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IN-VITRO COMPARISON OF THE MUCOLYTIC ACTIVITY OF SODIUM METABISULFITE, N-ACETYLCYSTEINE AND DITHIOTHREITOL

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ABSTRACT

Using the *in vitro* mucin model, sodium metabisulfite was shown to exhibit considerable mucolytic activity. Compared on a molar basis, it is less powerful in viscosity reduction than dithiothreitol but stronger than N-acetylcysteine, which is currently the most often used mucolytic agent. Combinations of sodium metabisulfite and N-acetylcysteine at relatively low concentrations exhibited equivalent and pronounced mucolytic activity as that produced by a high concentration of N-acetylcysteine. The possibility of combining two or more mucolytic agents at low concentrations to give a less toxic but, nevertheless, effective product is raised. Finally, the *in vitro* mucin model appeared to be incapable of distinguishing highly

mucolytic solutions, and caution must be exercised in utilizing this model for testing such solutions.

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Mucolytic substances can decrease the viscosity of pulmonary mucous secretions, and are thus useful as adjuvant therapeutic agents in patients who have chronic or acute bronchopulmonary diseases such as emphysema and cystic fibrosis. Although there is an apparent clinical need for these drugs, only a very limited number of mucolytic products are presently available[1]. It is, therefore, useful to identify and evaluate potentially beneficial substances for their mucolytic activity.

The viscosity of pulmonary mucous secretions is mainly a function of the concentration of mucoprotein present with deoxyribonucleic acid concentration playing a minor role[2]. An apparent correlation between the breaking of disulfide bonds and the reduction in viscosity of mucoprotein solutions has been demonstrated[2]. Thus, compounds such as N-acetylcysteine and dithiothreitol, which can participate in a sulfhydryl exchange reaction through their free thiol (-SH) group, have shown good mucolytic activity[3]. However, because of the strong S-nucleophilicity of the -SH group, these compounds, particularly dithiothreitol, are potentially toxic substances. The sulfite functional group is a somewhat weaker S-nucleophile than the -SH group[4] and may, therefore, be less toxic. Sulphite com-

pounds, however, may still possess sufficient mucolytic activity to elicit beneficial clinical responses. In this study, the mucolytic activity of a sulfite compound, viz.: sodium metabisulfite, is compared to those of two well-established mucolytic agents, N-acetylcysteine and dithiothreitol, using an *in vitro* mucin model. The effectiveness in viscosity reduction of combinations of sodium metabisulfite and N-acetylcysteine is also examined.

EXPERIMENTAL

The *in vitro* model used was essentially identical to that developed by Davis *et al*[5] with minor modifications. Thirty grams of gastric mucin (ICN-K &K Laboratories, Inc., NY) was dissolved in 300 ml of 0.1 M tris buffer adjusted to pH 8.0. The resulting viscous solution was divided into three 100 ml portions, each of which was placed in a 250 ml beaker covered with parafilm and refrigerated overnight. A period of at least 12 hours was allowed for hydration of the mucin and for air bubbles which had been incorporated during mixing to escape. The beakers were then placed in a water bath (37°C) and allowed to equilibrate for one hour after which the initial viscosity was measured. The viscometer used was a Brookfield Model RVT fitted with spindle T-A rotated at 100 rpm. N-acetylcysteine (Eastman Kodak Co., NY), dithiothreitol (J. T. Baker Chemical Co., NJ) and sodium metabisulfite (Fisher Scientific Co., NJ)

were used without purification. The agent being tested for mucolytic activity was dissolved in 5 ml of water and introduced to the mucin solution via a pipet. The resultant solution was then mixed with a three-blade mechanical stirrer at 250 rpm for two minutes. The time at which mixing ended was designated as t=0. Viscosity was measured at t=5, 15, 30, 45, 60, 75, 90, 105, 120, 135 and 150 minutes. When a combination of mucolytic agents was tested, the agents were separately dissolved in water and added simultaneously to the mucin solution.

RESULTS AND DISCUSSION

The Davis *in vitro* mucin model for screening mucolytic activity has been shown to be useful in predicting *in vivo* results[1]. The present procedure differed from the Davis method in two ways: (i) a Brookfield Model RVT viscometer was used instead of a Ferranti-Shirley viscometer, thus (ii) necessitating the use of a larger volume of mucin solution. However, as shown in Table 1, our viscosity data on previously

TABLE 1

Comparison of the Present Procedure with the Davis Mucin Model

	Mean % Reduction in Viscosity	
Mucolytic Agent	Present Study	From Davis[1]
Urea 4M	15.3	17
N-acetylcysteine (20%)	58.3	61
H_2O	22.4	19

tested mucolytic agents such as urea and N-acetylcysteine correlated well with those published by Davis[1]. Thus, our modifications did not appear to have affected the validity of the Davis model.

Fig. 1 shows some typical viscosity curves obtained after the addition of different concentrations of mucolytic agents. The control solution, distilled water, showed a decrease in viscosity of approximately 20% from 20 minutes to 150 minutes post mixing. This viscosity reduction perhaps represented

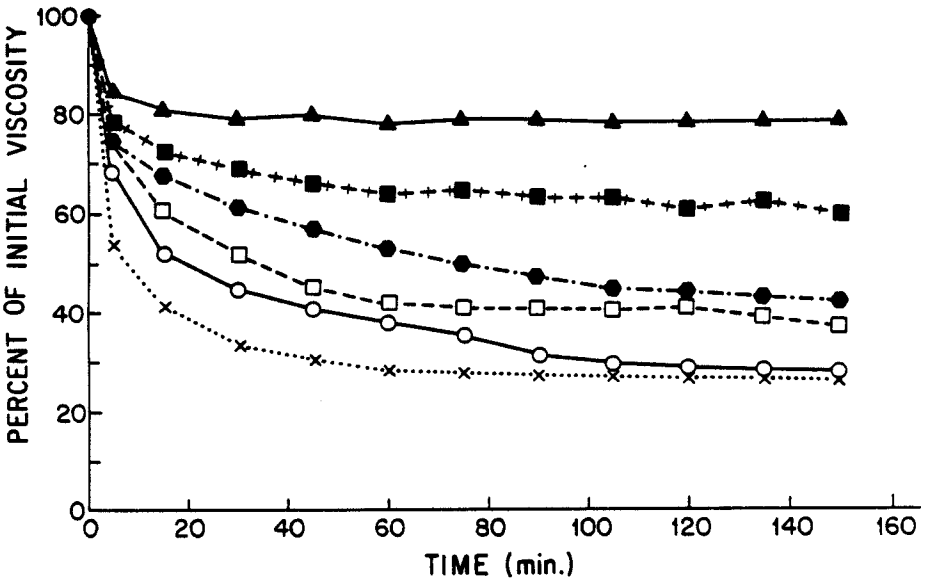

Fig. 1. Some typical viscosity curves after addition of mucolytic agents: (▲) water, (■) 0.1 M sodium metabisulfite, (⬢) 1.23 M N-acetylcysteine, (□) 0.5 M sodium metabisulfite, (○) 0.5 M sodium metabisulfite plus 0.61 M N-acetylcysteine, (X) 0.1 M dithiothreitol.

that caused by dilution of the mucin. In almost every case, the decrease in viscosity leveled off at about 150 minutes, and this "plateau" value was thus chosen to compare the effectiveness of different mucolytic agents (Table 2). Each value of mean percent reduction in initial viscosity listed is the mean of at least three separate determinations.

TABLE 2

Viscosity Reduction by Different Mucolytic Agents

Mucolytic Agent	Concentration in molar	Mean % reduction of initial viscosity @ 150 min. ± S.D.
N-acetylcysteine	2.45	74.3 ± 5.6
	1.84	67.1 ± 1.3
	1.23	58.3 ± 0.6
	0.61	53.8 ± 2.9
	0.31	44.2 ± 2.3
	0.15	34.3 ± 0.4
Dithiothreitol	0.10	73.1 ± 1.2
	0.05	23.0 ± 2.8
	0.01	24.0 ± 0.8
Sodium metabisulfite	1.00	71.0 ± 0.5
	0.50	62.6 ± 6.1
	0.25	51.4 ± 3.0
	0.10	41.1 ± 4.0

On examination of Table 2 it is apparent that viscosity reduction is dependent on the concentration used in all three mucolytic agents. At 0.15,M N-acetylcysteine produced a significantly larger decrease in viscosity than that observed for water. This viscosity reduction increased with increasing concentrations of the drug. Dithiothreitol showed very pronounced viscosity lowering at 0.1 molar, consistent with literature reports. However, at concentrations of 0.05 and 0.01 molar, the reduction in viscosity of mucin was not significantly different from control. Sodium metabisulfite exhibited considerable mucolytic activity and appeared to be a better mucolytic agent than N-acetylcysteine at comparable concentrations. Thus, for example, at 1.0 molar sodium metabisulfite, the mean % reduction of initial viscosity observed at 150 minutes was 71, compared to a value of about 58 produced by a 1.23 molar (20 % w/v) solution of N-acetylcysteine. Similar superiority in viscosity reduction by sodium metabisulfite was observed at lower concentrations. It appears, therefore, that sodium metabisulfite could be a useful mucolytic agent, and *in vivo* testing of its effectiveness may be warranted.

It is of interest to examine the mucolytic activity of combinations of mucolytic agents. If mucolytic activity is additive whilst toxicity is not, it may be possible to produce an effective mucolytic by combining sub-toxic concentrations of

two or more mucolytic agents. Combinations comprising of a fixed concentration of N-acetylcysteine, 0.61 molar (10% w/v) and varying concentrations of sodium metabisulfite (0 to 1 molar) were, therefore, tested for viscosity reduction. The results are shown in Table 3. At 0.10 molar sodium metabisulfite, no additive lowering of viscosity was observed. However, in the presence of sodium metabisulfite concentrations greater than 0.25 molar, the viscosity reduction produced by a 0.61 molar N-acetylcysteine solution increased from 54% to about 75%. This lowering of viscosity is equivalent to that produced by a 2.45 molar (40% w/v) solution of N-acetylcysteine. It seems, therefore, that equivalent mucolytic activity can be achieved through combination of relatively low concentrations of two mucolytic agents, thus possibly obviating the use of high (and potentially toxic) concentrations of one single drug when high mucolytic activity is desired.

TABLE 3

Viscosity Reduction by Combinations of N-acetylcysteine and Sodium Metabisulfite

Concentration of N-acetyl cysteine in molar	Concentration of Sodium Metabisulfite in molar	Mean % reduction of initial viscosity @ 150 min. ± S.D.
0.61	1.00	74.9 ± 1.9
0.61	0.50	72.3 ± 1.3
0.61	0.25	71.4 ± 0.4
0.61	0.10	44.7 ± 0.6
0.61	—	53.8 ± 2.9

It is noteworthy that of all the systems tested, none could produce a reduction in viscosity greater than 75% of the initial value. Interestingly enough, each mucolytic agent and the combination tested gave a lowering of viscosity in the general range of 70-75% at the maximum drug concentrations (Tables 2 and 3). It is possible that the present *in vitro* mucin model may not respond adequately in its lower viscosity limit to allow differentiation between highly mucolytic solutions. Other tests may have to be employed in conjunction with this technique when newer and more powerful mucolytic drugs are tested.

CONCLUSION

Using the *in vitro* mucin model, sodium metabisulfite was shown to be a more effective mucolytic agent than N-acetylcysteine when compared on a molar basis. Combinations of sodium metabisulfite and N-acetylcysteine at low concentrations produced similarly high mucolytic activity as that obtained from a 40% solution of N-acetylcysteine. The possibility of combining two or more mucolytic agents at relatively low concentrations to avoid toxicity is raised. The *in vitro* mucin model for mucolytic screening may have an apparent limitation in that it may fail to distinguish between highly mucolytic solutions. Alternate techniques may have to be utilized in conjunction with the mucin model when screening highly mucolytic compounds.

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