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What is This?
In vitro Virucidal Effectiveness of a 0.12%-Chlorhexidine Gluconate Mouthrinse

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The purpose of this work was to assess the in vitro antiviral effectiveness of a mouthrinse (Peridex) containing 0.12% chlorhexidine gluconate (CH) on several viruses that are associated with the oral cavity. These included herpes simplex virus (HSV), cytomegalovirus (CMV), influenza A, parainfluenza, polio, and hepatitis B (HBV).

Materials and methods.

Mouthrinses assayed.—A mouthrinse (Peridex) containing 0.12% chlorhexidine gluconate (CH) and a placebo containing only excipients, no CH, were assayed.

Cells and viruses.—Rabbit kidney (RK) cells were prepared from the kidneys of New Zealand white rabbits. The other tissue cultures, human foreskin fibroblast (HFF), rhabdomyosarcoma (RD), African green monkey kidney (CV-1), and Madin Darby canine kidney (MDCK) are continuous lines. Tissue cultures were maintained in Earle’s Basal Medium (MEM) containing 2-10% heat-inactivated (35°C, 30 min) fetal bovine serum, penicillin (100 units/mL), streptomycin (50 μg/mL), and L-glutamine (2 mmol/L).

Clariﬁed pools of herpes simplex virus type 1 (McIntyre strain) were evaluated on RK cells, cytomegalovirus (strain AD169) on HFF cells, polio type 1 (Chat strain) on RD cells, influenza (strain A/Bethesda 1/85) on MDCK cells, and parainfluenza type 3 (strain HA-1) on CV-1 cells. The titer (in plaque-forming units, PFU/mL) of each virus assayed is listed in the Table.

Viral assays.—Preliminary studies indicated that the mouthrinse containing 0.12% CH was toxic for all the tissue culture cells at a dilution of 1:10, and for the RD cell line at a dilution of 1:100 when incubated for two h. Therefore, a modification of the procedure of Bailey and Longson (1972) was used, in which a mixture of virus + CH mouthrinse or placebo was diluted >1:100 and incubated for one h with tissue-culture cells. The fluid was removed, and the cells were washed once with phosphate-buffered saline (PBS). Under these conditions, the viruses could infect the tissue-culture cells with no evidence of cell toxicity. For the virus assay, 0.1 mL of a virus suspension was mixed with 0.1 mL of mouthrinse, placebo, or PBS at ambient temperature. After 30 s, five min, and 15 min, an aliquot of 0.1 mL was removed and diluted 1:100, 1:1000, and 1:10,000 in PBS in 1.0-mL volumes. Immediately, duplicate 0.2-mL aliquots of each dilution were removed and assayed on the appropriate tissue culture monolayer for one h at 37°C. Each tissue culture was washed with 5 mL of PBS. An agarose overlay containing 0.18% agarose, 2% fetal bovine serum, and penicillin (100 units/mL) plus streptomycin (50 μg/mL) (Gibco, Grand Island, NY) in Minimum Essential Medium (Flow Laboratories, McLean, VA) was applied to each tissue culture, except for influenza, which contained an overlay with 3 μg/mL trypsin and no fetal bovine serum. Plates were incubated for three to five days at 37°C in a 5% CO₂ atmosphere, stained with 1% crystal violet, and enumerated for plaques. Under these conditions, no cytopathic effects were observed for the mouthrinse or the vehicle. The assays were repeated on two separate days.

HBV-DNA polymerase assays.—Since HBV does not grow in tissue culture, inactivation of the virus was tested by the assay of the virus-associated DNA polymerase activity during contact with active and placebo mouthrinses. A Dane particle concentrate, rich in DNA polymerase, was prepared from HBe antigen-positive carrier plasma by centrifugation over a sucrose gradient and was suspended in 1/10 of the original volume in

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Results.

_Virucidal activity of CH._—Virucidal activity of the CH mouthrinse and vehicle are presented in the Table as percentage reductions in virus concentration averaged over the two experiments. The virucidal effect occurred quite rapidly, with a 30-second exposure showing reductions of 59% for parainfluenza and of 99.7%+ for CMV. Generally, virucidal effectiveness increased with time. The best example of this was parainfluenza, which showed a 59% reduction at 30 s, a 91% reduction at five min, and a 99% reduction at 15 min. The percentage of reductions at 15 min ranged from >98% for influenza to >99.9% for HSV. The placebo had virtually no virucidal effectiveness, except against CMV, which showed a 70% reduction at 15 min. Neither the CH-containing mouthrinse nor the placebo was effective against the polio virus.

_DNA polymerase activity assays for HBV._—This indicated that exposure of HBV to the placebo had little effect on DNA polymerase activity. However, exposure to the 0.12%-CH mouthrinse significantly reduced HBV-DNA polymerase activity in 30 s (85% reduction), compared with the placebo. After 15 min of exposure to the CH mouthrinse, HBV-DNA polymerase activity was decreased 99%, compared with the placebo.

**Discussion.**

This work confirmed the antiviral effectiveness of chlorhexidine against HSV (Bailey and Longson, 1972; Rodgers et al., 1989) and extended the range of effectiveness to other enveloped viruses, including influenza, parainfluenza, and CMV. Moreover, we demonstrated that the antiviral effect of the 0.12%-chlorhexidine mouthrinse occurred rapidly, so that by 30 s, the titers of influenza, HSV, and CMV were reduced by at least 97%. This activity was, however, time-dependent. This was best demonstrated with parainfluenza, where inactivation was 59% at 30 s and increased to 99% after 15 min. Thus, not all enveloped viruses exhibited the same susceptibility to the chlorhexidine mouthrinse. In fact, CMV was inactivated 70%
after prolonged exposure to the placebo. These different effects may be explained in part by subtle chemical or physical differences in the membranes of the enveloped viruses.

The chlorhexidine mouthrinse also demonstrated antiviral activity against HBV, which also has a lipoprotein envelope. Exposure of a concentrate rich in Dane particles, the infectious unit of HBV, resulted in reduced activity of DNA polymerase, an enzyme contained within the core of HBV. Only non-enveloped viruses such as polio and adenovirus (Bailey and Longson, 1972) appear resistant to the effects of chlorhexidine. The activity of CH against rotavirus (Rodgers et al., 1985) suggests that an interaction with surface glycoproteins may account for some of the activity of chlorhexidine on this virus and, therefore, may allow chlorhexidine to be effective against rotaviruses. Rotaviruses are non-enveloped viruses that contain surface glycoproteins that are essential for infectivity. Thus, the antiviral effects of chlorhexidine on enveloped viruses are consistent with the antibacterial effects of chlorhexidine on the cytoplasmic membrane of bacteria (Hennessey, 1977).

In addition to the in vitro antibacterial activity of chlorhexidine, recent studies have shown that use of a chlorhexidine mouthrinse will reduce the number of oral bacteria (Briner et al., 1986; Ferretti et al., 1987). This report demonstrates the in vitro activity of a chlorhexidine mouthrinse for several enveloped viruses commonly excreted from the oral cavity, and suggests the need for in vivo evaluation(s) for exploration of the possibility that viruses in the oral cavity might also be reduced in a similar way. This proposition is supported by the recent work of Park and Park (1989), who showed significant, but somewhat lower, antiviral effectiveness of 0.1% CH against HSV-1 in tissue culture, and that topical 0.2% CH reduced development of skin viral lesions and viral titers in vivo in HSV-1-infected mice.

REFERENCES


