

Sensitization of guinea pigs with chlorhexidine

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Haugen, E. & Johansen, J. R. Sensitization of guinea pigs with chlorhexidine. *Acta Odont. Scand.* 32, 173—175, 1974.

Guinea pigs were immunized with autologous serum exposed to chlorhexidine gluconate. Injections were given every second day for one week. After four weeks the animals were given a booster-dose and were tested for antibodies using the cutaneous anaphylaxis test. The titre of chlorhexidine-treated serum showing a positive reaction was 1:1000 in immunized animals. Control animals showed positive reaction in a dilution of 1:10 but negative in 1:100.

Key-words: Chlorhexidine; immunology; animal studies

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The bis-biguanide chlorhexidine (Hibitane®), is a widely used disinfectant. It has been used successfully in urology, gynecology, ophthalmology and in local treatment of burns and scalds.

Chlorhexidine was first used in dental practice for disinfecting the oral mucosa prior to administration of local anesthetics (Cawson & Curson, 1959). It has also been used for sterilisation of the endodontic field (Birch & Melville, 1961), infected root canals (Atkinson & Hampson, 1964), and for disinfecting the oral mucosa before surgery. Chlorhexidine administered as a dentifrice, mouthrinse or applied topically to the tooth surfaces inhibits the formation of dental plaque in vivo and in vitro (Løe & Rindom Schiøtt, 1970; Gjermo & Rölla, 1971; Turesky, Glickman & Sandberg, 1971).

Information about the penetration of chlorhexidine through the oral mucosa is sparse. It has been postulated that only minor if any part of locally applied solu-

tions of chlorhexidine-salts penetrated the mucosa (Magnusson & Heyden, 1973). Since measurable amounts of chlorhexidine salts have been recovered from the urine, it is evident that penetration must take place in some part of the digestive tract (Winrow, 1973).

At physiological pH chlorhexidine is positively charged and forms precipitates with macromolecules from serum and saliva (Hjeljord, Rølla & Bonesvoll, 1973). It has been shown by Tolo & Rölla (1972) that proteins from serum have altered mobility in disc electrophoresis when serum was treated with chlorhexidine. These proteins could be used to sensitize rabbits against the chlorhexidine-protein complex. The rate of penetration of mucosa by protein complexes has been reported to vary in sensitized and non-sensitized animals (Tolo, 1973).

Rabbits are generally considered as good producers of antibodies in contrast to guinea pigs (Ovary, 1958). The aim of

the present study was to assess the possibilities of sensitization to chlorhexidine in the guinea pig.

MATERIALS AND METHODS

Ten albino guinea pigs weighing about 450 g were used. The day the experiment started 3.5 ml of blood was drawn from each animal by heart puncture. The blood was stored at room temperature for 20 min, then at 6°C for 2 h and centrifuged at 2800 g for 10 min. The supernatant was recentrifuged for 10 min. The final supernatant was stored at 6°C for 24 h.

The next day 0.2 ml serum was mixed with 0.2 ml chlorhexidine solution of 0.2 % concentration for 10 min. The commercial preparation of chlorhexidine gluconate (Imperial Chemical Industries, England) was used, diluting the 20 % solution in distilled water. Distilled water was chosen since this solvent is used in clinical tests for plaque prevention. Six of the animals were given subcutaneous injections with 0.4 ml of the chlorhexidine-serum mixture and equal amounts of Freund's complete adjuvant (Difco, U.S.) in the abdomen. Four guinea pigs served as controls. The six test animals were injected every second day for one week. After three weeks the animals received the same amount as a booster-dose and four days later they were tested for antibodies to the mixture of serum-chlorhexidine. The cutaneous anaphylaxis test was used (Ovary, 1958).

A serial dilution from 10 to 1000 of the injected mixture was prepared for each animal. The fur was removed from the abdomen with electric clippers taking care not to irritate the skin and provoke un-specific reactions. An injection of 1.0 ml Evans blue was slowly given intracardially,

whereafter 0.1 ml of the dilution of chlorhexidine treated serum was injected into the skin. On the other side of the abdomen isotonic saline and autologous serum were tested. The reaction was read after 15 min. A blue spot with a diameter of 5 mm was considered a positive reaction.

RESULTS

All animals survived the experimental procedures. The test animals showed a positive reaction to a dilution of chlorhexidine treated serum/saline 1:1000. Control animals showed positive reactions in a dilution of 1:10 but negative in 1:100. Injections with isotonic saline and autologous serum were negative in all animals.

DISCUSSION

A great variety of substances, including even autologous serum, are able to provoke an increase in the permeability of the small vessels, if the substances are injected into the skin and the concentration is above a certain threshold value (Ovary, 1958).

The cutaneous anaphylaxis test of actively sensitized guinea pigs is considered sensitive to prove the presence of antibody in the serum. It is required that control animals should not show any coloured spots for the injected concentration of antigen solution.

The result from this investigation showed that guinea pigs could be sensitized to chlorhexidine when serum was exposed to chlorhexidine and the mixture was injected, even though these animals are »poor» producers of antibodies (Ovary, 1958).

There are no reports of allergic reactions to chlorhexidine used as a wound dressing or in disinfection of hands or

operation sites (Grant & Findlay, 1957; Lowbury & Bull, 1964). Lindhe *et al.*, (1970) did not find any sign of inflammation in the oral mucosa in hamsters after topical application of chlorhexidine daily during 12 weeks. Neither has any allergic reaction been reported to application of chlorhexidine after gingivectomy (Lindhe *et al.*, 1970; Asboe-Jørgensen *et al.*, 1972). These findings do, however, not outrule the possibility of sensitization to chlorhexidine, since no refined parameters such as cutaneous tests were applied.

Molecules of amphetamine, norephedrine, chlorpheniramine, nicotine (Beckett & Triggs, 1967), lidocaine, prilocaine (Bergmann *et al.*, 1969), and albumin (Tolo, 1971) have been reported to penetrate the oral mucosa. Due to its surface active properties, chlorhexidine is supposed to bind to mucin and hydroxyapatite and form »reservoirs» of chlorhexidine in the oral cavity (Rölla, Löe & Rindom Schiøtt, 1970). However, penetration of small amounts of protein-chlorhexidine complexes may still be possible. Therefore, chlorhexidine may also have a sensitizing potential when applied topically in the oral cavity. Thus further study of a possible penetration of the oral mucosa seems mandatory in the evaluation of chlorhexidine as a disinfectant for prolonged use.

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