

Fecal Peritonitis in Rats

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Abstract

In this study, cecal ligation and puncture were performed in 75 Sprague-Dawley rats. Peritoneal lavage was performed using 10 ml of povidone-iodine as 1% concentration in group A, while the same volume of 50% of honey diluted in distilled water was used in group B. Group C was used as a control. The overall mortality was 32% in group A (8/25), 12% in group B (3/25) and 60% in group C (15/25). Tissue toxicity was found to outweigh bactericidal effects. Honey was tested for its broad-spectrum antibacterial activity without adverse effects. So, we concluded that honey might be used as a peritoneal lavage solution with no side effects.

Keywords: Peritonitis; Experimental; Cecal-ligation & puncture; Peritoneal lavage; Honey

Introduction

Peritoneal infections are classified as primary, secondary or tertiary (Osztrógonác *et al.*, 1996). The common etiologic entities of secondary peritonitis include perforated appendicitis, perforated gastric and duodenal ulcer disease, perforated colon caused by diverticulitis, volvulus, cancer or strangulation of the small bowel (O'Sullivan *et al.*, 1996). The resulting peritonitis is almost always polymicrobial, containing a mixture of aerobic and anaerobic bacteria with a predominance of gram-negative organisms (Barrera *et al.*, 2011). Furthermore, the presence of fecal material in the abdominal cavity would promote the release of inflammatory cytokines from mast cells, macrophages, and other cell types (Pastores *et al.*, 1996, Neumann *et al.*, 1999).

A picture similar to fecal peritonitis in man was studied; standard inoculum of *Escherichia coli*, *Bacteroides fragilis* and *Enterococcus faecalis* in a sterile rat feces-barium sulfate suspension adjuvant was put in the peritoneal cavity of rats (Linhares *et al.*, 2001), while in other studies, bacterial peritonitis was induced in rats using cecal ligation and

puncture (Rittirsch *et al.*, 2009). In generalized peritonitis, evaluating aggressive, one-stage surgical management may be efficiently controlled by combining peritoneal debridement, fecal diversion, and drainage of the pelvis (Parc *et al.*, 2000), a good peritoneal lavage seemed to be as effective as any combination of antibiotic treatment (Simopoulos *et al.*, 1994). On the other hand, the use of povidone iodine for peritoneal lavage in colorectal surgery is controversial (Whiteside *et al.*, 2005). Due to its antibacterial activity (Saber, 2010), authors in the present study used diluted honey in comparison with povidone-iodine solution as a peritoneal lavage to study its effect in cases of fecal peritonitis.

Materials and methods

Animals

A total of 75 healthy male Sprague-Dawley rats having average weight 250-300 grams were divided into three groups (25 rats for each): honey, povidone and control groups. Rats were obtained from the documented animal house of the Faculty of Veterinary Medicine, Suez-Canal University, Egypt and were housed and fed a standard laboratory diet and water *ad libitum*. The local ethics committee for the use of laboratory animals ap-

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proved all experimental procedures. Appropriate animal care and use were performed according to implementation and compliance with the Animal Welfare Act.

Anaesthesia

General anaesthesia was induced with intramuscular Ketamine (50 mg/kg) and Xylazine (6 mg/kg), and conducted by a team work of the Department of Surgery and Anaesthesiology, Faculty of Veterinary Medicine, Sues-Canal University, Egypt.

Surgical procedure

Under a strict antiseptic condition, all surgical procedures were performed. The peritoneal cavity was entered through 3 cm lower midline abdominal incision and the voluminous cecum was easily identified in the right iliac fossa. Then cecal ligation and puncture were performed as previously described (Rittirsch *et al.*, 2009). Cecal puncture involved a through and through puncture with a needle. A double puncture was then made with a 20-gauge needle. No drain was left and the abdominal wound was closed in two layers with continuous 3/0 Vicryl semisynthetic sutures. Postoperative antibiotics (Ceftriaxone, Sandoz Egypt) were given intramuscularly as 25 mg/Kg b.w. /24 hours as recommended. Relaparotomy was performed after the scheduled 8-hour period with drainage only without lavage in case of group C. Peritoneal lavage using three ml of povidone-iodine (White-side *et al.*, 2005) as 1% concentration was performed in group A, while the same volume of 50% of honey diluted in distilled water was used in group B. After that, the abdominal wound was closed in two layers with continuous 3/0 Vicryl semisynthetic sutures without drains.

Post-operative period

All rats were observed in the postoperative period,

and data was collected day by day and saved as software files for later evaluation. After the scheduled 8 hours all rats were reopened for second-look laparotomy and peritoneal lavage in both honey and povidone groups were performed. After two weeks (Saber, 2010), the survived rats were opened again to detect any residual intraperitoneal infection as localized abscess formation or generalized peritonitis.

Statistical analysis

The statistical tests were run on a compatible personal computer using the Statistical Package for Social Scientists (SPSS) for windows 11. Chi-square distribution was used for studying the frequencies of mortality. The values were expressed as mean \pm standard errors. The mean values of the groups were compared by one-way analysis of variance and paired comparisons of the groups were done using the paired student t test. $P < 0.05$ was considered significant.

Results

All rats showed pictures of intra-abdominal sepsis as lethargy with diminished motor power, loss of appetite, loss of eye luster and pilo-erection. No mortality occurred within the first eight hours of induction of peritonitis. Relaparotomy findings showed evidence of fecal peritonitis with offensive peritoneal fluid, and the whole abdominal contents were amalgamated together within the fibrinous coagulum.

Mortality rate

The overall mortality was 32% in group A (8/25), 12% in group B (3/25) and 60% in group C (15/25) ($P < 0.5$) (Tables 1, 2).

Table 1. Showing the mortality in the three groups of study in relation to the postoperative period

	Mortality			
	3-5 Days	5-7 Days	7-10 Days	Total
Group A	2	2	4	8
Group B	1	1	1	3
Group C	7	4	4	15

Table 2. Showing Chi-Square Tests for the mortality of the three groups

Test	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	12.834	2	.002

Comparing the results between two of the three groups revealed that the distribution was significant in case of groups A and C, but insignificant distribution was noted in case of group A and B.

Group A (Povidone)

Eight rats (32%) of this group were died by the end of seven days after lavage. All rats showed less lethargic with more or less better motor activity within the first two days, then two died by the end of the 3rd post-relaparotomy and lavage day. Another two rats died in the 5th day and the remaining four rats died in the 7th day.

Group B (Honey)

Three rats (12%) of this group were died by the end of ten days after lavage. All rats showed less lethargic with more or less better motor activity within the first two days, then one rat died by the end of the 4th post-relaparotomy and lavage day. Another one died in the 7th day and the last rat died in the 10th day.

Group C (Control)

Fifteen rats (60%) of this group were died by the end of seven days. All rats showed signs of toxemia and sepsis. Seven rats died by the end of the day corresponding to 3rd post-relaparotomy day. Another four rats died in the 5th day and the remaining four rats died in the 7th day.

After two weeks

No generalized peritonitis was seen in any of the survived rats after the second look laparotomy. Seven out of the 17 survived rats in group A, and five out of the 10 survived rats in group C, showed localized abscess formation in the form of pelvic, pericecal and right subphrenic abscesses. Eight out of the 22 survived rats in group B, showed localized abscess formation in the form of pelvic and pericecal abscesses.

Discussion

To study the efficacy of several commonly used peritoneal lavage solutions in the treatment of experimental fecal peritonitis; lethal peritonitis was created in 75 rats by inducing cecal ligation and puncture that came in agreement with many other published works of the same interest (Hubbard *et al.*, 2005). Fecal peritonitis was induced in previous works using standard inoculum of *Escherichia coli*, *Bacteroides fragilis*, *Enterococcus faecalis* (Linhares *et al.*, 2001), or multibacterial fecal specimen was installed in the abdominal cavity (Hendriks *et al.*, 2010). In the present study, during the second-look laparotomy, the whole abdominal contents were amalgamated together within the fibrinous coagulum at re-operation up to eight hours after cecal ligation, and perforation, showed that it was very enough for peritonitis to develop as reported in previous studies (Güllüoğlu *et al.*, 2002, Brocco *et al.*, 2008). Treatment of fecal peritonitis includes administration of antibiotics, physical removal of contaminants, and restoration of gastrointestinal integrity (Saber *et al.*, 2011). The temporal relationship of parenteral antibiotics and peritoneal irrigation using saline solution or lidocaine reduced the bacterial growth and the concentration of endotoxin in abdominal exudate, the plasma endotoxin concentration, and mortality (Brocco *et al.*, 2008).

Intraperitoneal lavage with the povidone-iodine solution has been reported by some to be beneficial in the treatment of peritonitis and by others to cause local and toxic side effects on blood pH as metabolic acidosis due to decrease in base excess (Lores *et al.*, 1981). It was reported that lavage with povidone-iodine increased the total peritoneal cell count; peritoneal bactericidal activity and peritoneal phagocytic activity and any detrimental effects of povidone-iodine lavage should be attributed to systemic rather than local toxicity (Egger and Baumann, 1988, Rittirsch *et al.*, 2009). In the present study, we noticed that in povidone-iodine treated rats there was no significant difference regarding mortality when compared with the honey lavage group ($P > 0.05$). Due to the following ex-

perimental and clinical studies scarring (El-Mezien *et al.*, 1991, El-labban and Abu Aly, 1992, Saber, 2010), honey surpasses povidone iodine when used as a peritoneal lavage solution.

Application of honey to severely infected cutaneous wounds was capable of clearing infection from the wound and improving tissue healing owing to physicochemical properties, stimulation of the immune response and antibacterial actions (Saber, 2010). The clinical observations on application of honey, reported that infection is rapidly cleared, inflammation, swelling and pain are quickly reduced, odour and sloughing of necrotic tissue are induced, granulation and epithelialization are hastened, and healing occurs rapidly with minimal scarring (El-Mezien *et al.*, 1991, El-labban and Abu Aly, 1992). No adverse effects have been noted in any of the studies in which honey has been applied topically to experimental wounds (El-labban and Abu Aly, 1992, Saber, 2010). All bacterial strains showed similar sensitivity to honey with minimum inhibitory concentrations below 10% concentration (Lusby *et al.*, 2002). It was found that honey could stimulate white blood cells or macrophages to produce cytokines, interleukin-1, and interleukin-6 and tumour necrosis factor alpha, which is known to be involved in inflammatory process (Tonks *et al.*, 2003, Saber, 2010).

The physicochemical properties of honey not only contribute to its antibacterial properties but also to its wound healing capabilities. Honey provides glucose supply for leucocytes and may modulate the activation of immunocompetent cells within the wound (Gencay *et al.*, 2008). The antioxidant and inflammatory activities of honey are both local and systemic, as honey diminishes the negative effects of obstructive jaundice on the hepatic ultrastructure and decreases the risk of pancytopenia in patients who are at high risk of developing neutropenia as a result of chemotherapy (Zidan *et al.*, 2006).

Regarding the systemic effects of the lavage solutions, 1% povidone-iodine intrauterine infusion in healthy mares altered progesterone concentrations and could reduce embryo survival (Kalpokas *et al.*, 2010). The intraoperative povidone-iodine lavage affected thyroid function and could result in changes to some thyroid hormone levels due to systemic absorption (Findik *et al.*, 2010) and Iodine-induced hypothyroidism in patients who gargle routinely with povidone iodine is well known (Sato

et al., 2007).

Logically, secondary peritonitis is associated with a high mortality rate and if not treated successfully leads to development of abscesses, severe sepsis and multi-organ failure. Source control and adjunctive antibiotics is the mainstay of treatment (Saber *et al.*, 2011). In our experiment, we didn't insert intraperitoneal drains to allow time for the lavage solution to act. But the no-drain policy seems to be insufficient and add for the residual peritoneal infection.

Conclusion

Peritoneal lavage using honey and 1% povidone-iodine in the treatment of fecal peritonitis together with antibiotics reduced mortality and residual abscess formation. Povidone-iodine is not free from undesired systemic effects and no such effects have been noted in any of the studies in which honey has been applied topically or systemically. So we could conclude that honey might be used as a peritoneal lavage solution at a concentration of 50% in cases of septic peritonitis owing to its bactericidal effects, the physical hygroscopic action and the very minimal or no side effects.

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