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Characterisation of bronchoconstrictor responses to sodium metabisulphite aerosol in atopic subjects with and without asthma

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ABSTRACT Inhalation of sodium metabisulphite is thought to induce bronchoconstriction by release of sulphur dioxide. We sought to establish the reproducibility of the airway response to inhaled sodium metabisulphite given in increasing doubling concentrations (0·3 to 160 mg/ml) to 13 asthmatic and five atopic non-asthmatic subjects and the contribution of cholinergic mechanisms to this response. In 15 of the 18 subjects bronchoconstriction was sufficient to allow calculation of the dose of metabisulphite causing a 20% reduction in the forced expiratory volume in one second (FEV_1) from baseline values (PD_{20} metabisulphite). The 95% confidence limit for the difference between this and a second PD_{20} metabisulphite determined 2–14 days later was 2·5 doubling doses. The difference between repeat PD_{20} metabisulphite measurements was unrelated to the number of days between challenges or change in baseline FEV_1. Ten subjects returned for a third study 3–120 days after the second challenge; variability in PD_{20} metabisulphite did not differ from that seen between the first and second challenges. PD_{20} methacholine was determined between the two metabisulphite challenges and found to correlate with PD_{20} metabisulphite ($r = 0·71$). Inhaled ipratropium bromide 200 μg given in a randomised, placebo controlled, crossover study to 10 subjects increased PD_{20} methacholine 42 fold but had no significant effect on the response to metabisulphite. A single inhalation of the PD_{20} metabisulphite in five subjects induced maximal bronchoconstriction 2–3 minutes after inhalation, with a plateau in FEV_1 lasting a further four minutes before recovery. A further single inhalation of the same PD_{20} dose 43 minutes later produced a 27% (SEM 4%) smaller fall in FEV_1 than the first inhalation. These results show that metabisulphite PD_{20} values measured over days and weeks show similar reproducibility to those reported for histamine inhalation and that PD_{20} metabisulphite correlates with methacholine responsiveness. Most of the bronchoconstriction is not inhibited by antimuscarinic agents; the underlying mechanisms require further investigation.

Introduction

Inhaled aerosols of the preservative agent sodium metabisulphite seem to cause bronchoconstriction in a large proportion of asthmatic subjects: in one study of 0·5 and 5 mg/ml aerosols six out of eight unselected asthmatic patients showed bronchoconstriction.[1] This effect is attributed to sulphur dioxide generated by the solutions. Oral ingestion of metabisulphite can cause severe and even life threatening asthma,[2,3] but this is rare in comparison with the response to inhaled aerosols. Inhaled sulphur dioxide gas and metabisulphite aerosol may have a common mode of action as bronchoconstriction caused by both agents is inhibited by inhaled cromoglycate and, in some studies at least, by anticholinergic drugs.[4,5] The mechanism whereby sulphur dioxide and metabisulphite cause bronchoconstriction, however, remains unknown.

Expensive measuring equipment is needed to administer sulphur dioxide safely to asthmatic patients. In contrast, the methods required to administer metabisulphite aerosols are cheap, simple, and similar to methods already used in methacholine and histamine challenges.[5] We report the reproducibility of cumulative dose-responses to inhaled metabisulphite in a group of atopic subjects with and without asthma over a few days and weeks. We compared the responses to metabisulphite with those to methacholine and

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the effect of pretreatment with inhaled ipratropium bromide on the response to inhaled metabisulphite. We ascertained the time course of bronchoconstriction after a single dose of inhaled metabisulphite and sought to establish whether short term tachyphylaxis occurred with a further single inhalation.

Methods

SUBJECTS

Thirteen asthmatic (nine men, four women) and five non-asthmatic subjects were initially assessed for inclusion in the study. All subjects were atopic to common environmental allergens based on positive results to skin prick tests and were unaware of any previous adverse reactions to sulphites. They were all non-smokers.

All subjects gave informed consent to a protocol approved by the Brompton Hospital Ethics Committee. Subjects had not had an upper respiratory tract infection for four weeks before the study. They abstained from using sympathomimetic and anticholinergic drugs and sodium cromoglycate for eight hours and from taking drugs or drinks containing xanthines for 24 hours before each day's testing.

One non-asthmatic and two asthmatic subjects failed to show a reduction in forced expiratory volume in one second (FEV_1) of 20% after the highest dose of metabisulphite. The remaining 15 subjects (nine men, six women, aged 30 (3) years (range 20–52), FEV_1 89% (4%) predicted) took part in the rest of the study. Four of these subjects were atopic but non-asthmatic and were receiving no treatment. All the asthmatic subjects were taking inhaled sympathomimetic agents, six inhaled corticosteroids, one inhaled ipratropium bromide, and one inhaled sodium cromoglycate.

DOSE-RESPONSE STUDY FOR INHALED METABISULPHITE

Pulmonary function was measured with a dry wedge spirometer (Vitalograph, Buckingham). Sodium metabisulphite solutions (Sigma, Poole) diluted with normal saline in doubling concentrations (0·3–160 mg/ml) were prepared freshly each day. Aerosols were delivered from a nebuliser attached to a breath actuated dosimeter (MEFAR, Brescia, Italy). The nebuliser delivered 6·7 μl/puff, in particles with a mass median aerodynamic diameter of about 4 μm. The dosimeter was set to nebulise for one second with a pause time of 10 seconds at a pressure of 22 lb/in^2 (152 kPa). Subjects were asked to inspire slowly from functional residual capacity to total lung capacity over five seconds and to hold their breath for five seconds after the nebuliser was triggered.

After measurement of baseline FEV_1 (best of three measurements) the subject inhaled four breaths of normal saline (diluent). After four minutes the best of two FEV_1 measurements was obtained and used as baseline FEV_1. If the response to saline was more than a 10% fall from control FEV_1 the test was discontinued. Four breaths of consecutive doubling concentrations for metabisulphite, starting at 0·3 mg/ml, were inhaled every five minutes and FEV_1 was measured four minutes after each inhalation. The test was discontinued when the FEV_1 had fallen by more than 20% of the control value after saline or when the subject had inhaled the highest dose of metabisulphite (160 mg/ml).

Log dose-response curves were constructed and the dose of metabisulphite needed to cause a 20% fall in FEV_1 calculated by linear interpolation (PD_{20} metabisulphite, μmol).

REPRODUCIBILITY OF PD_{20} METABISULPHITE AND RELATION TO PD_{20} METHACHOLINE

PD_{20} metabisulphite was determined in the 15 subjects at the same time of day on two non-consecutive days, six (range 2–14) days apart. A methacholine challenge was also performed. On a separate day between the first two metabisulphite challenges, consecutive doubling concentrations of methacholine (Sigma, Poole) (0·06–128 mg/ml in normal saline) were administered at four minute intervals to all subjects with the same nebuliser-dosimeter system as in the metabisulphite challenge. PD_{20} methacholine (μmol) was determined as for PD_{20} metabisulphite. Seven asthmatic and three atopic non-asthmatic subjects (aged 28 (2) years, range 20–44) underwent a third metabisulphite challenge 32 (11) days (range 3–120) after their second metabisulphite challenge.

EFFECT OF IPRATROPIUM ON METABISULPHITE INDUCED BRONCHOCONSTRICTION

The 10 subjects who had previously performed three metabisulphite challenges and one methacholine challenge attended the laboratory on three more occasions over a period of two weeks. On the first day PD_{20} methacholine was determined 45 minutes after inhalation of 200 μg ipratropium bromide (Boehringer Ingelheim, Bracknell) delivered from a metered dose inhaler through a spacer device (Volumatic, Allen and Hanburys). At the remaining two visits PD_{20} metabisulphite was determined 45 minutes after randomised double blind administration of ipratropium bromide or placebo in the same manner as described above.

TIME COURSE OF RESPONSES TO SINGLE DOSE OF METABISULPHITE

Five asthmatic subjects (two women) inhaled four breaths of the concentration of metabisulphite immediately above their previously determined mean PD_{20} metabisulphite. FEV_1 was measured every min-

ute for five minutes and every five minutes thereafter until it had returned to within 5% of the baseline value; this took 43 (9) minutes. To determine whether there was a tachyphylactic response to the bronchoconstrictor effect of inhaled metabisulphite a further identical dose of metabisulphite was then inhaled and FEV_1 measured every minute until maximum bronchoconstriction had again occurred.

ANALYSIS

Data on PD_{20} metabisulphite and PD_{20} methacholine were log transformed for statistical analysis and expressed as geometric means (geometric standard errors). All other data are expressed as means (standard errors). Paired comparisons of FEV_1 and log PD_{20} data were made with Student's paired *t* test. All linear correlations were made with the least squares method. PD_{20} metabisulphite measurements on all three open challenge days were compared with PD_{20} metabisulphite on the placebo challenge day with analysis of variance for repeated measures.[6] The presence and duration of the plateau in the time course of bronchoconstriction by metabisulphite were established also with analysis of variance for repeated measures.

Results

Metabisulphite challenge was as simple to perform as challenge with methacholine. All subjects noticed mild irritation and cough when inhaling concentrations of metabisulphite greater than 20 mg/ml. Challenges with both metabisulphite and methacholine took 30 to 45 minutes to complete, and the induced bronchoconstriction was quickly reversed with an inhaled sympathomimetic agonist. None of the subjects reported irritation or wheezing in the hours or days after challenge.

All but two asthmatic subjects and one non-asthmatic subject showed a dose dependent decrease in FEV_1 with metabisulphite challenge, achieving a 20% reduction in FEV_1 after inhaling up to 160 mg/ml of metabisulphite. Inhalation of higher concentrations was not possible because of cough and irritation.

REPRODUCIBILITY OF METABISULPHITE RESPONSES

FEV_1 values before the first metabisulphite challenge (3·31 (0·25) litres) did not differ significantly from those before the second (3·30 (0·26) litres). The geometric mean PD_{20} for the first and second test, 1·31 and 1·79 μmol, did not differ significantly. The absolute mean difference between the first and second PD_{20} metabisulphite measurements was 0·9 (0·2) doubling dose intervals; the 95% confidence limit for the difference between the two PD_{20} measurements was 2·5 doubling dose intervals. There was a 32 fold to 64 fold range of sensitivity to inhaled metabisulphite between subjects, non-asthmatic subjects being less sensitive to metabisulphite than those with asthma (fig 1). Differences in PD_{20} metabisulphite between days related neither to the between days difference in baseline FEV_1 values ($r = 0{\cdot}089$; NS) nor to the number of days between tests ($r = -0{\cdot}015$; NS).

FEV_1 before the third metabisulphite challenge (3·32 (0·30) litres) did not differ significantly from baseline FEV_1 values before the previous two challenges. The mean difference between the second and third PD_{20} metabisulphite measurements, 1·1 (0·3) doubling dose intervals, did not differ significantly from the mean difference between the first two measurements in these 10 subjects (1·0 (0·3) doubling dose intervals). Mean PD_{20} for the third test ($n = 10$) was 1·75 μmol.

RELATION BETWEEN PD_{20} METABISULPHITE AND PD_{20} METHACHOLINE

The range of sensitivity to methacholine between subjects (0·01 to 2·48) was 256 fold, and greater than the range for metabisulphite (0·3–7·04). There was, however, a significant linear correlation between the mean of two PD_{20} metabisulphite measurements and PD_{20} methacholine ($r = 0{\cdot}714$, $p < 0{\cdot}05$) in the 15 subjects (fig 2). The molar potency of metabisulphite was about six times less than that of methacholine.

EFFECT OF IPRATROPIUM BROMIDE ON PD_{20} METABISULPHITE AND PD_{20} METHACHOLINE

There was no significant difference in baseline FEV_1

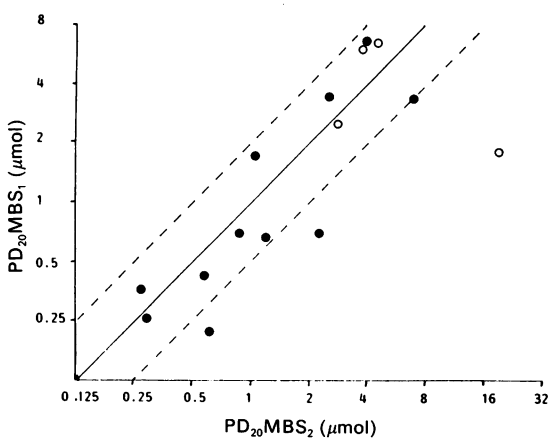

Fig 1 *Relation between first ($PD_{20}MBS_1$) and second ($PD_{20}MBS_2$) PD_{20} metabisulphite measurements in 11 asthmatic (●) and four non-asthmatic (○) atopic subjects. Measurements were taken a mean of six days apart. Solid line is line of identity; interrupted lines indicate one doubling dose interval each side of this. Lower sensitivity to metabisulphite was found in the non-asthmatic subjects.*

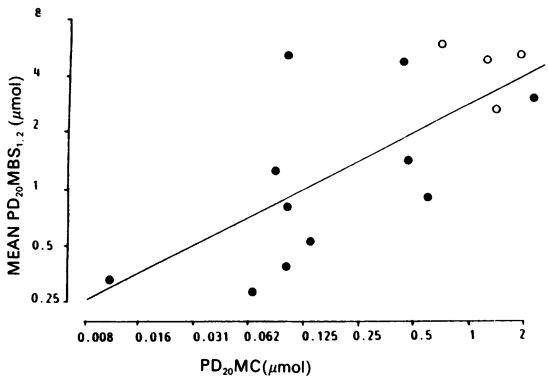

Fig 2 *Relation between mean of two PD_{20} metabisulphite ($PDMBS_{1,2}$) and a single PD_{20} methacholine measurement in 11 asthmatics (●) and four non-asthmatic (○) atopic subjects. Regression line is indicated by solid line ($r = 0.714$; $p < 0.05$). Molar sensitivity to metabisulphite was six fold less than to methacholine.*

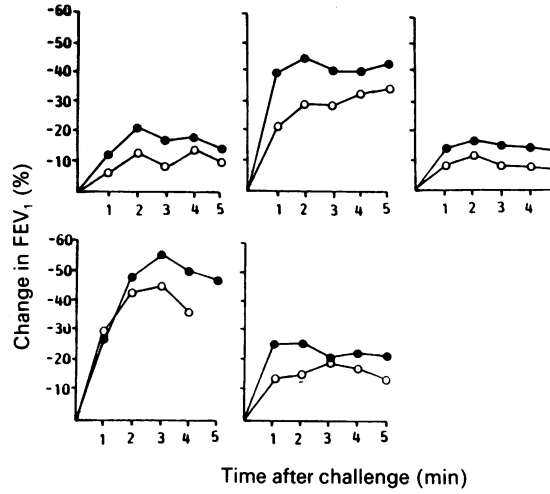

Fig 3 *Comparison of initial time course (5 min) and peak responses to a single dose of metabisulphite (●) followed by the same dose given 43(9) minutes later (○) in five asthmatic subjects. Response is plotted as percentage change in FEV_1 compared with baseline FEV_1 immediately before each challenge. Peak response occurred within 2–3 minutes with a subsequent plateau. The second response was 27% (4%) less than the first.*

before metabisulphite challenge with placebo (3·27 (0·33) litres) and ipratropium bromide (3·37 (0·31) litres) or before methacholine challenge between non-treated (3·34 (0·26) litres) and ipratropium bromide pretreated (3·31 (0·30) litres) days. Ipratropium caused significant bronchodilatation before both methacholine (14·8% (3·7%) increase in FEV_1) and metabisulphite (10·1% (3·3%) increase in FEV_1) challenges; placebo caused a smaller (4·8% (3·7%) but significant increase in FEV_1. PD_{20} metabisulphite was not significantly different after placebo compared with the previous three open challenges.

PD_{20} methacholine increased about 42 fold after ipratropium (from a geometric mean of 0·40 μmol to 17·8 μmol). Pretreatment with ipratropium caused no significant change in PD_{20} metabisulphite compared with placebo (geometric mean 2·3 (1·4) μmol after placebo, 2·6 (1·5) μmol after ipratropium).

TIME COURSE AND TACHYPHYLAXIS AFTER SINGLE DOSE INHALATIONS

The time course of response to a single inhalation of metabisulphite on two occasions 43 (9) minutes apart, are shown for each subject in figure 3. Maximum bronchoconstriction occurred two minutes (range 1–3) after inhalation. Analysis of variance for repeated measures[6] showed no significant change in FEV_1 between three and seven minutes, with significant recovery occurring thereafter. The second response was less than the first in all subjects, the lowest FEV_1 being 2·47 (0·5) litres after the first inhalation and 2·62 (0·5) litres after the second ($p < 0.05$). When expressed as a percentage of baseline FEV_1 the second response was 27% (4%) less than the first response.

Discussion

In this group of atopic subjects with and without asthma bronchial responsiveness to sodium metabisulphite varied within one doubling dose in most subjects over a period of several days. Variation in responsiveness was unrelated to the time period between tests. PD_{20} metabisulphite correlated with PD_{20} methacholine, but like PD_{20} methacholine varied widely between subjects. We found that cholinergic blockade with inhaled ipratropium bromide had no effect on responsiveness to metabisulphite despite a 42-fold decrease in responsiveness to methacholine. Although we found some short term tachyphylaxis to metabisulphite at 45 minutes, which was similar to that found for some hours after inhalation of sulphur dioxide,[4,7] the effect was small.

The reproducibility of PD_{20} metabisulphite was similar to that reported for histamine over the same time.[8,9] It is not known whether environmental influences such as viral infections, immunisation, allergen and isocyanate exposure, and smoke pollution[10–12] affect responsiveness to metabisulphite in the same way as to methacholine, but the correlation between the two measurements suggests that they might. Intrasubject variation in PD_{20} metabisulphite, like PD_{20} histamine,[13] was unrelated to small changes in baseline airway calibre.

The mechanism of metabisulphite induced bronchoconstriction remains uncertain. Fine *et al* showed that the response to sulphite aerosols is not due to the pH of the solution or to sulphite ions but to the sulphur dioxide gas generated or the bisulphite ions in the aerosol, or both. These agents, which are highly reactive in aqueous solution, may exert their effects by altering membrane receptor function, as has been shown for presynaptic and postsynaptic cholinergic transmission in vitro.[15,16] Such alterations may not necessarily be confined to cholinergic receptors.

Pretreatment with anticholinergic drugs has produced variable effects on sulphur dioxide induced bronchoconstriction, from clearcut inhibition in normal[17] and asthmatic subjects[18] to less impressive and variable inhibition.[19,20] In one study inhaled atropine methonitrate inhibited only the milder degrees of sulphur dioxide induced bronchoconstriction in a group of hyperreactive normal and asthmatic subjects.[4]

Using a method similar to our own for a 35% reduction in specific airways conductance, Dixon and Ind found a small (1·4 doubling dose interval) inhibition of the response to inhaled metabisulphite in atopic subjects given inhaled oxitropium.[5] The greater degree of bronchoconstriction in this study (reduction in FEV_1 of 20%) may explain why we were unable to achieve any inhibition with ipratropium bromide. The lack of effect cannot be attributed to an insufficient dose of ipratroprium, as 100 μg is as effective as 200 μg or the addition of 2 mg of atropine[21] in inhibiting sulphur dioxide induced responses and the 200 μg dose was effective in reducing the response to methacholine challenge.

The small or variable response to anticholinergic agents suggests that most of the response to inhaled metabisulphite is not mediated through parasympathetic nervous pathways. Metabisulphite or sulphur dioxide may cause the release of secondary mediators, though the potent histamine receptor antagonist terfenadine provides no protection.[5] Nedocromil sodium[5,22] and sodium cromoglycate[23] are, however, potent inhibitors of bronchoconstriction induced by metabisulphite and sulphur dioxide, presumably by a mechanism unrelated to inhibition of histamine release from mast cells. Bradykinin shows a similar pattern of activity being inhibited by cromoglycate[24] but little affected by antihistamine pretreatment.[25] As cromoglycate inhibits afferent nerve transmission,[26,27] the effects of metabisulphite and bradykinin may be mediated through non-cholinergic excitatory neural pathways.[28] Metabisulphite might also exert its effects by causing the release of bradykinin. Finally, although the mechanisms underlying bronchoconstriction induced by sulphur dioxide and metabisulphite are likely to be similar, different distributions of sulphur dioxide gas and metabisulphite aerosol within the lung may be another reason why responses to the two agents differ.

Irrespective of the mechanism underlying sulphite induced bronchoconstriction, challenge with increasing doubling doses of inhaled sodium metabisulphite aerosols is a simple and reproducible tool for studying bronchoconstriction mediated by non-cholinergic pathways in asthmatic subjects.

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