INVESTIGATION OF THE ACTIVITY AND PATHOGENECITY OF STAPHYLOCOCCUS AUREUS ENTEROTOXIN C BY LIGATED ILEAL LOOP ASSAY IN RABBITS

Basil A. Abbas  Mohammed H. Khudor  Hasan I. Idbeis

Department of Microbiology, College of Veterinary Medicine, University of Basrah, Iraq.

Keywords; Staphylococcus, Enterotoxin, Haemorrhage.

(Received 12 November 2013) (Accepted 19 May 2013)

ABSTRACT

Three enterotoxigenic isolates of Staphylococcus aureus previously isolated from contaminated milk and evaluated for their enterotoxin producing ability and histopathological changes by the ligated rabbit ileal loop assay. The results of this assay revealed that crude toxin obtained by these isolates caused fluid accumulation in rabbit ileal loops. Fluid aspirated from the loops was bloody and the histopathological changes in sections were characterized by moderate to severe haemorrhage, erosion and inflammatory cells, in addition there was distortion and shift of villi. This finding established that staphylococcal enterotoxin that associated with vomiting and diarrhea, which often abate within 24 hrs., there was potential risk for more serious disturbances such as inflammation, tissue damage and toxic shock.

Key words: S. aureus, enterotoxin, ileal loop, rabbits

INTRODUCTION

Staphylococcus aureus produced large numbers of extracellular proteins and toxins. The most important toxins are called staphylococcal enterotoxins (1). Staphylococcal enterotoxins (SEs) are a family of structurally related proteins that are produced by S.aureus (2). The enterotoxin family now contains 17 toxins. The SE family is divided into the classical enterotoxins SEA to SEE and a group of recently discovered toxins SEG to SER, in addition the SEC has three antigenically distinct subtypes: SEC1, SEC2, SEC3, and SEG have a variant form called SEGv (3,1).

Many SEs are responsible for food poisoning, acute illness, fever, erythematous lesions, and hypotension (1). It is estimated that about 5% of food poisoning cases in which none of the classical enterotoxins were detected can, however, be attributed to new enterotoxins (3,4). Since S. aureus may produce a large variety of
enterotoxins but 95% of poisoning outbreaks are caused by classical enterotoxins: A, B, C, D, and E (5).

The SEs are generally heat resistant and a heat denatured enterotoxin can be renatured by prolonged storage or in the presence of urea. Toxins remain active even after boiling for 30 min. and they are stable at 121 °C for 28 min. The SEs also are resistant to most proteolytic enzymes such as pepsin or trypsin thus keep their activity in the digestive tract after ingestion and all are capable of causing food poisoning (2, 3).

MATERIALS AND METHODS

Bacterial strains and Ligated rabbit ileal loop assay

Three S. aureus strains harboring Sec gene detected by PCR previously isolated from milk (6) were used in this study for evaluation of their enterotoxin-production ability and histopathological changes made by the ligated rabbit ileal loop assay (7).

Enterotoxin production

Cultures for enterotoxin production were initially prepared using nutrient broth. Ten milliliter aliquots of sterile nutrient broth, in sterile tube, were inoculated each with approximately 10⁵ cells/ml according to Mc Farland standard (8), and incubated at 37 °C for 48 hrs. Subsequently, the S. aureus strains were cultured in 10 ml of milk at pH 8 and pH 4 that pasteurized by heating to 80 °C for 30 min. Cultures were incubated as above nutrient broth cultures. Following 48 hrs. incubation of the nutrient broth and milk cultures, cell free cultures supernatants were collected by centrifugation at 5000 rpm followed by filtration through 0.20 μm Milllex syringe filters. The cell free filtrates were then used as crude toxin preparation (6,7).

Assay for enterotoxin activity

One to 1.5 kg body weight female rabbits were starved for 24 hrs. with water supplied and libitum. Each rabbit was anesthetized with two ml. of ketamin injection and secured in dorsal recumbency. Following a midline incision, starting from the rectal end, the ileum was divided into segments of 5 cm. in length with string ligatures. The crude toxin preparation (0.5 ml.) was injected into different segments. Uninoculated broth and sterile saline were injected into some segments to serve as negative controls. The incisions were then sutured and the animals allowed recovering from anesthesia (6, 7).

Post–mortem examination

After 7 hrs., test animals were killed and opened immediately for examination. The gross appearance of the loops was noted, and if either the control loop contained
fluid, all tests in that rabbit were considered invalid. The length (centimeters) and volume (milliliters) of each test loop was measured. For positive loops, the volume of fluid recovered by aspiration was used to determine the dilatation index (DI) estimated as the ratio of volume of fluid to length of ileal segment. A DI > 0.2 was taken as positive. The test was done in triplicate animals (7).

RESULTS

Diarreagenic microorganisms including S. aureus are tested by ligated ileal loop assay. Three strains of PCR positive S. aureus isolated from contaminated milk were evaluated for their enterotoxin-producing ability and histopathological changes by the ligated rabbit ileal.
Cell-free culture supernatants (crude toxin preparations) of the *S. aureus* strains caused fluid accumulation when injected into rabbit ileal segments, indicating enterotoxin activity. Dilatation index (DI) values ranged from 0.2 to 0.48. Moreover, there was a dark-reddish colouration of the positive ileal loops (Figure 1) and the aspirated fluid from such segments appeared bloody.

Histopathological changes in sections collected from the rabbit ileum were characterized by circulatory disturbances and inflammatory changes. Sections of the intestine collected from untreated (control) rabbits showed mucosae (including glands) and submucosae with normal histomorphology (Figures 2), while sections from rabbit’s ileum inoculated with crude toxin preparations showed moderate to severe haemorrhage, erosion and inflammatory cells. In addition, there was distortion and shift of villi with the presence of some intestinal glands in mucosal area (Figures 3, 4, 5).

**Fig (1)** Ligated segments of rabbit ileal loop after injection with crude preparations of staphylococcal enterotoxin (SE) produced under different growth conditions. 1 – 6, SE produced at pH 8; 7-11, SE produced at pH 4 – there was change to a brownish colouration with less fluid accumulation than the previous pH; 12, segment inoculated with sterile saline (control).

**Fig (2)** Section of control rabbit ileum showing normal villus (arrow) and intestinal gland. 125X H&E.
Fig (3) Section of rabbit ileum inoculated with crude staphylococcal enterotoxin pH 4, showing A) Erosion in the mucosal layer B) presence of intestinal glands and some of the villi which show destruction and sloughing C) Oedema 125X H&E.

Fig (4) Section of rabbit ileum inoculated with crude staphylococcal enterotoxin pH 8, showing A) large areas of hemorrhage in the wide area of mucosal erosion and complete absence of the villi B) moderate inflammatory reaction. 125X H&E.
DISCUSSION

In the present study the crude toxin preparation of enterotoxigenic *S. aureus* can elicit positive ileal loops of the rabbits with dilatation index (DI) values that ranged from 0.2 to 0.48. Moreover, there were dark-reddish colorations of the positive ileal loops and the aspirated fluid from such segments appeared bloody. This result agreed with (7) who found the crude toxin preparation of enterotoxigenic *S. aureus* can elicit positive ileal loops of the rabbits with the dilation index (ID) that ranged from 0.2 to 0.57 (ml/cm).

Koupal and Deibel, (9) recorded the culture supernatants of the enterotoxigenic *S. aureus* can elicit positive ileal loops of the rabbits with dilation index (ID) ranged from 0.52 to 57ml/cm.

The accumulation of the fluid in the intestine occurs because the intestinal epithelial cells form a barrier between the luminal contents and the sub epithelial region and SEs act as superantigen which causes down regulate of intestinal barrier function and increase epithelial permeability (10).

Histopathological changes in sections collected from the rabbit ileum were characterized by circulatory disturbances and inflammatory changes these include, moderate to severe haemorrhage, erosion, inflammatory cells, In addition, there was distortion and shift of villi with the presence of some intestinal glands in mucosal area.
Similar findings were obtained by (7) with the exception that degenerative and necrosis were not detected in the present study, this may be contributed to the strains variation among different S.aureus isolates (11, 5). Bhunia, (1) documented the SEs elicit damage to the intestinal epithelial cells resulting in the destruction of intestinal villi and inflammatory changes.

Also similar findings were obtained by Kuroishi et al., (12) who elucidated mechanisms by which SEC induced inflammatory changes in bovine mammary glands. The SEC-inoculated mammary glands exhibited interstitial inflammation, with epithelial cell degeneration and the migration of polymorphonuclear neutrophils.

Although our study describes histological changes in a rabbit model, there is documented evidence that the clinical syndromes in some animal models simulate human enterotoxicosis (13).

اختبار فعالية وإمراضة المكورات العنقودية الذهبية الفارسة للذئاب المعوية نوع C

باسم عبد الزهرة عباس محمد حسن خضر حسن اكريم ادبيس
فرع الاحياء المجهرية كلية الطب البيطري ,جامعة البصرة

الخلاصة

اختبرت ثلاث عزلات موجبة لفحص البلمرة المتعدد (تمتلك أنواع المكورات المعوية C نوع) وقومت قدرتها على إنتاج الخفافيش المعوية وإحداث التغيرات النسيجية باستخدام طريقة الأمعاء المعوية المعدة للأرانب ولاحظ أن الخفافيش المنزوعة لهذه العزلات يصبح ثروت دموية في العقد اللئامية للأرانب كما أظهرت المقاطع النسيجية وجود نزف شديد وتداخلات وتشوهات شديدة في الغدد المعوية وانحراف في الغدد المعوية.
REFERENCES


