Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(3):402-404

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Postoperative anaerobic sepsis could be combated by prophylactic vaccination

Medhat Khafagy[1]*, Ahmed M. Mayla[2]*, Ayatallah Khafagy[3], Nabil Baraya[1], Gamal Emira[1], Mohamed El-dweik[4], Wael Abdelgawad[1], Moataz Gadalla[5], Manar M. Moneer[6], Mostafa Elberry[2] and Ahmed Orabi[7]

[1]Surgery Department, National Cancer Institute, Cairo University, Cairo, Egypt
[2]Cancer Biology Department, National Cancer Institute, Cairo University, Cairo, Egypt
[3]Obstetrics and Gynaecology Department, Inova Medical Center, Fairfax, VA,
[4]Experimental Surgery, National Cancer Institute, Cairo University, Cairo, Egypt
[5]Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, Baltimore, MD,
[6]Cancer Epidemiology and Biostatistics Department, National Cancer Institute, Cairo University, Cairo, Egypt [7]Faculty of Veterinary Medicine, Cairo University, Cairo, Egypt

---

ABSTRACT

Postoperative sepsis is the leading cause of morbidity and mortality after major surgery, resulting in hefty financial costs in hospitals all over the world. 40 Fischer rats were injected intra-peritoneally with 0.05 ml Covexin 10 which contains toxoids from different Clostridial species, 2 weeks prior to caecal ligation and puncture. Another 40 Fischer rats, as a control, underwent caecal ligation and puncture without vaccination. 16 of 40 vaccinated rats died (40%), peritoneal fluid cultures from the dead rats grew E. coli only, and 36 of 40 control rats died (90%), peritoneal fluid cultures from the dead rats were: 19 grew E. coli and C. perfringens; 12 grew E. coli,C. perfringens, and enterococci; 3 grew E. coli only; 1 grew E. coli, and enterocci; and 1 grew no organisms. Prophylactic vaccination with clostridial toxoids proved effectiveness in preventing anaerobic infection, and reduce mortality in rats that underwent caecal ligation and puncture; the gold standard model for polymicrobial sepsis. Currently, a pilot study is underway in which human patients who will undergo major surgery are prophylactically vaccinated with clostridial toxoids, in an attempt to eradicate postoperative anaerobic sepsis.

Key words: Postoperative, Anaerobic sepsis, Vaccine, Clostridia toxoids, Covexin.

INTRODUCTION

Postoperative sepsis is the leading cause of morbidity and mortality after major surgery, resulting in hefty financial costs in hospitals all over the world **[1]**. Different micro-organisms can be cultured from abdominal fluid obtained from Fischer rats with intra-abdominal infection resulting from a perforated digestive tract. Sepsis is a serious medical condition characterized by dysregulated systemic inflammatory responses followed by immunosuppression **[2]**. To study sepsis, diverse animal models have been developed. Polymicrobial sepsis induced by caecal ligation and puncture (CLP) is the most frequently used model because it closely resembles the progression and characteristics of human sepsis **[3]**.

Prophylactic antibiotics against anaerobic organisms e.g. metronidazole and clindamycin phosphate, are used routinely in most major surgery but the problem is that anaerobic infection occurs in media deficient in oxygen like crushed tissues and in haematomas and seromas or ligated ischaemic tissues. But unfortunately, enough blood level of antibiotics is not reached because of the ischaemic nature of previous sites. Vaccination with toxoid of *Clostridium difficile* was successful in treatment of clostridium associated chronic diarrhoea**[4]**.

We evaluated a cohort of rats with abdominal sepsis with the aim of obtaining more insight into the type of microorganisms involved and the efficacy of pre-treatment vaccination **[5]**.

EXPERIMENTAL SECTION

Polymicrobial sepsis was induced using caecal ligation and puncture (CLP) in 80 Fisher rats. After deep anaesthesia, midline laparotomy was performed with exposure of caecum followed by ligature and many transverse perforations were made, with a 14 G needle, for induction of sepsis. After surgery, the caecum of the animals was replaced into peritoneal cavity, which was closed in two layers with 3.0 vicryl suture **[3]**.Fischer rats were divided into 2 equal groups. The control group animals received vehicle (saline solution) and the animals of the second group received the Covexin10 (Schering-Plough Animal Health, Ltd.) at a dose of 0.05 mL per animal, two weeks before they underwent identical laparotomy and CLP.

Bacterial identification

Bacterial identification was performed on peritoneal fluids aseptically taken from dead animals. Samples were inoculated on cooked meat media preheated till 80 °C and were incubate anaerobically at 37 °C for 24 hr then were spread on neomycin blood agar media and were incubate anaerobically for 48 hr for *Clostridium* isolation also the same samples were inoculated on MAcConkey's agar and Kennel fecal agar for *E.coli* and *Enterococus* isolation respectively**[6]**.

Statistical methods

Data was analysed using IBM SPSS Advanced Statistics version 20.0 (SPSS Inc., Chicago, IL). Numerical data were expressed as median and range. Qualitative data were expressed as frequency and percentage. Chi-square test was used to examine the relation between qualitative variables. For quantitative data, comparison between two groups was done using Mann-Whitney test. All tests were two-tailed. A p-value < 0.05 was considered significant.

RESULTS

Thirty six of 40 control rats died (90%), peritoneal fluid cultures from the dead rats were: 19 grew *E. coli* and *C. perfringens*; 12 grew *E. coli*, *C. perfringens*, and enterococci; 3 grew *E. coli* only; 1 grew *E. coli*, and enterocci; and 1 grew no organisms (Table 1). 16 of 40 vaccinated rats died (40%), peritoneal fluid cultures from the dead rats grew *E. coli* only (Table 2). Living at the end of the experiment on control group is 48 days and experiment on vaccinated group is 50 days. The cut-off point of survival is 50 days for vaccinated and control groups.

Only 4 rats survived in control group at 50 days and 24 rats survived in same period (Table 3). Only the control group grew anaerobic organisms and vaccinated group grew aerobic organisms (Table 4).

Table 1: Types of organisms from peritoneal fluid after caecal ligation and puncture in fisher rats (control group)

Isolated Organisms	Number of Rats (n = 40)	Percent
Escherichia coli	3	7.5%
E. coli and C. perfringens	19	47.5%
E. coli, C. perfringens and Enterococci	12	30%
E. coli, and Enterococci	1	2.5%
No organisms	1	2.5%
Total of dead rats	36	90%

Table 2: Types of organisms from peritoneal fluid after caecal ligation and puncture in fisher rats (vaccinated group).

Isolated Organisms	Number of Rats (n = 40)	Percent
Escherichia coli	16	40%
E. coli and C. perfringens	0	0%
E. coli, C. perfringens and Enterococci	0	0%
E. coli, and Enterococci	0	0%
No organisms	0	0%
Total of dead rats	16	40%

Table 3: Survival after caecal ligation and puncture in vaccinated and control *

	Number of rats survived	Total number of rats	Survival percent
Control	4	40	10%
Vaccinated	24	40	60%

****** p value < 0.001***

A rat may harbour both aerboes and anaerobes

Table 4: Type of infection in vaccinated and control group **

Type of infection	Anaerobic **	Aerobic ***
Vaccinated Group	0% (0/40)	40% (16/40)
Control Group	77.5% (31/40)	10% (4/40)

******* p value < 0.001*
**** p value < 0.001*
A rat may harbour both aerobes and anaerobes

DISCUSSION

The vaccinated rats in this study showed good immunity to anaerobic organisms, and the peritoneal fluid of the dead animals showed only aerobic organisms. Vaccination with anaerobic toxins was successful in preventing anaerobic peritonitis in those rats. The type of organisms recovered from peritoneal fluid in dead unvaccinated rats (control) showed that most of them grow anaerobic organisms, 31 of 36 rats (86.1%).

The study showed that mortality from peritonitis due to perforated viscous was due mainly to anaerobic infection in rats. These findings may have an impact on morbidity and mortality after operation on large bowel or biliary tract where there is an incidence of leakage from anastomosis of bowel. Prophylactic vaccination with anaerobic toxins in such patients may lower morbidity and mortality of patients who develop peritonitis after such operation from leakage of gastrointestinal contents in peritoneal cavity.

There was no report in the literature about the use of clostridial toxoid except in *Clostridium difficile* associated diarrhoea (CDAD) and it was effective in treating this illness. Patients discontinued treatment with oral vancomycin without any further recurrence. A *C.difficile* toxoid vaccine induced immune responses to toxins A and B in patients with CDAD and was associated with resolution of recurrent diarrhoea. The results of this study support the feasibility of active vaccination against *C.difficile* and its toxins in high-risk individuals. Currently, a pilot study is underway in which human patients who will undergo major surgery are prophylactically vaccinated with clostridial toxoids, in an attempt to eradicate postoperative anaerobic sepsis.

REFERENCES

[1] RL Nichols;JW Smith. *Clin Infect Dis*,**1994**;18 (Suppl 4):S280-6.
[2] J Ruiter; J Weel; E Manusama; WP Kingma;PH van der Voort. *Infection*, **2009**;37(6):522-7.
[3] JB Silva; SK Oliveira; IA Campos; CH Carvalho-Júnior; C CoutinhoTda;TG Silva. *Braz J Infect Dis*, **2013**;17(1):20-6.
[4] L Dejager; I Pinheiro; E Dejonckheere;C Libert.*Trends Microbiol*,**2011**;19(4):198-208.
[5] S Sougioultzis; L Kyne; D Drudy; S Keates;S Maroo; C Pothoulakis; PJ Giannasca;CK Lee; M Warny; TP Monath;CP Kelly. *Gastroenterology*,**2005**;128(3):764-70.
[6] K Tonguthai; S Chinabut; T Somsiri; P Chanratchakol; S Kanchanakhan.Diagnostic Producer for Finfish DiseasesAquatic Animal Health Research Institute, Bangkok, Thailand,**1999**; 152.