INTERFERENCE WITH HEALING OF RAT SKIN INCISIONS TREATED WITH CHLORHEXIDINE

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Abstract. Chlorhexidine is a common wound antiseptic. Its effects on the healing of incisional wounds in rat skin were studied using a tensiometric technique. The breaking load was significantly lower after 7 days in wounds that, immediately after infliction, had been exposed to chlorhexidine 0.02% or 0.1% for 5 minutes and then sutured, as compared with control wounds treated with saline or chlorhexidine’s vehicle. The same result was obtained when chlorhexidine-exposed incisions were closed with a non-suture technique as compared with sutured control incisions. Pending further knowledge, the results suggest caution in the use of chlorhexidine in man on wounds with critical circulation where additional tissue injury may be dangerous.

Chlorhexidine (1,1-hexamethylenediamine-5-(4-chlorophenyl)biguanide) is an antibacterial compound active against both gram-positive and gram-negative bacteria even in the presence of body-fluids (8, 23). The chlorhexidine salts most frequently used in medicine are the digluconate, acetate and diacetate. In Swedish hospitals, the use of chlorhexidine as an antiseptic has increased during recent years at the expense of quaternary ammonium compounds (9). It is used clinically for disinfection of hands and operation sites (21), in the treatment of burns and scalds (16, 20), in urology and gynaecology (2, 15) and by dentists in caries and periodontitis (11). Chlorhexidine (5% in a tincture or cream) has been recommended for use in bacterial and mycotic skin diseases (24). It is also marketed as a first-aid wound treatment at home.

An important requirement of a wound disinfectant is not to produce additional tissue injury of such magnitude as to significantly impair regeneration. Chlorhexidine has been reported to exert cytotoxic effects on living epithelial cells in vitro (17), to induce microvascular disturbances when administered into the tissues of the hamster cheek pouch (19) and in some patients taking daily mouth washes with chlorhexidine desquamations and soreness of the oral mucosa developed (12). In spite of such evidence of chlorhexidine’s potential to cause tissue injury, there is a general clinical impression that it is non-irritant for topical use in wounds (3, 7, 13, 16, 22, 32). This discrepancy may be explained by the presence of a threshold value of tissue injury in the wound that must be exceeded before the repair process is impaired, as suggested by Rydberg & Åhrén (29). Another possibility is that the methods used in clinical practice to record the healing are not sensitive enough. Gross examination and/or histopathologic investigations will reveal obvious deviations from normal wound healing, but these methods may be too crude to detect minor variations.

The aim of this study was to elucidate the effect of chlorhexidine on wound healing, as reflected by the gain of breaking load in sutured or taped skin incisions. The determination of the breaking load is regarded as the most informative indicator of the progress of repair in a closed incisional wound (27).

MATERIAL AND METHODS

Sixty-five male white rats (Sprague-Dawley, about 300 g) were used. They were kept in separate cages, and allowed free access to pellets and water.

The chlorhexidine solutions were prepared from Hibitane® (Imperial Chemical Industries Ltd., Macclesfield, England). They were sterile, isotonic and freshly prepared. The compositions are given in Table I. The concentrations chosen are those used clinically at this hospital: 0.1% for disinfection of the skin in the genital region and urethral meatus, and 0.02% for irrigation of the
bladder. Chlorhexidine is not recommended for use in wounds at Sahlgren's Hospital, but other hospitals in Sweden use a 0.05% sterile isotonic solution of chlorhexidine for a period of 5 min before the surgical incision. The solution was instilled into the wound cavity (test wound) and the incision was then sutured with continuous through-and-through sutures including the skin and the subcutaneous muscle. The sutures were placed 5 mm apart and 5 mm from the wound edge, using black braided silk No. 000 and curved triangular needles no. 18. It took about 5 minutes to suture the wound. The antiseptic solution was evacuated before the sutures were tightened and tied. A contralateral incision was then inflicted, filled with the control solution (vehicle or saline) and sutured in the same way as the test wound. Wound dressings were not used.

Wound closure with surgical tape. Silk sutures in a wound add to the tissue damage from operative trauma and antiseptics (29). If wound closure with sutures is replaced with approximation of the wound edges with surgical tape, postoperative inflammation is less pronounced and the wound heals faster (6). In order to investigate whether the effect of chlorhexidine on wound healing manifests itself even in the absence of sutures, a non-suture technique was used in 10 rats for closure of the test wound. These wounds had been exposed to 0.1% or 0.02% chlorhexidine, as shown in Table 111.

Following shaving of the animal's back, the skin was epithilated by a hair remover cream (SurgeX®). Infliction of wounds, exposure to the solutions and suture of the control wound (saline exposed) have been described above. The test wound was closed by 6 strips, each 12.7 mm wide, of a microporous surgical adhesive tape (Steristrips®) and then 2 strips were placed along the incision above the transversal strips. Finally Tensoplast® was wrapped around the animal's body.

Table I. Composition of chlorhexidine solutions and the vehicle (used as control)
breaking load of that wound. Differences in strength be­
tween test and control wounds of each animal are given
in per cent of the value for the control wound (34). Sta­tistical analysis was carried out according to Student’s

RESULTS
The results from the breaking load determinations
of wounds are presented in Tables II and III.

Sutured wounds. Chlorhexidine in concentra­
tions of 0.02 % and 0.1 % resulted in significantly
lower breaking load values than the corresponding
control wounds treated with saline. Wounds ex­
posed to the vehicle only were also weaker than
control wounds exposed to saline, but this differ­
ce was not as pronounced as when chlor­
hexidine was compared with saline. When chlor­
hexidine (0.02 % and 0.1 %) treated wounds were
compared with the corresponding control wounds
treated with vehicle only, the former still had
significantly lower breaking load values.

Tape-closed test wound, sutured control wound.
The strength over skin incisions exposed to 0.1 %
or 0.02 % chlorhexidine for about 5 minutes and
then closed with surgical tape was lower after
7 days of healing than that of corresponding con­
trol wounds treated with saline and closed with
silk sutures. The differences are significant.

DISCUSSION
This study has shown that for healing periods of
7 days the breaking load of chlorhexidine-treated
wounds is lower than that of symmetrical incisions
also closed by continuous silk suture but exposed
to saline. This can partly be attributed to chlor­
hexidine’s vehicle, a sodium acetate–acetic acid
buffer which also turned out to retard the healing
processes of wounds as determined by the break­
ing load. However, even when chlorhexidine was
compared with its vehicle, the impairment of the
healing process caused by the antiseptic was
significantly greater.

The use of stitches in these experiments adds
to the trauma in the wound region, but this
factor is equal in both test and control wounds.
When the suture closure of the test wound was
replaced with a non-suture technique, the tissue
injury resulting from the operative trauma and
the antiseptic was still enough to impair the re­
pair process significantly compared with the con­
trol (saline) wound closed with conventional
suture technique.

The formulations and concentrations of chlo­
hexidine chosen for this study were those supplied
by the hospital’s pharmacy for clinical use. Its
action time in the experimental wounds, about
8 minutes, does not exceed the calculated ap­
proximate time of action of cationic detergents
in a surgical wound (4). When chlorhexidine is
applied by a layman to an open wound as a lotion
or in a cream base without subsequent water or
saline irrigation, its action time may be even
longer.

The experimental wound model used in this
study, where test and control wounds are inflicted
simultaneously and symmetrically in the same
animal by a thoroughly standardized technique,
has been used before to investigate the effect of
topical antiseptics on the repair process. The
cationic detergents benzalkonium chloride and
cetylpyridinium chloride (28), dequalinium chlo­
ride (25) and a cationic triphenylmethane dye,
crystal violet (26), in concentrations used clinically
all turned out to retard the healing processes in
incisional wounds closed by silk suture. These
results are probably also significant for the heal­
ing of open wounds, since the differences between
the wound-healing processes in wounds healing
by primary and secondary intention are con­
sidered to be quantitative rather than qualitative
(1, 31). This information from animal studies
cannot be directly translated to the conditions of
clinical use in man, and it is possible that the
toxic effects of the bioassayed substances are
stressed compared with clinical conditions. There
is, however, a varying individual susceptibility to
irritants; some people are more sensitive in this
respect than others. Clinical observations have
confirmed the capacity of the agents studied to
influct tissue damage in man under certain condi­
tions (5, 14, 33).

The application of topical antiseptics in infected
wounds may be justified if the advantages of
degerming outweighs the risk of additional tissue
damage. On the other hand, recently acquired
wounds without clinical signs of infection are
often almost ritually treated by the layman with
antiseptics, which are considered of dubious value
in this type of wound (10). Under these circum­
stances, the use of an antiseptic with a cytotoxic
potential will bring the risk of negative effects to
the healing process without adding substantial advantage. This study has demonstrated that chlorhexidine under certain experimental conditions can induce tissue injury of such a significance as to impair wound healing. Extrapolation of the results from our studies in one animal species to the varying clinical situations in man must be done with caution (vide supra). It has been reported that instillation of chlorhexidine into human operation and traumatic wounds did not produce manifest harmful effects (7, 13, 32), which however does not exclude a subthreshold influence on the repair process. If further injury is superimposed on such a wound, the cumulative effect may manifest itself as a clinically detectable impairment of healing. Examples of possible such injurious factors are, in varying degrees and combinations, disturbed circulation, bacterial infection and mechanical tissue injury.

Pending further knowledge, we suggest that chlorhexidine should be used with caution on those wounds caused by disturbed circulation where additional tissue injury may be dangerous, and on tissue structures whose function may be restricted by excessive scar tissue, e.g. exposed tendons and tendon sheaths. Occlusive dressings may increase penetration into the tissues and consequently contribute to the development of untoward effects.

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REFERENCES


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