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The effect of inhaled heparin on bronchial reactivity to sodium metabisulphite and methacholine in patients with asthma

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The effect of inhaled heparin on bronchial reactivity to sodium metabisulphite and methacholine in patients with asthma. I. Pavord, T. Mudassar, J. Bennett, P. Wilding, A. Knox. ©ERS Journals Ltd 1996.

ABSTRACT: Inhaled heparin inhibits the early response to allergen and exercise-induced asthma, probably by inhibiting mast cell mediator release. Recent animal studies suggest that heparin might also inhibit cholinergic neurotransmission in asthma by restoring inhibitory M_2 receptor function. We have tested the hypothesis that heparin inhibits neurally-mediated bronchoconstriction in asthma by examining the effect of inhaled heparin on bronchial reactivity to sodium metabisulphite. We also examined the effect of inhaled heparin on methacholine-induced bronchoconstriction to exclude a direct effect on airway smooth muscle.

Eleven patients with mild asthma inhaled nebulized heparin (1,000 U·kg^{-1}) or placebo (normal saline) in a randomized, double-blind fashion, 10 min before a challenge with sodium metabisulphite. Nine patients were also challenged with methacholine after the same dose of heparin or placebo.

Inhaled heparin did not significantly change forced expiratory volume in one second (FEV_1), nor did it alter the provocative dose of sodium metabisulphite or methacholine required to cause a 20% fall in FEV_1 (PD_{20}). Geometric mean sodium metabisulphite PD_{20} was 2.54 and 2.15 μmol after placebo and heparin, respectively (mean difference -0.24 doubling doses; 95% confidence interval (95% CI) -0.64–0.17). Geometric mean methacholine PD_{20} was 1.00 and 1.51 μmol after placebo and heparin, respectively (mean difference 0.6 doubling doses; 95% CI -0.25–1.5).

Thus, heparin inhaled at doses sufficient to inhibit allergen and exercise-induced bronchoconstriction has no effect on the response to sodium metabisulphite and methacholine challenge in asthma. This argues against an inhibitory effect on neural pathways or airway smooth muscle.

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Inhaled heparin has been shown to inhibit both the early response to allergen and exercise-induced bronchoconstriction in subjects with asthma [1, 2]. The mechanism of action is unclear, although studies on isolated human mast cells *in vitro* suggest that heparin inhibits mast cell mediator release [3, 4]. The inhibitory effect of heparin in the airway might extend to neural pathways, since animal studies show that heparin reverses allergen-induced inhibitory M_2 receptor dysfunction [5].

The function of the M_2 inhibitory receptor function is impaired in asthma and this may be partly responsible for bronchial hyperreactivity to irritant stimuli, such as sulphur dioxide and sodium metabisulphite [6]. We have tested the hypothesis that heparin restores M_2 autoreceptor function and, thus, inhibits cholinergic neurotransmission in asthma by examining the effect of inhaled heparin on sodium metabisulphite-induced bronchoconstriction. We also examined the effect of inhaled heparin on methacholine-induced bronchoconstriction to exclude a direct effect on airway smooth muscle.

Methods

Subjects

Eleven subjects (8 males and 3 females) with mild stable asthma but no other important illnesses were recruited from the City Hospital asthma register. Subjects were nonsmokers, with a forced expiratory volume in one second (FEV_1) greater than 60% predicted and a provocative dose of sodium metabisulphite causing a 20% fall in FEV_1 (PD_{20}) <8 μmol (table 1). All subjects participated in the first study and the first nine participated in the second (table 1). Subjects were treated with salbutamol as required and four were taking additional regular inhaled corticosteroids (beclomethasone dipropionate <1,000 μg·day^{-1}). Bronchodilator medication was withheld for 6 h before each visit; inhaled corticosteroid treatment was not interrupted. Subjects gave signed consent to participate after full written and verbal explanation of the study. The study protocol was approved by the City Hospital Ethics Committee.

Table 1. – Characteristics of subjects studied

Subject No.	Sex	Age Yrs	Treatment	FEV_1 L	FEV_1 % pred
1	M	36	S,B	3.40	81
2	M	34	S,B	4.20	95
3	F	39	S,B	2.25	74
4	M	23	S	4.70	104
5	M	42	S	3.30	82
6	M	33	S	2.75	66
7	F	31	S,B	3.40	108
8	M	33	S	4.05	90
9	M	33	S	4.30	96
10	F	41	S	2.45	95
11	M	27	S	5.05	112
Mean		34		3.62	91

Subjects Nos. 10 and 11 participated in the first study only. S: salbutamol; B: beclomethasone dipropionate; M: male; F: female; FEV_1: forced expiratory volume in one second; % pred: percentage of predicted value.

Measurements

FEV_1 was measured on a dry bellows spirometer (Vitalograph, Buckingham, UK) as the higher of two successive readings within 100 mL. Sodium metabisulphite challenge was performed by a method based on that described by NICHOL *et al*. [7]. Serial dilutions, over the range 0.6–160 $mg \cdot mL^{-1}$, were made up in normal saline each day. Aerosols were delivered from a nebulizer attached to a breath-actuated dosimeter (MEFAR, Brescia, Italy); the nebulizer was set to nebulize for 1 s with a pause time of 6 s at a pressure of 22 $lb \cdot in^{-2}$ (152 kPa) and delivered 6.5 $\mu L \cdot puff^{-1}$. Subjects inhaled doubling doses (0.03–64 μmol) of sodium metabisulphite in 1–4 breaths by inspiring rapidly from functional residual capacity to total lung capacity, holding their breath for 3 s and exhaling slowly for 3 s. FEV_1 was measured 2 min after each set of inhalations. The challenge was discontinued when the FEV_1 had fallen by 20% or more, or when subjects had inhaled the highest cumulative dose of sodium metabisulphite (128 μmol).

Methacholine challenge was performed by a similar method. Serial dilutions of methacholine (Sigma, Poole, UK) were made up in normal saline over the range 0.42–27 $mg \cdot mL^{-1}$. Doubling doses (0.02–5.12 μmol) were administered *via* the breath-actuated dosimeter every 2 min as in the metabisulphite challenge, except that the output was 10 $\mu L \cdot puff^{-1}$. FEV_1 was measured 2 min after each inhalation.

Protocol

Subjects attended on four separate occasions at the same time of day. Heparin sodium (Monoparin; CP Ltd, UK) 1,000 $U \cdot kg^{-1}$ (maximum 80,000 U) diluted to 4 mL in normal saline or placebo (4 mL normal saline) were administered in random order and double-blind *via* a jet nebulizer (output 0.2 $mL \cdot min^{-1}$; mass median diameter 5.3 μm), with the subjects inhaling through a face-mask at tidal volume until the nebulizer was dry. FEV_1 was measured before, immediately after and 10 min after inhalation. The sodium metabisulphite or methacholine challenges proceeded immediately after the 10 min measurement.

Analysis

FEV_1 before and after inhalation of heparin or placebo and change in FEV_1 following heparin and placebo were compared within subjects by paired t-test. Sodium metabisulphite and methacholine PD_{20} values were calculated by linear interpolation of the cumulative log dose-response curve. The PD_{20} values were log transformed for analysis and expressed as geometric mean values. The differences in PD_{20} between heparin and placebo for sodium metabisulphite and methacholine were compared by a paired t-test and expressed as doubling doses with 95% confidence intervals (95% CI). The study had 90% power to detect a difference in sodium metabisulphite PD_{20} of one doubling dose and 80% power to detect the same difference in methacholine PD_{20}.

Results

Inhaled heparin was well-tolerated and caused no significant change in FEV_1 in either study. The combined mean (SEM) FEV_1 was 3.62 (0.27) and 3.61 (0.21) L, respectively, before and 10 min after inhaled normal saline, and 3.60 (0.28) and 3.60 (0.26) L, respectively, before and 10 min after inhaled heparin. In a preliminary study of two subjects, there was no evidence of prolongation of the activated partial thromboplastin time (APPT) after inhaled heparin (1,000 $U \cdot kg^{-1}$).

Inhaled heparin had no significant effect on the position of the dose-response curve to sodium metabisulphite or methacholine. The geometric mean sodium metabisulphite PD_{20} was 2.15 and 2.54 μmol after inhaled heparin and placebo, respectively (mean difference -0.24 doubling doses; 95% CI -0.64–0.17; p=0.67) (fig. 1). The geometric mean methacholine PD_{20} was 1.51 and 1.00 μmol after inhaled heparin and placebo, respectively (mean difference 0.6 doubling doses; 95% CI -0.25–1.5; p=0.21) (fig. 2).

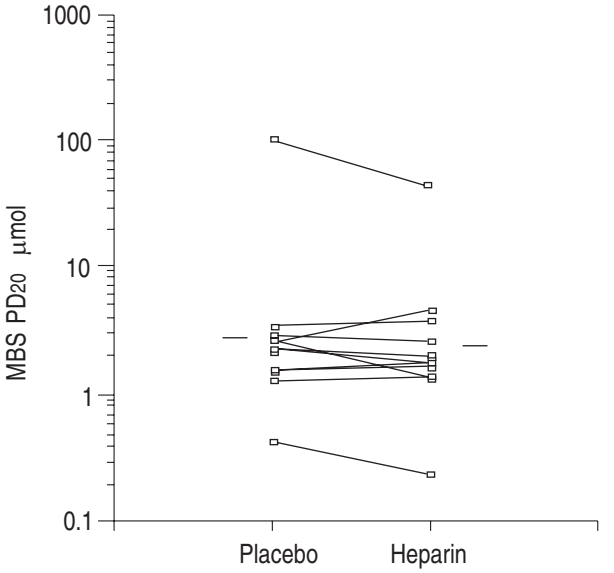

Fig. 1. – Individual sodium metabisulphite (MBS) PD_{20} values after inhaled heparin and placebo. Horizontal bar=geometric mean. PD_{20}: provocative dose causing a 20% fall in forced expiratory volume in one second.

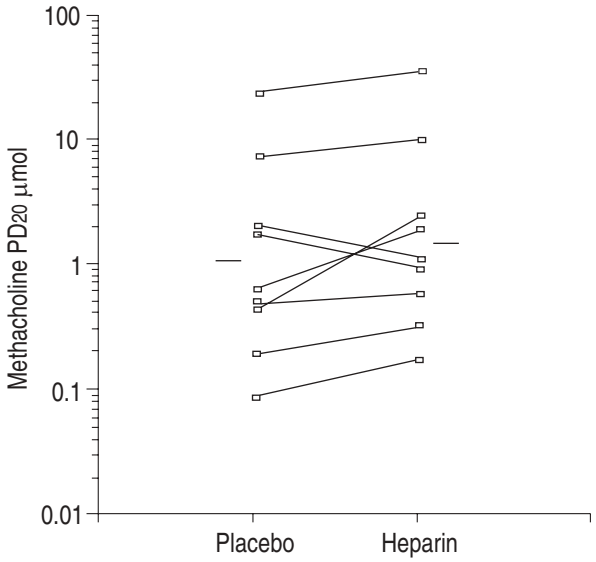

Fig. 2. – Individual methacholine PD_{20} values after inhaled heparin and placebo. Horizontal bar=geometric mean. PD_{20}: provocative dose causing a 20% fall in forced expiratory volume in one second.

Discussion

We have shown that heparin inhaled at a dose that is the same [2] or greater [1] than has been shown to inhibit allergen and exercise-induced bronchoconstriction in man [1, 2], and produces maximum inhibition of allergen-induced bronchoconstriction in allergic sheep [8], has no effect on the response to sodium metabisulphite or methacholine.

The lack of effect of inhaled heparin against the response to the directly acting spasmogen methacholine is in agreement with previous studies showing that heparin has no effect on histamine and carbachol-induced bronchoconstriction in sheep [8] or histamine-induced bronchoconstriction in subjects with asthma [2]. Ours is the first study to examine the effect of inhaled heparin on the neurally-mediated bronchoconstrictor response to sodium metabisulphite, although intravenous heparin has previously been shown to inhibit the enhanced bronchoconstrictor response to direct vagal stimulation in allergen challenged guinea-pigs [5].

Our interest in the effects of inhaled heparin on sodium metabisulphite-induced bronchoconstriction was stimulated by reports that intravenously applied heparin restored the allergen-induced M_2 autoreceptor dysfunction in sensitized guinea-pigs [5]. The probable mechanism is binding and neutralization of the polycationic endogenous M_2 receptor antagonist major basic protein [9]. Since hyperresponsiveness (in asthma) to irritant stimuli, such as inhaled sodium metabisulphite or sulphurdioxide, is thought to be partly due to M_2 receptor dysfunction [6] and this dysfunction might be due to the effects of major basic protein, we hypothesized that heparin would inhibit the response to sodium metabisulphite in subjects with asthma.

Our failure to show an inhibitory effect of inhaled heparin on sodium metabisulphite-induced bronchoconstriction is unlikely to be due to the timing of the challenge after heparin inhalation, since time course studies have shown that the inhibitory effect of inhaled heparin on allergen-induced bronchoconstriction in *Ascaris suum* sensitive sheep is maximal less than 20 min after inhalation [4]. Our negative findings may reflect problems with access of inhaled heparin to cholinergic nerve endings. Previous studies [5] showing an inhibitory effect of heparin on cholinergic neurotransmission in guinea-pigs have used intravenous heparin (2,000 $U \cdot kg^{-1}$), where more complete access of heparin to cholinergic nerve endings might occur. Another possible explanation is that the mechanisms of M_2 autoreceptor dysfunction in asthma and animal models differ.

Heparin has a number of anti-inflammatory effects including, inhibition of mast cell mediator release [3, 4], inhibition of aspects of lymphocyte activation [10], and limitation of the injurious effect of major basic protein [9], and it has been suggested that it serves a defensive role in the airways [11]. Our study suggests that this defensive role does not extend to inhibition of neurally-induced bronchoconstriction at least if heparin is administered by inhalation.

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