

# Effect of methacholine induced bronchoconstriction on the pulmonary distribution and plasma pharmacokinetics of inhaled sodium cromoglycate in subjects with normal and hyperreactive airways

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**ABSTRACT** Inhalation treatment may be less effective in the presence of bronchoconstriction because of the reduced penetration of drugs into the airways. The effect of bronchoconstriction on the lung deposition and plasma pharmacokinetics of inhaled sodium cromoglycate was examined. Ten subjects attended the laboratory on three occasions. On the first occasion a bronchial provocation test was performed to determine the concentration of methacholine required to reduce the forced expiratory volume in one second (FEV<sub>1</sub>) by 20% (PC<sub>20</sub>). On the two subsequent occasions subjects inhaled either saline or their PC<sub>20</sub> methacholine, followed five minutes later by an aerosol containing sodium cromoglycate and stannous phytate labelled with technetium-99m. Twenty minutes later a gamma emission lung scan was performed to determine the intrathoracic deposition of the nebulised aerosol. The central:peripheral (C : P) ratio of lung deposition was then calculated. Measurements of FEV<sub>1</sub> were made and blood samples taken for analysis of plasma sodium cromoglycate concentration at intervals for four hours. Methacholine led to a 23.4% (SEM 0.6%) lower FEV<sub>1</sub> and a 2.8 times higher C : P ratio than those observed after saline. There was a direct correlation between log PC<sub>20</sub> methacholine and the increase in the C : P ratio ( $r = 0.81$ ). Despite these changes with methacholine, the plasma pharmacokinetics of inhaled sodium cromoglycate were not significantly different after methacholine and after saline, except that the maximum concentration achieved (C<sub>max</sub>) was increased. These observations suggest that the area of cromoglycate deposition and the anatomical site are less important in determining the plasma pharmacokinetics of cromoglycate than is the total dose delivered to the lung.

## Introduction

In 1967 Howell and Altounyan first reported that inhaled sodium cromoglycate administered as a dry powder via a propeller device was efficacious in the treatment of chronic asthma.<sup>1</sup> Delivery of drugs for asthma directly to the airways introduces a degree of therapeutic selectivity by achieving a high local concentration of drug for the desired pharmacological effects with minimum systemic side effects.<sup>2</sup> Inhalation treatment for patients with airways disease is not,

however, without its problems. Good technique is necessary for optimal drug delivery from metered dose and dry powder inhalers, and specialised equipment is necessary for nebulised solutions. In addition, the degree of bronchoconstriction may reduce the penetration of drug into the more peripheral airways.<sup>3-5</sup> Changes in bronchial blood flow or the presence of mucosal oedema or excess mucus may decrease the passage of inhaled drugs to small airways, or alter drug clearance from their sites of deposition.

The introduction of a specific radioimmunoassay for sodium cromoglycate has made it possible to examine the pharmacokinetics of this drug.<sup>6</sup> After inhalation of cromoglycate as a dry powder from a Spinhaler plasma drug concentrations increase rapidly to reach a peak at 10-20 minutes, followed by a

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gradual decline. The rate of the decrease in serum drug concentrations is less than that seen after intravenous administration<sup>7-9</sup> and the absorption of sodium cromoglycate from the bronchial mucosa is rate limiting. The fall in plasma cromoglycate concentrations is therefore determined by the rate of drug absorption from the airways (absorption rate limited or flip flop kinetics).<sup>7-9</sup> Thus the plasma cromoglycate concentration at any time is determined by the amount of drug remaining in the lung—that is, the site of action. In 1973 Benson and coworkers<sup>10</sup> showed that when patients with airways obstruction inhaled sodium cromoglycate as a dry powder urinary excretion of unchanged drug correlated with peak inspiratory flow rate. Similarly, in non-asthmatic subjects with normal baseline airway calibre a single inhalation of sodium cromoglycate powder produced peak plasma concentrations and an area under the plasma concentration-time curve (AUC) that related directly to the flow rate used to inspire the drug.<sup>9,11</sup>

It is well established that bronchoconstriction has a large effect on the distribution of inhaled drugs in the airways, causing more central deposition.<sup>3-5</sup> Benson *et al.*<sup>10</sup> and Neale *et al.*<sup>8</sup> have suggested that absorption of sodium cromoglycate after inhalation occurs at two rates—possibly relating to absorption from two anatomical sites, alveoli and airways. As bronchoconstriction would influence the penetration of the drug to each of these sites we have investigated the influence of methacholine induced bronchoconstriction on the deposition of sodium cromoglycate aerosol within the lung and its relation to plasma drug pharmacokinetics in 10 atopic subjects.

## Methods

The study was approved by the Southampton university and hospitals ethical committee and all subjects gave their written informed consent. Ten symptom free volunteers (five men and five women of mean age 23 (SEM 1) years) were studied outside the pollen season. Subjects had to have normal baseline lung function ( $FEV_1 > 90\%$  of predicted), and a bronchoconstrictor response to inhaled methacholine at a concentration of 64 mg/ml or less. All subjects were atopic (a  $> 3$  mm skin weal response to at least one common allergen) and none was a smoker or taking regular medication. Two subjects had mild seasonal asthma requiring occasional inhaled salbutamol (Nos 9 and 10) but were symptom free at the time of study, and eight had seasonal allergic rhinitis.

Subjects attended the laboratory on three occasions, separated by at least 10 days. On the first occasion two baseline measurements of  $FEV_1$  were made five minutes apart and a mean value was calculated. A

methacholine bronchial provocation test was then undertaken. Methacholine was administered as an aqueous aerosol generated from a starting volume of 4 ml by an Inspiron Mini-neb nebuliser (Bard International Ltd, Sunderland) driven by compressed air at a flow rate of 9 l/min. Subjects inhaled five deep breaths from functional residual capacity (FRC) to total lung capacity (TLC) of the aerosol through a mouthpiece while wearing a noseclip.<sup>12</sup> Under these conditions the aerosol mass median particle diameter is 4.2  $\mu\text{m}$  (Malvern HSD 2600 laser particle sizer, Malvern Instruments Ltd, Malvern, Worcestershire). Three minutes after aerosol inhalation two further measurements of  $FEV_1$  were made one minute apart, the maximum value being used for analysis. Increasing doubling concentrations of methacholine (0.03–64 mg/ml, non-cumulative) were administered at five-minute intervals until the  $FEV_1$  had fallen by 20% from the mean baseline value. The cumulative methacholine concentration that reduced  $FEV_1$  by 20% of baseline ( $PC_{20}$ ) was derived by linear interpolation from the log concentration-response curves.

On the two subsequent visits subjects inhaled a random order saline (placebo) and the previously determined  $PC_{20}$  methacholine, and  $FEV_1$  was measured before and at 5, 15, and 25 minutes after each inhalation. At 5 minutes, when the fall in  $FEV_1$  had previously been shown to be maximum, subjects inhaled a solution containing sodium cromoglycate (30 mg in 3 ml, Fisons Pharmaceuticals, Loughborough) mixed with stannous phytate labelled with technetium-99 m (<sup>99m</sup>Tc) (4 mg in 1 ml, 200 MBq/ml). The aerosol was generated by an Inspiron nebuliser operated under the same conditions as for the saline and methacholine inhalations with the exception that nebulisation was triggered to occur for 3.5 seconds during inspiration by a Rosenthal-Frenc dosimeter (Laboratory for Applied Immunology Inc, Maryland, USA). The cromoglycate-<sup>99m</sup>Tc stannous phytate solution was inhaled during 60 breaths of tidal breathing over six minutes. The nebuliser was weighed before and after nebulisation and found to deliver a mean calculated cromoglycate dose of 6.8 (SEM 0.4) mg.

A gamma emission lung scan (large field of view gamma camera, Siemens, Holland) was undertaken on both days 20 minutes after inhalation of the radiolabelled sodium cromoglycate solution to define the intrathoracic deposition of the nebulised particles. The lung scans were digitised and stored on magnetic tape for later computer analysis. The lung scans obtained on the day that saline was inhaled were divided into central and peripheral areas. The "central" area was delineated as two rectangles centred over the mid point of each hilum with the dimensions of one third of the width by one third of the height of

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the visible scintigram plus the trachea and upper mediastinum. The lower mediastinum was excluded and the central area on the anterior scan of the left lung was adjusted to exclude the cardiac shadow. The "peripheral" areas of the scan were delineated with a dimension of one third of the width of each lung from the visible peripheral border. The mean number of counts per pixel in the central and peripheral areas were obtained and a central peripheral (C : P) ratio was calculated for each scan. The same central and peripheral boundaries were applied to the lung scans obtained after inhalation of methacholine and once again C : P ratios were calculated.

Blood for measurement of plasma sodium cromoglycate concentrations was taken from an indwelling Teflon cannula inserted into an antecubital vein and kept patent with heparised saline. Blood samples of 10 ml were taken before and at 1, 2, 5, 10, 15, 30, 60, 90, 120, 150, 180, 210, and 240 minutes after sodium cromoglycate administration. Blood was transferred to glass lithium-heparin tubes and the plasma was separated by centrifugation at 5000 g and stored at  $-40^{\circ}\text{C}$ . Sodium cromoglycate was measured in plasma by a specific radioimmunoassay<sup>6</sup> with a limit of detection of 0.7 ng/ml.<sup>9</sup> All samples were assayed in triplicate and a standard curve was constructed for each set of test samples. The coefficients of variation of reference plasma samples to which 3 and 5 ng/ml of cromoglycate had been added were 6% and 11%.

#### DATA ANALYSIS

Baseline values of FEV<sub>1</sub> on the saline and methacholine study days were compared by means of Student's *t* test for paired data. The gamma emission scintigrams were quantified in arbitrary units and the saline and methacholine study days were compared in terms of the integrated areas and C : P ratios by the Wilcoxon signed rank test. The relation between the

C : P ratio and PC<sub>20</sub> was examined by least squares linear regression analysis.

The time courses of the plasma concentrations of sodium cromoglycate were analysed by non-linear least squares regression analysis (NON-LIN). The data were fitted to a single compartment model with first order absorption kinetics. The plasma half life for the initial increase in concentration of cromoglycate (initial  $t_{1/2}$ ), the terminal half life (terminal  $t_{1/2}$ ), and the AUC between 0 and 240 minutes were derived from the computer analysis. Values for the AUC were calculated to infinity by dividing the final plasma concentration by the terminal slope. The maximum plasma concentrations of cromoglycate (C<sub>max</sub>) and the time to maximum (T<sub>max</sub>) are the observed values. The pharmacokinetic parameters on the saline and methacholine study days were compared by Student's paired *t* test.

#### Results

For all the subjects studied the geometric mean PC<sub>20</sub> for methacholine was 23.6 mg/ml with a range of 0.85–82.3 mg/ml. Five subjects had a PC<sub>20</sub> methacholine of 8 or less (geometric mean PC<sub>20</sub> 1.7, range 0.85–3.9 mg/ml), and five had values over 8 mg/ml (geometric mean PC<sub>20</sub> 40, range 27–82 mg/ml) (table 1).

There was no significant difference between mean (SEM) baseline measurements of FEV<sub>1</sub> on the saline (4.21 (0.28) l), and methacholine (4.20 (0.30) l) study days. After administration of the saline aerosol there was no significant change in FEV<sub>1</sub> measured at 5, 15, and 25 minutes. After the PC<sub>20</sub> methacholine dose mean FEV<sub>1</sub> had fallen by 23.4% (0.6%) from baseline values five minutes after challenge ( $p < 0.001$ ) and it remained low at 15 minutes (23.9% (0.9%)). Inhalation of radiolabelled sodium cromoglycate aerosol

Table 1 Central:peripheral ratio of sodium cromoglycate–technetium-99m labelled stannous phytate when administered after inhaled saline or methacholine

Subject no	Age	Sex	FEV <sub>1</sub> (% pred)	PC <sub>20</sub> methacholine (mg/ml)	C : P* saline	C : P* methacholine	Change in ratio†
1	32	M	125	59.00	0.40	2.00	5.0
2	22	F	105	31.00	0.30	1.00	3.3
3	22	F	95	26.00	0.60	2.40	4.0
4	22	F	104	82.00	0.60	3.00	5.0
5	22	M	96	27.00	0.60	3.60	6.0
6	22	F	115	3.90	0.60	0.70	1.2
7	22	M	105	3.40	3.80	8.20	2.2
8	22	M	98	1.40	0.70	0.80	1.1
9	21	M	120	0.90	0.60	1.30	2.2
10	22	F	112	0.85	0.80	1.70	2.1

\*The ratio of central to peripheral aerosol distribution.

†Change in ratio =  $\frac{\text{C : P methacholine}}{\text{C : P saline}}$

Table 2 Mean (SEM) indices of plasma pharmacokinetics of inhaled sodium cromoglycate in the bronchoconstricted and unstricted state

	Unstricted		Bronchoconstricted	
	All subjects	Subjects 1-5	All subjects	Subjects 1-5
Cmax (ng/ml)	7.7 (1.0)*	7.6 (1.5)	10.6 (1.3)*	11.4 (2.7)
Tmax (min)	31 (8)	35 (15)	23.0 (5.0)	14.5 (5)
AUC (ng min/ml)	1687 (266)	1076 (37)	1918 (480)	1370 (49)
Initial t <sub>1/2</sub> (min)	5.4 (2.6)	2.9 (1.3)	5.8 (2.5)	3.1 (0.5)
Terminal t <sub>1/2</sub> (min)	166 (35)	216 (73)	120 (20)	139 (49)

\*p < 0.03.

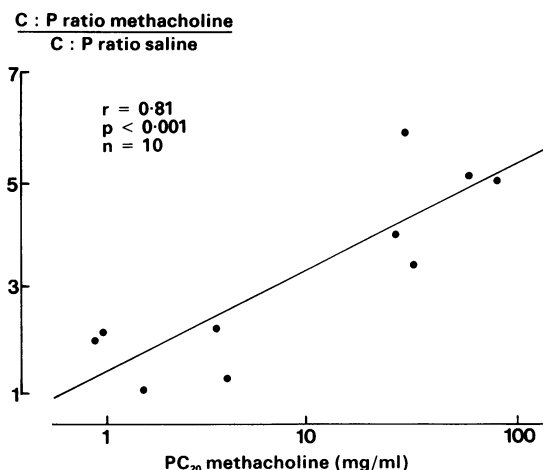


Fig 1 Relationship between airway reactivity to methacholine ( $PC_{20}$ ) and the change in central to peripheral lung deposition (C:P) ratio.

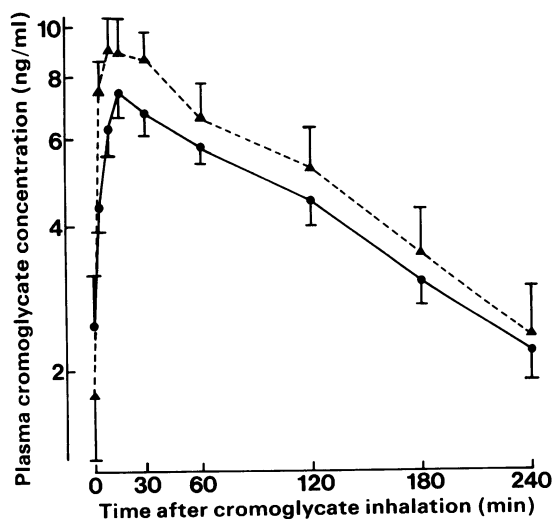


Fig 2 Plasma concentration-time curves for sodium cromoglycate following inhalation in the bronchoconstricted (---  $\blacktriangle$ ) and unstricted (—  $\bullet$ ) state.

caused no significant change in FEV<sub>1</sub> after either saline or methacholine when measured five minutes after administration. In all subjects bronchoconstriction after methacholine was associated with increased central and decreased peripheral deposition of radiolabelled cromoglycate aerosol, the geometric mean C:P ratio being 0.68 (range 0.3-3.8) after saline and 1.87 (0.7-8.2) after methacholine ( $p < 0.005$ ) (table 2). A significant correlation was observed between the fold increase in C:P ratio and the previously determined  $\log_{10} PC_{20}$  methacholine ( $r = 0.81$ ,  $p < 0.01$ ) (fig 1).

On the day that subjects inhaled saline followed by sodium cromoglycate—<sup>99m</sup>Tc labelled stannous phytate the plasma profile of cromoglycate showed the characteristic absorption limited pharmacokinetics (fig 2). The mean (SEM) pharmacokinetic indices were: Cmax 7.8 (1.0) ng/ml, AUC 1687 (266) ng/min,  $t_{max}$  31 (8) min, initial  $t_{1/2}$  5.4 (2.6) min, and terminal  $t_{1/2}$  166 (35) min, (table 2). The plasma profile when cromoglycate was given after methacholine was not significantly different from that observed after inhaled saline with the exception of Cmax, which was significantly higher (10.6 (1.3) v 7.8 (1) ng/ml,  $p < 0.03$ ) (fig 2). No relationship could be established between any of the calculated indices of sodium cromoglycate pharmacokinetics and the starting methacholine responsiveness or the distribution pattern of aerosol within the lung. The pharmacokinetic data for subjects with a  $PC_{20}$  methacholine of 1 mg/ml or less did not differ significantly from those of subjects with a  $PC_{20}$  over 8 mg/ml, despite the much greater shift in C:P ratio after methacholine in the latter group.

## Discussion

The plasma pharmacokinetics of inhaled sodium cromoglycate administered as an aerosolised solution (in both normal and hyperresponsive asthmatic and non-asthmatic subjects) fits a simple, one compartment model in which the terminal decrease in plasma concentration is absorption rather than excretion limited.<sup>7,9,11</sup> A more complex model including two absorption rates to fit plasma concentration-time data

after inhalation of sodium cromoglycate has been proposed recently by Neale *et al.*,<sup>8</sup> but this was not found to be necessary for the analysis of the present pharmacokinetic data or for similar data obtained previously after inhalation of dry powder sodium cromoglycate.<sup>9</sup> Reduction of the FEV<sub>1</sub> by over 20% by inhalation of methacholine before inhalation of a radiolabelled sodium cromoglycate aerosol greatly influenced the regional deposition of the drug in favour of the central airways. Surprisingly, however, this alteration in regional deposition produced no discernible effect on cromoglycate pharmacokinetics apart from a small but significant increase rather than decrease in C<sub>max</sub>.

The absorption of inhaled sodium cromoglycate from the bronchial mucosa is complex and may be an important determinant of its therapeutic efficacy in asthma. Neale *et al.*<sup>8</sup> studied the plasma kinetics of sodium cromoglycate after both intravenous dosing and inhalation from a spinhaler. The latter resulted in wide variation between individuals, and to fit the data to a pharmacokinetic model two absorption rates had to be incorporated. They suggested that the rapid and slow increases in plasma sodium cromoglycate they observed may relate to differing anatomical sites of drug absorption within the lung, the initial rapid absorption being from the alveolar capillary bed from small particles penetrating to the lung periphery and a slower phase representing absorption from the airways mucosa.<sup>10</sup> If initial rapid absorption occurred through the alveoli then deposition of more aerosol in more central airways would be expected to diminish both the initial plasma half life of the drug and its maximum concentration and prolong the terminal half life, which is absorption rate limited. Despite the appreciable fall in FEV<sub>1</sub>, however, and the increased central deposition of sodium cromoglycate after inhalation of methacholine in the present study the initial plasma half life and the terminal half life were unchanged and the maximum concentration was increased rather than decreased. Moreover, in the absence and in the presence of bronchoconstriction the amount of cromoglycate being deposited in the lungs as reflected by the AUC was similar. These findings, and the observation that the plasma pharmacokinetics of inhaled sodium cromoglycate is almost identical after direct bronchoscopic instillation of the drug into a segmental bronchus,<sup>9</sup> support the view that there is a single absorption compartment within the lung for cromoglycate. The high early plasma peak of sodium cromoglycate after inhalation of the dry powder described by Neale *et al.*<sup>8</sup> was not seen in this or our previous studies on cromoglycate pharmacokinetics.<sup>9,11</sup> The high early peak probably relates to the more rapid of the two absorption rates proposed by Neale *et al.*<sup>8</sup> Its absence in the present

study after saline inhalation when considerable peripheral aerosol deposition occurred is difficult to explain, but may indicate a difference in the permeability characteristics of the airways in different subjects.

In our study the total amount of sodium cromoglycate absorbed into the circulation, as reflected in the AUC, was not reduced when the drug was administered in the bronchoconstricted state. We interpret this as implying that the area of cromoglycate deposition within the bronchial tree is not a major determinant of its plasma pharmacokinetics following inhalation. Consequently sodium cromoglycate absorption from the lung periphery must contribute a negligible amount to the total amount of absorbed drug.

An unexpected finding was the significant positive correlation between PC<sub>20</sub> methacholine, an index of non-specific airways responsiveness, and the increase in the central to peripheral ratio of radiolabelled aerosol deposition. Thus in those subjects with increased bronchial responsiveness the peripheral airways contribute more than the central to the reduction in FEV<sub>1</sub> induced by methacholine whereas the reverse is true of subjects with airways responsiveness falling within the normal range. McFadden and Lyons<sup>14</sup> have reported that peripheral airways in asthma are more responsive to cholinergic stimuli than are central airways, though whether this reflects a greater peripheral distribution in muscarinic cholinergic receptors<sup>15</sup> or increased smooth muscle receptor-contraction coupling is not known.

It has been reported that the efficacy of aerosolised sodium cromoglycate in protecting against exercise provoked bronchoconstriction in asthma is related to the total plasma concentration of the drug (AUC) and therefore the total dose delivered to the lung.<sup>16</sup> If this relationship were to be extrapolated to the present study, the total dose of cromoglycate reaching the lung would be the important determinant of efficacy rather than the pattern of distribution within the lung. Many asthmatic patients who use sodium cromoglycate for maintenance treatment of their disease have varying degrees of airways obstruction with attendant airflow limitation. In these conditions any drug delivered by inhalation is likely to be deposited in the central rather than the peripheral airways. As we have observed almost identical cromoglycate plasma pharmacokinetics irrespective of the state of bronchoconstriction, possibly cromoglycate deposited centrally is distributed peripherally via the bronchial circulation, which thus extends its area of pharmacological action within the bronchial tree. To establish whether this occurs in reality, further studies are required to relate sites of deposition of sodium cromoglycate to its protective

effect against challenge and subsequently to clinical efficacy.

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