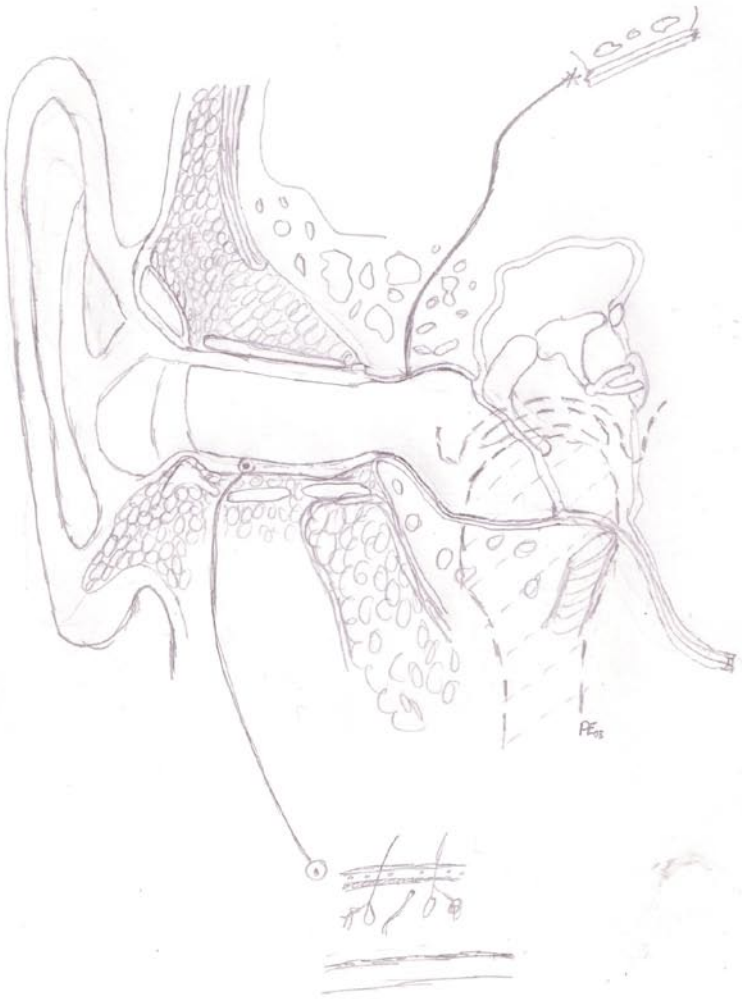
EXTERNAL OTITIS AND ITS TREATMENT. IS A GROUP III STEROID WITHOUT ANTIBIOTICS SUFFICIENT THERAPY? – EXPERIMENTAL AND CLINICAL STUDIES

By
Per Emgård



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Som med vederbörligt tillstånd av Rektor vid Umeå Universitet för avläggande av doktorseksamen i medicinsk vetenskap offentligen försvaras i Sal B (Rosa salen, 9 tr) Tandläkarhögskolan, Norrlands Universitetssjukhus, fredagen 28 oktober kl 09.00.

Av

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To

Mari, for her support and love

“If you repair or rebuild something, it will rarely be perfect, but it will be better than before!”

My wife, Mari

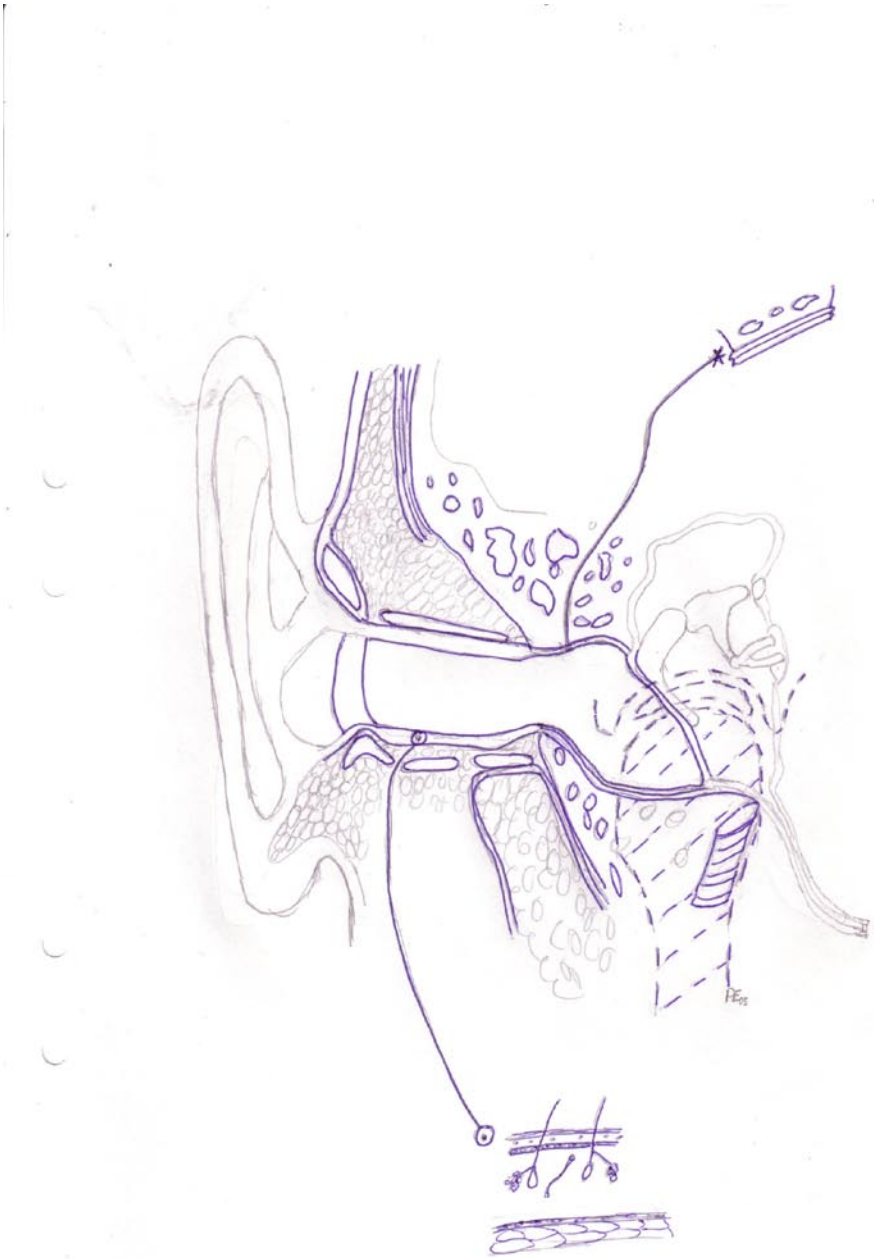

Fig: Visual mind map
Remember; Full thickness skin with adnexa and with cartilage beneath, in 2/3 of the EAC.
The skin is thin and with a bony support, in the most medial part of the EAC. Channels of Santorini. Parotid gland and the mandibular joint in the floor of the bony EAC, with the Carotid artery close to middle ear cavity. The surface of the tympanic membrane is not perpendicular in the medial-lateral projection. The EAC is curved and with narrow parts

ABSTRACT

External otitis and its treatment. Is a group III steroid without antibiotics sufficient therapy? – Experimental and clinical studies
Per Emgård, Department of Otorhinolaryngology, University of Umeå and Ystad Hospital, Umeå and Ystad, Sweden

External otitis is one of the most common ear, nose and throat (ENT) diagnoses in out-patient clinics. The clinical course of external otitis includes itching, pain, redness, swelling and effusion of the external auditory canal (EAC) with normal tympanic membrane status. The inflammatory condition is often associated with infection by bacteria, e.g. *Pseudomonas aeruginosa*, or skin bacteria such as Staphylococcus species. Fungi are present only in a low percentage of cases and if present *Candida albicans* infection is the most frequent in northern countries such as Sweden and the UK. Topical therapy is recommended in most countries and dominates the therapy in most studies. Topical drugs used are usually a combination of antibiotics and a steroid. However, external otitis is treated with surprisingly many strategies – eleven different ones in Sweden, for example, and 18 in the UK.
The aims of the present studies were to –

- establish an animal model, infected and uninfected, suitable for testing various treatment strategies of external otitis; and
- perform a clinical study in patients to elucidate whether a group III steroid alone is as efficient for treatment of external otitis as is the commonly used topical drug containing a combination of a steroid and antibiotics.

The animal model was established through mechanical irritation of the external ear canal skin of Sprague-Dawley rats. An evaluation scale for characterization of the clinical status of the ear canal was introduced, recording redness, swelling and occurrence of effusion in a standardized way. Specimens of the ear canal skin were analysed by histological techniques. A topical solution of 0.05% bethametasone dipropionate (BD) was compared with a 1% hydrocortisone solution with antibiotics oxytetracycline and polymyxin B added (HCPB), administered in the external otitis model infected or non-infected with bacteria (*P. aeruginosa*) and a fungus (*C. albicans*).
The same drugs were tested in a randomized parallel-group multi-centre study in 51 patients. The clinical status of the external otitis patients was evaluated on a similar scale as used in the animal model. Early normalization of the ear canal skin status and frequency of relapses during the 6-month follow-up period were used as end-points of the study.
The studies showed the following:

- An animal model for external otitis, infected or uninfected, could be established.
- A new scale for evaluation of the external ear canal status with regard to redness, swelling and occurrence of effusion was introduced for the animal model as well as for the investigations in patients.
- Treatment with a group III steroid topical solution without antibiotics was superior to treatment with a group I steroid with antibiotics added in achieving resolution of external otitis.
- The effectiveness of the topical drugs in the clinical studies in external otitis patients was similar to that in animal external otitis models.

We conclude that a group III steroid solution cures external otitis more effectively than does a solution containing a group I steroid combined with antibiotics, whether infected by bacteria or by fungi. No difference was evident regarding adverse effects. Furthermore, costs favour a solution without any antibiotic components. In view of these observations a group III steroid solution is preferred for remedy of external otitis in the clinical situation.

Key words: external otitis, external auditory canal (EAC), animal model, treatment, betamethasone, hydrocortisone, antibiotics, human study, *Pseudomonas aeruginosa, Candida albicans.*

ABBREVIATIONS

ABR	auditory-evoked brainstem response
BD	betamethasone dipropionate
C. albicans	*Candida albicans*
CFU	colony-forming unit
CRF	case record form
EAC	external auditory canal
ENT	ears, nose and throat
HC	hydrocortisone combined with oxytetracycline
HCPB	hydrocortisone with oxytetracycline and polymyxin B
P. aeruginosa	*Pseudomonas aeruginosa*
Staph. aureus	*Staphylococcus aureus*
Staph. auricularis	*Staphylococcus auricularis*
Staph. epidermidis	*Staphylococcus epidermidis*

ORIGINAL PAPERS

I Emgård P, Hellström S. An animal model for external otitis. Eur Arch Otorhinolaryngol 1997; 254: 115–119.

II Emgård P, Hellström S, Ohlander B, Wennmo C. Effects of betamethasone dipropionate plus an antihistamine in patients with external otitis. Curr Ther Res Clin Exp 1999; 60: 364–370.

III Emgård P, Hellström S. A topical steroid without an antibiotic cures external otitis efficiently: a study in an animal model. Eur Arch of Otorhinolaryngol 2001; 258: 287–291.

IV Emgård P, Hellström S, Holm S. External otitis infected with *Pseudomonas aeruginosa* or *Candida albicans* cured by use of a topical group III steroid, without any antibiotics. Acta Otolaryngol 2005; 125: 346–352.

V Emgård P, Hellström S. A group III steroid solution without antibiotic components – an effective cure for external otitis. J Laryngol Otol 2005; 119: 342–347.

INTRODUCTION

1. External otitis

1.1 Definition, diagnosis and treatment

In the present thesis "external otitis" is defined as an inflammatory condition located in the external auditory canal (EAC) with no affection of the tympanic membrane or to the bone or cartilage of the EAC, and with a duration of less than 30 days. When the condition is of longer duration external otitis is considered chronic. The status of the EAC is dominated by one or more of the three variables redness, swelling and effusion. Infection with bacteria and/or fungi is common.

Most often a variety of bacteria and fungi representing normal skin flora are present in the EAC (Brook et al, 1992; Clark et al, 1997; Feidt & Federspil, 1989). The most common type of acute external otitis is the diffuse variant, characterized by a generalized redness and swelling of the EAC skin. Less frequent and more difficult to examine is external otitis with otorrhea. The latter is most often caused by bacterial infection but occasionally when a whitish-yellow fluid of low viscosity appears in the EAC, fungi, mainly *Candida albicans*, are present (personal observation). The severe form of external otitis, the so-called "malignant external otitis" with engagement of cartilage and bone of the EAC, is dangerous with a tendency of a breakthrough towards the brain. Patients with this condition are rarely seen but must be recognized and are not to be misdiagnosed as the common uncomplicated external otitis. In Sweden the most common treatment of external otitis, whether infected or not, is mechanical cleansing followed by topical instillation of ear drops. The most frequently prescribed drug contains a group I steroid combined with two antibiotic components, terracortril and polymyxin B (Terracortril-polymyxin B®) (Medical Index Sweden, 1997). In other countries other otic solutions for treatment of external otitis are more common, such as

Corticosporin® in the US, consisting of neomycin, polymyxin B and hydrocortisone (Tong et al, 1996). In the UK Reilly and Skinner (1990) identified 18 different treatment modalities. A similar survey in Sweden identified eleven well-defined groups of topical and systemic agents as therapy for external otitis (Paper II). The cost and benefit of antibiotic components added to a steroid solution may be questioned, however (Reilly & Skinner, 1990).

1.2 Epidemiology

External otitis is the second most common complaint encountered in ears, nose and throat (ENT) out-patient care in the UK, according to Cassi et al (1997). In a UK study of external otitis the prevalence was 1.3% for female and 1.2 for male patients per year (Rowlands et al, 2001). Another study performed in 1999 in the US reports that in that year there were about 3.31 million consultations for external otitis, and in about 40% of them children were the patients. In Sweden, with 9 million inhabitants, about 250 000 out-patients annually present with external otitis as estimated from the number of prescriptions for ear drops (Medical Index Sweden, 1997). According to other studies, this could be an overestimation as all prescriptions of this drug are not used as topical treatment for external otitis. On the other hand, not every case of external otitis is medically treated. If we took the number of visits per capita with a diagnosis of external otitis in an agricultural area of southern Sweden and translated it to the total population of Sweden the figure of about 160 000 patient visits would be more accurate. Of these patients approximately 5% are referred to ENT specialists. The remainder are handled by primary health care practitioners (personal observation). In the study by Rowland et al (2001) 3% of patients were referred to secondary care. In a consensus panel report Hannley et al (2000) stated external otitis to affect 4/1 000 adults in the US. Despite some reports to the contrary (e.g. Rowlands et al, 2001; Hughes & Lee, 2001) the incidence of patients referred to an ENT department is not significantly higher during the

"swimming season" (personal observation). Summarizing all these studies to the inhabitants of Europe and North America one may assume an annual incidence of 1% for acute external otitis in out-patients. In countries with a tropical climate the incidence is supposed to be higher.

1.3 Pathogenesis

External otitis can be characterized into three stages (Lucente , 1995), (i) a pre-inflammatory stage; (ii) an acute stage; and (iii) a chronic condition. During the first, pre-inflammatory stage, damage of the skin caused by manipulation or a skin disease provides an oedema of the EAC skin, often with a moist surface and an opportunity for pathogens to enter the skin. Itching, evoked by histamine from the mast cells, and other inflammatory factors stimulate self-manipulation and further injuries to the skin. The EAC appears to be dry and red, or pale red, swollen and often moist dependent on the initial skin damage. If not treated with topical solutions (often containing steroids combined with one or several antibiotics/antimycotics) the condition enters the second stage of acute external otitis.

Acute external otitis is dominated by pain, itching, a more pronounced swelling and moisture and often otorrhea. The fluid from the EAC may mix with debris and when it does this it is associated with a foul smell. In severe cases the redness extends onto the pinna and the skin behind the auricle over the mastoid process. Palpable pre- and post-auricular lymph nodules may occur. If the otorrhea is whitish-yellowish and of high viscosity a *C. albicans* infection is often present (personal observations). Sometimes colonies of fungi are clearly visible in the otomicroscope, resembling thin cotton threads characteristic of Aspergillus species. If the EAC is purple severe itching often occurs, but the EAC is dry. In these cases untreated external otitis often becomes chronic.

External otitis is considered chronic if it persists for more than 30 days. The patient often has chronic itching in the EAC and manipulates the tragus or the EAC by him/herself by use of different tools such as matches or needles, further enhancing the inflammatory condition. In a study by Smith et al (1990) as many as 58% of patients with chronic external otitis presented with atopic contact dermatitis. Five per cent of all unselected chronic external otitis was found positive for nickel. In three out of nine patients with chronic external otitis, substances in the topical treatment drugs or vehicles group, e.g. hydrocortisone, were found to cause hypersensitivity (Wilkinson & Beck, 1993, Fränki et al. 1985, Sood et al.2002). Other skin diseases such as psoriasis, metabolic diseases such as diabetes, and malnutrition may also facilitate the chronic stage of external otitis.

1.4 Structure of the external ear canal

The external auditory canal in humans

In humans the EAC originates from the first ectodermal branchial groove between the mandibular and hyoid arches. The auricle itself also derives from the first branchial groove but also the first and second branchial arches contribute (Selesnick, 1996; Lucente et al, 1995).

From its beginning in the cavum auris of the auricle the EAC extends as an s-shaped channel about 25 mm long and 4–5 mm in diameter, and ends with the tympanic membrane as a cul-de-sac. The posterior and superior canal walls are approximately 6 mm shorter than are the anterior-inferior walls. This explains the oblique position of the tympanic membrane. In children the lateral, cartilaginous portion of the EAC is longer than the bony canal of the temporal bone. In adults the bony portion is slightly longer than the lateral cartilaginous part. The slightly curved EAC protects the tympanic membrane from both direct view and

manipulation. The glenoid fossa and the mandibular condyle create a convexity in the anterior wall that can limit the possibility of inspecting the tympanic membrane. The narrowest portion of the EAC is the junction between the cartilaginous and bony portions. The volume of the adult EAC is smaller than 1 ml, approximately 0.85 ml. The elastic cartilage surrounding the lateral part of the EAC is incomplete in its superior portion but bridged by dense fibrous tissue attached to the temporal bone. Antero-inferiorly the two fissures of Santorini allow for flexibility but sometimes also constitute a lymphatic passage of infectious material and of tumour cells from the parotid gland (Selesnick, 1996). The bony canal is a complete canal attached to the tympanic membrane.

Arterial supply to the lateral wall of the EAC is received from branches of the posterior auricular and superficial temporal arteries. Medially the deep auricular artery, a branch of the internal maxillary artery, supplies the EAC. The veins from the EAC drain the blood into the superficial temporal and posterior auricular veins. From there they join into the external jugular vein. Sometimes the posterior auricular vein joins into the mastoid emissary vein which is connected to the sigmoid sinus and the intracranial cavity. The lymphatic drainage of the EAC follows the veins into three locations, the parotid, the post-auricular and the mastoid regions.

The sensory innervation originates from four cranial nerves, No. V, VII, IX and X. The posterior wall is connected to the VIIth, IXth and Xth cranial nerves. The Vth cranial nerve, which lies anterior to the external ear, branches off into the auriculotemporal branch of the mandibular nerve. Arnold's nerve, the auricular branch of the vagal nerve, enters the jugular foramen through a foramen of its own into the canaliculus in the temporal bone, crosses the fallopian (facial nerve) canal and enters the posterior EAC through the styloid foramen or the tympanomastoid suture (Lucente et al, 1995). It is notable that manipulation of the EAC can cause nausea or coughing through stimulation of Arnold's nerve.

The Figure below illustrates the anatomical landmarks of the auricle and the EAC, with some indications of the surrounding structures (Fig. 1)

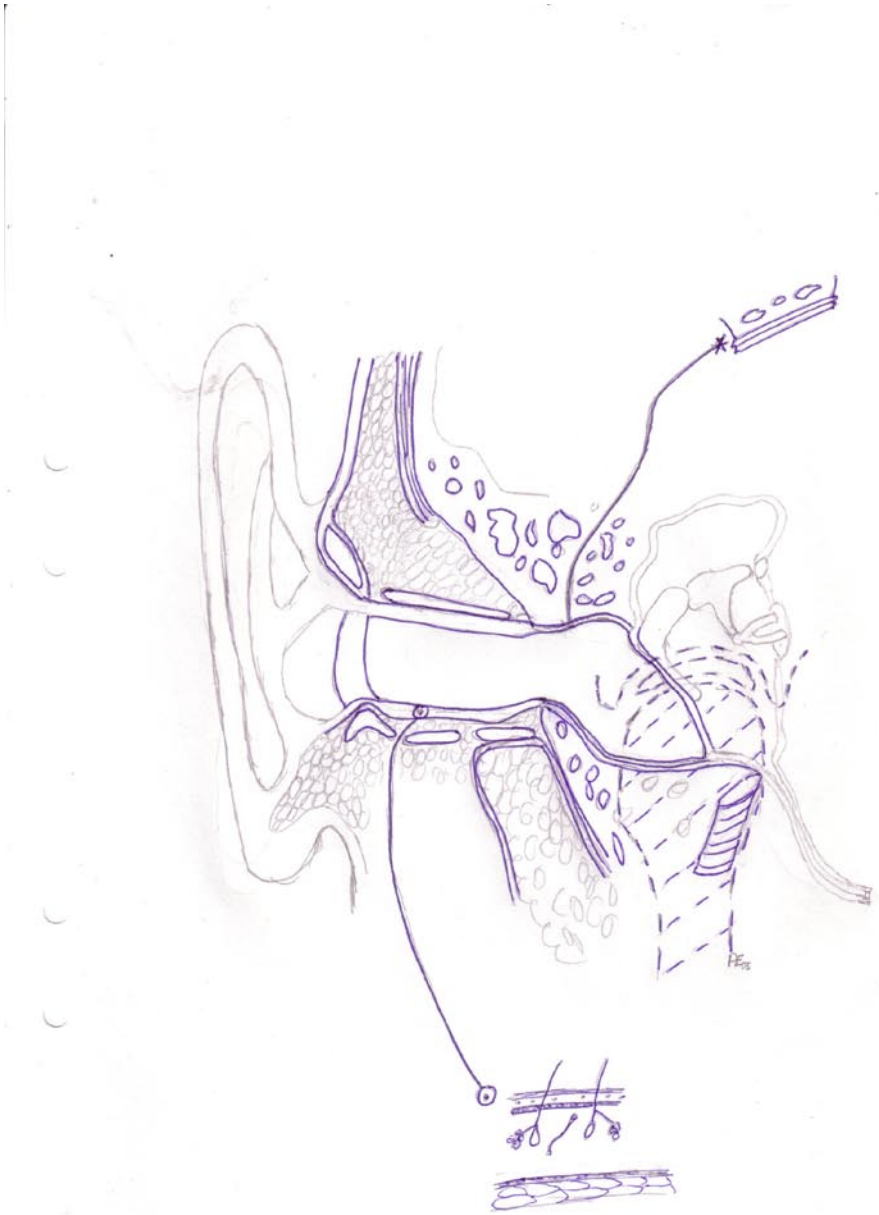

Fig: Visual mind map

The external ear

Histopathologically the EAC divides the skin of the “cul-de-sac” into a lateral portion with cartilage beneath the skin surface and a medial portion with a thin skin stretched directly over the periosteum of the bone. The EAC skin structure differs markedly between the lateral and the medial portion. The whole EAC is lined by a keratinizing stratified squamous epithelium. At the tympanic membrane the EAC skin continues as the external layer of the tympanic membrane (Lucente et al, 1995).

The skin of the lateral portion of the EAC is thicker than that of the medial portion, which is only 0.5–1.0 mm. The epidermis is composed of four layers, the basal cell (germinal), prickle cell (squamous), granular cell, and cornified layers. Hair follicles are almost solely located in the lateral third of the EAC. The hairs are of two types, tiny and large terminal hairs named “tragi”. In adult men the large hairs can be seen also in the proximal meatus and on the tragus and the antitragus of the auricle. Sometimes hair growth is extreme and makes investigation of the EAC and tympanic membrane difficult. The follicle canal of the larger hairs contains sebaceous glands and apocrine sweat glands open into its lumen. The secretory portions of these glands are rounded alveoli ranging from 0.2 to 2.0 mm in diameter. The central part of the alveoli contains large cells with fat droplets within the cytoplasm. The more fat the smaller the nuclei, which gradually shrink and disappear, with a breakdown of the cell into a fatty detritus mixed with horny scales which are excreted into the follicle canal and extruded onto the EAC surface. The apocrine glands also extrude their secretion into the follicle canal. Together with desquamated keratin cells this mixture of sebaceous fat and apocrine secretion

builds up the wax of the EAC. Because of the fatty acids in this material, and their acidity pH value, a bacteriostatic environment develops around the wax.

The skin of the medial portion of the EAC is approximately 0.2 mm thick and is loosely attached to the periosteum without any subcutaneous layer. This architecture makes the medial EAC more vulnerable than the lateral portion to trauma from instruments when cleansing. There are few small hairs in the osseous canal, and glands and sebaceous glands are infrequent or absent.

Structure of the external auditory canal in the rat

The EAC of the rat is almost solely surrounded by cartilage and a small medial bony structure only a few millimetres in size. The EAC is therefore very mobile and the tympanic membrane easy to observe. The keratinizing epithelium lining the EAC is thin, with little or no keratin on its surface. The epithelium towards the basal membrane has a smooth surface. The loose connective tissue layer contains vessels, fibroblasts, nerves and mast cells. Glands are absent in the most medial portions of the EAC. The normal skin of the lateral portion of the EAC is ≤80±29.7 µm thick while in the medial portion of the EAC the skin is only 60±15.2 µm thick (Emgård & Hellström, 2001, Paper III).

2.0 External otitis in animal models

Few animal models for external otitis have previously been described. In a study by King and Estrem (1990) guinea pigs were infected in the EACs with *Pseudomonas aeruginosa* suspension without any mechanical (i.e. through cleaning) or chemical stimulation. In another study Wright et al (2000) chemically stimulated the EAC in mice by use of topical application of tetradecanoylphorbol acetate (TPA). Two applications bilaterally into the EAC with a 24-hour interval between them caused inflammatory reaction of the skin. The EAC skin showed polymorphonuclear leukocyte infiltration, vascular dilation and thickening.

Ciprofloxacin/hydrocortisone suspension was used as topical treatment in this external otitis model.

Topical drugs for treatment of external otitis were evaluated in mice in a study by Sobol et al (2005). However, these authors tested the drugs not on EAC skin but on the shaved dorsum neck skin.

3 Microbiology in the healthy and inflamed external auditory canal

In a study by Roland and Stroman (2002) it is stated that in the past and until the end of World War II external otitis was considered a fungal infection. However, the fact that external otitis was shown to be the second most common disease among American troops in the South Pacific island of Guam led to investigations that verified the suspicion of a bacteriological infection. Since then various culturing data have been reported. Some of the significant studies were by Roland and Stroman (2002), Stroman et al (2001), Brook et al (1992), Dibb (1991), Lucente et al (1995), Maher et al (1982) and Mugliston and O'Donoghue (1985). The most frequent bacteria are *P. aeruginosa* and Staphylococci of different subgroups. With regard to fungi, the extensive study by Roland and Stroman (2002) shows only 49 samples of yeast and mould that were recovered from 2 048 ears with external otitis.

3.1 Bacteria

Few studies have been performed of the normal bacterial and fungal growth of the EAC. In a study on 310 samples from the EAC by Stroman et al (2001) the authors report 93% Gram-positive, 4.5% Gram-negative and 2.5% fungal isolates. Staphylococci constituted 63% of the bacteria. Of the Staphylococci *Staph. auricularis* contributed 21% and *Staph. epidermidis* contributed 17%. *Staphylococcus aureus* was not detected at all.

Coryneform bacteria (diphtheroides) were frequent, contributing 57 out of 310 positive samples. Of this bacterial group *Turicella otididis* represented 36 out of 57 samples while *Corynebacterium aureus* represented seven out of the 57. Gram-negative bacteria were found in 14 out of 310 samples while Pseudomonas of different species were found in four out of 14.

In a study by Roland and Stroman (2002) *P. aeruginosa* occurred in 1 089 isolates from 2 048 ears with external otitis. Altogether 543 isolates of different subtypes of Staphylococcus were found; *Staph. epidermidis* was detected in 257.

In a retrospective study of 46 patients with external otitis (Brook et al, 1992) aerobic bacteria only were obtained in 31 out of 46 patients (67%). Nineteen out of 45 isolates were *P. aeruginosa* while seven were *Staph. aureus* and five were *Staph. epidermidis*. Anaerobic bacteria only were isolated in 17% while Peptostreptococcus species were found in half of the cases. In a prospective study by Clark et al (1997) 26 patients with external otitis were studied. Anaerobic growth was found in two samples and aerobic bacteria were found in altogether 33 different species, 14 of which were *P. aeruginosa*.

In a prospective study on 226 patients with external otitis Dibb (1991) detected *Staph. aureus* in 34%, *P. aeruginosa* in 22% and *Streptococcus pyogenes* in 9% of cases. Unlike other investigators Dibbs excluded the normal flora from his results.

3.2 Fungi

Candida or Aspergillus of different species are the most common fungi in cultures from patients with external otitis. *Aspergillus* has been reported (Selesnick, 1996) to represent 80–90% of the fungi obtained from patients with external otitis. Aspergillus fungi are more common in countries with a tropical, subtropical or Mediterranean climate (Lucente et al, 1995), as found in Italy, Bahrain, Burma and Iraq, while in countries with temperate and cold

climates, such as the UK or Sweden, infections by Candida species are seen more frequently than are infections with Aspergillus species.

In their study Mugliston and O'Donoghue (1985) included also aural mycoses other than external otitis and report Candida species to be the most common yeast, involved in 636 out of 1 061 samples, followed by Aspergillus species, seen in 418 out of 1 061 samples. In the study by Roland and Stroman (2002) 1.7% of out-clinic patients with external otitis showed fungal growth. Aspergillus species occurred in 18 and Candida in 33 out of 51 samples.

3.3 Topical and systemic treatments and prevention of external otitis

In Sweden eleven different, well-defined treatment strategies for external otitis have been distinguished (Paper I). These include topical glucocorticoids with or without antibiotics, an acid with or without antibiotics, an ethanol mixture, antimycotics, oils and dyes. Furthermore, antimycotics and antibiotics alone, i.e. not combined with other drugs, have been tried in clinical practice. Both potential ototoxicity (Palomar,Palomar. 2001,Barlow et al.1994, Picket et al. 1997, Marsh, Tom. 1989, Russel et al 2001) and risk of bacterial drug resistance (Cantrell et al.2004) must be considered when topical treatment for external otitis is used. Reilly and Skinner (1990) report that in the UK 18 different types of aural antibiotic/steroid preparations are available for treatment of external otitis. In another survey conducted in the UK Rowlands et al (2001), investigating the General Practice Research Database, report that the first line of therapy for external otitis with a duration of 28 days or less was topical ear drops in 85% and oral antibiotics in 21% of cases. The most commonly used ear drop was a corticosteroid combined with an antibiotic in 64% and a steroid alone in 35%. Of the steroids used without antibiotics dexamethasone drops accounted for 85%. In another study performed by general practitioners 213 adults with external otitis divided into three groups were given either acetic acid, or acetic acid plus triamcinolone acetonide, or dexamethasone phosphate

sodium, neomycin sulphate or polymyxin B sulphate (Van Balen, 2003). Acetic acid alone proved less efficient than did acetic acid and steroid or steroid plus antibiotics. No difference was noted between the last two groups.

The question of the necessity of prescribing topical antibiotics for treatment of external otitis was studied in 39 patients by Tsikoudas et al (2002). The patients were given steroids or steroid and neomycin sulphate. The study showed no significant difference between the two treatment groups. Lancester et al (2003) investigated compliance in three groups with different topical medications, Gentisone HC® (hydrocortisone and gentamicin), Sofradex® (framycetin-gramicidin-dexamethasone in a spray) and Otimize® (dexamethasone, neomycin sulphate and glacial acetic acid). Sofradex® had the tendency to be significantly overdosed by patients, mainly due to an easy- handled delivery system and the low- viscosity of the drops are related. Their conclusion was that in order to have good compliance with topical treatment of external otitis there should be a reproducible volume of medication each time and the administration must be simple.

Another aspect of compliance is the degree of satisfaction with the treatment drug (Shikiar et al, 1999). One study showed that overall patient satisfaction related to four events: relief of symptoms, ability to return to normal activities, ease of administration and absence of medication side effects (Shikiar et al, 1999). The 41 patients studied by that group of authors received polymyxin/neomycin/hydrocortisone as topical treatment for external otitis. The patients completed a diary card and a satisfaction questionnaire and evaluated their pain on a visual analogue scale (VAS). Altogether 34 patients were possible to evaluate, 32 of whom reported a “plugged” feeling in the treated EAC. Twenty-six out of 34 complained of itching and 16 out of 34 had discharge from the treated EAC. The overall satisfaction correlated best to the simplicity of administration ($p<0.01$) and ability to work ($p<0.001$). An inverse correlation was observed when the grading of satisfaction was compared to the severity of

itching($p< 0.001$), i.e. great itching and low satisfaction or reversed. The severity of plugging was also inversely correlated to satisfaction. As expected, a higher level of pain created less satisfaction ($p<0.001$). Two other perhaps unexpected findings related to compliance were low cost ($p<0.05$) and good information ($p<0.05$). The authors concluded that "... a therapy that causes less itching and that relieves symptoms and brings cure quickly is likely to result in greater patient satisfaction" (Skikiar et al, 1999).

OBJECTIVES

The mayor aims of the present studies were:

- To develop an animal model, uninfected and infected, that would be suitable for testing various therapy strategies for treatment of external otitis;
-To perform a clinical study based on the animal experiments, in human patients in order to elucidate whether a group III steroid solution alone is as efficient for treatment of external otitis as is the topical drug, containing a suspension of a group I steroid and two antibiotics.

MATERIALS AND METHODS

1. Materials

The first three papers (Papers I–III) in this thesis represent studies of external otitis in an animal model. The following two papers (Papers IV and V) describe clinical studies of external otitis in humans. A new animal model for external otitis was created by mechanical irritation of the EAC in rats. The mechanically induced inflamed ear canal skin was infected

with *P. aeruginosa* and *C. albicans*. We chose *P. aeruginosa* because it is dominant amongst the bacteria associated with external otitis in humans.

The fungus *C. albicans* was considered easy to handle in a laboratory situation. In Sweden it is a frequent isolate from the human EAC subjected to external otitis. A multi-centre, prospective, randomized, double-masked placebo-controlled trial was performed as a pilot study to elucidate the efficacy of the group III steroid betamethasone dipropionate (BD) in treating external otitis. This human pilot study was followed by an open, randomized, parallel-group, multi-centre trial on the same subject.

1.1 Materials in animal studies

We used Sprague-Dawley rats weighing 250–300 g. Intravenous anaesthesia was induced by sodium methohexital (Brietal®, Lilly, Indianapolis, IN, USA). In all experiments a Zeiss otomicroscope was used for inspection of the EAC. The colour, swelling and effusion of the EAC were graded according to a standardized system developed for both the animal and the human studies. For histology, samples were taken from the lateral and medial portion of the EAC skin. The samples were embedded in plastic as well as paraffin for sectioning.

Plastic-embedded material: All skin specimens were fixed for at least 24 hours in a 3% glutaraldehyde solution in a 0.1 M cacodylate buffer with 4% polyvinylpyrrolidone and 0.002 M $CaCl_2$ added. Specimens were then post-fixed in 1% osmium tetroxide in the same buffer, followed by dehydration at increasing concentrations of acetone. Samples were then embedded in an epoxy resin (Polybed 812; Polysciences, Warrington, PA, USA) and sectioned in 1.0 µm sections using an ultramicrotome. The sections were stained with toluidine blue and analysed and photodocumented in a Zeiss Axiophot light microscope.

Paraffin-embedded material: After dissection, samples were transferred to saline and within 30 minutes placed in a mixture of 2% formaldehyde mixed with 0.5% glutaraldehyde in a 0.1

M phosphate buffer. The specimens were then fixed in a microwave oven set at 45ºC. After a rinse in 0.1 M phosphate buffer, specimens were dehydrated in a graded series of ethanol and embedded in paraffin wax. Paraffin sections 5 µm thick were stained in haematoxylin-eosin for routine examination. Adjacent sections were stained with a 0.5% toluidine blue solution (pH 2.0) for detection of mast cells. Other sections were also reacted with a biotinylated hyaluronan-binding protein probe for localization of hyaluronan. The probe was then visualized by the avidin-peroxidase technique (Hellström et al, 1990) and studied in a Zeiss Axiophot light microscope. The toluidine blue-stained sections were analysed for mast cells by use of a point-counting technique (Weibel, 1979). The morphometric measurements were made in three visual fields in each of at least two sections of each specimen using a graticule within the eyepiece of a light microscope. The measurements were made at an objective lens magnification of x 40.

1.2 Materials in human studies

The initial human pilot study consisted of 30 out-clinic patients from three different ENT departments in southern Sweden. The patients, aged 18–65 years, and of both sexes, were selected during the spring and early summer seasons. Patients treated with topical or systemic drugs for external otitis within 30 days before entering the study were not accepted. All patients had suffered at least one medically treated external otitis episode during the previous year. Patients with a known neoplasm, diabetes, multiple drug hypersensitivity, or lactose intolerance were excluded from the study, as were breast feeding and pregnant women and women planning to become pregnant. Only paracetamol was allowed for relief of pain. The treatment studies included topical treatment with BD (Diprosane®; Schering Plough, Inc., Heist, Belgium) in a 0.05% solution in combination with either systemic loratadine 20 mg or placebo.

The final human study was an open, randomized, parallel-group multi-centre trial performed at eight different ENT departments in Sweden covering both the northern and the southern regions. It included 51 patients of both sexes, aged 18–67 years. The inclusion and exclusion criteria were the same as in the previous pilot human study described above. Only paracetamol was allowed for pain relief.

The treatment for external otitis was topical treatment with BD (Diprosane®; Schering Plough, Inc., Heist, Belgium) in a 0.05% solution, or a solution of 1% hydrocortisone acetate combined with oxytetracycline and polymyxin B (HCPB) (Terra-Cortril polymyxin B®; Pfizer, Inc., Brussels, Belgium).

2. Experimental design

2.1 Experimental design in animal studies

Sprague-Dawley rats were used in all experiments. The animals were kept in cages and provided with water and food without restrictions. To initiate the inflammatory reaction of the EAC skin, anesthetized animals were mechanically stimulated in their right EAC by 400 rotations of a conical micropipette, at a speed of 80 rpm. This procedure was continuously observed under an otomicroscope. During anaesthesia caused by intravenous administration of sodium methohexital in the tail vein, bacteria, fungi and other substances were administered in later experiments. A numerical standardized grading system for determination of colour, swelling and effusion was also established in the animal model. The colour was graded as follows: 0 = normal; 1 = red; 2 = purple. With regard to grading of swelling, 0 = swelling absent or swelling with a diameter of at least 4 mm; 1 = swelling with a diameter of 3 mm or less; 2 = swelling with a diameter of 2 mm or less; and 3 = swelling with a diameter of 1 mm or less. The presence of effusion was classified as follows: 0 = dry; 1 = moist; 2 = fluid; and 3 = otorrhea.

In the first study (Paper I) no inoculation was performed. The animals were observed once daily on days 0–5, and again on days 7, 11 and 21. On days 3, 7 and 11 two rats each daily were sacrificed for histological sampling. The remaining animals were sacrificed on day 21.

In the second study (Paper II) the same external otitis model was used to test the treatments. The rats were divided into four different groups and treated topically, as follows: group A (n = 12) were treated with a group III steroid solution (BD); group B (n = 8) with hydrocortisone combined with oxytetracycline(HC) (Terra-Cortril®; Pfizer, Inc., Brussels, Belgium); group C (n = 8) with the same substance as group B but with addition of polymyxin B (HCPB) (Terra-Cortril Polymyxin B®; Pfizer, Inc., Brussels, Belgium); and group D (n = 12) with saline. All rats were observed daily during the first 7 days and again on days 10 and 20. Histological specimens of the EAC were collected on days 3, 7, 10 and 20.

The third animal experiment (Paper III) concerned external otitis infected with *C. albicans* or *P. aeruginosa*, and its treatment. A total of 77 animals were divided into three main groups, groups A, B and C. They were inoculated once and treated once daily for 7 days.

Group A (n = 36) were inoculated with *P. aeruginosa*, and treated with a group III steroid solution and divided into subgroups A1–A4; subgroup A1 (n = 8) were untreated controls while subgroup A2 (n = 11) were treated with 0.1 ml of 0.05% BD solution. Subgroup A3 (n = 11) were treated with 0.1 ml of HCPB; and subgroup A4 (n = 6) were treated with 0.1 ml of saline, at pH 5.0.

Group B (n = 35) were inoculated with *C. albicans* and treated in the following manner: subgroup B1 (n = 15) were untreated controls; subgroup B2 (n = 8) were treated with 0.1 ml of 0.05% (BD) solution; subgroup B3 (n = 8) were treated with 0.1 ml of HCPB; and subgroup B4 (n = 4) were treated with 0.1 ml of saline, at pH 5.0.

Group C (n = 6) were not infected, but within 1 minute post-stimulation were treated with 0.1 ml saline, pH 5.0, administered in the EAC.

2.2 Experimental design in human studies

The pilot study (Paper IV) was a prospective, randomized, double-masked trial consisting of 30 patients. All patients were given BD treatment. In addition, half of the patients were randomly assigned to concomitant treatment with loratadine while the remaining half of the patients received placebo.

On days 0, 3, 7, 11 and 21 the status of the EAC and auricle was examined and the colour, swelling and effusion were graded on a four-graded scale regarding colour and three graded scales for swelling and effusion. The overall condition of the patient was evaluated according to a similar four-graded scale.

In the second clinical study the design was an open randomized, parallel-group, multi-centre study involving 51 patients. The exclusive aim was to compare the clinical treatment efficacy of a topical solution containing 0.05% BD without any antibiotic component, in treating external otitis, with that of ear drops containing HCPB. The patients were randomized to two groups given either of the two above treatments. In this study as well as in the pilot study only senior ENT consultants were allowed to investigate the patients. Cultures were taken for fungi and bacteria on days 1 and 11. Twice daily during 2 weeks each patient recorded his or her symptoms, as well as consumption of paracetamol.

3. Microbiology

An animal model for infected external otitis (Paper III): To induce an external otitis condition with bacterial infection a 0.1 ml suspension of *P. aeruginosa*, at a concentration of 1.5×10^7 colony-forming units (CFUs)/ml, was used. The bacterial suspension was instilled after mechanical stimulation and the fluid allowed to rest in the EAC for 5 minutes before the animals woke up. The *P. aeruginosa* strains had been harvested from a sample of human

external otitis (Ao 7757) and preserved in a solution of tryptic soy broth (Acumedia, Baltimore, MD, US).

To induce an external otitis condition with a fungal infection a suspension of *C. albicans* at a concentration of 4.5 x 10^6 CFUs/ml was instilled in a volume of 0.1 ml into the mechanically stimulated EAC. The *C. albicans* strains had been obtained from the upper airway tract area of humans and preserved in a solution of Sabouraud broth (Lab M, Bury, UK).

Human cultures: In the human pilot study (Paper IV) cultivation of bacteria and fungi was performed in all cases with signs of infection. This meant that only 18 out of 30 patients were sampled. Culturing was performed in a routine manner at the various laboratories.

In the second human study (Paper V) EACs of all patients were cultured and the samples were specifically marked and handled by the various laboratories, according to a strict protocol. The bacteriological samples were taken with a slightly moist stick twisted three times in the EAC and then transported to the laboratory in a Copoban, Labdesign™, transportation tube. The samples were cultured by one primary draw and three draws on platelets with blood, CLED agar and haematin agar. If no growth was found after 1 day at 37ºC, or in a CO^2 environment at 37ºC (hematin agar), samples were incubated for 1 more day. On each platelet the number of colonies were counted for each of the following species: *Haemophilus influenzae, Streptococcus pneumococci, Moraxella catharralis*, *Streptococci* groups A, C and G, *Staph. aureus*, Enterobacteriacae, *P. aeruginosa*, G-negative rods.

The sampling of fungi was performed with a dry stick twisted three times in the EAC. Samples were placed into the Copan tube and transported. The culturing took place on Saboraud agar platelets with antibiotics added. Platelets were incubated for 10 days at a temperature of 25–30ºC.

Fungus colonies were identified and typed according to a simplified schedule revealing yeasts

such as *C. albicans* or others such as or *Aspergillus niger* and other Aspergillus species. Other moulds were not identified.

4. Statistical analyses

To test differences in thickness of the lateral portion of the rat EAC skin in Paper II, Student's *t*-test was used. In Paper IV the mean values of status were used to describe the EAC characteristics of the EAC- status. The Mann-Whitney non-parametric U-test was used to test for differences in the degree of inflammatory changes between the two groups. Also in this paper, Student's *t*-test was used to test for differences between the groups in terms of patient assessments. The difference was considered significant if $p \leq 0.05$. In Paper V when designing the clinical multi-centre study, we worked under the assumption of a true failure rate of 50% for the HCPB and 25% for the BD group. This made a number of 120 participants suitable with a power of 80% to reject the null hypothesis in the event of equal failure rates, performing a two-sided test at the 5% level. However throughout the study it became evident that it was not possible to recruit this number of patients within the time limits of the study. The strategy was changed and, according to the principle of intention to treat all randomized patients (n = 50) baseline and post-baseline evaluations were included in the analysis of the efficacy. In the analysis of safety data including all adverse events reported, all patients receiving at least one dose of study medication of either sort were included (n = 51). For variables of binary type – event or no event – including the primary efficacy variable, the two groups were compared using Mantel-Haenszels chi-squared tests, taking stratification by centre into account. With regard to variables of continuous type, analysis of variance was performed including treatment and centre effects. For ordinal variables, Wilcoxon's rank-sum tests were used. All tests were two-sided, and regarded significant at the 5% significance level. All data entered into the database were checked versus case record forms (CRFs) and

patient diaries by counter-proofreading. Furthermore, computerized checks of variable ranges were performed for all numerical variables in the CRFs and patient diary cards. An internal audit to scrutinize the results in the database was also performed, when two minor comments not affecting the analysis were noted.

RESULTS

1. An animal model for external otitis (Paper I)

Mechanical stimulation of the EAC, according to a standardized procedure, evoked inflammatory changes in the EAC skin. The alterations mimicked the status observed in the human EAC when subjected to external otitis. The mechanical stimulation of the EAC elicited redness, swelling and the leakage and accumulation of fluid in all twelve animals. We scored the various inflammatory changes by means of the grading scale. On day 1 colour, swelling and effusion were scored at 1.0, 1.7 and 1.9, respectively. On day 3 the inflammatory scores had started to decline and measured 0.9, 1.1 and 0.9, respectively. On day 7 the status started normalizing and no effusion was observed. On day 21 only minor inflammatory reactions were recorded, scoring 0.7, 0.7 and 0.2, respectively.

The predominant light microscopic findings were a swelling of the EAC skin and pronounced hyperkeratosis of the EAC. The swelling was caused by a massive oedema of the subepidermal connective tissue layer. Surprisingly few leukocytes were found. The mast cells decreased significantly. Also, an increased immunoreactivity to hyaluronan was observed in the connective tissue. The findings were most pronounced on day 3 but remained at day 7. By day 11 the oedema was reduced or normalized. Though the inflammatory changes still remained to some degree at an otomicroscopical level, the histological picture showed normalization. The left EAC skin of all animals appeared normal during the entire study, both

at the light microscopic and at the otomicroscopic level. The swelling as a result of the stromal oedema and inflammatory reaction was not accompanied by large masses of inflammatory cells. It is feasible to assume that we could have seen leukocytes had an earlier observation been performed. Probably the reduction in mast cells was the effect of cell destruction as the histamine granulas were released.

2. Experimental external otitis cured by a group III steroid solution without antibiotics (Paper II)

This study investigated the treatment effects of a group III steroid in comparison with a group I steroid combined with an antibiotic in the animal external otitis model previously developed. The study was performed in three topical treatment groups, with administration of 0.05% BD solution, HC and HCPB. A fourth group of external otitis animals was treated with saline, and served as controls. The same grading scale for determination of EAC variables colour, swelling and effusion as in Paper I was used. Immediately after the mechanical stimulation the EACs of all groups showed a similar grading score, viz. 2, 1 and 0, respectively. The scoring of the three parameters was summarized and the mean value of the EAC status 5 minutes after stimulation was 4.1–4.4. Effusion was observed about 1 hour after stimulation. The most marked improvement from day 1 to day 3 occurred in the BD group, but all groups had a tendency towards a lower grading by day 3. A complete recovery by day 5 was only demonstrated in the BD group. All groups except the HCPB-treated one had normalized by day 20.

The EAC skin of the animals with external otitis was thickened, with a less well-organized oedematous connective tissue layer and with the long axis of the fibroblasts irregularly oriented. The epidermal layer was covered with a hypertrophic layer of keratin and below the epidermal layer the basal layer appeared wavy. The skin reached its maximum thickness on

day 3, which was much thicker than in any of the treatment groups. All treatment groups, BD, HC and HCPB, exhibited a thin keratin layer although that of the HCPB-treated animals was the thickest. On day 3 the BD group had a significantly thinner epidermal skin layer ($p \leq 0.05$). Also, the oedema of the connective tissue layer was less pronounced in the steroid-treated groups than in the saline group. There were few inflammatory cells in the steroid-treated EACs and their number gradually decreased from day 3 to day 20. Mast cells sparsely occurred at any time point but likewise seemed to decrease throughout the study. In this study normalization occurred faster in the BD group and the animal model proved suitable for the treatment purpose.

3. A topical steroid without an antibiotic cures infected external otitis efficiently: a study in an animal model (Paper III)

In this study the external otitis model with otitis evoked by mechanical stimulation was combined with infection both bacterial and fungal. This infected external otitis model was then used for treatment studies.

The *P. aeruginosa*-infected animals (group A) were divided into four subgroups, viz. an untreated group serving as controls; a topical BD-treated group; a group in which HCPB was administered; and a saline of pH 5.0 group.

The *C. albicans*-infected animals (group B) were also divided into four subgroups, viz. an untreated group used as controls; and a topical BD; a topical HCPB; and a saline of pH 5.0 group.

A third group, group C, consisted of uninfected, saline-treated animals.

Five minutes after mechanical stimulation all EACs showed manifest external otitis with a mean total scoring of the EAC status of 3–4.

By day 2 only the status of the *P. aeruginosa*-infected EACs treated by BD had started to improve. On day 3 the other treatment groups also showed some improvement. At day 20 only the BD-treated group was cured.

All *C. albicans*-infected subgroups reached a similar high score during days 0–3, thus not indicating any improvement. On day 7, however, the *C. albicans*-infected animals treated with BD were graded as 0 while all other *C. albicans*-infected animals still showed pronounced inflammatory status with a score of 0.9–1.2. The *C. albicans*-infected EACs treated by HCPB did not improve at all from day 3 to day 10. At day 20 the *C. albicans*-infected groups treated with HCPB still remained diseased. The uninfected and saline-treated group demonstrated a tendency towards an improved status on day 7 and had reached complete recovery by day 20.

Three days after inoculation of the EAC with *P. aeruginosa* the untreated EACs exhibited a swollen keratinized epithelium layer and a massive inflammatory cell infiltration into an oedematous connective tissue layer. Frequent inflammatory cells covered the surface of the EAC skin. Both BD and HCPB treatment reduced the inflammatory reaction, with the most pronounced effect occurring in the BD group. By day 20 all groups had normalized.

The skin of the *C. albicans*-infected EACs was extremely thickened, which was mainly caused by a layer of oedematous connective tissue, which was also invaded by inflammatory cells. The structure of the basal membrane below the epidermal layer was irregular, with swollen keratinocytes. By day 3 the BD-treated group had almost normalized. Though they still had a slightly oedematous connective tissue layer there were few inflammatory cells. By day 20 the structure of the EAC skin in both the BD- and the HCPB-treated groups of the *C. albicans*-infected external otitis animals had normalized.

Bacteriological culturing for the *P. aeruginosa*-infected, non-treated EACs showed positive cultures for all animals at days 3, 5 and 7. On day 10, two out of four EACs still showed

positive cultures. The *C. albicans*-infected, non-treated animals also showed positive cultures on days 3, 5 and 7. On day 10, seven out of nine animals were positive and on day 20, two out of seven EACs were still positive.

In the BD and HCPB treatment groups all animals infected with *P. aeruginosa* showed negative cultures at days 10 and 20.

In the *C. albicans*-infected animals positive cultures were present at all times except in the BD-treated group on day 20. In the saline group (pH 5.0) all *P. aeruginosa*-infected EACs were culture-negative on days 10 and 20. By contrast, the *C. albicans*-infected EACs still remained infected when treated with saline.

In this work the animal model proved suitable for infection and treatment of external otitis. The benefit of adding an antibiotic to a topical drug containing a steroid was not obvious.

4. External otitis cured by a group III steroid solution with an antihistamine added (Paper IV)

The aim of this study was to elucidate the efficacy of group III steroid BD solution for treatment of external otitis. Furthermore, we wanted to investigate whether addition of an antihistamine, loratadine, could improve the treatment results. Thirty patients aged 18–62 years were enrolled in this pilot study. They were all treated daily for eleven days with BD but half of them were also exposed to daily treatment with 20 mg of loratadine. There was no difference in age or sex distribution between the groups. All patients completed the study. The status of all EACs was evaluated by the ENT surgeon according to a grading scale for colour, swelling and occurrence of effusion. A VAS grading of the ability to work, as well as of itching, pain, sleep disorders and overall condition expressed as healing was performed by each patient. On day 11 the EACs showed an almost normal status in all patients. At the end of the acute phase of the study, on day 20, all patients but one were cured (97%). Already on

day 3 the scores for swelling, extension of redness, effusion and colour had decreased markedly. All patients were able to work on day 3. No side effects of any of the treatments were reported. Only 18 patients with clinical signs of infection were cultured and 14 were positive. *P. aeruginosa* was found in five out of 14, *P. mirabilis* in three and *Staph. aureus* and a mixture of bacteria in two cases each. Two cases of fungi were found, both with *C. albicans*. It was concluded that the BD treatment without an antibiotic component did have 97% efficacy during the 20 days of evaluation. Addition of an antihistamine did not significantly improve the result. The improvement of the patients' status and symptoms was the same in patients considered infected and patients considered not infected.

5. External otitis cured by use of a topical group III steroid, without any antibiotics (Paper V)

The main purpose of this study was to evaluate the efficiency of treatment for external otitis with regard to a decreased number of relapses during a 6-month period post-treatment. Also, the study focused on the number of cured patients at the end of the treatment period and a few days on.

Fifty-one patients were enrolled in the study and divided into two treatment groups, a BD-treated (n = 26) and a HCPB-treated (n = 25) group. The same grading scale for evaluation of swelling, effusion and redness as in Paper IV was used. Patients had to fill in diary cards with a VAS grading of their symptoms.

In the BD group 58.3% were cured by day 11 and remained relapse-free for 6 months. The corresponding figure for the HCPB group was 27.3% ($p<0.03$).

The grading of the EAC status on days 4 and 11 in the BD and HCPB groups was in favour of the BD group ($p<0.01$). The VAS grading of the overall condition of the patients also favoured treatment with BD on day 4 ($p<0.01$) but on day 11 there was no statistically

significant difference between the treatment groups. The number of patients complaining of itching also differed between the groups ($p<0.01$), with less itching in the BD group. Regarding pain relief (paracetamol tablets), a difference was seen between the groups, with a higher consumption by the HCPB group than by the BD group (5.2 v. 1.8). The culturing on day 0 revealed that Staphylococcus strains predominated and occurred in 60% of patients (n=47). *P. aeruginosa* was the second most common bacterium and occurred in 20% of patients. Regarding fungi, *C. albicans* was the predominant fungus and occurred in eight out of altogether 15 positive fungal cultures on day 0. No growth was seen in 12% of the BD and in 9% of the HCPB groups at day 0.

On day 11 post-treatment, 40 cultures, 22 from the BD and 18 from the HCPB groups, were obtained. Growth of bacteria (no specification) was present in the BD group in 32% and in the HCPB group in 44%. Growth of fungi (no specification) represented 9% and 28%, respectively. No growth at all was observed in 59% of the BD-treated patients and in 28% of the HCPB-treated subjects. The less frequent growth of bacteria and fungi in the BD group compared with the HCPB group on day 11 was statistically significant ($p<0.03$ and $p<0.01$, respectively). To conclude, in this study the BD was superior or equal to HCPB in eradicating bacteria and fungi and the status evaluation and the patients' VAS grading were not in favour of adding the antibiotic HCPB steroid solution.

DISCUSSION

Is it a sign of high medical standards when a medical practitioner has 20 or so different therapies to choose from as alternative treatments in a patient with one specific diagnosis, in this case external otitis? This question could be combined with questions about the obvious risk of discomfort to a patient not given a simple, safe and cheap therapy.

External otitis is not a life-threatening condition but because of the great volume of patients involved it is associated with great cost to the health care system.

The wish to find an effective, simple, non-antibiotic and cheaper alternative to the commonly prescribed topical solutions or high-viscosity topical drugs combining steroids and antibiotics for treatment of external otitis was the background to the studies presented in Papers I–V. It is of utmost importance to find an evidence-based, cost-effective treatment for external otitis.

Questions asked concerning external otitis focus not only on pathogenesis, incidence, bacterial and fungal growth, resistance to antibiotics, ototoxic properties of vehicles or drugs in topical preparations, cleansing problems, wax, and relationship to skin diseases and contact allergy; they also focus on costs.

Papers I–III in this thesis revealed that an animal model for external otitis could be established and that this model is also suitable for evaluation of treatment strategies in both infected and uninfected external otitis. It was concluded that a group III steroid solution (BD) without antibiotics added was superior to a generally prescribed group I steroid combined with antibiotics (HCPB) for curing external otitis in both uninfected and infected animals.

There is always a fear of adverse effects of steroids. In this context it is of importance to state that no damage to the tympanic membrane or any signs of effusion in the middle ear occurred in either the BD- or the HCPB-treated animals. The efficacy of these drugs revealed in the animal model was then tested in clinical human trials (Papers IV and V). These showed that

external otitis patients topically treated with a group III steroid (BD) had fewer relapses during a 6-month period following an acute episode of external otitis, than did those treated with group I steroids combined with two antibiotics. It is noteworthy that most other clinical trials on external otitis have fairly short follow-up periods, lacking any long-term follow-up (Van Balen et al, 2003). Against the background of the above findings the eight questions listed below will be discussed.

Question I: Can an animal model for uninfected and infected external otitis be established?
In Papers I–III we report the development of an external otitis model, from its induction by mechanical stimulation with a 4 mm funnel rotated 400 turns in the EAC during 5 minutes, to infection of mechanically stimulated EACs with *P. aeruginaosa* and *C. albicans*. The EAC was treated with BD, HCPB and saline. To evaluate the EAC status we constructed a new standardized graded scale for use both in animal and in human studies. Describing the status of two different observations on a standardized scale is crucial in animal studies and is a problem in treatment of patients when practitioners alternate in acute out-clinic practice. The skerries in the diagnostic sea are plenty. Consequently three main landmarks of EAC status were set, viz. colour (with a score range of 0–2), swelling (0–3) and effusion (0–3).
Also, similarities in anatomy between rats and humans concerning the diameter of the EAC, epidermic structures and tympanic membrane are of importance in the model. The safety, by tail vein injections, of anesthetizing drugs, with no accidentally killed animals, also makes the studies easier to validate than had a few animals "disappeared" from each subgroup. The animal model using both infected and uninfected animals was scrutinized when making comparisons between the status and the time to full cure and/or culturing results. In conclusion, the model seemed to relevant to each stated purpose.

The scale was shown to be appropriate both for animal and for human studies. Use of a standardized scale for describing the status is crucial in animal studies for comparison of treatment results. It should be useful also in clinical practice, and properly used this will be an objective method for evaluating the efficacy of a treatment strategy.

Question II: Is it possible to standardize the evaluation of the status of external otitis by use of a graded scale? As part of this question we must also ask whether such graded scale could be used for studies in animal models as well as in humans.

One of the main difficulties in evaluating the results of previous clinical studies of external otitis relates to the degree of inflammatory changes or severity of the disease in treated patients. This has led to the development of the present standardized graded scale of colour, swelling and effusion to determinate the degree of inflammatory changes of the EAC skin. The graded scale also allowed the investigators to standardize the EAC status at the different ENT centres involved in these multi-centre studies. Thus the severity of the external otitis condition could be translated into figures which could be completed for each patient and animal before, during and after the treatment.

In the experience of the author the status of the EAC is rarely accurately described in the medical record at the start of medication. This makes it difficult for the practitioner, ENT surgeon or other doctor at the next consultation to objectively assess the status and degree of improvement. In the clinical trials in these studies the results were further strengthened by the subjective evaluation of the symptoms by the patient by use of a VAS. There was good agreement between the severity of external otitis and the estimation of the symptoms by the patient on the grading scale.

Question III: Is a topical group III steroid solution without an antibiotic at least as efficient in curing external otitis as a topical group I steroid suspension with two antibiotics added? The studies in the external otitis model showed that a group III steroid (BD) without antibiotic components definitely cured the inflamed external ear canal skin (Paper II).

In Paper III the efficacy of the treatment of external otitis in the rat model infected with a bacterium or a fungi was investigated. The finding in Paper III that also in an infectious condition, bacterial or fungal, treatment with a group III steroid was superior to treatment with a group I steroid with antibiotics added was intriguing. The observations in the clinical trials in Papers IV and V were in good agreement with the data obtained in the animal model. Thus BD showed better or similar efficacy as HCPB in eradicating both *P. aeruginosa* and *C. albicans.* The difference in efficacy between the drugs was not only recorded as alterations in EAC status but also applied to the degree of symptoms. Thus the itching was significantly lower ($p<0.01$) in the BD group in comparison with the HCPB group. However, it must be stressed that the design of the pilot study (Paper IV) has some weaknesses as the patient number was low and there were no clear-cut controls as the steroid treatment was evaluated in combination with another drug, loratadin. Furthermore, all ears were not systematically cultured.

Question IV: What is the role of infection in external otitis? Does infection have implications for the selection of treatment drugs? What do the treatment results tell us about the mechanisms behind the inflammation?

Many colleagues will disagree with the suggestion that a steroid can be used to treat an infectious inflammatory condition, in particular when fungi are involved. Obviously a group III steroid can cure culture-positive external otitis as well as or even better than a group I steroid with antibiotics added. This may be interpreted to mean that the major mechanism in

the pathogenesis of external otitis is inflammation. If the inflammatory reaction is pronounced the swelling of the EAC skin will allow a low-viscosity solution such as BD to penetrate into the lumen of the canal, while the high viscosity of the HCPB will be a hindrance. It is also feasible to assume that the event triggering the inflammatory reaction is the trauma to the EAC skin, e.g. induced by mechanical cleansing (irritation or extensive exposure to alkaline water). In the sequence of events the trauma will precede the invasion of microbes. The action of steroids in this condition can only be speculated on. The steroids will reduce the swelling and therefore increase the blood flow to the micro veins. This may lead to a wash-out of noxious substances from the diseased area. But also assumptions concerning changes in the acidity of the EAC environment should be considered. The mean pH value of the normal EAC is 5.7. In chronic external otitis the pH is elevated to 6.7 (Devesa et al, 2003). The mean pH value of the BD solution is 5.0. This slightly acidic solution could influence the survival of the infectious microbes. In particular *C. albicans* will have difficulties to survive in this milieu. A positive effect on resolution of external otitis was shown in the animal model by the acidic pH of 5.0 (Paper III). The BD solution also contains isopropyl alcohol and the role of alcohol in eradicating fungi has long been accepted. However, the effects of isopropyl alcohol on *C. albicans* have been tested in vitro and it has been shown that BD solution is more efficient than is isopropyl alcohol in eradicating *C. albicans* (Emgård, 1991, unpublished investigation).

The present results are supported by a study by Tsikoudas et al (2002), in which steroid solution alone was compared with a steroid solution with aminoglycoside added. It cannot be excluded that difference in viscosity may have influenced the results when comparing the effect of the BD and the HCPB solution. Topical ear drops with low viscosity, such as the BD solution, may quickly reach the surface of the EAC, in particular when administered in a swollen EAC.

Question V: What are the similarities in the structure of the EAC and reactions to external otitis when comparing the human with the animal model? Is it possible to transfer the treatment results obtained in an animal model to human conditions?

The diameter and length of the EAC in the rat and 5-year-old humans are surprisingly similar (personal experience). In the rat the bony canal is, however, very short and occupies a smaller part of the ear canal than it does in small children. In the lateral portion of the EAC of rats the skin is thick and contains hair follicles, the latter lacking in the medial portion towards the tympanic membrane, which also is much thinner. In terms of the histology of the EAC skin the rat and human resemble each other closely. The tympanic membrane of the rat is thinner than the human tympanic membrane and less conically shaped. In contrast to most other experimental animals the rat has an EAC which can be easily inspected through an ear speculum.

Both the status of the EAC when subjected to external otitis and the reaction to various topical treatments, whether infected external otitis or not, showed many similarities. In fact, treatment results with BD as well as HCPB were almost identical between the animal model and the patient material. This strongly supports the argument that the findings in this particular external otitis model can be transferred to the human condition. It should be mentioned that the efficacy of the tested drugs in eradicating *P. aeruginosa* and *C. albicans* was investigated elsewhere in an in vitro test (Emgård, 1991, unpublished investigation). "Artificial" EACs were pierced and applied to regular Agar gels for culturing of bacteria and fungi. In these moulded Agar gel EACs the microbes were treated with BD and HCPB. The study showed that BD more efficiently eradicated both *P. aeruginosa* and *C. albicans* than did HCPB.

The comparison between the treatment results in the animal model and the human studies may, however, suffer somewhat from the differences in the early phase after having administered the drugs. In humans the BD droplets may stay in place and be exposed to the EAC skin for a longer time than when allowed to wake up after the anaesthesia, and therefore there is a risk in animals that some of the applied drug will disappear immediately.

Question VI: Are there any severe adverse events when treating external otitis with a strong (group III) topical steroid solution?

There is always a "steroid fear" (Charman et al, 2000) amongst prescribers. Also, the possibility of a potentially ototoxic substance is a general fear. More rarely prescribers are aware of the sensitizing property of the steroid substance. Also, the placebo effect may diminish if the prescriber is in doubt of the prescribed drug.

Regarding local reactions the BD did not cause any damage to the tympanic membrane in either the animal model or the clinical studies. Neither did any animals treated with BD show any signs of systemic illness, e.g. weight loss, deteriorating fur quality, biting scars or vertigo. Furthermore, none of the patients in the clinical studies complained of any change in their hearing capacity after BD treatment. By contrast, HCPB often causes such complaints as owing to its high viscosity HCPB will often remain on the tympanic membrane days after being instilled into the EAC, and cause hearing impairment. This unwanted property of HCPB is also a problem when the patient uses a hearing aid. The greasy HCPB may plug the canal of the hearing aid. With its water-like appearance BD will have much shorter contact with the EAC skin and the tympanic membrane.

With regard to possible ototoxic properties of steroids, it is feasible to refer to the experimental studies by Spandow (1989). In Sprague-Dawley rats Spandow investigated the effect on the inner ear of various topical drugs used in otology. He showed that 2% of

hydrocortisone micronized in sterile water, administered once daily for 5 consecutive days caused impaired auditory-evoked brainstem response (ABR) thresholds in the high-frequency area. It must, however, be stressed that the steroid was administered directly into the middle ear cavity. In another experiment the same author instilled 70% isopropyl alcohol into the middle ear cavity and exposed the round window niche for 10 minutes (Spandow, 1989). No hearing impairment could be detected with this drug; however, after 2 hours of adherence longer latencies were visible in the ABR. In another study on inner ear effects of steroids triamcinolone was administered into the middle ear of chinchillas (Picket et al, 1997). No impairment of the action potentials from the round window membrane could be recorded. To the best of our knowledge no ototoxic effects of the steroids investigated in the studies discussed (Papers I–V) have been reported. The conclusion of all the experimental studies with regard to influence of steroids on the inner ear is that possible ototoxic effects must be considered, but as long as the drug is administered in the EAC, and the tympanic membrane is intact, the treatment is safe.

Question VII: When selecting treatment for external otitis should both medical aspects and economic considerations be taken into account?

The variation, in price per millilitre, of drug preparations with comparable therapeutic efficacy in treatment of external otitis was investigated in the UK (Reilly & Skinner, 1990). There was a 577% difference between the cheapest and most expensive drugs used. The more complicated topical drugs, e.g. steroids with antibiotics added, were more expensive than a drugs without antibiotics. In Sweden the difference in cost between HCPB and BD is less obvious. However, HCPB is not recommended in external otitis with a fungal infection, which is when more expensive topical drugs must be used.

When selecting a drug for treatment of external otitis the viscosity must also be considered as a preparation such as the high-viscosity HCPB will have a potential risk of occlusion of the EAC. Apart from viscosity the recommended number of applications per day will also affect the compliance, as shown in one study (Shikiar et al, 1999). Also, the fact that a topical drug is more expensive in pipettes than in a bottle but easier to medicate in the prescribed quantities using pipettes could be considered.

Question VIII: Could the present research inspire treatment strategies not yet accepted for group III steroid solutions?

Patients with chronic otitis media have thick mucosa in the middle ear cavity and a spectrum of high minimal inhibitory concentration (MIC) values for nearly all antibiotics. They are today prescribed HCPB, but perhaps BD without an antibiotic can be considered?

Another possible field of research is patients with grommets in the tympanic membrane and otorrhoea. The most common treatment in Sweden, to my knowledge, is HCPB. It is not certain that HCPB actually enters the middle ear cavity. As the viscosity of the HCPB is high I suggest using the low-viscosity BD solution as an alternative. Other patients, most often elderly patients with hearing aids and external otitis, often present as out-clinic patients, in my experience. To these patients, plugging of the hearing aid channels is a problem when using HCPB.

CONCLUSIONS

External otitis can be induced by mechanical stimulation of the lateral part of the EAC in rats.

This animal model proved useful when after stimulation the EAC was treated with BD or HCPB.

The BD-treated animals were cured as efficiently as were the HCPB-treated group.

The animal model infected by *P. aeruginosa* or *C. albicans* proved suitable for testing BD and HCPB treatments. The results still favoured BD treatment.

A similar result was obtained in animal and in human studies (Papers I–V).

In the human study the efficacy of treatment in the acute phase, at 20 days, and during a relapse-free period of 6 months significantly favoured BD treatment.

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REFERENCES

van Balen FAM, Smit WM, Zuithoff NPA, Verheij TJM. Clinical efficacy of three common treatments in acute otitis externa in primary care: randomised controlled trial. BMJ 2003 ; 327: BMJ.com 1-5

Barlow DW, Duckert LG, Kreig CS, Gates GA. Ototoxicity of topical otomicrobial agents. Acta Otolaryngol (Stockh) 1994; 115: 231-235

Brook I, Frazier EH, Thompson DH. Aerobic and anerobic microbiology of external otitis.
Clin Inf Dis 1992; 15:955-958

Cantrell HF, Lombardy EE, Duncanson FP, Katz E, Barone JS. Declining susceptibility to Neomycin and Polymyxin B of pathogens recovered in otitis externa clinical trials. Southern Med J 2004; 97: 465-471

Cassi N, Cohn A, Davidson T, Witten BR. Diffuse otitis externa: clinical and microbiologic findings in the course of a multicentre study on a new otic solution. Ann otol Rhinol Laryngol 1997;86(Suppl 39): 1-16

Charman CR, Morris A, Williams HC. Topical steroid `Phobia`in patients with atopic eczema. Br J Dermatol 2000; 142: 931-936

Clark WB, Brook I, Bianki D, Thompson DH. Microbiology of otitis externa. Otolaryngol
Head Neck Surg 1997; 116: 23-25

Devesa PM, Willis CM, Capper JWR. External auditory canal pH in chronic otitis externa 2003; 28:320-324

Dibb W L Microbial aetiology of otitis externa. J Inf 1991; 22:233-239

Emgård P, Hellström S, Holm S. In vitro study of an otitis externa model, in manuscript

Emgård P, unpublished investigation, in manuscript

Feidt H, Federspil P. Eigene Untersuchungen uber das aktuelle Spektrum der pathogenen Keime bei der Otitis externa und der chronischen Mittelohrentzundung. Laryngo Rhino Otol 1989; 68: 401-406

Fränki JE, Kalimo K, Tuohimaa P, Aantaa E. Contact allergy to various components of topical preparations for treatment of external otitis. Acta otolaryngol 1985; 100: 414-418

Hannely MT , Dennely III JC, Holzer SS. Consensus panel report, use of ototopical antibiotics in treating 3 common ear diseases Otol Head Neck Surg 2000; 122: 934-940

Hellström S, Tengblad A, Johansson C, Hedlund U,Axelsson E. An improved technique for hyaluronan histochemistry using microwave irradiation. Histochem 1990; 22: 677-682

Hughes E, Lee H J Otitis Externa . Pediatr Rev 2001; 22: 191-197

King III D M, Estrem SA. Gentamicin iontoforesis in the treatment of bacterial otitis externa in the guinea pig model. Laryngoscope 1990;100(10): 1112-1115

Lancester J, Mathews J, Williams R S, Thussey C, Kent S E. Comparision of compliance between topical aural medications. Clin Otolaryngol 2003; 28: 331-334

The external ear. Lucente E F, Lawson W, Novick N L. eds.WB Saunders Company, Philadelphia, US, 1995; pp1-17

The external ear. Lucente FE, Lawson W, Novick NL. Eds.(WB Saunders company): Philadelphia, US, Diseases due to infection 1995: pp 49-97

Maher A, Bassiouny A, Moawad M K , Hendawy D S Otomycosis: An experimental evaluation of six antimycotic agents. J Laryngol Otol 1982; 96:205-213.

Marsh RR, Tom LWC. Ototoxicity of antimycotics. Otolaryngol Head Neck Surg 1989; 100: 134-136

Medical Index Sweden, MIS 1997; Läkemedelsstatistik AB, Stockholm, Sweden

Mugliston T, O`Donoghue G. Otomycosis-A continuing probleme. J Laryngolog Otolog 1985; 99:327-333.

Palomar GV, Palomar AV. Are some drops ototoxic or potentially ototoxic? Acta otolaryngol 2001;121.565-568

Picket BP, Shinn JB, Smith MFW. Ear drop ototoxicity: reality or myth? A J Otology 1997; 18: 782-791

Reilly G, Skinner FR. Antibiotic/steroid ear drop preparations: a cost – effective approach to their use J Laryngol Otol 1990; 104: 862-864

Roland P S, Stroman D W. Microbiology of acute otitis externa. Laryngoscope 2002; 112: 1166-1177

Roland PS. External otitis: a challenge in management. Curr Inf Dis Rep 2000; 2: 160-167

Rowlands S, Devalia H, Smith C, Hubbard,Dean A. Otitis externa in UK general practice: a survey using the UK general Practice Research Database. Brit. J. Gen. Pract 2001; 51, 533-538

Russel PT, Church CA, Jinn TH,Kim DJ, John EO, Jung TK. Effects of common topical otic preparations on the morphology of isolated cochlear outer hair cells. Acta otolaryngol 2001; 121;135-139

Selesnick HS. Ed. The Otolaryngol Clin north America, The external auditory canal 1996; pp725-727

Shikiar R, Halpern M, McGann M, Palmer CS, Seidlin M. The relation of patient satisfaction with treatment of otitis externa to clinical outcomes: development of an instrument. Clin Therapeut 1999;21:1091-1104

Smith I M, Keay D G, Buxton P K. Contact hypersensitivity in patients with chronic otitis externa. Clin Otolaryngol 1990; 15: 155-158

Sobol S E, K Sundeep, Pavadia J K , Crombleholme T, Potsic P W. Effect of corticosteroid-antibiotic agents on granulation tissue in a murine model. Arch Otolaryngol head Neck Surg

131(4): 330- 335

Sood S, Strachan DR, Tsikoudas A, Stables GI. Allergic otitis externa . Clin Otolaryngol 2002; 27: 233-236

Spandow O. Inner ear influences of substances passing from the middle ear. Thesis: Umeå University 1989

Stroman DW, Roland PS, Dohar J, Burt Wayne 2001, Microbiology of Normal external auditory Canal. Laryngoscope 111: 2054-2059

Tong MCF, Woo JKS, van Hasselt CA. A double blind comparative study of ofloxacin otic drops versus neomycin-polymyxin B-hydrocortisone otic drops in the medical treatment of chronic suppurative otitis media. J. Laryngol Otol 1996; 110:309-314.

Tsikoudas A, Jasser P, England R J A. Are topical antibiotics necessary in the management of otitis externa? Clin Otolaryngol 2002; 260-262

Weibel E R. Stereological methods. Practical methods for mycological morphometry . Academic Press , London 1979;1: 101-161

Wilkinson S M, Beck M H. Hypersensitivity to topical corticosteroids in otitis externa. J Laryngol Otol 1993; 107: 597-599

Wright CG, Pawlowski KS, Roland PS, Meyerhoff W L. An animal model for external ear canal inflammation. Laryngoscope 2000;110: 1112-1118