Indian J Surg (March–April 2009) 71:73–77

ORIGINAL ARTICLE

The effect of Kombucha on post-operative intra-abdominal adhesion formation in rats

Hemmat Maghsoudi · Hussein Benagozar Mohammadi

Received: 23 June 2008 / Accepted: 19 January 2009

Abstract

Background Peritoneal adhesions are fibrous bands of tissues formed between organs that are normally separated and/or between organs and the internal body wall after peritoneal injury. The aim of the study was to investigate the effect of intra-peritoneal administration of Kombucha on intra-peritoneal adhesions.

Materials and methods Eighty Wistar rats were subjected to standardized lesion by scraping model and were randomly divided into two groups. Group I received no treatment, and Group II received 15 ml of Kombucha solution intra-peritoneally. On the post-operative 14th day adhesion intensity score, inflammatory cell reaction and number of adhesion bands were determined.

Results In the control group, there were no rats with grade 0 and I adhesions. In the group II, there were 26 rats (78.8%) with grade 0 -2 adhesions. Adhesion intensity was significantly less in group II ($P<0.0001$). Number of adhesion bands was significantly less in group II ($P<0.001$).

Conclusion it was concluded that intra-peritoneal administration of Kombucha might be useful for preventing peritoneal adhesions.

H. Maghsoudi · H. B. Mohammadi
Department of Surgery,
Faculty of Medicine,
University of Medical Sciences of Tabriz, Iran

H. Maghsoudi (✉)
E-mail: maghsoudih@yahoo.com

Keywords Peritoneal adhesions. Kombucha. Prevention

Introduction

Adhesion formation after surgery is a significant cause of morbidity [1, 2]. The formation of peritoneal adhesion is a specific peritoneal response to injury activating the cascades which leads to adhesion formation [3]. The inflammatory response, normally induced by infection or tissue injury, is crucial in controlling and eliminating infectious agents, as well as in promoting wound healing [4]. It is known that wound healing and adhesion formation has similar pathways, following the sequence of tissue inflammation, fibrin deposition, fibrin organization, collagen formation, and maturation [5]. Peritoneal adhesions are defined as pathological fibrin bands developed between any surfaces in the peritoneal cavity. For the development of adhesions between two surfaces inside the peritoneum, there must be peritoneal mesothelial damage on at least one surface [6]. A few hours after the mesothelial damage, fibrinouse exudate is released. When the exudates is absorbed, fibrous bands and newly formed capillary vessels remain in the site and these structures form the permanent fibrotic adhesion [6].

Post-operative peritoneal adhesions (PPA) develops after 90% of all laparotomies [7, 8]. Intestinal obstruction related to PPA develops in 1% of all surgical interventions and in 3% of all laparotomies [7]. The 15–20% of female infertility is caused by PPA [9, 10]. Also entero-cutaneous fistuli, intra-abdominal abscess, ureteral obstruction, and chronic abdominal pain may develop as a result of PPA [10]. Several agents have been used to solve this very serious problem of abdominal surgery [11, 12]. On this topic, biomedical companies also continue to search and there are several barrier products in the market too [12, 13, 16, 17].

Kombucha is an old folk remedy, which originated centuries ago somewhere in the Far East. It is a symbiotic culture of yeast and several different strains of bacteria grown on sweetened black or green tea. More recently a German microbiologist discovered small mobile bacteria inside the yeast cells (endosymbionts). Because of their small size and size variations he has not been able to determine the species. It is too early to make any statements about the significance of this discovery.

Kombucha looks like a white rubbery pancake. The culture is placed on or in the tea which is allowed to ferment for about 7–10 days. One drinks 4–8 oz. of the tea every day for detoxification of the body. It has a slightly sour to vinegary taste perhaps similar to a cider. The finished tea contains glucuronic acid, lactic acid, acetic acid, vitamins and other components. Glucuronic acid is used by the liver to detoxify certain compounds and is considered by many to be the main beneficial ingredient of Kombucha tea [14]. People from all over the world claim drinking Kombucha tea provides relief from many physical ailments. While it may not be the cure to all the ills of mankind, it is a traditional fermented beverage used in many cultures to promote well-being.

In this study, we planned to investigate the effect of Kombucha in preventing PPA developing after a wound healing process in rats.

Materials and methods

In this study, the Kombucha tea used was prepared as follow [14,15]:

Based on many years of experience, producing the best tasting Kombucha Tea, the following is a brief instructional guide on how to care for your Kombucha Mushroom. Included are: How to prepare, ferment the tea, how and when to harvest and how to store the mushroom.

Preparation

1. Remove and discard tea bags or empty tea ball.
2. Allow sweetened tea to cool to room temperature, even if it takes overnight.
3. When at room temperature, pour the sweetened tea into a One gallon, 5 inch diameter size jar. (This is where the tea will ferment.)
4. Before pouring the cooled tea into the jar, pour 8 oz. of the starter tea (which came with the mushroom) into the jar. Then pour the cooled tea into the Jar, to ensure an even mixture of the tea with the starter. For future batches you may use your own Kombucha tea starter. In the event that no starter is available to you, you may use 8 oz. of organic, raw and unfiltered apple cider vinegar as a starter.
5. Place the Kombucha mushroom on top of the sweetened, cool tea, making sure that the darker rougher side faces down.(Don't worry if it sinks to the bottom, it's O.K.)
6. Cover jar with a piece of loosely woven cloth (the mushroom has to "breath". Cotton or linen or paper towel will do just fine.)
7. Secure with a rubber band to keep cloth (or paper towel) in place and to keep out any insects or contamination.

The study was conducted in Tabriz University of medical sciences, faculty of medicine, Experimental animal Raising and Research laboratory. The rats were also obtained from this laboratory. After approval of the local Ethics committee, eighty 6 month-old, out-bred, male Wistar rats weighting 200 to 250g were used. The rats were kept in air-conditioned colony rooms and given standard rat chow diet and water and libitum. After overnight fasting, all animals were anesthetized with 25 mg/kg Ketamine and 4 mg/kg Xylazine. The abdomen was shaved and swabbed with a povidine iodine solution pre-operatively. In order to remove powder particles, operation gloves were washed thoroughly with saline. The same researcher performed all surgical procedures. A 5 cm midline incision was made and the abdomen was opened under clean surgical conditions. The terminal ileum and cecum of all animals were mobilized and placed onto wet gauze. Both sides of a 10 cm terminal ileum segment, just proximal to the cecum, and cecum were scraped and parietal peritoneum until there were serosal petechiae on the intestinal and peritoneal surfaces. Later the arteries of the scraped segments were clamped for 1 minute to induce transient ischemia (scraping model) [18].

In order to eliminate the possible differences between the rats, animals were randomly divided into two groups by using number random table, treated as follows: group I ($n = 40$) was the control group and received intra-peritoneal administration of 15 ml normal saline, group II ($n = 40$) received intra-peritoneal administration of 15 ml of Kombucha solution. The abdominal incision was then closed in two layers with 3–0 propylene suture. The animals were then placed on the regular pellet (state manufacturer) food. All rats were sacrificed on the 14th day after being anesthetized with overdose ethyl ether before re-laparotomy. Two observers, who were blinded to treatment groups, assessed the extent of adhesion formation. The frequency and intensity of adhesions were recorded and the mean of the two observers' scores was used for statistically analysis. Adhesions were graded as 0–3 according to their severity [19] (Table 1).

The injured terminal ileum and cecum walls with fibrous adhesions were excised to confirmation of the wall scraping and adhesion formation. The specimens were fixed in 70% alcohol, dehydrated and embedded in paraffin wax. Sections were cut at a thickness of 5 mm and stained with hematoxylin eosin.

Table 1 Adhesion grading according to Evans model [19]

Grade	
Grading of adhesions	
0	No adhesions
1	Spontaneously separating adhesions
2	Adhesions separating by traction
3	Adhesions separating by dissection

A Mann–Whitney U-Statistic as a non- parametric test was used to determine differences in adhesion grading according to severity. A Chi-Square test was used to analysis number of adhesion bands between groups. A P-value <0.05 was considered significant and <0.001 was considered highly significant.

Results

The grading of adhesions in each group is summarized in Table 2. There were no animal in grade 0 and 1 in the control group and seven and thirty-tree in grade 2 and 3 respectively, but group II (study group) contained six (18.2%) in grade 0, five (15.1%) in grade 1. In group II, there were 15 (45.5%) in grade 2 and 7(21.2%) in grade 3. Comparison of groups by Mann – Whitney U test indicated that severity of adhesion bands was highly significantly less in the group II ($P < 0.001$). In group I, all of animals had adhesion bands, but in group II, six rats had no adhesion band. Comparison of groups indicated that number of adhesion bands was highly significantly less in the group II ($P < 0.001$). The number of adhesion bands in each group is summarized in Table 3. Non-specific inflammatory changes were seen in the damaged cecal and terminal ileal walls in all specimens. The dominant inflammatory cells were polymorph nuclear neutrophils. In the group II, the inflammation was less sever and the dominant cells were macrophage and polymorph nuclear neutrophils fewer in number than control group.

Among the studied rats, seven rats in the group II died immediately after operation, but etiology was unknown. No infection (intra-abdominal and incisional) was observed in both groups.

Table 2 Adhesion grading of the groups ($P < 0.001$ between groups I and II according to Mann–Whitney U-test)

Grade	Group I	Group II
0	0	6(18.2%)
1	0	5(15.1%)
2	7(17.5%)	15(45.5%)
3	33(82.5%)	7(21.2%)
Total	40(100%)	33(100%)

Table 3 Number of adhesion bands according to number of cases of the groups ($P < 0.001$ between groups I and group II)

Number of adhesion bands	Group I (number of rats)	Group II (number of rats)
0	0	6
1	0	2
2	0	13
3	4	5
4	5	7
5	4	0
6	10	0
7	6	0
8	2	0
9	1	0
10	1	0
11	5	0
12	2	0
Total	40	33

Discussion

There two major strategies for adhesion prevention or reduction. Surgical trauma is minimized within the peritoneum by careful tissue handling, avoiding desiccation and ischemia, spare use of cautery, laser, and retractors. Fewer adhesions form with laparoscopic surgical techniques due to reduced tissue trauma. The second major advance in adhesion prevention has been the introduction of barrier membranes and gels, which separate and create barriers between damaged surfaces, allowing for adhesion-free healing. Modified oxidized regenerated cellulose and hyaluronic acid membranes or solutions have been shown to reduce adhesions in gynecologic patients, and being investigated for their ability to prevent adhesion formation in general surgical patients [20, 21].

Innumerable substances and methods have been used, either locally or systemically in an effort to reduce or prevent PPA formation, such as sodium citrate, heparin, dextran, prostigmine, olive oil, steroids and antihistamines [11].

Mechanical separation of the peritoneal surfaces used either organic (ox peritoneum) or bioabsorbable inorganic (Seprafilm1 Genzyme Co., USA) membranes [13]. But, unfortunately these materials and procedures had only limited success in abdominal surgery.

There are many experimental models for engendering peritoneal adhesions: the damaged uterine horn model, the ileal transection model, the large bowel anastomosis model, the peritoneal damage model, the bacterial peritonitis model, and the scraping model [7, 22–26].

The scraping model is very effective in engendering peritoneal adhesions because there are two stages in the

damage: direct mechanical intestinal wall damage from gauze scraping until petechial points appear, plus ischemic damage which is secondary to vascular clamping. For the reason that, this model mimics abdominal surgery, we have chosen it in our study.

Several researchers have used different criteria for grading adhesions [23, 25, 27–29]. The Evans model grades adhesions as 0–3 according to their severity [16]. We have adopted the Evans model in this study because of its simplicity and rationale. A large number of studies have described the agents used to prevent the formation and reformation of peritoneal adhesions. Kombucha is used in many medical research studies for many purposes, but it has never previously been tried in preventing PPA.

We wanted to test the effect of Kombucha on PPA due to its contents. Kombucha tea is an ancient drink from Manchuria that is cultivated from a culture formed from Bacterium Iylinum. The taste of Kombucha is slightly acidic in nature and has been known to taste like apple cider vinegar, slightly sweet, slightly sour.

In this study we observed that administration of 15ml of 0.1 mg/ml Kombucha solution significantly decreases the development of PPA. In our study, we showed that intraperitoneal administration of Kombucha, inhibits inflammation and altered the intensity and frequency of adhesion formation.

Conclusion

This study suggests that intra-peritoneal administration of Kombucha solution decrease peritoneal adhesion formation. In the light of the promising and encouraging results of our study, we are optimistic as regards the power of our innovation to contribute to the prevention and decrease of PPA. To our best knowledge there are no recognized limitations to this treatment. However, further large-scale experiments are needed before clinical trials can be performed. In addition, more studies are needed in order to understand the mechanisms of this prevention of PPA by Kombucha.

The mechanism is not clear, since, we do not have much information either on the properties of Kombucha or on the PPA process. More detailed studies are needed on these topics. Although the mechanism of action is not clear, administration of Kombucha intra-peritoneally significantly reduces the PPA.

Conflict of interest The authors do not have any disclosable interest

References

1. Ellis H, Moran B, Thompson J (1999) Adhesion related hospital readmissions after abdominal and pelvic surgery: a retrospective cohort study. Lancet 353:1476–1480
2. Cheong YC, Laird SM, Li TC, Shelton JB, Ledger WL, Cooke ID (2001) Peritoneal healing and adhesion formation/reformation. Hum Reprod 7(6):556–566
3. DiZerega GS, Campeau JD (2001) Peritoneal repair and postsurgical adhesion formation. Hum Reprod Update 7(6): 547–555
4. Milligan DW, Raftery AT (1974) Observations on the pathogenesis of peritoneal adhesions: a fight and electron microscopically study. Br J Surg 61:274–280
5. DiZerega GS. The peritoneum and its response to surgical injury. In: diZerega ZG, Malinak L, Diamond M, Linsky C, editors. Treatment of post-surgical adhesions. New York: Wiley Liss. pp 166–171
6. Nair S, Bhat I, Aurora A (1974) Role of proteolytic enzyme in the prevention of post-operative intraperitoneal adhesions. Arch Surg 108:849–853
7. Menzies D, Ellis H (1990) Intestinal obstruction from adhesions. How big is the problem? Ann R Coll Surg Engl 72: 60–63
8. Ellis H (1971) The cause and prevention of post-operative intraperitoneal adhesions. Surg Gynecol Obstet 133:497–511
9. Hers A, Diamond MP, DeCherney AH (1991) Adhesiolysis. Clin Obstet Gynecol 34:395–398
10. Afyez JA (1983) An assessment of the role of operative laparoscopy in tuboplasty. Fertil Steril 39:476–479
11. Ellis H (1982) The causes and prevention of intestinal adhesions. Br J Surg 68:241–243
12. Treutner KH, Schumpelick V (2000) Adhesion prevention.Wish and reality. Chirurg 71:510–517
13. Alponat A, Lakshminarasappa SR, Yavuz N, Goh PM (1997) Prevention of adhesions by Seprafilm, an absorbable adhesion barrier: an incisional hernia model in rats. Am Surg 63: 818–819
14. Frank GK (1996) Healthy beverage from the Far East. Its correct preparation and use. Ennsthaler. Steyr
15. Blance P (1995) Research on tea fungus. Draft of research article posted to Kombucha Discussion list
16. Larsson B, Svanberg SG, Swolin K (1977) Oxyphen-butazone, an adjuvant to be used in prevention of adhesion in operations for fertility. Fertil Steril 28:807–809
17. Luciano AA, Hauser, Benda J (1983) Evaluation of commonly used adjuvants in the prevention of postoperative adhesions. Am J Obstet Gynecol 146:88–91
18. Elkins TE, Bury RJ, Ritter JI (1984) Adhesion prevention by solutions of sodium carboxymethyl cellulose in rat. Fertil Steril 41:926–928
19. Evans DM, Mc Aree K, Guyton DP (1993) Dose dependency and wound healing aspects of the use of tissue plasminogen activator in the prevention of intra-abdominal adhesions. Am J Surg 165:229–232
20. Dijkstra FR, Nieuwenhuijzen M, Reijnen MM et al. (2000) Recent clinical developments in pathophysiology, epidemiology, diagnosis and treatment of intr-abdominal adhesions. Scand J Gastroenterol SUPPL 232:52
21. Cheong YC, Laird SM, Shellton JB (2002) The correlation of adhesions and peritoneal fluid cytokine concentrations: A pilot study, Hum Reprod 17:1039
22. Costain DJ, Kennedy R, Ciona C, Mc Alister VC (1997) Prevention of postsurgical adhesions with N,O-carboxymethyl chitosan: examination of the most efficacious preparation and the effect of N,O-carboxymethyl chitosan healing. Surgery 121:314–19

23 Kennedy R, Costain DJ, Mc Alister VC (1996) Prevention of experimental post-operative peritoneal adhesions by N,O-carboxymethyl chitosan. Surgery 120:866–870
24 Ozogul Y, Baykal A, Onat D, Renda N, Sayek I (1998) An experimental study of the effect of Aprotinin on intestinal adhesion formation. Am J Surg 175:137–141
25 Kagoma P, Burger SN, Seifter E, Levenson SM, Achilles A (1985) The effect of vitamin E on experimentally induced peritoneal adhesions in mice. Arch Surg 120: 949–951
26 Snoj M, Ar'Rajab A, Ahren B, Larsson K, Bengmark S (1993) Phospholipase-resistant phosphatidylcholine reduces intra-abdominal adhesions induced by bacterial peritonitis. Res Exp Med 193:117–122
27 Nagler A, Rivkind AI, Raphael J, Schaffer FL, Genina O (1998) Halofuginone—an inhibitor of collagen type I synthesis—prevents post-operative formation of abdominal adhesions. Ann Surg 227:575–582
28 Galili Y, Abraham RB, Rabau M, Klausner J, Kluger Y (1998) Reduction of surgery induced peritoneal adhesions by methylene blue. Am J Surg 75:30–32
29 Roussin M (1996) Out on the Kombucha range. Post to Kombucha Discussion list