Lack of chondrotoxicity of ofloxacin otic solution on the auditory ossicle cartilages of juvenile guinea pigs

Michiyuki Kato*, Kazumi Akahane and Kohji Shimoda

Drug Safety Research Laboratory, Daiichi Pharmaceutical Co., Ltd, 1-16-13 Kitakasai, Edogawa, Tokyo 134, Japan

The effect of 0.3% otic solution of ofloxacin on the cartilages constituting the epiphyses of the auditory ossicles and the wall of the auditory tube was histologically examined after 30-day repeated intratympanic administration to juvenile male guinea pigs aged 4 weeks. Ofloxacin showed no chondrotoxicity for the cartilages and, in some but not all animals, reduced haemorrhage and neutrophil infiltration in the tympanic cavity, compared with that of the control. Further, the articular cartilage of the humeral trochlea and femoral condyle also showed no change.

Introduction

Ototopical application of antibacterial agents is often used for the treatment of otitis media because of its ease of administration, high efficacy and safety in comparison with systemic administration. Ofloxacin has been developed as an otic solution and is widely used in Japan. The solution has been shown to be transferred to the mucosa of the tympanic cavity and to be found in otorrhoeic fluid at high concentrations and to have high efficacy for otitis media as well as otitis externa and myringitis.1,2 It has also been confirmed that otic ofloxacin has no deleterious effect on hearing and is transferred to the blood of patients only at very low levels, suggesting a low possibility of inducing systemic toxicity.3

However, quinolones, including ofloxacin, are well-known to induce cavitation and erosion in the articular cartilage of juvenile animals after systemic administration, and their use in children has generally been limited. The three auditory ossicles are linked by joints including cartilage in the tympanic cavity, and another cartilage is located close to the auditory tube; although ofloxacin otic solution has already been used in children, the toxicity of ofloxacin on these cartilages has not been examined in animals. We therefore conducted the present study to examine the toxicity of ofloxacin on the cartilages of juvenile guinea pigs which have been used extensively to evaluate ototoxicity.

Materials and methods

Ofloxacin synthesized at Daiichi Pharmaceutical Co., Ltd and commercial 5-ml vials of an otic solution of ofloxacin were used in the present study. The otic solution contained 0.3% ofloxacin and 0.0025% benzalkonium chloride.

Twenty-five male Hartley guinea pigs were purchased from Charles River, Yokohama, Japan, housed in wire-mesh cages in an air-conditioned room (temperature 23 ± 2°C, humidity 55 ± 15%, light cycle 12 h/day) and aclimatized to the environment. The animals were allowed free access to commercial laboratory chow (RC-4, Oriental, Japan) and tap water during the course of the study.

To examine the susceptibility to chondrotoxicity, 15 animals aged 3 weeks were given oral ofloxacin at 100, 300 or 900 mg/kg for 7 days, suspended in 0.5% sodium carboxymethylcellulose.

The remaining animals were used at 4 weeks of age for examination of the middle ear. Ofloxacin otic solution was administered into the left tympanic cavity at a fixed volume of 0.1 mL for 30 days. The tip of an indwelling feeding tube for infants (3Fr, outer diameter 1.0 mm; Atom, Tokyo, Japan) was cut to an appropriate length, and a disposable 27 gauge needle was introduced into the tube up to immediately behind the tip hole. The needle was connected to a disposable 1 mL syringe. The apparatus was sterilized with ethylene oxide gas and the test
solution was sterilized by filtration (pore size 0.22 μm) before use. The tube was inserted slowly and carefully into the left tympanic cavity of the guinea pig under light ether anaesthesia to perforate the tympanic membrane, and the test solution was then slowly administered. Control animals received saline.

On the day after last administration, the animals were killed by bleeding out under ether anaesthesia. The humeral trochlea, femoral condyle and osseous tissue including the tympanic cavity with auditory tube were removed, fixed in 10% buffered formalin, decalcified with formic acid, embedded in paraffin wax, sectioned, stained with haematoxylin and eosin and examined histologically. The osseous tissue was sectioned sequentially so that all ossicle joints and auditory tube were completely cut.

Three animals died after the first oral administration of ofloxacin 900 mg/kg on day 1; the residual two were killed on day 2 for examination. One control animal assigned to middle ear examination died from overdose of anaesthesia on day 12.

Results

Oral administration of ofloxacin at 300 and 900 mg/kg, but not at 100 mg/kg, induced cavitation or erosion in the articular cartilage of the humeral trochlea in two of five and two of two guinea pigs, respectively, but intratympanic administration of ofloxacin otic solution did not.

No change was seen in the cartilage of the auditory ossicles (malleus manubrium, incudomalleal fusion, incudostapedial joint and stapediovestibular joint) or the cartilage supporting the auditory tube in the ofloxacin and control groups. The following changes were commonly observed in both groups: haemorrhage and neutrophil infiltration with debris in the tympanic cavity; haemorrhage, inflammation, thickening and necrosis of frontal wall mucosa of the tympanic cavity; proliferation and protrusion of connective tissue from the surface of the cochlea and tympanic cavity wall, including inflammation, proliferating squamous cells, the auditory ossicles (particularly the malleus), ruptured tympanic membrane and debris; and periosteal inflammation and thickening of the auditory ossicles in a few cases. Among these, moderate or severe haemorrhage and neutrophil infiltration were seen in the tympanic cavity of the control group, and one animal showed focal thinning and perforation of the cochlear wall, which was accompanied by osteoclasts in the thickened peristeum. The degree of haemorrhage and neutrophil infiltration was reduced in three of five animals of the ofloxacin group, compared with those in the control (Table).

Discussion

Tissues of the tympanic cavity showed inflammatory and proliferative changes, which are thought to have been caused by infection following the tympanic membrane perforation made at administration. The degree of haemorrhage and neutrophil infiltration with debris in the tympanic cavity was lower in the ofloxacin group than in the control group receiving saline; this is thought to be due to the antibacterial activity of ofloxacin. In a previous study, mucosal inflammation was reported to be induced in control guinea pigs receiving vehicle alone by intratympanic administration for 7 days and to be decreased in severity in those receiving ofloxacin.3 Ofloxacin has been shown by autoradiography to be transferred into the perilymph of the cochlea after intratympanic administration to guinea pigs.4 However, no injury of the hair cells in the organ of Corti was shown by scanning electron microscopy after intratympanic administration of 0.3% or 0.5% ofloxacin solution to guinea pigs for 7 or 10 days.3,5 Moreover, these authors also showed that the electrocochleogram and auditory brainstem response were not affected by ofloxacin. Ciprofloxacin was also reported to have no effect on the hearing of guinea pigs after 5-day ototopical application.6 In contrast, gentamicin5 and neomycin,7 administered intratympanically have been shown to induce morphological changes in the hair cells of the organ of Corti in the cochlea, penetrating through the round window membrane.

In conclusion, 0.3% ofloxacin otic solution was shown to be safe in terms of chondrotoxicity on the cartilages of the middle ear and legs of juvenile guinea pigs.

Table. Histological findings in the middle ear of juvenile male guinea pigs (numbered 1 to 10) receiving intratympanic ofloxacin for 30 days

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>0.3% ofloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Tympanic cavity: haemorrhage and neutrophil infiltration with debris</td>
<td>3b</td>
<td>2b</td>
</tr>
<tr>
<td>Osicle cartilage</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: a. One animal died from overdose of anaesthesia. -, no change; 1, mild; 2, moderate; 3, severe.

b. Many neutrophils were included.
**Experimental ofloxacin chondrotoxicity**

**Acknowledgement**

We are grateful to Daiichi Pharmaceutical Co., Ltd for financial support.

**References**


Received 14 February 1996; returned 29 March 1996; revised 15 April 1996; accepted 19 August 1996