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The effect of various topical antibiotic and anti-bacterial agents on the middle and inner ear of the guinea-pig

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The topical application of antibiotics and anti-inflammatory agents is used to reduce inflammation of the middle ear mucosa and irradicate infection. Many of the antibiotic and anti-inflammatory preparations so used contain compounds known to be or are potentially ototoxic. Eighteen antibiotic, antibacterial, antifungal and anti-inflammatory compounds and four commonly used solvents were screened for the absence of ototoxicity and inflammation to the middle ear mucosa. All compounds were injected intra-tympanically and sensory hair cell loss recorded graphically. Inflammation of the middle ear mucosa was assessed macroscopically. Of the 18 compounds only three, penicillin, carbenicillin and nystatin were free of hair cell toxicity and inflammatory effects on the middle ear mucosa. Only one of the commonly used solvents was free of side effects.

Otitis media, especially in the chronic stages, can lead to sensory hair cell loss and deafness (Paparella & Brady, 1970; English, Northern & Fria, 1973), and many antibiotics used for its treatment can also cause sensorineural hearing loss (Ballantyne, 1970; Ajodhia & Dix, 1976). Any preparations used topically in the ear should contain compounds free from ototoxicity and inflammatory side effects. With this in mind, we have examined 18 compounds and four solvents used in otic products for their effects on the middle and inner ear. Initially only damage to the cochlea was used as the main criteria but it became apparent that examination of the middle ear mucosa was also necessary. Some of the compounds were known to be highly ototoxic, i.e. gentamicin, and were used to test the validity of the experimental conditions and technique. All compounds were administered by intra-tympanic injection using a similar method to that described by Kohenen & Tarkkanen (1969). Cochlea damage was assessed using the surface technique of Engström, Ades & Anderson (1966) and sensory hair cell loss recorded using cochleograms. The middle ear mucosa was examined macroscopically for inflammatory reactions.

MATERIALS AND METHODS

Materials

Antibiotics: gentamicin, chloramphenicol, bacitracin, tetracycline hydrochloride, oxytetracycline hydrochloride, chlortetracycline hydrochloride, penicillin G, carbenicillin, colimycin methane sulphate (Polymixin E).

Antibacterial bacteriostatic: chlorhexidine acetate, benzalkonium chloride, iodochlorhydroxyquinolone, gramicidin.

Antifungal: nystatin, amphotericin B, griseofulvin.

Anti-inflammatory: dexamethasone base, dexamethasone sodium phosphate.

Solvents: polyethylene glycol 400, propylene glycol, dimethyl formamide and isopropyl myristate.

Carbenicillin was administered as a 5·0% solution, chlortetracycline at 3·0%, all other compounds were given as a 1·0% solution or suspension either in water or solvent.

Animals. Groups of 6 or 8 male albino guinea-pigs, Dunkin Hartley strain from Charles River, Kent, were used; each animal had a starting body weight of 350–400 g.

Methods

Intra-tympanic administration. The method was similar to that described by Kohenhan & Tarkkanen (1969). Each guinea-pig was lightly anaesthetized with ether and laid on its side. A fine nylon tube, pre-sterilized in 70% isopropyl alcohol, attached to a No. 19 syringe needle was pushed through the tympanic membrane. Great care was taken to avoid movement or damage to the ossicles or rupture of the malleus artery. Any guinea-pig that showed signs of bleeding at this stage was discarded. A volume of 0·1 ml of the sterile test solution was then injected slowly into the middle

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ear. In each animal the left ear received the test solution and the right ear received 0·1 ml of the sterile vehicle and served as the control. Each animal received one dose once daily for five days. The equipment was, where possible, kept sterile throughout the dosing procedure. Some difficulties in dosing were found where the irritation produced an increase in mucus which filled the middle ear cavity.

Hair cell histology. Ten days after receiving the last dose of the test compound, the animals were killed by cervical disslocation and the bullae quickly removed, opened and the cochlea exposed. The round window and the apex were opened and a solution of veronal buffered 1·3% osmium tetroxide allowed to flow through the cochlea. Each bulla was then fixed for 1½ h at 4° in buffered 1·3% osmium tetroxide. After they had been washed in running tap water for 20 min and partially dehydrated in alcohol, the cochleas were dissected in 30% ethanol and between ¼–½ of a turn was removed from each of the four coils of the organ of Corti. Each sample was examined using phase contrast microscopy and cellular damage assessed and recorded using cochleograms according to the method of Engström & others, 1966). Each cochleogram consisted of four rows of fifty '○', each '○' representing one sensory hair cell. Each section of the organ of Corti was examined and the area showing the greatest damage was recorded.

Inflammatory effects. After the observance of many instances of inflammation in the test but not control ears, each drug was checked for any adverse effects on the middle ear mucosa. Any sign of redness, oedema, swelling, rupture of blood vessels, cholestaetoma, puss or calcinosis similar to that described by Hybasek (1971a,b) or Friedmann (1955a,b, 1957), was noted.

RESULTS

Antibiotic, antibacterial. The results are summarized in Table 1. Toxicity has been classified as mild, moderate or severe. Examples of these are given in Fig. 1. Only penicillin and carbenicillin were free from any signs of toxicity to the organ of Corti and irritancy to the middle ear mucosa. The toxicity shown by the known ototoxic antibiotics was of the same order as that reported by others; gentamicin, colimycin, tetracyclines (Kupper, Stupp & others, 1970; Wersäll, Lundquist & Bjorkroth, 1971), chloramphenicol (D'Anglo, Patterson & Morrow, 1967, Proud, Mittelman & Seiden, 1968). The toxicity produced by chlortetracycline and oxytetracycline was not due to the low pH of the solution. Physiological saline at pH 2·7 produced no loss of sensory hair cells. This is in agreement with the results published by Orsulakova & Stupp (1975). Iodochlorhydroxyquinolone, which was soluble in propylene glycol, caused moderate loss of sensory hair cells, usually of the basal turn, as did gramicidin and benzalkonium chloride. Chlorhexidine acetate produced hair cell loss when administered in distilled water. Bicknell (1971) reported extensive hearing loss in man after preoperative sterilization with chlorhexidine in 70% alcohol before myringoplasty.

Bacitracin showed no significant sensory hair cell loss but like nearly all of the compounds tested showed gross inflammatory effects. The irritancy and inflammation shown by most compounds was unexpected. Gentamicin produced inflammation and thick mucoid secretion in every animal treated. Iodochlorhydroxyquinolone produced the worst effects on the middle ear mucosa which was always inflamed and new bone growth was seen; the effects were similar to those described by Friedmann (1957) after intra-aural infection, and those described by Hybasek (1971a,b) after administration of various agents to induce calcinosis within the middle ear. The tetracycline group, chloramphenicol, gramicidin and benzalkonium chloride all induced mucoid secretion and new bone growth.

Table 1. *The effect of antibiotic and antibacterial compounds on the organ of Corti and the Middle ear mucosa.*

Compound	Degree of hair cell loss	Inflammation Present + Absent ○
Gentamicin sulphate	+ + +	+
Tetracycline HCl	+ +	+ +
Oxytetracycline HCl	+ +	+ +
Chlortetracycline HCl	+ +	+ +
Chloramphenicol	+	+ + +
Colimycin	+ + +	○
Bacitracin	○	+ +
Benzalkonium chloride	+ + +	+ + +
Iodochlorhydroxyquinolone	+ +	+ + +
Chlorhexidine acetate	+ + +	+ +
Penicillin G	○	○
Carbenicillin	○	○
Gramicidin	+	+ + +

Hair cell loss: minor +, moderate + +, major + + +.
Inflammation: none ○, slight mucoid secretion or redness +, middle ear filled with mucus + +, middle ear full of pus, mucus and new bone growth + + +.

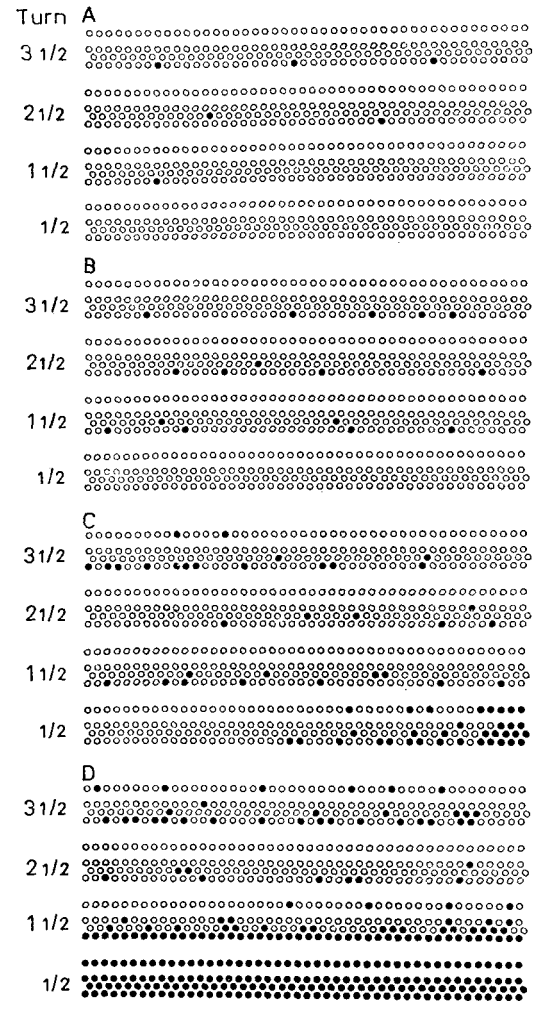

FIG. 1. A: Control, B: Minor damage, C: Moderate damage, D: Major damage. A shows the maximum amount of damage present, B, C, D show the minimum amount of damage found. Open circles represent apparently normal cells. Closed circles represent cells which are unmistakenly damaged.

Colimycin although extremely toxic caused no irritancy to the middle ear.

Anti-fungal agents. Nystatin and amphotericin B were free of toxic effects to the organ of Corti. Griseofulvin caused sensory hair cell loss. Griseofulvin and amphotericin B also caused irritancy to the middle ear mucosa (Table 2).

Anti-inflammatory agents. Dexamethasone base given as a suspension in water and dexamethasone sodium phosphate, soluble in water, were both found to be free of toxic effects and irritancy to the middle ear mucosa.

Table 2. *The effect of anti-fungal compounds on the organ of Corti and the middle ear mucosa.*

Compound	Degree of hair cell loss	Inflammation Present + Absent ○
Nystatin	○	○
Amphotericin B	○	+ + +
Griseofulvin	+ +	+ + +

Solvents. Polyethylene glycol 400, propylene glycol and a PEG 400–water mixture 33–67% all caused sensory hair cell loss and all induced inflammation of the middle ear mucosa (Table 3). This is in agreement with Morizono & Johnstone (1975) who reported the ototoxicity of chloramphenicol ear drops solubilized in propylene glycol. Dimethyl formamide was extremely toxic, being stopped after two doses of 0·1 ml because the animals developed torticollis indicating vestibular damage. Isopropyl myristate produced no sensory hair cell loss and it was also non-irritant to the mucosa.

Table 3. *The effect of various solvents on the organ of Corti and the middle ear mucosa.*

Compound	Degree of hair cell loss	Inflammation Present + Absent ○
Distilled water	○	○
0·9% Sodium chloride	○	○
Polyethylene glycol 400	+ +	+ + +
Propylene glycol	+ +	+ + +
Dimethyl formamide	+ + +	N.D.
PEG 400–water 33%–67%	+	+ + +
Isopropyl myristate	○	○

DISCUSSION

The results show that different types of antibiotic, antifungal and antibacterial compounds can cause sensory hair cell loss when instilled into the middle ear. Some of the compounds produced bad inflammatory reactions to the middle ear mucosa. This finding was unexpected because it has not been previously reported.

No specific degree of toxicity or inflammatory response was investigated because non-toxic or non-irritant compounds were required. This made it difficult, in some cases, to determine whether the damage to the organ of Corti was due to the

inflammatory reaction, the compound or to ototoxic or inflammatory solvents.

Of the compounds tested only nystatin and dexamethasone, base or salt, could be used in an otic product. The penicillins although free from toxicity and inflammation can cause hypersensitivity reactions (De Weck, 1972) and are therefore not suitable.

The middle ear mucosa is of such sensitivity that hyperplasia is liable to occur after an irritant substance or infection (Friedmann, 1955a,b; Sadé & Weinberg, 1969). We therefore suggest that any substance for topical use in the ear should be screened not only for ototoxicity but also for irritant properties which might lead to changes to the middle ear mucosa. The use of viscous ear drops containing polyethylene glycol and propylene glycol may cause failure of the cilia within the middle ear and this in turn would lead to an increase in mucus and other substances that could not be removed via the eustachian tube.

From the findings it is suggested that great care should be exercised in the use of compounds within the middle ear. They should preferably be used only where there is no possibility of them passing through the tympanic membrane and entering the middle ear chamber where they can perfuse the round window into the cochlea. It is possible that in the presence of active discharge the tissues are less at risk from ototoxic action than when the ear is dry.

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