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Comparison of sulphur dioxide and metabisulphite airway reactivity in subjects with asthma

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Abstract

Background **– In asthmatic subjects bronchoconstriction is induced by inhalation of the common food preservatives sulphur dioxide (SO_2) and metabisulphite (MBS). SO_2 and MBS challenges share many similarities, but it is not known whether they are equivalent. In this study of subjects with mild clinical asthma equivalence was assessed by comparing SO_2 and MBS reactivity by estimating the total dose of SO_2 inhaled during SO_2 and MBS challenges, and by calculating SO_2 uptake during both challenges. In addition, as the MBS solutions inhaled were acidic and hyperosmolar, the effect of these factors on MBS responsiveness was investigated.**

Methods **– Fifteen subjects were challenged on separate days with doubling (0·5 to 8·0 ppm) concentrations of SO_2 gas inhaled during three minute periods of isocapnic hyperventilation and MBS administered in doses ranging from 0·1 to 12·8 μmol using the Wright protocol. On two other days SO_2 and MBS challenges were preceded by a challenge with phosphate buffered saline (PBS) solutions of pH and osmolarity similar to MBS solutions. Response was measured as the dose or concentration causing a 20% fall in FEV_1 (PD_{20} or PC_{20}).**

Results **– All subjects reacted to MBS and 14 responded to SO_2. Geometric mean histamine PD_{20} was 1·61 μmol (95% confidence interval 0·72 to 3·60). MBS and SO_2 airway responsiveness were not significantly related. Estimates of the mean concentration of SO_2 inhaled during SO_2 and MBS challenges differed, as did estimates of the mean SO_2 uptake during both challenges. MBS and SO_2 reactivity were not affected by prior challenge with PBS solutions.**

Conclusions **– SO_2 and MBS challenges are not comparable. MBS reactivity was not affected by the hyperosmolar, acidic nature of its solutions.**

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Sulphiting agents such as sodium metabisulphite (MBS) and sulphur dioxide (SO_2) are commonly used as food and wine preservatives. In subjects with asthma ingestion of foods and beverages containing these agents can provoke bronchoconstriction,[1] but bronchoconstriction develops more frequently following inhalation of either SO_2 gas[2] or metabisulphite aerosols[3] which are commonly used in the laboratory to assess sensitivity to sulphiting agents. Many characteristics of the airway responses to SO_2 and MBS are similar,[4] so the mechanism by which inhaled MBS provokes bronchoconstriction has been attributed to SO_2 released from MBS aerosols, and SO_2 and MBS challenges have been considered to be equivalent. However, the effect of inhaled MBS may not be solely due to liberated SO_2. In solution MBS also converts to bisulphite, another potent bronchoconstricting stimulus, and MBS induced bronchoconstriction may be caused by the bisulphite ions in the aerosols acting alone, or together with generated SO_2.[5] Some of the mechanisms by which SO_2 and MBS provoke bronchoconstriction appear to be similar, but there are also differences. Nedocomil sodium inhibits bronchoconstriction induced by both SO_2[6] and MBS,[4] but while anticholinergic agents have no effect on the response to MBS,[7] in at least 30% of asthmatic subjects SO_2 induced bronchoconstriction is cholinergically mediated.[2,8]

The aim of this study was to determine whether the bronchoconstrictions induced by inhalation of SO_2 and MBS were similar. This was first examined by comparing the provocative concentration of SO_2 and dose of MBS which caused FEV_1 to fall by more than 20% from baseline. Secondly, to determine whether SO_2 and MBS challenges were equivalent in terms of the amount of SO_2 inhaled the concentration of SO_2 delivered during an SO_2 challenge, and the concentration released and inhaled during an MBS challenge, were compared. Thirdly, the uptake of SO_2 gas during MBS and SO_2 challenges was estimated and the values compared. Lastly, as the MBS challenge protocol used in this study involved dissolving it in acidic, hyperosmolar solutions, it was important to determine whether these properties of MBS solutions affected responses to MBS, and therefore the comparison between MBS and SO_2 airway responses.

Methods

DOSE-RESPONSE STUDIES

Fifteen clinically stable subjects (seven women, eight men) aged 18–53 years were studied (table 1). Twelve subjects were atopic on skin prick testing and all were non-smokers. Four subjects were taking regular inhaled steroids (beclomethasone 400–1000 μg daily) and all used a β_2 agonist as required. Baseline

Table 1 Anthropometric details, baseline forced expiratory volume in one second (FEV_1) expressed as percentage of predicted value measured on the first study day, provoking concentration of sulphur dioxide (SO_2) and provoking doses of metabisulphite (MBS) and histamine that caused a 20% fall in FEV_1

Subject no.	Sex/Age (years)	Treatment	Baseline FEV_1 (%pred)	SO_2 PC_{20} (ppm)	MBS PD_{20} (μmol)	Histamine PD_{20} (μmol)
1	F/22	S	83	2·9	2·5	5·6
2	M/31	S,B	80	2·4	1·7	0·3
3	F/29	S	90	2·8	3·8	0·1
4	M/21	S,B	92	1·0	4·5	0·6
5	F/28	S,B	99	5·8	3·2	2·1
6	F/35	S	79	2·8	5·6	7·4
7	F/21	S	100	14·5	6·1	0·8
8	M/30	S	112	5·4	6·2	3·5
9	M/21	S	80	6·2	8·6	0·5
10	F/51	S,B	85	14·5	2·7	7·8
11	M/53	S	86	6·2	6·4	1·8
12	F/18	S	100	23·5	4·0	2·9
13	M/30	S	102	16·0	12·0	6·2
14	M/32	S	102	17·0	8·6	0·7
15	M/26	S	85	8·0	7·9	7·2
Mean	30		91			
Geometric mean				6·17	4·47	1·61

S = salbutamol; B = beclomethasone dipropionate.

forced expiratory volume in one second (FEV_1) was above 75% of predicted values in all subjects. All subjects had a histamine PD_{20} of less than 7·8 μmol with a geometric mean of 1·61 μmol (95% CI 0·72 to 3·60). Aerosol bronchodilators were withheld for at least six hours before testing. Informed consent to the protocol, which was approved by the medical ethics review committee of the Royal North Shore Hospital, was obtained from all subjects.

Spirometric parameters were measured by a Vitalograph dry spirometer (Vitalograph, Buckingham, UK). During both SO_2 and MBS challenges FEV_1 was measured before challenge and at one and two minutes after each dose. Measurements were made in duplicate and if values differed by more than 100 ml a third measurement was taken. The highest of two or three measurements was taken.

Sulphur dioxide challenge

Subjects were challenged with SO_2 during sequential three minute periods of eucapnic hyperpnoea separated by three minutes. After measurement of baseline FEV_1 subjects first inhaled a partially humidified air control, followed by doubling concentrations (0·5, 1·0, 2·0, 4·0, and 8·00 ppm) SO_2. An additional 8·0 ppm SO_2 was administered to three of the 15 subjects to enable measurement of a response to SO_2. The challenge was stopped when FEV_1 fell by 20% or more of the control response, or the highest concentration was inhaled.

Sulphur dioxide (100%) was delivered via a Nupro dual double pattern metering valve and 60 μm filter to a stainless steel chamber where it was continually mixed with partially humidified air before being stored in a 100 l Seran bag (Aspec, Ann Arbor, Michigan, USA). End tidal carbon dioxide was maintained at normal resting levels during hyperpnoea by adding 4–5% carbon dioxide to the bag gas mixture. Subjects inhaled the gas mixture using a noseclip via a two way Hans Rudolf valve. The air temperature and humidity of the inspired gas mixture, which were maintained at 65% relative humidity and 27°C, were measured with a Novasina temperature and humidity probe (Novasina, Zurich, Switzerland) with the probe placed in the inspiratory port of the Hans Rudolf valve. The inspired SO_2 concentration was continuously measured with an electrochemical cell SO_2 analyser (Draeger, Sweden) through a port proximal to the Hans Rudolf valve. A Fleisch No. 3 pneumotachograph and differential pressure transducer (P K Morgan, UK) measured air flow which was digitally integrated to obtain ventilation ($\dot{V}E$). A constant $\dot{V}E$ was maintained by subjects breathing in time to a metronome and inhaling a constant tidal volume, with each subject being cued by watching their respiration on a visual display unit. Subjects inhaled a constant tidal volume of either 1·0 or 1·5 l depending on total lung capacity.

Metabisulphite challenge

Metabisulphite challenges were administered with the protocol described by Wright *et al.*[4] Sodium metabisulphite solutions were made up in phosphate buffered saline (PBS) in concentrations of 6·2, 12·5, 50, and 100 mg/ml. The doses of MBS administered were 0·1, 0·2, 0·4, 0·8, 1·6, 3·2, 6·4, and 12·8 μmol. Aerosols were delivered with De Vilbiss No. 40 hand held nebulisers (DeVilbiss Corporation, Somerset, Pennsylvania, USA) and all challenges were performed within 30 minutes of preparing the solutions. After inhaling a control aerosol of PBS, increasing doses of MBS were inhaled at three minute intervals. The challenge ended when FEV_1 fell by 20% or more from the control measurement, or when the maximal dose had been administered. The pH and osmolarity of the MBS solutions were 6·56 and 415 mosmol in the 6·25 mg/ml solution, 6·26 and 520 mosmol in the 12·5 mg/ml solution, 5·43 and 1160 mosmol in the 6·4 mg/ml solution, and 4·95 and 1960 mosmol in the 12·8 mg/ml solution.

PBS and histamine challenges

Phosphate buffered saline challenges involved inhalation of solutions of increasing osmolarity, pH and titratable acidity, equivalent to the MBS solutions, using the MBS challenge protocol described above. The osmolarity and pH of the MBS and control PBS solutions are shown in table 2.

Histamine challenges were carried out as described by Yan and coworkers.[9] Histamine

Table 2 Characteristics of metabisulphite (MBS) and equivalent phosphate buffered saline (PBS) solutions

Solutions	pH	Osmolarity (mosmol)
MBS 6·25 mg/ml	6·56	415
PBS equivalent	6·78	382
MBS 12·5 mg/ml	6·26	520
PBS equivalent	6·24	510
MBS 50 mg/ml	5·43	1160
PBS equivalent	5·38	1150
MBS 100 mg/ml	4·95	1960
PBS equivalent	4·75	1990

solutions (3·1, 6·0, 25, and 50 mg/ml) were administered via DeVilbiss No. 40 hand held nebulisers in doses ranging from 0·03 to 7·8 μmol histamine. The test was stopped when there was a fall in FEV_1 of 20% or more, or after 7·8 μmol histamine had been administered.

Test procedure

Subjects attended the laboratory five times. At visit 1 they were evaluated by performing baseline pulmonary function, a histamine challenge test, and skin prick tests to common inhaled allergens including *Dermatophagoides pteronyssinus*, cat and dog dander, *Alternaria* and *Aspergillus* moulds, and rye, prairie and timothy grasses. At visit 2 an MBS challenge was performed immediately after a PBS challenge. At visits 3, 4, and 5 subjects were randomly challenged with MBS or SO_2 or, on the other day, a PBS challenge was performed followed immediately by an SO_2 challenge. Challenge with PBS solutions before MBS and SO_2 challenges was performed to determine whether the acidic, hyperosmolar properties of the PBS solutions caused bronchoconstriction, and also whether these solutions potentiated the airway response to MBS. It was expected that a bronchoconstrictive effect due to the properties of the PBS solutions would be identified by challenges performed before both MBS and SO_2. The administration of a PBS challenge before an SO_2 challenge was included in order to determine whether PBS solutions potentiated the bronchoconstrictive response, as it was possible this would be missed when a PBS challenge preceded an MBS challenge which involved adminstration of MBS dissolved in the hyperosmolar, acidic PBS solutions.

STUDIES OF CONCENTRATION OF SO_2 INHALED DURING SO_2 AND MBS CHALLENGES

To calculate the concentration of SO_2 inhaled during both challenges it was necessary to determine whether SO_2 and MBS challenges were cumulative or non-cumulative in effect. Three subjects with controlled asthma were challenged with MBS on two consecutive days and on two other consecutive days SO_2 challenges were performed. On the first day of a set of challenges an SO_2 or MBS challenge, as described above, was performed. On the second day the final SO_2 concentration or dose of MBS which caused a 20% fall in FEV_1 on the first day was given.

Measurement of SO_2 gas produced by each MBS solution

The concentration of SO_2 generated by each concentration of MBS was measured by squeezing one puff of an MBS solution into a three litre syringe. The concentration of SO_2 was measured with an electrochemical SO_2 analyser (Draeger, Sweden) and SO_2 concentrations greater than 10 ppm were diluted with air to obtain a measurement. Measurements were made on three separate occasions and each time the amount of SO_2 released from each MBS solution was measured three times. Mean values were calculated.

Calculations to determine the concentration of SO_2 gas inhaled during SO_2 and MBS challenges

The concentration of SO_2 delivered during inhalation of SO_2 was calculated by multiplying the SO_2 concentration (ppm) inhaled by the ventilation (l/min) maintained during inhalation by the duration of inhalation of SO_2 (minutes). The amount of SO_2 delivered during inhalation of a dose of MBS was estimated by multiplying the concentration of SO_2 (ppm) released from the MBS solution by the number of inhalations involved in administering the dose of MBS.

UPTAKE OF SO_2 DURING SO_2 AND MBS CHALLENGES

Experiments to estimate the in vivo uptake of SO_2 gas with each dose of MBS and each concentration of SO_2 were performed by three non-asthmatic, non-atopic subjects. After inhalation of a dose of MBS or concentration of SO_2 subjects exhaled via a mouthpiece into a 750 ml container in which the sample line and sample return line of an electrochemical SO_2 analyser (Draeger, Sweden) were placed. Measurement of SO_2 concentration was made immediately after exhalation. Measurement of the amount of SO_2 exhaled following inhalation of a dose of MBS was made after each puff of an aerosol and after the final inhalation involved in administration of the dose. The amount of SO_2 exhaled during SO_2 challenges was measured after subjects inhaled SO_2 for three minute periods of eucapnic hyperventilation. Three sets of measurements were recorded by each subject for each dose of MBS and each SO_2 concentration. Mean values were calculated.

These in vivo measurements were used to calculate the uptake of SO_2 which occurred during SO_2 and MBS challenges. SO_2 uptake was estimated by the following equation:

$$SO_2 \text{ uptake} = 1 - \frac{(SO_2 \text{ concentration exhaled})}{(SO_2 \text{ concentration inhaled})}$$

in which SO_2 concentration inhaled was the concentration either generated by MBS solution or administered during SO_2 challenge, and unity represented total uptake.

To calculate SO_2 uptake in each subject the estimate of the total concentration of SO_2 inhaled by a subject was multiplied by the appropriate SO_2 uptake fraction, as calculated above.

ANALYSIS OF DATA

A two way analysis of variance was used to determine if there were any differences in baseline FEV_1 on each study day and the FEV_1 measured before SO_2 and MBS challenges on

Table 3 Forced expiratory volume in one second (FEV_1) in asthmatic subjects for metabisulphite (MBS) challenge and MBS preceded by phosphate buffered saline (PBS) challenge (PMBS): baseline and after PBS challenge values

	MBS challenge FEV_1 (l)	*PMBS challenge FEV_1 (l)*	
Subject no.	*Baseline*	*Baseline*	*After PBS*
1	3·55	3·50	3·55
2	3·35	3·35	3·40
3	3·30	3·35	3·35
4	4·35	4·40	4·45
5	3·40	3·30	3·20
6	2·50	2·55	2·50
7	3·20	3·20	3·25
8	4·20	4·20	4·10
9	3·20	3·10	3·05
10	2·00	2·00	2·00
11	2·70	2·80	2·75
12	3·75	3·75	3·70
13	4·05	4·05	4·00
14	3·90	3·90	3·85
15	3·90	3·85	3·85
Mean	3·42	3·42	3·40
SD	0·65	0·65	0·65

those days when subjects were first challenged with PBS. The effect of eucapnic hyperventilation of humidified air on pulmonary function during SO_2 challenges was evaluated by paired *t* tests.

Dose-response curves were plotted for each challenge, showing the change in FEV_1 against the log of the dose of MBS and against the log of the cumulative concentration of SO_2 or cumulative dose of histamine. The PD_{20} or PC_{20} was obtained by linear interpolation. When the fall in FEV_1 was less than 20% the maximum dose of MBS or maximum cumulative concentration of SO_2 was recorded as the PC_{20} or PD_{20} value. The PD_{20} and PC_{20} values were log transformed and expressed as geometric mean. The differences in PC_{20} and PD_{20} resulting from challenges with SO_2 and SO_2 preceded by PBS (PSO_2) and from challenges with MBS and MBS preceded by PBS (PMBS), were expressed as fold differences with 95% confidence intervals (CI).

The method of Bland and Altman[10] was used to compare PC_{20} values obtained after challenges with SO_2 and PSO_2 challenges and to compare PD_{20} values recorded following MBS and PMBS challenges. The relation between SO_2 and MBS airway reactivity was compared by linear regression. Paired *t* tests were used to determine if there was any difference between the concentration of SO_2 inhaled during SO_2 and MBS challenges, and between the uptake of SO_2 gas during SO_2 and MBS challenges. The association between histamine and SO_2, and between histamine and MBS, was assessed by linear regression. Significance was taken at the 5% level.

Results

SO_2 AND MBS STUDIES

There was no significant difference between baseline mean (SD) FEV_1 on the different study days (tables 3 and 4) and these values did not differ from the mean baseline FEV_1 of 3·40 (0·59) l recorded before histamine challenge.

All subjects responded to inhalation of MBS during both MBS and PMBS challenges. Inhalation of acidic, hyperosmolar PBS had no significant effect on baseline pulmonary function (table 3). MBS PD_{20} values ranged from 1·7 to 12·0 µmol with a geometric mean of 4·47 µmol (95% CI 3·16 to 6·31) (table 1). PMBS PD_{20} values ranged from 1·3 to 12·0 µmol with a geometric mean of 4·47 µmol (95% CI 3·07 to 6·50). The mean change between MBS and PMBS PD_{20} of 0·99 (95% CI 0·79 to 1·25) fold differences was not significant (fig 1).

One subject (no. 15) did not respond to inhalation of SO_2, either during SO_2 or PSO_2 challenges. This subject was assigned a value of 23·5 ppm for both challenges. There was no significant difference in mean FEV_1 before and after inhalation of the humidified air control which preceded SO_2 challenges on both study days (table 4), nor did the mean change in FEV_1 after inhalation of humidified air differ significantly on the two days. On the SO_2 study day the mean difference between prechallenge FEV_1 and FEV_1 measured after inhalation of the humidified control was 0·023 l (95% CI −0·02 to 0·06) compared with a mean difference of −0·003 l (95% CI −0·04 to 0·03) measured on the PSO_2 study day. The mean $\dot{V}E$ recorded during SO_2 challenges on SO_2 and PSO_2 challenge days of 40·6 (12·34) l/min and 40·8 (10·46) l/min, respectively, were similar.

Baseline FEV_1 did not change significantly

Table 4 Forced expiratory volume in one second (FEV_1) in asthmatic subjects for sulphur dioxide (SO_2) challenge and SO_2 preceded by phosphate buffered saline (PBS) challenge (PSO_2): baseline, after PBS challenge, and after inhalation of humidified air control values

	SO_2 challenge FEV_1 (l)		*PSO_2 challenge FEV_1 (l)*		
Subject no.	*Baseline*	*After air*	*Baseline*	*After PBS*	*After air*
1	3·50	3·45	3·40	3·35	3·35
2	3·35	3·40	3·30	3·20	3·20
3	3·30	3·35	3·50	3·50	3·55
4	4·45	4·50	4·50	4·50	4·50
5	3·35	3·40	3·20	3·30	3·20
6	2·50	2·45	2·60	2·65	2·65
7	3·25	3·15	3·00	3·10	3·00
8	3·95	4·15	4·00	4·00	4·00
9	3·20	3·20	3·10	3·15	3·15
10	2·10	2·10	2·10	2·15	2·15
11	2·70	2·70	2·80	2·75	2·70
12	3·70	3·80	3·70	3·70	3·75
13	3·85	3·90	4·00	4·00	4·15
14	3·85	3·90	3·80	3·80	3·85
15	3·65	3·60	3·80	3·80	3·80
Mean	3·38	3·40	3·39	3·40	3·40
SD	0·60	0·64	0·62	0·60	0·63

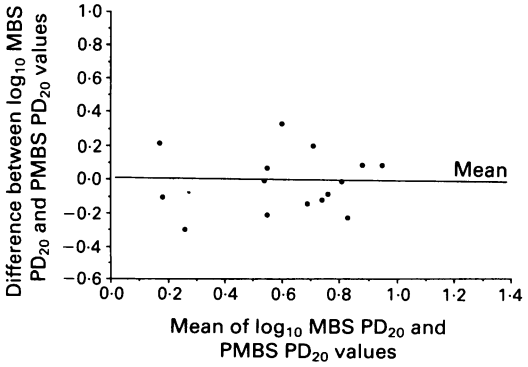

Figure 1 Relation in each subject (n=15) between the mean of log_{10} PD_{20} values following challenge with metabisulphite (MBS) and challenge with phosphate buffered saline followed by MBS (PMBS), and the difference between log_{10} PD_{20} MBS and log_{10} PD_{20} PMBS. PD_{20} MBS is the dose of MBS producing a 20% fall in FEV_1.

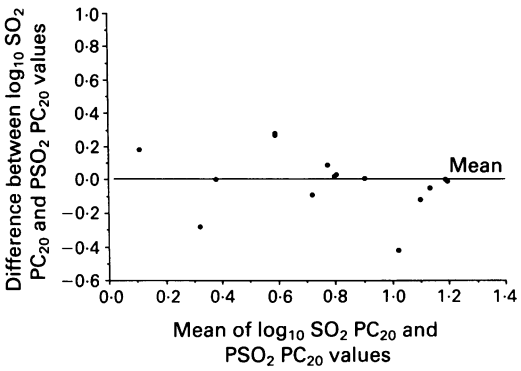

Figure 2 Relation in each subject (n=15) between the mean of log_{10} PC_{20} values following challenge with sulphur dioxide (SO_2) and challenge with phosphate buffered saline followed by SO_2 (PSO_2), and the difference between log_{10} PC_{20} SO_2 and log_{10} PC_{20} PSO_2. PC_{20} SO_2 is the cumulative concentration of SO_2 producing a 20% fall in FEV_1.

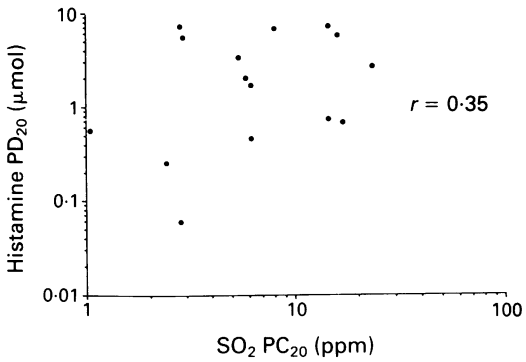

Figure 4 Relation between the PC_{20} for sulphur dioxide (SO_2) (ppm) and the PD_{20} for histamine (μmol) in 15 asthmatic subjects.

following challenge with PBS (table 4). Sulphur dioxide PC_{20} values ranged from 1·05 to 23·5 ppm with a geometric mean of 6·17 ppm (95% CI 3·77 to 10·01) (table 1), and PSO_2 PC_{20} values ranged from 1·5 to 23·5 ppm with a geometric mean of 6·08 ppm (95% CI 3·95 to 9·35). The mean change between SO_2 and PSO_2 PC_{20} of 0·99 (95% CI 0·78 to 1·25) fold differences was not significant (fig 2).

The correlation between MBS PD_{20} and SO_2 PC_{20} was not significantly related ($r=0·42$, $p>0·05$) (fig 3). Responsiveness to histamine did not correlate significantly with responsiveness to either SO_2 ($r=0·35$, $p>0·05$ (fig 4) or to MBS ($r=0·47$, $p>0·05$) (fig 5).

CONCENTRATION OF SO_2 INHALED DURING SO_2 AND MBS CHALLENGES

The effect of MBS did not appear to be cumulative. When three subjects inhaled increasing doses of MBS FEV_1 fell by a mean of 25·6 (4·3)% at the dose of MBS which caused FEV_1 to fall by more than 20% from baseline. This change in FEV_1 was similar to the mean change in FEV_1 of 24·3 (2·3)% which was recorded when only the final MBS dose was inhaled. In contrast, the effect of SO_2 appeared cumulative. When three subjects inhaled SO_2 the fall in FEV_1 at the concentration of SO_2 which caused FEV_1 to fall by more than 20% from baseline was a mean of 22·8 (2·6)%. This change differed significantly from a mean fall in FEV_1 of 9·3 (0·6)% which occurred when only the final concentration of SO_2 was inhaled.

The mean concentrations of SO_2 released by the 6·2, 12·5, 50, and 100 mg/ml solutions of MBS were 1·3 (0·14) ppm (range 0·9–1·4), 1·92 (0·13) ppm (range 1·8–2·1), 18·24 (1·65) ppm (range 17–21·0), and 51·25 (3·89) ppm (range 48–54), respectively. As MBS did not act cumulatively, the concentration of SO_2 inhaled by each subject during an MBS challenge was calculated using only the concentration of SO_2 generated by the dose of MBS which caused FEV_1 to fall by 20% from baseline. As SO_2 challenges were cumulative, the concentration of SO_2 inhaled by each subject was the sum of all the SO_2 inhaled during the doses prior to and including the SO_2 concentration which caused FEV_1 to fall more than 20% from baseline.

Using these results it was estimated that, during MBS challenges, the total SO_2 concentration delivered ranged from 45 to 381 ppm, with a mean of 168 ppm (95% CI 119 to 217). This was significantly different ($p<0·0001$) from the total concentration of SO_2 inhaled during SO_2 challenges which ranged from 300 to 2325 ppm with a mean of 957 ppm (95% CI 564 to 1350).

UPTAKE OF SO_2 DURING SO_2 AND MBS CHALLENGES

In vivo experiments confirmed that uptake of SO_2 generated by each dose of MBS was almost complete. The SO_2 concentration

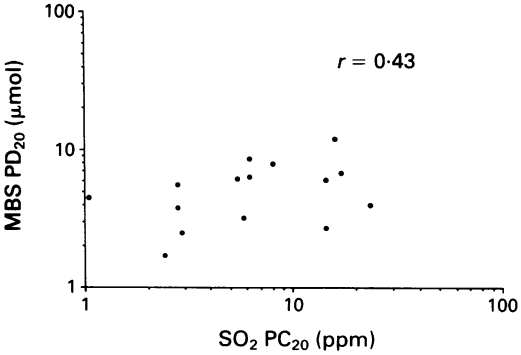

Figure 3 Relation between the PC_{20} for sulphur dioxide (SO_2) (ppm) and the PD_{20} for metabisulphite (MBS) (μmol) in 15 asthmatic subjects.

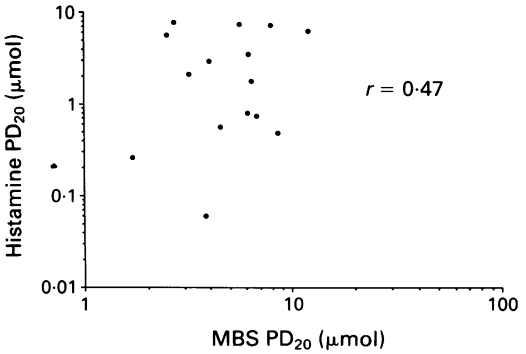

Figure 5 Relation between the PD_{20} for metabisulphite (MBS) (μmol) and the PD_{20} for histamine (μmol).

measured on exhalation after inhalation of each MBS dose was 0·5 ppm. When a dose of MBS involved more than one inhalation the concentration of SO_2 in exhaled samples was the same, either when measured after each inhalation or when measured after the final inhalation. The estimated uptake of SO_2 was 60% for a 0·1 µmol dose of MBS, 75% for 0·2, 0·4, and 0·8 µmol doses, and between 95% and 97% for 1·6, 3·2, 6·4, and 12·8 µmol doses of MBS. Uptake of SO_2 was 80% for all SO_2 concentrations.

When these results were used to estimate SO_2 uptake it was calculated that, during MBS challenges, SO_2 uptake ranged from 43 to 377 ppm with a mean of 165 ppm (95% CI 116 to 214). This differed significantly ($p < 0·001$) from the uptake of SO_2 during SO_2 challenges, when estimates ranged from 140 to 1860 ppm with a mean of 765 ppm (95% CI 450 to 1080).

Discussion

Although inhalation of nebulised MBS is thought to provoke bronchoconstriction via generated SO_2, no relation between SO_2 and MBS airway responsiveness was found in this study. However, as all subjects recruited reacted to relatively high doses of both MBS and SO_2, airway reactivity to MBS and SO_2 may be related in subjects more sensitive to both agents. In addition, as a small number of subjects were studied, the failure to find a relation between SO_2 and MBS responsiveness may have been due to a type II error.

The properties of aerosols which can cause airway narrowing in asthmatic subjects include the osmolarity, pH, and titratable acidity.[11 12] Bronchoconstriction is provoked by inhalation of hyperosmolar solutions[12] and by inhalation of acidic solutions, with buffered acidic solutions inducing more severe airway narrowing than unbuffered solutions.[11] The MBS solutions administered in this study were hyperosmolar and acidic and were also buffered by phosphate saline. However, when subjects inhaled PBS solutions of osmolarity, pH, and titratable acidity equivalent to the MBS solutions, no bronchoconstriction was observed. These results are supported by the findings of Wright *et al*[4] who partially investigated whether the properties of MBS solutions affected MBS responses. In their study five asthmatic subjects did not bronchoconstrict after challenge with saline solutions of osmolarity equivalent to the MBS solutions.

The acidic, hyperosmolar properties of the PBS solutions did not appear to potentiate airway responsiveness. There was no difference between SO_2 PC_{20} values obtained when an SO_2 challenge was performed alone or preceded by a PBS challenge and, similarly, an initial PBS challenge did not affect MBS responsiveness. It is most likely that the properties of the solution in which MBS was dissolved did not affect the response to MBS because the quantity of aerosol adminstered was so small. In studies investigating the bronchoconstrictive potential of hyperosmolar aerosols[12 13] and acidic aerosols[11] the minimum volume inhaled has been 2 ml. The mean output of DeVilbiss nebulisers used in this study was 0·018 ml per puff and, therefore, during an MBS challenge the greatest amount of aerosol administered was only 0·14 ml.

The lack of a relation between SO_2 and MBS airway responsiveness and the lower estimates of the amount of SO_2 inhaled and absorbed during MBS challenges suggest that MBS induced bronchoconstriction is not solely due to generated SO_2. When MBS is dissolved in solution it reacts chemically to form bisulphite and sulphite and SO_2 is generated. These substances enter into equilibrium with each other, with more acidic solutions favouring generation of SO_2. Bisulphite and SO_2 are potent bronchoconstricting agents, whereas sulphite has only a weak effect.[5] During MBS challenges aerosolised bisulphite and generated SO_2 are highly reactive and it is likely that these bronchoconstricting stimuli continue to interact after inhalation. Bronchoconstriction could result from bisulphite ions deposited directly in the airways or formed locally from dissolved SO_2 gas and from SO_2, either inhaled or generated from bisulphite in the airways. In contrast, during SO_2 challenges, when a constant concentration of SO_2 is inhaled, SO_2 is quickly absorbed in the aqueous environment of the airways.[14] At the pH of human airways, which averages 6·6,[15] it is likely that most of the inhaled SO_2 rapidly converts to bisulphite.[16] Thus, both SO_2 and bisulphite probably play a part in SO_2 and MBS induced bronchoconstriction, but the contribution of each stimulus to each challenge differs. Such a difference may underlie the lack of a relation between SO_2 and MBS challenges.

Sulphur dioxide is almost completely absorbed when inhaled via the nose,[14] but when inhaled via the mouth absorption of SO_2 is altered by the concentration of SO_2 administered and, more importantly, by the rate of administration.[14 18] When 1 ppm and 10 ppm SO_2 were administered to rabbits SO_2 absorption decreased from 99·5% to 96·3%, but following a tenfold increase in the rate of administration SO_2 absorption fell to 66%.[19] In our study it was estimated that 80% of each concentration of SO_2 inhaled was absorbed. This uniform amount of absorption was most probably due to the rate of administration of SO_2 which was inhaled at an average $\dot{V}E$ of 40 l/min. Factors which could influence absorption of SO_2 or bisulphite ions generated during MBS challenges have not been investigated but, in our study, in vivo experiments indicated that more SO_2 was absorbed at the higher MBS concentrations. One factor which could have affected our estimates of SO_2 absorption in both MBS and SO_2 challenges was desorption of SO_2 from the mucosal surfaces of the upper airway. This begins immediately after ceasing exposure to SO_2 and about 15% of the inspired concentration is desorbed over 30 minutes.[14 19] It is unlikely, however, that SO_2 desorption significantly contributed to exhaled SO_2 measurements performed in our study as SO_2 concentrations were sampled over a matter of seconds.

The lack of correlation between airway responsiveness to histamine and to either MBS or to SO_2 has been observed in other studies.[4 20] We also confirmed that MBS did not act cumulatively, which Wright *et al*[4] had clearly demonstrated in 11 asthmatic subjects. However, increasing concentrations of SO_2 were found to be cumulative in effect. This difference was studied in only a small number of asthmatic subjects because we had previously observed, in 15 asthmatic subjects using specific airway resistance to measure the airway response (unpublished data), that SO_2 acted cumulatively when administered using the protocol described in this study. It is possible that this differing characteristic of MBS and SO_2 responses relates to the duration of exposure to SO_2 as, during SO_2 challenges SO_2 was inhaled continuously for three minute periods, whereas inhalation of MBS involved only three second breath holds.

In conclusion, MBS and SO_2 airway responsiveness were not related in subjects with asthma. Although it is not clear whether bronchoconstriction provoked by inhalation of MBS is due to the effect of generated SO_2 or bisulphite ions,[5] the difference between the estimated amounts of SO_2 inhaled during SO_2 and MBS challenges, the differing estimates of SO_2 uptake during both challenges, and the lack of a relation between MBS and SO_2 airway reactivity all indicate that MBS induced bronchoconstriction involves mechanisms additional to the effect of generated SO_2.

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