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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-hexamethylenebis[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 1. 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

Table I. *Composition of chlorhexidine solutions and the vehicle (used as control)*

Chlorhexidine 0.1 %	
Chlorhexidine acetate	1 g
Sodium acetate for infusion	21 g
Acetic acid	ad pH 7.9
Distilled water	ad 1 000 ml
Chlorhexidine 0.02 %	
Chlorhexidine acetate	0.2 g
Sodium acetate for infusion	21 g
Acetic acid 5 M	q.s.
Distilled water	ad 1 000 ml (pH 7.0–8.5)
Vehicle	
Sodium acetate Nord. Reagens	21 g
Acetic acid 5 M	q.s.
Distilled water	ad 1 000 ml (pH 7.3–7.4)

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 diacetate for this purpose (9). When chlorhexidine is acquired without prescription for home-treatment of wounds, it is usually dispersed as a lotion or cream with 0.05–0.1 % of the antiseptic.

In the control wounds, sterile saline or the vehicle (a sterile aqueous solution of a sodium acetate–acetic acid buffer) was used.

Wound closure with sutures. The backs of the animals were shaved with an electric razor under ether anesthesia. The positions of the incisions and stitches were drawn on the skin in order to achieve uniformity. Under surgically clean conditions a 6 cm long incision was made 1.5 cm from and parallel to the midline. The incision was perpendicular to the skin surface and included the skin and the underlying subcutaneous muscle. The wound margins were elevated with small hooks, about 3 ml of the test 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 stitches 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.

Table II. *Influence of chlorhexidine on the breaking load of sutured skin wounds in the rat after 7 days of healing*

No. of animals	Test wound	Control wound	Rel. difference %[a] (mean ± S.E.M.)	Significance
15	Vehicle	Saline	−11.4 ± 2.8	$0.01 > p > 0.001$
10	Chlorhexidine 0.1 %	Saline	−37.7 ± 2.8	$p < 0.001$
10	Chlorhexidine 0.1 %	Vehicle	−33.1 ± 4.9	$p < 0.001$
10	Chlorhexidine 0.02 %	Saline	−23.9 ± 2.5	$p < 0.001$
10	Chlorhexidine 0.02 %	Vehicle	−12.4 ± 3.9	$0.05 > p > 0.01$

[a] Differences between test and control wounds are expressed in per cent of the control wound's breaking load.

Table III. *Effect of chlorhexidine on the breaking load of tape-closed skin incisions after 7 days of healing. Control wounds closed with silk sutures*

No. of animals	Test wound	Control wound	Rel. difference %[a] (mean ± S.E.M.)	Significance
4	Chlorhexidine 0.1 %	Saline	−42.2 ± 5.5	$0.01 > p > 0.001$
6	Chlorhexidine 0.02 %	Saline	−24.5 ± 7.5	$0.05 > p > 0.01$

[a] Differences between test and control wounds are expressed in per cent of the control wound's breaking load.

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 III.

Following shaving of the animal's back, the skin was epilated 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 (Steri-strips®) and then 2 strips were placed along the incision above the transversal strips. Finally Tensoplast® was wrapped around the animal's body.

Tensiometry. After 7 days, the sutures or the tape strips were removed and the breaking load of each wound was measured *in situ* with a special type of tensiometer originally described by Sandblom et al. (30) and as modified by Holm-Pedersen & Zederfeldt (18). Three determinations were made on each 6 cm long wound, and the arithmetic mean of these was taken as the

breaking load of that wound. Differences in strength between 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 *t*-test.

RESULTS

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

Sutured wounds. Chlorhexidine in concentrations of 0.02% and 0.1% resulted in significantly lower breaking load values than the corresponding control wounds treated with saline. Wounds exposed to the vehicle only were also weaker than control wounds exposed to saline, but this difference was not as pronounced as when chlorhexidine was compared with saline. When chlorhexidine (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 control 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 chlorhexidine's vehicle, a sodium acetate–acetic acid buffer which also turned out to retard the healing processes of wounds as determined by the breaking 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 repair process significantly compared with the control (saline) wound closed with conventional suture technique.

The formulations and concentrations of chlorhexidine chosen for this study were those supplied by the hospital's pharmacy for clinical use. Its action time in the experimental wounds, about 5 minutes, does not exceed the calculated approximate 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 chloride (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 healing of open wounds, since the differences between the wound-healing processes in wounds healing by primary and secondary intention are considered 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 inflict tissue damage in man under certain conditions (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 circumstances, 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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