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THE EFFECT OF SODIUM 2-MERCAPTO-ETHANE SULPHONATE AND HYPERTONIC SALINE AEROSOLS ON BRONCHIAL CLEARANCE IN CHRONIC BRONCHITIS

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1 The efficacy of a mucolytic agent, 2-mercapto-ethane sulphonate, administered in the form of an aerosol was evaluated in a group of eleven patients with chronic bronchitis in a controlled, double-blind, crossover study.

2 Saline aerosol isotonic (1.21M, 7.1%) to the drug was used as a placebo.

3 Approximately 1 ml drug/placebo was inhaled by the patients twice a day for 3 days and a final dose was given on the mornings of the drug/placebo trial runs.

4 There was no improvement in this group of patients in lung function or subjective well being attributable to the drug.

5 The viscosity of sputum, dry macromolecular weight and N-acetyl neuraminic acid/fucose ratio remained unaltered throughout the study.

6 An enhancement of tracheobronchial clearance was obtained following the administration of either placebo (31%) or drug aerosols (24%) Statistical significance ($P < 0.01$) was only achieved for the placebo and was attributed to an increase in sputum volume.

Introduction

The efficacy of mucolytic drugs has in the past been evaluated by their effect, singly or in combination on clinical state and subjective assessment (Aylward, 1973), lung function (Clarke, Craig & Makin, 1972), rheological and chemical properties of sputum (Dippy & Davis, 1969; Havez, Degand, Roussel & Randoux, 1970; Marriott & Richards, 1974) and clearance of bronchial secretions (Thomson, Pavia, Jones & McQuiston, 1975). All four methods have been employed in a study, reported below on the efficacy of a mucolytic agent, sodium 2-mercapto-ethane sulphonate, which was administered in the form of an aerosol to patients with chronic bronchitis. Saline having the same tonicity (1.21 M; 7.1%) as the drug under investigation was used as a placebo.

Methods

Eleven patients (three female) with chronic bronchitis (Medical Research Council, 1965) took part in the study. All were stable throughout the trial period. Their mean (±s.d.) age was 62.8 ± 8.0 years and height 1.67 ± 0.03 m. There were ten smokers who had smoked on average 38.0 ± 12.3 pack-years.

The ventilatory capacity of the patients when first seen was assessed by a dry bellows spirometer (Vitalograph®) and the Wright peak flow meter. Their mean (±s.d.) ventilatory indices together with the percentages of predicted (Cotes, 1975) in parentheses were: forced expiratory volume in 1 s (FEV_1), 1.29 ± 0.48 l (50 ± 7%); forced vital capacity (FVC), 2.76 ± 0.74 l (81 ± 6%); FEV_1/FVC, 47 ± 4% (67 ± 5%) and peak flow rate (PFR), 202 ± 22 l/min (48 ± 19%).

The sputum produced when the patients were first seen was classified in the manner prescribed by the Medical Research Council (1965) as M1 (mucoid; white) in four patients; M2 (mucoid; white with a hint of yellow) in one patient; P1 (purulent; less than $\frac{1}{3}$ yellow) in one patient; P2 (purulent; $\frac{1}{3}$–$\frac{2}{3}$ yellow) in two patients and P3 (purulent; more than $\frac{2}{3}$ yellow) in another; two patients produced no sputum during the trials.

Although the patients were not chosen specifically because of difficulty in coughing phlegm, we have

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shown that patients who do not complain of this are capable of responding to expectorant and mucolytic drugs (Thomson, Pavia & McNicol, 1973; Thomson, Pavia, Gregg & Stark, 1974). Informed written consent was obtained from each patient.

Tracer techniques

The radioaerosol technique has been fully reported by Thomson & Short (1969). Polystyrene particles (5.0 ± 0.7 μm) permanently tagged with the gamma emitting radionuclide ^{99m}Tc (Few, Short & Thomson, 1970) were generated by a spinning disc (May, 1949) and inhaled via the mouth by the patients seated upright. Each patient inspired from functional residual capacity (FRC), known volumes of aerosol in a series of single breaths which were automatically cut off by solenoid valve at the preset volume (0.3 l). Thereafter there was an obligatory 3 s breath-holding pause before exhalation. The average flow rate during inhalation was measured by pneumotachygraph. There was no statistically significant difference between the group mean ($\pm$ s.d.) of the individual average flow rates during the radioaerosol inhalations for the control (42 ± 6 l/min), placebo (40 ± 5 l/min) and drug runs (39 ± 4 l/min).

The patients washed out their mouths and swallowed some water after the inhalation of the radioaerosol to remove any particles present in the oropharynx or oesophagus. The initial depth of deposition of the particles was recorded by rectilinear gamma scanning (Dawson, Douglas, Pavia, Reeves, Short & Thomson, 1971) across the right lung at one inch intervals. The rate of clearance from the lungs was subsequently monitored by two opposing scintillation detectors (NaI (Tl) crystals, each 3.8 cm diameter × 2.5 cm thick), the output of which was averaged. One detector was at the sternum and the second axially opposite in the seated patients. The degree of collimation was such that the field of view of the detectors included most of both lungs but virtually excluded the stomach. Counts were made at frequent intervals (20–30 min) for 100 s during the 6 h observation period; all counts were corrected for radioactive background and decay. In all runs the counts were expressed as percentages of the first count to adjust for unavoidable differences in the initial radioactive lung burden; the burden did not exceed 30 μCi of ^{99m}Tc in any one run.

The patients were under continuous observation and did not smoke for one hour prior to the inhalation of the radioaerosol or throughout the 6 h experimental period.

During the trial runs the times of all coughs and sputum samples were noted and the type, total weight and radioactive content of the samples were ascertained. The data of the various parameters measured in this study were not normally distributed. A non-parametric method, the Wilcoxon test for pair differences (Snedecor & Cochran, 1968), has been applied throughout this study to analyse the data.

Rheological and chemical studies

The viscosity was measured with a Ferranti-Shirley cone and plate viscometer using a 200 g.cm torque spring and a 7 cm diameter cone. The specimens were tested over a speed range 0–1800 s^{-1} and a sweep time of 120 s as described by Palmer, Ballantyne, Diament & Hamilton (1970) and Charman & Reid (1972). The viscosity (poise) was calculated at 1350 s^{-1} and each value represents the mean of two aliquots. The sputum samples were tested, without pretreatment, within 1–3 h after collection.

The remainder of the specimen was stored at −20°C for chemical analysis that included estimation of dry macromolecular weight, fucose (Gibbons, 1955) and N-acetyl neuraminic acid (Warren, 1959).

Experimental design

Existing therapy was maintained with the exception of mucolytic and expectorant drugs which were discontinued for 1 week before and throughout the trial period. The control runs were done first and then in a random double-blind crossover manner each of the eleven patients was allotted placebo or drug treatment subject to half receiving one treatment first. The three runs in each patient were one week apart. Both treatment aerosols were inhaled twice daily (in the morning and early evening) on the three days before the trial runs.

The treatment runs were identical to the control runs save that final doses of both treatment aerosols were inhaled 30 min after the inhalation of the radioaerosol.

Treatment aerosol

Hypertonic saline (1.21 M) or drug (Mistabron®) aerosol was produced by a MistO_2gen® ultrasonic generator (type: EN140) and of this air mixture 100 l were inhaled directly from the generator by the seated patients wearing nose clips. It was estimated from the amount of saline or drug left in the reservoir and tubing that 1.9 ± 0.9 ml (s.d.) of placebo/drug entered the patients' mouths. Mercer, Goddard & Flores (1968) reported that the mass median diameter of the droplets produced by the generator was 6.5 μm. The mean ($\pm$ s.d.) time taken to inhale the treatment aerosol was 10.6 ± 3.2 min. Asmundsson, Johnson, Kilburn & Goodrich (1973) measured the airway deposition from an ultrasonic generator and their findings indicate that less than 1 ml of solution is deposited on the airway during our 10 min aerosol inhalation.

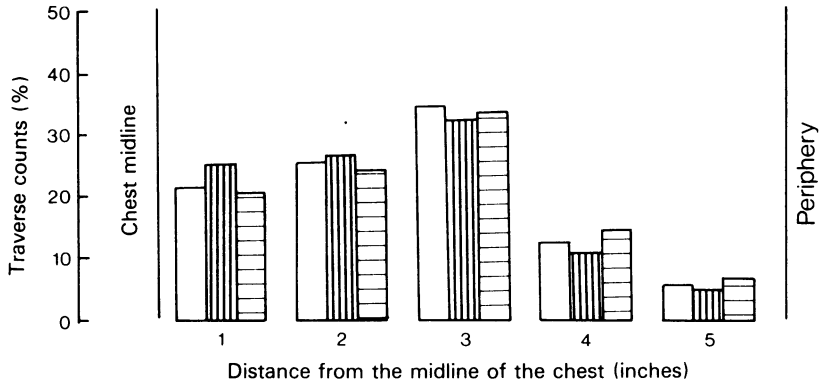

Figure 1 Initial lateral distribution of inhaled radioaerosol across the right lung. The heights of the columns represent for control (□), placebo (saline) (▥)and drug (▤) runs the means of the individual counts for each traverse of the scan expressed as percentages of the total scan counts.

Results

Lung scans

Figure 1 shows the mean initial lateral distribution of the radioaerosol particles across the right lung for the eleven patients for the control, placebo and drug runs. The counts for each traverse have been expressed as a percentage of the total traverse count for each individual and the mean percentages for all patients are shown as the height of the columns in Figure 1. An index of penetration (Thomson & Pavia, 1974) is obtained from the ratio of the sums of the means of traverses 4 and 5 to those of 1 and 2. These mean ratios ($\pm$s.d.) were 0.40 ± 0.22, 0.31 ± 0.18 and 0.52 ± 0.31 for the control, placebo and drug runs respectively. There was no significant difference in the initial lateral deposition of the radioaerosol between the control and placebo or between the control drug run.

Lung clearance

Figure 2 shows the mean clearance curves from whole lung counts at half-hourly intervals for the eleven patients for the control, placebo and drug runs. All three curves are of the expected exponential type. The mean retained lung burden was lower in the placebo and drug than in the control runs at all post-treatment counts. The differentiation between the curves took

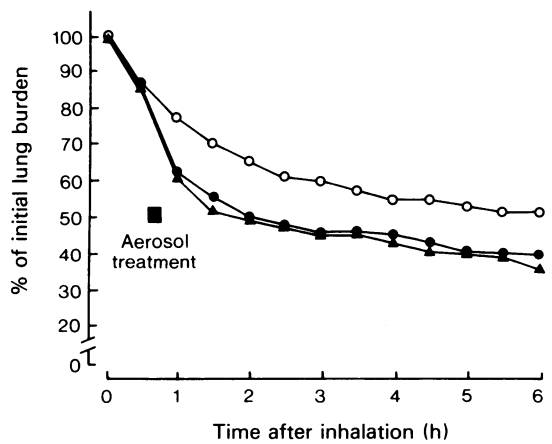

Figure 2 Mean clearance curves from whole lung counts for eleven patients for control (○), placebo (saline) (▲) and drug (●) runs.

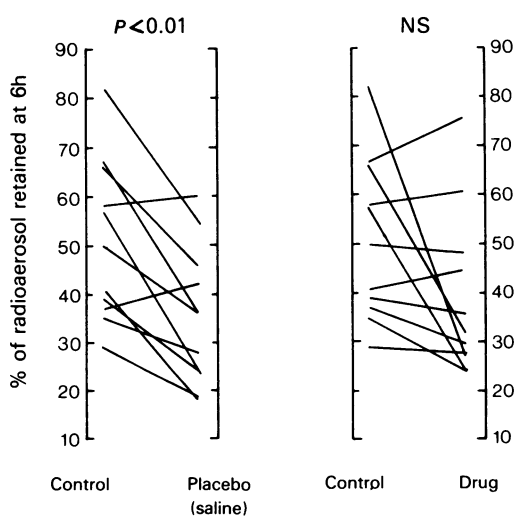

Figure 3 Individual (eleven patients) paired differences for the amount of radioaerosol retained in the lungs at 6 h as a % of the initial value between (a) control and placebo runs and (b) control and drug runs.

place between 0–1.5 h: thereafter (between 2–6 h) the three graphs were almost parallel. Figure 3 shows the paired differences for the amount of radioaerosol present in the lungs at 6 h expressed as a percentage of the initial value between (a) control and placebo runs and (b) control and drug runs. The mean retention was higher in the control runs (51%) compared to the placebo (35%) and drug runs (39%) although statistical significance was only found in the control-placebo comparison ($P<0.01$). A comparable analysis at 2 h showed substantially the same results.

Cough and sputum

Table 1 shows the mean (±s.d.) number of coughs and the weight, radioactive content and specific activity (gamma counts/g) of sputum voided during the control, placebo and drug runs. The difference in the number of coughs was not significant between the three runs. However, the mean weight and radioactive content of sputum was statistically ($P<0.02$) significant from the control in the placebo runs only. There was no difference in the specific activity of sputum between the three runs.

Rheology and chemistry

It was only possible to carry out rheological and chemical data in nine out of the eleven patients. Levels of viscosity and concentrations of dry macromolecular weight, fucose, N-acetyl neuraminic acid (NANA) and NANA/fucose ratio for control, placebo and drug periods are given in Table 2. The mean levels of viscosity for the group during drug and placebo were higher than during control periods but the difference did not reach significance. Although the concentrations of NANA and fucose, particularly fucose, were higher during the drug period the differences were not statistically significant. Dry macromolecular weight and NANA/fuscose ratio changed very little throughout the study.

Subjective assessment

The answers to questions about change in breathlessness, quantity and type of sputum, and difficulty in expectorating were equivocal for the placebo and drug runs.

Lung functions

Table 3 shows the mean (±s.d.) FEV_1, FVC, FEV_1/FVC and PFR for the control runs as well as those after 3 days of placebo and drug treatments. There was no significant difference between the three runs for any of the ventilatory indices which were obtained before the inhalation of the radioaerosol.

Discussion

The visco-elastic properties of mucus which are essential for efficient functioning of the mucociliary system are determined by the degree of crosslinking between macromolecules (King, Gilboa, Meyer &

Table 1 Mean and s.d. of number of coughs and sputum weight, radioactivity and specific activity for the eleven patients for control, placebo (saline) and drug runs.

	Number of coughs			*Sputum weight (g)*			*Sputum gamma counts × 10^3*			*Sputum specific activity (10^3 counts/g)*		
	C	*P*	*D*	*C*	*P*	*D*	*C*	*P*	*D*	*C*	*P*	*D*
Mean	92	91	99	7.9	12.0*	7.8	117	257*	117	14.49	40.63	16.03
s.d.	112	116	154	10.3	12.6	8.5	127	225	141	18.59	57.81	15.49

C: Control; P: Placebo; D: Drug.
*$P<0.02$ for change from control run.

Table 2 Mean ± s.d. of levels of viscosity, concentrations of dry macromolecular weight, fucose, neuraminic acid (NANA) and NANA/fucose ratio

Trial run	*Control*	*Placebo*	*Drug*
Viscosity (poise)	0.65 ± 0.39	0.75 ± 0.47	0.81 ± 0.47
Dry macromolecular weight (mg/ml)	23.13 ± 16.78	24.82 ± 14.75	23.61 ± 13.00
Fucose (μmol/ml)	3.82 ± 2.64	4.62 ± 3.64	6.12 ± 4.32
NANA (μmol/ml)	2.57 ± 1.11	2.98 ± 1.61	3.24 ± 1.30
NANA/fucose ratio	0.73 ± 0.28	0.77 ± 0.39	0.72 ± 0.25

There was no statistical significance for any of the variables between any two trial runs

Table 3 Mean and s.d. FEV_1, FVC, FEV_1/FVC and PFR before the control, placebo (saline) and drug runs

	FEV_1 (l)			*FVC (l)*			*FEV_1/FVC (%)*			*PFR (l/min)*		
	C	*P*	*D*	*C*	*P*	*D*	*C*	*P*	*D*	*C*	*P*	*D*
Mean	1.3	1.3	1.3	2.8	2.7	2.7	47	49	47	202	187	182
s.d.	0.5	0.5	0.6	0.7	0.8	0.9	13	11	12	71	60	65

C: Control; P: Placebo; D: Drug.
There was no statistical significance for any of the ventilatory indices between any two trial runs.

Silberberg, 1974), notably between glycoprotein subunits and between glycoprotein and plasma type glycoproteins such as IgA (Roberts, 1976; Creeth, Bhaskar, Horton, Das, Lopez-Vidriero & Reid, 1977).

Like acetylcysteine (Hurst, Shaw & LeMaistre, 1967) sodium 2-mercapto-ethane sulphonate has a mucolytic action *in vitro* (Hirsch, Kory & Hamilton, 1966) by virtue of its reactive sulphydryl group. It is said to reduce sputum viscosity by breaking the long glycoprotein chains at their disulphide bonds. Hirsch, Zastrow & Kory (1969) graded it third most active of a series of compounds which they examined by their 'consistometer'. The present trial indicates that it accelerates the clearance of mucus from the lungs of chronic bronchitics but that its efficacy is no greater than that of saline of comparable tonicity. However the mechanism by which clearance was accelerated may not have been the same in drug and placebo. In the present study sodium 2-mercapto-ethane sulphonate had no effect on sputum viscosity and a similar negative finding has also been reported by Matthew (personal communication) in children with cystic fibrosis treated over a period of 2 months. More sputum was produced ($P<0.02$) over the 6 h trial period in the placebo (saline) than in the control runs (12.6 *v.* 7.8 g). Moreover the radioactive content of sputum was higher ($P<0.02$) in the placebo runs (257×10^3 gamma counts) compared to the control run (117×10^3 gamma counts). Hypertonic saline may therefore have acted by increasing mucus production as a result of an increase in serum transudate component (see below) whereas it appears that the drug may have enhanced mucociliary clearance. This is supported by the finding that sputum produced after inhaling the drug contained more fucose — a marker of mucus — than either placebo or control sputum.

A placebo aerosol was used in the present trial in addition to a control without aerosol for the following reasons. Even slight contact of the mucus membrane of the respiratory tract eg. by cotton wool (Hilding & Hilding, 1962), may provoke increased glandular secretions. The impact of aerosol particles on the membrane might have had the same effect and if so this could complicate the interpretation of the results had an aerosol placebo not been used. Also Marin, Davis & Nadel (1976) have demonstrated that the net effect of ion fluxes across the living mucous membrane can produce an osmotic gradient which would lead to the transfer of water into the mucous layer (transudation). The addition of water to mucus could enhance ciliary clearance by reducing its viscosity or, conceivably, improve the efficiency of coughing by mobilising sticky mucus (Hilding, 1943). It may be relevant that the addition of salts reduces the drag efficiency of aqueous polymer solutions (Little & Patterson, 1974). A further non specific effect of even non-irritant aerosols is short lived bronchoconstriction (Widdicombe, Kent & Nadel, 1962) which may improve the efficiency of cough by increasing air velocity and stability of airway walls although it is unlikely to affect ciliary clearance appreciably. Thus in addition to the control run without aerosol, placebo isotonic to the drug (1.2.1 M) was required to control these possible non-specific effects of the drug. For this purpose sodium chloride although not ideal, had the same cation as the drug and was not more irritant as judged by the absence of coughing during administration.

To avoid missing any transitory beneficial effect, a final drug dose and necessarily also that of the placebo was given on the morning of the trial. If the effect of the drug was cumulative the airways should have been more patent after 3 days' treatment permitting greater penetration of the radioaerosol into the lung (Thomson *et al.*, 1974); in the event the index of penetration was higher in the drug than in the control or placebo runs but the difference was not statistically significant.

In conclusion the enhancement of clearance of lung secretions attributable to the hypertonic saline aerosol appears to be related to an increase in sputum volume. Although on average the tracheobronchial mucociliary clearance after the drug administration was faster than the control run it failed to reach statistical significance and it did not appear to be related to changes in the chemical or rheological properties of sputum. One cannot exclude from the present data a possible enhancement of ciliary beat, changes in the elastic properties of mucus or in the thickness of the sol or periciliary layer favouring a more efficient mucociliary clearance mechanism as a result of administering the drug.

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