Description

This invention relates to the use of ofloxacin or a salt thereof for preparing a topical preparation in the form of a solution for treating otopathy, the use of S-(-)-ofloxacin being excluded.

Conventionally employed topical preparations for treating otopathy include solutions containing antibiotics, such as fradiomycin, kanamycin, chloramphenicol, and cefmenoxime.

However, it has been pointed out that these preparations have ototoxicity as side effects or therapeutic effects thereof tend to be decreased due to emergence of resistant microorganisms.

EP-A-274 714 which is a document in the sense of Art.54 (3) EPC discloses the use of ofloxacin for treating and preventing an infection in the auricular cavity in the form of a hydrogel or cream formulation.

EP-A-275 515 which is also a document in the sense of Art. 54(3) EPC discloses aqueous pharmaceutical compositions containing S-(-)ofloxacin.

H.N.O.,34, 1986, pages 511 - 514, refers to the use of the gyrase inhibitor ciprofloxacin in the local treatment of chronic bacterial infections in radical middle-ear cavities. Ciprofloxacin was applied in the form of a solution and it was found that ciprofloxacin is a suitable agent for locally treating otopathy.

Lab. med., 10, 1986, pages 82 - 83, discloses the oral use of ofloxacin and ciprofloxacin in the treatment of otopathy.

Infection, 14 (suppl. 4), 1986, pages S324 - S326, refers to the oral use of ofloxacin in the treatment of otopathy. On the basis of the preliminary results reported, ofloxacin was found to be a highly efficient oral substance.

The present invention relates to the use of ofloxacin or a salt thereof for preparing a topical preparation in the form of a solution for treating otopathy, the use of S-(-)-ofloxacin being excluded.

Ofloxacin is 9-fluoro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-2,3-dihydro-7H-pyrido[1,2,3-de][1,4]-benzoxazine-6-carboxylic acid. S-(-)-ofloxacin is one of the optical isomers thereof.

The salts of ofloxacin include acid addition salts formed with organic or inorganic acids, e.g., hydrochloric acid, and salts formed from the carboxyl group and an alkali metal, e.g., sodium and potassium.

The otopathy on which the preparation of the present invention is effective includes inflammatory otopathy, such as otitis media and otitis externa, and particularly purulent otitis media, circumscript external otitis and diffuse external otitis.

Dose forms of the topical preparation of the present invention include sprays, otic solutions, e.g., intratympanic injections and ear drops.

The solutions can be prepared by dissolving ofloxacin in water, physiological saline, or an appropriate buffer, adding, if desired, antiseptics, e.g., methyl p-hydroxybenzoate and benzalkonium chloride, to the ofloxacin aqueous solution, and adjusting the solution to a pH in a neutral region. The concentration of ofloxacin in the aqueous solution usually ranges from 0.05 to 2% w/v, and preferably from 0.1 to 1% w/v.

In administration of the ofloxacin solution, several 0.5 ml-doses per day are applied to the external auditory canal by spreading, spraying or instillation, or intratympanically injected through a puncture of the tympanic membrane.

Ofloxacin is of high safety. Acute toxicity (LD_{50}) of ofloxacin was found to be 5450 mg/kg (p.o.) in mice, 200 mg/kg or more (p.o.) in dogs, and from 500 to 1,000 mg/kg (p.o.) in monkeys.

The preparation according to the present invention exhibits marked improvements over the conventional drugs in terms of not only ototoxicity but also tissue distribution and excellent therapeutic effects on otopathy, particularly otitis media and otitis externa. Accordingly, the preparation of the present invention is useful as a topical preparation for treating otopathy.

The present invention is now illustrated in greater detail with reference to the following Examples and Test Examples.

EXAMPLE 1

Ofloxacin was dissolved in distilled water for injection, and the solution was adjusted to a pH of from 6.0 to 7.0, followed by bactericidal filtration to prepare a 0.5% ofloxacin aqueous solution.

EXAMPLE 2

Ofloxacin was dissolved in physiological saline, and the solution was treated in the same manner as in Example 1 to prepare an ofloxacin solution.

EXAMPLE 3

Ofloxacin was dissolved in physiological saline, and the solution was treated in the same manner as in Example 1 to prepare a 0.3% ofloxacin aqueous solution.

TEST EXAMPLE 1

1) Test Method:

Fifteen white guinea pigs were divided into the following two groups: 10 in an ofloxacin group topically administered with a 0.3% ofloxacin solution (hereinafter referred to as "0.3% OFLX group") and 5 in a control group administered with physiological saline.

Before testing, the auditory brain-stem response (hereinafter referred to as "ABR") of each animal was determined to obtain a response threshold. A Logon sound wave having a frequency of 10,000 was used as a stimulating sound.

The left tympanic membrane was punctured under general anesthesia, and 0.2 mℓ of the ofloxacin solution of Example 3 or 0.2 mℓ of the control solution (physiological saline) was injected into the tympanic cavity once a day for consecutive 7 days. ABR was measured after 10 days from the final day of injection.

2) Result:

The change between the ABR threshold before the administration and that after the administration was shown in Table 1 below.

TABLE 1

Change of ABR Threshold		
	Threshold (dB, Mean ±S.E.)	
	Before Admin.	After Admin.
0.3% OFLX Group	30 ± 1.3	34.5 ± 1.4
Control Group	30 ± 1.6	35 ± 1.6

As is apparent from Table 1, the threshold changes in both of the 0.3% OFLX group and the control group were slight (4.5 dB and 5.0 dB, respectively), indicating that the reduction of acoustic acuity due to the administration of ofloxacin was negligible.

TEST EXAMPLE 2

1) Test Animal and Administration Route:

Twenty-nine guinea pigs showing normal Preyer's reflex (body weight: 250 to 350 g) were divided into the following three groups; 12 in a group receiving topical administration of 0.5% ofloxacin solution (hereinafter referred to as "0.5% OFLX group"); 13 in a group topically administered with gentamicin (hereinafter referred to as "GM group"); and 4 in a control group administered with physiological saline.

After ketalar and a muscle relaxant (xylazine) were intramuscularly administered, an incision of 2 mm in diameter was made at the middle ear from the posterior portion of the ear of the guinea pig, and a tube of the same diameter was inserted into the tympanic cavity through the opening and sealed and fixed with an adhesive. The incision wound was temporarily sutured and closed. Immediately after the operation, ABR was recorded. From the same day, 0.2 mℓ of a 4% gentamicin solution, 0.2 mℓ of the ofloxacin solution of Example 2, or 0.2 mℓ of the control solution (physiological saline) was injected through the tube into the tympanic cavity. The injection was made once a day for consecutive 10 days. On the 10th day, ABR was again recorded, and the animals were killed.

2) Method of Observation:

i) Measurement of ABR:

An ear drum lead was used for the measurement of ABR. The ground electrode was placed on the foreleg of the same side as the injected ear of the guimia pigs. A clicking sound was given to the external auditory canal from a tube via a crystal receiver. The auditory response was given as a reaction wave, and an average of 200 measurements obtained by a computer was recorded by an XY recorder. The ABR threshold in the individual animal was obtained from the resulting electrocochleogram.

ii) Morphological Observation

a. Microscopic Observation:

A sample tissue (middle ear mucosa) was fixed in formalin using the auditory bulla in a conventional method. After decalcification, the sample was embedded in paraffine and sliced. The paraffin section was stained with hematoxylin and eosin and microscopically observed.

b. Scanning Electronmicroscopic (SEM) Observation:

A sample tissue (cochlea) was thoroughly washed in a jet stream of physiological saline and fixed in a 2.5% glutaraldehyde solution for 12 hours using a conventional method. Then, the tissue was again washed with a phosphate buffer solution (pH 7.4) by shaking, fixed in a 1% osmic acid solution for 1 hour, and electrically stained with osmium tannate. The tissue was dehyrated with an alcohol using a conventional method and dried at a critical point, followed by sputtering with platinum ions. The sample thus prepared was observed under a Hitachi S-570 Model scanning electronmicroscope.

iii) Determination of Distribution of Ofloxacin

Distribution of the drug to the serum, brain tissue, middle ear mucosa, and cochlear perilymph was examined in the control group and the 0.5% OFLX group. A blood sample was collected from the carotid artery, and the brain tissue was excised immediately after decapitation. The middle ear mucosa was stripped off after thorough washing of the middle ear cavity with physiological saline. Thereafter, the cochlear perilymph was collected by a glass capillary.

The concentration of the drug in the tissue was biologically assayed according to a paper disc method using B. subtilis ATCC 6051 and E. coli Kp as test microorganisms.

The serum was used as untreated for the test. The brain tissue was homogenized together with an equal weight of a 0.1M phosphate buffer solution (pH 7.0) in a glass-made homogenizer to prepare a 50% homogenate solution.

Sampling of the perilymph and the middle ear mucosa was carried out within the same time zone, and the samples were pooled in the cold. After measuring the volume of the perilymph the sample was ten-fold diluted with a phosphate buffer and tested. On the other hand, after weighing and homogenizing, the mucosa sample was tested.

3) Result:

i) Change of Acoustic Acuity:

The change of ABR thresholds are shown in Table 2 below.

TABLE 2

ABR Threshold Change	
	Threshold Change (dB, Mean ±S.E.)
0.5% OFLX Group	-7.9 ± 2.8**
GM Group	-38.5 ± 5.8*
Control Group	-15.0 ± 5.8

Note: ** P <0.01 vs. GM group
* P <0.05 vs. control group

As can be seen from Table 2, reduction of acoustic acuity of an average of 15 dB was noted in the control group, while the GM group showed a rise in ABR threshold of 38.5 dB, that is, remarkable reduction of acoustic acuity. On the other hand, the 0.5% OFLX group showed an extremely small rise in ABR threshold, i.e., reduction in auditory acuity, averaging 7.9 dB. Therefore, it was confirmed that ofloxacin did not have any ototoxicity.

ii) SEM Observations on Cochlear Hair Cell:

The SEM picture of the cochlea in the control group or 0.5% OFLX group revealed that the hair of the outer and inner hair cells at the basal turn and the second or third turns suffered form no disturbance.

On the other hand, the SEM picture of the cochlea of the GM group revealed that the outer and inner hair cells suffered from disturbance of the row of the hair cells or disappearance of the hair cells from the basal turn through the third turn. There was observed a tendency that the disturbance of the inner hair cells became serious toward the upward turn. On the other hand, the outer hair cells did not show such a tendency, however, a stronger disturbance of the outer hair cells was noted at the third turn thereof. Also, when SEM was also carried out on 6 ears on the opposite side of the animals of the GM group, a slight disturbance of the outer hair cells was observed in every case, in spite of that GM was administered directly to these ears.

iii) Observations of Middle Ear Mucosa:

Considerable inflammation, such as remarkable cellular infiltrate in the mucosa or thickening of the mucous periosteum, were observed in the control group. To the contrary, such inflammation was not observed in the middle ear mucosa of the 0.5% OFLX group and the GM group.

iv) Distribution of Ofloxacin:

The results are shown in Table 3.

TABLE 3

Concentration of OFLX in Various Tissues After OFLX Admin.						
	No. (hr)	Time from Admin.	Ofloxacin Concen. (μg/mℓ or μg/g) Mid. Ear			
			Serum	Brain	Mucosa	Lymph
OFLX Group	1	1-1.5	<0.19	<0.20	78.70	<1.00
	2	"	0.91	<0.20		
	3	"	<0.19	<0.20		
	4	"	<0.19	<0.20		
	5	1.5-2	<0.19	<0.20	45.85	3.80
	7	"	1.20	<0.20		
	9	"	<0.19	<0.20		
	10	"	<0.19	<0.20		
	6	2-2.5	<0.19	<0.20	40.85	<1.00
	11	"	<0.19	<0.20		
	12	"	0.19	<0.20		
	13	"	0.39	<0.20		
Control Group	1			<0.20		
	2			<0.20		

As can be seen from Table 3, the ofloxacin concentration in the serum was less than 0.19 μg/mℓ in most of the cases of the OFLX group. Therefore, ofloxacin did not distribute to tissues other than the middle ear. In particular, from the fact that the concentration of ofloxacin in the brain was less than 0.20 μg/g in all cases, the same level in the controls. Therefore, it was considered that side effects on the central nervous system, which are liable to be induced by the use of new quinolone-type antibacterial agents, were not involved at all in case of administration of ofloxacin to the ear. On the other hand, distribution of ofloxacin to the middle ear mucosa, which is of the most important from the standpoint of effectiveness, was higher than 40 μg/g,

As above demonstrated, ofloxacin, when topically administered into the ear, caused substantially no ototoxicity and exhibited excellent distribution to the desired middle ear mucosa without showing substantially no distribution to tissues, particularly to the brain, other than the desired tissues. Thus, the ofloxacin topical preparations for otopathy according to the present invention are believed to be of extremely high clinical use.

TEST EXAMPLE 3

It is known that otitis media and otitis externa are induced through bacterial infection. Hence, antibacterial activities of ofloxacin on all the bacteria isolated from the lesion of a patient suffering from purulent otitis media were assayed in comparison with other drugs for otopathy. The results obtained were shown in Table 4 below.

TABLE 4

MIC_{80} on Test Microorganisms

Drug	MIC_{80} (μg/mℓ)	Total Number of Strains	Composition of Microorganisms (%)				
			S. aureus	S. epidermidis	P.aeruginosa	Proteus	Other Microorganisms
Ofloxacin	1.56	174	33.3	11.3	14.5	11.1	S.pneumoniae Corynebacterium anaerobic bacteria
Cefmenoxime	25	237	37.7	10.77	13.5	13.1	Streptococcus A. calcaeticus Peptostreptococcus
Chloramphenicol	50	237					
Fradiomycin	100	237					

As is apparent from Table 4 above, ofloxacin exhibited more excellent antibacterial activities on bacteria causing purulent otitis media than other conventional drugs.

* C L M S *

Claims

1. Use of ofloxacin or a salt thereof for preparing a topical preparation in the form of a solution for treating otopathy, the use of S-(-)-ofloxacin being excluded.

Patentansprüche

1. Verwendung von Ofloxacin oder einem Salz davon zur Herstellung eines topischen Präparates in Form einer Lösung zur Behandlung von Otopathie, wobei die Verwendung von S-(-)-Ofloxacin ausgenommen ist.

Revendications

1. Utilisation de l'ofloxacine ou d'un de ses sels pour préparer une préparation topique sous la forme d'une solution pour le traitement des otopathies, l'utilisation de la S-(-)-ofloxacine étant exclue.