Hydrogen peroxide *vs* normal saline lavage in experimental fecal peritonitis[1]

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The authors compared survival in experimental fecal peritonitis between rats undergoing lavage with a dilute solution of hydrogen peroxide and animals treated with normal saline. Using a rat model of human fecal peritonitis, they compared surgical peritoneal lavage with the two test solutions and a control group not subjected to lavage. Two trials compared different strengths of peroxide antiseptic solutions. Five or eight animals were used in each test group. In both trials, more rats survived that had lavage with normal saline than those treated with peroxide or those in the control group. Hydrogen peroxide appears to have significantly greater toxicity than benefit in this model of fecal peritonitis. In fact, survival in the control group was not statistically different from that in the peroxide treatment group.

Index terms: Peritoneum, lavage • Peritonitis

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Fecal peritonitis remains a severe problem in modern surgery. Despite peritoneal toilet and surgical drainage, antibiotics, and supportive care, mortality and morbidity after fecal soilage of the peritoneal cavity remains very high. Hau and Simmons[1] reported a mortality rate of 30–50% for peritonitis in general. Fecal soilage causes a very severe polymicrobial peritonitis. Laparotomy, drainage of purulent fluid with or without fecal diversion, peritoneal lavage, and repair of viscus perforations and systemic antibiotics are among the established methods of treatment. Intraoperative lavage of the peritoneal cavity with a variety of solutions has been reported in both clinical and experimental surgical studies.[2–5] Some types of antisep-

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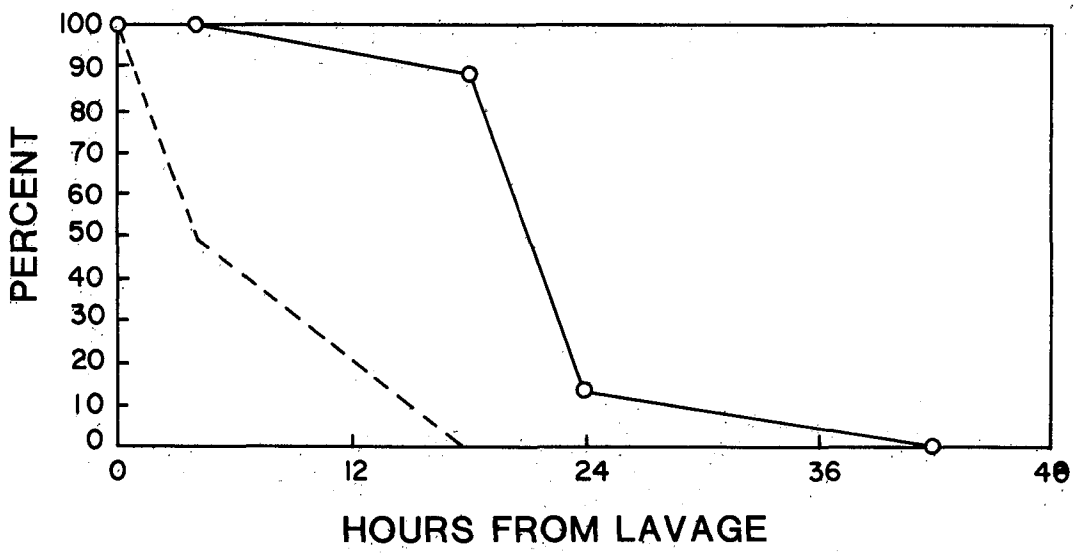

Fig. 1. Percent of animals surviving trial one. Group 1: normal saline 0.3 mL inoculum —O—, N = 8. Group 2: 1.5% peroxide 0.3 mL inoculum ---, N = 8.

tics have been tested in nonoperative animal peritonitis.[6]

Previous experimental studies suggest that antibiotic solutions used in peritoneal lavage have little or no benefit over parenteral antibiotics.[2] Platt et al tested povidone iodine (Betadine), taurolin, noxythiolin, and chlorhexidine gluconate as intraperitoneal antiseptics.[6] They found no beneficial effect from any of the antiseptics except chlorhexidine gluconate. They used a murine model in which a pure, quantitated culture of *E coli* was injected into the peritoneal cavity followed by the test solutions. Chlorhexidine significantly reduced the early mortality of these mice; however, after five days, chlorhexidine-treated animals showed much less benefit.

Despite wide and successful use as a skin antiseptic and local wound dressing, povidone iodine did not show benefit in the abovementioned study or its references.[3,6] This may be a result of rapid local absorption and inactivation of iodine within the peritoneal cavity. Thus, to date, no effective antiseptic to control peritonitis has been identified for use in peritoneal lavage.

Antibiotic solutions for peritoneal lavage have theoretical benefits but have failed to show dramatic therapeutic advantages in controlled studies.[2] The clinical studies to date have usually been poorly controlled. There are definite potential problems with antibiotic lavage, particularly when aminoglycosides have been used, because of the unpredictable absorption of these nephrotoxic and ototoxic agents.

In addition to drainage of purulent fluid and grossly visible contaminants encountered in the fecally soiled peritoneum, lavage with normal saline has been advocated. Very-large-volume lavage with warm saline has become a popular clinical maneuver. The sequelae of fecal peritonitis often include the formation of abscesses with mixed bacterial flora. Anaerobic species including *Bacteroides* are frequently encountered since these bacteria are the most common organisms normally found in human feces.

We postulated that the antiseptic effect of hydrogen peroxide, particularly its generation of high local oxygen levels, could have a beneficial effect on fecal peritonitis. In a search of the English language literature, we found no previously published studies on such use of hydrogen peroxide solutions. If effective in treating peritonitis, use of this common local antiseptic in peritoneal lavage would represent a significant therapeutic advance in the care of these severely ill surgical patients. We thus decided to undertake a controlled trial of hydrogen peroxide lavage in an animal model of human fecal peritonitis. Normal saline lavage was chosen for a comparison treatment because of its clinical use in comparable situations. We chose a well-described model using rats and an inoculum of human feces.[7] This model does not violate the animals' viscera and produces a microbial flora akin to that observed in humans by means of the human feces in the inocula. This model has also been used successfully by other investigators.[2,7] We

Table 1. Pathologic examination of histologic sections

Group	Organ	Description
Group #1		
½ strength H_2O_2 treatment, animal dead at 4 hours	Intestine	Normal histologic appearance
Group #2		
Normal saline treatment, animal dead at POD #1, 0.3 mL inoculi	Intestine	Acute peritonitis on serosa
	Spleen	Normal appearance
Group #3		
¼ strength H_2O_2 treatment, animal dead at POD #6	Intestine	Acute peritonitis on serosa with intact mucosa, esophagogastritis, junction same appearance
	Mesentery	Fat necrosis
	Liver	Acute peritonitis liver capsule, parenchyma unremarkable
Group #4		
Normal saline treatment, animal dead at POD #4, 0.15 mL inoculi	Intestine	Acute peritonitis on serosa with some fat necrosis visible
	Liver	Near normal appearance

have only slightly modified the model and procedures used by previous investigators.

Materials and methods

This rat model of fecal peritonitis is a slight modification of that first described by Nichols et al.[7] The animals used were young, male Sprague Dawley rats weighing 200–220 g. The fecal inoculum was prepared in an anaerobic chamber and consisted of fresh human feces from a healthy volunteer. This was mixed 1:1 by volume with thio culture medium. One gram of barium sulfate was then added per 10 mL of inoculum mixture. The inoculum was thoroughly mixed and sealed in test tubes. These were frozen at −70°C until just before use.

All operative procedures used ether as inhaled anesthesia. Animals were removed from their cages and anesthetized in an ether jar. They were removed from the jar and their abdominal areas were shaved. Supplemental ether was given during the procedures. A lower abdominal midline incision approximately 1 cm long was then made through the fascia into the peritoneal cavity.

Inoculum thawed in a water bath just before the procedure was then measured into plain gelatin capsules with a small calibrated syringe. This capsule with the measured volume of inoculum was then implanted in the animal's peritoneal cavity in the lower abdomen. The wound was closed in two layers with nonabsorbable suture. The rat was then transferred back to its cage.

All rats were housed in standardized cages with four rats to a cage. They were fed water and rat chow ad libitum. No evidence of aggressive behavior was noted between animals. Animals were housed so that those in a particular cage all received the same treatment regimen. The animals were not made to fast before or after surgery.

Four hours after a rat's initial surgery, the animal was reanesthetized and the incision reopened. The incision was lengthened to 3 cm to expose the entire abdomen. Grossly visible particles of inoculum were removed, and the peritoneal cavity lavaged with the test solutions in either two or three aliquots as specified for each trial. Animals were assigned to treatment groups arbitrarily. After each aliquot was instilled, fluid from the abdominal cavity was aspirated with a clean syringe. The incision was then closed again in two layers with the deep layer consisting of peritoneum, muscle, and fascia while the superficial layer included subcutaneus tissue and skin. Nonabsorbable sutures were used in the deep layer and either nonabsorbable sutures or staples in the superficial layer. Animals were then marked with an ear punch for identification and transferred back to their cages. Control animals had capsules of inoculum placed but no further treatment, i.e., surgery or anesthesia. Any animal dying during one of the surgical procedures or not recovering from the ether was excluded from analysis. Animal survival was checked frequently.

After death, each animal was examined and its abdomen opened and inspected. Representative animals from each treatment group had tissues from the abdominal viscera preserved and histologic examination performed. The main basis of comparison used between groups was survival.

In the first trial, 0.3 mL of inoculum was placed in the gelatin capsule. Sixteen rats were used, with eight receiving lavage with normal saline and eight receiving lavage with dilute peroxide.

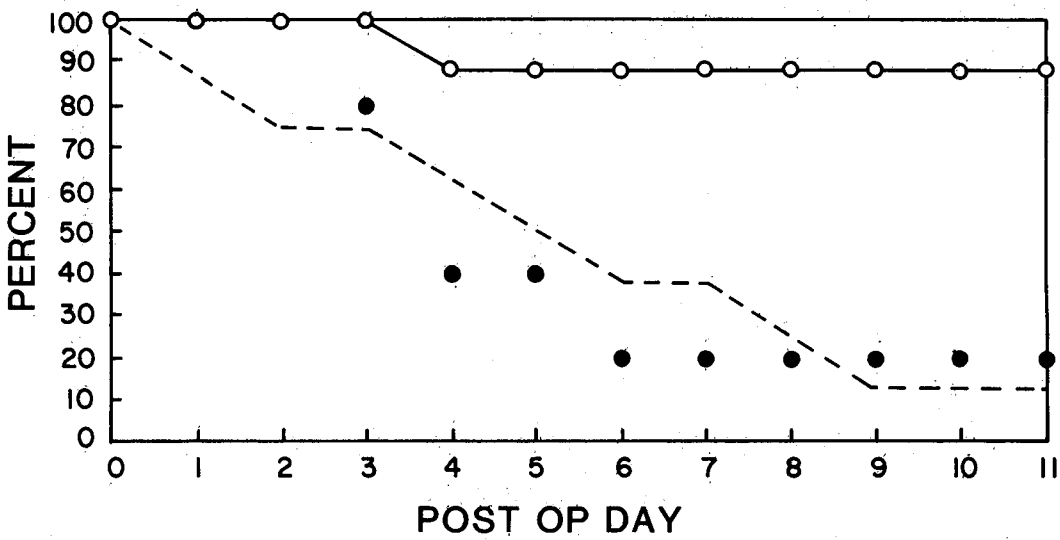

Fig. 2. Percent of animals surviving trial two. Group 3: normal saline 0.15 mL inoculum —O—, N = 8. Group 4: 0.75% peroxide 0.15 mL inoculum ---, N = 8. Group 5: control (no lavage) 0.15 mL inoculum ●, N = 5.

The normal saline was instilled in two aliquots of 5 mL each (25 mL/kg). The hydrogen peroxide was 3% hydrogen peroxide solution with water diluted 1:1 with normal saline to give a 1.5% solution. Five mL (25 mL/kg) was instilled into the abdomen and then aspirated. A second aliquot, also 5 mL, of normal saline was then instilled to rinse the peritoneal cavity.

In the second trial, 21 rats were used. All received 0.15 mL of inoculum. Five served as a control group and only had a capsule of inoculum placed intraperitoneally without treatment. Eight animals received lavage with 6 mL (30 mL/kg) of normal saline as the first aliquot. This was followed by 6 mL (30 mL/kg) of 3% hydrogen peroxide solution diluted 1:3 with normal saline to give a 0.75% solution of peroxide. A third aliquot of 6 mL normal saline was then used to rinse the abdomen. Eight other animals received the same inoculum and lavage with three aliquots of 6 mL (30 mL/kg) of normal saline. There were thus five different groups of animals with a total of 37 animals in our study. Surviving animals were killed with a massive dose of pentobarbital (Nembutal) and cultures taken of their abdominal cavities. Animals in the second trial that survived 10 or 11 days were killed and cultures taken of the peritoneal fluid. A culture from the inoculum was also obtained at the end of the study. All microbiologic identification was done using standard clinical procedures by our Department of Microbiology.

Results

No wound dehiscence was noted during the study. Animals surviving to 10 days did not show signs of illness. The results of the first trial are shown in *Fig. 1.* No animals survived past 48 hours with this large fecal inoculum. According to published results by Nichols,[7] this inoculum is equivalent to the LD_{100} or lethal dose. Animals treated with 1.5% dilute peroxide solution seemed to do worse than those treated with normal saline. The percentage of animals surviving at each interval is represented with a small dot or a square. Observations were made at 4 hours, 18 hours, 20 hours, 24 hours, and 42 hours. The differences in the survival between these two groups were compared using the Breslow test,[8] which allows for comparison of the entire curve rather than just the end point. Because no animals survived, no cultures were taken. Tissue was collected for histologic examination and is described in *Table 1* as one-half strength peroxide– and normal saline–treated animals. There is a significant difference between the two groups' survival curves with a $P < 0.001$.

In the second trial, 9 of 21 animals survived the trial period of 10 or 11 days (necropsies of 3 animals were performed on day 10 in the normal saline group). This size inoculum in Nichols' work caused greater than 70% mortality in untreated animals. Our results are represented in *Fig. 2.* Animals were observed each day and

Table 2. Culture results

Source of Specimen	No. of Organisms Identified Per Animal	Identities
Fecal flora inoculum (1)	13	1. *Escherichia coli* 2. *Klebsiella pneumoniae* 3. *Staphylococcus aureus* 4 & 5. Enterococci (2 species) 6. *Streptococcus bovis* 7–11. *Bacteroides* (5 species) 12. *Peptococcus* 13. Anaerobic gram-negative cocci, unidentified species
Peroxide treatment animal (1) one survivor Group 3	3	1. *E coli* 2. Enterococcus 3. *Clostridium* species
Normal saline treatment animals surviving (7) Group 4	4.4 avg. (31 total isolated)	1. Enterococcus 2. *E coli* 3–5. *Clostridium* (3 species) 6. *Staphylococcus aureus* 7. *Streptococcus bovis* 8. *Lactobacillus* 9. Group B *Streptococcus* 10. *Staphylococcus epidermidis* 11. *K pneumoniae* 12. Alpha *Streptococcus* not Group D 13. *Actinomyces* 14. *Fusobacterium nucleatum*
Control animals survivors (1) Group 5	9	1. *E coli* 2. *Providencia rettgeri* 3. *Proteus mirabilis* 4. Enterococcus 5 & 6. *Lactobacillus* (2 species) 7. α-hemolytic *Streptococcus* not Group D 8. *Streptococcus bovis* 9. *Clostridium sordellii*

deaths noted. In *Table 1*, 0.75% hydrogen peroxide solution is noted as one-quarter strength H_2O_2. The total lavage per kilogram of rat body weight is also noted with 30 mL/kg in each aliquot for a total lavage of 90 mL/kg. The group survival curves were again compared using the Breslow test. The animals treated with normal saline lavage had significantly better survival than either those treated with dilute hydrogen peroxide or the control group. The *P* value was equal to 0.01 between normal saline and peroxide and 0.02 between normal saline and control groups. The difference between the control and peroxide groups in the second trial was not significant, with a *P* value equal to 0.88. This may be due to the low statistical power of this comparison using only small numbers of animals. The Breslow test requires large sample sizes for validity, but our statistician thought it offered great advantages over other statistical methods that compare survival only at selected times.

The results of microbial cultures are represented in *Table 2*. Intraperitoneal cultures demonstrated that, even in surviving animals, human fecal flora, including anaerobic species, may be recovered using this model. The presence of *Bacteroides* and other anaerobic species in the inoculum cultured at the conclusion of the experiment shows that the mixed fecal flora survive preparation and storage for considerable periods of time at −70°C. It is difficult to say much about the effect on microbial flora of the different treatments with such poor survival in control and peroxide groups. Tissue obtained from animals dying during the experiment was preserved in formalin. Sections with permanent hematoxylin and eosin stains were prepared and reviewed with one of our staff pathologists. The microscopic findings are presented in *Table 1*. No specific microscopic findings were noted. No distinction could be made between the cellular response to bacterial sepsis and the response that might have resulted from chemical peritonitis, i.e., from exposure to dilute hydrogen peroxide.

Discussion

This study was conducted to test the hypothesis that peritoneal lavage with dilute hydrogen peroxide solutions might be preferable to lavage with normal saline alone in fecal peritonitis. This was based on the theoretical killing of anaerobic bacteria present in human feces by the peroxide's high oxygen concentration and the liberation of free radicals. The animal model chosen has previously been used to study related treatment techniques.[2] This model has several advantages over others that have been proposed. First, the rat's enteral tract is not violated. Second, the inoculum consists of human flora rather than the rat's own, which might be affected differently by treatment. Third, this model's microbial flora has been documented to retain anaerobic bacteria, which are thought to play an important role in the pathogenesis of human peritonitis and abdominal abscesses.

The microbiologic culture results are interesting, but with only one surviving animal in the peroxide treatment group in the second trial, it is impossible to say what effect the dilute peroxide had on the bacteria. It should be noted that bowel or fecal microorganisms were indeed recovered in all animals whose peritoneal fluid was cultured. This is despite the apparent good condition of most of these animals and careful technique to avoid contamination of the cultures before sampling the peritoneal cavity.

Histologic examination of tissues revealed that peroxide did not seem to cause necrosis of any abdominal organ or tissue but did seem to cause some injury. Alternatively, the peroxide may not have prevented injury by the bacterial peritonitis. The findings on examination of peroxide-treated animals likely represent chemical peritonitis. There was no specific indication of this except a nonspecific cellular inflammatory response. The normal saline–treated animals in the second trial seemed to resume eating and drinking more rapidly than peroxide-treated animals. The cages used in this study made measuring food and water intake impossible, so it remains a subjective observation.

The difference in survival between normal saline–treated animals in the first trial and those in the second is undoubtedly due to the two changes. First, animals in the second trial had larger volumes of the lavage fluid instilled into the abdominal cavity for greater mechanical cleansing and, second, animals in the second trial all had smaller inocula. The 88% survival of animals treated with normal saline in group 4 contrasts with the 20% survival of control animals in group 5. This closely agrees with the survival reported by Nichols confirming that surgical toilet and lavage increases survival.[7]

It should be noted that one investigator who used a different animal model observed no change in survival with saline lavage *v* simple surgical toilet,[5] so it is difficult to credit the increased survival of group 4 to saline lavage with complete confidence. A simple trial using Nichols[7] model to compare surgical toilet alone to lavage and surgical toilet would be valuable.

This study involved relatively small numbers of animals, but the results of both trials agree. Dilute hydrogen peroxide solution used as a peritoneal lavage shows no beneficial effects. Peroxide is definitely inferior to normal saline as a lavage solution, whereas saline combined with surgical toilet appears to improve survival. Peroxide does not show promise as a treatment solution for lavage. Further studies comparing lavage and surgical toilet between normal saline and chlorhexidine gluconate would seem to be indicated based on the results of Platt et al.[6] The model described by Nichols et al[7] seems most satisfactory for such an investigation.

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