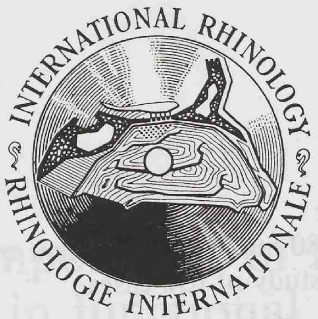

RHINOLOGY

Supplement No. 4 1988

INFLAMMATION OF THE UPPER RESPIRATORY TRACT

ROLE OF PNEUMOREL® 80 mg

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

CONTENTS

(Contents to be continued next page)

Under the auspices of the European Rhinologic Society

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Rhinology, Suppl. 4, 3–11, 1988

The importance of antibiotic treatment in functional and aesthetic rhinosurgery

W. Pirsig and J. Schäfer

Our knowledge about the normal microflora and potential pathogens in the nose and their influence on wound healing after septorhinoplasty is rather insufficient. Therefore the value of the 'protective umbrella' of antibiotic prophylaxis in rhinosurgery is still a matter of discussion.

Some try to prevent infectious complications by antibiotics in each case of rhinoplasty, others consider the antibiotic prophylaxis as overtreatment. Only two studies have approached the subject of preventative antibiotics in rhinosurgery in a prospective manner. Eschelman et al. (1971) found three infections among 110 patients who underwent septal surgery or rhinoplasty. These infections were proportionately distributed in the three groups of patients treated with penicillin (n = 40), ampicillin (n = 36) or placebo (n = 36). Weimert and Yoder (1980) evaluated the efficacy of ampicillin and erythromycin in 75 patients with submucous septal resection or rhinoplasty and compared the frequency of infections with a placebo group of 99 patients. Only 4/174 developed minor infections. The rate of infections observed in both studies was about 2.5% in both the placebo and antibiotic groups. Therefore the following conclusion can be drawn: there is no need for antibiotic prophylaxis in simple septoplasty or rhinoplastic operations. Our own experiences in about 2000 patients with this type of septorhinoplasty in the last years support this conclusion.

Among our patients with rhinosurgical treatment most infectious complications were observed in those who had had previous operations on their noses. Therefore we thought that this group of patients for revision rhinoplasty is especially apted to study the prophylactic value of antibiotics.

Our study was designed to answer the following questions:

1. What is the pre- and postoperative microflora of the nose of patients with previously operated noses and which are the potential pathogens in the nasal cavities?
2. Which are reliable clinical and laboratory signs of disturbances in wound healing following rhinosurgery?

3. What is the rate of nasal infections after revision-rhinosurgery?
4. Does the administration of antibiotics prevent these nasal infections after revision-rhinosurgery?
5. Is there an influence of the long-standing packing on the microflora in the nasal cavities?

To all these questions there are only very incomplete data in the literature (Schäfer, 1987).

PATIENTS AND METHODS

100 adult patients (71 men, 29 women) with a mean age of 31 ± 12 years were hospitalized for revision-rhinosurgery to reestablish nasal breathing and to improve the cosmetic nasal appearance. Patients were allocated treatments in a random binary sequence to receive either no antibiotic (n = 52) or propicillin (n = 48). 3 mega units propicillin were given transorally/day for 12 days, starting six hours postoperatively. All patients received the antihistamine Clemastinhydrogenfumarat transorally for the time of the nasal packing. The mean age (30 ± 11 y vs. 32 ± 13 y), the body weight (71 ± 12 kg vs. 68 ± 12 kg), the length (173 ± 8 cm vs 173 ± 10 cm), and the time of surgery (93 ± 17 min vs. 90 ± 19 min) showed no significant differences between both groups. In the same way the type of nasal deformities and the degree of difficulty to correct them was almost equally distributed for both groups (Table 1). All patients had had nasal operations before, 40% had been previously operated for more than once. Twelve patients had undergone several operations because of their lip-palate-cleft nasal deformity. Rhinosurgery was performed under general anaesthesia by one surgeon. In most cases a complete rhinoplasty with septal reconstruction, osteotomies, hump and wedge resections, and cartilage transposition was done. In 38 patients of the propicillin group and in 37 of the placebo recipients free transplants from the nose (cartilage and bone in 60 patients), ear, and rib (in 15 patients) were used for reconstruction (Table 2). Transplants and reconstructed caudal septum were fixed by mattress sutures and/or guide sutures with 4-0 plain cat which were removed after seven to twelve days postoperatively. As a rule a loose petroleum jelly gauze coated with ointment containing oxytetracycline and polymyxin B, was left as anterior packing in the nasal cavities for six days on the average (range 2–10 days). External fixation of the nasal structures was performed by plaster of Paris.

Preoperative swabs were taken from the mucosa above the posterior half of the inferior turbinate under direct vision and with a sterile long-bladed nasal speculum. Postoperative swabs were collected from the mucous of the posterior end of the nasal packing after removal. The swabs were plated on to nutrient agar without delay. A transport medium for anaerobic cultures was used (Transwab – Mast Diagnostica, Schubertstr. 3, Hamburg 76). Routine cultures for wound swabs at our hospital were performed. These consist of a thioglycollate

Table 1. Rhinosurgical procedures in 100 patients with revision rhinoplasty.

procedure	antibiotic	placebo	total
Closure of septal perforation	8	5	13
Wedge resection	16	22	38
Dorsum sutures	17	9	28
Columella sutures	24	26	50

Table 2. Distribution and donor-sites of patients with free cartilaginous transplants.

free transplant	antibiotic	placebo	total
Nose: cartilage	20	11	31
bone	1	3	4
both	10	15	25
Ear cartilage	4	1	5
Rib cartilage	2	4	6
Preserved cartilage	1	3	4
Total	38	37	75

broth for enrichment, sheep blood agar plates for general use, Schaedler agar plates for cultures of anaerobes, chocolate agar for recovery of Haemophilus and Neisseria species, MacConkey agar plates for recovery of aerobic and facultatively anaerobic gram-negative bacilli, a plate for species identification of staphylococci and a plate with enterococcus-selective media.

RESULTS

Microflora of the nose

The distribution of aerobic bacteria and Candida cultured from the nasal cavity of 100 patients preoperatively and postoperatively in the two treatment groups is listed in Table 3a.

As to the numbers of different microorganisms in the nasal cavity of the individual patient there was a range of "no growth" on one end and "eight types of bacteria" on the other end. These data for our 100 patients are summarized in Figure 1, which shows a clear trend toward "no" or "reduced numbers" of different microorganisms in the postoperative nasal swabs as this has been reported by Herzon (1971). Whether this shift to single microorganisms is caused by the local regimen of oxytetracycline and polymyxin B, has to be investigated in further studies. Candida was cultured only from postoperative swabs.

For comparison (Table 3b) we cite the distribution of aerobic bacteria cultured from the nasal cavities in 97 healthy young men from Finland (Savolainen et al. 1986). Concerning the three most frequent aerobic bacteria the flora of the

Table 3a. Aerobic bacteria and Candida species from the nasal cavity in 100 patients for revision-rhinosurgery.

bacteria	preoperative swabs n = 100	postoperative propicillin group n = 48	swabs placebo group n = 52
Staphylococcus epidermidis	69	25	23
Staphylococcus aureus	42	3	6
Corynebacterium species	21	1	–
Streptococcus viridans	13	4	6
Neisseria species	11	1	–
Proteus species	8	5	5
Streptococcus pneumoniae	5	1	–
β-hemol. Streptococcus gr. A/B	5	–	–
Escherichia coli	5	–	–
Acinetobacter species	4	3	2
Enterobacteritiae	1	6	1
Haemophilus influenzae	1	–	–
Enterococci	1	–	–
Candida species	–	6	9

Table 3b. Aerobic bacteria from the nasal cavity in 97 healthy young men (Savolainen et al.).

bacteria	%
Staphylococcus epidermidis	79
Staphylococcus aureus	34
Corynebacterium species	41
Moraxella species (Neissericiae)	3.5
Branhamella cat (Neissericiae)	3
Proteus mirabilis	1.5
Streptococcus pneumoniae	0.5
α-hemol. Streptococcus gr. A/B	4
Escherichia coli	1
Enterobacter cloacae	1.5
Haemophilus influenzae	5

nasal cavity in our patients is rather similar to the flora isolated from the healthy men. Staphylococcus epidermidis and aureus, and diphtheroids are the most frequent aerobes in both investigations. A remarkable difference concerns the potential pathogens in the nose: Savolainen cultured 5.5% (Haemophilus influenzae, Streptococcus pneumoniae), while we found 24% potential pathogens (Streptococcus viridans, Streptococcus pneumoniae, β-hemolytic Streptococci group A/B, Haemophilus influenzae). Interestingly, Slavin et al. (1983) found Streptococcus viridans in 17.3% of their 52 patients for rhinoplasty. Thus patients with obstructive nasal deformities which need to be operated seem to have more of the so-called pathogenic bacteria than a normal population. In our patients postoperatively Staphylococcus aureus and Coryne-

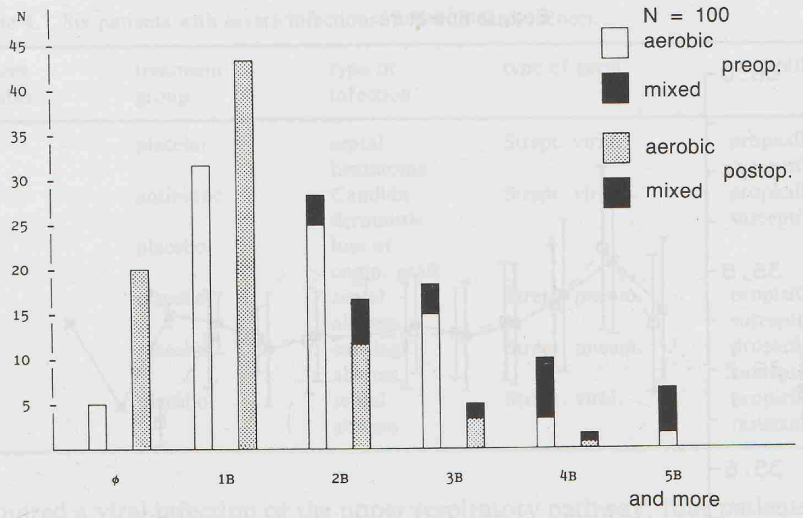

Figure 1. Numbers of patients (N) who had no (Ø), one bacterium (1B), two bacteria (2B) and more in their nasal swabs pre- and postoperatively.

bacteria had almost vanished from the nasal cavities independent from the treatment modality. Staphylococcus epidermidis, however, was isolated from the nasal swabs in almost the same quantity as preoperatively.

Nasal infections

To evaluate disturbances of wound healing body temperature was measured every day and, in addition, the degree of headache, nasal pain, and sneezing were estimated by the patient. Erythrocyte sedimentation rate and white blood count were registered preoperatively, on the first postoperative day, when nasal packing was removed, and when sutures were cut. A second physician, other than the surgeon, checked the patient's condition in the postoperative period and recorded local signs for nasal infection: turbid nasal secretion; reddening, pain, and swelling of the nasal linings; bleeding of the non-injured nasal mucosa when packing was removed; pustulosis of nasal skin; infection of the suture-canals. For the determination of differences between propicillin and placebo as to their clinical efficacy Fisher's exact 2×2 test for significance was used.

Surprisingly none of these single criteria or their combination was able to indicate in a reliable way a nasal infection. In Figures 2 and 3 representative examples for this finding are shown.

18% of the patients demonstrated signs of minor or severe nasal infections postoperatively. Among 12 patients with signs of minor infections three had

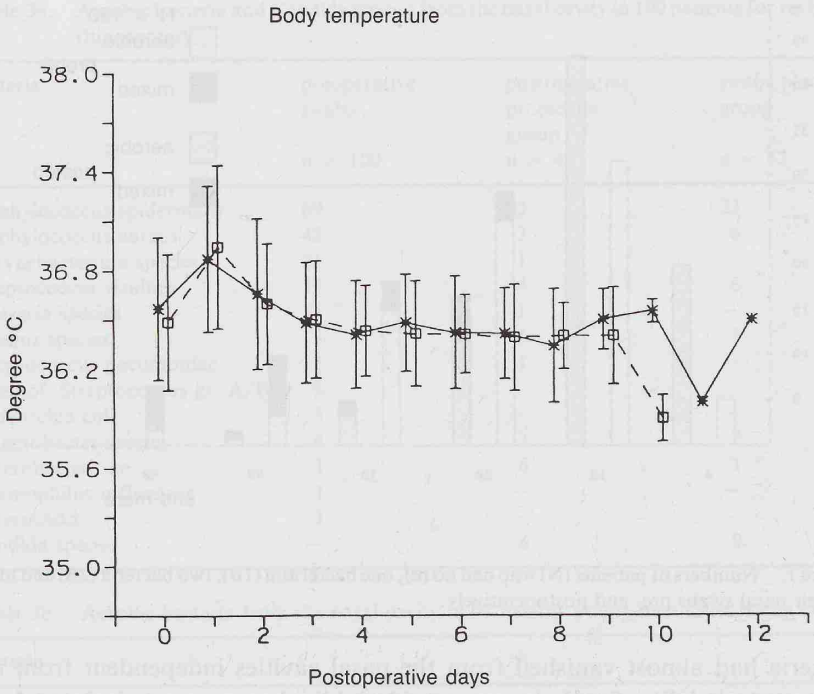

Figure 2. Mean values of body temperature with standard deviation in patients of placebo group (□) and penicillin group (*).

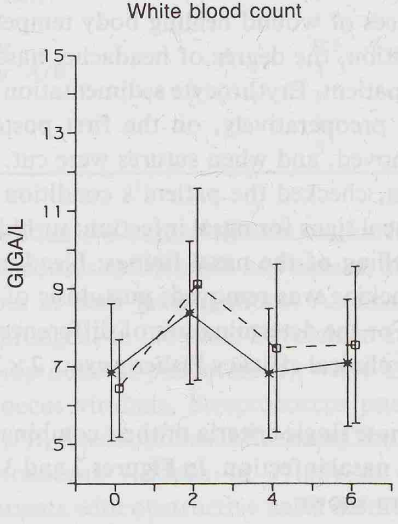

Figure 3. Mean values of white blood count with standard deviation in patients of placebo group (□) and penicillin group (*). 0 = preoperatively; 2 = first postoperative day; 4 = removal of nasal packs; 6 = removal of sutures.

Table 4. Six patients with severe infections: 5/6 with Streptococci.

patient number	treatment group	type of infection	type of germ	susceptibility
8	placebo	septal hematoma	Strept. virid.	propicillin susceptible
15	antibiotic	Candida dermatitis	Strept. virid.	propicillin susceptible
23	placebo	loss of comp. graft	?	
64	placebo	septal abscess	Strept. pneum.	propicillin susceptible
88	placebo	cartilage abscess	Strept. pneum.	propicillin susceptible
107	placebo	septal abscess	Strept. virid.	propicillin resistant

acquired a viral infection of the upper respiratory pathway, four patients had Streptococci, and three had Proteus or E. coli in their preoperative nasal cultures. There were six severe nasal infections (Table 4). In five of these six patients Streptococci viridans (3×) or pneumoniae (2×) were cultured from preoperative nasal swabs.

But does that mean, that these microorganisms are the potential pathogens? In the literature (Dalton and Nottebart, 1986) a large number of microorganisms are considered to be pathogenic, many of them we found in the normal nose.

Value of antibiotic prophylaxis

Did the administration of antibiotics prevent these nasal infections after revision-rhinosurgery? Among the six patients with severe nasal infections only the patient with Candida dermatitis was in the antibiotic group, while the other five patients belonged to the placebo group. There were no infections in 44 patients of the antibiotic group and in 38 of the placebo recipients. Mild or severe infections were observed in four patients of the propicillin group and in 14 of the placebo group. The last difference is significant ($p < 0.01$).

Our rates of mild infections (12%) and severe infections (6%) are much higher than those registered by the authors of the two other prospective studies (Eschelman et al. 1971; Weimert and Yoder, 1980), who reported of 2.7 respectively 2.3% infections. This is probably due to different surgical procedures: these authors had no patients with previous rhinosurgery. In our series 75% of the patients had free transplants.

Influence of nasal packing

Table 5 summarizes the data about the duration of the nasal packing in the

Table 5. Nasal packing vs. rate of infection in 100 patients.

nasal packing		infections		
days	patients	mild	severe	%
2	2	–	1	
4	5	1	–	13.6
5	15	–	2	
6	65	10	3	4.6
7	7	2	–	
9	5	1	–	0
10	1	1	–	

cavities of our 100 patients and the frequency of mild and severe infections of the nose. Severe infections were observed in the group where the packing was left in place for two to five days (3/22) and in the main group (3/65), while no remarkable infection occurred in the group with longstaying packing. Furthermore we did not see any case of the so often cited toxic shock syndrome (Breda et al., 1987).

CONCLUSIONS

In patients with revision-rhinoplasty especially when free transplants are used, the prophylactic administration of antibiotics may prevent severe infections in most cases. There is no influence of long staying nasal packing on the frequency of nasal infections in patients with revision-rhinosurgery. We learnt from studying the literature and by interpretation of our results that a number of questions concerning the clinical importance of rhinosurgery and nasal infections cannot be answered with the current state of knowledge. There is the problem to define the pathogenic microorganisms in the nasal cavities. As we can find up to eight different microorganisms in the nose of a single patient among which three are possibly pathogenic, how can we decide which germ was responsible for the nasal infection? More information may be available if we start to determine the quantity of the different microorganisms in the nasal cavities. There is the problem of different modifications to apply the prophylactic antibiotic. Studies are necessary to show whether a pre-, peri- or postoperative admission of the antibiotic is most effective and whether or not the spectrum of the antibiotic should be broader than e. i. penicillin. A third problem concerns the antimicrobial compounds included in the ointment which the nasal packing is coated with. Should the antimicrobial spectrum correspond to the systemic antibiotic or should it be additional? More studies have to be performed before we will understand the value of antibiotic prophylaxis in rhinosurgery.

REFERENCES

1. Breda SD, Jacobs JB, Lebowitz AS, Tierno PM. Toxic shock syndrome in nasal surgery: A physiochemical and microbiologic evaluation of MEROCEL and NuGAZE nasal packing. Laryngoscope 1987; 97: 1388–1381.
2. Dalton HP, Nottebart HC. Interpretative Medical Microbiology. New York: Churchill Livingstone, 1986.
3. Eschelman LT, Schleuning II, Brummett RE. Prophylactic antibiotics in otolaryngologic surgery: A double-blind study. Trans Am Acad Ophthal Otolar 1971; 75: 387–394.
4. Herzon FS. Bacteremia and local infections with nasal packing. Arch Otolaryngol 1971; 94: 317–320.
5. Savolainen S, Ylikoski J, Jousimies-Somer. The bacterial flora of the nasal cavity in healthy young men. Rhinology 1986; 24: 249–255.
6. Schäfer J. Antibiotic prophylaxis in Oto-Rhino-Laryngology. Hyg Med 1987; 12:391–394.
7. Slavin SA, Rees T, Guy CL et al. An investigation of bacteremia during rhinoplasty. Plast Reconstr Surg 1983; 71: 196–198.
8. Weimert TA, Yoder MG. Antibiotics and nasal surgery. Laryngoscope 1980; 90: 667–672.

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Sinus pathology and respiratory tract disease

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The relationships between sinus and respiratory tract pathology are multiple and correspond to very different contexts.

The sinus disease may sometimes be discovered during the assessment of respiratory tract disease for which it constitutes either a cause or an aggravating factor. This is especially the case in lung abscess, chronic bronchitis and bronchiectasis.

Sinus disease may also accompany respiratory tract disease without being the cause. This is essentially the case in asthma.

Lastly, sinus and respiratory tract disease can be observed simultaneously in the course of systemic diseases: Kartagener's syndrome or, more generally, primary ciliary dyskinesia syndrome, Wegener's granulomatosis and infectious diseases occurring in immunodepressed subjects.

We shall successively review each of these different manifestations.

SINUS DISEASE AS AN AGGRAVATING FACTOR OF RESPIRATORY TRACT DISEASE

The frequency of sinusitis associated with lung abscess has been diversely estimated to be between 4.6% (Kourilsky and Decroix, 1960) and 10.5% (Milleron, 1974) of cases. A certain number of old studies have clearly demonstrated that the mechanism of such abscesses is simple: septic particles originating in the sinuses penetrate into the bronchial tree by inhalation then stagnate in the lower bronchial territories (this explains the preferential involvement of the dorsal segments of the upper lobes and the apical segments of the lower lobes which are in low position at night). This bacterial stagnation is promoted by certain cofactors which decrease mucociliary clearance: anaesthetic sleep, acute alcohol intoxication, smoking, low temperature are the most important.

The place of sinus infection in the pathogenesis of acute infectious episodes of chronic bronchitis is less clearly defined. It is always difficult to prove that a case of chronic sinusitis is the cause of episodes of secondary infection in patients with chronic bronchitis. It is generally accepted that about 10% of sub-

jects with chronic bronchitis suffer from associated chronic sinusitis (Mounier-Kuhn, 1973) and appropriate treatment of these infections significantly decreases the number of infectious episodes.

The place of sinus infections in the course of bronchiectasis has also been the subject of a number of studies. The incidence of this association is estimated to be between 18 and 36%. This incidence appears to be higher in the case of bilateral bronchiectasis (41% vs 13%) (Bourcereau, 1971). It is absolutely essential to correctly treat the sinus infection before resection of the bronchiectasis. The incidence of postoperative congestion is higher when the sinus infection has not been treated (25% vs 50%) (Bourcereau, 1971).

Lastly, it should be noted that chronic cough may be simply related to chronic sinusitis, without any detectable lesion of the sub-glottal airways and resolves completely when effective treatment of sinusitis is instituted.

SINUS DISEASE MAY ACCOMPANY RESPIRATORY TRACT DISEASE WITHOUT BEING THE CAUSE

This is essentially the case in asthma. Chronic allergic sinusitis frequently accompanies asthma; a classical context is that of Widal's triad which actually corresponds to the association of nasal polyposis, sinusitis, hypersensitivity to aspirin, and asthma. The pathophysiology of such lesions has been the subject of numerous papers and is still partially obscure at the present time.

The important question in practice is to determine whether or not, in this infection, nasal sinus surgery, in particular polypectomy, is likely to aggravate the asthma.

The data in the literature concerning this point are very controversial:

A number of authors have reported since 1920 that surgery may aggravate asthma or may even reveal asthma in a subject who has never had any previous episodes (Vander Veer, 1920; Samter and Lederer, 1958; Samter and Beers, 1967).

Other authors have reported that, on the contrary, surgery may have beneficial effects in the majority of patients (Weille, 1936; Weille and Richards, 1951; Brown et al., 1979).

English (1986) has a remarkable experience of this problem extending over the period 1969 to 1982 in 205 male and female subjects (including 91.2% of adults). All of the subjects in this study suffered from steroid-dependent asthma. Following surgical treatment (polypectomy, ethmoidectomy, Caldwell-Luc, etc.), the great majority of patients were improved. Eighty-two patients were no longer steroid-dependent; out of 62 patients with unstable asthma prior to the operation despite the prescription of continuous corticosteroid and bronchodilator treatment, only six cases persisted after the operation (Table 1).

No postoperative deaths were reported.

However, an episode of bronchospasm, resolving in response to appropriate medical treatment, was observed in 40% of cases.

Table 1. Naso-sinusal surgery and Widal's triad (English, 1986).

pre-operative		post-operative
0	Class I	82
72	Class II	93
71	Class III	24
62	Class IV	6

Class I: Sensitivity to bronchodilators (BD) used occasionally. No corticosteroids (CS)
Class II: Sensitivity to BD used daily. Occasional use of CS.
Class III: Permanent steroid dependence.
Class IV: Mediocre action of all drugs.

This debate therefore appears to be finally settled; nasal sinus surgery may improve rather than aggravate these patients with Widal's triad. However, it must be performed under excellent conditions: bronchodilator treatment must be systematically prescribed and well conducted; pre- and post-operative corticosteroid therapy must be systematically proposed. Sinusitis and polyposis should be treated simultaneously.
It is probably because these precautions are not always respected that this favourable opinion of nasal sinus surgery in asthmatics has not yet been accepted by all authors.

SINUS DISEASE AND RESPIRATORY TRACT DISEASE MAY BE OBSERVED SIMULTANEOUSLY IN THE COURSE OF SYSTEMIC DISEASES

Kartagener's syndrome, associating situs inversus, chronic sinusitis, bronchiectasis (Figure 1) and male sterility, is part of the more general context of ciliary diseases. It is the complete form of an entity defined by Afzelius under the term of primary ciliary dyskinesia.
The alteration in beating of the ciliated cells of the respiratory epithelium is responsible for microbial stasis and naso-sinus and bronchial infections, while immobility of the flagella of the spermatozoon is responsible for male sterility. This more or less complete defect of ciliary activity is due to a structural abnormality of the cilia, which lack the dyneine arm (Eliason et al., 1977) (Figure 2). The association of sino-pulmonary infections and sterility is not reserved to the immotile cilia syndrome. It may also be observed in other very different clinical contexts such as mucoviscidosis or Young's syndrome (Table 2).
Wegener's granulomatosis is a vasculitis which associates, in the typical forms, respiratory (Figure 3), naso-sinusal and renal lesions. These manifestations may be accompanied by renal, articular, cutaneous or ocular manifestations (Table 2). The clinical picture is often far from being as complete as these typical syndromes and the pulmonary lesion is frequently isolated. In the forms presenting early in the course of the disease, the naso-sinusal manifestations

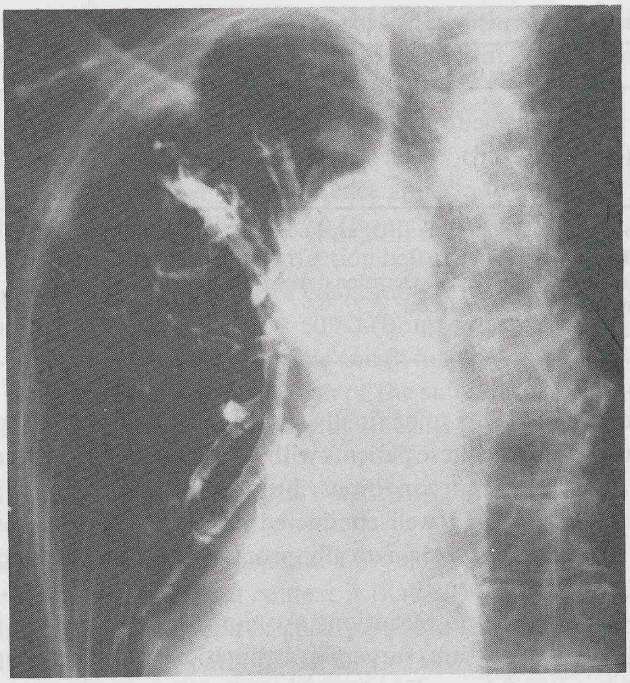

Figure 1. Bronchiectasis and situs inversus (Kartagener's syndrome).

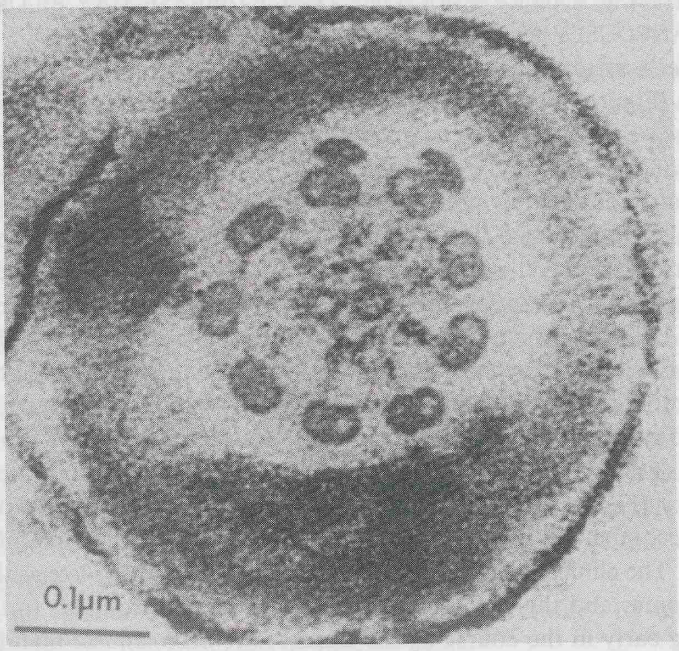

Figure 2. Section of a cilium under the electron microscope: absence of the dyneine arm.

Table 2. Sino-pulmonary infections and sterility (Handelsman et al., 1984).

	ultrastructure of spermatozoa and cilia	vas and epididymis	sperm count	pancreas	sweat
Immotile cilia syndrome	Abnormal	Normal	Immobile	Normal	Normal
Mucoviscidosis	Normal	Malformations	Azoospermia	Abnormal	Abnormal
Young's syndrome	Normal	Obstruction	Azoospermia	Normal	Normal

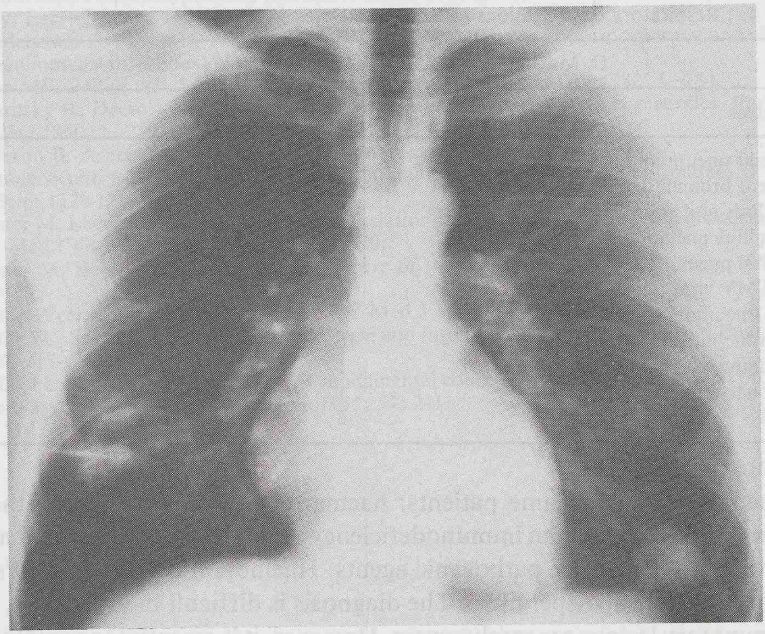

Figure 3. Large bilateral opacities, some of which are excavated (under the right hilum) with thin walls (Wegener's granulomatosis).

are only observed in about one half of cases (Kahn and Peltier, 1982).

The diagnosis of Wegener's granulomatosis is particularly important in that this disease was formerly fatal in almost every case, but is now virtually curable as a result of well-conducted treatment, generally consisting of corticosteroids and cyclophosphamide.

Immunodepression states frequently present an association of respiratory tract and sinus diseases, which have many points in common (Berlinger, 1985). They

Table 3. Clinical manifestations of Wegener's granulomatosis (%).

	Walton (1958) N = 56	Fauci (1973–1978) N = 21	De Remee (1975) N = 50
Fever		78	
Respiratory signs	100	100	70
Naso-sinusal signs	89	94	70
Renal signs	90	83	46
Articular signs	34	56	
Cutaneous signs	46	44	
Nervous signs	29	33	
Ocular signs	41	28	

Table 4. Respiratory complications after bone marrow graft.

C. Maynaud and C. Darne, Tenon 1987 (N = 59)		
Diagnosis	(%)	
Bacterial pneumonia	12	(20.3)
Purulent bronchitis	2	
Bronchitis and sinusitis	11	(18.6)
Aspergillus pneumonia	3	
Parasitic pneumonia	2	
Non-CMV viral pneumonia	1	
CMV Pneumonia	4	
Idiopathic interstitial pneumonia	16	(27.1)
Drug-induced pneumonia	1	
Pulmonary tuberculosis	1	
Others	6	

frequently affect the same patients: haematological disease, chemotherapy, organ transplants, human immunodeficiency virus (HIV) infection and are frequently due to the same pathogenic agents: Haemophilus influenzae, Pseudomonas aeruginosa, Aspergillus. The diagnosis is difficult in both cases, as the inflammatory lesions are rarely severe. However, it is essential to rapidly establish the diagnosis as these ENT and respiratory tract infections are equally severe: both require well-conducted, sometimes aggressive treatment, particularly surgery in the case of sinus infections. Amongst the respiratory complications observed after bone marrow graft, sinusitis and bronchitis, frequently due to Gram negative organisms and often recurrent, may themselves constitute a predisposing factor for episodes of GVH (graft versus host) disease (Darne, 1987).

These are the principal circumstances in which naso-sinusal and respiratory tract lesions are observed together. They sometimes constitute very close cause-and-effect relationships or may only correspond to a simultaneous observation

of two distinct manifestations. However, these associations always raise diagnostic problems and frequently difficult therapeutic problems which require close collaboration between pneumological and ENT specialists.

REFERENCES

1. Berlinger NT. Sinusitis in immunodeficient and immunosuppressed patients. Laryngoscope 1985; 95: 29–33.
2. Bourcereau J. Étude statistique de 206 interventions chirurgicales pour dilatation des bronches. Thèse, Paris 1971; 1–77.
3. Brown BL, Harner SG, Van Dellen RG. Nasal polypectomy in patients with asthma plus sensivitity to aspirin. Arch Otolaryngol 1979; 105: 413–416.
4. Darne C. Complications respiratoires après greffes de moëlle osseuse. Analyse de 59 observations et revue de la littérature. Thèse, Paris 1987; 1–62.
5. Eliason R, Mossberg B, Camner P, Afzelius BA. The immotile-cilia syndrome. N Engl J Med 1977; 297: 1–6.
6. English GM. Nasal polypectomy and sinus surgery in patients with asthma and aspirin idiosyncrasy. Laryngoscope 1986; 96: 374–380.
7. Handelsman DJ, Conway AJ, Boylan LM, Turtle JR. Obstructive azoospermia and chronic sinopulmonary infections. N Engl J Med 1984; 310: 3–9.
8. Kahn MF, Peltier AP. Maladies dites systémiques. Ed Flammarion 1982; 1–784.
9. Kourilsky R, Decroix G. Les suppurations bronchiques pulmonaires et pleurales. Paris, Ed Baillère 1960; 1–259.
10. Milleron B. Aspects actuels des abcès du poumon. Thèse, Paris 1974; 1–82.
11. Mounier-Kuhn P. Place et rôle de l'infection haute dans les bronchites chroniques. J Agreg 1973; 6: 117–121.
12. Samter M, Lederer FL. Nasal polyps: their relationship to allergy, particularly bronchial asthma. Med Clin North Am 1958; 42: 175–179.
13. Samter M, Beers RF. Concerning the nature of intolerance to aspirin. J Allergy 1967; 40: 281–293.
14. Vander Veer A. The asthma problem. NY Med J 1920; 112; 392–399.
15. Weille FL. Studies in asthma: XIX. The nose and throat in 500 cases of asthma. N Engl J Med 1936; 215–235.
16. Weille FL, Richards AB. Influence of fundamental concepts in allergy upon special problems in otolaryngology. Arch Otolaryngol 1951; 54: 231.

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Efficacy of Pneumorel® 80 mg (fenspiride) in the treatment of chronic sinusitis

Double-blind placebo-controlled study

G. Cuénant

Chronic sinusitis is defined as chronic inflammation of the cavities associated with the nasal fossae. The major symptoms are nasal obstruction and anterior and posterior rhinorrhoea. Pain is generally absent or reflects secondary infection or an acute episode. X-rays of the sinuses remain an essential complementary examination in the positive and differential diagnosis.

Inflammation of the mucosa of the sinus cavities follows the usual course of this disease: oedema with exudate which mixes with the mucus, modifying the volume and viscosity of the secretions. Varying degrees of obstruction of the pathways of drainage of the sinuses by these inflammatory phenomena result in intra-sinusal stasis of pathological secretions. Stasis promotes secondary infection and progression towards chronicity.

Consequently, it is logical to treat this type of disease from two sides:

- eliminate the agent responsible (whether it is specific to the subject: anatomical or external: allergies, pollution, infections),
- and specifically treat the inflammation.

Pneumorel® 80 mg, which essentially exerts its action on the primary, vascular phase of inflammation (Evrard et al., 1986), has been demonstrated to be an effective treatment of inflammation of the respiratory tract mucosa (Fraysse and Furia, 1984; Receveur, 1985; De Fenoyl, 1985; Cuénant, 1985). In chronic bronchitis, it markedly improves the capacity for drainage of the respiratory tracts by increasing the rate of mucociliary transport (Olivieri and Del Donno, 1987). In the ENT sphere, by reducing oedema, Pneumorel® 80 mg reduces congestion and restores normal volume and viscosity of the secretions, thereby counteracting stasis and preventing the development of chronic sinusitis.

It is therefore important to study the efficacy of Pneumorel® 80 mg on the inflammatory condition of the sinus mucosa in the absence of secondary infection or acute exacerbation.

This double-blind, placebo-controlled study was conducted on an outpatient

basis in patients with chronic sinusitis present for more than six months.

OBJECTIVE OF THE STUDY

The study was conducted in 42 patients with chronic sinusitis in order to evaluate the efficacy of Pneumorel® 80 mg administered at a dosage of three tablets per day for one month. Two groups of subjects were constituted by randomisation: 21 patients received Pneumorel® 80 mg and 21 patients received placebo. Various criteria were evaluated for each patient:

1. Descriptive variables: sex, age, height, weight.
2. Clinical examination performed on day 0, on the 10th day and on the 30th day of treatment (nasal obstruction, rhinorrhoea and pain: scored from 0–3 according to the severity of the symptom for each sinus).
3. Radiological examination of the facial sinuses on day 0 and on the 30th day of treatment (scored according to the degree of the thickening of the sinus mucosa):

0 corresponding to a sinus with normal mucosa
1A corresponding to thickening with low liquid level
1B corresponding to thickening with high liquid level
2A corresponding to frame-shaped thickening less than 2 mm
2B corresponding to frame-shaped thickening greater than 2 mm
3 corresponding to complete clouding of the sinus

For each of these clinical and radiological examinations, two measurements were recorded for each variable, the first scoring the severity for the right sinus and the second for the left sinus. As these two measurements could not be dissociated clinically, the statistical analyses concerned the sum of the two measurements.

PATIENTS AND METHODS

1. Study population

Forty-two patients were included into the study, 21 patients in each group. The population consisted of 25 women and 17 men. Six subjects from the active treatment group and seven subjects from the placebo group were withdrawn from the study with no significant difference between the two groups.

A. Withdrawals from the active treatment group

Four subjects were withdrawn from the study before D10: treatment failure, one; side effects, one (the patient suffered from tachycardia, vertigo and drowsiness); two patients were lost to follow-up.

Two subjects were withdrawn from the study between D10 and D30: treatment failure, one; side effects, one (gastric pain).

B. Withdrawals from the placebo group.

Five subjects were withdrawn from the study before D10: side effects, one

Table 1. Study of the homogeneity of the population on D0.

parameters	initial characteristics		statistical analysis
Sex	43% men 57% women	with Pneumorel® 80 mg	
	38% men 62% women	with placebo	p = 0.753
Age (years)	37.8 ± 2.8 39.3 ± 1.7	with Pneumorel® 80 mg with placebo	p = 0.663
Height (cm)	167.2 ± 1.6 164.9 ± 1.7	with Pneumorel® 80 mg with placebo	p = 0.347
Weight (kg)	64.6 ± 2.2 60.7 ± 2.5	with Pneumorel® 80 mg with placebo	p = 0.239
Clinical signs	– nasal obstruction (*) – rhinorrhoea (*) – pain (*)		p = 0.614 p = 0.428 p = 0.121
Radiological signs	Thickening of the sinus mucosa(*)		p = 0.110

(*) Rank sum.

(gastric pain); an episode of secondary infection, one; treatment failure, one; two patients were lost to follow-up.

Two subjects were withdrawn from the study between D10 and D30: treatment failure, one; an episode of secondary infection, one.

Analysis of the course of the subjects between D0 and D10 and between D0 and D30 concerned the entire population included in the study with the exception of the patients lost to follow-up; the patients withdrawn from the study because of side effects, treatment failure or an episode of secondary infection were counted as failures. Nineteen patients in each group were therefore included in the analysis.

2. Homogeneity of the population on D0

The homogeneity of the two groups on D0 was confirmed in terms of the principal criteria of the study: sex, age, height, weight, nasal obstruction, rhinorrhoea, pain, thickening of the sinus mucosa. The two groups were comparable in terms of all of these criteria (Table 1).

3. Methodology

Clinical evaluation: Each subject was seen as an outpatient prior to starting treatment. The initial clinical and radiological assessment was therefore per-

formed on D0. In addition to radiological clouding of at least one sinus, the patients also presented at least one of the following three signs: nasal obstruction, rhinorrhoea or pain. The patients included in the study then received three tablets per day (either placebo or Pneumorel® 80 mg) as single-agent therapy for 30 days.

A second clinical and radiological assessment was performed on the 10th day (D10), and a third was performed at the end of the study on the 30th day (D30).

Statistical analysis: An initial analysis compared the course of the two groups during treatment.

Clinical signs:

1. For each criterion, the subjects presenting the sign on at least one occasion during the study were included in the analysis.
2. The comparison of the variations in each sign was analysed between D0 and D10 and between D0 and D30 by tests derived from the χ^2 test (G test).
3. The analysis was interpreted in terms of improvement or failure. The patients were 'improved' or 'greatly improved' depending on the magnitude of the variation of the severity of the clinical sign on D10 or D30 (improved: decrease in the score by 1 or 2 points; greatly improved: decrease in the score by more than 2 points).
4. The failures corresponded to treatment failures (persistence or deterioration of the sign), whether or not they were withdrawn from the study, together with the withdrawals from the study for intolerance or for an episode of secondary infection.

Radiological signs: Comparison of the variations between D0 and D30 was performed by means of the Mann-Whitney test.

A second analysis concerned the intra-group variation. For the clinical parameters: Friedman's non-parametric test followed by a multiple comparisons test was performed at times D0, D10 and D30. For the variation in the 'sinus' parameter between D0 and D30, Wilcoxon's non-parametric test was performed in each group.

RESULTS

Comparison of the variations in the two groups over the 30 days of treatment and results of the clinical course for each symptom

A. VARIATION BETWEEN D0 AND D10

The comparison of the variations in the clinical signs between D0 and D10 is illustrated in Table 2.

1. Nasal obstruction.

73.6% of the patients in the Pneumorel® 80 mg group (i.e., 14 out of 19 subjects) obtained improvement in this symptom during the first 10 days of treatment, compared with 33.3% in the placebo group (i.e., six out of 18 subjects).

Table 2. Comparison of the course between D0 and D10.

	obstruction		rhinorrhoea		pain	
	Pneumorel® 80 mg	placebo	Pneumorel® 80 mg	placebo	Pneumorel® 80 mg	placebo
Failure	5 26.4%	12 66.7%	5 26.3%	13 72.2%	6 40%	12 66.6%
Improved	7 36.8%	6 33.3%	5 26.3%	3 16.7%	5 33.4%	5 27.7%
Greatly improved	7 36.8%	0 0%	9 47.4%	2 11.1%	4 26.6%	1 5.7%
Total improvement	73.7%	33.3%	73.7%	27.8%	60%	33.4%
	p = 0.002		p = 0.011		p = 0.158	

Furthermore, in the Pneumorel® 80 mg group, there were as many 'greatly improved' patients as 'improved' patients (36.8%), while none of the patients in the placebo group were 'greatly improved'. The comparison of the variations in the two groups was statistically highly significant (p = 0.002).

2. Rhinorrhoea

73.7% of the patients in the Pneumorel® 80 mg group (i.e., 14 out of 19 subjects) obtained improvement in this symptom during the first 10 days of treatment, compared with 27.8% in the placebo group (i.e., 5 out of 18 subjects). Interestingly, in the Pneumorel® 80 mg group, there were more 'greatly improved' patients (47.4%, i.e., 9 subjects) than 'improved' patients (26.3%, i.e., 5 subjects). The comparison of the variations in the two groups was statistically significant (p = 0.011).

3. Pain

60% of the patients in the Pneumorel® 80 mg group (i.e., 9 out of 15 subjects) obtained improvement in this symptom during the first 10 days of treatment, compared with 33.4% in the placebo group (6 out of 18 subjects). The comparison of the variations in the two groups was not statistically significant (p = 0.158).

B. VARIATION BETWEEN D0 AND D30

The comparison of the variations in the clinical signs between D0 and D30 is presented in Table 3.

1. Nasal obstruction

68.4% of the patients in the Pneumorel® 80 mg group (i.e., 13 out of 19 subjects) obtained improvement in this symptom by the end of treatment, compared with 38.9% in the placebo group (i.e. 7 out of 18 subjects). Furthermore, in the Pneumorel® 80 mg group, there were more 'greatly improved' patients than 'improved' patients, in contrast with the placebo group. The comparison of the variations in the two groups was statistically significant (p = 0.07).

Table 3. Comparison of the course between D0 and D30.

	obstruction		rhinorrhoea		pain	
	Pneumorel® 80 mg	placebo	Pneumorel® 80 mg	placebo	Pneumorel® 80 mg	placebo
Failure	6 31.6%	11 61.1%	7 38.9%	11 64.8%	7 43.8%	12 70.6%
Improved	5 26.3%	5 27.8%	0 0%	3 17.6%	4 25%	5 29.4%
Greatly improved	8 42.1%	2 11.1%	11 61.1%	3 17.6%	5 31.2%	0 0%
Total improvement	68.4%	38.9%	61.1%	35.2%	56.2%	29.4%
	p = 0.07		p = 0.007		p = 0.015	

Table 4. Comparison of the course of the radiological examination between D0 and D30.

	D0–D30	
	Pneumorel® 80 mg group	placebo group
Improvement	9 64.3%	3 20%
Failure	5 35.7%	12 80%
	n = 14 patients	n = 15 patients

2. Rhinorrhoea

61.1% of the patients in the Pneumorel® 80 mg group (i.e. 11 out of 18 subjects) obtained improvement in this symptom by the end of treatment, compared with 35.2% in the placebo group (i.e. 6 out of 17 subjects); the 11 patients in the Pneumorel® 80 mg group were all 'greatly improved'. The comparison of the variations in the two groups was statistically highly significant (p = 0.007).

3. Pain

56.2% of the patients in the Pneumorel® 80 mg group (i.e. 9 out of 16 subjets) obtained improvement in this symptom by the end of treatment, compared with 29.4% in the placebo group (5 out of 17 subjects). In the Pneumorel® 80 mg group, there were more 'greatly improved' patients than 'improved' patients, while none of the patients in the placebo group were 'greatly improved'. The comparison of the variations in the two groups was statistically significant (p = 0.015).

C. RESULTS OF THE RADIOLOGICAL COURSE

The course of the radiological examinations in terms of improvement in the films between D0 and D30 demonstrated that in 64.3% of the patients in the

Table 5. Variation between D0 and D30. Comparison of the variations in each group.

			probability	
Clinical examination	Obstruction	Pneumorel® 80 mg	$p < 0.001$	D0 ≠ D10 and D30**
		Placebo	$p = 0.077$	
	Rhinorrhoea	Pneumorel® 80 mg	$p < 0.001$	D0 ≠ D10 and D30**
		Placebo	$p = 0.360$	
	Pain	Pneumorel® 80 mg	$p = 0.012$	D0 ≠ D10 and D30*
		Placebo	$p = 0.269$	
Radiological examination	Sinuses	Pneumorel® 80 mg	$p < 0.01$	D0 ≠ D30*
		Placebo	$p > 0.05$	NS

* $p < 0.05$ ** $p < 0.01$

Pneumorel® 80 mg group (i.e. 9 out of 14 subjects) a radiological improvement was observed at the end of treatment compared with 20% in the placebo group (3 out of 15 subjects) and this improvement was statistically significant ($p = 0.018$) (Table 4). Furthermore, there were no cases of deterioration in the Pneumorel® 80 mg group in contrast with the placebo group (two deteriorations). For the radiological examination, as for the clinical examination, therefore, a marked improvement was observed with Pneumorel® 80 mg in comparison with placebo after one month of treatment.

Intra-group variation D0/D10/D30

Overall, for all of the parameters, the variation within the groups was only significant between D0 and D30 in the group treated with Pneumorel® 80 mg. More precisely, an improvement was observed with Pneumorel® 80 mg, which was significant by the 10th day and which remained stable for one month for the parameters 'obstruction' and 'rhinorrhoea', while the improvement in pain was only significant between D0 and D30.

A similar significant improvement in the radiological examination of the sinuses was observed between D0 and D30 with active treatment. In the placebo group, no statistically significant variation was observed between D0/D10 and D30 for any of the parameters considered (Table 5).

ACCEPTABILITY

The acceptability of Pneumorel® 80 mg at the dosage of three tablets per day for one month was perfectly satisfactory.

DISCUSSION AND CONCLUSION

This study was designed to evaluate the efficacy of Pneumorel® 80 mg in the treatment of chronic sinusitis in a population of 42 patients. This study was

Table 6. Analysis of the study population includes withdrawals which are counted as failures

1. Nasal obstruction

	D0–D10		D0–D30	
	Pneumorel® 80 mg	placebo	Pneumorel® 80 mg	placebo
Failure	5 26.4%	12 66.7%	6 31.6%	11 61.1%
Improved	7 36.8%	6 33.3%	5 26.3%	5 27.8%
Greatly improved	7 36.8%	0 0%	8 42.1%	2 11.1%
Total improvements	73.7%	33.3%	68.4%	38.9%
	p = 0.002		p = 0.07	

2. Rhinorrhoea

	D0–D10		D0–D30	
	Pneumorel® 80 mg	placebo	Pneumorel® 80 mg	placebo
Failure	5 26.3%	13 72.2%	7 38.9%	11 64.8%
Improved	5 26.3%	3 16.7%	0 0%	3 17.6%
Greatly improved	9 47.4%	2 11.1%	11 61.1%	3 17.6%
Total improvements	73.7%	27.8%	61.1%	35.2%
	p = 0.011		p = 0.007	

3. Pain

	D0–D10		D0–D30	
	Pneumorel® 80 mg	placebo	Pneumorel® 80 mg	placebo
Failure	6 40%	12 66.6%	7 43.8%	12 70.6%
Improved	5 33.4%	5 27.7%	4 25%	5 29.4%
Greatly improved	4 26.6%	1 5.7%	4 31.2%	0 0%
Total improvements	60%	33.4%	56.2%	29.4%
	p = 0.158		p = 0.015	

conducted in outpatients under double-blind, placebo-controlled conditions at the dose of three tablets per day for one month. The overall results were extremely satisfactory as demonstrated by the statistical analysis for each symptoms (Table 6).

1. Nasal obstruction: 68.4% of patients in the Pneumorel® 80 mg group were improved, compared with 38.9% in the placebo group.
2. Rhinorrhoea: 61.1% of patients in the Pneumorel® 80 mg group were improved, compared with 35.2% in the placebo group.
3. Pain: 56.2% of patients in the Pneumorel® 80 mg group were improved, compared with 29.4% in the placebo group.

X-rays of the sinuses demonstrated similar results, as an improvement or return to normal of the films of at least one of the affected sinuses was observed in 64.3% of patients treated with Pneumorel® mg. In contrast, in the placebo group, only 20% of patients were improved and two patients deteriorated.

This study is particularly interesting in view of the fact that this disease is common and often difficult to treat. A large number of drug treatments have been proposed, but their efficacy is not always convincing, particularly in cases of chronic sinusitis with no mechanical cause. Consequently, before undertaking surgical treatment, one should consider using Pneumorel® 80 mg, the value of which has been demonstrated in this study, conducted in cases of chronic sinusitis, in the absence of secondary infection and in patients in whom previous treatments had failed.

REFERENCES

1. Cuénant G. Intérêt de Pneumorel® (fenspiride) dans le traitement de l'inflammation chronique de la muqueuse respiratoire (à propos de 30 cas de sinusites chroniques). Cahiers ORL 1985; 20: 528–539.
2. De Fenoyl O. Pneumorel® 80 mg (fenspiride) dans le traitement de l'inflammation de la muqueuse respiratoire. Gaz Méd 1985; 92: 54–56.
3. Evrard Y, Lhoste F, Advenier C, Duhault J. Le fenspiride et le système broncho-pulmonaire: nouvelle approche pharmacologique. Sem Hôp Paris 1986; 62 (19): 1375–1381.
4. Fraysse B, Furia E. Intérêt du fenspiride (Pneumorel® 80 mg) dans le traitement pré-opératoire des otites séro-muqueuses chroniques. Vie Méd 1984; 23: 943–948.
5. Olivieri D, Del Donno M. Étude de l'action du fenspiride Pneumorel® 80 mg sur la clairance mucociliaire chez le bronchitique chronique. JIM 1987; 96.
6. Receveur M. Intérêt du Pneumorel® 80 mg dans le traitement de l'inflammation de la muqueuse respiratoire en pathologie ORL aiguë. Trib Méd 1985; 129: 47–48.

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Value of fenspiride (Pneumorel® 80 mg) in the preoperative treatment of chronic open tympanum otitis*

Double-blind placebo-controlled study

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INTRODUCTION

The aim of the present study was to evaluate, by means of a double-blind placebo-controlled trial, the action of fenspiride (Pneumorel® 80 mg) on inflammation of the mucosa of the middle ear in adult subjects with chronic otitis associated with an inflammatory reaction of the mucosa and tympanic perforation requiring surgical treatment, without any known immunological disorder and in whom the severity of the tubal obstruction was measured by manometry, and the condition of the mucosa was evaluated by otoscopy.

Fenspiride hydrochloride (Pneumorel® 80 mg) is a disubstituted piperazine, which possesses well-established pharmacological properties on the vascular phase of inflammation: the action of fenspiride on the mucosa is reflected by a reduction in oedema and inflammatory exudate (Evrard et al., 1986).

A preliminary open pilot study suggested the favourable action of fenspiride in patients with chronic otitis associated with tympanic perforation, with improvement in otorrhoea, the otoscopic appearance of the mucosa and the course of Rinne's test (Fraysse and Furia, 1984).

MATERIAL AND METHOD

Study population

This study was conducted in a population of 40 subjects randomised into two groups. Adult patients (over the age of 14 years) of either sex with inflammation of the mucosa of the middle ear secondary to chronic otitis with tympanic perforation, without any known immunological disorder, who were to undergo surgical treatment upon completion of the present study, and in whom the severity of the tubal obstruction (without qualification) was measured by manometry and the condition of the mucosa was evaluated by otoscopy, were included in this study.

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Children aged 14 years or less, pregnant women, patients with proven liver or renal failure, history of allergy, immunological disorders likely to have induced or caused persistence of the chronic otitis or a contraindication to surgery were not included in the study. Similarly, patients receiving systemic corticosteroid therapy not suspended for at least three months prior to the start of the study were also not included.

Lastly, patients receiving the following drugs also failed to satisfy the inclusion criteria:

- systemic anti-inflammatory agents,
- any drugs modifying respiratory tract secretions (mucolytics, "muco-regulators", liquefiers, mucus inhibitors),
- antihistamines, antiallergics, vasoconstrictors,
- antibacterial and anti-infectious agents (other than those permitted by the present protocol) and not suspended for at least one week before the study.

Patients not completing the whole course of treatment, patients requiring one of the drugs listed in the criteria of non-inclusion during the study and patients with severe adverse effects requiring discontinuation of treatment were excluded from the study.

Description of the study and experimental design

The experimental design was that of a double-blind placebo-controlled study conducted in two parallel groups. The total duration of the study was two months, with an acceptable range of ± 15 days, in view of the problems related to organisation of the operations.

The study was conducted according to the following plan:

1. T0 recruitment, first assessment – randomisation, start of treatment with Pneumorel® 80 mg or placebo
2. T1 second assessment, preoperative (T1 = T2−1 to 4 days), continuation of treatment
3. T2 (T2 = T0 + 45 to 75 days) third assessment, intraoperative.

At the end of the period T0 → T2, during the eight days preceding the operation, two 20-minute sessions per day of hydrocortisone + soframycin aerosol, constituting a systematic and standard preoperative preparation, were added to the treatment with Pneumorel® 80 mg or placebo.

Pneumorel® 80 mg or placebo was administered at a dosage of three tablets per day: one tablet with each of the principal meals.

The active drug and the placebo were supplied in the form of tablets with an identical appearance, as the treatments were administered under double-blind conditions after randomisation according to random numbers tables.

Assessments performed

Three assessments were performed during the study. The following parameters

were evaluated at T0, at the time of inclusion:
- qualitative and quantitative appearance of otorrhoea,
- inflammatory appearance of the mucosa of the middle ear on otoscopy,
- audiometric Rinne's test determined at the four frequencies usually used (250, 1,000, 2,000 and 4,000 Hz),
- pneumatisation of the mastoid (radiography of the petrous bone with Schuller's view).

The second, preoperative, assessment included scoring of the otorrhoea, otoscopy and audiometry. Lastly, the third, intraoperative, assessment included anatomo-functional quantification of the inflammatory condition of the mucosa on an intraoperative histological specimen examined by light microscopy.

Scores

The criteria evaluated at the three assessments were scored as follows:

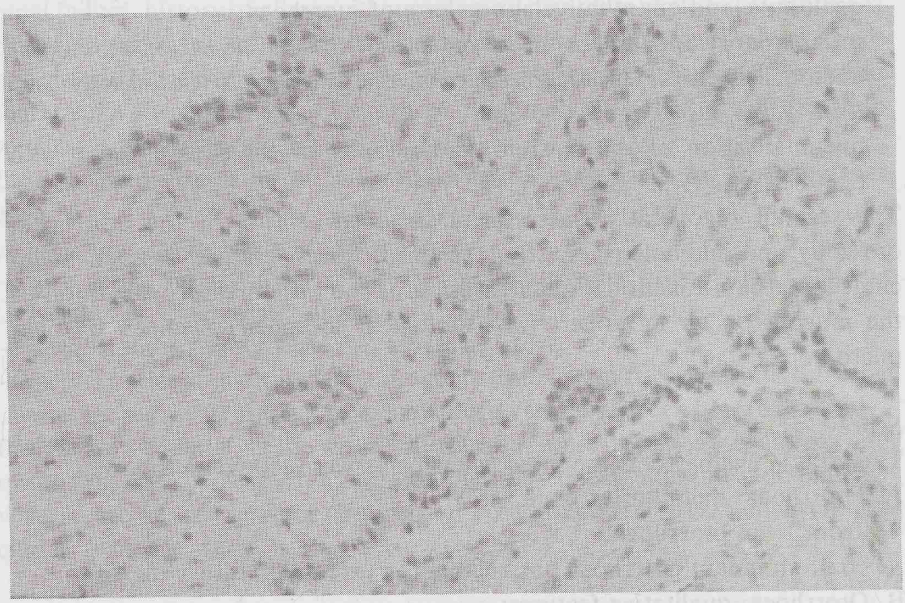
A. Otorrhoea: quantitative features:
   from 1: no otorrhoea
   to 4: abundant otorrhoea obstructing the external auditory meatus

B. Otorrhoea: qualitative features:
   1 = no otorrhoea
   2 = clear fluid
   3 = mucoid appearance
   4 = purulent otorrhoea

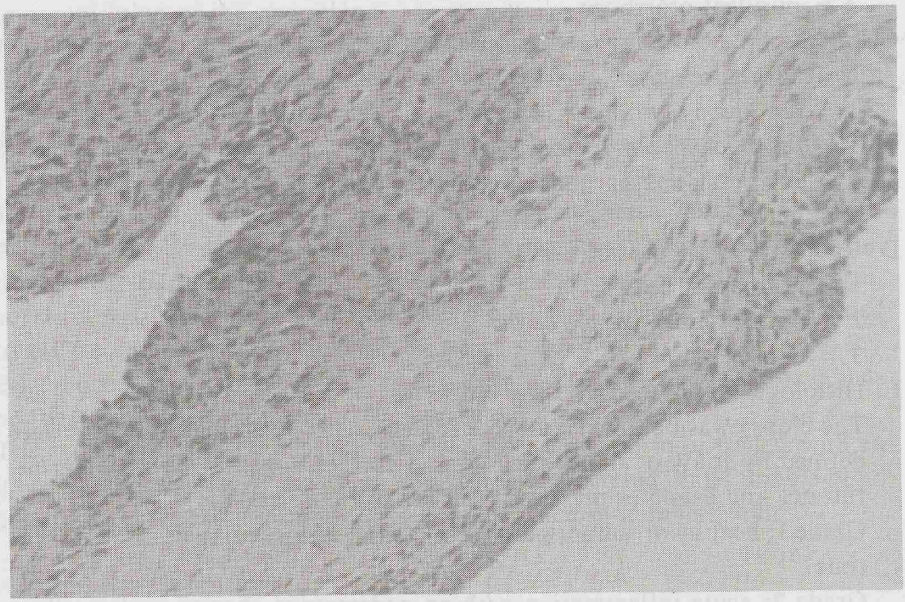
C. Microscopic otoscopy (condition of the mucosa):
   1 = normal mucosa
   2 = red mucosa without thickening
   3 = red thickened mucosa
   4 = polypoid secretory mucosa with thickening as far as the residual tympanum

D. Radiography of the petrous bone:
   1 = normal air cells
   2 = opacity but presence of air cells
   3 = condensation of the entire mastoid

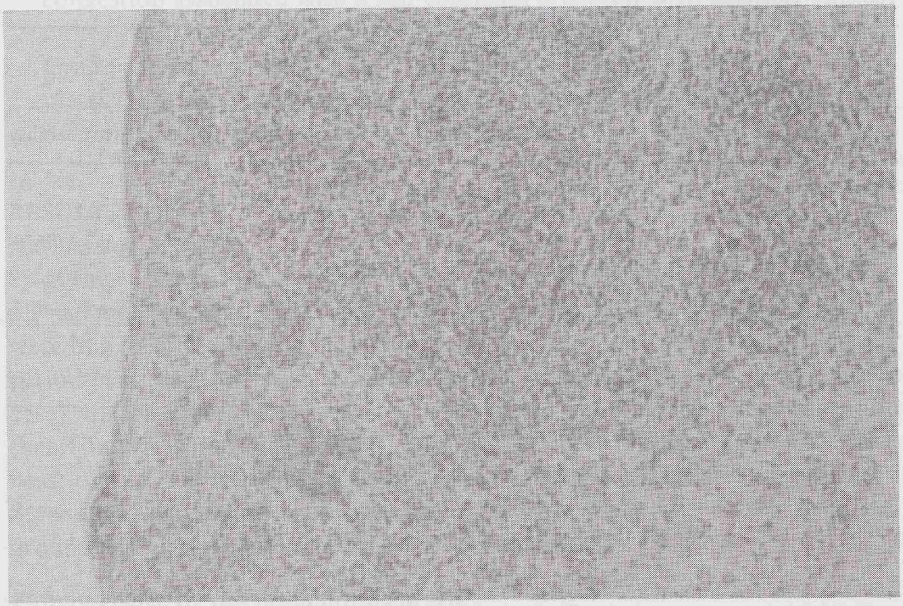
E. Histological appearance of the biopsy:
   The biopsy was obtained with fine forceps. Three histological grades were defined by study of the epithelium and chorion together with cellular infiltrates and exudates present at this level (Figure 1a–d):
   Grade 1: healthy or subnormal mucosa without thickening or cellular infiltrate.
   Grade 2: acute inflammation with an oedematous chorion and capillary



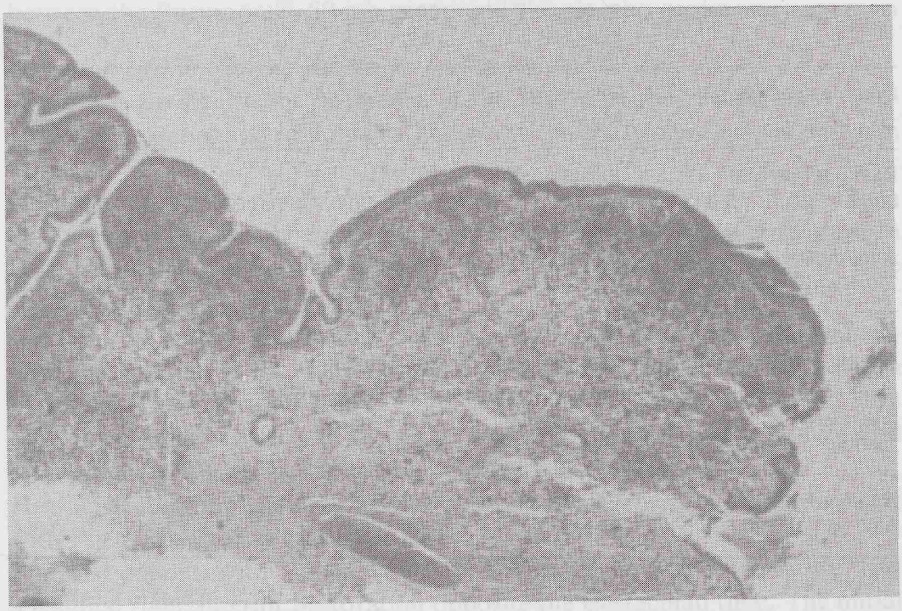
A. Grade 1. Subnormal mucous membrane with cylindrical-cubical epithelium and submucosa without inflammation.



B. Grade 2. Same with submucosal inflammation and numerous polymorphonuclears.



C. Grade 3. Hyperplastic mucosa with malphigian metaplasia. Dense submucosal infiltration with numerous plasmocytes.



D. Grade 3. Polypoid mucosa with cylindrical epithelium and dense connective tissue inflammation.

Figure 1. Histological changes of inflammation.

Table 1. Homogeneity at T0.

	n = 13 with Pneumorel® 80 mg n = 15 with placebo		
variables	initial characteristics		probability
Sex	Pneumorel®	61.5% of men 38.5% of women	p = 0.619
	Placebo	46.7% of men 53.3% of women	
Age	Pneumorel® Placebo	29.7 ± 4.3 37.3 ± 4.2	p = 0.220
Diagnosis	Pneumorel®	38.5% cholesteatomas (CHOL) 61.5% chronic otitis with tympanic perforation alone (COTP)	p = 0.934
	Placebo	40% CHOL 60% COTP	
Quantitative otorrhoea	Pneumorel® Placebo	199* 207*	p = 0.629
Qualitative otorrhoea	Pneumorel® Placebo	204* 202*	p = 0.475
Otoscopy-endoscopy	Pneumorel® Placebo	192* 214*	p = 0.872
Radiology petrous bone	Pneumorel® Placebo	185.5* 220.5*	p = 0.890
Audiometry 250 Hz (dB of loss)	Pneumorel® Placebo	26.9 ± 4.0 29.0 ± 4.6	p = 0.739
Audiometry 1000 Hz (dB of loss)	Pneumorel® Placebo	25.4 ± 4.1 26.3 ± 3.9	p = 0.869
Audiometry 2000 Hz (dB of loss)	Pneumorel® Placebo	21.9 ± 4.2 16.7 ± 3.1	p = 0.317
Audiometry 4000 Hz (dB of loss)	Pneumorel® Placebo	24.2 ± 2.9 23.0 ± 3.8	p = 0.804

* Rank sum.

congestion associated with a sub-epithelial predominantly neutrophil polymorphonuclear cell infiltrate. There is little change in the epithelial layer. Grade 3: prolonged subacute inflammation. The epithelial layer is hyperplastic. The chorion is polypoid with a dense infiltrate of variable numbers of lymphocytes, plasma cells and neutrophil polymorphonuclear cells.

RESULTS

Number of patients

28 of the 40 patients initially recruited completed the study: 13 in the Pneumorel® 80 mg group and 15 in the placebo group. The withdrawals from the study were due to the following reasons: operation not performed during the study period (four cases), refusal of operation (one case), patient not reviewed (one case), discontinuation of treatment (one case), intercurrent contraindication (heart failure with placebo, one case), adverse effects (bitter taste and tiredness: one case with the active drug) and incomplete case files (two cases).
Statistical analysis did not reveal any significant difference between the two groups in terms of withdrawals from the study.

Homogeneity of the populations at T0

As shown in Table 1, the two groups were perfectly homogeneous at T0: regardless of the criterion considered, there was no significant difference at T0 between the Pneumorel® 80 mg group and the placebo group.

Variation of the clinical, otoscopic and radiological signs

There was no significant difference in the course of the clinical signs, otoscopic appearance and radiological signs between the two groups, as shown in Table 2.
Only four of the 28 patients who completed the study had a purulent otorrhoea at T0: two in the placebo group and two in the Pneumorel® 80 mg group. In these four cases, the purulent nature of the otorrhoea had resolved at the time of the preoperative examination.
It should be noted, although it obviously does not have any statistical significance because of the small sample size, that the two patients treated with placebo had persistent otorrhoea, scored as 3 (mucoid appearance) and that in the two patients treated with the active drug, the otorrhoea had resolved and in one patient the perforation had completely healed.
Healing of the tympanic perforation was observed during prolonged treatment with Pneumorel® 80 mg in three cases:
The first patient, with a history of tympanoplasty two years previously, had a residual right anterior perforation opposite the Eustachian tube. The initial examination did not reveal any otorrhoea and the mucosa appeared to be normal on otoscopy. Closure of the perforation was observed after seven weeks of treatment.

Table 2a. Comparison of the variations with each treatment.

n = 13 with Pneumorel® 80 mg
n = 15 with placebo

variable		characteristics	variation (*)	test of the treatment × time interaction
Quantitative otorrhoea	Pneumorel®	Deterioration (T2–T0 > 0) for n = 1 subject Improvement (T2–T0 < 0) for n = 5	p = 0.219	
		Stability (T2–T0 = 0) for n = 7		p = 0.580
	Placebo	Deterioration (T2–T0 > 0) for n = 0 Improvement (T2–T0 < 0) for n = 3	p = 0.250	
		Stability (T2–T0 = 0) for n = 12		
Qualitative otorrhoea	Pneumorel®	Deterioration (T2–T0 > 0) for n = 1 subject Improvement (T2–T0 < 0) for n = 5	p = 0.219	
		Stability (T2–T0 = 0) for n = 7		p = 0.311
	Placebo	Deterioration (T2–T0 > 0) for n = 0 Improvement (T2–T0 < 0) for n = 2	p = 0.500	
		Stability (T2–T0 = 0) for n = 13		
Otoscopy-endoscopy	Pneumorel®	Deterioration (T2–T0 > 0) for n = 0 subject Improvement (T2–T0 < 0) for n = 3	p = 0.250	
		Stability (T2–T0 = 0) for n = 10		p = 0.565
	Placebo	Deterioration (T2–T0 > 0) for n = 1 Improvement (T2–T0 < 0) for n = 7	p = 0.070	
		Stability (T2–T0 = 0) for n = 7		

Table 2a. Continued.

n = 13 with Pneumorel® 80 mg
n = 15 with placebo

variable		characteristics	variation (*)	test of the treatment × time interaction
Radiology petrous bone	Pneumorel®	Deterioration (T2–T0 > 0) for n = 0 subject Improvement (T2–T0 < 0) for n = 3 Stability (T2–T0 = 0) for n = 9	p = 0.250	p = 0.608
	Placebo	Deterioration (T2–T0 > 0) for n = 0 Improvement (T2–T0 < 0) for n = 2 Stability (T2–T0 = 0) for n = 13	p = 0.500	

* Nil differences (stability) ignored.

Table 2b. Comparison of the variations with each treatment.

n = 13 with Pneumorel® 80 mg
n = 15 with placebo

variable	characteristics			variation (*)	test of the treatment × time interaction
		T0	T1		
Audiometry 250 Hz (dB of loss)	Pneumorel®	26.9 ± 4.0	25.4 ± 3.2	p = 0.613	p = 0.684
	Placebo	29.0 ± 4.6	29.3 ± 3.7	p = 0.922	
Audiometry 1000 Hz (dB of loss)	Pneumorel®	25.4 ± 4.1	21.9 ± 3.7	p = 0.377	p = 0.388
	Placebo	26.3 ± 3.9	27.3 ± 3.5	p = 0.719	
Audiometry 2000 Hz (dB of loss)	Pneumorel®	21.9 ± 4.2	17.3 ± 3.2	p = 0.097	p = 0.020 S at T0: p = 0.304 at T1: p = 0.325
	Placebo	16.7 ± 3.1	22.3 ± 3.6	p = 0.094	
Audiometry 4000 Hz (dB of loss)	Pneumorel®	24.2 ± 2.9	18.8 ± 2.9	p = 0.042 S↘	p = 0.367
	Placebo	23.0 ± 3.8	21.0 ± 4.2	p = 0.479	

(*) Nil differences (stability) ignored.

The second patient had a left anterior tympanic perforation, complicating the course of seromucous otitis. The initial examination revealed moderately severe purulent otorrhoea, red, thickened, inflammatory mucosa and tubal obstruction. Healing was obtained after three months of treatment.
Lastly, the third case was that of a patient with a mucous polyp descending into the external auditory meatus through a posterior marginal tympanic perforation. On otoscopy, the rest of the tympanum appeared to be thickened. After two months of treatment, the otorrhoea and the polyp had resolved and the perforation had healed.
In these three cases, the perforations were small and the inflammatory phenomena in the tympanic cavity and especially in the Eustachian tube prevented spontaneous healing.

Functional course (audiometry)
Analysis of the variation in Rinne's test (determined by the difference between air conduction and bone conduction) demonstrated a significant improvement in favour of the Pneumorel® 80 mg group (Table 2b):
At 2000 Hz: the difference in the losses in dB decreased from 21.9 ± 4.2 to 17.3 ± 3.2 in the Pneumorel® 80 mg group and, in contrast, increased from 16.7 ± 3.1 to 22.3 ± 3.6 in the placebo group. The difference in the variation between the two groups was significant ($p = 0.020$).
At 4000 Hz: A significant improvement ($p = 0.042$) was observed in the Pneumorel® 80 mg group, while no significant improvement was observed in the placebo group.

Variation in the histological appearance of the mucosa
Three patients were not operated in the Pneumorel® 80 mg group because of complete healing in response to medical treatment.
The distribution of the biopsies according to the histological classification defined above is seen in Table 3.

Table 3. Distribution of the biopsies according to the histological classification.

	Pneumorel® 80 mg group	Placebo group
Complete healing	3	0
Subnormal mucosa (without inflammation)	4	1
Chronic inflammation	0	2
Grade 2 without infiltrate	0	1
Grade 2	3	3
Grade 3	2	2

Statistical analysis performed with Fischer's exact test, comparing the numbers of normal and subnormal samples with the numbers of inflammatory samples, revealed a significant difference in favour of Pneumorel® 80 mg ($p = 0.031$).

DISCUSSION AND CONCLUSION

This double-blind placebo-controlled study demonstrated a favourable action for fenspiride (Pneumorel® 80 mg) on the inflammatory condition of the mucosa of the middle ear in patients with chronic otitis with tympanic perforation, requiring tympanoplasty.

The preparation of these patients by two months of treatment with Pneumorel® 80 mg at a dose of three tablets per day induced, in comparison with placebo, a significant audiometric improvement at frequencies of 2000 and 4000 Hz and a marked regression in the inflammatory condition of the mucosa.

Although the variation in the clinical signs was not significantly different between the two groups, two points should nevertheless be stressed:

1. The four subjects with purulent otorrhoea showed resolution of this purulent condition, probably related to the administration of soframycin. However, the two subjects receiving placebo had persistent mucoid otorrhoea, while the otorrhoea dried up in the two patients treated with the active drug.
2. Furthermore, in three patients from the Pneumorel® 80 mg group, the tympanic perforation healed with medical treatment alone, while none of the patients treated with placebo obtained spontaneous healing.

This study also confirmed the good acceptability of Pneumorel® 80 mg, as there was only one withdrawal from the study due to the development of a feeling of tiredness and a bitter taste. This good systemic acceptability allows the long-term prescription of this drug in this type of chronic disease in adults and older children.

In conclusion, this study demonstrates the value of the anti-inflammatory action of Pneumorel® 80 mg in the medical treatment of chronic otitis and in the preparation for surgery when tympanoplasty is indicated.

REFERENCES

1. Evrard Y, Lhoste F, Advenier C, Duhault J. Le fenspiride et le système bronchopulmonaire: nouvelle approche pharmacologique. La Semaine des Hôpitaux 1986; 62: 1375–1381.
2. Fraysse B, Furia F. Intérêt du fenspiride (Pneumorel® 80 mg), dans le traitement préopératoire des otites séromuqueuses chroniques. La Vie Médicale 1984; 2.

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Value of Pneumorel® 80 mg in the treatment of chronic otitis in adults

Multicentre study of 696 cases

L. Sallebert and D. Schutz

INTRODUCTION

Chronic otitis is a result of complex histological mechanisms combining various degrees of infection, inflammation, epithelial or connective tissue metaplasia, fibrosis and mucosal or bony involvement.

The common denominator remains, above all, chronic inflammation associated with tubal dysfunction. All forms of tubal pathology affect the middle ear, inducing a modification in tympanic compliance, effusion and interference with auditory transmission. Tubal dysfunction and inflammation are self-perpetuating, resulting in chronicity of the disease.

Fenspiride (Pneumorel® 80 mg) is an original molecule characterised by a high specificity for the ciliated mucosa of the airways and by an action on the primary, vascular, phase of inflammation in this mucosa. Clinically, its efficacy has been extensively demonstrated in bronchial pathology. In the ENT sphere, its efficacy has been particularly demonstrated in chronic sinusitis and in preparation for tympanoplasty. The results obtained by Fraysse (1987) in this last indication suggested the particular value of Pneumorel® 80 mg, not only in preparation for surgery, but also in the medical treatment of chronic otitis in adults. A large-scale multicentre study was conducted under clinical practice conditions in order to confirm these concepts.

MATERIAL AND METHOD

The aim of this clinical study was therefore to evaluate the action of Pneumorel® 80 mg on the clinical signs of inflammation of the mucosa of the middle ear in adult subjects with chronic otitis present for more than six months, without any known immunological disorders. The study was conducted by 80 ENT specialists throughout France in a total of 696 cases. The subjects included were patients of either sex over the age of 14 years. The patients had suspended any treatments likely to interfere with Pneumorel® 80 mg at least eight days before inclusion into the study, i.e. in practice, modifiers of

respiratory tract secretions, antihistamines and other anti-allergic agents, as well as vasoconstrictors. Any corticosteroid therapy prescribed for a period of at least 15 days had to be stopped for at least three months prior to inclusion. If an episode of secondary infection occurred during the study, antibiotic therapy, strictly limited to beta-lactams or macrolids, could be prescribed. However, the various assessments could not be performed during the acute episodes.

The following subjects were not included into the study:

- children aged 14 years or less,
- pregnant women,
- subjects with mastoiditis requiring specific medical or surgical treatment,
- subjects with severe visceral disorders, especially patients with liver or renal failure,
- subjects in whom the otitis was accompanied by a history of allergy or immunological disorders,
- subjects receiving a drug prohibited in the inclusion criteria, as well as those for whom there were good reasons to suggest that they would not return for the last examination.

The patients included in the study were treated for three months by Pneumorel® 80 mg, at a dose of three tablets per day, in three divided doses. The study, conducted according to an open protocol, included three assessment times: on inclusion into the study (T0), after 45 days of treatment with Pneumorel® 80 mg (T1) and after 90 days of treatment with Pneumorel® 80 mg (T2, end of study).

A clinical examination and otoscopic examination were performed at each time: the clinical examination defined the site of the chronic otitis, evaluated earache and the qualitative and quantitative features of otorrhoea and assessed any associated ENT pathology as well as the severity of the hearing impairment.

The otoscopic examination evaluated the condition of the tympanum (shape, colour, appearance) when it was closed and the inflammatory state of the mucosa of the middle ear when the tympanum was open (the mucosa was classified into four grades: normal, erythematous, thickened erythematous, polypoid secretory).

At the beginning and at the end of the study, threshold pure tone audiometry was used to determine the audiometric Rinne's test and the hearing loss at frequencies of 500, 1,000, 2,000 and 4,000 Hz. Impedancemetry was only performed when the tympanum was closed.

The side effects occurring during the study were systematically recorded. All of the withdrawals from the study, whether or not they could be attributed to treatment, were thoroughly documented.

Statistical analysis of the results was performed after stratification of the population, which was subdivided into two subpopulations according to the condition of the tympanum at the time of inclusion into the study. The tympanic membranes subsequently evolved, either by maintaining their initial condition, i.e. open or closed, or by changing condition. In view of the sample size, these latter were only counted: however, it should be noted, without pre-empting the presentation of the results, that 29 tympanic membranes which were open on inclusion into the study closed in the course of the three months of treatment with Pneumorel® 80 mg.
The variation in the quantitative variables was submitted to analysis of variance. The ordinal qualitative variables were analysed either by Friedman's test or by Wilcoxon's test. Cochran's test was applied to the non-ordinal qualitative variables.

RESULTS

1. Population studied

The study recruited a total of 696 cases, representing 840 diseased ears at T1, distributed as follows:
378 cases of unilateral chronic otitis with open tympanum
174 cases of unilateral chronic otitis with closed tympanum
44 cases of bilateral chronic otitis with open tympanum
84 cases of bilateral chronic otitis with closed tympanum
16 cases of bilateral chronic otitis with one tympanum open and the other closed.
630 ears maintained the same condition of the tympanum at the three times of the study: open (352 ears) or closed (278 ears).
The statistical analysis was performed on this population, considering, for each criterion, the number of cases in which the data were present at the three times of the study.
The overall course of the cases of bilateral otitis was analysed separately to respect the orthodoxy of statistical analysis: the parameters of the two ears of a same subject cannot be considered to be independent. The 696 subjects in the study presented the following characteristics (Table 1).

2. Concomitant treatments

Treatments suspended: 115 patients suspended a drug prior to inclusion into the study. 86% of the drugs suspended corresponded to drugs prohibited by the protocol.
Permitted drugs maintained during the study: 96 patients continued their treatment in parallel with the study, but only 17 patients, i.e. 2.4% of the population included in the study, received an ENT treatment permitted by the protocol.

Table 1. Characteristics of the population on inclusion into the study.

Sex	Male 371	Female 323 (2 missing data)
Age	43 ± 1.4 yrs	
Weight	65.3 ± 1,2 kg	
Height	167 ± 0.8 cm	
ENT history		
Non-surgical	433 patients, i.e. 62.2% of the total population	
Surgical	76 patients, i.e. 10.9% of the total population	
including:		
tympanoplasty	46 patients, i.e. 6.6% of the total population	
transtympanic drain	17 patients, i.e. 2.4% of the total population	
cholesteatoma	3 patients, i.e. 0.4% of the total population	
other	10 patients, i.e. 1.4% of the total population	

Treatments prescribed during the study: a treatment was prescribed during the study in 113 patients. In 104 cases, it corresponded to standard antibiotic treatment for acute ENT infection permitted by the protocol (β-lactams, macrolids).

Associated ENT diseases at T0: 142 patients (i.e. 20.4% of the total population) suffered from rhinopharyngitis at T0; 118 patients (i.e. 16.9% of the total population) suffered from sinusitis; another ENT disease was observed in 53 patients, i.e. 7.6% of the total population.

3. Variation in the cases of unilateral otitis

The various clinical and complementary criteria were studied by distinguishing the cases of otitis with an open tympanum from those with a closed tympanum.

Earache: of the 350 patients with an open tympanum and in whom earache was present at T0 and scored at the three times of the study, 32.3% were improved by T1 and 40.3% were improved after three months of treatment with Pneumorel® 80 mg. This progression was highly significant ($p < 0.001$) (Figure 1). The results were even better in the 173 ears with a closed tympanum, with improvement in earache in 48% of ears after 90 days of treatment with Pneumorel® 80mg ($p < 0.001$) (Figure 2).

Otorrhoea: of the 350 ears with an open tympanum and scored for this criterion at the three times of the study, the qualitative and quantitative variation in otorrhoea was very significant between T0 and T1 and between T0 and T2 ($p < 0.001$).

The percentage of ears improved in terms of the two aspects of this criterion were virtually identical at the two times studied, as illustrated by Figure 3.

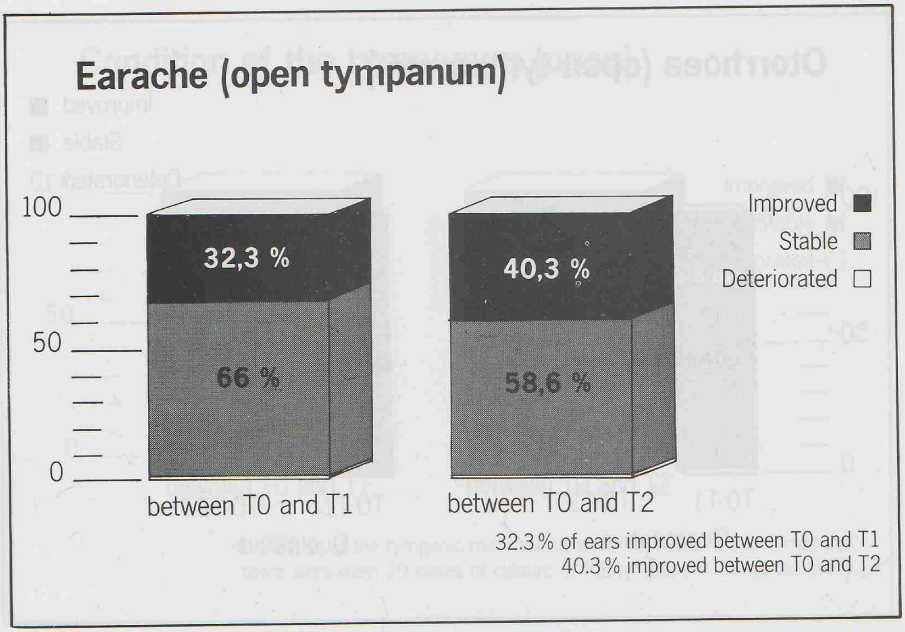

Figure 1. Outcome of earache in open tympanum otitis between T0 and T1 and between T0 and T2.

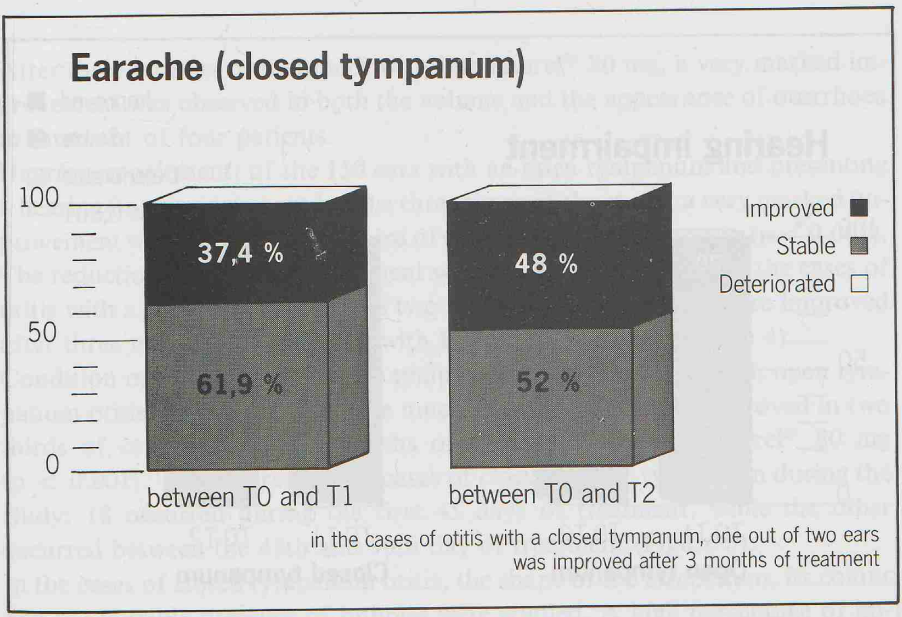

Figure 2. Outcome of earache in closed tympanum otitis between T0 and T1 and between T0 and T2.

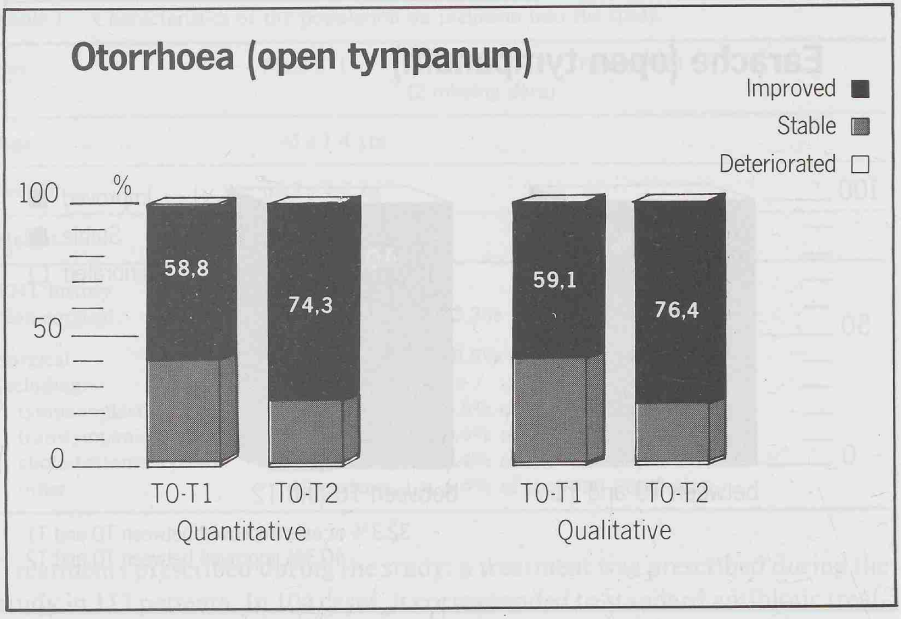

Figure 3. Quantitative and qualitative outcome of otorrhoea (open tympanum otitis) between T0 and T1 and between T0 and T2.

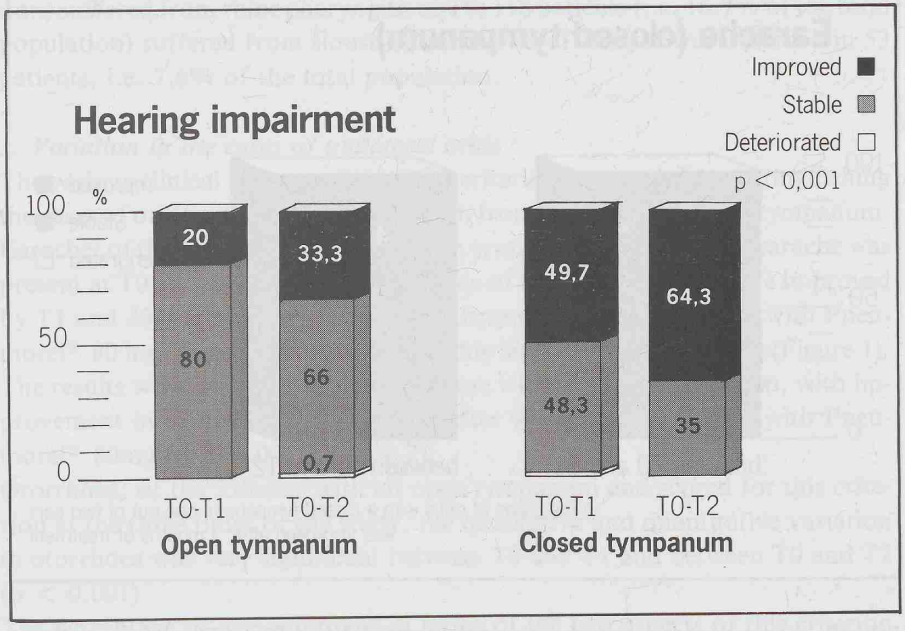

Figure 4. Outcome of hearing impairment between T0 and T1 and between T0 and T2.

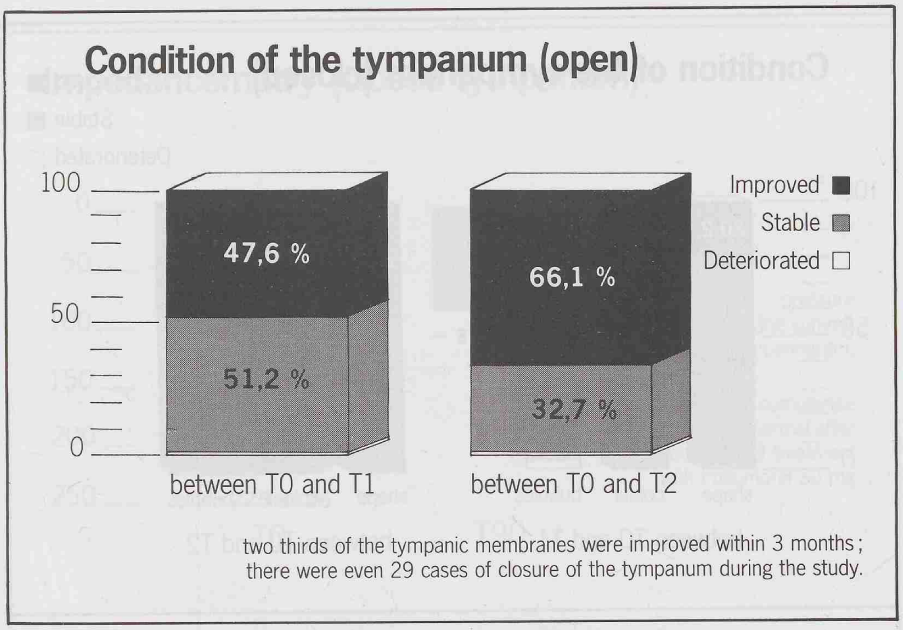

Figure 5. Outcome of the condition of the tympanum in open tympanum otitis between T0 and T1 and between T0 and T2.

After three months of treatment with Pneumorel® 80 mg, a very marked improvement was observed in both the volume and the appearance of otorrhoea in three out of four patients.

Hearing impairment: of the 150 ears with an open tympanum and presenting a hearing impairment scored at the three times of the study, a very marked improvement was observed in one third of subjects after three months ($p < 0.001$). The reduction in hearing impairment was much more frequent in the cases of otitis with a closed tympanum, as two thirds of these subjects were improved after three months of treatment with Pneumorel® 80 mg (Figure 4).

Condition of the mucosa and the tympanum: of the 432 ears with open tympanum otitis, the condition of the mucosa was considerably improved in two thirds of cases after three months of treatment with Pneumorel® 80 mg ($p < 0.001$). There were even 29 cases of closure of the tympanum during the study: 18 occurred during the first 45 days of treatment, while the other occurred between the 45th and 90th day of treatment (Figure 5).

In the cases of closed tympanum otitis, the shape of the tympanum, its colour and the possible presence of bubbles were studied. A high percentage of improvements was observed for each of these criteria (Figure 6). However, the results were much better at T2 than at T1, which means that treatment must

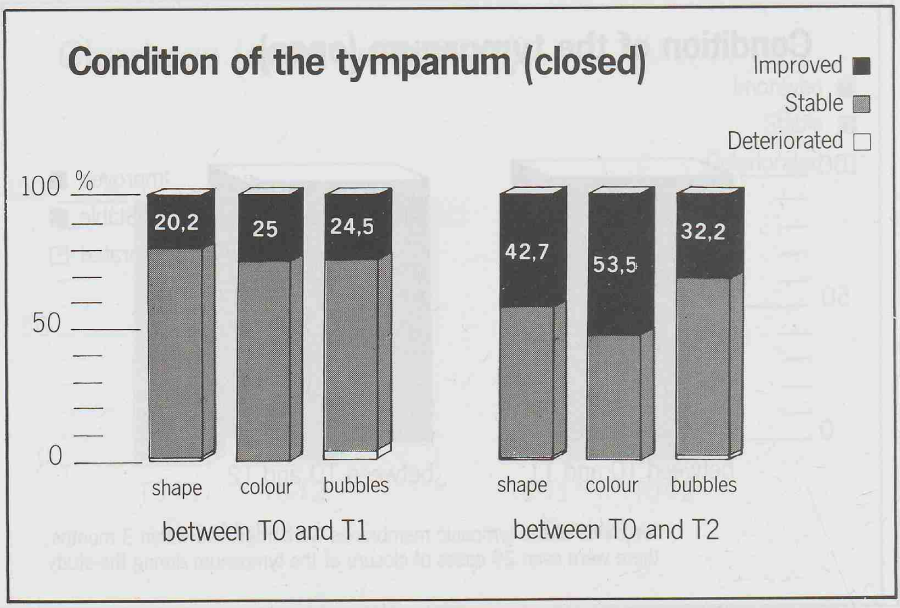

Figure 6. Outcome of the condition of the tympanum in closed tympanum otitis between T0 and T1 and between T0 and T2.

be administered for a sufficiently long period of time in order to obtain improvement in the appearance of the tympanum. The clinical significance of resolution of bubbles is a topic of debate. A correlation study by Spierman's test revealed that the resolution of these bubbles accompanied the improvement in the shape and colour of the tympanum, which suggests that this resolution of bubbles constitutes an improvement.

Impedancemetry (on a closed tympanum): three criteria were studied during measurement of impedancemetry in ears with a closed tympanum at T0 and T2: the height of the peak, the deviation towards negative pressures and the pressure necessary at the summit of the tympanogram. These three parameters were very significantly improved between the start and the end of the study ($p < 0.001$).

Of the 164 studied ears, the pressure at the summit of the tympanogram was very significantly decreased by Pneumorel® 80 mg between T0 and T2 ($p < 0.001$), which reflects the reduction in the pressure in the middle ear and therefore a return towards normal (Figure 7).

At T0, the mean pressure necessary at the summit of the tympanogram was -225 ± 26.5 mm, H_2O, while it was only -87.5 ± 20.6 mm H_2O at T2, which can be considered to be the limit of normal.

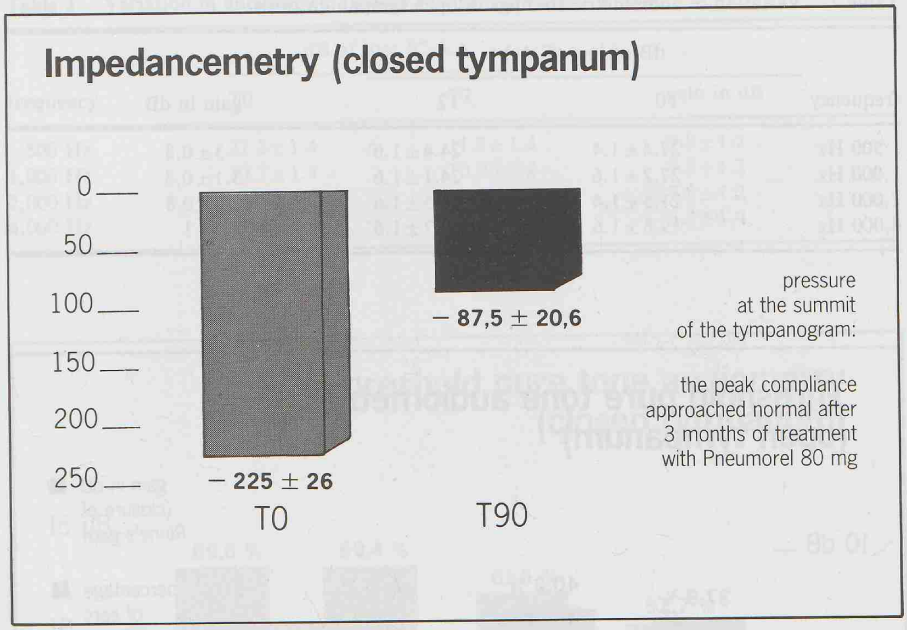

Figure 7. Outcome of peak pressure on the tympanogram after 90 days of treatment with Pneumorel® 80 mg.

For 27 patients suffering from closed tympanum otitis, a peak was observed on the tympanogram at T2, while the curve was flat at T0. This result can be interpreted to be an improvement in serous otitis, regressing to the stage of tubal catarrh after three months of treatment with Pneumorel® 80 mg.

Audiometric gain: of the 300 ears with an open tympanum for which audiometry was performed at the two times of the study at T0 and at T2, bone conduction was obviously not modified, but a minor but significant gain was observed in air conduction: 27 to 40% of the ears were improved, depending on the frequency considered and the mean gain, calculated for all of the ears, was 2 to 3 dB, depending on the frequency (Table 2) (Figure 8). The gain was obviously higher for each of the ears actually improved.

The audiometric improvement was obviously much greater in the 250 ears studied in which the tympanum was closed: the mean gain in dB in these ears was virtually 10 dB at 500 Hz and at 1,000 Hz (Table 3) (Figure 9) and two thirds of these ears presented an audiometric improvement with a decrease in the difference between the loss in air conduction (A.C.) and bone conduction (audiometric Rinne's test).

Associated ENT diseases: the course of ENT diseases associated with open tympanum or closed tympanum chronic otitis was determined on the basis of

Table 2. Variation in audiometric findings in open tympanum otitis.

frequency	dB of loss (C.A.) T0	T2	gain in dB
500 Hz	27.4 ± 1.4	24.4 ± 1.6	3 ± 0.8
1,000 Hz	27.2 ± 1.6	24.1 ± 1.6	3.1 ± 0.8
2,000 Hz	21.5 ± 1.4	19.5 ± 1.6	2 ± 0.8
4,000 Hz	19.8 ± 1.6	17.7 ± 1.6	2.1 ± 1

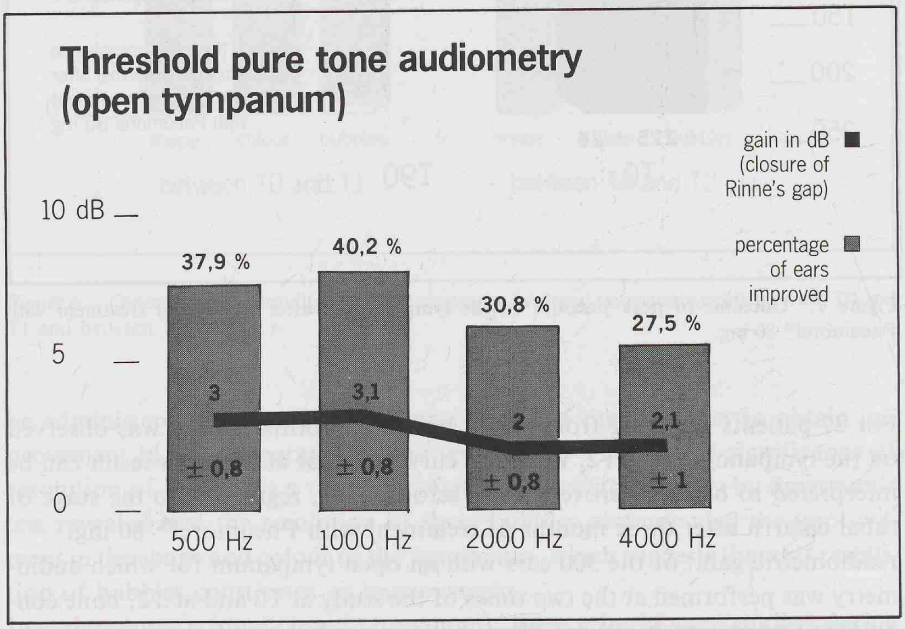

Figure 8. Outcome of threshold pure tone audiometric and audiometric Rinne's test findings after 90 days of treatment with Pneumorel® 80 mg in open tympanym otitis.

the clinical history and examination performed at the three times of the study. Three months of treatment with Pneumorel® 80 mg very significantly improved the cases of rhinopharyngitis, sinusitis and other ENT diseases present during the study ($p < 0.001$), as illustrated in Figure 10.

4. Variation in the cases of bilateral otitis

As the two ears of the same subject could not be considered to be independent, the cases of bilateral otitis were analysed separately. However, comparable results to those obtained in the cases of unilateral otitis were observed.

Table 3. Variation in audiometric findngs in closed tympanum otitis.

frequency	dB of loss (C.A.) T0	T2	gain in dB
500 Hz	21.2 ± 1.4	11.3 ± 1.4	9.9 ± 1.2
1,000 Hz	20.7 ± 1.4	10.9 ± 1.4	9.8 ± 1.2
2,000 Hz	16 ± 1.4	8.2 ± 1.2	7.8 ± 1.2
4,000 Hz	14.8 ± 1.6	8.6 ± 1.4	6.3 ± 1.4

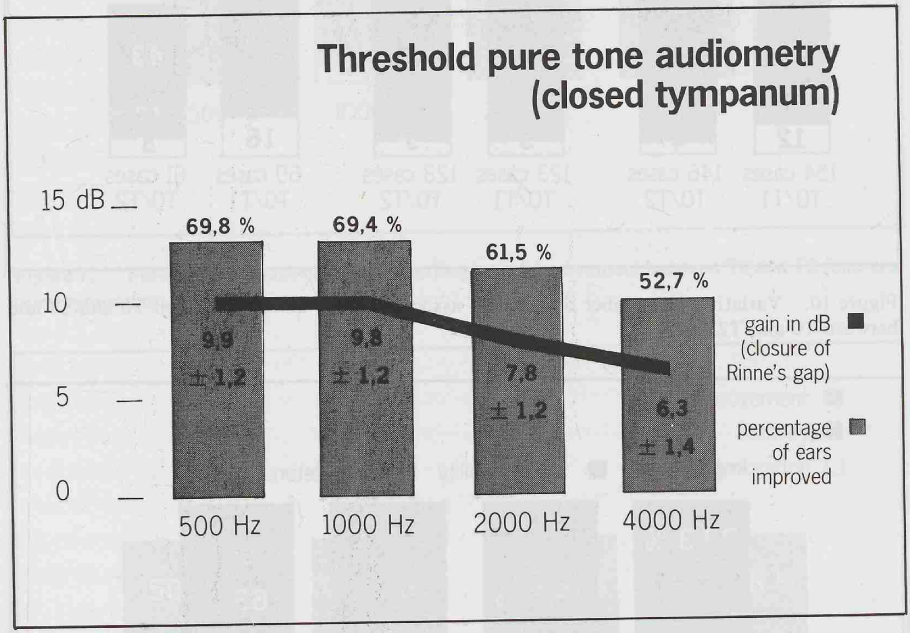

Figure 9. Outcome of threshold pure tone audiometric and audiometric Rinne's test findings after 90 days of treatment with Pneumorel® 80 mg in closed tympanum otitis.

Bilateral open tympanum otitis: after three months of treatment, Pneumorel® 80 mg relieved earache in one out of three patients and qualitatively and quantitatively improved otorrhoea in almost three out of four patients. The condition of the middle ear mucosa was improved in two thirds of patients (Figure 11).

An improvement in audiometric findings was observed in almost one out of three patients at the various frequencies studied, as illustrated by Figure 12.

Bilateral closed tympanum otitis: when the tympanic membranes were closed, earache was improved in one out of two patients after three months of treat-

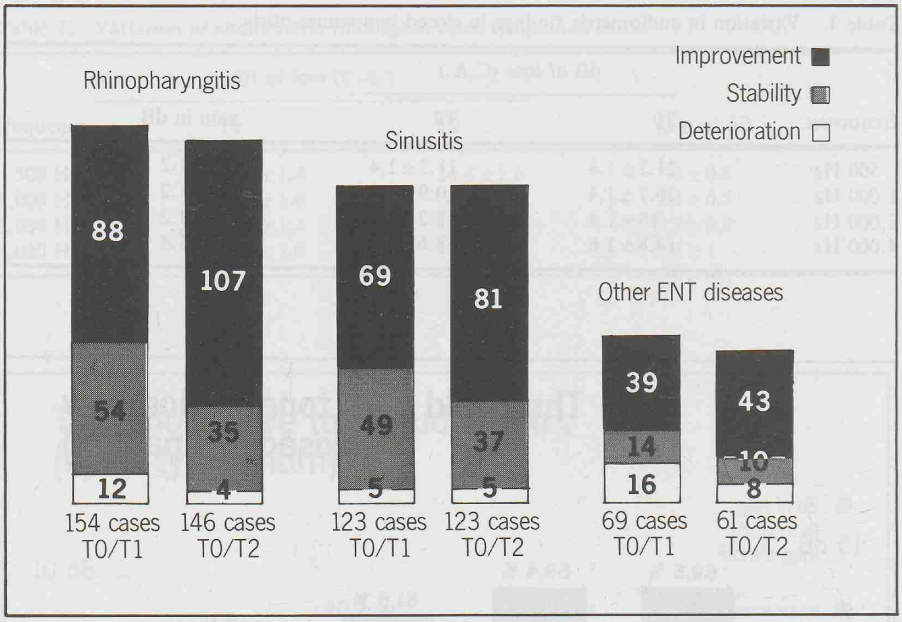

Figure 10. Variation (in number of cases) of associated ENT diseases between T0 and T1 and between T0 and T2.

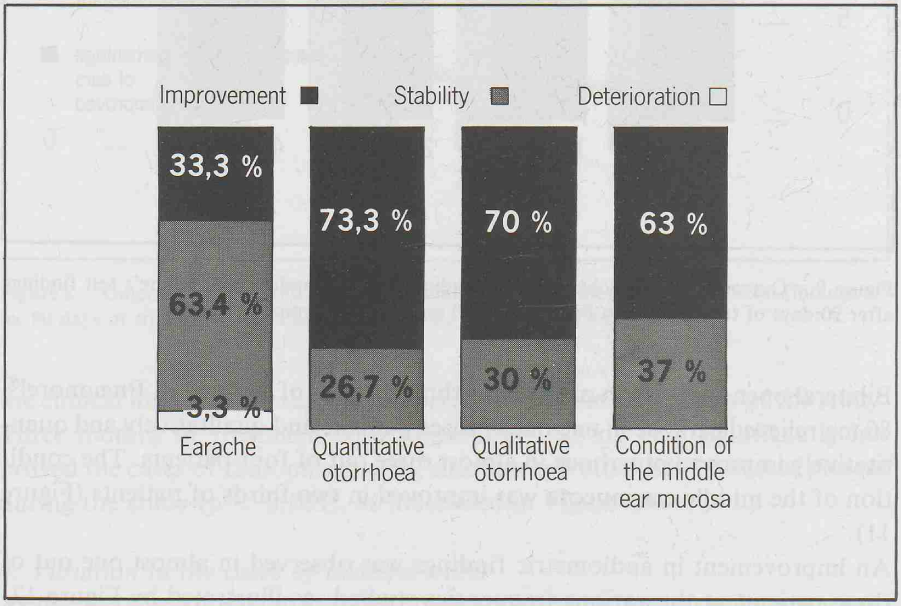

Figure 11. Variation in the cases of bilateral open tympanum otitis after 90 days of treatment with Pneumorel® 80 mg.

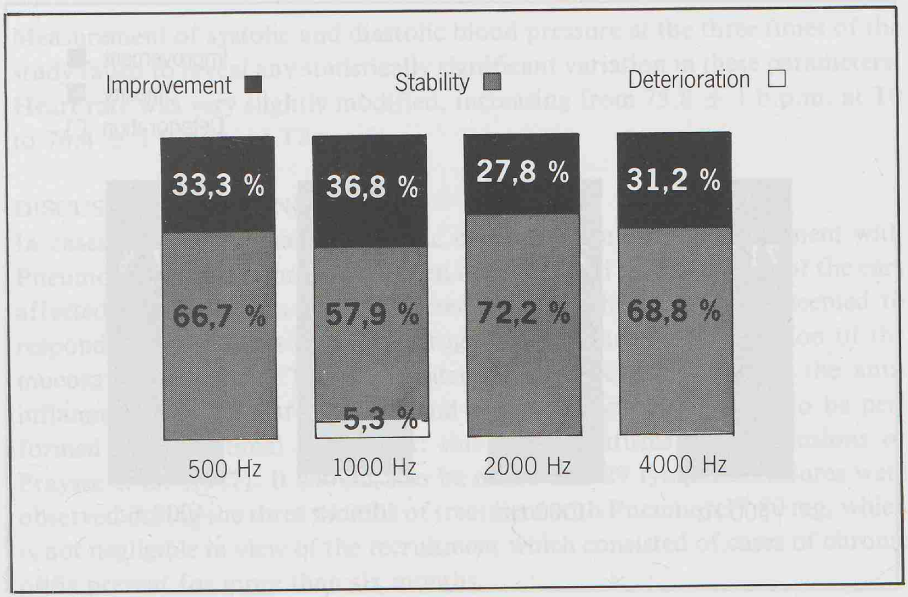

Figure 12. Percentage of patients with an audiometric improvement between T0 and T2 (bilateral open tympanum otitis).

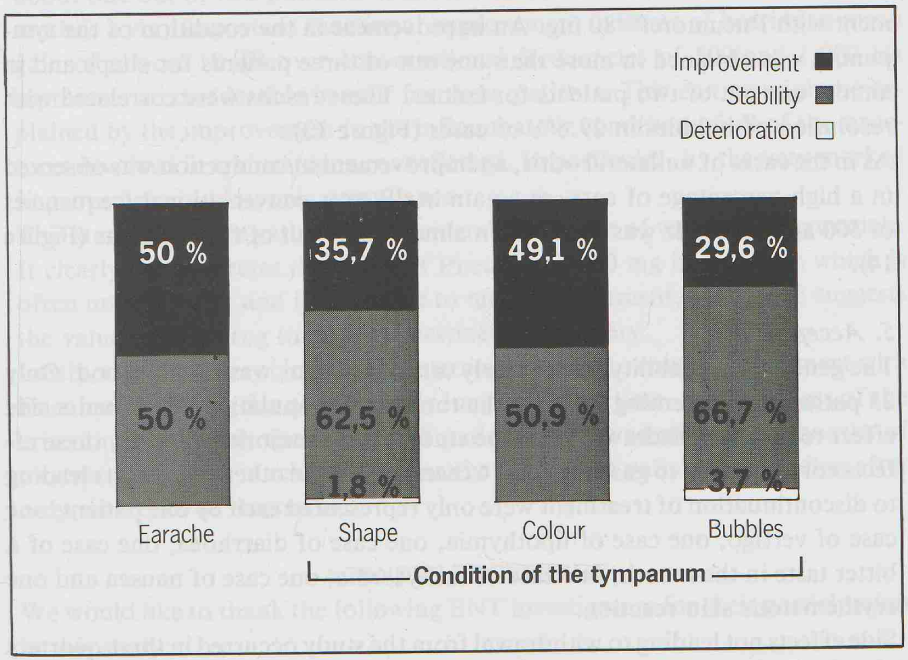

Figure 13. Variation in the cases of bilateral closed tympanum otitis after 90 days of treatment with Pneumorel® 80 mg.

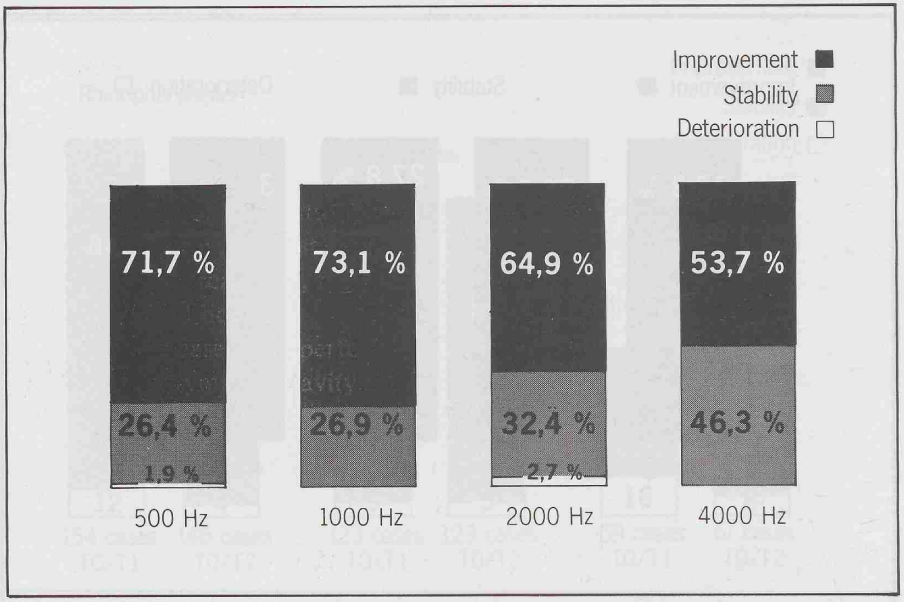

Figure 14. Audiometric variation in cases of bilateral closed tympanum otitis after 90 days of treatment with Pneumorel® 80 mg.

ment with Pneumorel® 80 mg. An improvement in the condition of the tympanum was obtained in more than one out of three patients for shape and in almost one out of two patients for colour. These results were correlated with resolution of bubbles in 29.6% of cases (Figure 13).

As in the cases of unilateral otitis, an improvement in conduction was observed in a high percentage of ears, as a gain in dB over conversational frequencies of 500 and 1,000 Hz was observed in almost three out of four patients (Figure 14).

5. Acceptability

The general acceptability, particularly cardiovascular, was usually good. Only 23 patients, representing 3.3% of the total study population developed a side effect requiring withdrawal from the study. In the majority of cases, these effects corresponded to gastric pain (16 cases), while the other side effects leading to discontinuation of treatment were only represented each by one patient: one case of vertigo, one case of lipothymia, one case of diarrhoea, one case of a bitter taste in the mouth, one case of tachycardia, one case of nausea and one erythematous skin reaction.

Side effects not leading to withdrawal from the study occurred in three quarters of cases during the first 15 days of treatment; they were always moderate and usually resolved without discontinuation of treatment.

Measurement of systolic and diastolic blood pressure at the three times of the study failed to reveal any statistically significant variation in these parameters. Heart rate was very slightly modified, increasing from 75.8 ± 1 b.p.m. at T0 to 76.4 ± 1 b.p.m. at T2.

DISCUSSION AND CONCLUSION

In cases of open tympanum chronic otitis, three months of treatment with Pneumorel® 80 mg improved between one third and three quarters of the ears affected, depending on the parameter studied. The signs which seemed to respond best to the action of the drug were otorrhoea and condition of the mucosa on otoscopy. These are interesting results which reflect the anti-inflammatory action of the drug and allow a surgical operation to be performed under optimal conditions: this study confirms the conclusions of Fraysse et al. (1987). It should also be noted that 29 tympanic closures were observed during the three months of treatment with Pneumorel® 80 mg, which is not negligible in view of the recruitment which consisted of cases of chronic otitis present for more than six months.

In cases of closed tympanum chronic otitis, three months of treatment with Pneumorel® 80 mg improved earrache and the condition of the tympanum in about one out of two patients. It should be noted that the action of the drug induces a very important functional improvement in terms of hearing: a mean gain of almost 10 dB over conversational frequencies of 500 and 1,000 Hz represents a considerable benefit for these patients. This can certainly be explained by the improvement in the inflammatory condition of all of the structures involved in transmission, as reflected, in particular, by the very marked improvement in tympanic compliance.

This study therefore gathered a considerable amount of valuable information. It clearly demonstrates the value of Pneumorel® 80 mg in a disease which is often unresponsive and inaccessible to medical treatment, but it also suggests the value of this drug in patients destined for surgery.

Lastly, this study very clearly demonstrates the need to continue treatment with Pneumorel® 80 mg for a sufficiently long period of time, as, regardless of the criterion considered, the number of patients improved after three months of treatment was very much greater than the number of patients responding after only six weeks of treatment.

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REFERENCES

1. Fraysse B, Calvet H, Faure Ph, Schutz D. Intérêt du fenspiride (Pneumorel® 80 mg) dans le traitement préopératoire de l'otite chronique à tympan ouvert. Étude en double aveugle contre placebo. Cahiers ORL 1987; 22: 597–604.

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Recent data on the pharmacology of fenspiride

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INTRODUCTION

The relationship between inflammation and the bronchospastic reaction has been demonstrated both during immediate IgE-mediated hypersensitivity reactions and during delayed allergic reactions.

IgE-mediated hypersensitivity involves the action of mast cells and non-mast cells such as basophils, macrophages, lymphocytes, eosinophils and platelets. The activation of these cells induces the release of numerous mediators with a direct action on smooth muscle fibers and/or pro-inflammatory effects. Amongst these mediators, histamine induces bronchoconstriction and vascular hyperpermeability, chemotactic factors induce an influx of inflammatory cells and PAF-acether increases airways resistance, modifies vascular permeability and induces an influx of inflammatory cells. Arachidonic acid derivatives, including prostaglandins (PGD_2) and leukotrienes ($LTC_4 - LTD_4 - LTB_4$) are also proinflammatory and bronchoconstrictor derivatives.

Delayed allergic reactions involve an increase in airways resistance occurring several hours after contact with an antigen, either in the nose, the bronchi or the skin. These reactions consist of a combination of infiltration of the bronchial mucosa by inflammatory cells and bronchial hyperactivity (Tonnel et al., 1986). Pneumorel® (fenspiride) possesses anti-bronchoconstrictor properties; it counteracts bronchoconstriction induced by contracturant agents and allergic causes, and exerts an anti-inflammatory and anti-exudative effect (Duhault et al., 1972). In the present study, the properties of fenspiride on bronchospasm, contraction of lung fragments, and production by this tissue of malondialdehyde (MDA), a break down product of membrane phospholipids during inflammatory reactions, were confirmed in the previously sensitised guinea pig.

METHODOLOGY

The injection of albumin in a guinea pig sensitises the animal to this protein. The basophils and mast cells of this animal therefore present anti-albumin immunoglobulins at their surface. Renewed contact with albumin, either by

aerosol in vivo, or directly onto the lung in vitro, induces degranulation of the cells (basophils and mast cells) with production and release of mediators. This results in a measurable bronchopulmonary contraction.

Induction of bronchospasm in the guinea pig (in vivo)
Bronchospasm was induced by an ovalbumin aerosol in female Hartley Charles River guinea pigs (300 g) after passive or active sensitisation according to the following protocol:
1. Passive sensitisation: After verification of the sensitivity of the guinea pigs by means of a histamine test, an intravenous injection of rabbit serum containing titrated anti-ovalbumin antibodies was administered 24 hours before induction of anaphylactic shock.
2. Active sensitisation: In this case, the sensitisation was induced by two subcutaneous injections of 0.5 ml of a suspension of ovalbumin (20 μg/ml) and alumina (2 mg/ml) at an interval of 15 days. Anaphylactic shock was induced one week after the second sensitisation injection.
3. Anaphylactic shock: Anaphylactic shock was induced by submitting the sensitised guinea pigs to a 0.25% ovalbumin aerosol for 10 minutes. The time to appearance of the first cough, dyspnoea and coma was recorded.
4. Treatment with fenspiride: Fenspiride (5, 10 and 20 mg/kg) was administered orally 45 minutes before the aerosol. When fenspiride was administered intraperitoneally (1, 2.5 and 10 mg/kg), treatment was given 20 minutes before the aerosol.
5. The percentage protection due to treatment was evaluated in terms of the time to appearance of the same signs in the non-treated control animals according to the formula:

$$\% \text{ protection} = \frac{Tr - Te}{600 - Te} \times 100$$

in which:
Te = mean reaction time in seconds (severe dyspnoea) of the control guinea pigs treated with the vehicle.
Tr = mean reaction time in seconds (severe dyspnoea) of the guinea pigs treated with the test drug.
600 seconds corresponds to the period of maximal exposure if no respiratory impairment is observed.

Induction of an anaphylactic reaction in the isolated guinea-pig lung (in vitro)
1. Active sensitisation: The lungs of guinea pigs previously sensitised to bovine albumin contract when placed in the presence of the antigen in vitro. This experiment studied the protective effect of fenspiride against induction of con-

traction and the relaxation of lungs previously contracted by the allergen.
2. Preparation of the lung fragment: Male albino guinea pigs (400 g) were sacrificed six weeks after active sensitisation. The heart-lung block was removed and the right lower lobe was placed in a Petri dish containing Krebs-Henseleit solution. A strip of tissue was carefully sliced and the lung fragment was connected to a tension transducer. The preparation was calibrated with 3.45 M potassium chloride. The contraction obtained under these conditions represented the maximal contraction of the organ (100% KCl). Anaphylactic shock was induced by addition to the bath of 0.1 ml of a solution of bovine albumin at a concentration varying according to the desired severity of shock. For each animal, a strip of lung parenchyma was exposed to the antigen in the absence (control) or in the presence of the test drug.
3. Induction of contraction and relaxation:
a) Demonstration of the preventative effect on contraction: the strip of lung parenchyma was incubated in the presence of the drug for 30 minutes prior to the addition of bovine albumin which induced contraction, the rate and course of which were recorded until maximal response.
b) Demonstration of a relaxant effect. In this case, when the level of bovine albumin induced contraction was reached the preparation was rinsed and the test drug was added to the isolated organ chamber. The relaxation of the organ was then monitored for a period of 60 minutes.
The contraction and relaxation observed after bovine albumin were measured on the traces and expressed as a percentage of the maximal response obtained with KCl (100%).
The amplitude of the contraction and the slope of contraction were measured in order to assess the effect on induction of contraction. The slope of contraction reflected the rate at which the maximal effect was obtained.
The half-relaxation time and the residual contraction at 60 minutes were measured in order to assess the effect on relaxation. In every case, each parameter was measured on the control lung strips and the homologous strips placed in the presence of the test substance.
Fenspiride (10^{-5} M) was studied in two different situations: 1) shock induced by the addition of 1 μg/ml of bovine albumin, 2) more severe shock induced by 10 μg/ml of bovine albumin. Mepyramine (10^{-5} M) was used as the reference substance.

Study of malondialdehyde release in vitro
After sensitisation of the guinea pigs according to the method described above, the lungs were removed at sacrifice and sliced into strips which were then incubated in Tyrode solution at 37°C. A series of three tubes was used for each preparation:

Table 1. Percentage protection exerted by various doses of fenspiride against ovalbumin-induced bronchospasm in the guinea pig.

passive sensitisation			active sensitisation		
dose mg/kg IP	n	% protection	dose mg/kg IP	n	% protection
1	8	5	5	6	41*
2.5	8	16	10	6	51*
5	7	44*	20	6	81**
10	7	87**			
ED_{50} = 5 mg/kg IP			ED_{50} = 9 mg/kg PO		

(Mann-Whitney test *: $p \leq 0.05$; **: $p \leq 0.01$, significance in relation to controls). n = number of animals per group; ED_{50} = Effective dose 50.

1. Tissue suspension + buffer = absolute control
2. Tissue suspension + ovalbumin = control
3. Tissue suspension + ovalbumin + drug

Fenspiride (3.10^{-4} M, 8.10^{-4} M and $1.7.10^{-3}$ M) was added to the incubation medium just before induction of anaphylactic shock.

Anaphylactic shock was induced as described above by the addition of ovalbumin (20 μg/ml). The incubation was stopped after 15 minutes with trichloracetic acid and aliquots were collected for determination of MDA production by thiobarbituric acid spectrophotometry (Ohkawa et al., 1979). MDA production during shock was assessed in relation to the controls not subjected to shock. The effect of fenspiride on MDA production during anaphylactic shock was evaluated in relation to the controls and to indomethacin ($1.4.10^{-5}$ M).

RESULTS

Protective effect of fenspiride on bronchospasm in the guinea pig

The results obtained with fenspiride are presented in Table 1.

Regardless of the method of sensitisation, fenspiride exerted a protective effect on ovalbumin-induced bronchospasm. This effect was proportional to the dose administered. The effective dose 50 (ED50) was 5 mg/kg IP following passive sensitisation and 9 mg/kg PO following active sensitisation.

Effect of fenspiride on anaphylactic shock induced in the isolated guinea pig lung

1. Prevention of contraction: The activity of fenspiride was compared to that of mepyramine at the same concentration of 10^{-5} M (Table 2). The amplitude of contraction was decreased by about 20% with the two substances. The rate at which maximal contraction was obtained was slowed by 50% with fenspi-

Table 2. Effect of fenspiride and mepyramine on contraction and relaxation of guinea-pig lungs during bovine-albumin (BOV)-induced anaphylactic shock.

		contraction		relaxation	
group	BOV µg/ml	amplitude (%/100% KCl)	slope	½ relaxation time (min)	residual contraction at 60 minutes (%/100% KCl)
Control n = 6	1 µg	72 ± 3	46 ± 1	19 ± 3	17 ± 5
Fenspiride 10^{-5}M n = 6	1 µg	53 ± 4***	24 ± 2***	10 ± 2*	0*
Control n = 6	1 µg	70 ± 7	42 ± 3	23 ± 9	15 ± 10
Mepyramine 10^{-5}M n = 6	1 µg	46 ± 5***	13 ± 1***	24 ± 15 NS	12 ± 7 NS
Control n = 6	10 µg	91 ± 4	51 ± 2	65 ± 18	46 ± 8
Fenspiride 10^{-5}M n = 6	10 µg	88 ± 6 NS	32 ± 4***	33 ± 12***	31 ± 10**
Control n = 6	10 µg	73 ± 7	42 ± 5	55 ± 16	39 ± 9
Mepyramine 10^{-7}M n = 6	10 µg	68 ± 9 NS	19 ± 2***	34 ± 12 NS	24 ± 7 NS

*: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$ in relation to controls. Student's t test for paired series.

ride, which was a highly significant effect. This parameter was decreased by 70% with mepyramine.

When the severity of shock was increased (Table 3), the effect of fenspiride was no longer significant on the amplitude of contraction, but remained very intense on the rate of contraction (−38%).

2. Relaxation of previously contracted lung strips: Fenspiride exerted a potent relaxant effect (Table 2): the half-relaxation time was decreased by 50% and the residual contraction was nil and even 17% less than that of the controls. In contrast, mepyramine had no effect on relaxation: the parameters measured did not differ from those measured in the controls.

The severity of bovine albumin induced shock (Table 3) did not modify the

Table 3. Effect of fenspiride and mepyramine on contraction and relaxation of guinea-pig lungs during bovine-albumin-induced shock. Results expressed as a percentage difference in relation to controls for n ± 6 in each group.

parameters	characteristics	bovine albumin 1 μg/ml		bovine albumin 10 μg/ml	
		fenspiride 10^{-5}M	mepyramine 10^{-5}M	fenspiride 10^{-5}M	mepyramine 10^{-7}M
Contraction	amplitude	−19% ***	−25% ***	−4% NS	−5% NS
	slope	−48% ***	−70% ***	−38% ***	−53% ***
Relaxation	½ relaxation time	−50% *	+5% NS	−48% ***	−38% NS
	residual contraction at 60 minutes	−17% *	−3% NS	−25% **	−15% NS

*: $P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$ – Student's t test for paired series).

effect of fenspiride on the relaxation time or on the residual contraction. This result differed from that obtained previously in the study of prevention of contraction.

Malondialdehyde (MDA) release

Ovalbumin-induced shock led to an increase in MDA production of the order of 50%. Pre-treatment with fenspiride or indomethacin completely inhibited this increase. After pre-treatment, the MDA levels did not differ from basal values. The decrease in MDA production in response to fenspiride is clearly demonstrated in Tables 4 and 5. When placed in contact with the lung strips, fenspiride exerted a significant effect proportional to the dose present in the incubation medium.

DISCUSSION

Fenspiride protects the sensitised guinea pig against albumin-aerosol-induced bronchospasm. An identical effect was observed whether sensitisation was passive (administration of antibody) or active (administration of antigen inducing antibody production by the animal itself) and whether fenspiride was administered orally or parenterally.

Fenspiride also exerted a protective effect when anaphylactic shock was induced in vitro in previously sensitised guinea-pig lungs. This effect was demonstrated on several parameters:

Fenspiride decreased the amplitude of bronchopulmonary contraction and

Table 4. Effect of fenspiride on in vitro malondialdehyde production by sensitised guinea-pig lung. Results expressed in nM of MDA/g of fresh tissue.

sensitised guinea pigs	nM of MDA/ g of tissue	10.4 ± 0.8 (n = 16)	9.96 ± 0.8 (n = 7)	9.99 ± 0.75 (n = 8)
Sensitised guinea pigs + ovalbumin in vitro (20 μg/ml)	nM of MDA/ g of tissue	14.6 ± 0.9^{+++} (n = 16)	12.3 ± 1.07^{++} (n = 7)	12.0 ± 0.9^{++} (n = 8)
Sensitised guinea pigs + fenspiride in vitro + ovalbumin in vitro (20 μg/ml)	Fenspiride concentration	$1.7.10^{-3}$M	$8.4.10^{-4}$M	$3.4.10^{-4}$M
	nM of MDA/ g of tissue	10.1 ± 0.7*** (n = 16)	9.1 ± 0.7* (n = 7)	10.1 ± 0.9* (n = 8)
	% reduction in relation to sensitised controls	−31%	−26%	−15%

Results expressed in nM of MDA/g of fresh tissue. m ± s.e.m. of n samples. Student's t test for paired series.
$^{++}$ $P < 0.01$; $^{+++}$ $P < 0.001$ in relation to absolute controls
* $P < 0.05$; *** $P < 0.001$ in relation to sensitised controls.

Table 5. Malondialdehyde production by guinea-pig lung in vitro. Effect of fenspiride and indomethacin.

	n	nM of MDA/g of tissue	
Sensitised guinea pigs	7	7.94 ± 0.62	$P < 0.01$
Sensitised guinea pigs + ovalbumin in vitro	7	14.5 ± 1.65	$P < 0.001$
Sensitised guinea pigs + indomethacin $1.4.10^{-5}$M + ovalbumin (20 μg/ml)	7	5.11 ± 0.31	$P < 0.01$
Sensitised guinea pigs + fenspiride $1.7.10^{-3}$M + ovalbumin (20 μg/ml)	7	8.23 ± 0.49	

(m ± s.e.m. of n samples)
(Student's t test for paired series).

slowed its rate of appearance. An analogous effect was observed with mepyramine, a reference antihistamine.

When the lung was already contracted, fenspiride accelerated the relaxation of the lung fragment. In contrast with fenspiride, mepyramine had no effect on this parameter. This confirms the previous studies reviewed in Evrard et al. (1986), which demonstrated that fenspiride exerted a non-specific effect against various contracturant agents and not only against a single contracturant agent, histamine, as in the case of mepyramine.

Lastly, fenspiride inhibits the production of pro-inflammatory derivatives released during anaphylactic shock induced in vitro in guinea-pig lungs. A similar type of effect was observed with indomethacin, a reference anti-inflammatory agent.

CONCLUSION

All of these results therefore demonstrate that fenspiride acts at various levels on the processes involved in motor activity and inflammation of the broncho-pulmonary tract.

REFERENCES

1. Duhault J, Le Douarec JC, Mayeroff C, Boulanger M, Regnier G. Étude des propriétés anti-allergiques et corticosécrétoires du chlorhydrate de phénéthyl-8-oxa-Idiaza-3,8-spiro(4,5)décanone-2 (fenspiride, JP428). Arzneimittel-Forsch 1972; 22: 1947–1952.
2. Evrard Y, Lhoste F, Advenier C, Duhault J. Le fenspiride et le système broncho-pulmonaire: nouvelle approche pharmacologique. Semaine des Hôpitaux 1986; 62: 1375–1381.
3. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analyt Biochem 1979; 95: 351–358.
4. Tonnel AB, Wallaert B, Prin L, Ramon Ph, Bart F. Mécanismes de l'asthme allergique. Rev Mal Resp 1986; 3: 5–12.

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Fenspiride and relaxation of tracheobronchial musculature. Mechanism of action

C. Advenier

INTRODUCTION

Fenspiride (Pneumorel®) possesses anti-inflammatory and anti-bronchoconstrictor properties which justify its use in respiratory tract diseases with an inflammatory and allergic component both in ENT and in pneumology (Duhault et al., 1972). Both of these disciplines are concerned with one of the subdivisions of the respiratory tract: the first deals with the upper airways while the second deals with the actual respiratory system, the thorax and lungs. However, this type of separation is purely arbitrary as, in addition to the anatomical continuum existing between the nose, pharynx, larynx, trachea and bronchi, these structures also have certain histological and functional factors in common. An example of these common functions is that of air filtration by the airways which is mediated by two cell types present throughout the entire respiratory tract mucosa: 1) mucus-secreting goblet cells, which are found in the nasal, pharyngeal and tracheobronchial airways and 2) ciliated epithelial cells which allow migration of mucus carrying impurities as a result of their regular ciliary beating. These cells are situated in the paranasal sinuses and in the tracheobronchial passages. Another example is that of defence against external agents which, although exerted by different mechanisms in different territories, leads to an identical result. In response to chemical, mechanical or allergic irritation, the upper and lower airways react by increased resistance to airflow. In the nasal mucosa, this reaction is simply the result of a vasomotor effect; in the tracheobronchial tract, an inflammatory reaction of the respiratory mucosa is associated with contraction of the smooth muscle fibres of the trachea and bronchi.

The innervation of the entire respiratory tract is principally sympathetic and parasympathetic. However, the fact that smooth muscle fibres are absent in the nose and that, on the contrary, they have a predominant activity at the tracheobronchial level, explains why parasympathetic stimulation induces vasodilatation in the upper airways and bronchoconstriction in the lower airways and that essentially α-adrenergic sympathetic stimulation in the nose

induces vasoconstriction, while essentially β-adrenergic stimulation in the bronchi induces bronchodilatation.

These data demonstrate that although the functions of the upper and lower airways are very similar, they are sometimes exerted by different mechanisms. The essential difference resides in the absence of smooth muscle fibres in the nasal fossae. This implies that, in the nasopharynx, fenspiride exerts a purely anti-inflammatory effect while, in the trachea and bronchi, it exerts a combination of anti-inflammatory and anti-bronchoconstrictor effects. This last aspect is the subject of the present study, which attempts to define the mechanism of action of fenspiride on tracheobronchial musculature by in vitro studies on guinea-pig trachea and on fragments of human bronchus.

SPASMOLYTIC ACTIVITY OF FENSPIRIDE

Isolated guinea-pig trachea

Tracheas were removed from urethane-anaesthetised guinea pigs and were placed in a chamber containing Krebs' solution. Contractions were induced by different spasmogenic agents and were measured by means of a strain gauge connected to an amplifier and to a recorder.

The following constricting agents were tested: Acetylcholine (ACh): 2.10^{-5} M, Histamine (Hist): 2.10^{-5} M, Serotonin (5-HT): 2.10^{-5} M, Potassium chloride (KCl): 3.10^{-2} M, Tetraethylammonium (TEA): 10^{-2} M, Calcium (Ca^{++}): 0.01 to 3 mM, Substance P(SP): 3.10^{-7} M, Neurokinin A (NKA): 3.10^{-8} M, Leukotriene D_4 (LTD_4): 10^{-8} M.

The activity of fenspiride was compared to that of theophylline. In certain cases, it was also compared to that of verapamil, isoprenaline and mepyramine. Different doses of these agents were studied either on induction of constriction (preventative effect) or on the previously constricted trachea (curative effect).

The results obtained are presented in Tables 1 and 2. This comparative study demonstrated that, like theophylline or isoprenaline, fenspiride exerts an essentially equivalent action against the various constricting agents. Its action contrasts with that of specific antagonists like mepyramine in relation to histamine or verapamil in relation to depolarising agents (KCl, TEA) and calcium. Fenspiride therefore exerts a spasmolytic action on tracheal smooth muscle opposing that of various constricting agents. Qualitatively and quantitatively, its action resembles that of theophylline.

Human bronchus

Fragments of human bronchus obtained from lobectomy specimens were submitted to the same protocol as the guinea-pig trachea preparations.

In this case, the constricting agents used were: Histamine (Hist): 10^{-5} M,

Table 1. – log EC_{50} of fenspiride and various reference products with respect to constricting agents (mean ± s.e.m.) – n.d. = not determined. (C. Advenier et al., 1984).

Substance studied	Constricting agent: ACh 2.10^{-5}M	Hist 2.10^{-5}M	5-HT 2.10^{-5}M	KCl 3.10^{-2}M	TEA 10^{-2}M	Ca^{++}
Fenspiride	3.39 ± 0.07	5.32 ± 0.24	3.56 ± 0.07	3.26 ± 0.06	3.27 ± 0.02	3.70
Theophylline	3.51 ± 0.06	4.08 ± 0.10	3.69 ± 0.15	2.94 ± 0.08	2.89 ± 0.09	3.61
Isoprenaline	6.81 ± 0.11	7.66 ± 0.11	7.31 ± 0.08	7.02 ± 0.08	n.d	7.59
Verapamil	4.17 ± 0.11	4.73 ± 0.16	5.74 ± 0.23	6.05 ± 0.06	6.34 ± 0.07	6.55
Mepyramine	3.92 ± 0.16	8.87 ± 0.12	5.01 ± 0.21	5.34 ± 0.24	4.97 ± 0.05	5.05

Table 2. – log EC_{50} of fenspiride and theophylline with respect to substance P, neurokinin A and leukotriene D_4 (means ± s.e.m.).

Substance studied	Constricting agent SP 3.10^{-7}M	NKA 3.10^{-8}M	LTD_4 10^{-8}M
Fenspiride	3.58 ± 0.08	3.28 ± 0.10	3.64 ± 0.12
Theophylline	4.08 ± 0.10	3.63 ± 0.09	3.76 ± 0.14

Table 3. – log EC_{50} of fenspiride and theophylline with respect to histamine, carbachol, potassium chloride and leukotriene D_4 (means ± s.e.m.).

Substance studied	Constricting agent Hist 10^{-5}M	Carb 10^{-6}M	KCl 3.10^{-2}M	LTD_4 10^{-8}M
Fenspiride	4.49 ± 0.14	3.76 ± 0.10	2.91 ± 0.16	3.15 ± 0.18
Theophylline	4.09 ± 0.16	3.79 ± 0.08	3.83 ± 0.12	4.10 ± 0.17

Carbachol (Carb): 10^{-6} M, Potassium chloride (KCl): 3.10^{-2} M Leukotriene D_4 (LTD_4): 10^{-8} M, and the activity of fenspiride was compared to that of theophylline.

The results are presented in Table 3 and are illustrated by Figure 1. Once again, they show that fenspiride has a similar activity to that of theophylline and that it is not specific for a particular constricting agent.

MECHANISM OF ACTION

Absence of β-stimulant effect

B-mimetic agents such as isoprenaline constitute some of the most potent bronchodilators. It was therefore logical to start by determining whether fenspiride belonged to this class of compounds.

It has been clearly demonstrated that fenspiride is not a β-stimulant agent; its bronchodilator effects are not inhibited by propranolol either in vitro or in vivo in the guinea pig and the cat. Binding studies have also demonstrated that fenspiride does not bind to β-adrenergic receptors in rat lungs (Evrard et al., 1986).

Stimulation of cyclic AMP

Like the xanthines, fenspiride inhibits phosphodiesterase, the enzyme which catabolises cyclic AMP. On rat lung homogenate (Vigano et al., 1978) fenspi-

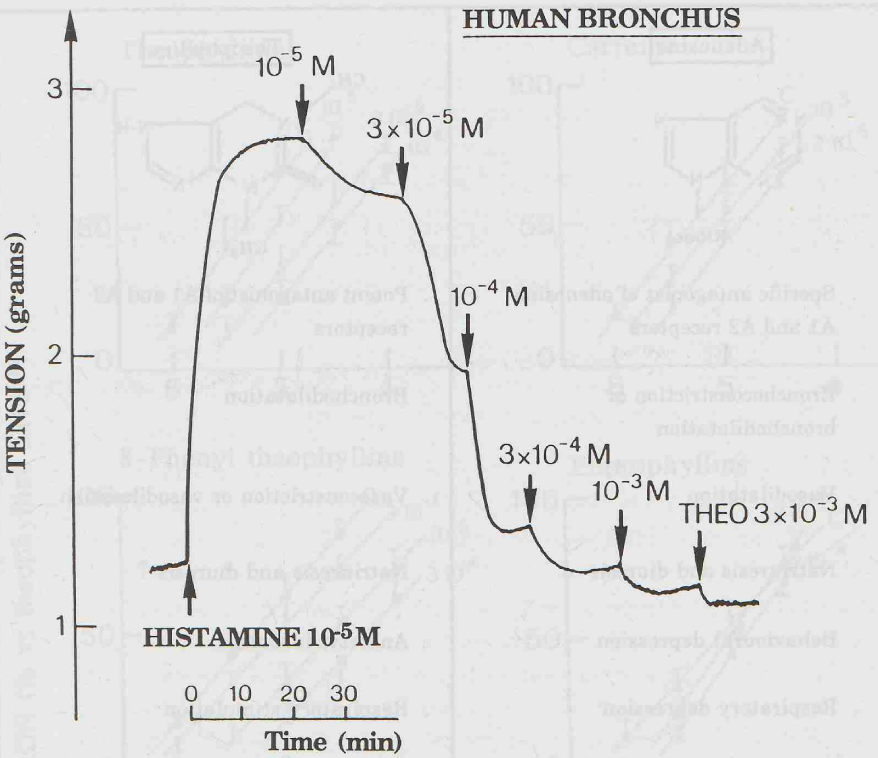


Figure 1. Relaxation by fenspiride increasing concentrations (10^{-5} M to 10^{-3} M) of a human bronchus strip previously contracted by histamine. Theophylline is added at the end of the experimentation to determine 100% of relaxation.

ride significantly increased the cyclic AMP level as a result of inhibition of phosphodiesterase. A similar effect was observed with fenspiride, aminophylline and papaverine. As cyclic AMP is involved in numerous cellular processes, this effect of fenspiride may be involved in its antibronchoconstrictor and anti-inflammatory activities.

FENSPIRIDE–ADENOSINE INTERACTION

In addition to the cholinergic and adrenergic innervation mentioned above, "non-adrenergic, non-cholinergic" nerves have also been demonstrated but the corresponding neurotransmitter has not yet been identified. Several substances have been proposed, including: VIP (vasoactive intestinal peptide), substance P, other neuropeptides or adenosine ... Adenosine occupies a particular place amongst these substances, as it has been suggested that the bronchodilator effects of xanthines are due to their antagonistic effect towards adenosine. In reality, although theophylline is a potent antagonist of adenosine

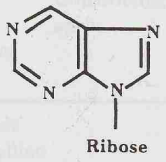
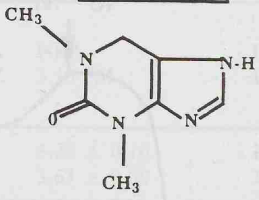
Adenosine	Theophylline
	
. Specific antagonist of adenosine A1 and A2 receptors	. Potent antagonist of A1 and A2 receptors
. Bronchoconstriction or bronchodilatation	. Bronchodilatation
. Vasodilatation	. Vasoconstriction or vasodilatation
. Natriuresis and diuresis ↓	. Natriuresis and diuresis ↑
. Behavioural depression	. Anxiety, insomnia
. Respiratory depression	. Respiratory stimulation
. Anticonvulsant effect	. Convulsion
. Gastric hyposecretion	. Gastric hypersecretion

Figure 2. Comparative effects of adenosine and theophylline (based on Persson, 1987).

A1 and A2 receptors, it is now generally accepted that this effect does not explain the effect of xanthines on the bronchi as, firstly, adenosine has a variable effect on tracheobronchial musculature and its contracturant effect is relatively minimal in comparison with its relaxant effect and, secondly, enprofylline, which is a xanthine with superior bronchodilator properties to those of theophylline, is not an adenosine receptor antagonist.

Although the anti-adenosine action of theophylline cannot explain its bronchodilator action, this antagonist may be responsible for other pharmacological properties of xanthines, as these substances have renal, behavioural, neurological and gastrointestinal effects strictly opposed to those of adenosine (Figure 2) and these effects are not observed with enprofylline.

It was therefore interesting to investigate whether the behaviour of fenspiride

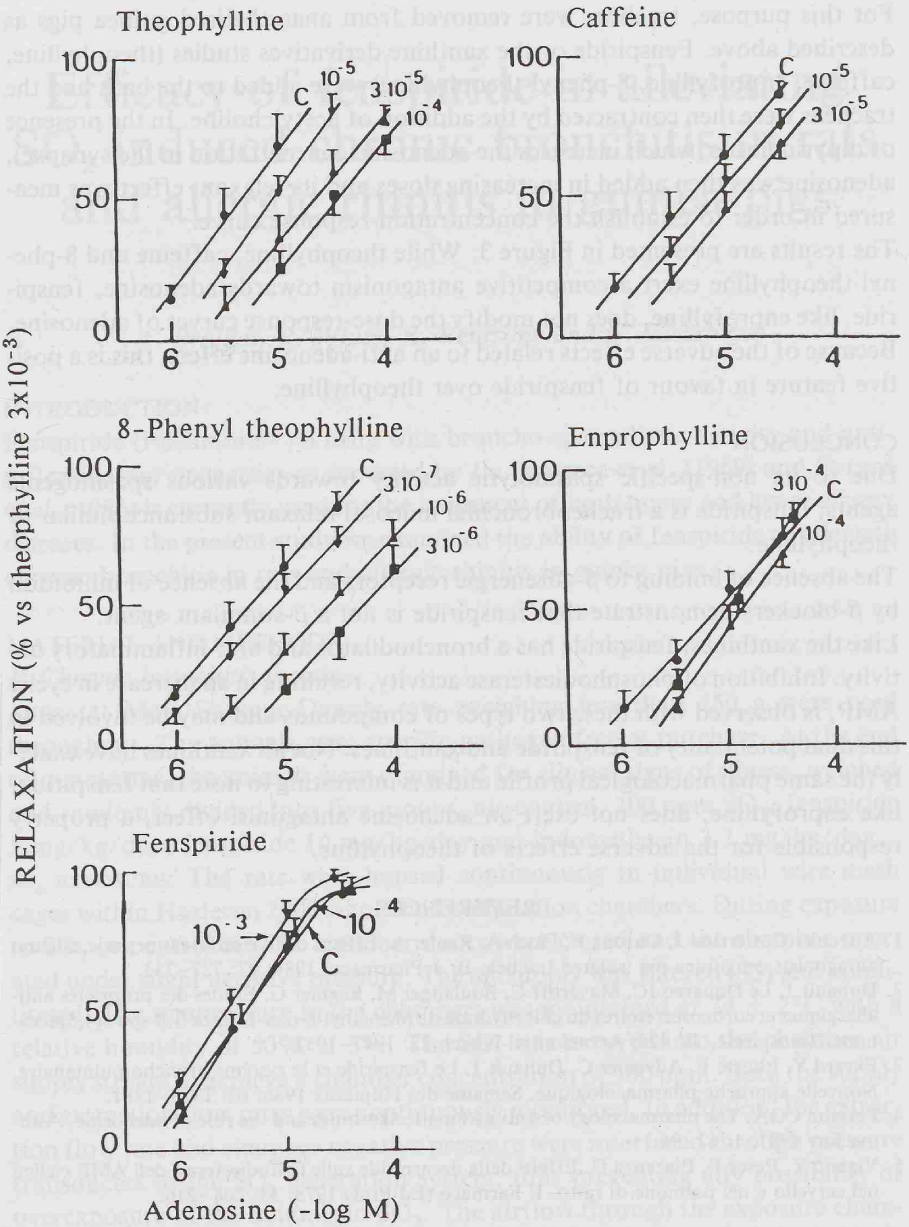


Figure 3. Concentration – relaxant action curves of adenosine on isolated guinea-pig trachea in the absence and in the presence of various substances.

towards adenosine resembled that of theophylline or enprofylline.
For this purpose, tracheas were removed from anaesthetised guinea pigs as described above. Fenspiride or the xanthine derivatives studies (theophylline, caffeine, enprofylline, 8-phenyl-theophylline) were added to the bath and the tracheas were then contracted by the addition of acetylcholine. In the presence of dipyridamole (which increases the adenosine concentration in the synapse), adenosine was then added in increasing doses and its relaxant effect was measured in order to establish the concentration-response curve.
The results are presented in Figure 3. While theophylline, caffeine and 8-phenyl-theophylline exert a competitive antagonism towards adenosine, fenspiride, like enprofylline, does not modify the dose-response curves of adenosine. Because of the adverse effects related to an anti-adenosine effect, this is a positive feature in favour of fenspiride over theophylline.

CONCLUSION

Due to its non-specific spasmolytic activity towards various spasmogenic agents, fenspiride is a tracheobronchial mucosal relaxant substance similar to theophylline.
The absence of binding to β-adrenergic receptors and the absence of inhibition by β-blockers demonstrate that fenspiride is not a β-stimulant agent.
Like the xanthines, fenspiride has a bronchodilator and anti-inflammatory activity. Inhibition of phosphodiesterase activity, resulting in an increase in cyclic AMP, is observed with these two types of compounds and may be involved in this dual potentiality of fenspiride and xanthines. Not all xanthines have exactly the same pharmacological profile and it is interesting to note that fenspiride, like enprofylline, does not exert an adenosine antagonist effect, a property responsible for the adverse effects of theophylline.

REFERENCES

1. Advenier C, Cerrina J, Duroux P, Floch A, Renier A. Effects of five different organic calcium antagonists on guinea-pig isolated trachea. Br J Pharmacol 1984; 82: 727–733.
2. Duhault J, Le Douarec JC, Mayeroff C, Boulanger M, Regnier G. Études des propriétés anti-allergiques et corticosécrétoires du chlorhydrate de phénéthyl-8-oxa-1-diaza-3,8-spiro(4,5)décanone-2(fenspiride, JP 428) Arzneimittel-Forsch 22: 1947–1952.
3. Evrard Y, Lhoste F, Advenier C, Duhault J. Le fenspiride et le système broncho-pulmonaire. Nouvelle approche pharmacologique. Semaine des Hôpitaux 1986; 62: 1375–1381.
4. Persson CGA. The pharmacology of anti-asthmatic xanthines and the role of adenosine. Asthma Rev 1987, 1: 62–94.
5. Vigano Y, Pesce E, Piacenza G. Effetti della decaspiride sulle fosfodiesterasi dell AMP ciclico nel cervello e nel polmone di ratto. Il Farmaco (Ed Prat) 1978; 33: 208–216.

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Efficacy of fenspiride in alleviating SO_2 induced chronic bronchitis in rats and allergic rhinitis in guinea pigs

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INTRODUCTION

Fenspiride (Pneumorel®) a drug with broncho-spasmolytic activity and anti-inflammatory properties as reported by Le Douarec et al. (1969) and Evrard et al. (1986) is currently used for the treatment of both upper and lower airway diseases. In the present study we examined the ability of fenspiride to alleviate chronic bronchitis in rats and allergic rhinitis in guinea pigs.

MATERIAL AND METHODS

1. Chronic bronchitis in rats

ANIMALS: Male Sprague-Dawely rats, weighing less than 250 g were used throughout. The animals were specific pathogen-free at purchase. At the end of quarantine, the animals were examined for clinical signs of illness, weighed and randomly divided into five groups, air-control, 300 ppm SO_2, fenspiride 5 mg/kg/day, fenspiride 10 mg/kg/day and indomethacin 3.7 mg/kg/day.

SO_2 EXPOSURE: The rats were housed continuously in individual wire mesh cages within Hazleton 2000 whole body inhalation chambers. During exposure to SO_2 or control air, the chamber doors were closed and the chamber operated under slight negative pressure. The air supply was filtered (S3) and conditioned. The temperature in the chambers was maintained at 24 ± 2°C with a relative humidity of 50% ± 5%. The SO_2 was metered into the chamber air supply stream to achieve a chamber concentration of 300 ppm. Both the supply and extraction flow rates were continuously monitored. In addition, the extraction flow rate and chamber negative pressure were interfaced through pressure transducers to the SO_2 generation system, thus preventing any possibility of overexposure of the animals to SO_2. The airflow through the exposure chambers was regulated to 25 m^3/h which corresponded to approximately 15 air changes per hour.

The animals (except the air controls) were exposed by whole body exposure to an atmosphere containing 300 ppm SO_2 for three hours a day, five days a week for four weeks.

SO_2 MONITORING AND ANALYSIS: The SO_2 exposure concentration was achieved by metering a known flow of SO_2 gas through a flow indicator (rotameter) into the air supply tube at a point several meters upstream from the chamber entry. The SO_2 was completely mixed with the air before entering the chamber. The SO_2 concentration was measured in the inhalation chamber by means of a mobile sampling probe.

DRUG TREATMENT: Fenspiride and indomethacin were dissolved in normal saline and administered once daily (seven days a week) by gavage. On those days corresponding to SO_2 inhalation, the drugs were administered just prior to the SO_2 exposure. Fenspiride was provided by Servier while indomethacin (Indocid® forte – 25 mg/kg of Indocid forte = 3.7 mg/kg indomethacin) was provided by Merck, Sharp and Dohme.

HISTOPATHOLOGY: At the end of exposure, the animals were anaesthetized with Vetanarcol® and sacrified by exsanguination. The thoracic cavity was opened, the lungs and trachea removed. The excised lungs were inflated with fixative. After fixation, the trachea was removed from lung and embedded in parafin. The trachea was sectioned longitudinally and examined for both goblet cell density and epithelial thickening. Epithelial thickening was evaluated in the upper third, middle third and lower third of the trachea, using a calibrated eye piece in the microscope.

The left lung was embedded and then sectioned along the axial plane to include the bifurcation of the main airways. After staining with AB-PAS, the peripheralization and hyperplasia of goblet cells were scored on both the trachea and lung sections. Goblet cell scores were obtained using an image analyser and a projection microscope.

TRACHEAL SECRETIONS: The day following the last SO_2 exposure, the animals were injected i.p. with 1 mCi/kg ^{35}S. After four hours the animals were anaesthetized with urethane and continuous tracheal lavages performed following the method described by Marriott et al. (1982). The radioactive perfusates were dialysed to remove all non-incorporated radioactivity, and concentrated with Aquacide® III. The concentrated fluids were evaluated for total radioactivity and total protein content.

PULMONARY HEXOSE AND FUCOSE: The day following the last SO_2 exposure, the animals were sacrified by exsanguination under anaesthesia. Total lung (and trachea) lavages were performed using physiological saline. The lavage fluids were clarified by centrifugation. The supernatant were lyophilized and reconstituted to 10% of the original volume. Total hexose and fucose were determined on each sample by the method of Winzler (1959) and Gibbons (1955) respectively.

B. Allergic rhinitis in guinea pigs

ANIMALS: Female Pirbright white guinea pigs, weighing approximately 250 g were used throughout. At the end of quarantine, the animals were examined for clinical sign of illness, weighed and randomly divided into four groups, positive controls, fenspiride 5% w/v, fenspiride 20% w/v and mepyramine 1.5% w/v.

ACTIVE SENSITIZATION: Sensitization was accomplished actively via vaccination according to Andersson (1981). The animals received on day 0 an intraperitoneal injection of saline (0.5 ml) containing 1 μg of ovalbumin (OA), grade V, Sigma) together with 100 mg Al $(OH)_3$ (Merck) as an adjuvant. Booster injections (1 μg OA + 100 mg Al $(OH)_3)_3$ were made intraperitoneally on days 7 and 14.

MEASUREMENT OF NASAL RESISTANCES: 4–5 weeks after the first vaccination, the female guinea pigs were anaesthetized with urethane solution and two polyethylene canulas inserted into the trachea. One was inserted caudal to permit spontaneous ventilation, the other cephalad through which air supplied from a compressed air tank was directed retrograde through the nasal cavities and nose. The oesophagus was ligated and the mouth sealed by filling oral cavity with pliable modeling plastic material. The pressure in the nasal cavities was visualized by a water manometer and recorded (X.Y Rhode Schwartz) via a pressure transducer (H.P. 267 B) and electronic amplifier (H.P. 8805 B).

INTRANASAL CHALLENGE AND DRUG TREATMENT: The guinea pigs were challenged topically with 1 ml ovalbumin solution (1%, w/v, water) which was allowed to remain in contact with the nasal mucosa for two minutes. Ten minutes before the antigen challenge, the guinea pigs were treated by a two minutes contact time intranasal application of either fenspiride solution (5% and 20%, w/v, water) or mepyramine solution (1.5% w/v, water). Positive controls received intranasal application of water only. Fenspiride was provided by Servier while mepyramine maleate was purchased from May Baxter Limited.

EFFECT OF DRUG TREATMENT ON NASAL RESISTANCE: The pressure in the nasal cavities was recorded for a twenty minutes period before and after the ovalbumin challenge. Areas under the pressure (cm H_2O)/time (min.) curves which correspond to nasal resistances, were estimated using a MOP image analyser (Kontron). Changes in nasal resistance were expressed as the percentage of the prechallenged control values.

C. Statistics

The data generated by this study were evaluated using the unpaired double tailed-t-Test (level of signifiance compared to the drug free control solvent group).

Table 1. Effect of oral daily treatment of fenspiride and indomethacin on respiratory tract goblet cell hyperplasia induced by subacute SO_2 intoxication in rats.

Units: Goblet cells number/mm groups	localization		
	trachea	proxi. airways	distal airways
Air-solvent control	10.9 ± 3.3***	2.9 ± 2.7***	0.6 ± 1.8
SO_2-solvent control	44.0 ± 20.8	12.1 ± 5.3	0.9 ± 1.4
SO_2 – 5 mg/kg Fenspiride	29.0 ± 10.5	6.6 ± 3.0*	0.0 ± 0.0
SO_2 – 10 mg/kg Fenspiride	37.0 ± 26.0	8.3 ± 4.5	0.0 ± 0.0
SO_2 – 3.7 mg/kg Indomethacin	22.2 ± 13.3*	8.9 ± 4.6	1.7 ± 2.0

(*) Level of signifiance compared to the SO_2-solvent-control. Unpaired t Test.

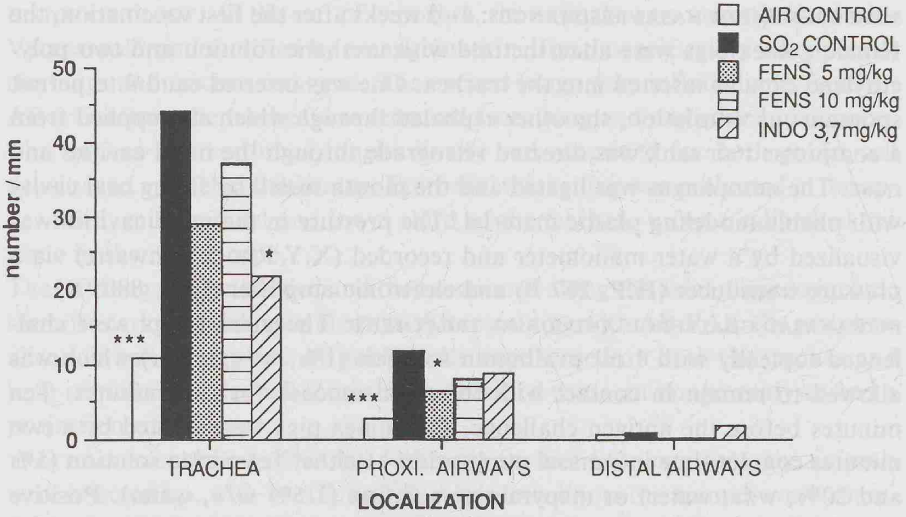

Figure 1. Effect of oral daily treatment of fenspiride and indomethacin on respiratory tract goblet cell hyperplasia induced by subacute SO_2 intoxication in rats.
* = $0.01 < 2P < 0.05$; ** = $0.001 < 2P < 0.01$; *** = $2P < 0.001$.

RESULTS

A. Chronic bronchitis in rats

GOBLET CELL DENSITY: The SO_2 exposure produce a generalized increase in the density and distribution of the goblet cells in the conducting airways. The protection due to the daily treatment with fenspiride was evident at all levels of respiratory tract and statistically significant for 5 mg/kg in the proximal airways. Indomethacin was generally less active than fenspiride as controlling goblet cell hyperplasia (Table 1, Figure 1).

EPITHELIAL HYPERTROPHY: Chronic inhalation of an irritant gas such as SO_2

Table 2. Effect of oral daily treatment of fenspiride and indomethacin on tracheal epithelial thickening induced by subacute SO_2 exposure in rats.

Units: Epithelial thickening (μm) groups	trachea localization		
	high	middle	low
Air-solvent control	22.1 ± 5.7	21.6 ± 4.6	20.9 ± 4.3
SO_2-solvent control	28.6 ± 9.5	25.3 ± 7.8	24.0 ± 6.4
SO_2 – 5 mg/kg fenspiride	29.3 ± 5.3	27.3 ± 5.8	27.5 ± 6.1
SO_2 – 10 mg/kg fenspiride	30.8 ± 7.0	25.9 ± 7.2	25.9 ± 6.4
SO_2 – 3.7 mg/kg indomethacin	20.1 ± 5.2*	18.9 ± 3.5*	20.1 ± 4.9

(*) Level of signifiance compared to the SO_2-solvent-control. Unpaired t Test.
* = $0.01 < 2P < 0.05$; ** = $0.001 < 2P < 0.01$; *** = $2P < 0.001$.

induced epithelial hypertrophy along the trachea. Daily treatment with indomethacin partially protected the trachea of the exposed animals from epithelial hypertrophy while fenspiride was ineffective (Table 2).

TRACHEAL SECRETION: Tracheo-bronchial secretions are rich in proteins, carbohydrate and mucus polysaccharides. The amount and composition of these secretions change as a result of chronic airways irritation. Animals exposed to SO_2 gas had substantially more 35S metabolically labelled tracheal secretion than did the air controls. SO_2-10 mg/kg and SO_2-5 mg/kg fenspiride significantly reduced the amount of metabolic labelling compared to the SO_2 alone. Indomethacin also reduced metabolic labelling but was less effective than fenspiride (Table 3, Figure 2).

PULMONARY HEXOSE AND FUCOSE: The pulmonary lavage fluids of animals subacutely exposed to SO_2 gas showed an increase in total hexose and total fucose compared to the air controls. Both fenspiride-SO_2 groups showed a reduction in pulmonary hexose, an effect statistically significant at the high dose. Although less active, similar results were observed with indomethacin (Table 4).

B. Allergic rhinitis in guinea pigs

Guinea pigs actively sensitized by low dose of antigen together with $Al(OH)_3$ developed an immediate hypersensitivity reaction manifested by a consistently marked increase in nasal resistance (17.9 ± 8.5%) when they are challenged 4–5 weeks later by an intranasal application of ovalbumin. Topical application of fenspiride solutions (5% and 20%) and mepyramine solution (1.5%) both significantly reduced the increase in nasal resistance induced by ovalbumin challenge in sensitized guinea pigs. Fenspiride was less active than the antihistaminic drug mepyramine tested in the same conditions (Table 5, Figure 3).

Table 3. Effect of oral daily treatment of fenspiride and indomethacin on the increase of 35S metabolically labelled tracheal secretions induced by subacute SO_2 exposure in rats.

groups	total protein (mg)	total radioactivity (DPM)
Air-solvent control	0.41 ± 0.13*	1694 ± 562
SO_2-solvent control	0.63 ± 0.16	2432 ± 786
SO_2 – 5 mg/kg fenspiride	0.57 ± 0.19	1462 ± 461*
SO_2 – 10 mg/kg fenspiride	0.40 ± 0.22	1309 ± 376*
SO_2 – 3.7 mg/kg indomethacin	0.55 ± 0.22	2061 ± 788

(*) Level of signifiance compared to the SO_2-solvent-control. Unpaired t Test.

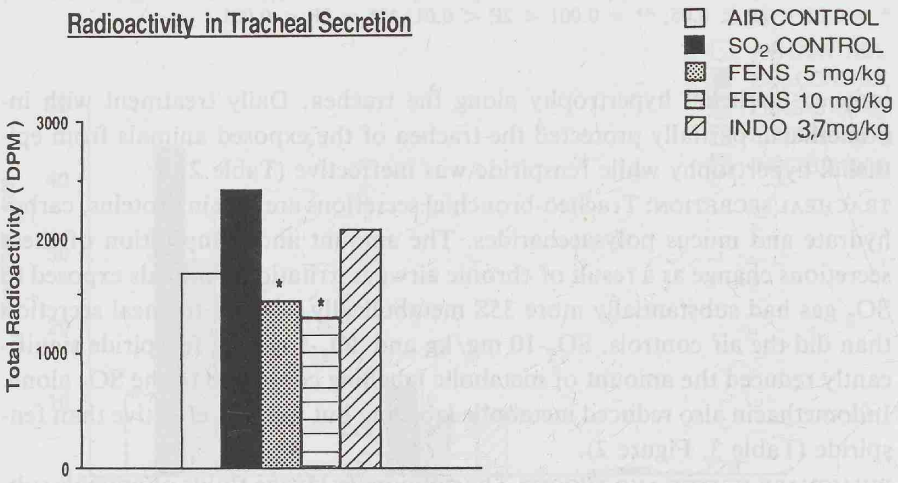

Figure 2. Effect of oral daily treatment of fenspiride and indomethacin on the increase of 35S metabolically labelled tracheal secretions induced by subacute SO_2 exposure in rats.
* = 0.01 < 2P < 0.05; ** = 0.001 < 2P < 0.01; *** = 2P < 0.001.

Table 4. Effect of oral daily treatment of fenspiride and indomethacin on pulmonary hexose, fucose and protein changes induced by subacute SO_2 exposure in rats.

groups	hexose (µg)	fucose (µg)	protein (mg)
Air-solvent control	572 ± 138	84 ± 9***	1.61 ± 0.36
SO_2-solvent control	738 ± 211	147 ± 38	2.24 ± 0.59
SO_2 – 5 mg/kg fenspiride	697 ± 81	140 ± 57	2.08 ± 0.53
SO_2 – 10 mg/kg fenspiride	513 ± 68*	136 ± 41	2.39 ± 0.51
SO_2 – 3.7 mg/kg indomethacin	503 ± 130*	113 ± 12	2.28 ± 0.41

(*) Level of signifiance compared to the SO_2-solvent-control. Unpaired t test.
* = 0.01 < 2P < 0.05; ** = 0.001 < 2P < 0.01; *** = 2P < 0.001.

Table 5. Effect of intranasal two minutes contact treatment of fenspiride and mepyramine on nasal resistance increase induced by intranasal application of ovalbumin in allergic guinea pigs.

groups	percentage of variation in nasal resistance	percentage of protection
Positive controls	17.93 ± 8.51 (10)	–
Mepyramine (1.5%)	1.33 ± 8.51 (6)**	92.60%
Fenspiride (5.0%)	6.79 ± 6.24 (6)*	62.11%
Fenspiride (20.0%)	3.29 ± 7.26 (8)**	81.70%

Mean ± S.D. ( ): n. of animals
Percentage of protection in comparaison to positive controls.
(*) Level of significance compared to the positive controls. Unpaired t test.

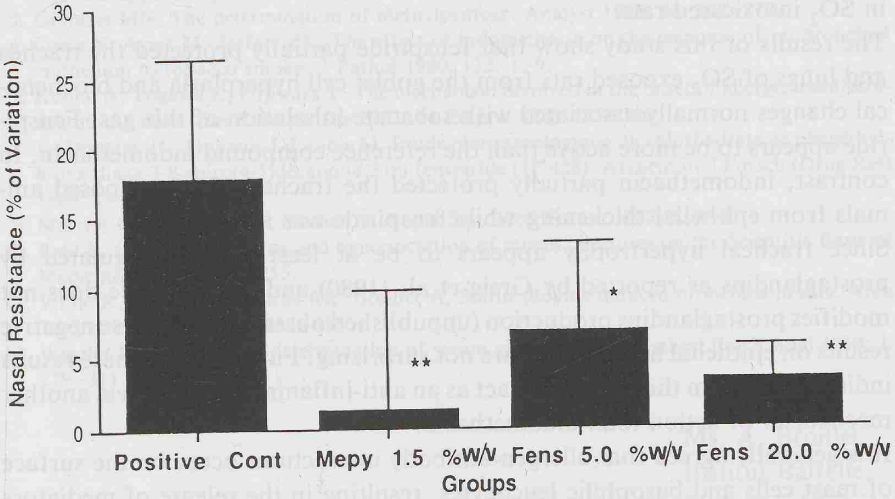

Figure 3. Effect of intranasal two minutes contact treatment of fenspiride and mepyramine on nasal resistance increase induced by intranasal application of ovalbumin in allergic guinea pigs. * = $0.01 < 2P < 0.05$; ** = $0.001 < 2P < 0.01$; *** = $2P < 0.001$.

DISCUSSION

Fenspiride (Pneumorel®) has been described to possess anti-inflammatory and broncho-spasmolytic activities. In the current study, we examined the ability of fenspiride to alleviate SO_2 induced chronic bronchitis in rats and ovalbumin induced allergic rhinitis in actively sensitized guinea pigs.

Chronic bronchitis is an inflammation of the large conducting airways due to persistent irritation which is characterized by a moderate cellular infiltration and a hypersecretion of mucus. It is this overproduction of mucus which is primarily responsible for the symptomatology, associated with the disease as

reported by Reid (1959). Mucus hypersecretion results from the inhalation of irritants such as NH_3 vapour, SO_2 gas, ozone, H_2SO_4 and cigarette smoke as described by Coles et al. (1979). In addition, the mucus secreting surface (in this case the respiratory epithelium) and the tracheal/bronchial submucosal glands seem able to modulate (increase) the amount of mucus produced by increasing both the number of goblet cells and the size of the submucosal glands.
In the present study, chronic bronchitis was induced in rats by exposing them to 300 ppm SO_2 for three hours a day; five days a week for four weeks. This dose level and exposure regimen produced primarily large airways disease as reported by White et al. (1986). The pathological changes induced by SO_2, observed in this study, were limited to the mucus producing cells and tissues.
In the examination of the efficacy of fenspiride, we investigated the effect of this compound on the mucus production and on bronchial-tracheal secretion in SO_2 intoxicated rats.
The results of this study show that fenspiride partially protected the trachea and lungs of SO_2 exposed rats from the goblet cell hyperplasia and biochemical changes normally associated with subacute inhalation of this gas. Fenspiride appears to be more active than the reference compound indomethacin. In contrast, indomethacin partially protected the trachea of SO_2-exposed animals from epithelial thickening while fenspiride was ineffective.
Since tracheal hypertrophy appears to be at least in part regulated by prostaglandins as reported by Greig et al. (1980) and as fenspiride does not modifies prostaglandins production (unpublished observations), these negative results on epithelial hypertrophy are not surprising. Furthermore, these results indirectly confirm that fenspiride act as an anti-inflammatory drug via another mechanism of action than indomethacin.
It is generally agreed that allergen-antibody interactions occur on the surface of mast cells and basophilic leucocytes, resulting in the release of mediators (such as histamine) which are responsible for the symptoms of allergic rhinitis in type 1 allergy as described by Eccles et Mygind (1985). Konno et al. (1983) reported that the nasal symptoms associated with mediators release are due to both local and reflex actions, as the mediators may act directly on glands and blood vessels, and also trigger reflex changes in autonomic activity by stimulation of sensory nerves ending.
Female guinea pigs actively sensitized by low dose of antigen together with $Al(OH)_3$, developed an allergic rhinitis characterized by a marked nasal resistance increase when they are challenged by intranasal application of ovalbumin 4–5 weeks after the first vaccination.
Fenspiride and mepyramine maleate both prevented significantly the allergic rhinitis in the sensitized guinea pigs when applied topically in the nasal cavities 10 minutes prior to the antigen challenge.

The protective effect of mepyramine is related to its specific anti-H1 receptor antagonist activity while most probably fenspiride may interfere on the allergic response via another mechanism of action.
In conclusion, the results of this study support fenspiride effectiveness in the treatment of both upper and lower obstructive respiratory diseases.

REFERENCES

1. Andersson P. Antigen – induced bronchial anaphylaxis in actively sensitized guinea pigs. The effects of booster injection and cyclophosphamide treatment. Int Archs Allergy Appl Immunol 1981; 64: 249–258.
2. Coles SJ, Havine LR, Reid L. Hypersecretion of mucus glycoproteins in rats airways induced by tobacco smoke. Am J Pathol 1979; 94: 459–471.
3. Eccles R, Mygind N. Clin Rev Allergy 1985; 3: 501–516.
4. Evrard Y, Lhoste F, Advenier C, Duhault J. Le fenspiride et le système broncho-pulmonaire: nouvelle approche pharmacologique. La Semaine des Hôpitaux 1986; 62(19): 1375–1381.
5. Gibbons MN. The determination of methylpentose. Analyst 1955, 80: 268–277.
6. Greig N, Ayers M, Jeffery PK. The effect of indomethacin on the response of rat bronchial epithelium to tobacco smoke. J Pathol 1980, 132: 1–9.
7. Konno A, Togawa K, Fujiwara T. The mechanism involved in the onset of allergic manifestation in the nose. Eur J Respir Dis 1983; 64 (Suppl. 128): 155–156.
8. Le Douarec JC, Duhault J, Laubie M. Étude pharmacologique du chlorhydrate de phénéthyl-8-oxa-diaza-3-8-spiro(4-5)decanone-2 ou fenspiride (JP 428). Arzneimittel Forsch (Drug Res) 1969; 19: 1263–1271.
9. Marriot C, Readman AS, Turner N. Royal Soc. Med Forum 1982; 5: 4–8.
10. Reid L. Chronic bronchitis and hypersecretion of mucus. Lectures on the Scientific Basis of Medicine 1959; 8: 235–255.
11. White R, Zoppi A-L, Haroz RK, Broillet A. Sulfur dioxide induced bronchitis in rats. Arch Toxicol 1986; Suppl. 9: 431–435.
12. Winzler RJ. Methods of determination of serum glycoproteins. Method Bioch Anal 1959; 2: 279–311.

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Mediators of inflammation and antagonism of experimental pleurisy in the rat by fenspiride

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INTRODUCTION

Pneumorel® (fenspiride) exerts an anti-inflammatory activity, especially in the upper and lower respiratory tracts (Semaine des Hôpitaux, 62. 1375–1381, 1986). We conducted a study to elucidate its mechanism of action and to determine whether it antagonises the effects of PAF-acether, a new potential mediator of inflammation, or those of zymosan, a suspension of yeast membranes which induces experimental inflammation with the participation of complement and neutrophils. Our results demonstrate that fenspiride is not a specific antagonist of PAF-acether, but that it possesses a marked anti-inflammatory activity in zymosan-induced pleurisy, which suggests an action at the level of the interaction between neutrophils and inflamed vessels.

MATERIAL AND METHODS

In vivo study

1. Bronchoconstriction: Guinea pigs of both sexes (400–500 g) were anaesthetised by an intraperitoneal injection of 40 mg/kg of sodium pentobarbital. The trachea was cannulated and connected to a Palmer respiratory pump which ventilated the animal at a flow rate of 1 ml/100 g. The two carotid arteries were catheterised to allow recording of the blood pressure and collection of blood samples for determination of the fall in the circulating platelet and leukocyte counts. The intravenous injection of 2 mg of pancuronium allowed muscular relaxation of the animal. Bronchial reactivity was tested by three to five intravenous injections of serotonin (3 to 6 μg) at ten minute intervals. The guinea pigs were treated with propranolol (1 mg/kg I.V. then 3 mg/kg I.P.) in order to inhibit the bronchodilator effect induced by endogenous catecholamines following administration of bronchoconstrictor agents. PAF-acether was infused via the intravenous route at a dose of 3, 30 or 44 ng/kg over 60 minutes. One group of animals was pretreated by an infusion of fenspiride at the dose of

33 mg/kg or 100 mg/kg for 10 minutes, five minutes before the start of the PAF-acether infusion. The number of circulating cells was measured one minute before and 2, 10, 30 and 60 minutes after administration of PAF-acether. Fenspiride was also administered under identical conditions to counteract bronchoconstriction induced by a secretion-stimulating tripeptide, f-methionyl-phenylalanine (fMLP) at doses of 3 and 30 μg/kg and bronchoconstriction induced by histamine, serotonin and acetylcholine at respective doses of 10, 5 and 30 μg/kg at 60-minute intervals by I.V. bolus injection.

2. Experimental pleurisy in the rat: Pleurisy was induced by intra-pleural injection of PAF-acether (1 μg), serotonin (100 μg) or zymosan (1 mg) in a final volume of 0.1 ml to Wistar rats of both sexes (weighing about 200 g), without anaesthesia. The animals were sacrificed with either, 30 minutes, one hour and four hours respectively after this injection. The pleural cavity was opened and washed with 3 ml of normal saline containing 20 U/ml of heparin. The volumes of exudate were measured, the protein content was assessed by Biuret's technique and the inflammatory cells were counted under a microscope using Neubauer's chamber. Fenspiride was administered orally at doses of 5, 15, 30, 60 and 100 mg/kg one hour before zymosan and at a dose of 100 mg/kg one hour before serotonin and PAF-acether. In several experiments, the thromboxane B_2 content was also evaluated by radioimmunoassay (see below).

In vitro study

1. Sensitisation procedure: An antigen solution was prepared by placing 200 μl of a 1 mg/ml solution of ovalbumin in contact with 500 μl of 40 mg/ml aluminium hydroxide gel for one hour at room temperature. The volume of the mixture was then made up to 10 ml with 0.9% sodium chloride and 0.5 ml of this preparation was injected subcutaneously into guinea pigs of both sexes (300–500 g). This procedure was repeated 14 days later and the animals were used between the 7th and the 10th day after the last injection.

2. Perfused lungs: The sensitised guinea pigs were anaesthetised by sodium pentobarbitone (40 mg/kg). The treachea was cannulated and connected to a Palmer respiratory pump, which ventilated the animal at a frequency of 60 cycles/minute with a flow rate of 1 ml/100 g. The animal was exsanguinated via the carotid artery and, after midline thoracotomy, the pulmonary artery was cannulated and perfused with 50 ml of Krebs' solution containing 2.5 g/l of bovine serum albumin (BSA). The lungs were then rapidly transferred to a plastic chamber, where they were perfused at a flow rate of 8 to 10 ml/min with Krebs-BSA solution maintained at 37°C by a thermostat and oxygenated by a

mixture of 95% O_2 and 5% CO_2. After a 10-minute equilibration period, two injections of PAF-acether (1 and 100 ng), followed by a single dose of ovalbumin (1 μg), were administered into the pulmonary artery in a volume of 0.1 ml at 10-minute intervals. Fenspiride was then added to the Krebs-BSA solution at the concentration of 10^{-4} M and the lung was stimulated as described above.

3. Radioimmunoassay of thromboxane B_2 (TXB_2): One ml samples of pulmonary perfusates were left to stand for 60 minutes at room temperature to allow total transformation of TXA_2 into stable TXB_2. These samples were then stored at −20°C. In order to perform this assay, 100 μl aliquots of each perfusate were incubated overnight at 4°C with radioactive-iodine-labelled TXB_2 and with anti-TXB_2 serum in phosphate buffer (0.1 M, pH 7.4) containing bovine gamma globulins (0.3% w/v). On the following day, the free and bound fractions were separated by precipitation with polyethylene glycol 6000 (30%) and centrifuged at 4°C for 10 minutes at 4,000 r.p.m. The supernatant was discarded and the radioactivity present in the centrifugation pellet, corresponding to the free fraction, was counted for 1 minute with a Kontron counter.

4. Histamine assay: 0.8 N perchloric acid was added to 1 ml aliquots of perfusate, which were then centrifuged for 10 minutes at 4,000 r.p.m. Histamine was assayed in the supernatant by spectrofluorometry.

EXPRESSION OF THE RESULTS AND STATISTICAL ANALYSIS

In the perfused lungs, the basal value of TXB_2 released (1.74 ± 0.82 ng/ml) was not significantly affected by the presence of fenspiride at a concentration of 10^{-4} M (0.64 ± 0.25 ng/ml). We therefore subtracted the values for TXB_2 obtained before each stimulation from the values obtained after the various stimuli with PAF-acether or the antigen. We evaluated the mean values ± S.E.M. for the groups of control lungs and lungs treated with fenspiride. Statistical analysis of the results was performed by means of Student's t test.

MATERIALS

Fenspiride batch no. 29336; pancuronium (Pavulon™) and heparin (Organon); sodium pentobarbitone (Clin Midy); PAF-acether (hexadecyl derivative, Bachem, stored in a 0.9% NaCl solution containing 0.1% bovine albumin (Sigma)); bovine gamma globulins (fraction 2. Sigma); labelled TXB_2 (NEN); anti-TXB_2 antibody (Institut Pasteur); scintillation liquid ACS II (Amersham). Krebs' solution contains: NaCl: 118 mM, KCl: 4.7 mM, $CaCl_2$. H_2O: 2.5 mM, $MgSO_4$ $7H_2O$: 1.2 mM, $NaHCO_3$: 25 mM, glucose: 5.6 mM, bovine serum albumin: 2.5 g/l.

Table 1. Effect of fenspiride (33 mg/kg/10 min) on bronchoconstriction (BC), blood pressure (BP), thrombocytopenia and leukopenia due to PAF-acether (30 ng/kg/min) in guinea pigs pretreated with propranolol. The high dose of PAF-acether (30 ng/kg/min) very rapidly induced the death of the animals and consequently, the values corresponding to times 30 and 60 min were not determined (n = number of animals used).

		decreased platelet (%)	decreased leukocyte (%)	decreased BP (%)	BC (cm H_2O)
PAF-acether (n=5)	2 min	18 ± 2	30 ± 6	55 ± 5	25 ± 1
	10 mn	51 ± 6	56 ± 4	66 ± 4	
Fenspiride + PAF-acether (n=4)	2 mn	32 ± 12	30 ± 9	50 ± 9	23 ± 2
	10 mn	43 ± 11	46 ± 8	58 ± 15	

Table 2. Effect of fenspiride (33 mg/kg/10 min) on bronchoconstriction, leukopenia and thrombocytopenia induced by fMLP in the guinea pig in vivo.
Two doses of fMLP were administered to guinea pigs at intervals of 45 minutes in the form of an I.V. bolus (3 μg/kg in the first injection and 30 μg/kg in the second injection). (* $P < 0.05$).

		decreased platelet count		decreased leukocyte count		bronchoconstriction (cm H_2O)	
		1st inj.	2nd inj.	1st inj.	2nd inj.	1st inj.	2nd inj.
without fenspiride n = 3	0.5 mn	10 + 3	9 + 4	54 + 8	36 + 13	11 + 3	16 + 3
	1 mn	8 + 6	13 + 8	47 + 4	37 + 12		
	10 mn	6 + 1	–	38 + 2	–		
	30 mn	6 + 3	–	16 + 8	–		
with fenspiride (33 mg/kg/10min) n = 3	0.5 mn	9 + 7	16 + 8	53 + 11	57 + 15	10 + 2	20 + 2
	1 mn	9 + 6	14 + 7	64 + 4*	59 + 5		
	10 mn	7 + 6	–	39 + 5	–		
	30 mn	5 + 5	–	9 + 3	–		

RESULTS

1. In vivo bronchoconstriction

In a preliminary study, it was found that, at the limit of the doses tolerated by the guinea pigs, i.e. 100 mg/kg infused over ten minutes, fenspiride induced intense hypotension accompanied by thrombocytopenia and leukopenia (expressed as a % of the fall in blood pressure and fall in platelet and leukocyte

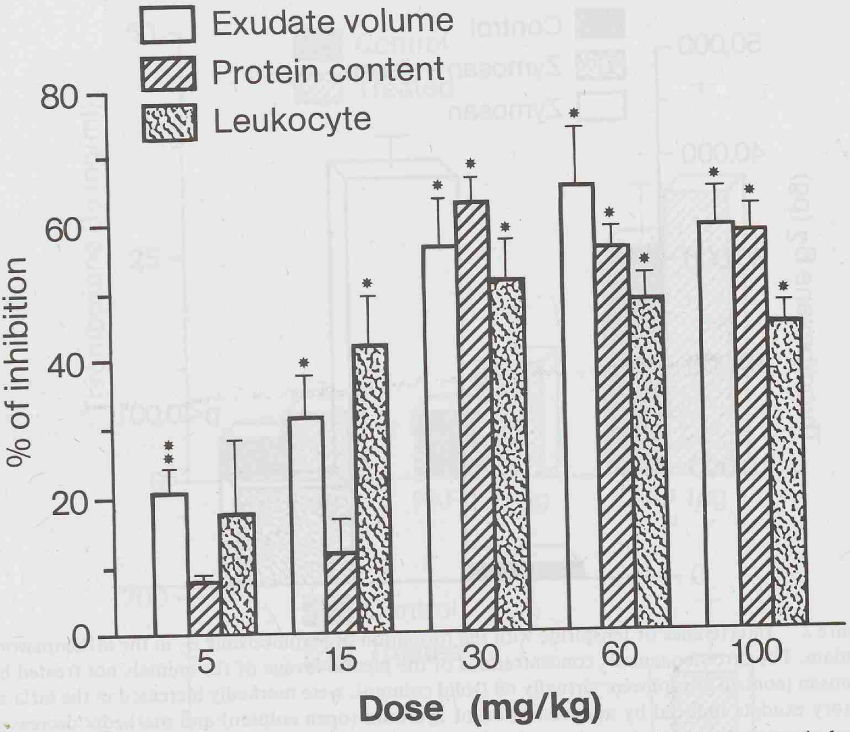


Figure 1. Interference of fenspiride, administered at the doses indicated, with zymosan-induced pleurisy. The columns express the percentage inhibition of the volume of exudate (open columns), their protein content (hatched columns) and the increase in the number of leukocytes present (dotted columns) by the doses of fenspiride indicated.

counts, respectively: 56 ± 7,23 ± 1 and 18 ± 2; n = 3). Under these conditions, the dose of fenspiride used in this study was 33 mg/kg. This dose suppressed bronchoconstriction induced by serotonin and by histamine (5–15 μg/kg of each), without modifying that due to acetylcholine.

When administered to the guinea pig at the dose of 33 mg/kg, five minutes before starting the PAF-acether infusion, fenspiride did not prevent PAF-acether-induced thrombocytopenia, leukopenia or bronchoconstriction (Table 1). Similarly, fenspiride did not inhibit the bronchoconstrictor and leukopenic effects induced by fMLP (Table 2).

2. Influence of fenspiride on experimental pleurisy in the rat

As shown in Figure 1, fenspiride, administered orally at doses of 5 to 60 mg/kg, induced dose-dependent inhibition of the three parameters of zymosan inflammation evaluated: the volume of inflammatory exudate, its protein content and the increase in the number of leukocytes present. The dose of 100 mg/kg was no more effective than that of 60 mg/kg. Figure 2 shows that fenspiride very

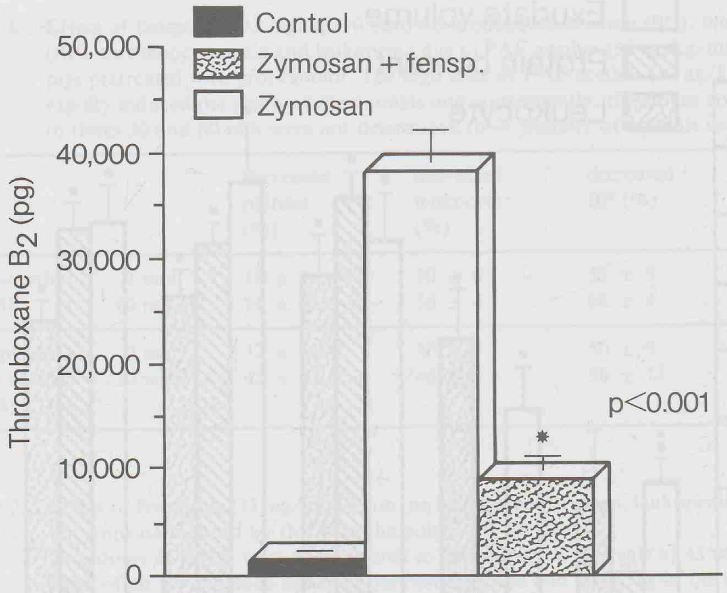


Figure 2. Interference of fenspiride with the formation of thromboxane B_2 in the inflammatory exudate. The thromboxane B_2 concentrations of the pleural lavage of the animals not treated by zymosan (control group) were virtually nil (solid column), were markedly increased in the inflammatory exudate induced by administration of zymosan (open column) and markedly decreased when zymosan was administered to animals protected by fenspiride, administered at the dose of 60 mg/kg (dotted column).

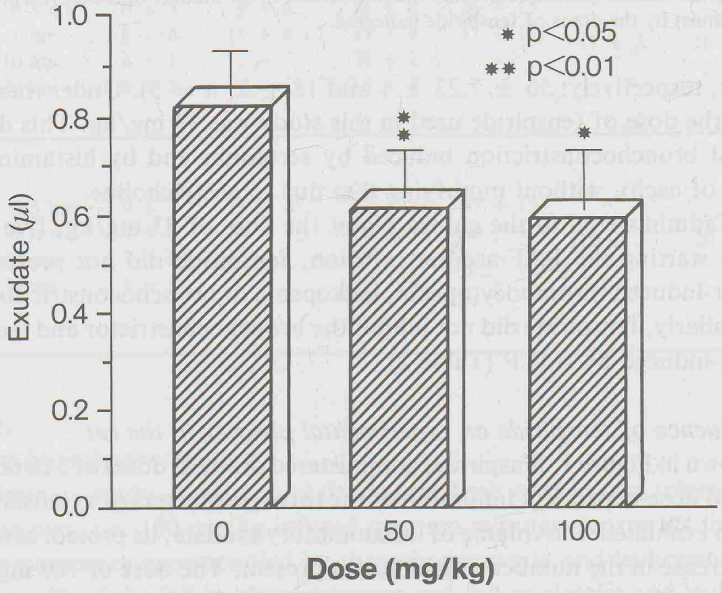
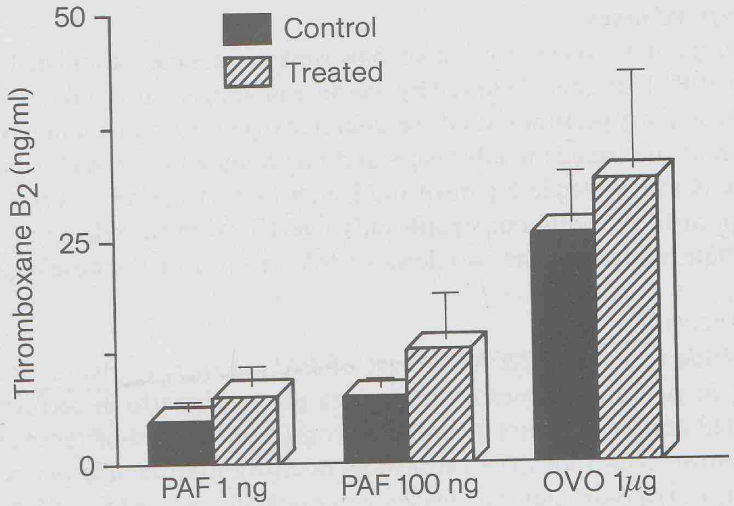


Figure 3. Partial interference of fenspiride with the inflammatory exudate induced by intrapleural administration of PAF-acether. The dose of fenspiride are indicated below each column.



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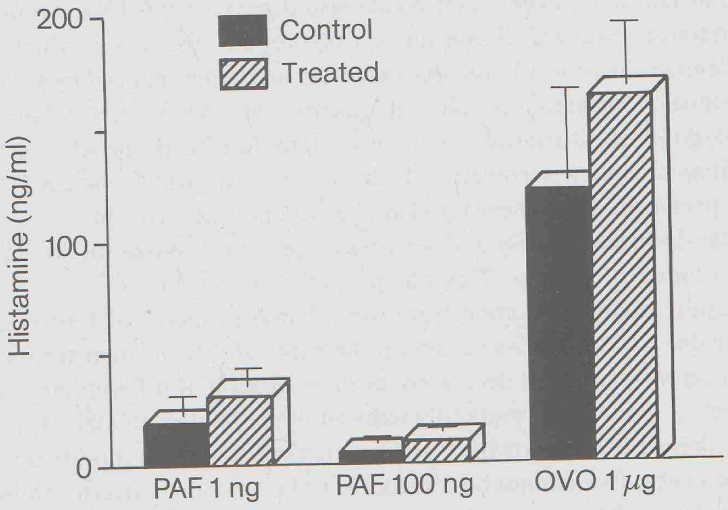

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Figures 4 and 5. Release of thromboxane B_2 (Figure 4) and histamine (Figure 5) induced by two successive injections of PAF-acether, followed by one injection of antigen (ovalbumin) to previously sensitised isolated guinea-pig lungs. The solid columns correspond to the control lungs and the open columns correspond to the lungs perfused with 10^{-4} M of fenspiride.

significantly reduced the TXB_2 content in the inflammatory exudate induced by intra-pleural administration of zymosan. Fenspiride, at a dose of 100 mg/kg, did not reduce serotonin-induced pleurisy (not reported), but partially decreased the volume of exudate formed during pleurisy induced by intra-pleural injection of PAF-acether (Figure 3).

3. Perfused lungs

The lungs of actively sensitised animals were successively stimulated with two doses of PAF-acether, followed by one dose of antigen, at 10-minute intervals. The pulmonary perfusion fluid was collected over the first six minutes after injection of the stimulant substances and one minute before each stimulation. The results presented in Figures 4 and 5 show that fenspiride, at a final concentration of 10^{-4} M, did not significantly interfere with the release of TXB_2 or histamine induced by the two doses of PAF-acether or the dose of antigen.

DISCUSSION

Fenspiride is not a direct antagonist of PAF-acether, as the effects of this agent, in vivo in the anaesthetised guinea pig and in vitro in perfused lungs, persisted despite the administration of considerable doses of fenspiride.

In contrast, fenspiride demonstrated an intense anti-inflammatory activity in the rat, as zymosan-induced pleurisy was markedly reduced for all three components evaluated: volume of exudate, protein content and number of infiltrated inflammatory cells. Fenspiride also decreased the TXB_2 content in the inflammatory exudate. Zymosan is a membrane suspension which activates complement; its pro-inflammatory effect is markedly reduced by PAF-acether antagonists. Repeated intra-pleural injections of PAF-acether induce so-called homologous desensitisation, i.e. in relation to the effects of PAF-acether itself, as well as crossed heterologous desensitisation towards zymosan (Martins et al., in press). PAF-acether may also play an important role in the induction of zymosan-induced pleurisy and we could also expect fenspiride to block PAF-acether-induced pleurisy. This was partially confirmed.

Our results therefore demonstrate the original property of fenspiride which may be due to a protective action on the permeability of the pleural cavity, as exudation was markedly decreased, or on migration of inflammatory cells, the numbers of which were markedly reduced in the treated animals. Inasmuch as the synthesis of thromboxane B_2 by isolated lungs was not modified by fenspiride, this substance cannot be considered to be a cyclooxygenase inhibitor, like aspirin, but rather another type of anti-inflammatory drug.

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