Effects of Topical Mitomycin on Inner Ear: A Light and Electron Microscopic Study

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ÇANKAYA, H., EGELI, E., KUNTSAL, L., OZBEK, H. and İÇLİ, M. *Effects of Topical Mitomycin on Inner Ear: A Light and Electron Microscopic Study.* Tohoku J. Exp. Med., 2002, **197** (2), 81-86 — Providing maintenance of myringotomy patency without use of ventilation tubes in the treatment of secretory otitis media has been one of the important study areas. For this reason, laser and Mitomycin C (MMC) are used together in experimental studies. But there has been no ultrastructural studies concerning whether leakage of MMC to middle ear during application of this procedure has an ototoxic effect or not and if so, to what extent. In this study, we searched the ultrastructural changes which occurred in the middle ear by direct applications of MMC to the middle ear for different time durations. The study was carried out over thirty adult guinea pigs without ear diseases. Bilateral myringotomy was performed and MMC was applied only to the right middle ear of each guinea pig. The first group received MMC once for 10 minutes, the second group received it once for 20 minutes, and the third group took it each day for 10 minutes during a one week period. The left ears of the samples were accepted as the control group. On the 8th day, sacrifice was carried out. After electron and light microscopy examination, significant changes in the inner ear were observed in the third group though no significant change was observed for the first and the second groups. As a result it was concluded that the application of MMC to the middle ear once for a short duration causes no toxic effect on the inner ear. ——— mitomycin; secretory otitis media; myringotomy; ototoxicity; inner ear

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Secretory otitis media is a frequently observed disease in childhood. In its treatment, ventilation tubes are used to provide aeration of the middle ear, and this procedure is the most frequent operation carried out during childhood periods (Facione 1991). Since ventilation tubes cause complications such as tympanosclerosis, tympanic membrane atrophy and chronic otorrhea; alternative treatment modalities have been searched. In particular, long term maintenance of the formed perforations without inserting ventilation tubes has become an important purpose for the authors carrying out studies on this subject (Estrem and Batra 1999).

MMC, an antineoplastic drug, has been used topically in ophthalmology for glaucoma, pterygia, conjunctival and corneal lesions, and in otolaryngology for endoscopic dacryocystorhinostomy, laryngeal and tracheal stenosis, glottic and subglottic stenosis, endolymphatic shunt procedure (Agarwal et al. 1997; Gebhart 1998; Yazawa et al. 1999; Selig et al. 2000; Garret et al. 2001; Rahbar et al. 2001). The proposed mechanism of action is through cross-linking of DNA, thus effectively preventing the replication of fibroblasts and epithelial cells. MMC is active against all cells, regardless of cell cycle phase, and has been safely used topically in human beings since 1983 (Estrem and Batra 1999). In recent years, especially the studies of Estrem who carried out his studies using laser and MMC, provide important advances in the role MMC plays in treatment of secretory otitis media (Estrem and Batra 1999; Estrem and Vanleeuwen 2000; Estrem and Baker 2000). Since there were few studies evaluating the effect of MMC on inner ear, the methods, which prevent or restrict the passage of MMC to the middle ear to the utmost, have been used in the experiments (Estrem and Batra 1999).

The purpose of this study is to search ototoxic effects of short-duration or long-duration MMC application among guinea pigs.

MATERIALS AND METHODS

The study was carried out over thirty adult guinea pigs, 300–400 g in weight and without ear diseases. For all experimental methods, permission was obtained from Yüzüncü Yıl University Medical Ethic Board. Under ketamine anesthesia, myringotomy was carried out by using otomicroscope for both ears of the guinea pig. Then, gelfoam soaked with 0.2 mg/ml MMC was applied to the right middle ear of samples via myringotomy passage and MMC in forms of drops was added to it in a way that will not run over the tympanic membrane.

Animals were divided into three groups which of them including right ears of ten animals (Group I, II and III). The left ears of the all thirty animals carrying out only myringotomy and not given any drugs was accepted as a control group (Group IV). Groups were formed as below, Group I received a single application for 10 minutes then gelfoam was taken out, and the middle ear was aspirated. Group II received a single application for 20 minutes then gelfoam was taken out and the middle ear was aspirated. Group III received 10 minute daily applications for seven days. At the end of the eight day of the beginning of the study sacrifice was accomplished with intraperitoneal injection of 100 mg sodium pentobarbital, followed if necessary by a 50 mg intracardiac injection after loss of consciousness in the all groups. Both temporal bones were removed. After bulla was opened, cochlea was removed.

For the electron microscopic examination, it was fixed in glutaraldehyde 2.5% in phosphate buffered saline, pH 7.2.

The specimen was placed in a large volume of fixative for 24–72 hours.

Post fixation was carried out with 1% osmic acid. The pieces dehydrated in alcohol series were blocked with Epon 812, and then thin slices were taken choosing proper blocks. Thin slices were contrasted with lead citrate

and uranyl acetate and examined under Jeol 100C electron microscope.

For the light microscopy examination, cochlea was fixed with 10% buffered formalin. After routinely done follow-up processes, it was embedded in paraffin.

The slices taken were stained with hematoxylin and eosine and also thin slices from the side were removed from the specimens prepared for electron microscopy and stained with toluidine blue and examined under light microscope.

RESULTS

Light microscopy: In the control group, nerve cells found in spiral ganglion were observed normal in appearance. Ganglion cells were observed with big and euchromatic nucleus with evident nucleolus (Fig. 1). Nissl bodies in perikaryons were shown in dark color. Surrounding the nerve cells, satellite cells were found with a big diameter, a big nucleus and apparent nucleolus (Fig. 2). Cytoplasm was rich in granular endoplasmic reticulum and ribosomes. Mitochondria, golgi complexes, and lysosomes are other organelles observed in cytoplasm. In Group I and Group II, no significant effect was observed on spiral ganglion cells, but

in Group III much influence was observed. It was seen that nerve cells of spiral ganglion lost their structural integrity and ganglion cells lost their typical features (Fig. 3).

It was observed that cell bodies were shrunken and their nucleus became small and hyper chromatic. In this group, infiltrations formed by cells such as lymphocytes and erythrocytes were observed over the organ of Corti (Fig. 4). When electron micrographs of the same experimental group were examined, heavy degeneration signs were detected in most of the spiral ganglion cells (Figs. 5a and 5b). In some cells nuclei were small and pyknotic. In a lot of cells, enlargement of cisterna of endoplasmic reticulum together with swelling in mitochondria and deformities in their cristae were observed.

DISCUSSION

When topically applied to the middle ear, medication can contact the round window membrane and thereby gain access to the membranous labyrinth. Most topical otic preparations contain a variety of ingredients that have well documented ototoxicity in various animal models (Morizono 1990). Mitomycin C is an antineoplastic chemotherapeutic agent. In the

Fig. 1. Light micrograph of control group shows nerve cells in spiral ganglion with normal appearance ($\times 500$).

Fig. 2. Electron micrograph taken from control group (N, nucleus; No, nucleolus; GER, granular endoplasmic reticulum; r, ribosome; Mi, mitochondrion; Sc, satellit cell) ($\times 5200$).

Fig. 3. Light micrograph of group III shows damaged on nerve cells of spiral ganglion ($\times 500$).

literature there is no chemotherapeutic agent searched for its ototoxicity, when applied topically to ear. Rather, in literature, ototoxic effects of chemotherapeutics can occur when applied systemically, were searched. The most commonly used major ototoxic chemotherapeutic agent is cisplatin, the first major platinum coordination complex. Cisplatin ototoxicity may be a two fold phenomenon in the inner ear, involving injury to both the stria vascularis and the organ of Corti (Schweitzer 1993).

Kohn et al. (1988) demonstrated with light and transmission electron microscopy that cisplatin produced toxicity in both organ of Corti and the stria vascularis in guinea pigs treated with cisplatin.

Damage to the stria vascularis consisted of non uniform injury to one third of the stria vascularis affecting most commonly the marginal cells, presence of lipid bodies, fragmentation of nuclei, dilatation of endoplasmic reticulum, destructive process in the mitochondria, and vacuolization of marginal cells.

Fig. 4. Light micrograph of group III shows infiltration on organ of Corti ($\times 310$).

Fig. 5. Electron micrograph of group III shows (a) Degeneration findings on spiral ganglion cells, n, nucleus; ger, granular endoplasmic reticulum ($\times 5200$). (b) Widening on granular endoplasmic reticulum, swelling in mitochondria and impairment on its cristae. Ger, granular endoplasmic reticulum; mi, mitochondrion ($\times 11\,000$).

Results of Group III in our study resemble findings of Kohn's study.

Ultrastructural study about ototoxicity of

MMC does not exist in the literature. Although Yazawa et al. (1999) reported that they carried out animal experiments for ototox-

icity before application of MMC for endolymphatic sac operation, there is no detailed study about this subject. Additionally Jassir et al. (2001) reached the conclusion that MMC application together with laser was safe in their distortion product otoacoustic emission measurements. But this is not a study searching ototoxicity. In our study, we searched ototoxicity of MMC that is directly applied to the middle ear. Although in experimental studies high doses such as 2 mg/ml have also been used, we preferred to apply most frequently used MMC concentrations of otolaryngology and ophthalmology procedures (Agarwal et al. 1997; Rubinfeld and Stein 1997).

In our study, single dose 10 or 20 minute duration applied guinea pigs had no significant distortion either at light or transmission electron microscopy findings when compared to the control group. But significant distortions either at light or transmission electron microscopy called attention to Group III, in which repeated MMC applications were carried out. This result practically shows that there will be no important ototoxic effect on occasion of MMC leakage to middle ear, applied after myringotomy. Furthermore, in secretory otitis media, we can theoretically assume that Mitomycin C can be used to prevent adhesion of graft to middle ear used in diseases such as adhesive otitis media. But this hypothesis is open for further research.

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