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Measurements of chlorhexidine, p-chloroaniline, and p-chloronitrobenzene in saliva after mouth wash before and after operation with 0.2% chlorhexidine digluconate in maxillofacial surgery: a randomised controlled trial

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Abstract

Chlorhexidine gluconate is used to prevent the accumulation of dental plaque and gingivitis, infection of the surgical site, and ventilator-associated pneumonia in maxillofacial surgery, but it is not clear whether the metabolites of chlorhexidine are detectable in the patient's saliva at clinically relevant concentrations. Forty-three patients who had orofacial operations were randomised to use a 0.2% chlorhexidine gluconate (n = 23), or an octenidine-based, chlorhexidine-free (n = 20), mouthwash once preoperatively and three times daily for five postoperative days. After the first, 8.7 (23.3) mg/L chlorhexidine (0.7%-2.5% of the total amount used) was measured in saliva. The concentration increased to 15.2 (6.2) mg/L after the second rinse (first postoperative day), and peaked at 29.4 (11.2) mg/L on the fourth postoperative day. It remained detectable for up to 12 hours after the last one, but was not detectable in serum or urine at any time. The potentially carcinogenic metabolite p-chloroaniline was detectable in saliva at higher concentrations in the chlorhexidine group (0.55 mg/L) than the octenidine group (0.21 mg/L), and p-chloronitrobenzene was detected in both groups in only minimal concentrations (0.001-0.21 mg/L). Chlorhexidine gluconate mouthwashes do increase the concentration of p-chloroaniline, but a single use seems to be safe. Whether prolonged exposure over many years may have carcinogenic potential is still not clear. Based on the hitherto unknown kinetics of p-chloroaniline in saliva, the recent recommendation of the Federal Drug Administration (FDA) in the USA to limit the use of a chlorhexidine gluconate mouthwash to a maximum of six months seems to be justified.

Keywords: Chlorhexidine; p-chloroaniline; antiseptic mouthwash; saliva; absorption

Introduction

Antimicrobial mouthwashes may often be used for long periods of time and, in vitro, there are mixed results for the mutagenicity of chlorhexidine digluconate.[1–3] However, the incidence of micronuclei has been found to increase signif-

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icantly in human lymphocytes after exposure to 0.5 mg/ml (0.05%).[4] In further experimental studies, dermal application of an 0.5% solution 0.2 ml twice daily for 28 days induced a considerable increase in chromosomal aberrations in the bone marrow in mice, and daily oral use of 0.12% solution 3 ml for eight days in rats increased damage to DNA in white cells and kidney cells.[5] Although this suggests that chlorhexidine gluconate has general mutagenic potential, we know of no specific clinical data in humans.

The antiseptics octenidine dihydrochloride and chlorhexidine are structurally identical with the exception of the substituent p-chloroaniline within the chlorhexidine molecule, and we know of no data that have shown that octenidine has any mutagenic potential.[6] It is therefore possible that the experimental mutagenic and precarcinogenic potency of chlorhexidine gluconate or chlorhexidine is a result only of its metabolite p-chloroaniline, which is known to be a carcinogen.[7] It has been detected in stored antiseptics that contain chlorhexidine gluconate, presumably through hydrolysis,[8–12] but we know of no work that suggests that it is present in saliva after the use of a mouthwash that contains chlorhexidine gluconate. Because p-chloroaniline may be metabolised to p-chloronitrobenzene under oxidative conditions, the aim of this study was to measure both substances with an improved analytic method[13] after the use of a chlorhexidine gluconate mouthwash.

Methods

After approval by the Ethics Committee of the University of Greifswald (Trial registry number: III UV 56/04) the study was done as a single-centre, blinded, randomised controlled trail using a chlorhexidine gluconate or an oxtenidine (control) mouth wash solution. After they had given written informed consent, adult patients over 18 years old of both sexes and with no ethnic limitations who were to have oral operations were included. Exclusion criteria were simultaneous participation in another study, pregnancy or lactating mothers, pre-existing or concurrent treatment with chlorhexidine gluconate, drug or alcohol misuse, or sensitivity to one of the test compounds.

The endpoints were defined as the occurrence of unwanted side effects or toxicologically relevant appearance of p-chloroanaline that exceeded the Biological Limit (Biologischer Arbeitsstoff-Toleranz-Wert, BAT) values for p-chloroaniline of > 1 mg/L of unbound substance in urine or > 100 mg/L in serum. Serum creatinine concentrations were measured to calculate the glomerular filtration rate in all patients.

Preoperatively, one of the two randomly-assigned antiseptic mouthwashes (15 ml) was used for a 30-second rinse. During the first five postoperative days, the same solution and procedure was used three times daily at 06:00, 12:00, and 21:00 hours.

Test compounds

The chlorhexidine gluconate mouthwash was 0.2% w/w chlorhexidine gluconate, 0.5% w/w peppermint essence, and 30% w/w sorbitol in purified water. Octenisept® (Schülke & Mayr GmbH, Norderstedt, Germany) contained 0.1% w/w octenidine hydrochloride and 2% w/w 2-phenoxyethanol,[14] and served as the control mouthwash. They were placed into identical glass flasks by the same pharmacy to mask the study solutions.

Preparation of the samples

Samples of saliva were collected in 5 ml specimen tubes (Sarstedt AG & Co, Nümbrecht, Germany) before and after the preoperative oral rinse, and on the five postoperative days in the morning before the rinse, immediately after the rinse, and 30 minutes and 60 minutes thereafter. The final sample of saliva was obtained 12 hours after the last elective oral rinse on the morning of the sixth postoperative day with no further rinse. All patients were asked not to swallow for one minute after the rinse. Blood and urine samples were taken preoperatively and before each rinse on the mornings of the five following postoperative days. Samples taken before the preoperative rinse were used as the baseline measures.

Analysis of the samples

Chlorhexidine was analysed as triazine derivate[13,15] using high performance liquid chromatography (HPLC, Liquid Chromatograph 1090 Series II with diode array detector, Hewlett Packard, USA). Chlorhexidine standard or sample 1 ml was added to 1 N sodium hydroxide 1 ml, followed by extraction with dichloromethane 4 ml. Trifluoroacetic anhydride 0.1 ml was then added to 3 ml of the extract and heated to 75 °C for one minute. The cooled content was then added to methanol/2 % formic acid 100 μl, and 20 μl were injected into the HPLC after incubation for two hours at room temperature.

Both p-chloroaniline and p-chloronitrobenzene were analysed after derivatisation[15] by gas chromatography-electron capture detection (GC-ECD, GC 5890 Series II, Hewlett Packard, USA). Gas chromatography variables were: column DB 624 (J&W Scientific, USA, Folsom), 30 m x 0.32 mm, 1.8 μm surface thickness; carrier gas: nitrogen 5.0; temperature 250 °C; temperature programme 100 °C (1 minute), 20 °C/minute up to 200 °C, 200 °C (14 minute); split off time 0.5 minute; column pressure 45 kPa; flow 1.16 ml/minute; septum wash 3.2 ml/minute; overall flow 51 ml/minute; aux gas 50 ml/minute; and ECD gas 6.3 ml/minute. Retention times were 12.3 minutes for p-chloronitrobenzene and 16.3 minutes for p-chloroaniline.

Statistical analysis

Power analysis was made using G-Power (Heinrich-Heine-University Düsseldorf, Germany). We aimed for a power (1-