whereas the control (n=10) received the equal volume of normal saline. After 7 d, the degree of intra-abdominal adhesions was evaluated by using the score method of ultrasonography and traditional dissection.

RESULTS: Compared with the control, both the ultrasonography and traditional dissection scores in Group AMD were significantly decreased that marked as 2.0±1.25 *vs* 3.3±0.82 and 1.91±0.83 *vs* 3.3±0.82 respectively (P<0.01).

CONCLUSION: The combined therapy of AMD is an effective way to prevent intra-abdominal adhesion, and ultrasonography is an useful tool to diagnose intra-abdominal adhesion.

Wang XC, Gui CQ, Zheng QS. Combined therapy of allantoin, metronidazole, dexamethasone on the prevention of intra-abdominal adhesion in dogs and its quantitative analysis. *World J Gastroenterol* 2003; 9(3): 568-571
http://www.wjgnet.com/1007-9327/9/568.htm

INTRODUCTION

Intra-abdominal adhesions are almost inevitable to some extent after major abdominal surgery. Weibel and Majno reviewed 289 subjects at post-mortem who had had previous laparotomies, and 67 % of the patients showed adhesions; after multiple operations, the incidence rose to 93 %[1]. However, there has been little advances in the treatment and prevention of this complication in recent years. Numerous attempts with agents and surgical techniques often obtain conflicting results[2,3]. The cause may be mainly, at least partly, due to multi-factor in the adhesion etiology and multi-pathway in the adhesion mechanism[4-6], which made the adhesions difficult to be prevented by using a single drug or certain measures. Besides, the evaluation of intra-abdominal adhesion was based classically on traditional dissection method, which was impossible to be applied to the clinical settings. In this study, we employed a new reproducible animal model of intra-abdominal adhesion cased by multi-factors, and a new evaluation method by ultrasonography to assess the effects of combined AMD (allantoin, metronidazolem and dexamethasone in combination) therapy.

MATERIALS AND METHODS

Materials

Allantoin (Alt) powder with the purity of 99.6 % was obtained from Jiangsu Huanghai Pharmaceutical Factory. Metronidazole (Met) powder was from Tianjing Hebei Pharmaceutical Factory with the purity of 99.85 %. Dexamethasone (Dex) powder was purchased from Roussel Uclaf Co and the purity is 99.6 %. They were dissolved and mixed with 5 % GS, the ratio of Alt: Met:Dex is 50:32:1. The HEWLETT-PACKARD Sonos-2000 Color Ultrasonic Doppler Method Diagnostic Equipment was employed in this study, using a real-time sonolayer SSA-270Aultrasound scanner (Toshiba, Tokyo, Japan) and a 3.5 MHz sector transducer.

Experimental animal

20 adult healthy dogs of both sexes, weighing from 7 to 10 kg were purchased from the Animal Center of Wannan Medical College.

Animal models

The experiment was carried out in clean but not sterile condition. Under 3 % sodium pentobarbital anesthesia (1 ml/kg iv), following shaving and skin disinfecting, the laparotomy was performed through a 5 cm, vertical, midline incision. 2 cm section from the cecal end was clamped and ligated, 1 cm of the section was cut, and the other 1 cm from the ligated site was kept. The cecum stump was closed with the purse-string suture. Then a 3×4 cm^2 patch of parietal peritoneum corresponding to the cecal was carefully stripped. In addition, both sides of peritoneum along the abdomen incision were scraped for an area of about 3×4 cm^2. Before skin closure of abdomen, the animals were randomly divided into 2 groups. The abdominal cavity in Group AMD were rinsed by 200 ml of AMD solution and with 50 ml left, whereas the control received the equal volume of normal saline. All the animals were fasted for 8 hours after operation.

Adhesion assessment by ultrasonography

At the seventh day after operation, all the dogs were reanesthesiaed. Selecting the skin point corresponding to the

appendix, 1 000 ml of normal saline was instilled into the abdominal cavity with a 12G needle to improve the acoustic window[7]. The whole abdomen was divided into four areas artificially by a horizon through nave and a vertical line through xiphoid (Figure 1), the number and density of adhesion sites were graded on the basis of ultrasonographic findings (Table 1).

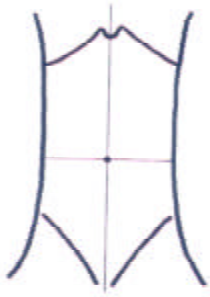

Figure 1 Abridged general view of dog abdomen subregion.

Table 1 Adhesion assessment by ultrasonography

Score	Description
1	Echogenic bands in one area
2	Echogenic bands in two areas
3	Echogenic bands in three areas or alveolate echogenic bands in one area
4	Massive agglutinating adhesion or adhesion between viscera and abdominal wall

Adhesion assessment by traditional dissection

After ultrasonic examination, the dogs were sacrificed and an autopsy examination was carried out with the attention to the number, density and site of the adhesion formation, which was scored by a modified scale by Swolin[8,9] (Table 2). The highest score for each dog was taken to be further processed.

Table 2 Adhesion assessment by traditional dissection

Score	Description
1	Filmy connections sparated spontaneously
2	Firm adhesions separated by gravity
3	Firm adhesions by traction
4	Dense adhesions requiring sharp dissection

Statistical analysis

Quantitative results were expressed as mean ± SD. Statistical analysis was performed using Student' s *t* test. $P<0.05$ was considered statistically significant.

RESULTS

One week postoperation animals in control group developed strip or round adhesions (Finger 2), attached either to the closed peritoneal defect or to the midline scar, or connected between the bowels. All the control animals had positive sonographic findings. Transabdominal sonogram clearly showed echogenic bands floating in the abdominal cavity like mice-tails (Figure 3). In more serious subjects, the adhesions were so dense that the sonogram showed alveolate echogenic masses (Figure 4); Some adhesions were formed between the organs in abdominal cavity (Figure 5). All the sonographic positive findings were proved to be the adhesion formation in laparotomy. The adhesion formed in Group AMD was significantly decreased compared with the control group as shown by both ultrasonography and traditional dissection score that marked as 2.0±1.25 *vs* 3.3±0.82 and 1.91±0.83 *vs* 3.3±0.82 respectively ($P<0.01$).

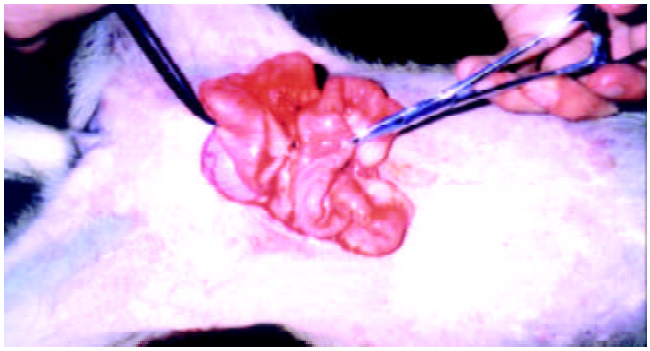

Figure 2 Adhesions between the bowls of animal in control group.

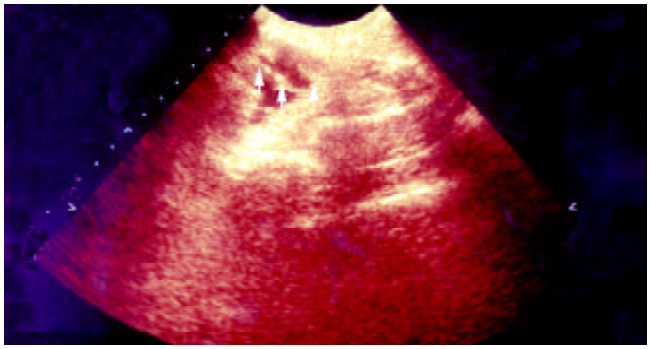

Figure 3A Transabdominal sonogram showed the adhesions between bowls, the adhesion looks like a mouse tail, and the score is 1.

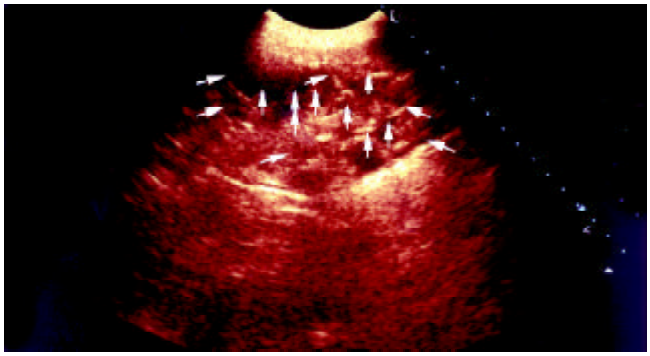

Figure 3B Transabdominal sonogram showed massive agglutinating adhesion between bowls, and the score is 3.

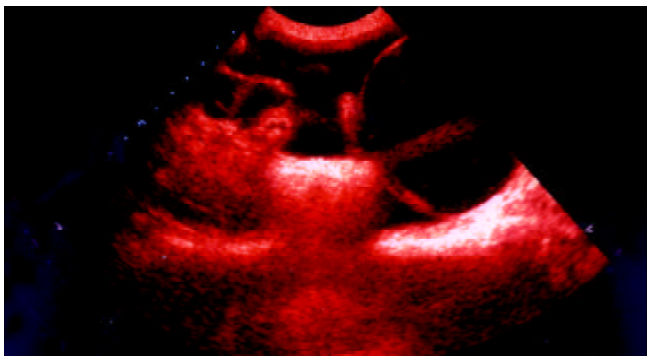

Figure 3C Transabdominal sonogram showed the adhesions between bladder and bowls, and the score is 4.

DISCUSSION

Because of the high incidence of intra-abdominal adhesion formation postoperation, plenty of investigations have been made for the past decades; However, up to the present, satisfactory results have not been achieved yet, which reflected such a fact that there still existed some obstacles waiting to be overcome.

The animal model of intra-abdominal adhesion was such a problem. Traditionally, the model was made mainly on mice, rats and rabbits by a series of America, British investigators in different means[10-12]; however, these small animals were quite different from human beings in phylogenesis, metabolism reactivity and so on. In addition, such model making laid much stress on mechanical trauma, many important factors, such as tissue ischemia, infection, inflammation and exudation[13-16] were severely neglected, which made the experimental results inconsistent with clinical settings. In the present, study a novel dog model of intra-abdominal adhesion caused by multi-factors was employed, which might reflect really the complexity of etiology and the nature of intra-abdominal adhesion formation.

There are a large number of substances used to combat adhesion formation at present[17-26], however, because of the multi-factors in the adhesion etiology and multi-pathways in the adhesion mechanism, the single use one or two of these agents could not turn out satisfactory results. Besides, the majority of these agents have been proven to be too toxic to be used[27-29].

The formation of intra-abdominal adhesion has been attributed to the local depression of plasminogen activator activity (PAA) for more than 3 decades[30-33]. This deficit permits the deposited fibrin on peritoneal surface to form fibrous adhesion. In this study, we used the combined AMD composed of allantoin, metronidazole and dexamethasone, which was proven to play a role of anti-inflammation, anti-bacteria and anti-exudation by prohibiting the fibrin rich exudate into the abdominal cavity and increasing the activity of endogenetic tissue plaminogen activator, to prevent intra-abdominal adhesion postoperation. According to our pilot study, the best proportion of these three drugs was 50:32:1, which made the effectiveness of the combination reinforced, whereas the toxicity didn't increase[34]. As for the impairment of the combination on wound healing, it was too slight to be noticed, for the intra-abdominal adhesion formed mainly in 6 hours postoperation, after that, the wound healing began to occur while the effect of the combination was gradually disappeared.

Intra-abdominal adhesion failed to be detected by routine ultrasonography. Lee *et al* identified instilling normal saline into abdominal cavity could diagnose the female pelvic lesions[7], we employed this method to perform an assessment for the intra-abdominal adhesion. It was demonstrated that this method was visible and accurate, the score of intra-abdominal adhesion was well in agreement with that done by the laparotomy. The most notable finding of sonography in the examination of intra-abdominal adhesion was the mouse-tail appearance, which was believed as the sign of adhesion band. In conclusion, we suggested that the combined AMD might be an effective way to prevent intra-abdominal adhesion, and the ultrasonography an useful tool to diagnose intra-abdominal adhesion, and their applications might be valuable to the clinical settings.

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Edited by Zhu L