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Does intratympanic xylitol administration have ototoxic effects in a mouse ear model?

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ABSTRACT

Objective: To research the ototoxicity of xylitol after intratympanic injection in mice ear model.
Methods: 24 female mice Balb/c mice (48 ears) included in the study. The mice were divided into 4 groups as 6 mice were found (12 ears) in each group. Solutions of 0.9% NaCl solution (Group A), 155 mg/ml (Group B), 310 mg/ml (Group C) and 620 mg/ml (Group D) xylitol, were applied into the middle ear cavity. Microscopic ear examination and auditory brainstem response test were done for each mouse before application of xylitol and on the 1st, 3rd and 10th day of injection.
Results: There are some statistically significant alterations found in the threshold values at 8000, 12000, 16000, 24000 Hz frequencies when each group were compared in itself on day 0, 1,3 and 10, which were independent from the increasing dosage.
Conclusion: According to our findings intratympanic xylitol injection does not have any ototoxic effect in the inner ear. To evaluate the effects of xylitol more clinical studies are need to carried out.

1. Introduction

Xylitol is a non-carcinogenic 5-carbon sugar alcohol which can be found in many vegetables, fruit and birch trees. It can be used as a low-calorie sweetener in gums and sugars, as a bulking agent in foods and as bacterial flora-regulator in dental products [1,2]. Focusing on childhood upper respiratory tract infections and in middle ear infections, the use of antibiotics has increased in recent years. This has led to the development of resistance to antibiotics whilst at the same time an increase in health spending thus creating an economic burden. In recent years in order to reduce the frequency of recurrence and to prevent the middle ear infections in children the chewing gum containing xylitol has been used as an alternative method. Furthermore syrup or lozenges that contain xylitol have been on trial [3,4]. In 2016 a meta-analysis study concluded that acute otitis media (AOM) occurrence can be reduced with the use of xylitol, however its preventive effect on AOM has not been clearly proved [5]. Although the effect of xylitol in preventing middle ear infections have been studied by many experts, there are few studies to determine the direct effects on the middle and inner ear. In this study the ototoxic effect of different concentrations of xylitol, after direct injection into the middle ear have been investigated with auditory brainstem response (ABR) testing.

2. Materials and methods

2.1. Study design

This study was approved by Near East University Research Animals Local Ethical Board (No: 2017/24). By taking into consideration the previous studies [6–8], twenty four female Balb/c mice were included in the study. Mice were transferred from Near East University Experimental Animals and Research Centre to the Department of Otorhinolaryngology clinics for four hours in appropriate transport boxes. Excluding these hours they were kept in the Research Centre at a temperature of 23 ± 2 °C, % 65–70 moisturized, 12 h daylight and 12 h dark room, they were fed with regular feed and tap water.

With an average weight of 25 g, ninety days, 24 female Balb/c mice were included in this study. The mice were distributed into 4 groups with 6 mice (12 ears) in each group. One mouse which was in the control group was excluded because of a middle ear infection, thus as a

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result a total of 46 ears were featured in the study. 10 ear in Group A were treated with saline solution, 12 ears in Group B were treated with 155 mg/ml xylitol solution, 12 ears in Group C were treated with 310 mg/ml xylitol solution, 12 ears in Group D were treated with 620 mg/ml xylitol solution.

Before intratympanic treatment (day 0) and in the post-treatment days of 1, 3 and 10 microscopic ear examination were done for each mouse before ABR test was carried out. Thereafter ABR tests were performed for each mouse.

2.2. Preparation of xylitol solution

The solubility of xylitol in water is approximately 625 mg/ml at 20 °C and its lethal dose (oral LD_{50}) in oral intake is 12.5 mg/kg [9]. In light of this information solutions were prepared as 155 mg/ml, 310 mg/ml and 620 mg/ml.

2.3. Intratympanic administration

Mice were examined with a microscope at 1.6 magnification (Opmi Pico, Zeiss®, Oberkochen, Germany) under intraperitoneal 10 mg/kg xylazine (DutchFarm®, International®, Nederhorst Den Berg Netherlands) + 100 mg/kg ketamine (Alfasan® Woerden, Netherlands) anesthesia before intratympanic saline or xylitol administration (day 0) to exclude any outer ear canal, tympanic membrane and middle ear pathology. At the same time ABR tests were only performed in laboratory animals with normal otological examination and they were accepted as day 0 values. Subsequently solutions of 0.9% NaCl solution (Group A), 155 mg/ml (Group B), 310 mg/ml (Group C) and 620 mg/ml (Group D) xylitol, were applied into the middle ear cavity through the inferior-posterior part of the pars tensa with 50 µl (Hamilton®, Reno Nevada, USA) 33 gauge needle (TSK® Laboratory®, Tochiki- Ken, Japan) injector under microscopic view. Approximately 5 µl of liquid injection was discontinued after the middle ear cavity and attic area was filled with fluid. After each intratympanic administration the head of the mouse was left to rest on the opposite ear for 5 min (Fig. 1).

2.4. ABR test

While the mice were under anesthesia, the needle electrodes (Model F-E2, Astro-Med Inc., Rhode Island) were placed on the ear with vertex (positive), ipsilateral (negative) and contralateral (earthing) and impedances were kept below 3Ω. During the test, EEG signals were checked for body movements/wakefulness and given an intraperitoneal administration in the maintenance dose of 100 mg/kg ketamine.

ABR measurements were performed using Intelligent Hearing System (IHS, Miami, FL, USA) hardware and Smart EP 5.10 version software system. The high pass filter for recording is set to 30 Hz and the low pass filter is set to 3000 Hz. Smart EP created 8000, 12000, 16000, 24000 and 32000 Hz tone pip stimuli with 1 ms rise/fall and 0 ms plateau using Blackman window. The stimuli are supplied with a high-frequency converter compliant with the IHS. The excitation speed is set to 31.1/sec, the analysis window is set to 10 ms, and the gain is set to 100.000. Starting at 70 dB SPL, reduced by 10 dB and applied to 10 dB SPL, the averaging was fixed as 512 samples in all records. The threshold value is defined as the level of low intensity that is reliably observed in the ABR. The most prominent waves for the mouse are II and III. (source), the lowest intensity level at which these waves are observed is considered as the threshold. Wave peak latencies in the 70 dB SPL are recorded for each frequency. The ABR test was repeated for each ear in each group before intratympanic administration (day 0) and at 1, 3 and 10 days after intratympanic administration.

2.5. Statistical analysis

All data obtained in the study were analyzed with Graph Pad Prism 7.0 software (San Diego, CA, USA). Group-to-group comparisons were made for each stimulus frequency on the 1st, 3rd, and 10th day, both in terms of hearing thresholds and II and III wave latencies. Analyses were carried out by 2-way ANOVA test and subsequently a Tukey Multiple Comparison test was applied to determine the significance of the results obtained. $p < 0.05$ value was accepted as statistically significant.

3. Results

3.1. Microscopic findings

In the microscopic view of the mice ear, perforation on the tympanic membrane, which arose after intratympanic injection, was present on day 1 and 3. But on the 10th day, we observed that all of the perforations were closed and the injection area had healed and lost its translucency.

3.2. ABR threshold values

3.2.1. 8000 Hz (threshold values)

The groups showed no statistically significant difference as compared to each other.

A statistically significant difference was found in ($p = 0.0049$) between the day 0 (31.667 dB ± 1.124) and day 3 (40 dB ± 2.31) in Group B (155 mg/ml xylitol) (Fig. 2).

3.2.2. 12000 Hz

The groups showed no statistically significant difference as compared to each other.

The increase at the threshold values on Day 1 (31.667 dB ± 1.667)

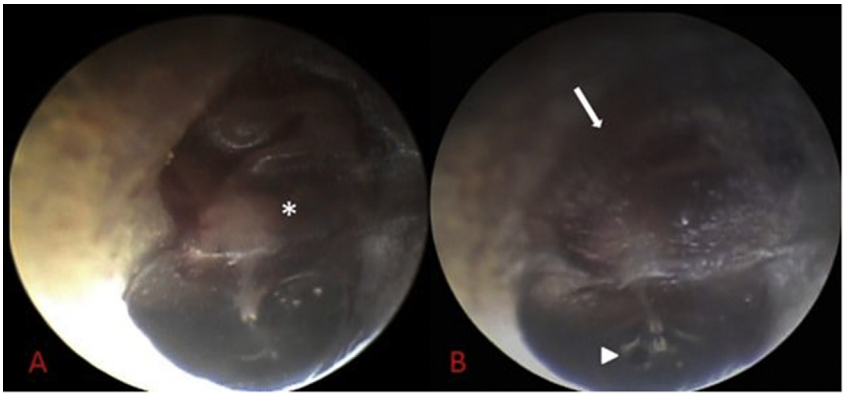

Fig. 1. (a) Mouse right ear before intratympanic administration, the asterisk indicates pars flaccida and attic appearance, **(b)** post-intratympanic appearance white arrowhead shows minimal post-injection perforation, white arrow shows pars flaccida.