Ototoxicity of Different Concentrations Povidone-Iodine Solution Applied to the Middle Ear Cavity of Rats

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Abstract To investigate the ototoxic effects of different concentrations of povidone-iodine solutions applied to the middle ear cavity of rats using distortion product otoacoustic emissions. 24 healthy 3–3.5-month-old adult female Sprague-Dawley rats were randomly divided into three groups. The group A ($n = 8$ ears) received 5 % povidone-iodine solution to the right ear, the group B ($n = 8$ ears) received 7.5 % povidone-iodine solution to the right ear and the group C ($n = 8$ ears) received 10 % povidone-iodine solution to the right ear. All animals received saline solution to the left ear as a control ($n = 24$ ears). The animals were tested before, 1 and 10 days after solutions administration to the middle ear. The resulting distortion product otoacoustic emissions were evaluated at 1.5, 2, 3, 4, 5, 6, 7, 8, 10 and 12 kHz. Statistically significant reductions in DP-gram amplitudes were noted at high frequencies (7, 8, 10, 12 kHz) in the group A at day 1 but this effect return at day 10. In group B and group C statistically significant differences were recorded for low and high frequencies (1.5, 2, 7, 8, 10, 12 kHz) according to the control group at day 1 and 10. 7.5 and 10 % povidone-iodine showed a significant ototoxic effect on day 1 and 10. But this toxic effect could not be elicited in 5 % povidone-iodine group on day 10. The present study revealed that commercially available high concentration povidone-iodine solution may cause significant ototoxic effects when applied topically through a perforated ear drum in rats. Based on results of this experiment, high concentration

povidone-iodine solutions should not be used for preoperative surgical site cleansing for otologic surgery.

Keywords Povidone-iodine · Concentration · Ototoxicity · Rat

Introduction

Wound infection is a possible complication for every surgical penetration [1–3]. To prevent this, the elimination of bacterial contamination of the operative site at the time of surgery remains a key factor [4, 5]. A wide variety of active chemical agents are found in these products, many of which have been used for hundreds of years, including alcohols, phenols, iodine, and chlorine [6]. As in any surgical case several antiseptic agents are utilized in head and neck surgical procedures to obtain a germ-free surgical site before the incision. However, the safety of these antiseptic agents is somewhat questionable. Ototoxicity of surface antiseptics was first suspected three decades ago. The suspicion arose as a result of reported sensorineural hearing losses following myringoplasty procedures when antiseptic chlorhexidine gluconate were used before surgery [7]. During the past 30 years, relatively little study has been directed to the potential ototoxicity of these commonly used agents [8].

Povidone-iodine is a broad-spectrum anti-septic solution, which is reportedly capable of killing bacteria on contact [9, 10]. Povidone-iodine was introduced into Anglo-American countries toward the late 1960s [11].

To our best of knowledge there is not previous report about potential ototoxicity of different concentrations povidone-iodine solutions if it penetrates into the middle ear cavity. In the present study, we investigated the ototoxic effects of different concentrations of povidone-iodine

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solutions applied into the middle ear cavities of rats by means of distortion product otoacoustic emissions (DPOAEs).

Materials and Methods

Animals and Groups

To evaluate the ototoxicity of different concentrations povidone-iodine (5, 7.5, 10 %) solutions (BATTICON[®] solution 5, 7.5, 10 %; Adeka, Samsun, Turkey) and 24 healthy 3–3.5-month-old adult female Sprague-Dawley rats weighting between 215 and 290 g were used. The study approval was obtained from the Institutional Animal Care and Use Committee. Animal care in this study was based on the criteria of the Ethics Review Committee for Animal Experiment and NIH Guidelines for the Care and Use of Laboratory Animals. The inclusion criteria for the rats in this study were normal otoscopic findings and normal replicable DPOAE measurement results before administration of any solutions on day 0. Rats were maintained under consecutive lights on and off periods for 12 h each; simulating the standard day and night rhythm at 24 ± 3 °C. The animals were fed with a standard commercial diet and given water ad libitum before and during the experiment. To study the effect of different concentrations povidone-iodine (5, 7.5, 10 %) solutions, animals were divided into three groups caged individually. Following myringotomy, the group A ($n = 8$ ears) received 5 % povidone-iodine solution to the right ear, the group B ($n = 8$ ears) received 7.5 % povidone-iodine solution to the right ear and the group C ($n = 8$ ears) received 10 % povidone-iodine solution to the right ear. In all animals saline solution was applied to the left ear as control ($n = 24$ ears). The amount of fluid necessary to fill the middle ear cavity was about 0.2 ml.

Anesthesia

The animals were anesthetized by xylazine (Rompun, Bayer Vital, Leverkusen, Germany) 5 mg/kg and ketamine (Ketalar, Eczacıbasi Warner Lambert, Istanbul, Turkey) 50 mg/kg given by intraperitoneal injection during solution injection to the middle ear and DPOAE measurements. The depth of anesthesia was determined with the pedal reflex, and in order to maintain anesthesia, a half dose of this initial cocktail was administered as required.

Distortion Product-Evoked Otoacoustic Emission Testing

All animals in this study had normal ear canals and tympanic membranes, based upon otomicroscopic examination, and

normal replicable DPOAEs before the administration of any solution on day 0. The recordings were performed in a quiet room. The DPOAEs were elicited from the each ear of experimental animals utilizing a standard commercial MI 34 OAE apparatus cochlear emission analyzer (MAICON Ltd., Berlin, Germany). Following anesthesia, the primary tones produced by two separate speakers were introduced into the animal's outer ear canal through an insert earphone probe using a plastic adapter that adapts and seals the probe in the outer ear canal. The stimulus consisted of two pure tones (F1 and F2; F1/F2 ratio = 1.22) at 70 dB SPL. In total, 1,000 acquisitions were analyzed. The resulting otoacoustic emissions were evaluated at 1.5, 2, 3, 4, 5, 6, 7, 8, 10 and 12 kHz.

Statistical Analyses

Datas of DP-gram amplitudes were expressed as mean $\pm$ SD. Statistical analyses were carried out using the SPSS[®] statistical package, version 18.0 (SPSS Inc., Chicago, IL, USA) for Windows[®]. The Kruskal-Wallis test was used for the between group comparison and the Mann-Whitney *U* test was used for sub-analyses. The Wilcoxon test was used in the analyses of intra-group repeated measurements. Results were considered statistically significant at $p < 0.05$.

Results

In total, 24 animals were studied successfully. Tables 1, 2, 3, and 4 show the results of the DP-gram results of the four groups corresponding to days 0 and 10. On day 0, prior to any solution, the initial DPOAEs measurement results gave similar values in the four groups ($p > 0.05$). In group A (5 % povidone-iodine administered group) statistically significant differences were recorded for frequencies of 7, 8, 10, 12 kHz according to the control group at day 1 ($p < 0.05$). In group B (7.5 % povidone-iodine administered group) and group C (10 % povidone-iodine administered group) statistically significant differences were recorded for frequencies of 1.5, 2, 7, 8, 10, 12 kHz according to the control group at day 1 ($p < 0.05$) (Fig. 1). Statistically ototoxic effect was much more significant in group C ($p < 0.05$). In group A, statistically significant differences were not recorded in any frequency according to the control group at day 10 ($p > 0.05$). In group B and group C statistically significant differences were recorded for frequencies of 1.5, 2, 7, 8, 10, 12 kHz according to the control group at day 10 ($p < 0.05$) (Fig. 2).

Discussion

Antiseptic solutions are chemical agents that slow or stop the growth of micro-organisms on external surfaces of the

Table 1 DPOAE amplitudes in control group ($n = 24$ ears)

	1.5 kHz	2 kHz	3 kHz	4 kHz	5 kHz	6 kHz	7 kHz	8 kHz	10 kHz	12 kHz
0 day	13.2 ± 1.13	13.6 ± 1.26	16.2 ± 1.37	16.2 ± 1.37	25.2 ± 2.54	28.2 ± 2.43	30.6 ± 1.91	32.4 ± 2.62	31.5 ± 1.92	33.1 ± 2.11
1 day	13.1 ± 1.44	14.0 ± 1.35	15.8 ± 1.59	17.1 ± 1.69	25.8 ± 3.01	28.8 ± 2.21	31.5 ± 1.78	31.3 ± 2.92	30.9 ± 2.45	31.9 ± 2.48
10 day	13.8 ± 1.59	14.1 ± 1.72	15.9 ± 1.70	17.3 ± 1.31	26.1 ± 2.45	29.1 ± 2.05	31.3 ± 1.74	31.7 ± 2.51	31.4 ± 2.31	32.3 ± 2.15

Table 2 DPOAE amplitudes in 5 % povidone-iodine administered group ($n = 8$ ears)

	1.5 kHz	2 kHz	3 kHz	4 kHz	5 kHz	6 kHz	7 kHz	8 kHz	10 kHz	12 kHz
0 day	12.4 ± 1.42	12.8 ± 1.37	13.4 ± 1.85	16.1 ± 1.75	23.8 ± 2.12	27.2 ± 2.21	29.7 ± 1.87	30.4 ± 2.46	32.3 ± 2.32	34.1 ± 2.42
1 day	13.2 ± 1.55	13.1 ± 1.41	13.5 ± 1.66	16.2 ± 1.37	24.2 ± 2.24	28.0 ± 2.39	25.6 ± 2.13*	27.4 ± 2.14*	28.4 ± 1.89*	30.6 ± 2.53*
10 day	12.7 ± 1.36	13.2 ± 1.57	13.6 ± 1.78	15.9 ± 1.69	23.9 ± 2.43	28.2 ± 2.43	30.5 ± 1.88	31.3 ± 2.18	31.7 ± 1.90	33.8 ± 2.49

* Statistically significant differences were recorded for frequencies of 7, 8, 10, 12 kHz according to the control group at day 1 ($p < 0.05$)

Table 3 DPOAE amplitudes in 7.5 % povidone-iodine administered group ($n = 8$ ears)

	1.5 kHz*	2 kHz*	3 kHz	4 kHz	5 kHz	6 kHz	7 kHz*	8 kHz*	10 kHz*	12 kHz*
0 day	12.9 ± 1.58	13.2 ± 1.45	12.9 ± 1.77	15.7 ± 1.87	21.9 ± 2.72	25.6 ± 2.47	28.7 ± 1.93	30.7 ± 2.17	32.9 ± 1.82	33.6 ± 2.44
1 day	9.9 ± 1.81	10.1 ± 1.81	13.2 ± 1.88	15.5 ± 1.93	22.1 ± 2.41	26.0 ± 2.26	24.1 ± 2.13	25.6 ± 2.29	27.5 ± 1.72	29.2 ± 2.21
10 day	10.2 ± 1.62	10.3 ± 1.71	13.1 ± 1.62	15.7 ± 1.74	23.0 ± 2.32	25.9 ± 1.98	23.5 ± 1.76	25.9 ± 2.29	26.8 ± 2.18	28.8 ± 2.51

* Statistically significant differences were recorded for frequencies of 1.5, 2, 7, 8, 10, 12 kHz according to the control group at day 1 ($p < 0.05$)

Table 4 DPOAE amplitudes in 10 % povidone-iodine administered group ($n = 8$ ears)

	1.5 kHz*	2 kHz*	3 kHz	4 kHz	5 kHz	6 kHz	7 kHz*	8 kHz*	10 kHz*	12 kHz*
0 day	12.1 ± 1.26	12.7 ± 1.52	13.1 ± 1.93	15.9 ± 1.67	20.8 ± 2.41	23.7 ± 2.06	27.2 ± 2.12	29.5 ± 2.27	31.7 ± 2.32	32.1 ± 2.56
1 day	9.5 ± 1.77	9.7 ± 1.79	12.9 ± 2.16	15.7 ± 1.93	21.2 ± 2.32	24.2 ± 2.24	22.6 ± 2.27	24.7 ± 2.38	26.1 ± 2.54	28.6 ± 2.48
10 day	9.8 ± 2.11	9.5 ± 2.02	13.0 ± 2.41	16.1 ± 2.17	22.3 ± 2.54	23.5 ± 2.17	22.4 ± 2.36	25.1 ± 2.43	25.8 ± 2.29	27.9 ± 2.33

* Statistically significant differences were recorded for frequencies of 1.5, 2, 7, 8, 10, 12 kHz according to the control group at day 1 ($p < 0.05$)

body which help to prevent surgical wound infections [2, 10]. A commonly used antimicrobial agent is povidone-iodine, a complex of iodine, the bactericidal component, with polyvinylpyrrolidone (povidone), a synthetic polymer [12]. The two federal agencies; The Food and Drug Administration and The Agency for Health Care Policy and Research have recommended the use of povidone-iodine solutions in wound treatment. The FDA has approved povidone-iodine for use in nonprescription first-aid antiseptic products [12, 13].

Povidone-iodine and their preparation are official in USA and European pharmacopoeia. It is recognized as an effective broad-spectrum biocidal agent with a proven invitro biocidal activity against bacteria, yeast, moulds, viruses, fungi, protozoa, actinomycetes and rickettsia [14]. Povidone-iodine is a loose complex of elemental iodine with a neutral, amphipathic organic compound, poly vinyl pyrrolidone, which serves as a sustained release reservoir of iodine [15]. Povidone-iodine has found broad application in medicine as a surgical scrub; for pre- and post-

operative skin cleansing; for the treatment and prevention of infections in wounds, ulcers, cuts and burns. For these purposes povidone-iodine has been formulated at different concentrations in solution, spray, surgical scrub, ointment, and swab dosage forms [12–15].

During the past 30 years, relatively small numbers of studies have been directed to the potential ototoxicity of these commonly used antimicrobial agents [16–18]. Most of these investigations referred the damage to the cochlear part of the inner ear, assessing it by pathological cochlear histology and/or abnormal responses in different electrophysiological tests. One of these studies evaluated damage by means of histological inspection and did not find pathological changes in guinea pig inner ears that were exposed to iodine in aqueous solution [7]. Morizono and Sikora [17], studied the ototoxicity of povidone-iodine preparations in chinchilla, and found reduction in compound action potential at 120 min after middle ear application. Perez et al. [8], studied povidone-iodine in rats and studied changes in both vestibular evoked potentials and auditory

Fig. 1 The DPOAEs measurement results of rats in three group in which 5, 7.5 and 10 % povidone-iodine solution were applied are shown at day 1

Fig. 2 The DPOAEs measurement results of rats in three group in which 5, 7.5 and 10 % povidone-iodine solution were applied are shown at day 10

brainstem responses. They found no apparent changes in either the vestibular evoked potentials or auditory brainstem responses with applications of 5 consecutive days. Ichibangase et al. [19], evaluated the ototoxicity of povidone-iodine preparation in infant, young and adult guinea pigs. They found mild hearing loss at 24 h and 7 days using 10 % solution, and no hearing loss with 5 % solution at 7 days. They concluded that the age of the animals influenced the outcome of the ototoxicity experiment and povidone-iodine preparations in the infants should be used with caution emphasizing that full strength povidone scrub solution should not be used for otologic surgery. A few reported studies about these products' vestibular ototoxicity and the evaluation of vestibular damage mainly based on description of histological findings, which is not an objective measurement of vestibular end organ function [16, 18].

The potential ototoxicity of iodine disinfectants has also previously been studied. In our study, statistically significant reductions in DP-gram amplitudes were noted at high frequencies (7, 8, 10, 12 kHz) in the group A at day 1 but this drop effect returned to normal at day 10. In group B and group C statistically significant differences were recorded

for low and high frequencies (1.5, 2, 7, 8, 10, 12 kHz) according to the control group at day 1 and 10. Our results showed that 7.5 and 10 % povidone-iodine concentrations showed a significant toxic effect on day 1 and 10. But this ototoxic effect was not observed in 5 % povidone-iodine group on day 10. We assume that different concentrations of povidone-iodine solutions penetrating through the round window membrane may be primary factor that is responsible for the different ototoxic effect patterns in these three groups.

To our knowledge, this is the first study to investigate the ototoxicity of different concentrations of povidone-iodine applied to the middle ear in animals. The present study revealed that that povidone-iodine in high concentrations cause significant ototoxicity in rats as showed by means of DPOAEs. Based on results of this experiment we conclude that, as precaution higher concentrations of povidone-iodine solutions should not be used for surgical site cleansing before otologic surgery.

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