Comparison of Modified Warm Water Infusion and Air Insufflation for Colonoscopy in A Rat Colon Cancer Model

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Abstract

Our aim is to compare the effect of warm water infusion and air infusion enteroscopy on the success rate of colonoscopy in a rat model of colorectal cancer. 60 rats with colorectal cancer were divided into 2 groups: the air infusion (AI) group taking air infusion enteroscopy and the warm water infusion (WWI) group taking warm water infusion enteroscopy. The success rate, testing time, pain score and diagnostic value of these two groups were evaluated. There are significant differences between two groups concerning the success rate and the average test time. In the WWI group, the success rate is 83.33% (25/30), and the average test time is 12.2±4.7 min, while in the AI group, the success rate is 56.67% (17/30), and the average test time is 17.6±5.8 min. Obvious lighter pain was observed in the WWI group than that of the AI group which reflected by pain score in the WWI group (1.7±0.8) compared with that of AI group (2.5±1.1) ($p<0.05$). There is no significant difference in the success rate of the colorectal cancer model in the WWI group (90.00%, 27/30) and the AI group (93.33%, 28/30). No significant differences were observed in sensitivity, specificity, correct diagnosis index, and misdiagnosis rate between two groups. Warm water infusion enteroscopy can increase the success rate of colonoscopy while reducing the testing time, and give less pain during colonoscopy than the air infusion enteroscopy.

Keywords: air Insufflation, colonoscopy, colon cancer model, rat, warm water Infusion

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Introduction

Colonoscopy is still the preferred method in diagnosis and treatment of colorectal cancer (Schattner and Gilad, 2002; Thanapirom et al., 2012). However, in clinic with poor tolerance to pain might negatively impact the technical performance of the examination (Rex and Khalfan, 2005). Moreover, patients often complain of abdominal pain, nausea and vomiting during colonoscopy. Traditional colonoscopy, a method of air injection, often causes intestinal flatulence and trigger spasm and junction loops to patients, resulting difficulties for colonoscopy performing (Kaminski et al., 2010). To minimize these drawbacks and improve the endoscopy techniques, several methods have been evaluated in recent years, such as warm water infusion methods (Lee et al., 2009) and carbon dioxide insufflation methods (Nakamura et al., 2014). However, few evidence was found about the comparison of warm water infusion with air insufflation methods concerning the successful rate, accuracy of diagnosis rate, sensitivity, specificity, correct diagnosis index and the rate of misdiagnosis of the two methods.

In this study, we aim to compare the difference between the two methods in a rat colon cancer model, and to evaluate whether warm water infusion method is a better choice than air infusion when performing colonoscopy.

Materials and Methods

Experimental groups: In this study, 60 Wistar rats were used to induce the colon cancer model and then divided into two groups: the WWI (warm water infusion) group and the AI (air infusion) group. Colonoscopy was performed with modified warm water infusion method and air infusion method, respectively. The pain score system is used to assess the discomfort of the two methods. The anatomy examination were performed to evaluate the successful rate, the accuracy of diagnosis rate, sensitivity, specificity, correct diagnosis index and the rate of misdiagnosis between the two groups. The warm water infusion method is easy to perform and can reduce the examining time as well as discomfort during the colonoscopy, and with the same diagnostic accuracy, sensitivity, the rate of misdiagnosis of air infusion methods.

Rat model of colorectal cancer: All experimental protocols were approved by the Animal Care and Use Committee of Suzhou University (No. 14-1017) and conformed to National Institutes of Health guidelines of China. 4-6 weeks old male Wistar rats with average weight of 122.7±12.4 g were used in this experiment. Rats model of colon cancer were set up using an enema by 4mg/ml MNU solution (N- methyl -N- nitrosourea, Sigma-Aldrich, St Louis, MO). The catheter was cut into 8-9 cm as an enema, and 0.5ml/100 g body weight dosage was injected 3 times a week. After the end of enema (6 weeks), rats were undergoing colonoscopy with Olympus Q260DL colonoscopy (Olympus, Tokyo, Japan) in strict accordance with product instructions.

Colonoscopy: All rats were anesthetized in a chamber using isoflurane (5%), sedation was maintained via continuous inhalation of 2% isoflurane. The AI group using air insufflation enteroscopy: all rats examined after using physiological saline enema at 37°C after emptying the gut. In order to make a clear observation of intestinal mucosal lesion, gas was used to provide clear vision after connected to the main injection pump. The colonoscopy was inserted into the ileocecum through the anus and intestinal cavity. The standard of success colonoscopy is the colonoscopy reach ileocecum within 10 min.

Rats in the WWI group were treated with modified method of infusion of warm water enteroscopy using Olympus OFP endoscopic water pump (Olympus, Tokyo, Japan), but the intestinal preparation was the same as control group. Rats were taken the left recumbent position, warm water were used to provide clear vision after connected to the endoscopic feeding pump.

Assessment of the colonoscopy: Body weight, constipation, success rate of colonoscopy and time of examination were compared between the two groups. Visceral pain in colonoscopy were compared according to the abdominal withdrawal reflex, and it was divided into 5 degrees: 0 degree, no behavioral reflection; 1 degree, only a slight head movements; 2 degree, mild abdominal contraction; 3 degrees, strong abdominal contraction; 4 degree, mandatory abdominal contraction. In order to assess the colorectal cancer model rats were dissected after colonoscopy, and the sensitivity, specificity, correct diagnosis index and the rate of misdiagnosis were used as evaluation indicators.

Statistical analysis: For statistical analysis, the SPSS 15.0 was used. Descriptive statistics were computed for all variables. Normally and abnormally distributed data were represented as Mean±SD, median and interquartile range. χ^2 and *t*-tests were used to compare proportions and means for normally distributed data, as appropriate; median values for abnormally distributed data were compared by using the Mann-Whitney rank sum test. For all tests, a $p<0.05$ was considered statistically significant.

Results

Procedural Data and the successful rate of the Colonoscopy on rats: In our study, colon cancer was successfully induced in 28 rats out of 30 rats in the WWI group and 27 rats out of 30 rats in the AI group according to the anatomy examination after Colonoscopy, and the success rate had no significant difference between the two groups. Parameters concerning the procedure are summarized in the **Table 1**. Both of the two kinds of colonoscopy methods show the lesions clearly (**Figure 1**). Significant differences can be seen in the success rate and the examine time between the WWI group and the AI group ($p<0.05$), because the success rate in the WWI group was 83.3% (25/30) while AI group was 56.67% (17/30). The time used during examination was 17.6±5.8 min and 12.2±4.7 min in the WWI and the AI groups,

respectively (*p*<0.05). No significant difference was observed between the two groups regarding the weight or the constipation. No significant difference was observed with regard to the overall difficulty of the examination, as rated by the endoscopists between the groups, as detailed in the Table 1. Taken together of the above results, the warm water infusion method is easy to perform and reduces examining time during the colonoscopy, which is consist with the recent report in human (Amato et al., 2013).

AI group **WWI group**
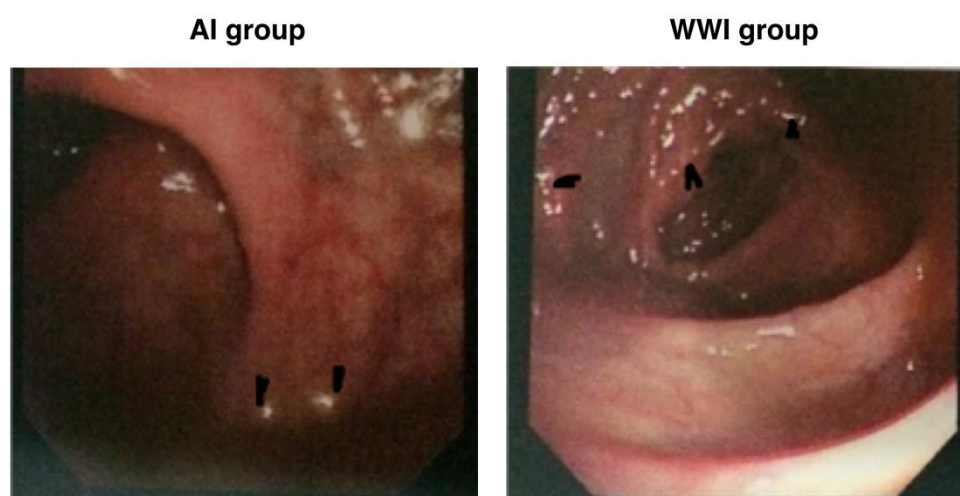
Figure 1 Image from colonoscopy at different group of Rats. Black arrow: colon cancer. Magnification: 40×.

Table 1 Parameters of the procedure and success rate between two groups.

	WWI	**AI**	*p*
weight	273.8±22.4	275.4±21.2	>0.05
Incidence of colorectal cancer (%)	93.33(28/30)	90.00(27/30)	>0.05
Successful rate (%)	83.33 (25/30)	56.67 (17/30)	<0.05
Time to cecum(min)	7 .26±1.30	9 .88±1.43	<0.05
Total procedure time (min)	12.2±4.7	17.6±5.8	<0.05
Constipation	10.00 (3/30)	13.33 (4/30)	>0.05
Difficulty examination rate (%)	4.00 (1/25)	5.88(1/17)	>0.05

The degree of Pain between WWI group and AI group: Differences in the pain degrees of two groups were assessed according to the body reaction during inspection. The degree of pain during the examination is shown in **Figure 2** and **Table 2**. The pain score was evaluated according to rats' pain abdominal withdrawal reflex when performing colonoscopy. The score of pain in the WWI group (1.7±0.8) was less than AI group (2.5±1.1), with significant difference (t=3.978, *p* < 0.05). Pain rating of the two groups have statistical difference (Z=5.472, *p* < 0.05): The rats in the WWI group which didn't show pain reflects were more than the AI group; a part of rats in the WWI group and the AI group showed slight head movement; only a small part of rats in the WWI group showed pain of degree 3; in the AI group a small part of rats showed pain of degree 4 while that was none in the WWI group. According to the above results, the warm water infusion can significant reduce the discomfort during the colonoscopy, which is consist with recent reports in human (Radaelli et al., 2010).
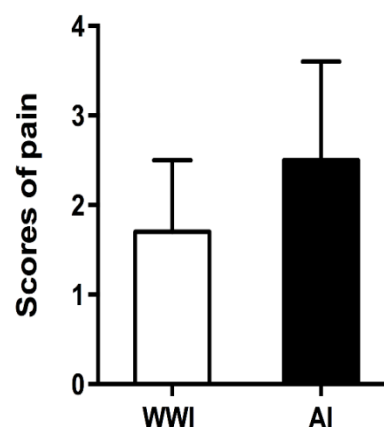
Figure 2 Assessment of pain between two groups.

Table 2 The differences on the degree of pain between two groups.

degree	behaviors	groups	
		WWI	AI
0	No behavioral reflection	11（36.67）	7（23.33）
1	Slight head movements	10（33.33）	9（30.00）
2	Abdomen did not leave the platform	8（26.67）	9（30.00）
3	Abdomen leave the platform	1（3.33）	3（10.00）
4	Body arched, abdominal high, pelvic erect and improve reflection.	0（0.00）	2（6.67）

The sensitivity, specificity, correct diagnosis index and the rate of misdiagnosis of the two groups: In order to evaluate the two kinds of endoscopic methods, we perform the anatomy examination after colonoscopy, and we assess the pathological changes of rat colon. Comparison of diagnostic accuracy, sensitivity, specificity, correct diagnosis index and the rate of misdiagnosis of the two groups can be seen in **Table 3**. There is no significant difference concerning the Polyp detection rate between the WWI group (86.66%) and the AI group (90.00%). There is no significant difference in the sensitivity between the WWI group (96.30%) and the AI group (92.86%). Lower correct diagnosis index can be seen in the WWI group compared to that of the AI group (0.63 vs.0.93, P>0.05). The misdiagnosis rates were same (6.67%) between the two groups. However, the WWI group has obvious lower specificity compared to the AI group (66.67% vs.100%, *p*<0.05), which indicate that warm water infusion method is as sensitive as air infusion method and with the same diagnostic accuracy of colon cancer.

Table 3 Differences on diagnostic accuracy, sensitivity, specificity, correct diagnosis index and the rate of misdiagnosis of the two groups.

	WWI	AI
Polyp detection rate (%)	86.66 (26/30)	90.00 (27/30)
Sensitivity (%)	96.30	92.86
Specificity (%)	66.67	100.00
Correct diagnosis index	0.63	0.93
Rate of misdiagnosis (%)	6.67	6.67

Discussion

Colon cancer is a common tumor occurring in the junction of the rectum and sigmoid colon. The occurrence and development of tumor is related with many factors, including physical, chemical and biological factors. The chemical factors is commonly used to induce colon cancer in experimental animal. In this study, the MNU solution was used to induce the enema colon cancer model in rats of the legal system. The anatomy examination were performed after colonoscopy and the colon pathology changes were observed in 28 rats in the WWI group, 27 rats in the AI groups, respectively. There was no significant difference between the two groups. In this study, success rate in the rat tumor model was 95% (57/60), suggesting a pretty higher efficiency of the MNU solution in inducing the colon cancer model in rats. MNU as a DNA alkylating agent, which can directly cause multiple organ cancer, is currently widely used to construct different experimental animal tumor model (Sharmila et al., 2014).

Manifested emaciation, abdominal distention, dyspepsia and bowel habits change were the early symptom of colon cancer patients. Since these symptoms are not typical, so the diagnosis is difficult (Zauber et al., 2012). Early treatment can significant improve the outcome of colorectal cancer patients, therefore the early diagnosis of colorectal cancer in patients is very important (Marques et al., 2014). At present, colonoscopy is a common method for diagnosis of colon cancer, it is sensitive, slightly invasive, and it can measure the tumor location, size and depth of tumor invasion. Moreover, colonoscopy also play an important role in the treatment of colon cancer (Amri et al., 2013). However, the procedure of colonoscopy is a great stimulation in patients, because inserting and tracing procedure can stimulate the sensory nerve in patient's visceral, and it may cause vagus excitement, nausea, vomiting and other discomfort. Even with the excitement of nerve, it often leads to intestinal spasm and resulted in abdominal pain and difficulty insertion (Park et al., 2010). The traditional colonoscopy, using the method of air injection, usually causes intestinal cavity, especially

product of gas and distortion in the sigmoid colon. The key factor of the successful colonoscopy is whether the insertion can smoothly through the sigmoid colon (Asai et al., 2015).

In this study, we use a modified warm water infusion method, and the results show that this method significantly increases the success rate, shortening the time of examination. Using of warm water can effectively extend the intestinal cavity, prevent intestinal cramps and elongated, conducive to colonoscopy smoothly through the intestinal tract. When rats were examined at the left recumbency, perfusion of warm water can also straightening of the sigmoid colon, reduce intestinal pull in the examination process, reduce pain and discomfort, and improve the inspection success rate. No statistical difference can be seen between the two groups of rats on weight, occurrence of constipation and other factors affecting the colonoscopy. According to the pain reflects during inspection, the pain scores in the WWI group were significantly lower than that of the AI group. Moreover, there is no significant difference can be seen in sensitivity, specificity, correct diagnosis index and the misdiagnosis rate between the two colonoscopy methods, ssuggesting that the modified warm water method has high application value in the diagnosis.

The present study provides the basis evidence for the application of a modified warm water infusion methods in the colonoscopy from a rat model. The modified warm water infusion colonoscopy method saves examination time, improve the success rate of colonoscopy and relieve discomfort during the examination.

Acknowledgements

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บทคัดย่อ

การเปรียบเทียบระหว่างการใช้น้ำอุ่นและการใช้ก๊าซสำหรับส่องกล้องตรวจลำไส้ใหญ่ในหนูขาวที่เป็นแบบจำลองของมะเร็งลำไส้ใหญ่

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วัตถุประสงค์เพื่อเปรียบเทียบผลของการใช้น้ำอุ่นและการใช้ก๊าซต่ออัตราความสำเร็จของการส่องกล้องตรวจลำไส้ใหญ่ในหนูขาวที่เป็นแบบจำลองของมะเร็งลำไส้ใหญ่ แบบแผนการทดลอง แบ่งหนูขาวที่เป็นมะเร็งลำไส้ใหญ่จำนวน 60 ตัวออกเป็น 2 กลุ่ม คือ กลุ่มที่ใช้ก๊าซและกลุ่มที่ใช้น้ำอุ่นในขณะส่องกล้องตรวจทางเดินอาหาร เพื่อประเมินอัตราความสำเร็จ ระยะเวลาในการส่องตรวจ คะแนนความเจ็บปวด และประสิทธิภาพของการวินิจฉัย โดยพบว่าอัตราความสำเร็จและค่าเฉลี่ยระยะเวลาในการส่องตรวจของหนูทั้งสองกลุ่มมีความแตกต่างกันอย่างมีนัยสำคัญ ในกลุ่มหนูใช้น้ำอุ่นพบอัตราความสำเร็จ 83.33% (25/30) และมีค่าเฉลี่ยระยะเวลาในการส่องตรวจ 12.2+4.7 นาที ในขณะที่กลุ่มหนูที่ใช้ก๊าซพบอัตราความสำเร็จ 56.67% (17/30) และมีค่าเฉลี่ยระยะเวลาในการส่องตรวจ 17.6+5.8 นาที หนูกลุ่มที่ใช้น้ำอุ่นมีความเจ็บปวดน้อยกว่าหนูกลุ่มที่ใช้ก๊าซ เมื่อเปรียบเทียบจากคะแนนความเจ็บปวดระหว่างหนูกลุ่มที่ใช้น้ำอุ่น (1.7+0.8) และหนูกลุ่มที่ใช้ก๊าซ (2.5+1.1) (p<0.05) อัตราความสำเร็จของแบบจำลองมะเร็งลำไส้ใหญ่ในกลุ่มหนูใช้น้ำ (90.00%, 27/30) และกลุ่มที่ใช้ก๊าซ (93.33%, 28.30) ไม่พบความแตกต่างอย่างมีนัยสำคัญ และไม่พบว่าหนูทั้งสองกลุ่มมีความไว ความจำเพาะ ดัชนีวินิจฉัยถูกต้อง และอัตราการวินิจฉัยผิดมีความแตกต่างอย่างมีนัยสำคัญ สรุป การส่องกล้องตรวจทางเดินอาหารโดยการใช้น้ำอุ่นสามารถเพิ่มอัตราความสำเร็จของการส่องกล้องตรวจลำไส้ใหญ่ โดยการลดระยะเวลาในการส่องตรวจ และลดความเจ็บปวดในขณะส่องกล้องตรวจได้ดีกว่าการใช้ก๊าซ

คำสำคัญ: air Insufflation colonoscopy colon cancer model หนูแรท warm water Infusion