An experimental study on surgical wound contamination from distant infective source in rabbits

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Summary

Surgical wound infections are usually encountered due to the endogenous rather than the exogenous infective sources. The objective of this study was to evaluate the effect of a distant inoculated infective source on the contamination of surgical wounds. Thirty White New Zealand rabbits divided randomly into three control and three treatment equal groups. In treatment groups 24 hrs before surgical intervention *Staphylococcus aureus* was injected subcutaneously in right thigh. In model animals, skin and muscles were incised 1 cm to the right of the vertebral column and sutured, immediately. The relative frequency of staphylococcal contamination of tissue specimens at 24 and 48 hrs after surgery in treatment groups were 20% and 60%, respectively. Statistical analysis did not show any significant differences in the rate of contamination between control and treatment groups at aforementioned times ($P>0.05$). However, comparison at 72 hrs after surgery, showed that the rate of contamination in treatment group is significantly more than control ones ($P = 0.004$). The presence of *S. aureus* in wounds of treatment animals at 72 hrs after surgery, suggested that microorganisms lodged in any part of body other than wound region could contaminate it, which could be important in wound healing.

Key words: Wound healing, *Staphylococcus aureus*, Rabbit

Introduction

It seems that post-operative wound infections are not primarily due to exogenous airborne bacteria entering the wound at the time of operation. Operative incisions are subject to infection from bacteria derived from sources both exogenous and endogenous to the patient. During clean operations, infection may be initiated by air or direct contact (Culver *et al*., 1991; Whyte *et al*., 1991; Whyte *et al*., 1992). Other possibilities include hematogenous spread, spread along indwelling devices (Pfaller and Herwaldt, 1988) or from post-operative manipulations. Despite a reduction of airborne bacteria by the use of various sterilization methods, the overall post-operative infection rate was not reduced (Jennings *et al*., 1977; Bitkover *et al*., 2000; Bowler *et al*., 2001).

Incisional infections can arise from endogenous bacteria by at least, two separate mechanisms. The first mechanism is by direct contact of bacteria within the body with the edges of the incision. This mechanism, clinically represented by gastrointestinal and genitourinary tracts surgeries. The second mechanism of endogenous infection is demonstrated by the patient who is sustaining a state of infection and bacteremia and who is at increased risk for the development of operative-wound sepsis, even at a site distant from the primary focus. This study was specifically directed to answering whether distant infective source could be able to change tissue culture results after 48, 72 and 96 hrs after inoculation *Staphylococcus aureus* and 24, 48 and 72 hrs after surgery in the rabbits.

Materials and Methods

Thirty White New Zealand rabbits of

both sexes (1750-2300 g) were provided by Razi Institute (Karadj, Iran). All animals were kept under the same condition. They were fed standard lab chow and given tap water ad libitum. The animals were divided randomly into three control (groups I, III and V) and three treatment (groups II, IV and VI) equal groups. In treatment groups 24 hrs before surgical intervention 5 ml/kg of the *S. aureus* (8×10^8 CFU/ml dilution) (Jennings *et al*., 1977) was injected subcutaneously in right thigh. Twenty-four hrs after inoculation in animals of both treatment and control groups, an area on the back was shaved and prepared with povidone-iodine and alcohol. Under general anesthesia induced by intramusculary injection of 3-5 mg/kg of xylazine HCl (Rompun®2%, Bayer Leverkusen, Germany) and 40 mg/kg ketamine HCl (Ketamine 10%, Aesco Boxtel, Holland) and using aseptic technique throughout, an incision with a length of 3 cm/kg body weight was made 1 cm to the right of the vertebral column. This was carried through the longissimus dorsi muscle, parallel to their bundles, to a depth of 3-5 mm. Hemostasis was effected by gentle blotting of the wounds with a dry, sterile gauze sponge. When bleeding ceased, the wounds were closed with simple interrupted sutures of monofilament nylon No. 3-0, 2 stitches/cm of incision length. Since the presence of suture material is known to lower the threshold of a wound to bacterial colonization (Jennings *et al*., 1977), these sutures were placed in the dermis to avoid inclusion of any of the underlying muscle which was subsequently to be biopsied. Following closure, the wounds were cleansed with povidone-iodine. The animals were returned to clean cages. Vital signs were assessed before and after operation.

Under light intramuscular general anesthesia, biopsy samples for bacteriological culture were obtained at 24 (groups I and II), 48 (groups III and IV) and 72 (groups V and VI), hrs post-operation from incised tissues. The dorsal wound was then prepared as described before and opened under aseptic conditions and biopsies were obtained from the previously sectioned muscle. All tissue specimens were immediately weighed and plated on nutrient agar for quantification of bacteria. Tissue specimens having up to 10^5/g bacteria were classified as contaminated.

To compare vital sign values in model animals in each group the paired Student's t-test and between various groups, ANOVA were used. The p-value less than 0.05 were considered as level of significance. Comparison of contamination rates in model animals of control and treatment groups was performed using Fisher's exact method.

Results

Of the 30 animals in this study, none of them died nor excluded from the analysis. In animals of treatment groups mean rectal temperature 24 hrs before and after inoculation of *S. aureus* were 38.42°C (± 0.11 SD) and 39.53°C (± 0.36 SD), respectively. The increase in rectal temperature 24 hrs after inoculation of *S. aureus* in treatment groups were statistically significant ($P<0.05$).

The relative frequency of staphylococcal contamination of tissue samples of surgical site at 24, 48 and 72 hrs after surgery in control and treatment groups was showed in Table 1. Statistical analysis did not show any significant differences in the rate of contamination between control and treatment groups at 24 and 48 hrs after surgery ($P>0.05$). However, comparison at 72 hrs after surgery showed that the rate of contamination in treatment group (5 positive sample of total 5 sample) is significantly more than control (0 positive sample of total 5 sample) ones ($P = 0.004$).

Table 1: The relative frequency of staphylococcal contamination in tissue samples of surgical site at 24, 48 and 72 hrs after surgery in study groups

Groups	Staphylococcal contamination (%)
I (Control 24 hrs)	0 (0 positive of 5 sample)
II (Treatment 24 hrs)	20 (1 positive of 5 sample)
III (Control 48 hrs)	0 (0 positive of 5 sample)
IV (Treatment 48 hrs)	60 (3 positive of 5 sample)
V* (Control 72 hrs)	0 (0 positive of 5 sample)
VI* (Treatment 72 hrs)	100 (5 positive of 5 sample)

*$p<0.05$

Discussion

Surgical wound contaminants are likely to originate from three main sources: (a) the

environment (exogenous microorganisms in the air or those introduced by traumatic injury), (b) the surrounding skin (involving members of the normal skin microflora such as *S. epidermidis*, micrococci, skin diphtheroids and propionibacteria) and (c) endogenous sources involving mucous membranes (primarily the gastrointestinal, oropharyngeal and genitourinary mucosae) and distant infective source (subcutaneous abscesses) (Bowler *et al*., 2001). In other words, it has become increasingly apparent that the predominant source of infection in operative wounds lies within the patient. (Robson *et al*., 1970a; Robson *et al*., 1970b; Jennings *et al*., 1977). Endogenous infection occurs by two routes: by direct implantation from a contaminated focus or by metastatic spread from distant foci by either hematogenous or lymphatic routes. The first wound is dirty at the time of incision; the second becomes so dirty, over a period of time. In both cases the surgeon is concerned with prevention of infection in wounds judged to be clean at the time of closure.

In this study the second route of endogenously acquired infection, has been studied in rabbits. In this situation, the exposure of the wound to the bacteria is sustained over period of study. Distant *S. aureus* abscesses produced a wound contamination rate with *S. aureus* of 20% at 24 hrs, 60% at 48 hrs and 100% at 72 hrs in animals of treatment groups. However, the contamination rates with *S. aureus* in animals of control groups were 0% at 24, 48 and 72 hrs. This is in agreement with the results of Jennings *et al*., (1977).

To date, widespread opinion among wound care practitioners is that aerobic or facultative pathogens such as *S. aureus*, *Pseudomonas aeruginosa* and beta-hemolytic streptococci are the primary causes of delayed healing and infection in both acute and chronic wounds. Although microorganisms are responsible for wound infection, widespread controversy still exists regarding the exact mechanisms by which they cause infection and also their significance in non-healing wounds that do not exhibit clinical signs of infection. Aerobic pathogens such as *S. aureus*, *P. aeruginosa* and beta-hemolytic streptococci are recognized for their ability to produce potentially destructive virulence factors (Heggers, 1998).

S. aureus excretes many bioactive proteins (toxins) including enzymes, such as lipase, esterase, deoxyribonuclease, staphylokinase (a plasminogen activator), hyaluronidase (spreading factor) and phospholipase (Hirsh and Zee, 1999). Production of coagulase by staphylococci is an important indicator of pathogenicity. Additional markers for pathogenicity are DNase activity and protein A production (Quinn *et al*., 2002).

In our study clinical assessment showed that in treatment groups there were a statistically significant increase in rectal temperature 24 hrs after inoculation of *S. aureus* ($P<0.05$). It has remembered that diagnosis of infection in infected wounds should be based primarily on clinical signs, such as heat, pain, erythema, edema, suppuration and fever; microbiological results may be helpful but can often be misleading, especially with polymicrobially infected wounds containing numerous potential pathogens (Trengove *et al*., 1996).

In two studies involving the microbiology of cutaneous abscesses, *S. aureus* was present as a pure culture in 24 to 29% of the infections (Brook and Finegold, 1981; Von Eiff *et al*., 1998). Other investigators have demonstrated that the resident microflora has little effect on the outcome of wound healing (Sapico *et al*., 1986; Handfield-Jones *et al*., 1988; Hansson *et al*., 1995).

The value of superficial cultures in wound assessment has been questioned and Robson (1997) stated that purulent wound fluid may fail to yield microorganism growth whereas biopsied tissue may yield significant numbers of bacteria if such cultures were performed routinely. Thus, the quantitative and qualitative aspects of wound microbiology are critical determinants in the development of infection.

The risk of surgical wound infections is generally based on the susceptibility of a surgical wound to microbial contamination. Clean surgery carries a 1 to 5% risk of post-operative wound infection and in dirty procedures that are significantly more susceptible to endogenous contamination, a

27% risk of infection has been estimated (Nichols, 1998).

With the exception of clean operative procedures, surgical wound infections are recognized as having a polymicrobial etiology, involving both aerobic and anaerobic microorganisms and intra-abdominal infections normally reflect the microflora of the resected organ (Bowler *et al*., 2001).

Minimizing the incidence of post-operative wound infection relies on adequate asepsis and antisepsis and preservation of the local host defences (Hunt, 1981). Asepsis involves the utilization of effective infection control procedures (e. g. air filtration, skin barrier garments, disinfection) to minimize exogenous microbial contamination during surgery. Antisepsis involves the use of skin antiseptics on the operative site and also, in the case of dirty surgical procedures, administration of prophylactic antibiotics at a time point just prior to surgery that will ensure adequate tissue levels of antibiotic during surgery. As a part of the surgical procedure, endogenous and exogenous microbial contamination must be minimized by ensuring good aseptic, skilled surgical techniques and minimizing the duration of surgery, also optimizing the local wound conditions (Bowler *et al*., 2001).

This primarily involves removing any devitalized tissue to re-establish blood flow to the wound area (Aldridge, 1994), thereby maintaining adequate perfusion to enable the delivery of immune cells, oxygen and nutrients and reducing the microbial load.

To prevent post-operative infections the following steps should be taken: (1) Proper prophylactic antibiotics should be administrated. (2) Efforts should be directed at preserving the integrity of the tissues by frugal use of electrocautery and careful wound closure. (3) The patient's skin should be meticulously prepared and draped. (4) Airborne contamination should be minimized by effective ventilation in the operating room, by use of occlusive scrub suits for the staff and occlusive drapings for the patient. (5) Skin conditions of staff and patients should be mentioned (Bitkover *et al*., 2000) and (6) special attention have to pay to combat with endogenous distant infective source.

In this study the presence of *S. aureus* in tissue samples of surgical wounds of rabbits, in treatment groups in comparison with control ones at 72 hrs after surgery, suggested that microorganisms lodged in any part of body other than wound region could contaminate surgical wounds and this fact should be considered in prevention of post-operation infection in this species.

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