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(57) Abstract

A pharmaceutical preparation for prophylaxis and/or treatment of acute otitis media (inflammation of the ear) and middle otitis (inflammation of the middle ear) is described, comprising at least one of the microorganism strains Streptococcus sanguis under Accession No. NCIMB 40104, Streptococcus sanguis under Accession No. NCIMB 40873, Streptococcus oralis under Accession No. NCIMB 40875, Streptococcus oralis under Accession No. NCIMB 40876 and Streptococcus mitis under Accession No. NCIMB 40874 and, optionally, other alpha streptococcus strains having essentially the same capacity of inhibiting Haemophilus influenzae, Streptococcus pneumoniae and Moraxella catharralis, in a pharmaceutically acceptable medium, in which the microorganism strains retain their viability.

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STREPTOCOCCUS PREPARATION

Field of the Invention

The present invention relates to a pharmaceutical preparation for prophylaxis and/or treatment of acute otitis media (inflammation of the ear) and middle otitis (inflammation of the middle ear).

Background Art

The normal microflora of bacteria on skin and various mucous membranes is most important to health. Disorders of this microflora can predispose the individual to the arising of various diseases. Clinical problems caused by disorders of the normal microflora are, for instance, acute inflammation of the ear (otitis media acuta). At the age of 3, about 50% of all children in Sweden have been affected by otitis at least once and, at the age of 7, about 70%. Acute otitis is most frequent at the age between 1 and 2 years. About one million medical treatments are made each year owing to acute otitis, which makes this disease the commonest affection that makes it necessary for small children to call on a doctor.

Acute otitis is frequently preceded by a virus-induced cold which is complicated by the fact that bacteria penetrate through the auditory tube into the middle ear and there cause an infection. The bacteria which as a rule cause acute otitis in children are Streptococcus pneumoniae (30-40%), Haemophilus influenzae (10-15%) and Moraxella catharralis (<10%), below referred to as otitis pathogens. Adherence of these bacteria to non-ciliated epithelial cells in nasopharynx, in the vicinity of the tubal orifice, i.e. that part of the auditory tube which leads to nasopharynx, is in a significant manner correlated to the disposition to otitis in children.

Acute otitis is currently treated with antibiotics. The drawback of this type of treatment is on the one hand that in many cases unsatisfactory results are produced

and, on the other hand, that treatment with antibiotics in itself is unsuitable owing to the risk of developing resistance.

In many countries, such as Spain and France, above all pneumococci have in recent years increased their resistance to penicillin and other common "ear antibiotics", which may result in the situation where we have no antibacterial preparations for these diseases. Children are already hospitalised for intravenous treatment with antibiotics. This involves a great risk to the child and great public expense. This situation also threatens Sweden since we are likely to import these bacterial strains from abroad. This has already occurred in some countries, for instance in Iceland. Intense research work to find a solution to this problem is in progress all over the world. Everybody agrees that we have to reduce the consumption of antibiotics for the purpose of reducing the tendency of these resistant bacteria spreading and surviving.

Serious complications in connection with inflammation of the ear are today unusual in Sweden. Middle otitis, inflammation of the middle ear, i.e. inflammation in the auditory tube (fluid in the middle ear), however, is a very common consequence of an inflammation of the ear. This often heals by itself but in many cases requires a surgical operation. Treatment of children suffering from an inflammation of the middle ear with a plastic tube is the commonest operation made by ear specialists and requires anaesthetic in most cases. These children must be carefully checked both before and after the operation. The follow-ups will as a rule continue for several years. In spite of adequate treatment, a small number of these children will have permanent troubles with chronical otitis (otitis media chronica). This may later require extensive surgical, hearing-promoting operations and/or hearing aids.

Although inflammation of the ear is a fairly banal affection in Sweden, the large amount of patients require great medical resources and also involve heavy expenses to society in the form of dropping off in production caused by absent parents in connection with diseases and calls on doctors.

In non-otitis-prone children, the nasopharyngeal flora is dominated by alpha streptococci whereas these are present in small amounts or not at all in otitis-prone children. There is today a lot that points towards there being in nasopharynx a natural homeostasis between the normal non-pathogenic flora and the potentially otitis-inducing bacteria (see Fujimori, I. et al: The nasopharyngeal bacterial flora in children with otitis media with effusion. Eur. Arch. Otorhinolaryngol. 1996; 253:260-263; and Bernstein, J.M. et al: Bacterial interference in nasopharyngeal bacterial flora of otitis-prone and non-otitis prone children. Acta oto-rhino-laryngol. belg 1994; 48:1-9).

Summary of the Invention

An object of the invention is to eliminate the problems that are associated with the different types of otitis.

This object is achieved by a pharmaceutical preparation of the type mentioned by way of introduction, which has the features defined in appended claim 1. Preferred embodiments of the pharmaceutical preparation are defined in the subordinated claims.

Among more than 300 different alpha streptococci isolated from the tubal orifice and the adenoid (lymphatic tissue in the nasopharyngeal space) of healthy children, five alpha streptococcus strains, all originating from the tubal orifice, have been selected to have a particularly good growth-inhibiting effect on otitis pathogens and a good adhesive capability in respect of adenoid epithelium. The isolation is made under anaesthetic and by using a specifically developed sampling

stick. Different types of alpha streptococci will be found, depending on whether samples are taken from the adenoid or the tubal orifice. Since these two areas are close to each other, sampling must be carried out with great accuracy. Four of these strains have been deposited on 19 March 1997 in National Collection of Industrial and Marine Bacteria Ltd (NCIMB), 23 st. Machar Drive, Aberdeen, AB2 1RY, Great Britain, viz. Streptococcus sanguis under Accession No. NCIMB 40873, Streptococcus mitis under Accession No. NCIMB 40874, Streptococcus oralis under Accession No. NCIMB 40875 and Streptococcus oralis under Accession No. NCIMB 40876, while one strain, Streptococcus sanguis under Accession No. NCIMB 40104, was deposited on 3 February 1989. These strains are typifieded according to API 20 Strep test, BioMerieux, France. The typification result is stated in Table 3.

The five above-mentioned microorganism strains can be present individually or in a combination of two or more in the pharmaceutical preparation. The Streptococcus oralis strain under Accession No. NCIMB 40876 has been found to be the most active against the otitis pathogens mentioned. Possibly, one or more other streptococcus strains having essentially the same capacity of inhibiting these otitis pathogens can also be included in the pharmaceutical preparation.

The pharmaceutically acceptable medium in which the strains are stored preferably consists of skim milk, NaCl or some other medium in which the bacteria retain their viability. Each of the microorganism strains in the preparation is present in the medium at a concentration of 10^4-10^{11} cfu/ml, preferably 10^5-10^{10} cfu/ml, and most preferably 10^7-10^9 cfu/ml (cfu = colony forming units). The selected alpha streptococci are first cultured in TY medium, washed in NaCl and diluted to a concentration of 10^9-10^{10} cfu/ml. The suspension is then frozen to -20°C or freeze dried. Administration to the patient is suitably carried out by using an atomiser, by means of which the

then thawed or dissolved preparation can be administered nasally. Administration is preferably carried out twice a day.

The pharmaceutical preparation according to the present invention can be formulated as a spray suspension for nasal administration, a freeze-dried powder, a lozenge, a freeze-dried tablet or gargle.

Experiments

a) An experiment was carried out for determining which alpha streptococci dominate the flora on the adenoid and in the tubal orifice of healthy children. The sampling was made under anaesthetic using a sterile sampling stick with a sterile plastic tube (Mülly suction catheter) for the sampling to take place in the correct position and not to cause contamination.

Alpha streptococci were isolated in 10 healthy children and typified according to API 20 Strep test, BioMerieux, France. The dominating alpha haemolytic streptococci (AHS) belonged to the strains Streptococcus sanguis, oralis, mitis, salivarius and intermedius (see Fig. 1).

b) An experiment was carried out to test the inhibiting capacity of the isolated alpha streptococci in respect of 25 different otitis pathogens, such as Streptococcus pneumoniae, Haemophilus influenzae and Moraxella catharralis. This experiment was evaluated by means of a modified agar overlay method. The result shows that alpha streptococci isolated from healthy children have a good inhibiting effect against otitis patogens, and that alpha streptococci isolated from the tubal orifice had a more inhibiting effect than those isolated from the adenoid (see Fig. 2 and Table 1).

c) A study was carried out to show that it is possible to find once more previously administered alpha streptococci in nasopharynx and in the tubal orifice. Two of the selected alpha streptococci were made resistant to an antibiotic, viz. erythromycin. These bacteria were

sprayed in nasopharynx of three healthy voluntary subjects. The cultivating samples were cultivated on blood agar plates with erythromycin to separate added alpha streptococci from the subject's own alpha streptococci. Erythromycin-resistant alpha streptococci were found in nasopharynx during spray treatment and up to 24 h after completed treatment (see Table 2).

d) A pilot test has also been performed on a 4-year-old boy suffering from recurrent otitedes (eight otitedes in one year). The boy was given alpha streptococci after being treated with penicillin. His nose was sprayed for 10 days and this was repeated once after one more week. No new otitedes occurred during the follow-up time of 5 months (December-April), when this patient should normally have had several recurrent otitedes.

TABLE 1

Average inhibiting activity of AHS from each child			
Patient	Age (year)	AHS from tubal orifice	AHS from adenoid tissue
F.H.	3	88	72
D.B	3.2	66	75
C. O-H.	3.5	67	41
D.A.	4	98	59
L.B-P.	4.5	88	84
C.L.	5	83	75
E.B.	5.8	100	96
J.O.	6.9	66	65
C.H.	9.1	78	26
L.H.	9.7	97	52
Average	5.5	83	64.5

TABLE 2

Patient	Test before treatment	Test during treatment	Test 24 h after completed treatment
No. 1	No growth	I.E.R.A.	I.E.R.A.
No. 2	No growth	I.E.R.A.	No growth
No. 3	No growth	I.E.R.A.	I.E.R.A.

I.E.R.A. = isolated erythromycin-resistant alpha streptococci

TABLE 3

RESULT IN TYPIFYING OF STREPTOCOCCUS STRAINS					
Test	S. sanguis NCIMB 40104 α89a	S. sanguis NCIMB 40872 α3	S. oralis NCIMB 40876 α4	S. mitis NCIMB 40873 α5	S. oralis NCIMB 40875 α6
VP	-	-	-	-	-
HIP	-	-	-	-	-
ESC	-	-	-	-	-
PYRA	-	-	-	-	-
αGAL	+	+	+	-	+
βGUR	-	-	-	-	-
βGAL	-	+	-	-	-
PAL	+	+	+	-	-
LAP	+	+	+	+	+
ADH	+	+	-	-	-
RIB	-	-	-	+	-
ARA	-	-	-	-	-
MAN	-	-	-	-	-
SOR	-	-	-	-	-
LAC	+	-	+	+	+
TRE	(+)	+	-	-	-
INU	-	-	-	-	-
RAF	+	+	+	-	+
AMD	+	-	-	-	+
GLYG	-	-	-	-	-
haemolysis	-	-	-	-	-
all have α haemolysis (green haemolysis)					

Alpha haemolytic streptococci having an inhibiting activity in relation to AOM (acute otitis media) pathogens in healthy children and children with recurrent AOM and secretory otitis media.

A study was carried out with a view to investigating the inhibiting activity of the normal epipharyngeal flora in relation to the three most frequent AOM pathogens in healthy children (n=19) and children with recurrent AOM (n=19) and secretory otitis media (SOM) (n=20). AOM pathogens and ten alpha haemolytic streptococci (AHS) were isolated from the tubal orifice and the adenoid. The sampling was performed under general anaesthesia. The method used to test the bacterial interference in vitro was a modified agar overlay method. Among healthy children, 52% had a non-typifiable Haemophilus influenzae, 21% Streptococcus pneumoniae and 36% Moraxella catarrhalis. In children with recurrent AOM, non-typifiable Haemophilus influenzae was found in 21% while 63% had Moraxella catarrhalis in the tubal orifice and 61% on the adenoid. 26% had Streptococcus pneumoniae in the tubular orifice and 22% on the adenoid. Moraxella catarrhalis was found in the tubal orifice of 73% of the children with SOM and in 86% of the samples from the adenoid. Moreover, group A streptococci (GAS) were found in all groups, 15% in healthy children, 15-22% in children with recurrent AOM and 31-40% in children with SOM. In healthy children, AHS with a strongly inhibiting capacity in relation to the AOM pathogens was found in the tubal orifice. AHS isolated from children with recurrent AOM or SOM had no inhibiting capacity or just a weak inhibiting capacity in relation to the AOM pathogens, especially the isolates from the adenoid in the AOM group. AHS had the best inhibiting capacity in relation to Moraxella catarrhalis. Inhibiting AHS in the tubal orifice may be important to prevent recurrent AOM.

Examination of the inhibiting capacity of isolated α streptococci in relation to otitis pathogens in children by means of the interference method

A = Adenoid T = Tubal orifice

	Healthy children		AOM children		SOM children	
	A	T	A	T	A	T
H. inf.	0.85	0.39	0.77	0.95	0.94	0.71
S. pneum.	0.37	0.30	0.54	0.67	0.37	0.49
M. cat.	0.35	0.14	0.27	0.60	0.19	0.21

Score: 0-2

0 = Inhibits completely

1 = Inhibits the growth partially

2 = No inhibition

CLAIMS

1. A pharmaceutical preparation for prophylaxis and/or treatment of acute otitis media (inflammation of the ear) and middle otitis (inflammation of the middle ear), characterised in that it comprises at least one of the microorganism strains Streptococcus sanguis under Accession No. NCIMB 40104, Streptococcus sanguis under Accession No. NCIMB 40873, Streptococcus oralis under Accession No. NCIMB 40875, Streptococcus oralis under Accession No. NCIMB 40876 and Streptococcus mitis under Accession No. NCIMB 40874 and, optionally, other alpha streptococcus strains having essentially the same capacity of inhibiting Haemophilus influenzae, Streptococcus pneumoniae and Moraxella catharralis, in a pharmaceutically acceptable medium, in which the micro-organism strains retain their viability.

2. A pharmaceutical preparation as claimed in claim 1, characterised in that it contains only the Streptococcus oralis strain under Accession No. NCIMB 40876.

3. A pharmaceutical preparation as claimed in claims 1 and 2, characterised in that the microorganism strains have been isolated from the tubal orifice and/or the adenoid, preferably the tubal orifice, in healthy children.

4. A pharmaceutical preparation as claimed in claims 1-3, characterised in that each microorganism strain in the preparation is present at a concentration of 10^4-10^{11} cfu/ml, preferably 10^5-10^{10} cfu/ml, most preferably about 10^7-10^9 cfu/ml, based on the pharmaceutically acceptable medium.

5. A pharmaceutical preparation as claimed in claims 1-4, characterised in that it is present in the form of a spray suspension for nasal

administration, a freeze-dried powder, a lozenge, a freeze-dried tablet or gargle.

6. A pharmaceutical preparation as claimed in any one of the preceding claims, c h a r a c t e r i s e d in that the pharmaceutically acceptable medium is skim milk or NaCl.

7. Use of a pharmaceutical preparation as claimed in claims 1-6 for prophylaxis and/or treatment of acute otitis media (inflammation of the ear) and middle otitis (inflammation of the middle ear).