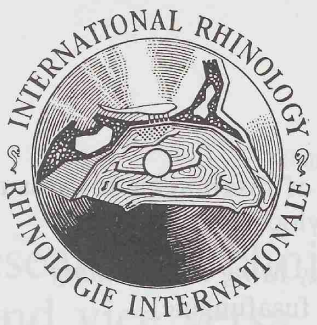

RHINOLOGY

Supplement No. 5 1988

LOCAL TREATMENT OF UPPER RESPIRATORY TRACT CONDITIONS

ROLE OF LOCABIOTAL® * AEROSOL (fusafungine)

Proceedings of an International Meeting
Athens, April 28–May 1, 1988

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Under the auspices of the European Rhinologic Society

Organized by Institut de Recherches Internationales Servier, France

Rhinology, Suppl. 5, 3–4, 1988

From basic research to clinical practice and vice versa

A.J. Coyas

The development of a drug must comply with extremely rigorous criteria. Before being made available to the clinician, a therapeutic molecule undergoes a long, perilous and ruthless course.
These essential steps confirm the reality of the findings observed with the substance and define its properties and expected effects.

It is a long way from the truth to imagine that everything has been discovered once the drug is in the hands of the therapist. Clinical experience is equally valuable, as it always enriches the scientific dossier and guides the clinician in his or her therapeutic choice.

To coincide with the international E.N.T. meeting organized by the European Rhinologic Society on the subject of Inflammatory Diseases of the Upper Respiratory Tract, we thought it essential to review recent studies concerning fusafungine (Locabiotal® Aerosol).

Using rhinomanometry in allergic rhinopathies as well as clinical and endoscopic examinations in rhinosinusitis, in which inflammation is frequently the predominant sign, the anti-inflammatory activity of fusafungine has now been able to be partly explained. Recent findings have shown that fusafungine regulates the production of superoxides by inflammatory cells such as macrophages.

In parallel, advances in the field of microbiology have furthered our understanding of the antibiotic activity of fusafungine. Legionella pneumophila, discovered barely a decade ago, escapes the activity spectrum of many antibiotics, but not that of fusafungine.

A surprising discovery was that certain nosocomial strains of Staphylococcus, already resistant to third generation cephalosporins, were still sensitive to fusafungine. This reflects the good antibacterial stability of fusafungine.

Investigators and clinicians met together in Athens to share their experience concerning fusafungine. The fruits of this meeting are presented in this issue. Fully accredited by research, fusafungine is today's modern answer, adapted to the challenge of respiratory tract infections.
Although science is at the service of medicine, the reverse is also true. Our duty is to continually investigate the drugs available with the imperative of leaving no stone unturned.

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Rhinology, Suppl. 5, 5–15, 1988

Functional surgery in inflammation of the nose and paranasal sinuses

E.H. Huizing

Functional surgery in inflammation of the nose and paranasal sinuses serves three goals: 1. eradication of disease, 2. improvement of breathing and 3. restoration of sinus ventilation and drainage.
Previously, surgery was the main mode of treatment in nasal and sinus infection. Today, medical therapy with antibiotics (systemic and local), corticosteroids and antihistamines has become our first choice. In patients with acute infections of the nose and sinuses without complications medical treatment is, indeed, our better therapy. In chronic sinus disease, however, surgery has remained indispensable, while in patients with recurrent acute infections, corrective surgery of the septum, the turbinates and sinusoidal ostia can be of great value in preventing upper respiratory infections. Thanks to the developments in rhinologic surgery during the last decades (re)creating anatomical and physiological normality has become possible.

WHAT HAVE BEEN THE MAIN CHANGES IN NASAL AND SINUS SURGERY DURING THE LAST FEW YEARS?

1. In septal surgery the old submucous septum resection has been completely abandoned and replaced, in the sixties, by a concept of septal reconstruction, or septoplasty as it is named by some authors.
2. Turbinate (micro)surgery, although already described long ago, has been reviewed thanks to the instrumental improvements of the last decades.
3. The Caldwell-Luc operation is carried out less and less and is nowadays reserved only for patients with severe antral polyposis.
4. Claoué's operation (inferior naso-antrostomy) is also less and less practised. Restoration of ventilation and drainage through the middle meatus is nowadays considered much more physiological.
5. Infundibulotomy with enlargement of the antral ostium has therefore replaced inferior nasal antrostomy in the majority of cases.
6. Endonasal anterior ethmoidectomy is often combined with infundibulotomy in order to restore drainage and ventilation through the middle meatus.

7. Endonasal (sub)total ethmoidectomy is nowadays considered an essential step in treating pathology of the maxillary and frontal sinuses. It makes a Caldwell-Luc operation, as well as the external approach to the frontal sinus, unnecessary.

SEPTAL SURGERY

Septal surgery is one of the procedures that may be beneficial in treating chronic upper respiratory infection. It may also be helpful in diminishing the number of recurrences of acute infection and the concomittant symptoms of frontal and facial headaches and sensations of frontonasal pressure. The new techniques enable us to correct both the major as well as the minor septal deformities in a more physiological way, while at the same time the incidence of complications such as septal perforations is greatly diminished.

MIDDLE MEATUS OBSTRUCTIVE SYNDROME

In a normal, healthy nose there should be no contact neither between the septum and the middle turbinate nor between the middle turbinate and the lateral nasal wall. Permanent contact between these surfaces enhances infection and may give rise to referred neuralgic pain and/or pressure sensations (Figure 1). At the contract area the mucosa becomes swollen and the cilia will disappear. Mucociliary clearance will be impaired and stagnation of secretion leads to infection and further swelling of the mucosa, resulting in polypoid degeneration, especially of the middle turbinate. The infundibulum will become blocked and consequently the drainage and ventilation of the maxillary and the frontal sinus and of ethmoidal cells become impaired. This sequence of events is generally accepted as the pathogenesis of sinusitis and of nasal and sinusoidal polyposis.

This Middle Meatus Obstructive Syndrome (Table 1) can have a number of causes. Usually many factors are involved: allergy, hyperreactivity, infection

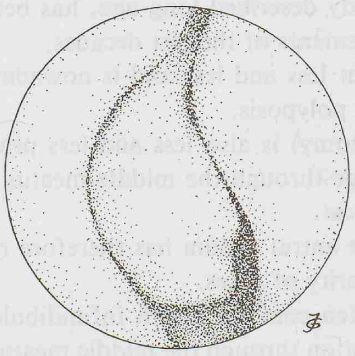

Figure 1. Middle Meatus Obstructive Syndrome on the right due to septal deviation and concha media bullosa.

Table 1. Frequent causes of Middle Meatus Obstructive Syndrome.

Septal Deviation Area 4
Middle Turbinate Pathology
– Concha Bullosa
– Concha Spongiosa
– Concha Polyposa
Middle Meatus Mucosal Pathology
– Mucosal Swelling
– Naso-ethmoidal Polyposis

and anatomical variations or deformities of the septum and the middle turbinate. Surgical correction of these anatomical variations and deformities can be of great help in treating chronic and recurrent upper respiratory infection. Careful examination of the middle meatus in these patients is therefore mandatory.

Septal deformations leading to the Middle Meatus Obstructive Syndrome may be: 1. a septal deviation in area 4 and/or 5, and 2. a septal spur and/or spine. Deviation of the septum in the region of the head of the middle turbinate is so common, as to be almost normal. The upper ventral part of the perpendicular plate is relatively thicker and at the transition of the cartilaginous septum and the perpendicular plate deflections and deformities are very common. Deformities in this area should be resected, thereby preserving the K-area. Repositioning, only, is usually not sufficient because of the excess of material and the thickness of the bone. The resected part of the bony perpendicular plate has to be thinned outside the nose and reimplanted (Huizing, 1974). This procedure has to be combined, in many cases, with surgery of the middle turbinate (see below).

Septal spurs and spines occur almost invariably in the lower nasal passage. They are well known and much less, often overlooked than the pathology above discussed. In contrast to what is generally believed, a septal spur and spine usually do not impair nasal breathing. However, as a consequence of their impact with the inferior turbinate they lead to mucosal swelling and impairment of mucociliary clearance. They can therefore, indirectly play a role in nasal and sinus infection and are better corrected.

The approach is through a caudal septal incision (hemitransfixion). In a unilateral vomeral spur a septal tunnel on one side may suffice, but usually bilateral tunnels are required. In the case of gross pathology they are to be combined with tunnels on the nasal floor (inferior tunnels). Spurs and spines are always made up of irreversibly deformed bone and cartilage and thus have to be resected. In reconstruction, care must be taken that the septal cartilage

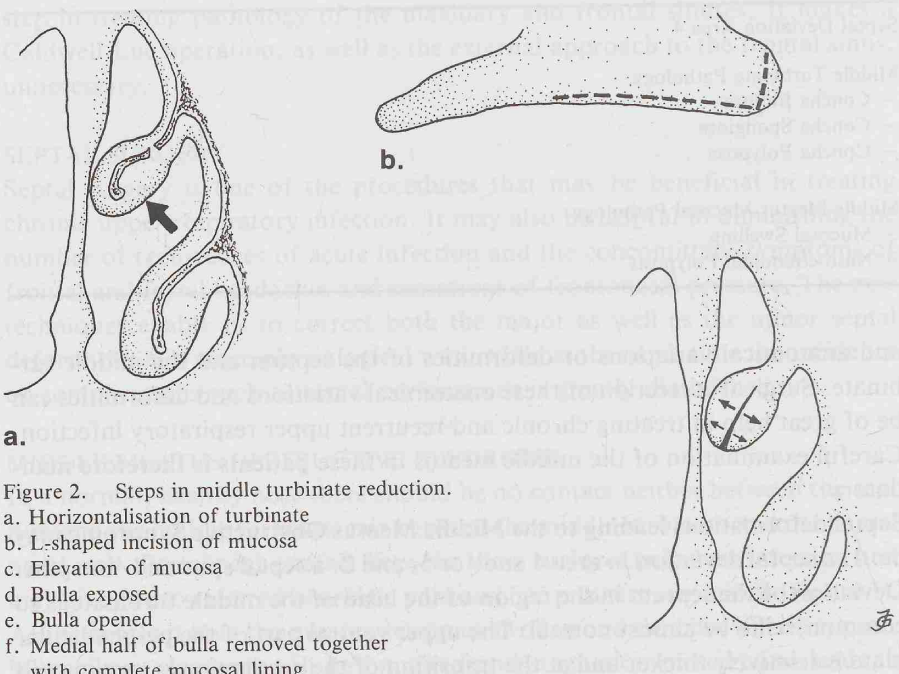

Figure 2. Steps in middle turbinate reduction.
a. Horizontalisation of turbinate
b. L-shaped incision of mucosa
c. Elevation of mucosa
d. Bulla exposed
e. Bulla opened
f. Medial half of bulla removed together with complete mucosal lining
g. Compression by Craig Forceps
h. and i. Trimming by special scissors

is not displaced posteriorly in order to avoid sagging of the dorsum. It is replaced in its original position and fixed by at least two septo-columellar sutures. Basal and posterior septal defects are reconstructed by reimplantation of small plates of bone (or cartilage) made from the resected material. In this way the original stiffness of the septum is retained, sagging is prevented and mucosal atrophy will not take place.

MIDDLE TURBINATE SURGERY

Anatomical variations and pathology of the middle turbinate are quite common. The skeleton of this concha may consist of a single bony plate, a more massive spongiotic bone or a bulla. Although the latter are normal variations, they can become an important factor in producing a Middle Meatus Obstructive Syndrome.

In clinical practice three types of pathologic middle turbinates can be distinguished: 1. the bullous type, 2. the spongiotic type, and 3. the hyperplastic mucosa type. Careful corrective surgery with reduction of the conchal volume and removal of polypoid degenerated tissue may be of great help in treating patients with recurrent and chronic upper respiratory infections.

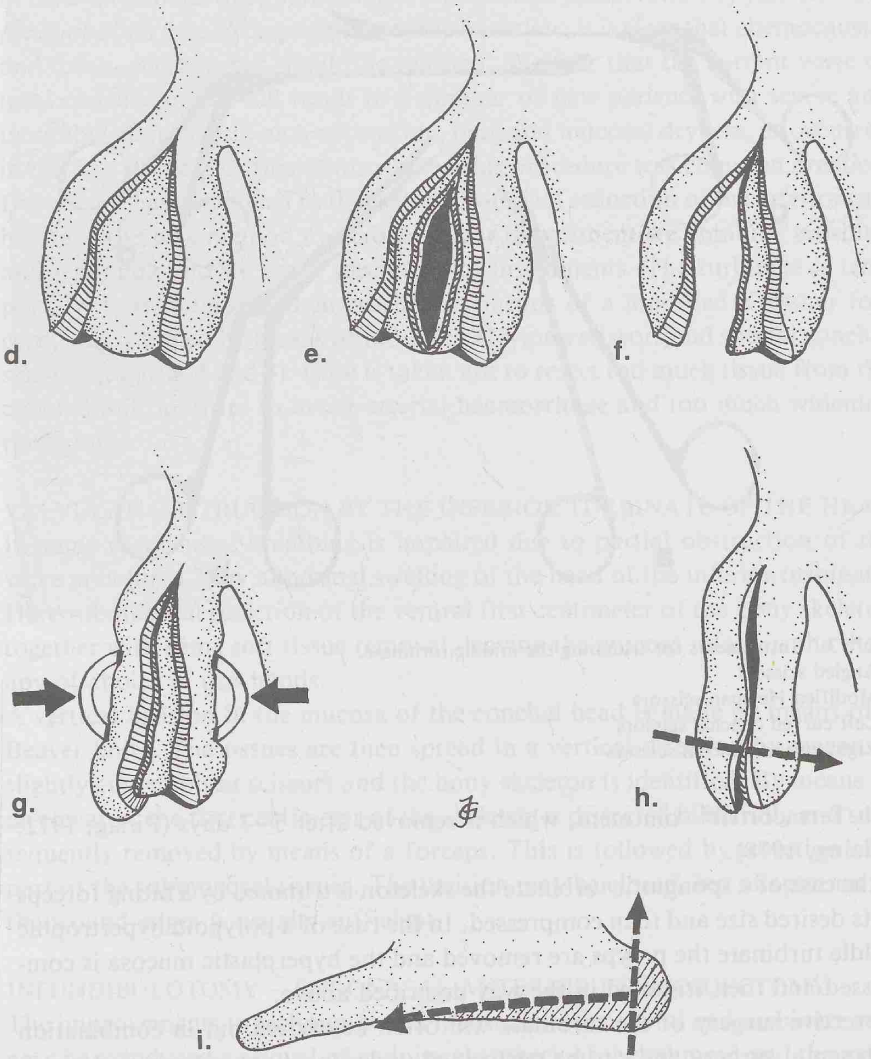

Figure 2d–i.

The bullous and spongiotic turbinate are treated by resection of a part of the skeleton. To this end, a L-shaped incision of the mucosa is made by means of a No. 64 Beaver blade. The mucoperiostium is then bilaterally elevated. The bulla is opened and one half of it is removed together with the total mucosal lining. The turbinate is then compressed by means of Craig forceps and subsequently trimmed by means of a set of special scissors (Figures 2 and 3). The reduced turbinate is kept in the desired position by application of a gauze strip

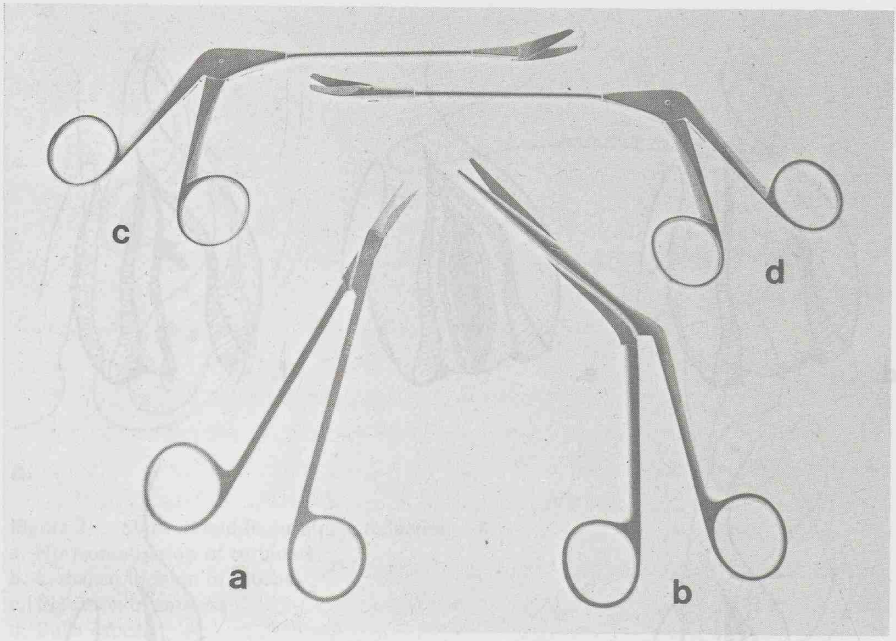

Figure 3. Instruments for trimming the middle turbinate.
a. Angled scissors
b. Modified Heyman scissors
c. Left curved conchal scissors
d. Right curved conchal scissors

with Terracortril® ointment, which is removed after 5–7 days (Pirsig, 1972; Huizing, 1978).

In the case of a spongiotic turbinate the skeleton is trimmed by a biting forceps to its desired size and then compressed. In the case of a polypoid hypertrophic middle turbinate the polyps are removed and the hyperplastic mucosa is compressed and then trimmed in the way described above.

Corrective surgery of the turbinate will often be carried out in combination with septal surgery and can be performed under local anaesthesia if desired.

INFERIOR TURBINATE SURGERY

Methods for reduction of a hyperplastic inferior turbinate have been a subject of dispute until the present day. A great variety of methods has been advocated, the most important being: lateralisation, electro-cauterisation, (sub)total conchotomy, application of chemocaustics, submucosal resection of the turbinate bone, intraconchal diathermy, cryosurgery, crushing and trimming and, most recently, laser surgery.

In our opinion the best method is the one which produces *the desired reduction*

in conchal volume in combination with maximal preservation of function i.e. functional surface. Accepting this as the basic law, it is clear that chemocaustic and total conchectomy should be rejected. We fear that the current wave of total conchectomies will result in a number of new patients with severe and incurable after-effects such as crusting, pain and mucosal dryness, as occurred in the first decades of this century when this procedure was common practice. Experience has convinced us that selected surgical reduction of the inferior turbinate is the best method available. In our department we combine crushing and trimming and we use a special set of instruments. The turbinate is temporarily horizontalised, then crushed by means of a modified Kressner forceps, and trimmed by means of modified Heyman scissors and special conchal scissors (Figures 4 and 5). Care is taken not to resect too much tissue from the conchal tail, in order to avoid arterial haemorrhage and too much widening posteriorly.

VALVULAR OBSTRUCTION BY THE INFERIOR TURBINATE OF THE HEAD

In some cases nasal breathing is impaired due to partial obstruction of the valve area (area 2) by abnormal swelling of the head of the inferior turbinate. Here submucosal resection of the ventral first centimeter of the bony skeleton together with some soft tissue removal, leaving the mucosa intact, is the therapy of choice in our hands.

A vertical incision in the mucosa of the conchal head is made by means of a Beaver knife. The tissues are then spread in a vertical direction by means of slightly curved blunt scissors and the bony skeleton is identified. By means of an elevator, the first centimeter of the skeleton is dissected bilaterally and subsequently removed by means of a forceps. This is followed by resection of a part of the submucosal tissues. The incision may be closed, but adaptation of the wound edges is usually sufficient.

INFUNDIBULOTOMY – ENDONASAL ANTERIOR ETHMOIDECTOMY

The improvements in techniques and instruments for septal and turbinate surgery have induced a revival of endonasal surgery of the ethmoid and the maxillary ostium (Wigand et al., 1988; Tos et al., 1989). Although much practised in previous decades, ethmoidectomy had become a more or less abandoned method which was brought into discredit because of serious complications and unsatisfactory results. By using new instruments and modern light sources such as the cold light speculum, the microscope and the nasal endoscope, these drawbacks could be conquered and several authors reintroduced endonasal ethmoid surgery again in the late sixties. Progress in this field was, among others, reported by Wigand (1981).

Straatman and Buiter (1981) reintroduced surgery of the antral ostium by

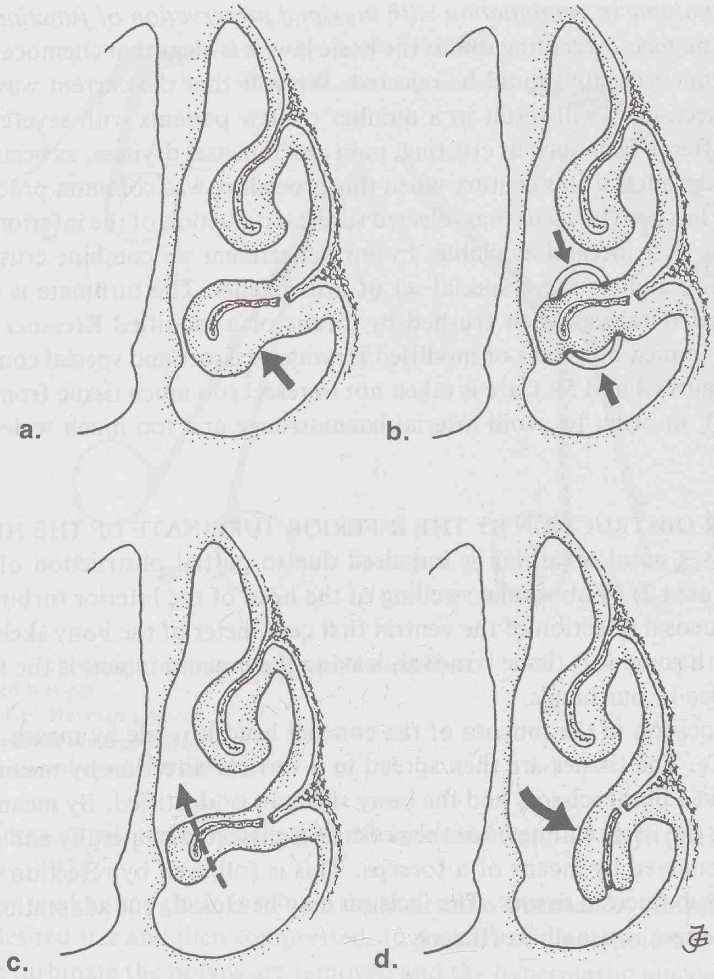

Figure 4. Steps in reduction of the inferior turbinate.
a. Horizontalisation
b. Crushing
c. Trimming
d. Lateralisation

indicating a method of creating a new ostium in the membranous fontanelle. Messerklinger, after having extensively studied the mucociliary pathways in the antrum and middle nasal passage, introduced the nasal endoscope in endonasal surgery (1972). From this work a technique was developed which is now called "Infundibulotomy" and which was recently widely propagated by

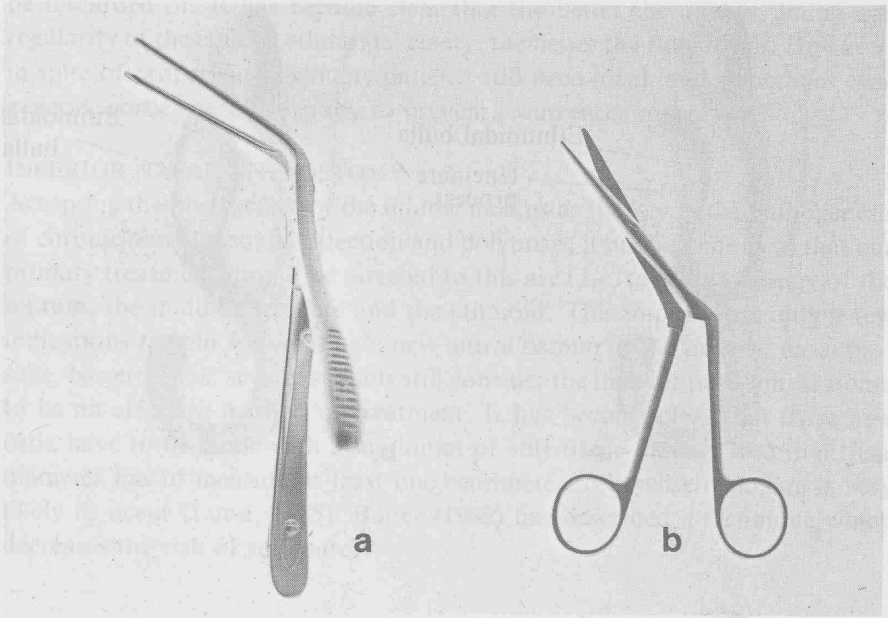

Figure 5. Instruments for reduction of inferior turbinate.
a. modified Kressner forceps for crushing
b. modified Heyman scissors for trimming

Messerklinger's pupil, Stammberger (1986) in Europe, and by Kennedy (1985) in the U.S.A.

Infundibulotomy implies the removal of the uncinate process and the ethmoidal bulla followed by widening of the maxillary ostium in a caudal and ventral direction by means of a backwards cutting punch. If necessary diseased ethmoidal cells are removed as well (Figure 6). The operation is carried out under endoscopic control. It should be emphasized however, that this is only possible in a bloodless field i.e. under local anaesthesia or under general anaesthesia with vasoconstriction and/or hypotension.

Infundibulotomy is only indicated in patients with impairment of ventilation and drainage of the sinuses and limited pathology of the middle meatus. In cases with extensive polyposis a subtotal or total ethmoidectomy is required.

SUBTOTAL ENDONASAL ETHMOIDECTOMY

Endonasal (sub)total or total ethmoidectomy has proved to be of great value in treating nasal and sinusoidal polyposis. It is generally accepted that resection of the diseased ethmoid is the key to successful treatment of this syndrome. All diseased ethmoidal cells are opened and resected under direct

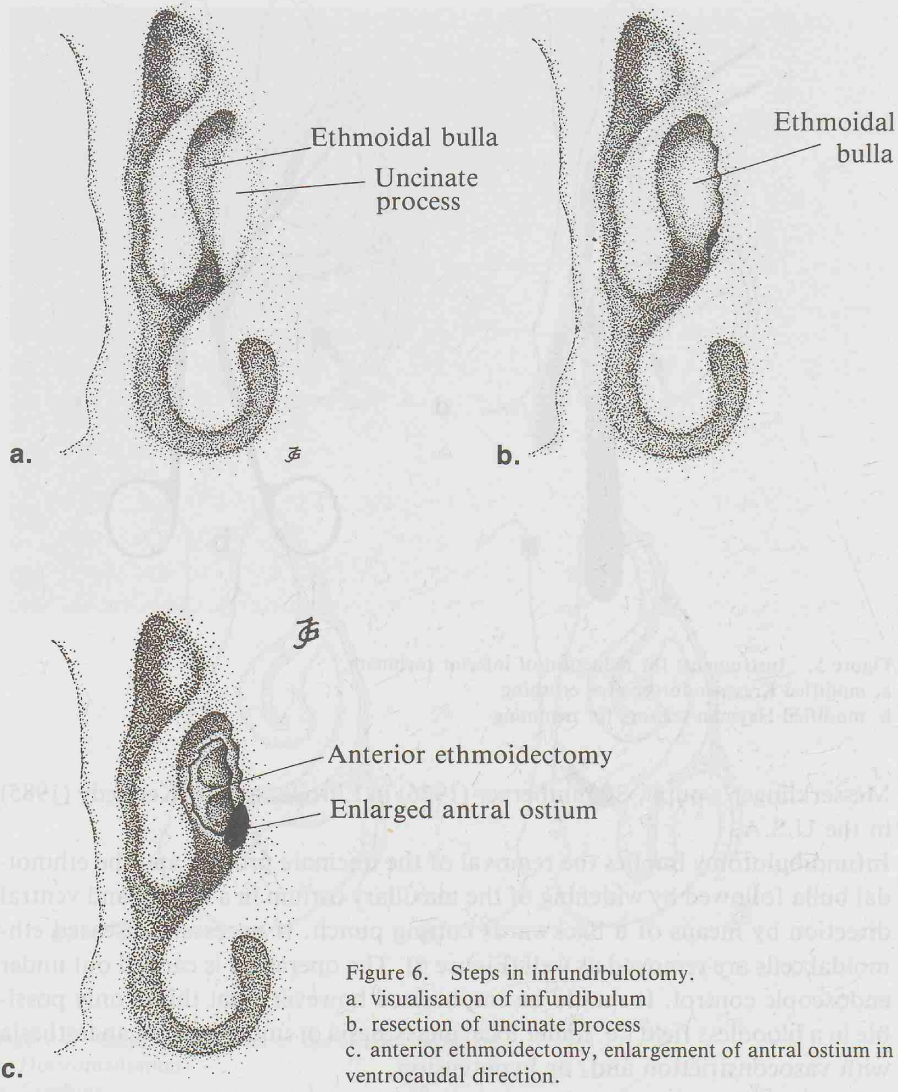

Figure 6. Steps in infundibulotomy.
a. visualisation of infundibulum
b. resection of uncinate process
c. anterior ethmoidectomy, enlargement of antral ostium in ventrocaudal direction.

vision, with or without magnification. Care is taken that a cavity with a regular lining is created. For this purpose forcepses, special curettes or a burr are used. The uncinate process is resected and the antral ostium is enlarged in a similar way as described above for infundibulotomy. The sphenoid sinus is opened and cleaned if necessary. The middle turbinate is trimmed or, in severe cases, removed. Ethmoidectomy generally restores the ventilation and drainage of the maxillary and frontal sinus. Unless the antrum at peroperative antroscopy appears to be filled up with polyps, a Caldwell-Luc operation can

be discarded of. It has become clear that the better the quality, lining and regularity of the created ethmoidal cavity, the better the final result. However, in spite of proper surgery many patients still need local, and sometimes even general, corticosteroid therapy to prevent recurrences of polyps.

INFERIOR NASAL ANTROSTOMY

Accepting the obstruction of the middle meatus as the key in the pathogenesis of chronic nasosinusoidal infection and polyposis, it has become clear that our primary treatment should be directed to this area i.e. to the pathology of the septum, the middle turbinate and the ethmoid. This implies that only a few indications remain for creating a new antral ostium in the inferior nasal passage. Nevertheless, several schools still consider the inferior naso-antral stoma to be an effective method of treatment. It has become clear that these new ostia have to be made with a minimum of soft tissue damage and that their diameter has to measure at least one centimeter, otherwise reclosure is very likely to occur (Lund, 1985). Buiter (1982) has described a technique which decreases the risk of reclosure.

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Role of free radicals in inflammation

B. Housset

Inflammation is a non-specific reaction involving a large number of mediators. Over recent years, these mediators have been shown to include free radicals. These substances, derived from molecular oxygen, are produced in the extracellular environment essentially by phagocytes (polymorphonuclear cells and macrophages) and may contribute to inflammatory lesions (Clark et al., 1985). After briefly defining the nature of these radicals, we will examine their mechanisms of production and elimination and their toxic effects, before discussing the various therapeutic possibilities.

NATURE OF FREE RADICALS

A free radical is defined by the presence of an unpaired isolated electron in an external orbit. This isolated electron confers a particular biological reactivity, as it tends to pair with an electron of an adjacent molecule, which in turn becomes a free radical. This is the first step in a chain reaction.

During mitochondrial respiration, the great majority of molecular oxygen is reduced tetravalently and simultaneously, resulting in the formation of one molecule of water. A small proportion of oxygen (about 10%) may be reduced univalently and sequentially, resulting in the successive formation of the superoxide radical, hydrogen peroxide and the hydroxyl radical (Boveris, 1977). The thermodynamic aspects of the formation of the hydroxyl radical are unclear. Divalent metallic cations (iron) would appear to be involved and, in the presence of hydrogen peroxide and the superoxide radical, would contribute to the formation of the hydroxyl radical (Halliwell and Gutteridge, 1984).

These free radicals are highly reactive and have a very brief half-life of the order of 10^{-6} seconds. However, this reactivity depends on the polar or non-polar nature of the medium in which these radicals are produced. Thus, in an aqueous medium, the superoxide radical is less reactive than in a hydrophobic medium. Moreover, hydrogen peroxide, which does not have the structure of a free radical, is minimally reactive, has a relatively long half-life and is therefore able to cross cell membranes. In contrast, the hydroxyl radical reacts immediately with any neighbouring molecule, which explains its extreme toxicity (Freeman and Crapo, 1982).

GENERATION OF OXYGEN-DERIVED FREE RADICALS

As we have seen, cellular respiration is responsible for the intracellular generation of free radicals. This is also the case for certain intracellular enzymes. During the inflammatory reaction, free radicals are released into the extracellular compartment by phagocytes. These cells possess a specialised enzyme system on the surface of their membrane which is able to form superoxide radicals from molecular oxygen (Klebanoff, 1980). The electron donor is NADPH, hence the name of NADPH oxidase. This is a complex transmembrane system involving several protein sub-units and containing cytochromes (Babior and Peters, 1981). Various congenital NADPH oxidase deficiencies have been described, the best known of which is responsible for familial chronic granulomatosis (Curnutte et al., 1974). This generation of free radicals is essential for the bactericidal action of these cells. In certain situations, the free radicals may overshoot their goal and induce tissue lesions. Many stimuli may lead to the activation of NADPH oxidase (phagocytosis, phorbol myristate, complement components, chemotactic factors such as FMLP) and their mechanisms of action are still poorly understood. However, recent results suggest a relationship between the activity of phospholipase A2 and activation of NADPH oxidase. Consequently, there would be a relationship between the release of arachidonic acid and the formation of superoxide radicals. The relationship between this system and cyclic AMP has not been elucidated. However, the substances which increase the intracellular levels of cyclic AMP appear to decrease the release of oxygen-derived free radicals. Together with superoxide radicals, elements of the myeloperoxidase system are also released into the extracellular environment, which, in the presence of a halide, generally chloride, leads to the formation of hypochloric acid and possibly singlet oxygen $^1O_2$. Singlet oxygen is an excited form of oxygen endowed with oxidant properties which may also contribute to tissue lesions.

In addition to phagocytes, which are the principal source of free radicals in the course of inflammatory reactions, endothelial cells may also release free radicals (Ratych et al., 1987). However, the pathophysiological role of this phenomenon has yet to be confirmed. Lastly, platelets also release free radicals during aggregation.

The reoxygenation of ischaemic tissue constitutes a particular situation. During ischaemia, cellular ATP is broken down to xanthine, while xanthine dehydrogenase is activated into xanthine oxidase. The introduction of oxygen results in the sudden formation of free radicals due to the activity of xanthine oxidase. These radicals appear to be incriminated in the pathogenesis of tissue lesions (McCord, 1985). These phenomena appear to be associated with activation of circulating leukocytes.

ELIMINATION OF FREE RADICALS (White and Repine, 1985)

The body possesses various defence mechanisms capable of dealing with the oxidative aggression induced by the extracellular release of free radicals.

Amongst the intracellular substances, three enzyme systems appear to be important:

1. The superoxide dismutases, which are present in two forms depending on the metal ion they possess (cuprozinc in the intracytoplasmic form and manganese in the essential intramitochondrial form). Superoxide dismutases accelerate the formation of hydrogen peroxide from the superoxide radical (dismutation).
2. Catalase takes over from superoxide dismutase to break down hydrogen peroxide to water.
3. Lastly, the glutathione peroxidase system detoxifies lipid peroxides. This ubiquitous enzymatic activity requires the presence of selenium.

Other intracellular substances also appear to be important in antioxidant defence, for example reduced glutathione, which readily donates an electron thereby preventing the propagation of the radical reaction, and vitamin E which is essentially fat-soluble, localised in cell membranes and partially limits lipid peroxidation. Many other antioxidant substances are present within the cell but appear to play a less important role.

Amongst the circulating substances which may have an antioxidant role, we should mention a form of superoxide dismutase described by Marklund and substances such as ceruloplasmin and vitamin C. However, the role of vitamin C is controversial as, at low concentrations, this substance, which is also located within the cell, may induce lipid peroxidation.

These various antioxidant substances are able to readily donate an electron, becoming, in turn, a free radical. This radical has little biological reactivity, which stops the propagation of the chain reaction. The antioxidant capacities of the body largely depend on the availability of the substrates necessary for the synthesis of these various substances (Chow, 1979). However, during acute oxidative aggression, the cell is able to regenerate reduced antioxidant substances at the cost of energy consumption. This may constitute an indirect mechanism of the cellular toxicity of free radicals.

EFFECTS OF FREE RADICALS

The toxicity of free radicals is essentially exerted on three types of molecules:

1. Polyunsaturated lipids, particularly those situated in the cell membrane. The presence of a double bond constitutes a weak point easily attacked by free radicals. The lipid radical formed subsequently reacts with molecular oxygen to form a lipid peroxide. These modifications in the lipid composition of membranes result in functional and structural alterations which may lead to cell rup-

ture and consequently cell death. Lipid peroxides are metabolised by the cell with the formation of toxic intermediate substances such as malondialdehyde. The complete metabolism of lipid peroxides results in the production of ethane and pentane, which are eliminated in expired air, where they can be measured, constituting an index of lipid peroxidation.

2. Proteins. Free radicals may interact with sulphydryl groups present in proteins. This may modify the secondary and tertiary structure of these proteins. The alteration in the protein configuration may be accompanied by functional modifications with, for example, inactivation of certain enzymes.

3. Nucleic acids. Free radicals may modify the bases and break the ribose-phosphate skeleton of deoxyribonucleic acid, leading to chromosomal breaks and possibly mutations (Hoffmann et al., 1984).

In the extracellular compartment, free radicals may also induce the formation of a potent chemotactic substance which, by attracting polymorphonuclear cells to the site of inflammation, amplifies the phenomena.

A more recent, and as yet poorly understood, aspect of the effect of free radicals seems to be activation of phospholipase A2 by hydrogen peroxide.

INVOLVEMENT OF FREE RADICALS IN THE COURSE OF CERTAIN INFLAMMATORY SYNDROMES

A large number of pathophysiological conditions are associated with the release of free radicals by neutrophil polymorphonuclear cells, for example: certain forms of non-cardiogenic pulmonary oedema (Tate and Repine, 1983), smoking, reperfusion ischaemic lesions (Menasche et al., 1986), certain joint diseases (McCord, 1974; Menander-Huber, 1981), pulmonary toxicity of oxygen (Fox et al., 1981; Housset and Junod, 1983). However, in all of these situations, the relative role of free radicals compared with other mediators is still poorly defined. Various studies using antioxidant substances suggest the important role of free radicals, raising the possibility of therapeutic impacts. The desired effect in this situation is to increase the antioxidant defence mechanisms, especially in the extracellular compartment, to act as a buffer between the leukocyte, source of free radicals, and the target tissue. Certain animal and human experiments demonstrate the protective effect of superoxide dismutase (SOD) (McCord, 1974; Menander-Huber, 1981). However, the practical value of this effect is limited by the short half-life of injected SOD and by the potential risk of infection associated with this type of treatment. The administration of catalase has only been studied in animals, but appears to be promising. Other antioxidant substances such as dimethylthiourea (Fox, 1984; Jackson et al., 1986), ebselen, a selenium derivative, or molecules with a free thiol group (N-acetylcysteine, glutathione) have been tested in animals and appear to at least partially prevent certain inflammatory lesions.

An indirect mechanism of protection against free radicals is the administration of iron chelators, which act by preventing the formation of the hydroxyl radical (Sinaceur et al., 1984). The current disadvantage of this type of molecule is the need to use the intravenous route and the difficulty of long-term treatment in chronic inflammatory diseases. Orally administered iron chelators are currently being studied (Hershko et al., 1984).
A final therapeutic approach is to increase the intracellular antioxidant defences. The use of liposomes, allowing the intracellular penetration of SOD and catalase, is partially effective in animals exposed to hyperoxia (Turrens et al., 1984). Other more indirect methods would consist of increasing the synthesis of antioxidant substances. In certain circumstances, this could be the mechanism of action of endotoxin (Frank and Roberts, 1979) or even Tumour Necrosing Factor (TNF) associated with interleukin 1. However, this is still at the level of basic research.

In conclusion, over recent years, free radicals have been recognised as new mediators of inflammation. Their place in relation to the already known mediators is still unclear. The interrelations which appear to exist between these radicals and arachidonic acid metabolism should be stressed. A number of recent studies suggest that this line of research may open up new therapeutic approaches to the inflammatory syndrome, in the near future.

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Value of local antibiotic therapy in respiratory tract infections

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The value of local antibiotic therapy is based on three facts:

1. The initial aggression is viral in the majority of cases, as reflected by the multifocal nature of the respiratory tract involvement.
2. The indications for systemic antibiotic therapy are rare apart from proven complications: otitis, purulent sinusitis and secondary bronchial infections (Bergogne-Bérézin, 1984).
3. However, viral infection creates favourable conditions for secondary bacterial infections according to a triple mechanism:

- Adherence of pathogenic bacteria (for example Pneumococcus) with formation of a polymerised substance elaborated by the microorganism which allows binding to epithelial cells. At this stage, whether or not these mechanisms are specific, they can be easily interrupted by simple lavage or, better still, local antibacterial treatment.
- This phase is followed by colonisation which is favoured by a number of factors: alcoholism, diabetes, viral infections, trauma and the presence of Staphylococcus aureus.
- The last phase, infection, occurs when the host's immune defences are surpassed. Local antibiotic therapy induces the very high local concentrations frequently required by the fact that pathogenic bacteria in the mucous membranes of the upper respiratory tract are surrounded by a very thick capsule.

Which bacteria are responsible? What complications may occur?

We shall consider four different situations: 1. common cold, 2. acute pharyngitis and tonsillitis, 3. acute viral laryngitis, 4. tracheitis, tracheobronchitis and viral bronchitis.

1. THE COMMON COLD

It is always of viral origin, in particular (Narcy, 1983): rhinoviruses in adults; in children: the syncytial virus, parainfluenza viruses, certain adenoviruses and, of course, rhinoviruses.

Clinically, it is a spontaneously resolving, usually afebrile, disease with an incubation period of two to four days. The syndrome consists of:

1. Nasal discharge, obstruction, sneezing, pharyngeal discomfort, cough, headache and general malaise.
2. Low grade fever lasting 24 hours is present in 10% of cases.
3. Rigors, odynophagia and adenopathy, when present, are due to another cause.

The course of the disease lasts seven days with a peak around the second or third day. After the third day, rhinopharyngeal secretions decrease and become viscous and frequently purulent. A minor, dry, non-productive cough often appears at this stage.

If the disease persists beyond the tenth or twelfth day, it can be considered to be due to another cause, in particular: mycoplasma infection, secondary bacterial infection or bacterial complication.

In children, this complication frequently consists of otitis; around the fourth and fifth days two organisms are particularly dangerous: Haemophilus influenzae, before the age of five years, and Streptococcus pneumoniae, in older children.

In adults, it tends to be sinusitis: either acute with fever, rigors and erythema of the cheeks and leukocytosis, or subacute, presenting simply with nasal discharge.

The bacteria responsible are: S. pneumoniae and H. influenzae (most frequently), penicillin-sensitive anaerobes and aerobic streptococci. S. aureus is less frequent and only encountered in 2% of cases. However, it should be stressed that, in the past, these figures were probably exaggerated (Kallings, 1983).

2. ACUTE PHARYNGITIS AND TONSILLITIS

The majority of cases of acute pharyngitis are viral. β-haemolytic streptococci are involved in 2 to 7% of cases, rarely exceed 10% and may be present in up to 25% of cases in certain epidemics in children between the ages of five and eight years. Other bacteria may also be involved: Mycoplasma pneumoniae and Neisseria gonorrhoeae (Dublanchet et al., 1986; Komaroff et al., 1983). The viruses most frequently isolated are Herpes simplex virus, Influenza viruses and Parainfluenza viruses. Adenoviruses 4, 3, 7 and 21 are found in military populations. Adenoviruses 1, 2 and 5 are also found in children. In adolescents and young adults: Herpes simplex virus and Epstein-Barr virus are the organisms most frequently isolated.

Mycoplasma pneumoniae is especially isolated from schoolchildren and student populations.

Streptococcus group A is predominant between the ages of five and fifteen years. It is transmitted by direct contact, very frequently within the family,

25% of which may be affected. Such epidemics may also be observed in young soldiers living in close contact. The peak incidence occurs principally at the end of winter and at the beginning of spring, but it may also occur during summer. The specific complications are: acute rheumatic fever and acute glomerulonephritis.

Signs suggestive of streptococcal infection are: severity of the pain, appreciable cervical adenopathy, exudative tonsillitis and leukocytosis exceeding 15,000. However, it must be remembered that acute exudative pharyngitis may be observed with Herpes simplex virus, Adenovirus, Epstein-Barr virus, Enterovirus infections and even diphtheria. Acute pharyngitis resolves spontaneously.

Local and regional complications: Acute otitis, sinusitis and adenitis by streptococcal spread are no longer seen. Peritonsillar abscesses (quinsy) present with oedema on the affected side and displacement of the tonsil towards the midline. In the majority of cases, streptococcus is not detected in the pus of the abscess and it is generally considered to be due to secondary infection with anaerobic bacteria. The treatment of choice is incision with perioperative penicillin cover.

3. ACUTE VIRAL LARYNGITIS

Acute viral laryngitis is an afebrile, spontaneously resolving disease characterised by hoarseness or, in the more severe cases, complete loss of the voice. In its isolated form, it is much less frequent than common rhinitis, but it may also be associated in 10 to 20% of cases or even more in the presence of viral pharyngitis.

The viruses most frequently encountered are Parainfluenza types 1, 2 and 3. In reality, the majority of respiratory tract viruses may probably be involved, but bacteria are not implicated.

4. TRACHEITIS, TRACHEOBRONCHITIS AND BRONCHITIS

These infections are due to Influenza type A or B viruses while Mycoplasma pneumoniae is essentially responsible for bronchitis (Smith et al., 1980).

Tracheitis

Tracheitis is characterised by paroxysmal, non-productive cough. Inspiration induces a feeling of discomfort, especially when the air is cold. Tracheal sensitivity can be demonstrated by pressing with the thumb just above the cricoid cartilage.

Bronchitis

Viral bronchitis does not require antibiotic therapy but this is not the case for

secondary bacterial infections which are difficult to prevent (Lebeau, 1983).

Tracheobronchitis

In view of the frequency of cough in the other infections, the diagnosis of tracheobronchitis requires the presence of the following symptoms: paroxysmal cough, tracheal sensitivity and abnormal auscultation, and the chest X-ray should obviously be normal.

THERAPEUTIC APPROACH

In general, the therapeutic approach is as follows:

1. Initially, the disease is due to viral infection; it is frequently multifocal and resolves spontaneously within five to ten days. However, in every case, the acute infection is subject to bacterial complications, situated distally (descending bronchial infection), but also proximally, in the upper airways (otitis, purulent sinusitis, peritonsillar abscess). Local antibiotic therapy is therefore useful provided, of course, that it is an antibiotic reserved for local use.
2. Occasionally, there is a risk of systemic complications. This is the case of infections occurring in certain particular clinical contexts, such as immunodepressed patients, in which systemic antibiotic therapy is required. This is also the case of streptococcal sore throat: Firstly, it is impossible to distinguish clinically between viral and streptococcal aetiology and secondly, laboratory results cannot be obtained within 24 hours. It is therefore logical to commence treatment immediately after obtaining the throat swab in all subjects presenting with severe dysphagia or in the presence of streptococcal risk. In other cases, it is possible to wait for the result of the swab, as prevention of the risk of rheumatic fever can be safely delayed for four or five days. The aim of treatment is to prevent peripharyngeal suppuration. In severe forms, antibiotic therapy may shorten the course of the disease and decrease the symptoms, provided that treatment is commenced within 24 hours.

CHRONIC AND RECURRENT INFECTIONS

Chronic and recurrent infections constitute a particular problem which always requires the help of an ENT specialist. We shall discuss the particular case of chronic pharyngitis.

Between 1980 and 1986, in our ENT department in Limoges, we saw 1,807 adult patients with sore throat. Almost 75% of them had already received at least two different antibiotics. Only 6% underwent tonsillectomy. One half of cases corresponded to pharyngeal paraesthesiae and the other half presented diffuse inflammatory pathology in which other factors were involved:

1. Various metabolic disorders: obesity, diabetes ...
2. Allergic context.

3. Prolonged viral infections such as infectious mononucleosis.

In all of these cases, the efficacy of local treatment has been proven for decades; either due to its mechanical effect, or to its emollient effect, and finally due to its anti-infectious activity.

In this type of situation, it is important to determine which patients really require systemic antibiotic therapy and which patients can be relieved by well-managed local treament.

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Bactericidal activity of fusafungine towards nosocomial methicillin-resistant staphylococcal strains

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There is renewed interest in aerosol treatments for respiratory tract infections, as the administration of aerosols results in effective local drug concentrations at the site of the infection, while limiting the systemic toxicity. This is particularly true for antibiotics which, when administered by aerosols, are concentrated in the respiratory mucosa without involving the anatomical, pharmacological and metabolic steps necessary for systemic antibiotic therapy. By applying them to the site of infection, antibiotics are able to act rapidly with low doses and without side effects. The therapeutic benefit of aerosol therapy is often spectacular both in acute and chronic diseases, especially in rhinitis, sinusitis, laryngitis, tracheitis, bronchitis and pneumonia. However, in order for this type of antibiotic therapy to be effective, it is essential to use molecules with a narrow spectrum adapted to the micro-organisms responsible for the infections and which do not induce either acquired resistance to this molecule nor crossed resistance with the commonly used antibiotics.

For this reason, we were particularly interested in fusafungine, a bacteriostatic antibiotic strictly intended for local use, extracted from Fusarium lateritium. We determined the in vitro spectrum of action of fusafungine on collection micro-organisms as well as on wild strains by classical determinations of the minimal inhibitory concentration (MIC) and the diameters of inhibition of diffusion according to various methods which we have validated. We also investigated the possible induction of acquired resistance to fusafungine or crossed resistances with other antibiotics commonly used in clinical practice (German-Fattal et al., 1988). The results presented here therefore concern the in vitro experimental studies. In contrast with in vivo studies, they do not take into account physical factors related to the aerosol itself, nor anatomical factors, nor individual factors. Furthermore, fusafungine was also studied in isolation, independently of its mode of administration and the substances associated in the pressurized preparation for therapeutic use. Consequently, we confirmed the therapeutic antibacterial activity of fusafungine by studying its

Table 1. Sensitivity of various nosocomial Gram positive cocci to fusafungine.

strain	origin	antibiotic resistance	MIC (mcg/ml)
S. aureus	eye	Pen G and M, Strepto, Kana, Tobra, Genta, Tetra, Linco, Erythro, Spira	16
S. aureus	sputum	Strepto, Kana, Tobra, Genta, Siso, Tetra, Mino, Erythro, Spira, Clinda, Linco, Chloramphenicol TSu	16
S. aureus	wound	Pen G and M, Carbeni, Ampi, Ami, Strepto, Kana, Tobra, Genta, Siso, Livido, Tetra, Erythro, Chloramphenicol	16
Strep. faecalis	ear	Strepto, Kana, Erythro, Spira, Linco, Tetra, Chloramphenicol, TSu	32
Strep. pyogenes	throat	Strepto, Kana, Tobra, Tetra, TSu	16
Gp B Strep.	throat	Strepto, Kana, Tobra, Tetra, Mino, Clinda, Chloramphenicol, TSu	8
Strep. pneumoniae	ear	Strepto, Kana, Tobra, Genta	16

Beta-lactams: penicillins (Pen) G and M, ampicillin (Ampi), carbenicillin (Carbeni). Aminoglycosides: streptomycin (Strepto), kanamycin (Kana), tobramycin (Tobra), gentamicin (Genta), sisomicin (Siso), lividomicin (Livido). Tetracyclines: tetracycline (Tetra), minocycline (Mino). Macrolides: erythromycin (Erythro), spiramycin (Spira). Lincosanides: lincomycin (Linco), clindamycin (Clinda). Phenicols: Chloramphenicol. Trimethoprim-Sulphamethoxazole (TSu).

in vivo efficacy in experimental pneumonia in the mouse by administration of the commercial aerosol preparation.

SPECTRUM OF ACTION OF FUSAFUNGINE

On the basis of pharmacological and clinical criteria, we were able to define the critical concentrations of 40 and 80 mcg/ml, representing, respectively, the limits for the categories of micro-organisms sensitive, intermediate and resistant to fusafungine.

In general, fusafungine exerts an excellent antibiotic activity on Gram positive bacteria. Fusafungine regularly exerts a bacteriostatic activity on Gram positive cocci, as the MIC for these organisms were equal to a maximum of 26 mcg/ml for the collection strains studied and for 90% of the wild strains (89/99). Furthermore, it was interesting to find that fusafungine was active on strains resistant to antibiotics classically administered systematically (Table 1). This demonstrates the therapeutic value of fusafungine and confirms the absence of crossed resistances.

Fusafungine also demonstrated an inhibitory activity on the Gram positive

Table 2. Antibacterial spectrum of action of fusafungine.

micro-organism	MIC (mcg / ml)
Gram positive cocci	26
Streptococcus mutans	30
Gram positive bacilli	26
Listeria monocytogenes	24
Gram negative cocci	
Branhamella catarrhalis	15
Veillonella parvula	> 100
Gram negative bacilli	> 100
Pasteurella multocida	40
Mycoplasma pneumoniae	18

bacilli studied (MIC = 26 mcg / ml), in particular Listeria monocytogenes. Similar results were observed on the Gram positive anaerobic cocci and bacilli studied (MIC between 15 and 30 mcg / ml), including Streptococcus mutans, the organism responsible for initiating dental plaque (Berenholc, 1985) and consequently the principal cariogenic bacterial species which must be eradicated from the buccal cavity.

Fusafungine was also found to be bacteriostatic against certain Gram negative cocci such as Branhamella catarrhalis, a saprophytic organism of the airways; the MIC for the collection strain studied was 15 mcg / ml. On the other hand, Veillonella parvula, an anaerobic, aerotolerant coccus, was resistant to fusafungine, as the MIC was higher than 100 mcg / ml (Table 2).

Similarly, collection strain and wild strain Gram negative bacilli were all found to be resistant to fusafungine, as the MIC were always higher than 100 mcg / ml. However, the collection strain of Pasteurella multocida studied demonstrated intermediate sensitivity as the MIC was 40 mcg / ml.

It is important to stress the activity of fusafungine on Mycoplasma pneumoniae, an organism which is becoming increasingly important in respiratory tract pathology and which is responsible for 20% of cases of pneumonia. It particularly affects schoolchildren, 35% of whom are symptomatic or asymptomatic carriers, adolescents and young adults (Foy et al., 1983). With an MIC of 18 mcg / ml, fusafungine is particularly valuable, as beta-lactams and cephalosporins are always inactive against this organism. Resistances to macrolides and doxycycline, antibiotics acting on protein synthesis, have also been reported (Stropler et al., 1980; Watanabe et al., 1983).

Lastly, yeasts belonging to the Candida genus have been shown to be fairly sensitive to fusafungine, as 8 of the 11 wild strains tested, including seven strains of C. albicans, presented a diameter of inhibition greater than the threshold

Table 3. Sensitivity to fusafungine of 11 nosocomial strains of Candida.

strain	diameter of inhibition*
Candida albicans	17.2 S**
Candida albicans	0
Candida albicans	11.1 S
Candida albicans	0
Candida albicans	0
Candida albicans	12.6 S
Candida albicans	11.1 S
Candida albicans	20.2 S
Candida albicans	19.5 S
Candida albicans	20.6 S
Candida tropicalis	15.4 S

* Diameter of inhibition for a load of 10 mcg of fusafungine per disk.
** Strain sensitive for a minimal diameter of inhibition of 11 mm.

diameter of 11 mm for disks containing 10 mcg of fusafungine. The MIC for the collection strain studied, C. albicans 646 CIP, was 26 mcg/ml (Table 3). This therefore limits the risk of secondary candidiasis, particularly during long-term treatments.

ANTIBACTERIAL ACTIVITY OF FUSAFUNGINE AEROSOL

In animals, fusafungine, delivered by aerosol under the conditions of administration normally used in clinical practice, was found to be effective in the treatment of experimental pneumonia induced by intranasal inoculation of Streptococcus pneumoniae (Figure 1). We observed a slowing of the course of the pneumonia associated with a significant extension of the survival time of the animals ($W = 187$, $p < 0.04$ on the Mann-Whitney and Wilcoxon tests), together with a significant reduction in the post-infectious mortality rate ($p < 0.05$ by Yates' corrected χ^2 test). Histological studies also demonstrated its efficacy in spontaneous acute pneumonia in the guinea pig and the mouse; all signs of infection resolved in the treated animals, with restoration of the parenchyma. These in vivo studies confirm the good diffusion of the aerosol preparation as far as the deep lung where it acts effectively.

The commercial fusafungine aerosol can be considered a bimodal type of micronised aerosol with a 0.7 μm fraction and a 3 μm fraction; 99% of the particles have a diameter less than 5.5 μm and the median diameter is 0.78 μm. These characteristics are particularly suitable for the deposition of the antibiotic in the distal airways: the terminal bronchioles, on the one hand, and the sinus cavities, on the other. However, when the patient is taught to force the ventilatory flow, it is possible to obtain a preferential tracheobronchial deposition. Rapid respiration leads to an immediate deposition of the particles in the buccal cavity and laryngotracheal junction, while slow inhalation via the

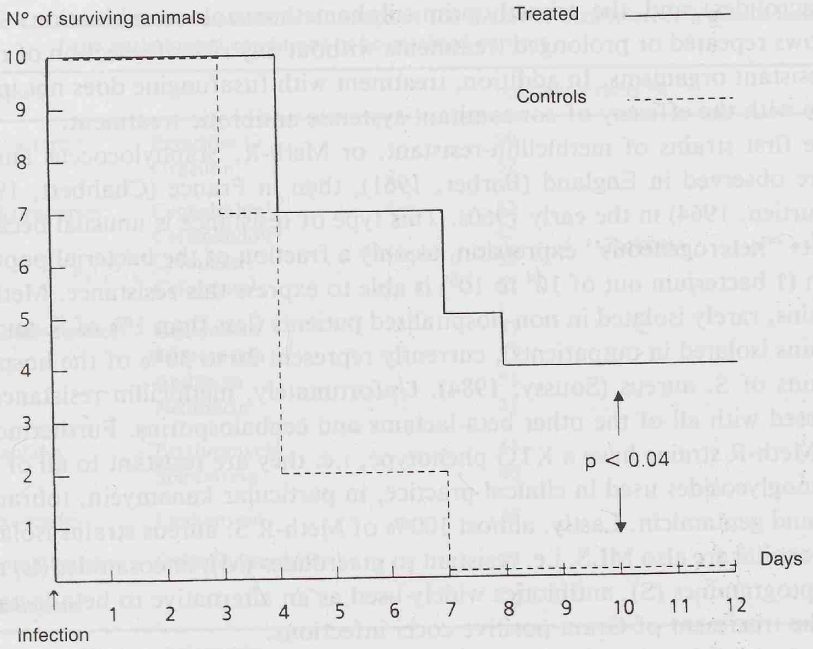

Figure 1. Actuarial survival curve of mice with experimental pneumonia produced by Streptococcus pneumoniae

mouth promotes the regular deposition at all levels of the respiratory tract (Stahlhofen et al., 1980). On the basis of physical and granulometric data, the proportion of the mass inhaled by aerosol deposited in the various airways in man was evaluated to be 58% in the lungs, 16% in the trachea and bronchi and 37% in the nasal region (Masse, 1984). A therapeutic session of the commercial aerosol preparation of fusafungine resulted in the following local concentrations: 80 mcg/ml in the lungs (deep lung, bronchioles and alveoli), 40 mcg/ml in the trachea and bronchi and 60 mcg/ml in the nose. The local concentrations obtained under normal conditions are therefore always higher than the MIC indicated in the spectrum of action of fusafungine.

STABILITY OF THE ANTIBACTERIAL ACTIVITY OF FUSAFUNGINE

Fusafungine has a stable spectrum of action. Fusafungine did not induce any direct bacterial resistance following repeated in vitro contacts simulating prolonged in vivo treatment. We also noted, in vitro, the absence of induction of crossed resistance with the following systemic antibiotics: penicillin G, cephacetrile (first generation cephalosporin), tetracycline and minocycline (tetracyclines), streptomycin (aminoglycoside), erythromycin and josamycin

(macrolides) and the trimethoprim-sulphamethoxazole combination. This allows repeated or prolonged treatments without any risk of selection of multiresistant organisms. In addition, treatment with fusafungine does not interfere with the efficacy of concomitant systemic antibiotic treatment.

The first strains of methicillin-resistant, or Meth-R, Staphylococcus aureus were observed in England (Barber, 1961), then in France (Chabbert, 1962; Courtieu, 1964) in the early 1960s. This type of resistance is unusual because of its "heterogeneous" expression, as only a fraction of the bacterial population (1 bacterium out of 10^4 to 10^6) is able to express this resistance. Meth-R strains, rarely isolated in non-hospitalised patients (less than 1% of S. aureus strains isolated in outpatients), currently represent 20 to 30% of the hospital strains of S. aureus (Soussy, 1984). Unfortunately, methicillin resistance is crossed with all of the other beta-lactams and cephalosporins. Furthermore, all Meth-R strains have a KTG phenotype, i.e. they are resistant to all of the aminoglycosides used in clinical practice, in particular kanamycin, tobramycin and gentamicin. Lastly, almost 100% of Meth-R S. aureus strains isolated in hospital are also MLS, i.e. resistant to macrolides (M), lincosanides (L) and streptogramines (S), antibiotics widely used as an alternative to beta-lactams in the treatment of Gram positive cocci infections.

Faced with this pejorative development of antibiotic resistance of nosocomial strains of S. aureus, we investigated whether a similar effect had developed with fusafungine since its introduction into clinical practice in the 1960s. We therefore studied the sensitivity of 169 strains of Staphylococcus isolated from hospitalised patients, including 106 strains of S. aureus, in relation to fusafungine and 22 systemic antibiotics commonly used in hospitals. 105 of these 106 strains (99%) were resistant to penicillin and 55 (52%) were resistant to oxacillin (Table 4). 52% of strains were therefore Meth-R, which is a much higher percentage than the isolation frequencies reported to date. These Meth-R strains were resistant to cephalosporins (47 to 52% of resistant strains), including cefotaxim, a third generation cephalosporin. As expected, these strains were also resistant to aminoglycosides (51 to 53% of resistant strains), to macrolides (47% of strains resistant to erythromycin and 43% resistant to spiramycin), to lincosanides (45% of strains resistant to lincomycin) and to streptogramine B (53% of resistant strains).

These data demonstrate that about 50% of the strains of S. aureus isolated from pathological conditions in hospitalised patients were Meth-R with a KTG and MLS phenotype. In contrast, 91% of the strains were sensitive to fusafungine with values for MIC less than 32 mcg/ml and 9% were resistant with values for MIC greater than or equal to 128 mcg/ml, although no relationship could be established with resistance to the antibiotics studied.

More importantly, during the first studies conducted on wild strains in the

Table 4. Heterogeneous methicillin resistance of 106 strains of Staphylococcus aureus isolated from pathological conditions in hospitalised patients.

antibiotic		resistant strains %
Beta-lactams:	Penicillin G	99
	Oxacillin	52
Cephalosporins:	Cephalothin[1]	52
	Cefamandole[2]	47
	Cefotiam[2]	55
	Cefotaxim[3]	52
Aminoglycosides:	Gentamicin	53
	Tobramycin	53
	Amikacin	53
	Netilmicin	51
Macrolides:	Erythromycin	57
	Spiramycin	45
Lincosanides:	Lincomycin	45
Streptogramines:	Streptogramine B	53
Fusafungine:		9

[1] First generation cephalosporin.
[2] Second generation cephalosporin.
[3] Third generation cephalosporin.

1960s, we found that 88% of S. aureus strains were sensitive to fusafungine, with values for MIC less than 40 mcg / ml (unpublished results). The sensitivity to fusafungine has therefore not really varied for nosocomial strains of S. aureus, although they are becoming more and more frequently resistant to systemic antibiotics. These results are therefore extremely important. They confirm the stability of the spectrum of action of fusafungine as well as the efficacy of fusafungine on bacterial strains which have become resistant to a large number of commonly used systemic antibiotics.

CONCLUSION

The spectrum of antibacterial activity of fusafungine covers all of the Gram positive bacteria usually encountered in tracheo-broncho-pulmonary infections. Fusafungine demonstrated a remarkable efficacy on strains of S. aureus isolated from hospitalised patients, 91% of which were sensitive to fusafungine, while 52% of them were methicillin-resistant and presented a KTG, MLS phenotype. The therapeutic value of fusafungine is reinforced by the fact that, under experimental conditions, it does not induce acquired resistance or crossed resistance with other systemic antibiotics. This allows the prescription of long-term or repeated treatments without any risk of selection of multiresis-

tant organisms and without interfering with systemic antibiotic therapy.

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Antiviral activity of fusafungine

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Fusafungine, the active ingredient of Locabiotal® Aerosol (Laboratoires Servier), is an antibiotic of fungal origin extracted from Fusarium lateritium. Its antibiotic properties were described by German (1984). Its spectrum of action is particularly adapted to the treatment of respiratory tract infections, especially those due to Gram positive cocci and bacilli. It also has the advantage of not inducing bacterial resistance (German, 1984). Only a few studies have been conducted on its action on the cells involved in the fight against viral infection (Nolibé et al., 1987). The aim of the present study was to determine the effects of fusafungine on natural killer cells (NK cells), which are present in large numbers in the lungs (Nolibé et al., 1981) and are known for their antiviral activity (Biron et al., 1983).

MATERIALS AND METHODS

Animals

Two-months old male OFA rats were obtained from Iffa Credo (Lyon). Wistar AG rats were obtained from CSEAL (Orléans la Source) for the tests requiring the use of a syngenic strain.

Exposure

Thirty animals were simultaneously exposed in a double enclosure described by André et al. (1988). The aerosol was produced by an Acorn generator containing the Locabiotal® Aerosol solution. The yield (quantity in the generator / quantity deposited in the lungs) was calculated by using a fusafungine solution in which the vehicle was labelled with Iodine 131 (Oris Industrie). The dose delivered was obtained by varying the duration of exposure.

Collection of cells

Alveolar macrophages were collected from isolated lungs by bronchial lavage with saline solution (6 × 5 ml). Pulmonary lymphocytes were obtained by perfusion of the pulmonary microcirculation with saline solution via the pulmonary artery (Nolibé et al., 1981). In order to avoid separation on ficoll, the first five millilitres of perfusate, with an excessively high red cell content, were discarded.

Measurement of NK activity

NK activity was measured in vivo by the test of pulmonary clearance of radiolabelled tumour cells (Fidler, 1970) and in vitro by the Chromium 51 release test (Heberman et al., 1975). In vivo, the number of viable cells was calculated by measuring the residual radioactivity present in the lungs 24 hours after intravenous injection of Iodine 125-labelled syngenic tumour cells. In vitro, the syngenic cell lines (pulmonary fibrohistiocytoma P77) and allogenic cell lines (lymphoma YAC-1 induced by Moloney's virus) were used as targets and the lymphocytes of the pulmonary circulation as effectors. The experiments were performed in triplicate for each effector-target ratio. The percentage lysis was calculated by means of the formula: (test cpm release − spontaneous cpm release / maximal cpm release − spontaneous cpm release) × 100.

Measurement of 2',5'-oligoadenylate synthetase activity

This activity was measured according to the technique described by Justesen et al. (1980) from cellular lysates in the presence of poly(I) and poly(C) and ^{32}P-ATP. The reaction products were analysed by thin layer chromatography, autoradiography and counting of the radioactivity corresponding to ATP and to 2',5'-oligoadenylates. The results were expressed in nanomoles of ATP / min / 10^7 cells.

RESULTS

Evaluation of the dose delivered

As previous experiments demonstrated that intratracheal injection could induce heterogeneous pulmonary perfusion, Locabiotal® Aerosol was therefore delivered by aerosol. In view of the properties of the solvent present in Locabiotal® Aerosol solution, it was necessary to evaluate, under these conditions, the yield of the generator, i.e. the fraction of the dose introduced into the generator which actually reaches the peripheral lung. Three groups of OFA rats (10 animals per group) were maintained for 30, 60 and 120 minutes respectively in an atmosphere saturated with Iodine-125-labelled Locabiotal® Aerosol. Five animals from each group were sacrified one and three hours respectively after the end of inhalation. The radioactivity present in the lungs was then evaluated in relation to the radioactivity dissipated by the generator. The yield of inhalation was evaluated to be 1.10^{-5} with a satisfactory reproducibility in the animals of the same group. As this yield remained constant for the different exposure times (Table 1), the dose delivered could be varied by varying the exposure time. Under the experimental conditions used, exposure times of 30 and 60 minutes corresponded respectively to doses of 10 and 20 μg / kg of fusafungine in the lungs. However, although 2 / 3 of the radioactivity inhaled was present in the lungs and 1 / 3 was present in the trachea after an

Table 1. Evaluation and distribution of the dose delivered by inhalation.

	exposure time		
	30 min	60 min	120 min
Yield ($\times 10^{-5}$)*	1.25	1.01	1.29
Lungs (%)	67	71	48
Trachea (%)	33	29	52

* ^{131}I-Locabiotal nebulised / ^{131}I-Locabiotal in lungs + trachea

Table 2. NK activity of pulmonary intracapillary cells against YAC-1 tumour cells.

treatment of the animals on D-1	% cytotoxicity for effector-target ratios			
	100:1	50:1	25:1	12.5:1
0	24.7 ± 1.4	19 ± 0.9	10 ± 0.3	5.7 ± 0.6
Fusafungine 10 µg / kg	39.9 ± 0.7	35.7 ± 0.2	29 ± 1.1	19.1 ± 1.2
Fusafungine 20 µg / kg	32.5 ± 0.4	23.8 ± 0.6	18.2 ± 0.8	9 ± 0.9
Poly(I) Poly(C_{12},U) 1 mg / kg	43.9 ± 0.9	39.7 ± 1.4	31.4 ± 0.3	22.1 ± 1.1

exposure time of 30 or 60 minutes, an increased proportion was detected in the trachea after longer exposure times (Table 1). In the animals sacrified three hours after exposure, the pulmonary load represented an average of 51% of the quantity present at one hour.

Effects of fusafungine on Natural Killer (NK) activity

Twenty-four hours after exposure to Locabiotal® Aerosol, the lymphocytes obtained by perfusion of the pulmonary capillaries were tested for their NK activity against P77 and YAC-1 tumour cells. Similar results were obtained with the two tumour cell lines used. For a dose of 10 µg / kg and with all of the effector-target ratios tested, a very significant increase in NK activity was observed, which represented 160 to 300% of the values obtained in the control animals (Table 2). Within the limits of the experiment, this effect was dose-dependent (Table 2).

Qualitative and quantitative analyses of the cells obtained by perfusion of the pulmonary circulation did not reveal any increase in the total number of cells collected or any increase in the percentage of large lymphocytes with azurophilic granulations (cells with NK activity).

The kinetics of NK stimulation indicated that the maximal increase was observed 24 hours after exposure. The duration of stimulation also appeared to depend on the dose delivered; 48 hours after inhalation, it was an average of

Table 3. Effect of fusafungine on lymphocyte 2′,5′ A synthetase activity.

	2′,5′ A synthetase*	
	controls	fusafungine**
Peripheral blood lymphocytes	0.10 ± 0.05	0.11 ± 0.06
Lymphocytes in the pulmonary circulation	0.02 ± 0.01	0.07 ± 0.02

* in nmoles of ATP / min / 10^7 cells; ** 10 μg / kg per inhalation.

50% for the dose of 10 μg / kg, while it was already no longer significant for the dose of 20 μg / kg.

These in vitro results were confirmed in vivo by the test of pulmonary clearance of radiolabelled tumour cells. A significant reduction in the number of viable tumour cells was observed 24 hours after treatment with fusafungine (Nolibé et al., 1987). Various parameters characteristic of NK stimulation by fusafungine were compared with those obtained after treatment with an interferon-inducing polynucleotide: poly(I) poly(C12.U). Although the increase in NK activity induced by the polynucleotide at all of the effector-target ratios was superior to that induced by fusafungine, the stimulation was of the same order of magnitude for the two substances (Table 2), followed the same kinetics and was confirmed in vivo (Nolibé and Thang, 1985). In view of the similarities recorded, the action of fusafungine can be considered to involve interferon-dependent stimulation.

The assay of 2′,5′ A synthetase was used to indirectly measure the level of circulating interferon. The peripheral blood lymphocytes separated on a ficoll gradient and tested 18 hours after the end of inhalation did not reveal any increase in 2′,5′ A synthetase (Table 3) in contrast with treatment by interferon inducers (Youn et al., 1983). In order to test the hypothesis of limited production at the site of deposition of fusafungine, the same assay was performed on lysates of lymphocytes extracted from the pulmonary microcirculation. A significant increase was observed, but it remained low in comparison with the production by peripheral blood lymphocytes (Table 3). Alveolar macrophages collected 1 to 15 hours after the end of exposure did not reveal any modification in their 2′,5′ A synthetase content induced by fusafungine, in contrast with poly(I) poly(C12.U), which induced a very significant increase by the twelfth hour after its administration (Table 4).

DISCUSSION

The stimulation of intrapulmonary NK activity induced by fusafungine has been previously described (Nolibé, et al., 1987). In this study, we demonstrate

Table 4. Comparison between fusafungine and Poly(I) Poly(C_{12},U) on alveolar macrophage 2′,5′ A synthetase activity

	2′,5′ A synthetase*					
	1 hr	3 hr	6 hr	9 hr	12 hr	15 hr
Controls	0.20	0.21	0.29	0.24	0.34	0.30
Fusafungine**	0.30 ± 0.14	0.32 ± 0.09	0.32 ± 0.07	0.28 ± 0.02	0.21 ± 0.07	0.30 ± 0.12
Poly(I) Poly(C_{12},U)***	0.31 ± 0.07	0.53 ± 0.02	0.58 ± 0.07		0.85 ± 0.10	1.24 ± 0.3

* in nmoles of ATP / min / 10^7 cells, at time t after treatment;
** 10 μg / kg per inhalation;
*** 1 mg / kg per intratracheal injection.

that not only this stimulation, but also its kinetics are dose-dependent. The stimulation, limited to 24 hours for the dose of 20 μg / kg, was still significant 48 hours after the dose of 10 μg / kg. Complementary studies will need to define these dose-response relationships. The two parameters of stimulation: intensity and kinetics can be used to define the optimal dose or the modalities of repeated treatment, if the stimulation is too brief.

The presence of a population of NK cells in the pulmonary capillaries quantitatively superior to that demonstrated in the peripheral blood (Nolibé et al., 1981) accentuates the potential value of stimulation of this function in the course of viral infections (Welsh et al., 1984). In particular, due to the rapidity of the response induced, fusafungine may constitute effective treatment by stimulating this first line of defence. NK activity is controlled by a large number of factors, the most important of which include interferon (Koren et al., 1981). In order to define the mechanism of action of fusafungine and bearing in mind that the stimulation obtained presents the same features as that induced by interferon-inducing polynucleotides, a comparative study was conducted over a period of 24 hours preceding the peak of maximal activity. As the level of circulating interferon was too low to be measured directly after injection of the polynucleotides (Hovanessian et al., 1982), its detection can be approached by measuring an enzymatic marker, 2′,5′ A synthetase (Justesen et al., 1980). In contrast with polynucleotides (Youn et al., 1983), fusafungine did not induce an increase in this marker in the peripheral blood. As fusafungine was administered by aerosol, we can propose the hypothesis of an increased production limited to the pulmonary compartment. Although an increase in 2′,5′ A synthetase was observed in macrophages after treatment with the polynucleotide, no modification was demonstrated in these cells after fusafungine aerosol. The intrapulmonary lymphocytes revealed a slight in-

crease in the 2′,5′ A synthetase content, but the production was very much less than that observed in the peripheral blood lymphocytes; the significance of this result has yet to be analysed.

All these data tend to exclude the role of interferon in the stimulation observed. However, it should be noted that, as the increase in 2′,5′ A synthetase occurs 15 to 18 hours after the peak of interferon production (Youn et al., 1983), it is possible that the measurements were obtained too early. Moreover, the correlation between interferon production and the presence of 2′,5′ A synthetase has only been clearly established for α and β interferons (Ferbus et al., 1987). The possibility of activation via interferon can therefore not be totally excluded, especially as interferon-lymphokinin synergies have been described at very low levels. This problem can only be resolved by studying the effect of an anti-γ-interferon serum on fusafungine-induced stimulation.

Another pathway of stimulation of the cytotoxic activity of NK cells via lymphokinins and activation of macrophages has also been reported (Trinchieri et al., 1984). This possibility is currently being investigated.

Finally, because of the anti-inflammatory properties of fusafungine (White et al., 1988) and the regulatory role of arachidonic acid metabolites on NK activity (Koren et al., 1981; Rossi et al., 1985), this mechanism should not be neglected in the interpretation of the stimulation obtained.

The respective roles of interferon and lymphokinins in the stimulation of NK activity by fusafungine should be defined in the course of future studies. However, in view of the large numbers of these cells in the lungs and the rapidity of the response, their stimulation by fusafungine may constitute an effective treatment, at least during the initial phase of pulmonary viral infections.

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In vitro and in vivo efficacy of fusafungine on Legionella pneumophila

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Infections of respiratory tract are among the most common indications for antibiotics. It is generally accepted that the concentration of antibiotic at the site of infection should exceed that needed to inhibit growth of the organisms. Local administration of antibiotics for the treatment of respiratory infections has the potential advantage of reduced systemic toxicity and increased drug concentration at the site of infection. Pulmonary drug delivery using aerosols may provide certain therapeutic advantages over systemic therapy (Molina et al., 1987).

Fusafungine is an antibiotic of fungal origin, extracted from Fusarium lateritium WR, strain 437; it is the active principle of Locabiotal (Servier Laboratories – France). This antibiotic possesses the original feature of being the only representative of its family used in therapeutics and it is only used by the local route in the form of an aerosol. No cases of acquired direct or crossed resistance to fusafungine have been reported to date.

Some people develop pneumonia after inhalation of Legionella pneumophila. Several factors are involved singly or in combination. When a sufficient number of virulent organisms reaches the respiratory tract beyond the ciliated epithelium of a susceptible host, acute alveolar infection develops. Legionella pneumophila, the etiologic agent of legionnaires' disease, is a facultative intracellular bacillus that evades host defences by parasitizing monocytes (Horwitz et al., 1980). It resists the bactericidal effect of immune serum, but its binding to monocytes is facilitated by specific antibody and complement. Erythromycin alone or in combination with rifampin is the currently accepted treatment of legionnaires' disease, though failure or delayed activity are observed, especially in immunocompromized patients. Thus, the activity of other antimicrobial agents has to be tested.

Evaluation of antimicrobial agents for the prevention or the treatment of legionnaires' disease is difficult because in vivo Legionella multiply intracel-

lularly and in vitro they grow only on special medium that modify many antibiotics' activity (Edelstein et al., 1980).

In this presentation, we studied two different aspects of fusafungine activity on virulent Legionella pneumophila: 1. in vitro activity on Legionella pneumophila culture to determine minimum inhibitory concentration (MIC), and 2. activity of this drug administered by aerosol route on in vivo Legionella pneumophila multiplication in guinea pigs.

IN VITRO ACTIVITY OF FUSAFUNGINE ON LEGIONELLA PNEUMOPHILA MULTIPLICATION

The present study concerned 27 different strains of Legionella pneumophila: 21 virulent strains of serogroup 1; four of serogroup 3 and two of serogroup 6. The evaluation of fusafungine activity on Legionella pneumophila used the usual methods (Barry, 1985): diffusion on solid medium, dilution on solid medium, dilution on liquid medium. The goal is the determination of minimum inhibitory (and bactericidal) concentration of fusafungine on Legionella pneumophila. Figure 1 shows that the distribution of mean inhibitory diameters of fusafungine obtained by diffusion method in solid medium was unimodal with 89% strains (inhibition diameters between 11 and 15 mm for a disc charge of 30 μg).

The minimum inhibitory concentration must be determined by several methods; in fact the poor solubility of fusafungine requires an organic solvant.

1. The critical concentration m′ can be determined experimentally by testing a bacterial isolate against several different concentrations of an antimicrobial agent, all applied to the same test plate. Under these conditions, the inoculum density, temperature of incubation, medium and timing of the experiment will all be constant factors. The critical concentration m′ represents a measure of the susceptibility of a test organism: m′ is similar to but not identical to the MIC as measured by dilution techniques that involve somewhat different test conditions. Figure 2 represents the determination of m′ for fusafungine on Legionella pneumophila. The corresponding volume to the critical charge is about 0.1 ml; under this condition the concentration value expressed per milliliter is tenfold, i.e. 17.6 mg / ml.

2. The dilution method in solid medium uses gelose base BCYE. We obtained a minimum inhibitory concentration of about 20 μg / ml whatever the organic solvant used to dissolve fusafungine.

3. The determination of fusafungine activity on Legionella pneumophila in liquid medium needs special growth conditions (yeast extract, ACES buffer, cetoglutaric acid; iron pyrophosphatic and L cystein). We obtained a minimum inhibitory concentration (MIC) of 4 μg / ml and a minimum bactericidal concentration (MBC) of 8 μg / ml. The MBC / MIC ratio equal to 2 is effective.

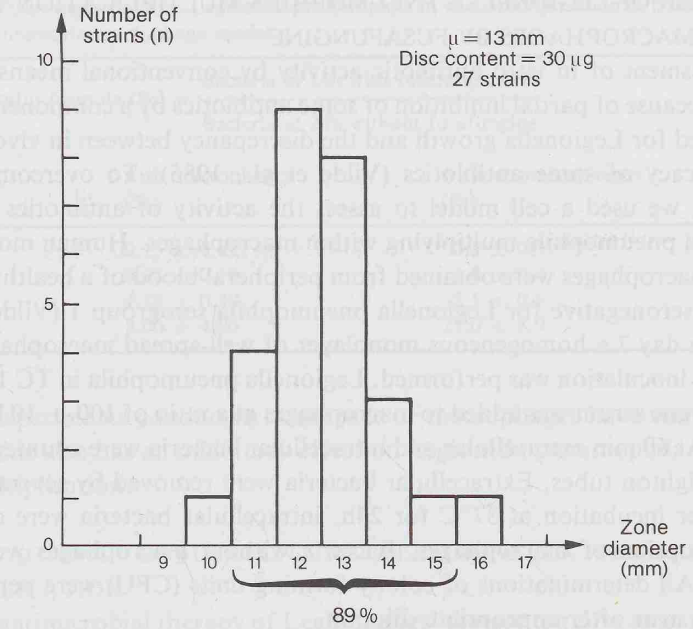

Figure 1. Distribution of inhibition diameter on 27 virulent strains of Legionella pneumophila (μ = mean inhibition diameter).

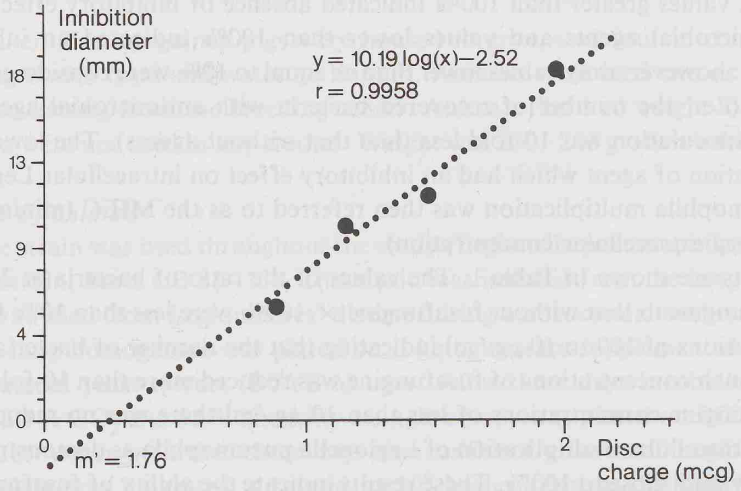

Figure 2. Determination of critical concentration (m') of fusafungine on Legionella pneumophila by linear regression and correlation.

INHIBITION OF LEGIONELLA PNEUMOPHILA MULTIPLICATION WITHIN HUMAN MACROPHAGES BY FUSAFUNGINE

The assessment of in vitro antibiotic activity by conventional means is misleading because of partial inhibition of some antibiotics by a component of the media used for Legionella growth and the discrepancy between in vivo and in vitro efficacy of some antibiotics (Vilde et al., 1986). To overcome these obstacles, we used a cell model to assess the activity of antibiotics against Legionella pneumophila multiplying within macrophages. Human monocyte-derived macrophages were obtained from peripheral blood of a healthy donor who was seronegative for Legionella pneumophila serogroup 1 (Vilde et al., 1985). On day 7 a homogeneous monolayer of well-spread macrophages was obtained; inoculation was performed. Legionella pneumophila in TC 199 with 10% immune serum was added to macrophages at a ratio of 100 ± 10 bacteria per cell. At 60 min extracellular and intracellular bacteria were counted in one of the Leighton tubes. Extracellular bacteria were removed by several washings. After incubation at 37°C for 24h, intracellular bacteria were counted after disruption of macrophages. Bacteria without macrophages were also counted. All determinations of colony forming units (CFU) were performed on BCYE agar after appropriate dilution.

The number of Legionella pneumophila 24h after macrophage infection in the presence of serial concentrations of antimicrobial agent, was compared with the number of Legionella pneumophila at 24h without agent. Results were expressed by the inhibition ratio (Legionella pneumophila at 24h with agent / Legionella pneumophila at 24h without agent) × 100%.

Thus, values greater than 100% indicated absence of inhibitory effect of the antimicrobial agents and values lower than 100% indicated an inhibitory effect: however, only values lower than or equal to 10% were considered inhibitory (i.e., the number of recovered bacteria with antimicrobial agents 24h after inoculation was 10-fold less than that without agents). The lowest concentration of agent which had an inhibitory effect on intracellular Legionella pneumophila multiplication was then referred to as the MIEC (minimum inhibitory extracellular concentration).

Results are shown in Table 1. The values of the ratio of bacteria at 24h with fusafungine to that without fusafungine × 100% were less than 10% for concentrations of 100 to 10 μg / ml indicating that the number of bacteria at 24h with such concentrations of fusafungine was reduced more than 10-fold. With fusafungine concentrations of less than 10 μg / ml there was no reduction of the intracellular multiplication of Legionella pneumophila as demonstrated by ratio values close to 100%. These results indicate the ability of fusafungine to penetrate inside the macrophages and to inhibit Legionella pneumophila multiplication.

Table 1. Fusafungine inhibits Legionella pneumophila in vitro multiplication in human derived monocyte-macrophage model.

$$\text{Inhibition ratio formula (\%)} = \frac{\text{Bacteria at 24h with fusafungine (F)}}{\text{Bacteria at 24h without fusafungine}}$$

F (μg/ml)	with macrophages (%)	without macrophages (%)
100	0.15 ± 0.05	0.5 ± 0.3
50	0.38 ± 0.10	1.0 ± 0.4
30	1.18 ± 0.30	2.5 ± 0.5
10	8.00 ± 4.00	25.0 ± 8.0

Similar experiments conducted in absence of macrophages have showed that fusafungine also has an inhibitory effect on Legionella pneumophila extracellular multiplication.

IN VIVO ACTIVITY OF FUSAFUNGINE ON LEGIONELLA PNEUMOPHILA MULTIPLICATION

Specific antimicrobial therapy of Legionnaires' disease requires an experimental animal model to compare in vivo data versus in vitro microbiologic determinations. We have used a previously described guinea pig model (Dournon et al., 1986) for the purpose of testing fusafungine aerosol in experimental prophylactic Legionella pneumophila disease.

Animals

Male Duken Hartley guinea pigs were housed in groups of four in conventional cages with food and water ad libitum. Animals were used two or four days after coming from the breeding center. Animals that had weight loss or diarrhoea were not used in any studies. Weight was 230–260 g when infected.

Infection of animals

The same strain was used throughout the study (Legionella pneumophila serogroup 1 strain, Paris CB 81–13). This strain was isolated from the lung of a patient who died from Legionnaires' disease during a nosocomial outbreak in Paris. A lung homogenate was plated on α-ketoglutarate-supplemented buffered charcoal yeast extract (BCYE-α) agar. After three days incubation at 35°C in 2.5% CO_2 and 95% humidity a single colony was plated on BCYE-α agar plates. After a further three days the confluent growth of Legionella pneumophila was harvested, adjusted to 10^9 bacteria/ml in distilled water and aliquoted at −80°C until used. Virulence of this twice BCYE-α agar passaged strain was assessed by its ability to multiply within human monocyte-

derived macrophages. Animals were infected intraperitoneally with 2×10^6 Legionella pneumophila in 2 ml distilled water. Infected animals were treated by fusafungine by aerosol route twice: 12h before and 12h after bacterial challenge.

Aerosol exposure system

Fusafungine aerosols were delivered two times in 5 to 10 minutes to each animal by a Bird system submitted to 500 mbar compressed air. It has been proved that with this sytem 25% of the particles have a diameter up to 5 μm, which is required for alveolar penetration. The guinea pig was placed in the plethysmograph (Battelle Center for Toxicology and Biosciences, Geneva – Switzerland). The ventilation was measured during the aerosol delivery.

The effectively inhaled amount was estimated to be equal to aerosol fusafungine concentration (mg/ml) × duration of aerosol (minutes) × animal ventilation (ml/min). The emerging guinea pig head was connected to the Bird nebulizer. The nebulizer reservoir was filled at 2 ml of fusafungine solution (Locabiotal – Servier). The amount of fusafungine nebulized solution was determined by weighing the reservoir before and after delivery. Male guinea pigs were randomly assigned to various groups.

Group 1: Guinea pigs with Legionella pneumophila infection without fusafungine treatment

A – no aerosol

B – saline aerosol

Group 2: Guinea pigs with Legionella pneumophila infection without fusafungine treatment

A – no aerosol

B – excipient aerosol

Group 3: Guinea pigs with Legionella pneumophila infection

A – treatment: 2 aerosols (12h before and 12h after infection); dosage = 3.8 mg/kg

B – same treatment; dosage = 6.2 mg/kg

Counting of Legionella pneumophila in lungs

Animals were sacrified for counting of Legionella pneumophila in lungs and in some cases in blood monocytes. Animals were anaesthetized with ether and bled by cardiac puncture with a 20 gauge, vacutainer multi sample needle into EDTA vacutainer tubes. The thoracic cavity was then aseptically opened and the right lower lung removed. The piece was rapidly washed, weighed, then homogenized in 10 ml of sterile twice-distilled water. A 1:100 dilution was obtained and 0.1 ml was placed on BCYE-α agar plates for counting of

Table 2. Clearance of Legionella pneumophila in lungs of guinea pigs with and without fusafungine aerosols (twice for 10 mn) at 24h (after inoculation).

		log CFU
Control (saline)	(n = 8)	5.4 ± 0.4
Excipient	(n = 4)	5.8 ± 0.5
F 5 mg/kg	(n = 8)	3.2 ± 0.6*

* $p \leq 0.01$.

Legionella pneumophila. Results were expressed as $\log_{10}$ colony forming units (CFU)/g of fresh lung.
The number of CFU indicated the degree of lung infection and allowed determination of the inhibition of bacterial multiplication by fusafungine (Table 2).

RESULTS

The guinea pigs of the three group were sacrificed 48h after bacteria inoculation and Legionella pneumophila were counted after tissue disruption. Results were expressed as the inhibition ratio (IR) = total CFU at 48h compared to total CFU at 48h without antibiotic × 100.

Group 1		Group 2		Group 3	
A	B	A	B	A	B
100	105	100	109	35	28

Values greater or equal 100% indicate no inhibitory effect, values lower than 100% indicate an inhibitory effect. Results with fusafungine administered by aerosol exposure are consistent with a marked effect in reducing the bacterial load; of special interest is the high clearance of Legionella pneumophila in lungs, but also in blood monocytes (data not shown) in group 3. A prophylactic effect of fusafungine aerosol on Legionella pneumophila lung multiplication is demonstrated in these experiments.

STIMULATION OF ALVEOLAR MACROPHAGE CHEMILUMINESCENCE AFTER TREATMENT WITH FUSAFUNGINE AEROSOL

Aerosol treatment with fusafungine was tested in guinea pigs and rats for its effect on kinetics of the induction of bronchoalveolar cells (alveolar macrophages, lymphocytes, polymorphonuclear leukocytes). Fusafungine led to an increase in the total number of bronchoalveolar cells.
The alveolar macrophages recruited in response to fusafungine were activated,

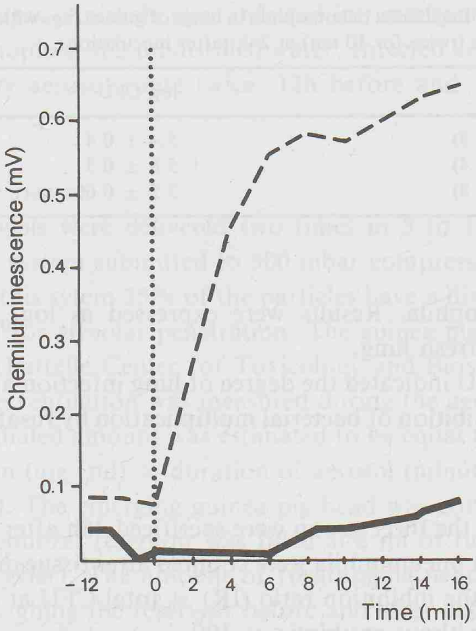

Figure 3. Luminol-dependent chemiluminescence (LDCL) response of alveolar macrophages from guinea pigs treated with fusafungine by aerosol route (3 mg/kg) (-----) and from control guinea pig (——). The magnitude of the response was calculated as the area under the response curve.

as indicated by luminol-dependent chemiluminescence in response to stimulation by opsonized zymosan (Figure 3).

The results suggest that activation of macrophages by fusafungine may partly explain the inhibition of lung multiplication of Legionella pneumophila.

CONCLUSIONS

Legionella pneumophila infections are highly representative of lung disease induced by intracellular pathogens. Fusafungine is effective in vitro on virulent Legionella pneumophila (especially strain 1). The determination of MIC by different ways shows effective MIC and a good MBC/MIC ratio (equal to 2). Fusafungine penetrates macrophages and limits the Legionella pneumophila intracellular multiplication. Fusafungine administered as Locabiotal is effective on guinea pig lung Legionella pneumophila multiplication. In addition, fusafungine increases luminol-dependent chemiluminescence from alveolar macrophage (with or without Legionella pneumophila). These results suggest that the inhibition of lung multiplication of Legionella pneumophila can be explained both by antibacterial activity and activation of macrophages by fusafungine.

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Fusafungine and inflammation

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The inflammatory reaction is the body's response to aggression, whether it is infectious, physical (heat, cold, electrical damage, irradiation) or mechanical. The inflammatory mechanism is stereotyped but, depending on the site of aggression, its intensity, duration and various constituent phenomena, inflammation develops at different rates and with different intensities: a simple, transient aggression triggers a brief response, while repeated and persistent attacks generally lead to sustained and chronic inflammation.

The reaction includes so-called "humoral" phenomena corresponding to haemodynamic modifications, alterations of vascular permeability, and so-called "tissue" phenomena, represented by cellular infiltration into the site of inflammation. The early and massive influx of cells coming from the periphery and migrating in the blood stream towards the site of inflammation consists of polymorphonuclear cells (neutrophils, basophils, eosinophils), platelets, mononuclear cells (lymphocytes, macrophages) and tissue mast cells. Macrophages involved in inflammatory reactions are derived from circulating blood monocytes which, once they have reached the site of inflammation, undergo structural, metabolic and functional modifications characteristic of the activated macrophage and develop the capacity to destroy foreign substances. These cells are involved in endocytosis.

In general, inflammation is a beneficial reaction of the tissues in response to aggression; it usually leads to elimination of the agent responsible and repair of the tissues. However, a large number of human diseases are due to uncontrolled inflammatory reactions which may lead to either tissue destruction or disorganised healing. The macrophage plays a key role in tissue restructuring under normal conditions as well as pathological conditions. The prolonged presence of highly active macrophages in the inflammatory focus probably contributes, to a great extent, to the associated pathology (Halliwell et al., 1986).

1. FREE RADICALS

During the 30 to 60 seconds following a stimulus, phagocytes start to produce

large quantities of free radicals (O_2.–); NAD(P)H oxidase catalyses the transformation of oxygen into superoxide, in which NADP(H) acts as the electron donor, leading to a sudden increase in cellular oxidation. The induction of this hyperactivity is independent of phagocytosis; it simply results from contact between the stimulus and the surface of the phagocyte (Damiani et al., 1980; Sherman et al., 1984; Baggliolini, 1984).

Although generally beneficial because of their bactericidal and healing action, the constant presence of free radicals, particularly when produced in large quantities, can induce severe or even irreversible damage. The body's natural defences, i.e. superoxide dismutase and catalase are overwhelmed (Diegelmann et al., 1981).

It is therefore not surprising that free radicals also lead to the destruction of tissues adjacent to their site of action. Depending on the extent of the deleterious effects of oxygen-derived free radicals, they may induce membrane peroxidation, destruction of connective tissue, cellular proliferation and fibrosis. Cell membranes, which are rich in unsaturated lipids, constitute an ideal target for oxygen-derived free radicals; we now know that these free radicals can cause destruction of collagen and glycosaminoglycans (Weissmann et al., 1979).

It was therefore interesting to determine whether fusafungine could prevent or neutralise the production of superoxide by inflammatory cells. In this case, this substance could probably also reduce or minimise the lesions and pathology associated with inflammation.

METHODS

Reference drugs

Non-steroidal anti-inflammatory (NSAI) agents, indomethacin and piroxicam, were used as reference drugs.

Macrophages

Peritoneal exudative macrophages were collected from the mouse peritoneal cavity by saline lavage according to the method described by Stewart et al. (1975). The exudative cells were collected three days after an intraperitoneal injection of 1.5 ml sterile 3% (w/v) thioglycolate. The macrophages obtained in this way were purified by adherence to plastic.

Zymosan

Zymosan particles were suspended in phosphate buffer at a concentration of 10 mg/ml, boiled for 20 minutes, washed three times and resuspended at a concentration of 50 mg/ml. They were then opsonised according to the method described by Jackson et al. (1975) by mixing one part of zymosan with

four parts of serum. The final suspension, at a concentration of 400 μg/ml, was presented to the test cells.

Superoxide production

The production of superoxides by mouse peritoneal exudative macrophages was studied by measuring the reduction of cytochrome C (according to the method described by Berton and Gordon, 1983). 1×10^6 peritoneal exudative macrophages per well (Costar 24-well plates) were cultured in an incubator with a humidified atmosphere (5% CO_2 and 95% ambient air). After 48 hours, the monocellular layer was washed twice with phosphate buffer. Cytochrome C (40 μM) was added in a volume of 0.5 ml. Samples of cells without cytochrome C or cytochrome C without cells were used as controls. Opsonised zymosan was used to stimulate the production of superoxides by the macrophages. Certain measurements were made in the presence of superoxide dismutase or catalase as controls. After 60 minutes incubation with the cells, the medium was collected and clarified by centrifugation. The supernatant was analysed to evaluate the reduction of cytochrome C by spectrofluorometry at 550 μ. The optical density (O.D.) of the sample was converted, after subtraction of the O.D. of the control, to the value corresponding to the quantity of reduced cytochrome C in nanomoles by means of the formula $E_{550} = 2.1 \times 10^{-4}\ M^{-1}$. Fusafungine was tested at concentrations of 10^{-6} and 10^{-7} M and was compared to indomethacin (10^{-4} M and 10^{-7} M) and to piroxicam ($10^{-5} - 10^{-7}$). All of the cultures were preincubated with the drugs for five minutes before addition of zymosan.

Statistics

The mean values, standard deviations and levels of significance between the control and treated groups were calculated on a Hewlett Packard 1000 computer using Student's t-test.

RESULTS

The results presented in Figure 1 show that superoxide dismutase inhibited the reduction of cytochrome C by stimulated macrophages, demonstrating the presence of $O_2.-$ in the culture medium.

At concentrations of 10^{-6} and 10^{-7} M, fusafungine significantly reduced the production of $O_2.-$ ($0.01 < 2P < 0.05$) by stimulated macrophages. Similar results were observed with piroxicam, while indomethacin did not reveal any inhibitory activity, confirming previous results (White, unpublished results).

2. PHAGOCYTOSIS

It has been known since 1883 (Metchnikoff) that phagocytosis plays a role in

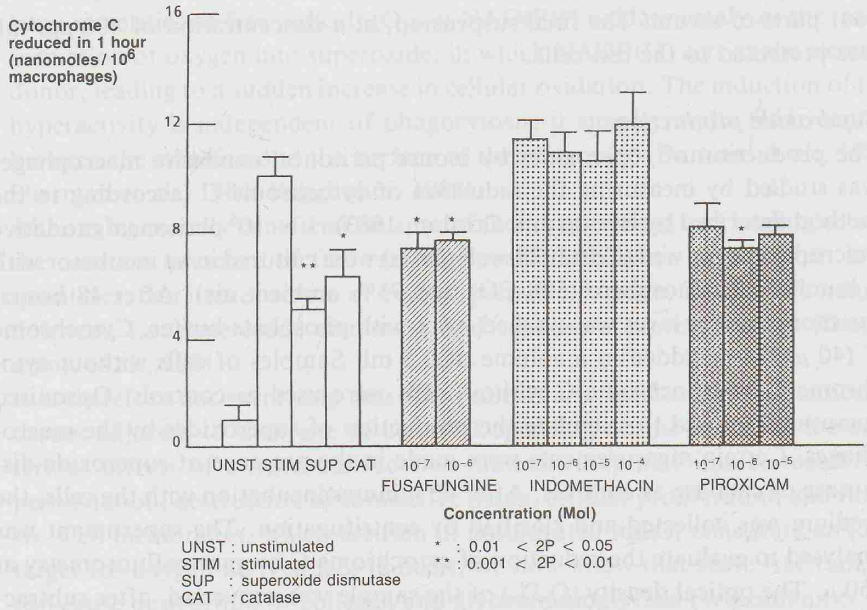

Figure 1. Effects of indomethacin, piroxicam and fusafungine on the production of superoxide by mouse peritoneal macrophages stimulated by zymosan.

the body's defences. The accumulation of numerous research studies has resulted in a large amount of information concerning phagocyte function. For example, we now know that a circulating phagocyte (neutrophil, basophil, eosinophil or monocyte) finds its site of impact by means of chemotaxis, as chemotactic substances are released in the vicinity of the site of inflammation. Phagocytes detect these substances and migrate towards their source. Phagocytosis commences as soon as these cells have reached their site of impact.

The "site of impact" is itself coated by special proteins produced by the infected organism which enable the phagocyte to identify it as the particle to be eliminated. These special proteins, known as opsonins, belong to the family of immunoglobulins and the complement system; they are thought to be recognised by specific receptors situated on the phagocyte membrane. Once it has been identified, the micro-organism coated with opsonins is enveloped by the phagocyte and is then destroyed (Baggliolini, 1984; Silverstein, 1977).

Most of the studies on phagocytosis are faced with certain problems, as it is very difficult or even impossible to differentiate between particles on the surface of the target cells and those inside the cell. Furthermore, it is often difficult and tiresome to count these particles. Labelling of the particles obviously simplifies the counting, but does not resolve the problem of defining the actual site of the particles (Silverstein, 1977).

Red blood cells were selected as particles, as they are relatively large, easily labelled and rapidly phagocytosed by macrophages. In contrast with many mammalian cells, macrophages are not lysed in distilled water, in which they can be immersed for several minutes without affecting their viability. Conversely, red blood cells (RBC) rapidly swell and burst in contact with distilled water. However, when they are enveloped by macrophages, they are protected against osmotic shock. In studies of phagocytosis, the RBC bound to the surface of the macrophages and the phagocytosed RBC can be distinguished from each other simply by placing the macrophages in distilled water; in this way, by lysing the RBC on the surface, we can be sure that all intact cells have been phagocytosed. Whole cells can be counted either directly or by means of a radioactive tracer.

METHODS

Macrophages

Peritoneal exudative macrophages were collected from 20 gram male DBA/2 mice by lavage of the peritoneal cavity with normal saline four days after an intraperitoneal injection of 1.5 ml of sterile 3% (w/v) thioglycolate. The macrophages obtained were then washed twice and cultured in Dulbecco's Modified Eagle's Medium (DMEM) on glass slides with a diameter of 12 mm, at a density of 10^6 cells per slide. After two hours, the cells were washed twice with DMEM and placed in the same medium containing 20% foetal calf serum and 1% pen-strep (GIBCO) (Stewart, 1975).

Labelling of the sheep red blood cells with ^{51}Cr

1 ml of sheep red blood cells was washed twice in phosphate buffer and resuspended in 10 ml of the same buffer. A 5 ml aliquot was collected, mixed with 500 μCi of $(Na)_2\ ^{51}CrO_4$ (200–400 Ci/g, New England Nuclear) in 500 μl of PBS and the mixture was incubated for 1 hour at 37°C. The ^{51}Cr-labelled cells were then washed twice in PBS to eliminate the unincorporated radioactivity (Henry, 1980).

Opsonisation

The ^{51}Cr-labelled sheep red blood cells were opsonised with heat-inactivated rabbit anti-sheep red blood cell serum. After one hour of incubation at 37°C, the cells were washed twice with PBS and resuspended in 5 ml of PBS at 4°C.

Preparation of the substances

The stock solutions of fusafungine and the reference products (indomethacin, piroxicam) were prepared by dissolution in DMSO at a concentration of 10^{-1} M. 10^{-7} M and 10^{-5} M solutions of fusafungine were prepared from the

stock solution by dilution in Dulbecco's Modified Eagle's Medium and were sterilised by filtration. The final solutions contained 0.1% DMSO. Colchicine was prepared in an identical way and tested at a concentration of 10^{-5} M.

Phagocytosis

After 24 hours, the macrophage cultures were placed in contact with 100 μl of labelled and opsonised sheep RBC and incubated in DMEM at 37°C. At regular intervals, over a period of 90 minutes, three of four slides were removed, rinsed in PBS at 4°C, then placed in distilled water (at 4°C) for 45 seconds to lyse the non-phagocytosed RBC.

The residual radioactivity was measured by a gamma counter. In the experiments designed to evaluate the effect of the drugs, the macrophages were preincubated for 10 minutes in the presence of the test products, which were also present during the phagocytosis periods.

Statistics

The mean values, standard deviations and levels of significance between the control and treated groups were calculated on a Hewlett Packard 1000 computer using Student's t-test.

RESULTS

In this study, the effects of fusafungine on phagocytosis were compared to those of two reference anti-inflammatory agents (indomethacin and piroxicam). Colchicine was used as a positive control; it depolymerises the components of the cytoskeleton of the cell and prevents phagocytosis. It was therefore not tested for the purpose of comparison.

After 45 minutes' incubation, the rate of phagocytosis was decreased with all concentrations of indomethacin tested and with piroxicam at concentrations of 10^{-6}, 10^{-5} and 10^{-4} M. Fusafungine had a very slight inhibitory effect at 10^{-5} M after 45 minutes, at the limit of significance ($0.01 < 2P < 0.05$).

None of the substances appeared to affect the total number of red blood cells phagocytosed (Figures 2 and 3). With the exception of 10^{-4} M of piroxicam, the number of RBC / 10^5 macrophages was the same in each group at the end of the experiment. The fact that the total number of RBC / 10^5 macrophages returned to control values after 90 minutes confirms the viability of the macrophages at the end of the experiment and that the reduced incorporation of labelled RBC was actually due to a slowing down in the reaction and not to death of the macrophages.

CONCLUSION

In summary, the inflammatory reaction is composed of an intricate network

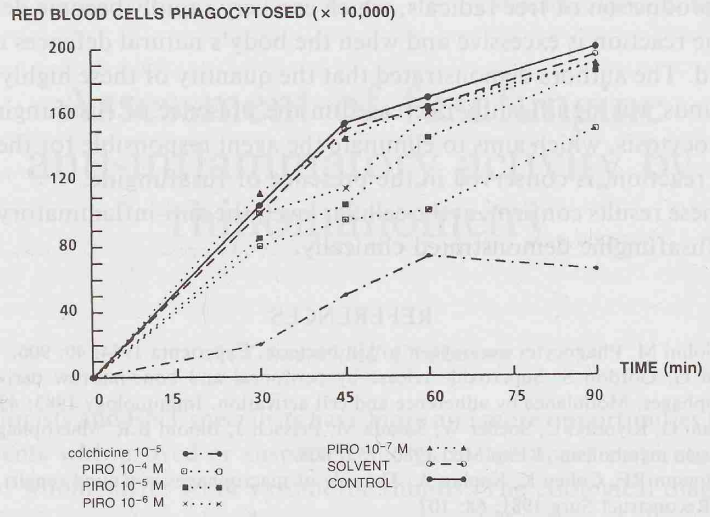

Figure 2. Effect of piroxicam on phagocytosis.

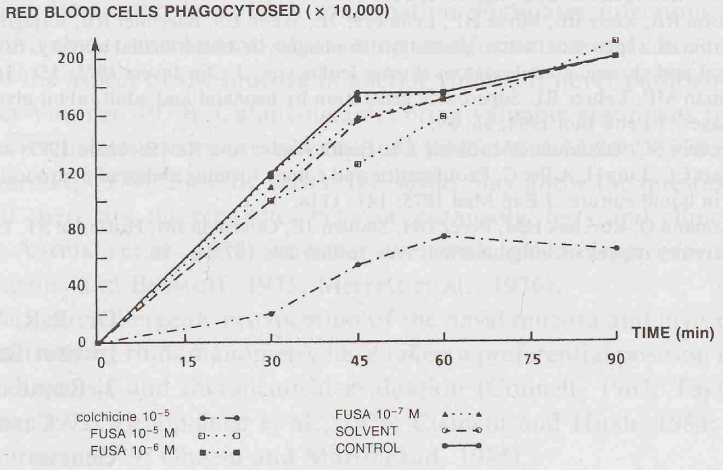

Figure 3. Effect of fusafungine on phagocytosis.

of phenomena which develop at different rates and different intensities. Although they have a beneficial effect when they develop normally, they can be responsible for pathological conditions when they are excessively intense or when the phenomenon is self-perpetuating. The authors wanted to study the effect of an anti-inflammatory agent on two of the processes occurring during the inflammatory reaction:

1. The production of free radicals, which can very rapidly become deleterious when the reaction is excessive and when the body's natural defences are overwhelmed. The authors demonstrated that the quantity of these highly reactive compounds was significantly decreased in the presence of fusafungine;
2. Phagocytosis, which aims to eliminate the agent responsible for the inflammatory reaction, is conserved in the presence of fusafungine.

All of these results confirm, at the cellular level, the anti-inflammatory properties of fusafungine demonstrated clinically.

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Assessment of fusafungine anti-inflammatory activity by rhinomanometry

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Allergologists and ENT specialists have more and more opportunities to examine patients with proved or suspected allergy of the upper respiratory tract; many of whom suffer from vasomotor rhinitis. The etiological diagnosis of such rhinitis, sometimes handicapping, is often difficult, and allergologic investigation is imperative in every case.

Clinical investigation may orientate, but most of the time it does not give conclusive diagnostic data; ENT examination eliminates infectious rhinitis and rhinitis due to mechanical obstruction, malformation and above all, polyps; but the aspect of the mucosa in allergic rhinitis is never pathognomonic (Moneret-Vautrin, 1979). Cutaneous tests bring valuable arguments for diagnosis, nevertheless, errors are not unusual (Daele and Melon, 1979; Girard and Mauroux, 1979). Specific serum IgE levels may allow the question to be settled if there are discrepancies between cutaneous tests and clinical data (Stenius-Aarniala et al., 1978); however, even with IgE levels, errors may happen (Huggins and Brostoff, 1975; Merrett et al., 1976).

For some years, allergenic provocation of the nasal mucosa and investigation of its reactivity by rhinomanometry have taken a preferential position in allergologic diagnosis and therapeutical evaluation (Connell, 1967; Taylor and Shivalkar, 1971; Holopainen et al., 1976; Clement and Hirsh, 1984; Girard and Mauroux, 1979; Ghaem and Martineaud, 1985).

The aim of the present study is to compare the results of allergenic provocation of the nasal mucosa, evaluated by rhinomanometry, before and after a ten-day topical treatment with fusafungine (Locabiotal® Aerosol).

MATERIAL AND METHODS

Allergenic provocation of the nasal mucosa

Nasal resistance was measured at rest, at first without any challenge, then after nebulizing 0.1 cm^3 of saline (NaCl: 154 $mmol.l^{-1}$) in each nostril in order to evaluate a possible effect of the solvent or a mechanical effect. Nasal resis-

tance measured in the latter condition was called initial resistance (Ri) and was used as baseline value. Allergen challenge was carried out based on a standardized protocol. In each nostril, 0.1 cm^3 of the allergenic solution was nebulized, the patient holding his breath. In order to avoid any inhalation of the product and any bronchial contamination, a nasal clip was immediately set. Fifteen minutes later, the clip was removed, the patient blew his nose, and after five to six minutes, the measurements were performed, one side after the other. The allergen was administered at increasing dosages until a positive response was obtained, i.e. an increase by about 100% of the total resistance for an airflow of 150 $cm^3.s^{-1}$; the shape of the dose response curve was also considered. The choice of allergen dosages was arbitrary, based upon the experience of the laboratory: 35, 70, 140 and 280 mcg (corresponding to the respective dilutions of 1/4000, 1/2000, 1/1000 and 1/500) successively, according to the responses observed, the doses being cumulative. The allergens used (house dust, acarien, grass pollen, cat dander) were obtained from the Institut Pasteur (Paris), purified, lyophilized and extemporaneously diluted.

Technical measure of nasal airway resistances (N.A.R.)

The principle of rhinomanometry is based upon the simultaneous determination of pressure and airflow changes in the nostrils throughout the ventilatory cycle (Masing et al., 1974; Graamans, 1981; Clement and Hirsh, 1984).

The method used was anterior rhinomanometry. The mouth being tightly closed, airflow and nasal anterior pressure were measured in one nostril, and posterior pressure in the opposite nostril carefully obstructed according to a technique previously described (Ghaem and Martineaud, 1985). Each nostril was investigated one after the other, and nasal airway resistance was determined by the ratio $\Delta P/\mathring{V}$: the ratio of the difference of pressure between anterior and posterior pressure to the airflow. Total resistance (Rt) was calculated from the resistance of the right and left nostrils (Rr and Rl) according to the equation):

$$\frac{1}{Rt} = \frac{1}{Rr} \frac{1}{Rl}$$

NAR is of mixed type and the resistance-airflow relationship is, according to Rohrer (1916):

$$R = K_1 + K_2 \mathring{V}$$

Determination of the resistance to laminar flow (K_1) and to turbulent flow

(K_2) allowed to calculate NAR at any time of the breathing cycle, for a given flow. The relationship resistance / flow could be established and both factors K_1 and K_2 were calculated from values registered during inspiration. In this study resistances were calculated at a flow of 0.15 $l.s^{-1}$, since in some patients, after allergen challenge, the maximum inspiratory flow was below 0.2 $l.s^{-1}$. NAR are expressed in $HPa.l.^{-1}s$.

Patients, treatment and study design

Drug and placebo were administered in double-blind random order. Fifty patients were included in the trial; one of them did not attend the second control, one other did not follow the course of his treatment. Therefore, the results concerned 48 patients, in equal proportion in both groups: 13 women and 11 men, mean age (27.0 ± 1.2 years) in the treated group, 14 women and 10 men (mean age 35.0 ± 2.1 years) in the placebo group. All patients had vasomotor rhinitis and the responsibility of the allergen was assessed by clinical investigation, positivity of cutaneous tests and of nasal provocation tests. Patients subject to bronchospasm were excluded.

The treatment consisted of four metered doses of fusafungine or placebo in each nostril four times a day, for 10 days. Patients were not allowed to take any other anti-inflammatory drugs, or vasoconstrictors. At the end of the treatment, and more than two hours after the last administration, patients were subjected to another nasal provocation test with saline. Thereupon, another provocation was performed with the same quantity of allergen than before treatment, and at the same hour of the day.

Statistics

The homogeneity of the groups was controlled with χ^2 or Barnett-Woolf G tests, when nominal variables were involved, and with Student's t-test when quantitative variables were involved. Homogeneity of responses before treatment and efficacy were studied by a two-way analysis of variance followed by Newmann-Keuls' test. Means are expressed with standard error of the mean.

RESULTS

When entering the study, both groups were statistically homogeneous for sex ratio, the implicated allergen and the duration of the allergenic disease (Table 1); the age difference between both groups was statistically significant.

The comparison of NAR at the same flow showed that (Figure 1):

1. Initial resistances were not significantly different in both groups (1.14 ± 0.06 group F and 1.10 ± 0.08 group P), and were not significantly modified after treatment (1.18 ± 0.08 group F and 1.20 ± 0.10 group P).

Table 1. Period of time of the evolution of the allergic condition divided over the two groups.

	number of patients	
period of time	fusafungine group	placebo group
< 5 yrs	16	12
5–9 yrs	4	4
10–14 yrs	1	2
> 15 yrs	3	6
Total	24	24

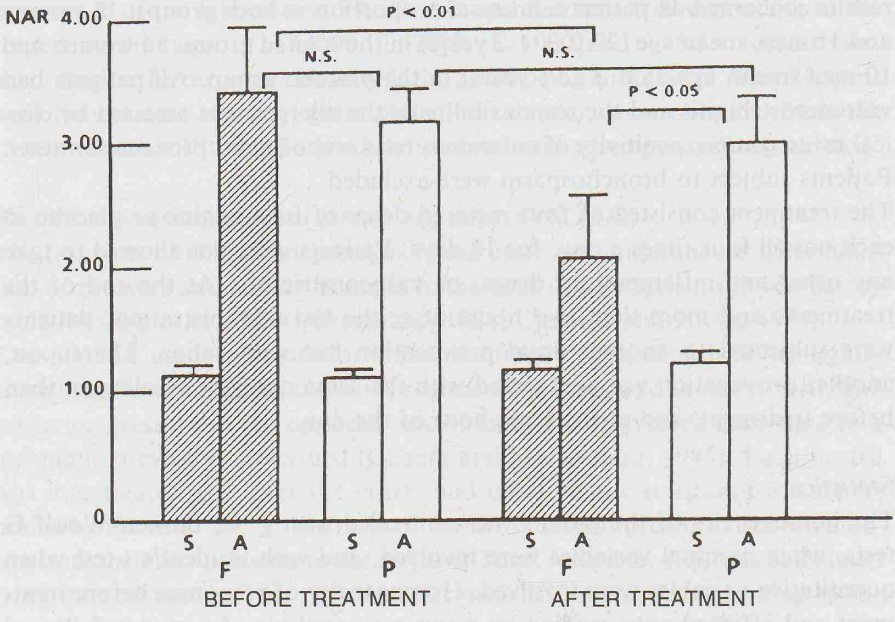

Figure 1. Nasal resistances in $HPa.l^{-1}.s$ calculated for a debit of 0,15 l/sec^{-1}.
S: serum; A: allergen; F: fusafungine group; P: placebo group; NAR: nasal airway resistance.

2. Before treatment, the responses to allergen challenge were not significantly different between both groups (3.40 ± 0.54 group F and 3.16 ± 0.26 group P).
3. After allergen challenge, the increase of NAR was not significantly different before and after treatment in the placebo group; on the contrary a statistically significant decrease of the nasal reactivity was observed after treatment with fusafungine ($p < 0.01$).
4. Nasal reactivity to allergen was significantly lower ($p < 0.05$) in the fusafun-

gine group than in the placebo group (2.07 ± 0.48 group F and 2.98 ± 0.36 group P).

DISCUSSION

Objective tests for investigation of the anti-inflammatory activity of topical drugs in humans are limited. Several methods have been described, such as subjective evaluation based on symptom scores, objective evaluation recording number of sneezes and volume of nasal secretions; the quantification of these techniques is difficult and unreliable (Daele and Melon, 1979). Measurement of nasal resistances by rhinomanometry allows the appreciation of the edema reactivity of the nasal mucosa to an allergenic provocation. As it is carried out during inspiration it respects physiological conditions; in this work, this test was used as a model for studying modifications in mucosa reactivity but did not imply intrinsic anti-allergenic properties of the drug. Fusafungine, a topical antibiotic with anti-inflammatory properties, might act on the nasal mucosa of allergic patients through both of these properties.

1. As an anti-inflammatory agent, fusafungine prevents congestion, edema, break of cilia of the respiratory epithelium and maintains granulocyte activity, as shown by histological studies in the animal (Du Boistesselin, 1969; Jousserandot, 1981). In the present study, the reduced sensitivity to allergens may be the witness of the anti-inflammatory activity of fusafungine allowing the mucosa to better resist aggression.
2. Furthermore, infection is frequent in atopic patients, hyperreactivity of their nasal mucosa increases after a bacterial or viral infection; the antibiotic effect of fusafungine eliminates infection, the second factor of aggression of the nasal mucosa.

CONCLUSION

Measurement of nasal resistances by rhinomanometry combined with allergenic provocation testing were used as an objective technique to assess the statistically significant decrease in hyperreactivity of the nasal mucosa after a ten-day treatment with fusafungine compared with placebo. This improvement with fusafungine may be related to its antibiotic and anti-inflammatory properties, competing against both of the main factors of hyperreactivity of the nasal mucosa: inflammation and infection.

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Value of Locabiotal® Aerosol in rhinosinusitis

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We studied the effect of Locabiotal® Aerosol as single-agent therapy in a randomised placebo-controlled double-blind trial in 40 rigorously selected patients. These patients suffered from rhinitis with bilateral and symmetrical sinus reactions, characterised by congestion of the middle meati, without frank pain, without fever, without radiological signs apart from oedema of the mucosa, not requiring systemic antibiotic therapy and suitable for treatment with a local antibiotic and anti-inflammatory agent.

Locabiotal® Aerosol contains a single active ingredient, fusafungine, which is both an antibiotic and an anti-inflammatory agent derived from a strain of fungus. Fusafungine exerts an antibacterial activity essentially against Gram positive cocci (Staphylococci, Streptococci, Pneumococci) (German-Fattal, 1987), but also against unusual organisms such as Mycoplasmas and Legionellas (Pocidalo, 1987). In addition, it also possesses an antifungal activity (Candida, Nocardia) (German-Fattal, 1987).

The anti-inflammatory activity has been evaluated, in particular, in an experimental model of acroleine-aerosol-induced tracheobronchial inflammation in the guinea pig. Fusafungine, administered prophylactically in the form of an aerosol, totally protected the mucosa against congestion and oedema and partially protected it against diapedesis. Fusafungine also prevents acroleine-aerosol-induced deciliation (Gremain et al., 1984). One of the explanations for the anti-inflammatory mechanism of action has been proposed by White (1987): fusafungine inhibits the production of superoxides by cultured macrophages placed in the presence of zymosan.

Examination of the nasal fossae, meati and rhinopharynx was performed by three types of endoscopic examination:

- simple rhinoscopy with Storz cold light frontal lamp,
- examination of the cavum with Bercyward's cold light epipharyngoscope,
- endoscopy of the nasal fossae using a 70° rigid endoscope with, if necessary, local anaesthesia with xylocaine without vasoconstrictor.

METHODOLOGY

This was a double-blind placebo-controlled study. 42 outpatients with acute inflammatory and possibly infectious rhinitis were included. Endoscopy of the middle meatus revealed a congested, oedematous ostium, reflecting early sinusitis without radiological or clinical signs such as fever or frank maxillary pain. Children under the age of 10 years were unable to be included, because the endoscopic examination was performed under local anaesthesia. Two subjects, one belonging to the Locabiotal® Aerosol group and the other to the placebo group, were excluded from analysis of the results. The mean age was 39.5 ± 3.9 years in the Locabiotal® Aerosol group and 41.7 ± 4.1 years in the placebo group; there were 12 women and 8 men in the Locabiotal® Aerosol group and 10 women and 10 men in the placebo group.

The patients included were randomised to receive either Locabiotal® Aerosol or placebo, which only differed from Locabiotal® Aerosol by the absence of fusafungine. Treatment was administered once every four hours; each treatment session consisted of six inspirations via the mouth and six into each nostril on the first four days, the four inspirations via the mouth and four into each nostril on the following six days. No other systemic, nasal or buccal antibiotic or anti-inflammatory treatment was permitted. The following treatments were coprescribed: anti-asthmatic treatment (four cases in the Locabiotal® Aerosol group, one in the placebo group), analgesics (one case in the Locabiotal® Aerosol group), antipyretic (one case in the placebo group) and local antibiotic for chronic otitis (one case in the Locabiotal® Aerosol group).

Clinical and endoscopic examinations, repeated on the third day of treatment, evaluated the course of the symptoms and assessed the efficacy of treatment. The same examinations were performed on the tenth day in order to evaluate the persistence of the improvement or the stability of the results obtained. These clinical and endoscopic examinations were performed by the same investigator throughout the study and for each case. In this way, any subjectivity likely to interfere in the evaluation of the results was identical for all patients. Statistical analysis therefore consisted of control of the homogeneity of the groups by Student's t-test for independent series for the parametric variables and by the χ^2 test for the non-parametric variables. The results were compared at the time of inclusion and on the third day of treatment by the χ^2 test in order to assess efficacy. Comparison of the results on the third and tenth days by the same test evaluated the subsequent course. The signs were recorded separately for each nostril and for each subject; the perfect symmetry of the response observed led to analysis based on a single nostril.

The two groups of patients presented similar pathology on inclusion into the trial. Three subjects in the placebo group had to leave the trial because of deterioration requiring systemic antibiotic therapy between the sixth and

eighth day. All of the subjects treated with Locabiotal® Aerosol completed the trial.

Overall, a significant improvement, in favour of Locabiotal® Aerosol, was observed by the third day for all of the signs, except for pain, which was rarely present on inclusion into the trial. The difference in the course was no longer significant between the two groups between the third and tenth day.

The local clinical signs consisted of nasal obstruction, nose running and pain. As frank pain was a criterion of exclusion, only three patients in the Locabiotal® Aerosol group and five patients in the placebo group complained of mild or moderate pain at the time of inclusion into the trial. Between inclusion and the tenth day, pain was aggravated in six cases in the placebo group and in two cases in the Locabiotal® Aerosol group. Nasal obstruction, slightly more severe before treatment in the Locabiotal® Aerosol group, was improved on the third day in 70% of subjects treated with Locabiotal® Aerosol compared with 20% in the placebo group ($p = 0.002$). Nose running decreased in 75% of subjects treated with Locabiotal® Aerosol on the third day and in only 25% of subjects treated with placebo ($p = 0.002$) (Figure 1).

The endoscopic examination evaluated the appearance of the nasal mucosa, the appearance and the quantity of secretions in the turbinates and cavum and the congestive and infectious appearance of the middle meatus.

The effect of Locabiotal® Aerosol was particularly marked on the appearance of the nasal mucosa. Whether it was oedematous or congested, the colour of the nasal mucosa rarely improved spontaneously as, in the placebo group, it remained red and oedematous in 19 cases and changed from red to oedematous in only one case. In the Locabiotal® Aerosol group, a considerable

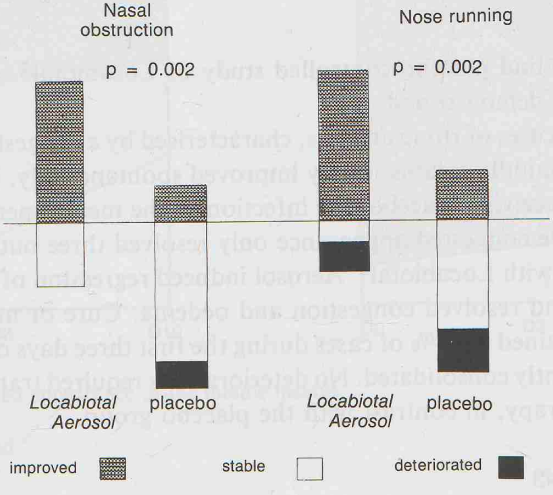

Figure 1. Course of the clinical signs on the third day (in %).

appreciable improvement was observed in 50% of subjects by the third day ($p < 0.001$) (Figure 2).

Nasal secretions were decreased by the third day in the turbinates in 70% of cases in the Locabiotal® Aerosol group and in 25% in the placebo group ($p = 0.013$) and in the cavum in 70% of cases in the Locabiotal® Aerosol group and in 30% in the placebo group ($p < 0.025$). The purulent nature of the secretions in the turbinates resolved in 11 out of 18 cases during treatment with Locabiotal® Aerosol; with placebo, it only resolved in three out of 18 cases ($p = 0.004$ at the third day). In the cavum, these proportions were 11 out of 18 in the Locabiotal® Aerosol group and only two out of 18 in the placebo group ($p < 0.001$ at the third day).

The congested appearance of the middle meatus was improved by the third day in 65% of subjects receiving Locabiotal® Aerosol and in only 5% of subjects with placebo ($p < 0.001$). By the third day of treatment, the meatus became normal or slightly congested in 12 subjects treated with Locabiotal® Aerosol and in three subjects receiving placebo. Infection in the meatus was improved in the same proportions ($p < 0.001$). It resolved with Locabiotal® Aerosol by the third day in eight cases and between the third day and the tenth day in two cases; with placebo, it only resolved in one case (Figure 3).

The treatments were generally well tolerated, particularly in the four patients in the Locabiotal® Aerosol group and one patient in the placebo group treated for asthma. One patient in the placebo group and three patients in the Locabiotal® Aerosol group reported slight irritation at the start of treatment, accompanied by sneezing in one case. In every case, these symptoms resolved with continuation of treatment.

DISCUSSION

This double-blind placebo-controlled study of Locabiotal® Aerosol in early rhinosinusitis demonstrated:

1. That these cases of rhinosinusitis, characterised by a congested, oedematous and infected middle meatus, rarely improved spontaneously. In particular, in the patients receiving placebo, the infection of the meatus persisted or deteriorated and the congested appearance only resolved three out of 20 cases.
2. Treatment with Locabiotal® Aerosol induced regression of the infection in the meatus and resolved congestion and oedema. Cure or marked improvement was obtained in 60% of cases during the first three days of treatment and was subsequently consolidated. No deteriorations required transfer to systemic antibiotic therapy, in contrast with the placebo group.

CONCLUSIONS

Early treatment of rhinosinusitis with Locabiotal® Aerosol, an antibiotic and

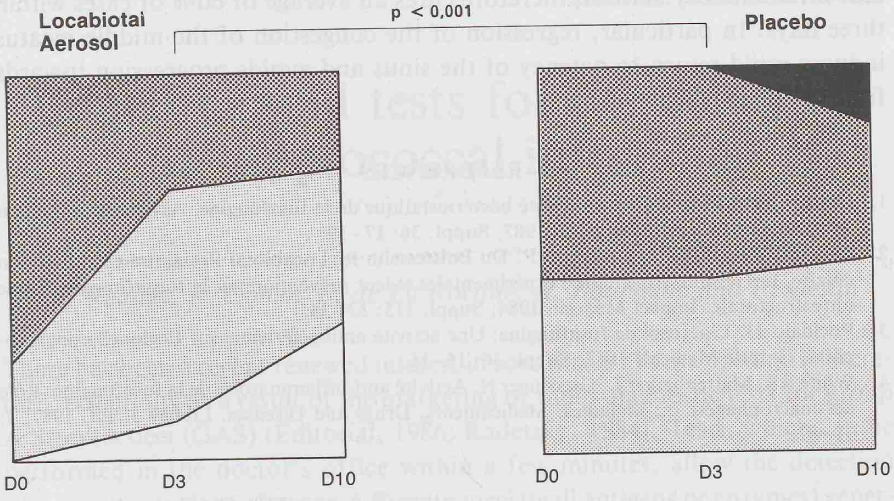

Figure 2. Appearance of the nasal mucosa.
□ normal
▨ oedematous
▩ red
■ withdrawals from the trial

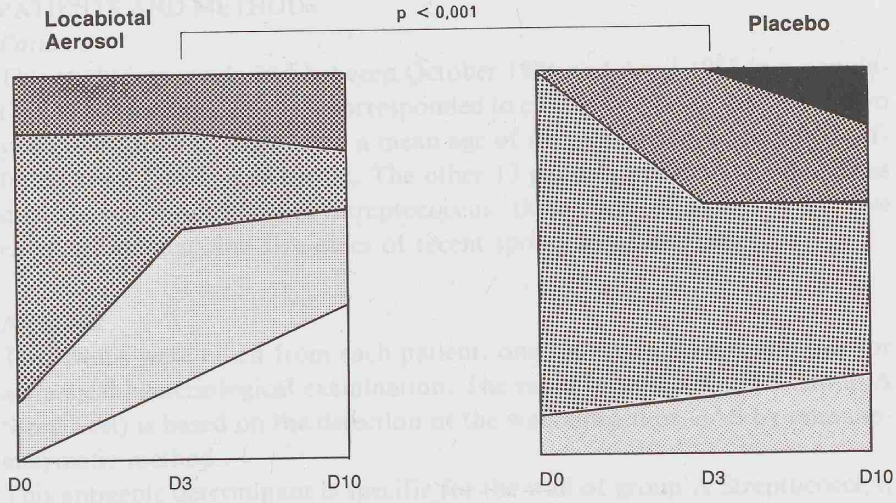

Figure 3. Congested appearance of the middle meatus.
□ normal
▨ slightly congested
▩ congested
▩ red
■ withdrawals from the trial

anti-inflammatory aerosol, therefore cures an average of 60% of cases within three days. In particular, regression of the congestion of the middle meatus induces rapid return to patency of the sinus and avoids progression towards frank sinusitis.

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Value of rapid tests for the detection of streptococcal infections

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There has been a recent renewed interest in sore throats in the paediatric literature, essentially as a result of the marketing of rapid diagnostic tests for group A Streptococci (GAS) (Editorial, 1986; Radetsky, 1984). Tests, which can be performed in the doctor's office within a few minutes, allow the detection of a specific antigen of group A Streptococci (wall antigens or enzymes) generally by means of monoclonal antibodies. The antigen-antibody reaction is visualised either by means of latex particles or by an immunoenzymatic method leading to a colour change. We report our experience with the Quidel Group A Strep Test.

PATIENTS AND METHODS

Patients

This study was conducted between October 1986 and April 1988 in a population of 105 patients. 92 cases corresponded to children between the ages of two years and fourteen years, with a mean age of six years and four months, suffering from febrile sore throat. The other 13 patients presented with various diseases possibly caused by Streptococcus: three cases of scarlet fever, five cases of impetigo and five cases of recent spontaneous otorrhoea.

Methods

Two swabs were taken from each patient: one for the rapid test and one for a classical bacteriological examination. The rapid test used (Quidel Group A Strep Test) is based on the detection of the wall antigen of GAS by immunoenzymatic method.

This antigenic determinant is specific for the wall of group A Streptococci; it is a polyrhamnose chain with N-acetyl glucosamine residues. This test is extremely simple to perform and was conducted in the doctors' offices. The test takes about 10 minutes to perform.

The classical bacteriological swab was obtained by means of Marion culture sticks with transport medium. The swabs were cultured within 1–18 hours:

either in a private laboratory or in the hospital laboratory. The medium used was Isovitalex + Negram and the culture was only performed under aerobic conditions.

RESULTS

In the patients with scarlet fever, impetigo or otorrhoea, we did not observe any difference in the results obtained with the rapid tests or with classical bacteriology (Table 1). In the 92 cases of sore throat, the rapid tests revealed 30 cases of Group A streptococcal sore throat, but, in comparison with the classical bacteriological identification, we obtained four false positives and one false negative. A total of 27 of the 92 cases of sore throat proved to be actually due to Group A streptococcal infection. On the basis of these results, we calculated four parameters to assess the value of these rapid tests:

1. The sensitivity, represented by the number of positive results common to the two tests over the number of positive bacteriological cultures:

$$\text{Sensitivity} = \frac{\text{Number of positive rapid tests} - \text{Number of false positives}}{\text{Number of positive cultures}} = 97\%$$

2. The specificity, represented by the number of negative results common to the two tests over the number of negative cultures:

$$\text{Specificity} = \frac{\text{Number of negative rapid tests} - \text{Number of false negatives}}{\text{Number of negative cultures}} = 94\%$$

3. Positive predictive value, corresponding to the ratio of positive results common to the two tests over the number of positive rapid tests.
4. Negative predictive value, corresponding to the ratio of negative tests for the two techniques over the number of negative rapid tests.

We obtained values of 89% and 99% respectively for these two parameters

DISCUSSION

Clinical features are insufficient to reliably distinguish between bacterial sore throat and viral sore throat. On the other hand they allow the detection of sore throats associated with a high risk of acute rheumatic fever (ARF): febrile sore throat occurring in a child over the age of 3–4 years. Two therapeutic approaches are based on this observation.

In France, the decision adopted has been systematic antibiotic treatment for all cases of febrile sore throat. The principal defect of this approach is the prescription of useless antibiotic therapy in at least three out of four cases, as, at most, GAS are responsible for 20 to 30% of cases of sore throat.

Table 1. Results of rapid tests and classical bacteriological swab.

	N	(+) rapid tests	classical G.A.S. bacterio.	false (+)	false (−)
Sore throat	92	30	27	4	1
Scarlett fever	3	3	3	0	0
Impetigo	5	2	2	0	0
Otorrhoea	5	2	2	0	0
Total	105	37	34	4	1

In the United States, the recommended approach is a systematic bacteriological swab. Antibiotics are only prescribed when a GAS is isolated on the throat swab. The essential defects of this approach concern the cost of the throat swab and the delay between the onset of clinical signs and the start of antibiotic treatment. Although this delay does not appear to prevent effective prophylaxis of ARF, early antibiotic treatment has been described to prevent from future complications as duration of symptomatology, frequency of local complication and decrease of surrounding people contamination (Breese et al., 1956; Chamovitz, 1960; Gerber et al., 1984; Nelson, 1984; Krober et al., 1985).

The value of the rapid test for GAS is therefore obvious:
This test is inexpensive (U.S. $ 1.50 to 3.00) compared with the cost of systematic antibiotic treatment for all cases of febrile sore throat.
It avoids three out of four cases of useless antibiotic therapy.
It would ensure better patient compliance. Sore throat should always be treated for 10 days, but the certainty of a streptococcal origin would give the doctor a greater feeling of conviction to ensure compliance with treatment over such a long period.
Lastly, in comparison with the classical bacteriological swab, this test constitutes a gain in time allowing earlier treatment and is less expensive. The current French social security code for a throat swab is B110, which corresponds to 205 francs.
However, this type of test has four sorts of disadvantages:
1. The specificity (false positive) in this study was 94% and 85 to 99% in the literature. However, this is a minor problem as, in the present study, it led to four cases of useless antibiotic treatment out of 105.
2. The sensitivity (false negative) is a much more serious problem. In this study, sensitivity was 97%, which can be considered to be good (68 to 99% in

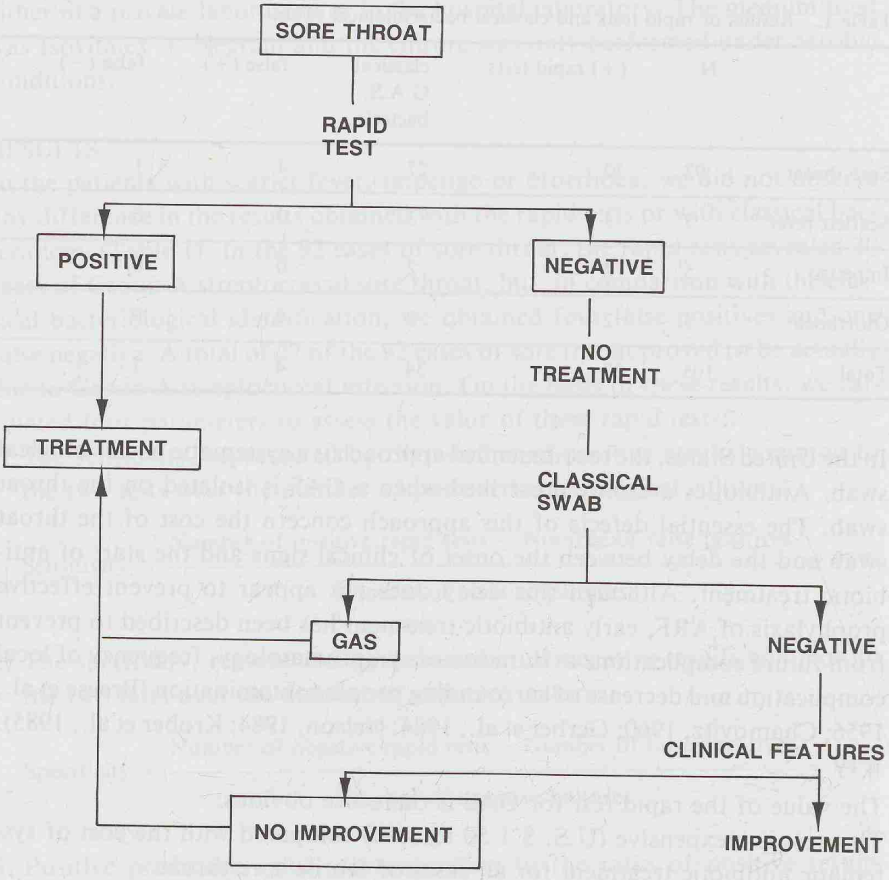

Figure 1. Decision flow diagram for sore throat.

the literature). If only the rapid test had been performed in this study, it would have led to the failure to treat one child with GAS on the classical bacteriological swab with a consequent risk of acute rheumatic fever. This risk must be considered in the light of the fact that many studies have shown that these false negative results correspond to patients with small quantities of GAS on the throat swab. These patients are frequently chronic carriers, but they may also develop true streptococcal sore throat with a rise in antibody rate (Otero et al., 1983; Gerber et al., 1984; Miller et al., 1984). We therefore believe that any negative rapid test should be followed by a classical bacteriological swab.

3. The failure to treat sore throat due to another bacterial species. We do not refer to Staphylococcus aureus, Haemophilus influenzae, Enterobacteria or alpha-haemolytic Streptococcus, frequently reported in the results of throat

swabs, but to Corynebacterium haemolyticum, which has been recently demonstrated to play a pathogenic role (Banck et al., 1986).

4. A final disadvantage in comparison with classical bacteriological swabs was recently reported by Pichichero et al. (1987). Children treated early at the time of the swab have a significantly higher incidence of late recurrences (beyond one month) than children treated after an interval of 48 hours (Pichichero et al., 1987).

On the basis of these data, we propose a decision flow diagram for sore throat, integrating the practice of rapid diagnostic tests (Figure 1). The essential obstacle to the diffusion of this type of test in France (they are very widely used in other countries) is legislative and economic: current legislation does not allow physicians who are not clinical pathologists to bill the patient for this type of test.

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