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Protective effect of loop diuretics, piretanide and frusemide, against sodium metabisulphite-induced bronchoconstriction in asthma

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ABSTRACT: We determined whether the loop diuretic, piretanide, had a similar inhibitory action against sodium metabisulphite (MBS)-induced bronchoconstriction in asthmatic subjects as frusemide and, if so, its duration of action.

In the first study, we compared the effect of inhaled placebo, piretanide (24 mg), or frusemide (40 mg), on the provocative concentration of MBS needed to cause a 20% fall in baseline forced expiratory volume in one second (FEV_1) (PC_{20}MBS) in 12 mild asthmatic subjects before, immediately after, and at 1.5, 3, 6, and 24 h, after inhalation. Both piretanide and frusemide induced a significant diuresis lasting at least 24 h. Frusemide caused a mean 3.8 fold (95% confidence interval: 2.3–6.3 fold), piretanide a 2.5 fold (1.8–3.4 fold) and placebo a 1.7 fold (1.5–1.9 fold) increase in PC_{20}MBS. The effects of frusemide and piretanide were significantly greater than that of placebo. At later time points, tachyphylaxis to the bronchoconstrictor effects of MBS was observed during the placebo limb. In the second study, we measured PC_{20}MBS at 90 min after inhalation of either placebo, piretanide (24 mg), or frusemide (40 mg). No significant difference in PC_{20}MBS was observed.

We conclude that piretanide in addition to frusemide significantly inhibits MBS-induced bronchoconstriction and that this action is short-lived over less than 90 min. Frusemide was more potent in inhibiting MBS-induced bronchoconstriction despite causing a smaller diuretic effect than piretanide. The basic mechanism of action of the loop diuretics in the airways remains unclear. *Eur Respir J., 1992, 5, 1184–1188.*

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The loop diuretic, frusemide, has been shown to be effective in preventing bronchoconstriction induced by several indirect bronchoconstrictor stimuli, such as exercise, distilled water, sodium metabisulphite (MBS) and allergen in patients with asthma [1–4]. The mechanisms by which frusemide affords such bronchoprotective effects are not known. Recent evidence suggests that frusemide can inhibit the release of mediators such as histamine and leukotrienes from immunoglobulin E (IgE)-stimulated human lung fragments [5]. In addition, frusemide has also been shown to inhibit contraction of guinea-pig airway smooth muscle, which is mediated through the release of either acetylcholine or tachykinins from airway nerves [6]. These mechanisms could, therefore, underline the protective effect of frusemide in the asthmatic airway.

Frusemide acts at the ascending loop of Henle in the kidney, to inhibit the Na/K/2Cl co-transporter, an action which explains its diuretic effect [7]. It is also active in inhibiting chloride ion transport across the airway epithelium [8, 9] but whether this inhibitory activity of frusemide underlies its beneficial effect in the airways is not clear. In patients with asthma, bumetanide, another loop diuretic with more potent inhibitory effects on the Na/K/2Cl co-transporter [10], has no significant effects against either MBS- or adenosine-induced bronchoconstriction [11]. Interestingly, in guinea-pig airways, neurally-induced contraction *in vitro* is inhibited by both frusemide and bumetanide [6]. In order to further examine whether the loop diuretics have a similar profile of bronchoprotective effects, we have now examined the effects of piretanide, a loop diuretic of greater potency than frusemide [12], against MBS-induced bronchoconstriction in stable asthmatic subjects. We have compared the effects of piretanide to that of frusemide, and have also examined the duration of effect of these diuretics in the airways.

Methods

The study was divided into two parts. In Study 1, we examined the duration of any potential inhibitory effects of frusemide, piretanide and placebo against

MBS-induced bronchoconstriction by performing repeated MBS challenges, before and after inhalation of these agents at several time points up to 24 h. Because of the development of tachyphylaxis, particularly after the third MBS challenge, we subsequently performed Study 2, in which we measured MBS challenge only once at 90 min after inhalation of either frusemide, piretanide or placebo.

Subjects

We recruited 16 mild asthmatic subjects for the two studies and eight took part in both (table 1). All were nonsmokers. All subjects responded to sodium metabisulphite inhalation, with a provocative concentration of sodium metabisulphite causing a 20% fall in forced expiratory volume in one second (FEV_1) (PC_{20}MBS) <40 mg·ml^{-1}. They were all on treatment with inhaled β_2-agonists as and when needed. None of the subjects was treated with inhaled or oral steroids or theophylline. All treatments were withheld for at least 12 h before attending the laboratory. All subjects participated with written consent to the protocol, which was approved by the Ethics Committee of the Royal Brompton and National Heart Hospitals.

Study design

Study 1. Each subject visited the laboratory at 8 a.m. on three separate days and the test days were separated by at least 48 h. On each test day, a baseline MBS challenge was performed one hour prior to inhalation of either placebo, frusemide or piretanide solution. These solutions were delivered according to a randomized, double-blind, cross-over protocol. MBS challenges were then repeated immediately after inhalation of test solution (5 min), and at 1.5, 3, 6, and 24 h after inhalation of test solution, in order to determine the duration of any effect.

The diuretic effect of each test solution was evaluated by asking the subject to empty his bladder one hour prior to inhalation of test solution. Urine was collected immediately prior to inhalation of test solution (baseline), and then at 0–1.5, 1.5–3, 3–6 and 6–24 h after inhalation.

Study 2. This study was performed after the results from Study 1 had shown that there was tachyphylaxis to repeated MBS challenges. A single MBS challenge was performed 90 min after inhalation of a 10 ml solution either frusemide, piretanide or placebo on 3 separate days. Each test-day was again separated by at least 48 h.

Administration of placebo, piretanide or frusemide

Frusemide solution was made up of 4 ml of frusemide injection B.P. (Antigen Ltd, Roscrea, Ireland; 10 mg·ml^{-1}) diluted to a final volume of 10 ml with 0.9% saline. Therefore, a dose of 40 mg of frusemide was delivered to the subjects. Piretanide solution was used as prepared for parenteral administration (Arelix, Cassella-Riedal, Frankfurt, Germany; 2.4 mg·ml^{-1}). In all, 24 mg of piretanide was delivered. The dose of frusemide used was similar to that used in previous studies [3, 11] and the dose of piretanide was chosen according to preliminary studies showing inhibitory effects at that dose. The placebo solution consisted of 10 ml of 0.9% NaCl solution.

The solutions were delivered *via* an ultrasonic nebulizer (DeVilbiss Ultraneb 99; DeVibliss Health Care UK Ltd, Middlesex, UK), set at a maximal output of 4 ml·min^{-1} with particle size of mass median aerodynamic diameter of 5.0 μ. Subjects wore a noseclip, and breathed tidally through a mouthpiece connected to the nebulizer for 10 min, which was the time necessary to nebulize all solutions to dryness.

Sodium metabisulphite challenge

MBS solutions (Sigma Chemical Co. Poole, UK) were freshly prepared in 0.9% saline in doubling concentrations (0.6–160 mg·ml^{-1}) 10 min before each challenge. Aerosols were delivered from a nebulizer attached to a dosimeter (Morgan Nebicheck Nebuliser Controller; PK Morgan Ltd, Kent, UK). The nebulizer had an output of 10 μl·puff^{-1}, with a particle size of mass median diameter of 3.5 μm. The dosimeter was set to nebulize for 1 s, with a pause-time of 10 s. The subjects then held their breath for 5–8 s after the nebulizer was triggered. Five inhalations at each

Table 1. – Baseline details of subjects

Study	Subjects	Age	Height	Sex	FEV_1 % pred			Baseline log PC_{20}MBS
	n	yrs	m	M:F	V1	V2	V3	mg·ml^{-1}
1	12	30±7	1.73±0.08	7:5	89±12	87±11	89±11	0.82±0.10
2	12	28±6	1.70±0.04	7:5	87±8	88±8	86±9	0.76±0.25

Values are mean±SEM. FEV_1: forced expiratory volume in one second; V: visit; PC_{20}MBS: provocative concentration of sodium metabisulphite needed to cause a 20% fall in baseline FEV_1.

doubling concentration were taken in succession, and 3 min after each set of inhalations at each doubling concentration, FEV_1 was measured using a dry wedge spirometer (Vitalograph, Buckingham, UK). The challenge started with inhalations of 0.9% saline (diluent) with subsequent measurement of FEV_1 which served as control. The challenge was discontinued when the FEV_1 had decreased by at least 20% of the post saline value, or when the subject had inhaled the top dose of MBS (160 mg·ml^{-1}). The PC_{20}MBS was then calculated by linear interpolation of the log-dose response curve.

Statistical analysis

Study 1. In order to examine the effect of each of the three treatments, we converted the data for each time-point after pretreatment as the change in log PC_{20}MBS from the baseline log PC_{20}MBS. For each time-point, we determined whether frusemide or piretanide had a significant protective effect compared to placebo by performing a two-way analysis of variance.

Study 2. A two-way analysis of variance was used to compare the effect of placebo, frusemide and piretanide on PC_{20}MBS. A p value <0.05 was considered to be significant.

Results

Study 1

There were no significant differences in the baseline FEV_1 and bronchial responsiveness to MBS over the three separate study days (table 1). Both loop diuretics had a significant diuretic effect, with piretanide being more effective than frusemide at the doses used (fig. 1). However, the duration of diuretic effect was similar for both diuretics and lasted for at least 3 h.

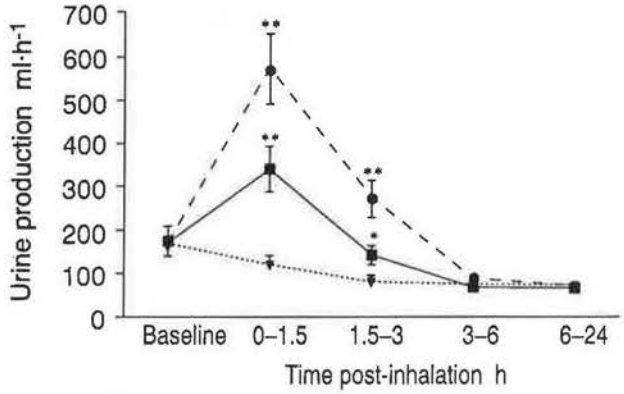

Fig. 1. – Time-course of diuretic effects of inhaled piretanide (24 mg), frusemide (40 mg) and placebo. Urine was collected one hour prior to inhalation, and after inhalation at 0–1.5, 1.5–3, 3–6 and 6–24 h. Piretanide and frusemide caused an increase in urine production at the 0–1.5 and 1.5–3 h periods, with piretanide being more potent than frusemide. Data are shown as mean±SEM. ●: piretanide; ■: frusemide; ▼: placebo; *: p<0.05; **: p<0.01, compared to placebo.

Immediately after inhalation, there was a significant change in mean log PC_{20}MBS after either piretanide or frusemide, when compared to placebo (fig. 2). The increase in mean log PC_{20}MBS was greater for frusemide (0.58±0.11, mean±SEM) than for piretanide (0.40±0.11). Following placebo, there was a significant increase in log PC_{20} by 0.23±0.03, which is approximately a 1.7 fold increase (p<0.001). In other words, frusemide induced a mean 3.8 fold (95% confidence interval: 2.3–6.3 fold), piretanide a 2.5 fold (1.8–3.4 fold) and placebo a 1.7 fold (1.5–1.9 fold) increase in PC_{20}MBS. No significant differences were observed at the other time-points between the three treatments (fig. 2). However, the persistent increase in PC_{20}MBS observed after placebo treatment, such as a mean log increase of 0.42±0.06 at 1.5 h, is highly indicative of increasing tachyphylaxis to the bronchoconstrictor effect of sodium metabisulphite. Therefore, it was not possible to determine the time-course of the protective effect of piretanide or frusemide using this protocol.

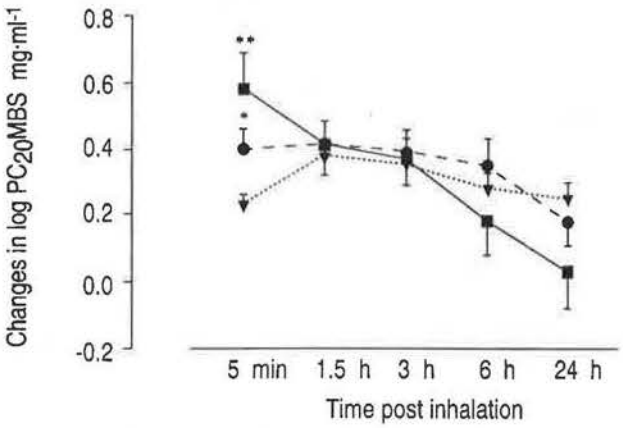

Fig. 2. – Time-course of the change in log PC_{20} to sodium metabisulphite (MBS) after inhaled piretanide (24 mg), frusemide (40 mg) and placebo. The change in log PC_{20} has been calculated from the PC_{20} measurement taken prior to inhalation of the loop diuretic or placebo. PC_{20}: provocative concentration causing a 20% fall in forced expiratory volume in one second from baseline: ●: piretanide; ■: frusemide: ▼: placebo; **: p<0.01; *: p<0.05.

Study 2. At 90 min after pretreatment with either placebo, piretanide or frusemide, there was no significant difference between the three measurements of PC_{20}MBS. Thus, the mean log PC_{20}MBS were: 0.76±0.08, 0.78±0.10, and 0.82±0.09 after placebo, piretanide and frusemide, respectively.

Discussion

In this study, piretanide, a loop diuretic closely related to frusemide, had protective effects against sodium metabisulphite-induced bronchoconstriction in patients with mild asthma. At the single inhaled doses administered, piretanide caused a significantly greater degree of diuresis than frusemide, but afforded a

smaller protective effect than frusemide. BIANCO *et al.* [13] found similar comparative effects of piretanide and frusemide in inhibiting the bronchoconstriction induced by nebulized water. We also found that the protective effect of both frusemide and piretanide to be short-lived, with no significant inhibition being found at 90 min.

Both loop diuretics caused an immediate onset of diuresis, maximal within 0–1.5 h after administration, which is likely to be due to the effect of swallowed diuretic of up to 90% of the dose delivered at the mouth. However, the diuretic effect did not correlate with the degree of inhibition of airway responsiveness to sodium metabisulphite. Such lack of correlation was also observed in a previous study, in which inhaled bumetanide at a dose that induced diuresis failed to inhibit airway responsiveness to sodium metabisulphite [11].

Two possibilities which are not mutually exclusive may underlie these findings. Firstly, the distribution and clearance of inhaled diuretic in the airways may be different from those of circulating diuretic in the renal tubules. Both frusemide and piretanide are rapidly absorbed after oral administration, to achieve peak plasma concentrations within an hour and to induce a rapid onset of diuresis [14]. In the kidney, there appears to be a concentrating mechanism within the nephron, which increases the concentration of loop diuretic within the proximal portion of the ascending loop of Henle. However, whether such a mechanism is present in the airways, and whether the concentration of loop diuretics persist for long periods within the airway wall, is not known. In addition, local metabolism of the individual loop diuretics studied within the airway cannot be excluded. Of interest, is the observation that both bumetanide and frusemide are active in inhibiting neurally-mediated bronchoconstriction in guinea-pig airways *in vitro* [6]. This would suggest that bumetanide may not achieve biologically significant levels within the airways *in vivo*.

Secondly, it is not clear whether the inhibitory effects observed in the airways and its diuretic actions share a common mechanism. The major mechanism underlying the diuretic effect of the loop diuretics we have studied (frusemide, bumetanide and piretanide) involves inhibition of the Na/K/2Cl co-transporter protein in the ascending loop of Henle, which prevents the reabsorption of sodium and chloride from the glomerular filtrate [15]. There is little information concerning the distribution of this co-transporter protein in the airways. The Na/K/2Cl co-transporter has been localized to the basolateral membrane of the airway epithelium [8, 9], but whether it is also present in other cell types of the airway is not yet known. Our current data may be taken as evidence against an inhibitory effect on the co-transporter as an important mechanism of action in the airways, because if piretanide and frusemide achieved optimal concentrations in the airways at the doses administered, piretanide should have had a greater inhibitory effect than frusemide in view of its greater diuretic activity.

Both loop diuretics, frusemide and bumetanide, can induce the release of prostaglandin E_2 (PGE_2) from renal tubules [16–18]. If PGE_2 were released from the airways, it could protect against bronchoconstrictor stimuli [19–21]. A cyclooxygenase inhibitor, flurbipropen, did not prevent the effect of frusemide against bronchial responsiveness to sodium metabisulphite [22]. However, using indomethacin as a cyclooxygenase inhibitor, PAVORD *et al.* [23] have shown that cyclooxygenase metabolites may be involved in the inhibition of exercise bronchoconstriction.

One property which appears to differentiate the three diuretics is that piretanide and frusemide induce vasodilation in the renal vasculature, whilst bumetanide does not [24, 25]. In man, forearm blood flow was significantly increased by frusemide but not by bumetanide at clinically-used oral doses [26]. Therefore, at the level of the airway submucosa, piretanide and frusemide may increase bronchial blood flow to increase the removal of inhaled constrictor substances, whilst bumetanide may not. However, this does not explain the differential protective effects of frusemide against methacholine and metabisulphite challenges [3]. In addition, frusemide has been shown not to increase bronchial blood flow in the conscious sheep [27]. Therefore, the mechanism underlying the bronchoprotective effects of frusemide and piretanide remains unclear.

The development of tachyphylaxis to the bronchoconstrictor effect of sodium metabisulphite, particularly at the third repeat challenge, is of interest and, unfortunately, did not allow us to study precisely the duration of the protective effects of frusemide and piretanide. However, in a second study, we found that the duration of protection lasted less than 90 min. We chose the initial protocol because previous studies have shown little refractoriness to MBS-induced bronchoconstrictor response when two successive challenges were separated by a period of one hour [28–30]. In our subjects, we even found a small but significant mean increase of 1.7 fold in PC_{20}MBS one hour after inhalation of placebo. The maximal increase in PC_{20} was of the order of 3.8 fold at the third and fourth repeat sodium metabisulphite challenge. No significant refractoriness was observed at the sixth repeat MBS challenge approximately 24 h later. Our data demonstrate that refractoriness to repeated sodium metabisulphite challenge occurs depending on the interval between successive challenges and on the number of challenges performed.

In summary, we have demonstrated that another loop diuretic piretanide, significantly protected against sodium metabisulphite-induced bronchoconstriction, thus sharing a similar property with that of the other loop diuretic, frusemide. However, this protective effect did not correlate with their diuretic potencies at the doses examined. It is unclear whether differences in clearance of metabolism of these inhaled diuretics

in the airways underlie this observation. Whether these loop diuretics possess similar mechanisms of effects in the airways as in the renal tubules is also not clear.

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References

1. Bianco S, Vaghi A, Robuschi M, Pasargiklian M. – Prevention of exercise-induced bronchoconstriction by inhaled frusemide. *Lancet,* 1988; ii: 252–255.
2. Robuschi M, Gamboro G, Spagnotto S, Vaghi A, Bianco S. – Inhaled frusemide is highly effective in preventing ultrasonically nebulised water bronchoconstriction. *Pulm Pharmacol,* 1989; I: 187–191.
3. Nichol GM, Alton EWFW, Nix A, *et al.* – Effect of inhaled frusemide on metabisulfite-induced bronchoconstriction and nasal potential difference in asthmatic subjects. *Am Rev Respir Dis,* 1991; 142: 576–580.
4. Bianco S, Pieroni MG, Refini RM, Rottolil Siestini P. – Protective effect of inhaled frusemide on allergen-induced early and late asthmatic responses. *N Engl J Med,* 1989; 321: 1069–1073.
5. Anderson SD, He W, Temple DM. – Inhibition by frusemide of inflammatory mediators from lung fragments. *N Engl J Med,* 1990; 324: 131.
6. Elwood W, Lotvall JO, Barnes PJ, Chung KF. – Loop diuretics inhibit cholinergic and non-cholinergic nerves in guinea-pig airways. *Am Rev Respir Dis,* 1991; 143: 1340–1344.
7. Burg M, Stoner L, Cardinal J, Green N. – Frusemide: effect on isolated perfused tubules. *Am J Physiol,* 1973; 225: 119–124.
8. Widdicombe JH, Nathanson IT, Highland E. – Effects of "loop" diuretics on ion transport by dog tracheal epithelium. *Am J Physiol,* 1983; 245: C388–C396.
9. Welsh MJ. – Inhibition of chloride secretion by frusemide in canine tracheal epithelium. *J Membr Biol,* 1983; 71: 219–226.
10. Cohen MR, Hirsch E, Vergona R, *et al.* – A comparative diuretic and tissue distribution study of bumetanide and frusemide in the dog. *J Pharmacol Exp Ther,* 1976; 197: 697–702.
11. O'Connor BJ, Chung KF, Chen-Worsdell Y, Fuller RW, Barnes PJ. – Effect of inhaled frusemide and bumetanide on adenosine 5 monophosphate and sodium metabisulfite-induced bronchoconstriction in asthmatic subjects. *Am Rev Respir Dis,* 1991; 143: 1329–1333.
12. Lawrence JR, Ansari AF, Elliott HL, *et al.* – Kinetic and dynamic comparison of piretanide and frusemide. *Clin Pharmacol Ther,* 1978; 23: 558–565.
13. Bianco S, Robuschi M, Vaghi A, *et al.* – Protective effect of inhaled piretanide on the bronchial obstructive response to ultrasonically nebulized H_2O. *Am Rev Respir Dis,* 1991; 143: A210.
14. Teredesai P, Puschett JB. – Acute effects of piretanide in normal subjects. *Clin Pharmacol Ther,* 1979; 25: 331–339.
15. Feig PU. – Cellular mechanism of action of loop diuretics: implications for drug effectiveness and adverse effects. *Am J Cardiol,* 1986; 57: 14A–19A.
16. Katayama S, Attallah AA, Stah RAK, Bloch DL, Lee JB. – Mechanism of frusemide-induced natriuresis by direct stimulation of renal prostaglandins E_2. *Am J Physiol,* 1984; 247: F555–561.
17. Miyanoshita A, Terada M, Endou H. – Furosemide directly stimulates prostaglandin E_2 production in the thick ascending limb of Henle's loop. *J Pharmacol Exp Ther,* 1989; 251: 1155–1159.
18. Craven PA, DeRubertis FR. – Calcium-dependent stimulation of renal medullary prostaglandin synthesis by furosemide. *J Pharmacol Exp Ther,* 1982; 222: 306–314.
19. Pasargiklian M, Bianco S, Allegra L. – Aspects of bronchial reactivity to prostaglandins and aspirin in asthmatic patients. *Respiration,* 1977; 34: 78–91.
20. Pasargiklian M, Bianco S, Allegra L. – Clinical, functional and pathogenetic aspects of bronchial reactivity to prostaglandins $F_{2\alpha}$, E_1 and E_2. *Adv Prostaglandin Thromboxane Res,* 1976; 1: 461–475.
21. Pavord I, Wisniewdki A, Mathur R, *et al.* – Effect of inhaled prostaglandin E_2 on bronchial reactivity to sodium metabisulphite and methacholine in subjects with asthma. *Thorax,* 1991; 46: 633–637.
22. O'Connor BJ, Ridge SM, Barnes PJ, Chung KF. – The role of cyclooxygenase products in the inhibition of sodium metabisulfite-induced bronchoconstriction by frusemide in asthma. *Am Rev Respir Dis,* 1991; 143: A210.
23. Pavord ID, Wisniewski A, Tattersfield AE. – Inhaled frusemide and exercise-induced asthma: evidence of a role for inhibiting prostanoids. *Am Rev Respir Dis,* 1991; 143: A210.
24. Friedman PA, Roch-Ramel F. – Hemodynamic and natriuretic effects of bumetanide and furosemide in the cat. *J Pharmacol Exp Ther,* 1977; 203: 82–91.
25. Omosu M, Fujimoto K, Sakaguchi T, *et al.* – Renal effects of piretanide in dogs. *Arzneim Forsch Drug Res,* 1983; 33: 1277–1280.
26. Johnston GD, Nicholls DP, Kondowe GB, Finch MB. – Comparison of the acute vascular effects of frusemide and bumetanide. *Br J Clin Pharmacol,* 1986; 21: 359–364.
27. Nichol GM, Parsons GH, Barnes PJ, Chung KF. – Metabisulfite aerosol increases bronchial blood flow despite anticholinergic, furosemide, nedocromil or antihistamine pretreatment. *Am Rev Respir Dis,* 1990; 141: A360.
28. Wright W, Zhang YG, Salome CM, Woolcock AJ. – Effect of inhaled preservatives on asthmatic subjects. I. Sodium metabisulfite. *Am Rev Respir Dis,* 1990; 141: 1400–1404.
29. Nichol GM, Nix A, Chung KF, Barnes PJ. – Characterisation of bronchoconstrictor responses to sodium metabisulphite aerosol in atopic subjects with and without asthma. *Thorax,* 1989; 44: 1009–1014.
30. O'Connor BJ, Chen-Worsdell M, Ridge SM, Fuller RW, Barnes PJ. – Inhaled sodium metabisulphite does not show immediate tachyphylaxis in asthma. *Thorax,* 1990; 45: 786.