CROMOGLYCATE: A HEALING AGENT IN ACUTE CHLORINE-INDUCED LUNG DAMAGE

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ABSTRACT

In the present study the effectiveness of sodium cromoglycate in treatment of alveolar damage induced by chlorine gas in rats was investigated. Chlorine was generated by chemical interaction between potassium permanganate and concentrated hydrochloric acid. The rats were exposed to sublethal dose of chlorine gas. Treatment with 2.5 mg of 1 ml nebulized sodium cromoglycate solution over 5 minutes was initiated 30 minutes after exposure followed by twice daily treatment for 21 days. Results of this study show that cromoglycate reduced alveolar thickness, septal rupture, hemorrhage and detachment of the epithelial lining of the bronchioles induced by chlorine gas.

Keywords: Chlorine gas, Lung damage, Rats, Sodium cromoglycate

INTRODUCTION

Pulmonary irritants are normally classified as primary and secondary. The primary irritants are considered to be sufficiently reactive in tissues and produce pulmonary effects that are so marked that the systemic effects are obscured or much less important. The effects of the secondary irritants on the tissues of the respiratory tract are less pronounced than their systemic effect (1).

The site of response in the respiratory tract is partly determined by the solubility of the gas in water. Highly water soluble gases such as NH_3, H_2SO_4 and HF are rapidly absorbed as they transverse the respiratory tract and exert their effects predominantly at the upper respiratory tract. In contrast, low water soluble compounds such as phosgene, nitrogen dioxide and ozone attain higher concentration at the parenchyma areas where their toxic actions are manifested. Compounds with intermediate solubility (Cl_2, SO_2 and Br_2) exhibit their effects in both upper respiratory tract and the lower lung tissues (1). The level of response depends on the concentration of the noxious compound, the duration of the exposure and the water content of the organ affected. Normally, low concentrations induce inflammatory responses while high concentrations induce corrosion of the alveoli (2).

With advances in the chemical technology in the last century, many groups of compounds have been produced and found their use in our lives. One group, which has found a wide spectrum of application, is the chlorinated compounds used in industry, domestic and in chemical warfare (3). In industry a bleaching agent in the form of sodium hypochlorite is a powerful disinfectant and a concentration as low as 0.6 to 9 mg/m^3 of chlorine sterilizes water. The toxic mechanism of action of chlorine in the living cells is believed to be due to production of reactive hypochlorite ions, hypochloric acid and the free chloride ion which interact as a powerful oxidizing agent with the sulfhydryl groups and sulfur bonds of the cellular proteins (4). While, for ethical reasons, there is no study in which the lethal level in humans has been definitely measured, Withers and Lees (1985) estimated 30 minute LC_{50} in humans, following accidental exposures to chlorine gas, at a standard activity according to regular, vulnerable and average population to be 250, 100 and 210 ppm respectively (5).

Previous studies have demonstrated that sodium cromoglycate to be an effective mast cell stabilizing agent and at the present it is commonly used as an anti-allergic agent in the prophylactic treatment of allergic diseases such as rhinitis and asthma (6). It has also been used orally in the treatment of ulcerative colitis and locally in the treatment of atopic dermatitis (7) with variable results. Since it is not absorbed in GI, formulations available are usually nebulization, or insufflation in powder or solution form. The mechanism of action of cromoglycate has been suggested to be via stabilization of mast cells and prevention of the release of a variety of chemical mediators from these cells including histamine, 5HT and a variety of potent pro-inflammatory leukotreins (8).

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Although sodium cromoglycate has been used for many years for prevention of asthma, especially in children (9), there is no report of its effectiveness in the treatment of alveolar damage. The aim of this investigation was to assess the effectiveness of sodium cromoglycate as an agent in treatment of chlorine-induced lung damage.

MATERIALS AND METHODS

Instruments and Materials

The nebulizer used was model ZT106d10 (Pari-Boy, Faber, Germany). The nebulized device uses vibration mechanisms and graded jet airflow for production of a fine mist. Eosin (article number 1345), hematoxylin (article number 4302). Potassium Permanganate and Hydrochloric acid were purchased from Merck, Germany. Male and female N-MRI rats of average weight of 200 g, purchased from Tehran Animal Centre (Tehran, Iran). The rats were housed in hard transparent plastic cages in rooms that was maintained at 27.5°C and humidity of 60±10% and had free access to food and water. The light cycle was on 12 hourly day/night bases and the lighting conditions were between 7 AM to 7 PM.

Standardization of the procedure for administration of chlorine gas

The animals were positioned in a specially adapted polystyrene container ensuring that the nebulized air was confined within the area where the animals were held and exposed to during the whole period of treatment with sodium cromoglycate solution (2.5 µg/ml in normal saline, nebulized over 5 minutes twice daily at 12 hourly intervals for 21 days post-exposure to a single sub-lethal chlorine gas exposure). A simplified diagrammatic representation of procedure is drawn and illustrated in Figure 1. Initial studies involved the selection of an appropriate sublethal dose of chorine gas, which induced sufficient alveolar damage. Basically, it involved administration of chlorine gas, produced by chemical interaction of potassium permanganate with concentrated hydrochloric acid. (Preliminary experiments showed that addition of 0.84 ml of concentrated HCl, to a cork-stopper beaker containing 200 mg of $KMnO_4$, and heating the mixture on a water bath at 70°C to be sufficient for production of sublethal toxic effects in rats after 7 minutes exposure to chlorine gas. The chlorine gas produced was administered via tubing connected to a glass cylinder (20 x 30 cm) where the rats were held under fan-operated hood chamber

Experimental protocol

The next stage was to test the effectiveness of sodium cromoglycate as an agent for treatment of the chlorine-induced lung damage in the rats. For this purpose, 36 rats were used and divided randomly into 3 groups of 12. The first group used as positive control to which chlorine was administered for a period of 7 minutes and during the subsequent 21 days nebulized with 1 ml normal saline for 5 minutes twice daily. The second group received nebulized sodium cromoglycate solution (2.5 mg /ml over 5 minutes twice daily for 21 days) starting 30 minutes after a 7-minute chlorine intoxication. The third group (placebo group) were not exposed to either chlorine gas or sodium cromoglycate, and instead received only nebulized normal saline twice daily for 21 days.

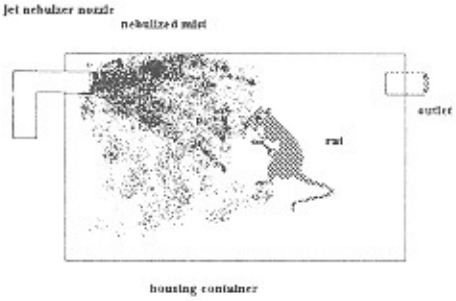


Fig. 1: Simplified diagrammatic representation of the nebulization procedure during which the rats were exposed to sodium cromoglycate solution (2.5 mg/Kg daily for 5 minutes for 21 days) post exposure to chlorine intoxication. The control rats were similarly treated with vehicle (normal saline).

Preparation of the histological samples

The animals were sacrificed by decapitation, 12 h after the last exposure to the nebulized solution. The lungs were removed, washed and samples from base of the lower left and right lobes were fixed in 10% formalin/normal saline solution for subsequent histological staining. The samples were then embedded in paraffin blocks, cut and stained with hematoxylin and eosin. The parameters of measurements selected for microscopic examination were: area of hemorrhage, thickness of the alveolar wall, rupture of the alveolar septa and detachment of the

epithelial lining of the bronchioles. Three slides were prepared from each animal, and 18 fields from each slide were examined by random selection (one field out of three viewed), with a total of 648 fields for each group. The samples were taken from the lower lobes of the right and left lungs. By the use of an eye piece graticule each field was divided into four equal areas and random serial reading along the tissue sample in a zig-zag manner was employed and by this way all the area of each slide was viewed and assessed. The presence of any parameters in the viewed fields in each of the proposed four areas was given a +1 score. The maximum possible score for each parameter was 648 x 4 = 2592. Each parameter was given a score ranged from 0 to +4, according to the presence or absence of the parameter to be measured in the microscopic field of assessment (+4, all the four areas in each field contained the parameter under examination).

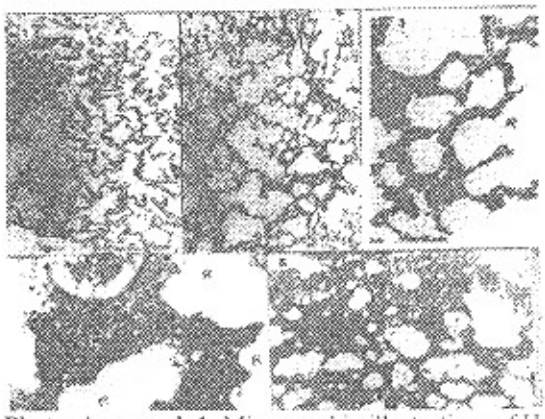
The effect of nebulization of sodium cromoglycate solution was expressed as the number of areas found to contain the parameter under study and as percentage relative to total proposed 2592 areas. The percentage reduction in the damage associated with each parameter was expressed by utilization of the following relationship: reduction%= (score for cromoglycate treated-score for positive control)/ score for positive control×100.

Statistical analysis

Non-parametric Mann-Whitney test was used to compare the results. Results from 12 animals in each group were presented as mean±SEM, a $P<0.05$ was considered as the level of significance. The percentage incidence and mean scores of hemorrhage, change of thickness the alveolar wall, rupture of the septum and detachment in the epithelium of the bronchioles in rats lung tissue from maximum possible score of 2592 in 648 microscopic field observations in the placebo control, the positive control chlorine-induced lung damaged and sodium cromoglycate treatment groups (n=12 in each group) (Mann-Whitney test, $^{**}P<0.01$ between the positive control and sodium cromoglycate-treated groups).

RESULTS

Since preliminary experiments showed that 10-min exposure to chlorine gas is the minimum period that causes fatal lung damage, for subsequent experiments, 7-min exposure was selected and considered sufficient to induce lung damage without immediate fatalities.



Photomicrograph 1. Micrographic illustrations of H & E. stained rat lung samples taken from normal control (1, x100), cromoglycate-treated (2 & 3, x 100 and x400) and positive control-chlorine exposed lungs (4 & 5, x400). Note the extensive hemorrhage (H), increase in thickness (T) and rupture of the septum in positive control, relative to cromoglycate-treated.

The results showed that sodium cromoglycate to be effective in reducing all the four parameters under investigation (Table 1). The extent of reduction in hemorrhage and alveolar thickness in cromoglycate treated group in comparison with the control group were 57.4% (score reduction from 1946 to 829) and 78.6% (score reduction from 2250 to 508) respectively. Also, sodium cromoglycate significantly reduced the rupture of the alveolar wall by 62.7%, (score reduction from 1871 to 697) and epithelial damage by 79.6% (score reduction from 2042 to 539) (Table1).

The photomicrographs in Figure 1 illustrate the histological findings for negative unexposed control, positive untreated control, and sodium cromoglycate treated groups and differences in the four parameters under consideration.

DISCUSSION

The results from these experiments showed that although sodium cromoglycate after a period of 21 days did not completely resolve the damages induced by chlorine gas, but it was capable in reducing damages associated with its inhalation in the alveoli significantly. In fact, 2 rats in the positive control group died before the completion of the experiments, on days 10 and 14, while non in the sodium cromoglycate group died during 21 days of the experiment. This observation could be a good indication of the effectiveness of cromoglycate in

Table 1. Changes in bronchioles of rat's lung tissue

Group	Hemorrhage (score)	Thickness of alveolar wall (score)	Rupture of the septum (score)	Detachment of epithelial lining of bronchioles (score)
Placebo Control	0	0	0	0
Positive Control	75.1 ± 9** (1946)	86.8 ± 10** (2250)	72.2 ± 8** (1871)	78.8 ± 9** (2042)
Sodium cromoglycate	32 ± 4 (829)	19.6 ± 3 (508)	26.9 ± 3 (697)	20.8 ± 2 (539)

reducing the degree of post-acute damages inflicted by the chlorine gas.

The percentage of damage induced by chlorine gas intoxication was found to occur in the following order: thickness> detachment> hemorrhage> rupture. This observation reflects the natural sequences of acute inflammatory responses, which is characterized by swelling and redness, due to protein and plasma exudation into the extracellular space. When the reaction is severe, as in this study, intense tissue reaction and vascular injury follows (10). On the other hand, the degree of damages following treatment with sodium cromoglycate was found to occur in almost in the reverse order as: thickness< detachment< hemorrhage< rupture.

The question that needs to be addressed is what mechanism governs these results. There is no simple and straightforward answer to this question as many factors are involved both in the initiation and resolution of inflammation. It is known that there is a close association between inflammation and resolution of the inflammatory responses. Under normal conditions, this results in complete healing and regeneration of the cellular components to full function. However, under pathological and abnormal conditions, full resolution is not accomplished and results in undesirable consequences of scar or abscess formation, or development of chronic inflammation, and fibrosis (11). In order that a tissue returns to its normal function during the resolution phase, all the process occurring during its development (12), must be resolved. The following events may be responsible for such resolution: removal of the inducing stimuli and nullifying generation of mediators, cessation of cellular migration from blood vessels, restoration of the normal microvascular permeability and their maturation into mature macrophages, and removal of extravascular fluid, proteins, bacterial and cellular debris (13). In addition, one of the hallmarks for tissue regeneration is the absence of damage to the extracellular matrix framework. All these events seem to set the stage for effective tissue repair. It is not as yet clear which of these mechanism(s) are modulated by sodium cromoglycate, but the present study demonstrated that this drug, directly or indirectly, via mast cell stabilization has an important role in the resolution of lung tissues. This assumption is supported by ample evidences that suggest the involvement of mast cells in many functions of the fibroblasts, the cells that are responsible for remodeling and wound repair. Therefore, it is also possible that stabilization of mast cells prepares the stage for more normal physiological balance and encourages resolution (14).

It has been shown by radiolabeling technique, that the phenomenon of alveolar macrophages plays an important role in the clearance of particles entering the lung (1). More recently it has been reported a fairly rapid recovery following accidental chlorine gas inhalation among people, which suggest that the lungs has an efficient clearance system when exposed to noxious stimuli (15). In an *in vivo* experiments (16) in which rats were exposed to phosgene, which is a noxious chlorinated agent, the extent of damage was dose dependent and resolution was found to parallel the reversal of polymorphonuclear leukocytes. On the other hand, the mast cells are known to release a variety of potent pro-inflammatory intermediates, of which the best known example is leukotriens (8), which singly and in combination with cellular components induce persistent inflammatory process. It may be inferred from these studies that sodium cromoglycate in addition to its mast cell stabilizing properties (6), may have a modulating action on this clearing process and, directly or indirectly, modifies the functional repairative activity of other cellular components of inflammation, notably the macrophages. Furthermore, since chlorine gas induces its toxic effects via activation of free-radical formation

(4), it is possible that sodium cromoglycate directly or indirectly, via inhibition of release of proinflammatory mediators, acts as a free-radical scavenging agent which facilitate the regeneration of the alveolar. Other effects attributed to sodium cromoglycate include direct action on the airway nerves (17) and antagonism of substance P (18). The mechanism(s) involved in the resolution of the alveoli may be a combination of all these factors.

CONCLUSION

The overall conclusion that may be drawn from this study together with results from other reports, is that sodium cromoglycate via mast cell stabilization or other actions related indirectly to this action may be involved in the modulation of mechanisms involved in the clearance and resolution of the damaged tissue via still unknown mechanism which deserve further investigations.

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