A pilot study on microbial load reduction in peritonitis with a neutral pH – super-oxidized solution

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Introduction

Intra-abdominal sepsis is a clinical disorder commonly found in Mexican general hospitals. It has been published that from 2% to 14.76 % of patients admitted to the emergency services of these Institutions present abdominal sepsis (Rodea, 1999; Bracho, 2002). The problem is that mortalities rates are higher in certain regions of the country due to the lack of fully equipped intensive care units. But despite major advances in surgery, antibiotics as well as nutritional, ventilatory and hemodynamic supports in developed countries, mortality rates remain in the range of 10-20%.

For these reasons, there has been an ongoing interest in reducing the mortality rates in peritonitis by improving source control. The latter includes an efficient eradication of infection, elimination of microbial contamination and restoration of the local environment (Holzheimer, 2001). Adjuvant surgical measures, then, aim at the reduction of the bacterial load in the peritoneal cavity. For this purpose, planned relaparotomy, relaparotomy on demand, and intraoperative peritoneal lavage (IOPL) are all used as necessary.

In severe peritonitis, for example, the use of multiple re-explorations and intra-operative lavage with large amounts of saline solution has been recommended to decrease the risk of postoperative infection. Yet, the benefit of adding antimicrobials or antiseptics to the lavage fluid remains controversial. Taurolidine, metronidazol and iodine povidone are some of the products so far tested. The bactericidal and anti-endotoxin product taurolidine, for example, has been shown to reduce the complication rates and length of hospital stay in patients with local peritonitis undergoing laparoscopy treatment (Gortz, 1997). Adjuvant metronidazol lavage has also been suggested as an efficient therapy for the treatment of intraperitoneal abcesses (Saha, 1996). However, no data has been further published on the efficacy and toxicity of these products. In addition, the use of iodine povidone for intraperitoneal lavage in colorectal surgery has been shown to lead to sclerosing encapsulating peritonitis (SEP) (Keating, 1997).

In an attempt to reduce chemical-related toxicity to the peritoneum, Inoue *et al*. (1997), performed lavages with an electrolyzed strong acid aqueous solution to

treat 7 patients with peritonitis and intraperitoneal abscesses. This was an antimicrobial solution that contained reactive oxygen species, high chlorine content (>600 ppm) and, therefore, a high oxidation-reduction potential (ORP). As expected, the four positive cultures found at entry became negative between days 3-7 after irrigation with the solution twice a day. Thus, the concept of using a super-oxidized solution (SOS) for source control in peritonitis resulted attractive.

Accordingly, the antimicrobial activity of a neutral-pH solution with a controlled amount of reactive species and low chlorine content (<70 ppm) was investigated in Mexican patients with acute generalized peritonitis (Microcyn, MCN®, Oculus Innovative Sciences, Petaluma CA). This SOS has been shown to exert a broad microbiocidal activity against bacteria, viruses, fungi and spores (Landa et al, 2005). MCN has also been shown to be safe in different biocompatibility tests including: skin and ocular irritation; acute dermal toxicity; skin sensitization; acute systemic toxicity (i.e. peritoneal); acute oral and inhalation toxicities; and genotoxicity micronucleus test (Gutiérrez 2006). The routine clinical use of MCN in diverse acute and chronic wounds, including burns and oral lesions for 12 months previous to the recruitment of patients with peritonitis, fully corroborated its safety.

Our main goal in this study was to evaluate the efficacy and safety of MCN for decreasing the microbial loads in patients with acute generalized peritonitis. Predicting factors of mortality, including type of microorganisms, APACHE II scale and Manheimm Peritoneal Index (MPI), were recorded in patients prospectively treated with saline solution and SOS for IOPL. Overall, MCN achieved a potent microbial load reduction and a shorter hospital stay in the study group in comparison to the historical control group. These results are encouraging and suggest that MCN® could be part of the comprehensive therapy of peritonitis. The benefits of this SOS in the clinical outcome of peritonitis are now being addressed in a controlled trial.

Patients and Methods

Patients

All patients admitted to the Hospital Ruben Leñero in Mexico City from June 2004 to January 2005 and with a diagnosis of acute generalized-secondary peritonitis were included in the SOS group. Patients that met the inclusion/ exclusion criteria gave informed consent prior to any study procedures. Secondary peritonitis was defined as the result of the loss of integrity of the gastrointestinal or genito-urinary tract leading to contamination of the peritoneal space (Laroche 1998). Retrospective analysis of paired-cases presenting similar peritoneal infections between 2003 and 2004 at the same institution was undertaken for the control group.

Scores on admission

The patient's history was recorded: the date of the onset of symptoms, the date and reason for the patient's admission, date of peritonitis diagnosis and etiology of peritonitis. The date and the type of surgical treatment of peritonitis were also recorded. Aerobic bacteria and fungi isolated from intra-operative peritoneal samples were also analyzed. The Acute Physiology And Chronic Health Evaluation score II (APACHE II) and the Mannheim Peritonitis Index (MPI) values were calculated for each patient upon admission (Knaus, 1991). These scales have been validated for stratifying patients into subsets of similar disease severity as well as for predicting overall mortality. According to MPI, the severity of peritonitis was classified in three different stages: Group-A patients with prognostically favorable peritonitis (MPI 0-20); group-B (MPI: 21-29) and Group C patients (MPI > 29) with intermediate-favorable and severe peritonitis, respectively (Billing, 1994; Bracho, 2002).

Treatment and follow up

Upon admission, all patients underwent open surgery and peritoneal lavage of all quadrants of the abdomen. Intraoperative peritoneal-culture samples were taken in both groups. The IOPL was performed with 10 L of saline solution in both groups and only followed by 5 L of SOS (Microcyn®) in the study group. The excess of SOS was removed and no further rinsing was conducted. The abdominal cavity was covered with a plastic mesh in both groups. However, in the study group, a dressing soaked in SOS was left on top of the mesh. The dressing was changed t.i.d. Empyric antimicrobial therapy was started in all patients with two antibiotics including clindamicine (600mg t.i.d.) and cefotaxime (1 g t.i.d.) or amikacin (15 mg/Kg day). Post-operative management in the control and study groups included daily irrigation of the mesh with 100 mL of saline solution or SOS t.id, respectively. In the latter case no further rinsing or lavage was conducted. Severe cases of peritonitis required re-laparotomy and IOPL every 72 hours.

Assessment of patient's outcome

Cultures of the peritoneal fluid for aerobic bacteria and fungi were taken every 72 hours in both groups for up to one week. The duration of length of stay in the hospital was recorded. The number of deaths within 30 days following the diagnosis of peritonitis was specified. For patients who had left before 30 days, these were considered as being still alive after 30 days if they had not been readmitted to our institution.

Super-oxidized solution

MCN is super-oxidized solution manufactured through the electrochemical treatment of pure water and sodium chloride (NaCl) in a multi-chambered cell. The final product has a release specification pH of 7.2 to 7.8, an oxidation reduction potential (ORP) of ≥ 800 mV. It contains 99.98% oxidized water and a limited number of oxygen- and chlorine-containing chemical compounds. The antimicrobial activity of this SOS has been recently reported (Landa *et.al*., 2005). Extensive safety and biocompatibility tests have also been performed by certified third-party laboratories, including: dermal, inhalatory, oral and systemic toxicities; skin sensitization; skin and ocular irritation; intracutaneous reactivity; wound healing and genotoxicity tests (Oculus IS files). For example, according to the International Organization for Standardization (ISO) document "Biological evaluation of medical devices – Part 1: Evaluation and Testing", this SOS does not induce irritation or sensitization. In another study, MCN-containing samples did neither reveal any evidence of cell lysis or toxicity (ISO 10993-5:1999; NAMSA, USA). Furthermore, the acute systemic toxicity of MCN in mice resulted in an LD50 greater than 50 mL/Kg after acute intraperitoneal administration. There were neither systemic effects nor pathological abnormalities in the peritoneal cavity of these animals (ISO 10993-11; Stillmeadow, Inc, USA). The mutagenicity potential of this SOS was also discarded in a micronucleus test after the intraperitoneal injection of the test article into mice (ISO 10993-3, 2003; Biomatech SAS, France). This SOS had been certified as an antiseptic and germicide by the Ministry of Health of Mexico previous to the start of the study.

Results

Patients´ data and treatment

Twenty consecutive patients were prospectively included in the SOS group (i.e. study group). Twenty control cases were selected from the medical records of the Institution and paired to the study group by age, sex and etiology of peritonitis (Fig. 1). Eight males and 12 females were included in the control group and 10 of each sex in the study group. Median age was 43.38 years for the control group and 45.77 for the study group. Upon admission the median MPI for both groups was 33.3 (range 1.3) and the APACHE II score was 3.66 and 4.33 for the control and study group, respectively. Altogether these data show that control and study populations were comparable in age, sex and prognostic factors at entry.

The anatomic origin and etiology of peritonitis was also similar for both groups (Table 1). Post-operative peritonitis was present in 19 and 17 patients of the control and study groups, respectively. All cases underwent surgical treatment followed by IOPL. The types of surgeries performed in control /study groups, were: appendicectomy (3/6), gastric resection (4/0), cholecystectomy (1/2), pancreatic necrosectomy (6/3), small bowel suture/ resection with anastomosis (4/3), Hartman's operation (1/1), colonic resection (0/1) and miscellaneous (1/4). The use of antibiotics was very similar in both groups. For control and study

groups, three antibiotics were administered in 16 and 15 patients and more than 3 antibiotics in 4 and 5 cases, respectively. Patients were kept at the ICU and were mechanically ventilated post-operatively.

Microbiological analysis and patients´ outcome

Peri-operative intra-abdominal samples were taken in all 40 patients (Table 2). The average numbers of microorganisms grown from these samples were 29 in the control and 30 in the study group. The microorganisms isolated are shown in Table 4. *Escherichia coli*, *Enterococcus*, *Staphylococcus aureus, Pseudomonas aeruginosa* and fungi were isolated from control and study groups in 3/6, 4/2, 10/8, 2/3 and 10/7 occasions, respectively. Positive cultures for *A. xilosoxidans* (1), *S. coagulase neg* (2) and *A. baumanii* (1) were only found in the study group.

A second intra-abdominal culture was taken during the first week after surgery. At this time, the average number of organisms isolated in the control group (24) was almost the same as in the peri-operative sample (29). Instead, there was a strong reduction in the number of positive samples in the study group. From 30 positive cultures in the peri-operative samples, only one remained positive for *S. aureus* and another one for *E. coli*.

To elucidate if the source control achieved with SOS was of any clinical benefit, the mortality rates and the hospital stay were analyzed. There were six deaths in the control group and 3 in the study one. All deaths occurred in the first 30 days after the first surgery and the calculated relative risk was higher for the control group (i.e. 3.3 versus 0). However, the sample size was too small to be of any statistical significance.

In the analysis of hospital days, the control group had a longer stay (31.9 days) in comparison to the study group (22.4 days). Even if a correlation was made between the length of hospital stay and each one of the most common isolated microorganisms, the length of stay was still higher in the control group. For example, the hospital days in cases with *C. albicans*, *E. coli*, and *S. aureus* for control / study groups, were: 19.4/6.3, 17.6/10.2 and 22.3/14.1, respectively. Unfortunately, there were few available or no positive cultures for *P. aeruginosa*, *A xilosoxidans* and *Enterococci* to compare the impact of these bacteria on the length of hospital stay between groups. However, as it is expected with these strains, their presence was associated with the longest hospital stay. Whether or not the clinical outcome and the length of stay were also significantly influenced by preexisting organ failure, duration of the peritonitis, presence anaerobic

bacteria or other variables, can not be discarded from our data and remains to be evaluated in future studies.

No local side effects were recorded with the use of SOS in the IOPL. Surviving patients in the study group were followed for 6 to 12 months. None of the patients in the SOS group presented intestinal occlusion or data suggesting sclerosing peritonitis in the follow-up period.

Discussion

The SOS herein tested has been widely used as a wound care product in Mexico since 2004. Diabetic foot ulcers, venous stasis ulcers, pressure sores, burns and, among other wound types, have all been successfully and safely treated with MCN . Now, this pilot study suggests that source control can be safely accomplished with the addition of SOS to the IOPL in patients with severe peritonitis.

It is clear that the pathophysiology of post surgical peritonitis is different from that related to trauma. Therefore, we predominantly included cases of post-operative peritonitis. Our control patients were then carefully matched to those in the study group in terms of age, primary causes and severity of the peritonitis, to properly evaluate the potential benefit of adding SOS to the standard treatment (i.e. surgery and IOPL).

It is also known that valuable scoring systems relying on systemic signs of the septic disease (e.g. APACHE II) seem to better differentiate the prognosis of the disease than more surgically oriented scores do (e.g. MPI). However, APACHE II and MPI scores were used in this study to help better match patients in both arms rather than for establishing a prognosis in each case. These data also showed that control and study populations were comparable in prognostic factors at entry.

The addition of antiseptics/ antibiotics to IOPL in severe peritonitis has been a controversial issue for many years. Some authors have found that bacteriological isolates from the inflamed peritoneal cavity do not necessarily correlate with the clinical course, and the occurrence of *Enterococci* may be slightly related to ongoing infectious complications (Berger 1998). Other studies using intra-abdominal administration of metronidazol, taurolidine, fibrinolytic agents or anti-cytokine antibodies, however, have shown some benefits in the outcome of these patients (Saha, 1996; Gortz, 1997). It has also been shown that higher IOPL-volumes of saline solution (20 L) are better than standard volumes (e.g. 10 L) for improving the prognosis. Unfortunately, most of these studies have not been properly controlled and it is difficult to draw any solid conclusions from them.

Yet, the use of a neutral pH-SOS in this study, showed good source control and clinical benefits in patients undergoing IOPL. Almost all cultures became negative in the week after surgery if the IOPL was conducted with SOS. This is expected as the SOS herein used is a broad-spectrum antimicrobial agent, as shown by Landa *et al* (2005).

Interestingly, the hospital stay was also reduced by 30% in the study group and the mortality was lower. Unfortunately the later data is not statistically significant due to the small sample size. Nonetheless, a reduced hospital stay suggests that a more efficient source control in the study group could have improved the outcome of the patients and allowed a faster discharge from the hospital. Unfortunately, it is impossible to establish a correlation between the bacteriological results and the clinical outcome according to the local and general sepsis scores obtained from this study. It is well known that pre-existing septic organ dysfunction and pre-existing co-morbidity are the main determinants of mortality rather than the bacteriological findings (Rodríguez, 1999; Holzheimer, 2001). Yet, it has also been established that source control in early stages of peritonitis improves the prognosis. Thus, these questions will need to be addressed in a future prospective, controlled study.

Despite the limitations of this report, the good source control herein obtained with the use of SOS was not associated with acute or chronic side effects. However, it must be underlined that SOS it is not meant to replace saline solution as the cornerstone of IOPL. On the contrary, SOS could become a useful adjuvant therapy in patients with peritonitis of any source. It requires no dilution or special handling or disposal. Furthermore, it is clear that SOS therapy will be a lot less expensive than any anti-cytokine antibody or antibiotic.

Altogether these results suggest that SOS therapy could positively impact the outcome of these patients. Yet, more studies are required to properly establish the safety and efficacy of SOS in peritonitis of different etiologies. A study on the cost / benefit ratio of this intervention is also mandatory.

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Figure 1.- Age groups in control and study patients with peritonitis

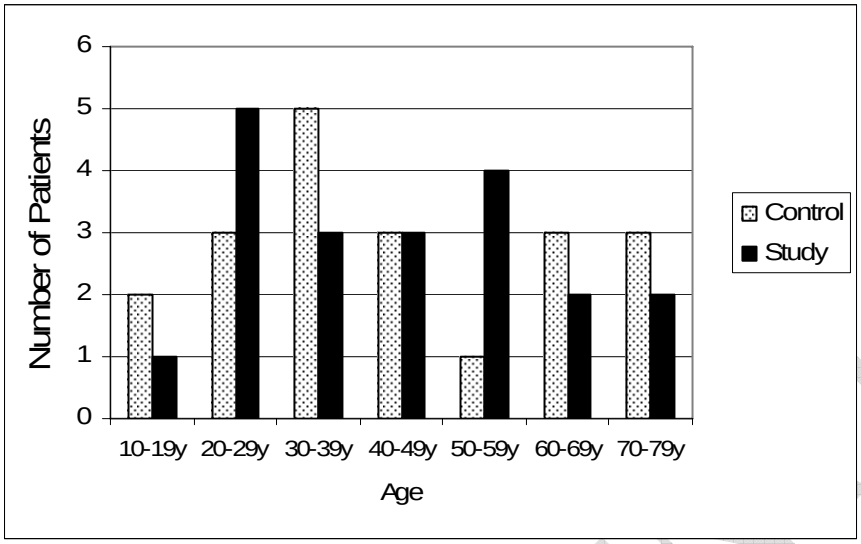

Table 1.- Anatomic origin or etiology of peritonitis

Diagnosis	Control	Study	Total	%
Appendicitis	3	6	9	23.0
Post-trauma	1	3	4	10.0
Pancreatitis	6	3	9	23.0
Cholecystitis	1	2	3	7.5
Colon cancer	0	1	1	2.5
Small bowel fistula	4	1	5	12.5
Diverticulitis	1	1	2	5.0
Gastric perforation	4	0	4	10.0
Organ perforation	0	2	2	5.0
Other	0	1	1	2.5
TOTAL	20	20	40	100.0

Table 2. Microorganisms isolated from intraperitoneal samples and length of hospital stay in patients with peritonitis. Samples were obtained in the peri-operative period and in the following week after intra-operative lavage with saline solution only (control group) or saline solution and SOW (study group). The average hospital stay was then analyzed for each microorganism isolated at entry and for the whole group.

	CONTROL GROUP			STUDY GROUP		
	Isolated strains (n)		Hospital	Isolated strains (n)		Hospital
Organism	**Peri-op**	**Post-op**	**Days**	**Peri-op**	**Post-op**	**Days**
C. albicans	10	7	19.4	7	0	6.3
E. coli	3	2	17.6	6	1	10.2
S. aureus	10	9	22.3	8	1	14.1
S. neg coagulase.	0	0	0	2	0	17.8
A. baumanii	0	0	0	1	0	22.4
E. faecalis	3	3	23.7	1	0	28.6
A. xilosoxidans	0	0	0	1	0	29.0
P. aeruginosa	2	2	24.0	3	0	33.9
E. cloacae	1	1	13.0	1	0	37.0
TOTAL	29	24	31.9	30	2	22.4