The influence on nasal mucosa of unpreserved and preserved nasal sprays containing disodium cromoglycate

N. Mygind, Copenhagen, A. S. Viner and N. Jackman, Loughborough, England

SUMMARY

The aim of the study was to determine whether the preservatives benzalkonium chloride and phenylethanol used in a 2% disodium cromoglycate nasal spray did or did not induce a chemical rhinitis. 16 patients with perennial rhinitis and 16 healthy volunteers entered in the trial. The measures were both clinical and histological. The results showed that the preservatives are not harmful to the nasal mucosa when applied in low concentrations. The evidence suggests that disodium cromoglycate plus preservatives is clinically more effective than disodium cromoglycate alone; this is contrary to the original premise that the preservatives act as chemical irritants.

INTRODUCTION

THE value of disodium cromoglycate (Lomudal (R), Intal (R)) administered in powder form in the treatment of perennial rhinitis has been reported by a number of investigators (Thorne and Bradbeer, 1972, Holopainen et al., 1973 and Sunderman and Crawford, 1973). Recently evidence has been presented that the same drug applied as a 2% solution may be beneficial, (Mygind et al., 1972 and Brain et al., 1974), but the evidence to date is not as conclusive as that produced with the powder formulation. There may be several reasons to explain this anomaly. It is possible that the actual dose of active drug delivered per application of the solution is clinically sub-optimal; however nasal challenge studies using 2% solution of disodium cromoglycate (D.S.C.G.) have demonstrated significant protection against a challenge with grass pollen (Taylor and Shivalkar, 1970 and Jenssen, 1973); these results are comparable to similar experiments carried out with a powder formulation (Engström, 1971 and Taylor and Shivalkar, 1971). In formulating an aqueous solution of D.S.C.G. it was necessary to include a preservative system in order to inhibit growth of bacteria. Laboratory tests showed that two preservative agents were required to achieve the criteria laid down by the Pharmacopeia of the U.S.A., (Eighteenth Revision); these were benzalkonium chloride and phenylethanol; the latter was specifically included to inhibit the growth or organisms of the pseudomonas species. In addition a chelating agent (E.D.T.A.) was added to the solution as it was found that D.S.C.G. in solution

reacts with minute traces of heavy metals forming a precipitate. Thus, the constituents of the solutions tested, with the concentrations, were:

disodium cromoglycate	2 %
benzalkonium chloride	0.01%
phenylethanol	0.4 %
E.D.T.A.	0.01%

A formulation incorporating all constituents has been used in clinical trials. From the data obtained it was noted that a number of subjects complained of nasal irritation after using the solution (Blair and Herbert, 1973); this occurred with both active and placebo both of which contained the preservatives. It was decided that a possible explanation for the somewhat inconclusive results in some trials could be due to the fact that the preservatives were acting as irritants and thus, inducing a mild chemical rhinitis. This study was therefore initiated to determine whether the preservatives in this formulation of D.S.C.G. did or did not induce a chemical rhinitis in both volunteers and patients.

Methods and material

16 patients with perennial rhinitis* and 16 healthy volunteers were entered in the trial randomly allocated to one of the following formulations:

1. DSCG 2%, benzalkonium chloride 0.01%, phenylethanol 0,4% & diluent
2. DSCG 2% & diluent
3. Benzalkonium chloride 0.01%, phenylethanol 0.4% & diluent
4. Diluent

The diluent used throughout was a 0.01% aqueous solution of E.D.T.A. The solution was delivered by a plastic squeeze bottle, and subjects were instructed to give two squeezes to each nostril 6 times a day. Trial treatment lasted for 4 weeks, and was preceded by a run-up period of one week, in which the subjects filled in diary-cards. This continued in the treatment period; a scale of 0-3 (none, mild, moderate, severe) was used to indicate severity of sneezing, blocking and running. These scores for nasal symptoms made it possible to quantify the changes in symptom intensity throughout the treatment. A second type of clinical measurement was an overall assessment of the irritancy of the solution made at the end of the trial on a four-point scale (0, +, ++, +++).

In addition to the clinical evaluation of the treatment, objective histological measures were made on biopsy specimens taken from the inferior turbinate, ½-1 cm behind the front edge. The biopsies were fixed in 5% glutaraldehyde, embedded in Epon, and 2μm thick sections cut with an ultramicrotome. Ten high power (X800) fields were examined on each section, and the results recorded were the averages over all fields. This procedure was followed at the beginning and at the end of the trial. The sections were examined blindly, and eighteen histo-

* By perennial rhinitis is meant a chronic disease, characterized by sneezing, watery rhinorrhoea and nasal blockage due to a swollen mucous membrane.

Table I. Trial design, showing numbers of subjects and allocation to treatments

			Formulation
16 perennial rhinitis patients	8 given D.S.C.G.	4 given preservatives	1
		4 not given preservatives	2
	8 not given D.S.C.G.	4 given preservatives	3
		4 not given preservatives	4
16 healthy volunteers	8 given D.S.C.G.	4 given preservatives	1
		4 not given preservatives	2
	8 not given D.S.C.G.	4 given preservatives	3
		4 not given preservatives	4

logical measures were made. Further details of the pre-trial histology are given elsewhere (Mygind et al., 1974).

The statistical design was a 2^3 factorial experiment with 4 replicates. The three factors and each of their two levels being: A. Type of subject (allergic/normal), B. D.S.C.G. (present/absent), C. benzalkonium chloride and phenylethanol (present/absent). To achieve the design a total of 32 subjects needed to be entered in the trial. To incorporate factors B and C, four solution formulations were necessary (Table I).

The diary card scores were analysed in two stages. First, the pre-treatment week's total scores were examined with a 2-factor Analysis of Variance for the correct selection of subjects, i.e. that patients did in fact differ from volunteers, and for successful random allocation of treatment, i.e. that treatment groups did not differ among themselves in terms of symptomatology. Second, each subjects scores were totalled variable by variable for the month of trial treatment, and from these totals were subtracted the pre-trial week's total multiplied by four. Thus, three data sets consisting of score changes (after treatment — before treatment) were obtained and these were submitted to an appropriate Analysis of Variance, which, in the event of significant F-ratios for interactions, was followed by t-tests using standard errors derived from the Analysis of Variance. Concerning the histological measurements the analysis was again in two stages. First, the baseline measures were examined for the same factors as were the diary card pre-treatment scores; second, the post-treatment readings were subtracted from the pre-treatment readings and these differences analysed. For both stages the same Analysis of Variance as were used for the diary card scores were applied to sixteen of the measures. The level of significance used throughout in statistical testing was 5%.

The volume of results of all statistical calculations are too extensive to be presented here. Those especially interested can obtain detailed results from one of the authors (N.J.).

RESULTS

Changes of clinical measures

Volunteers had zero or very minimal symptoms; the changes in nasal scores throughout the treatment occurred predominantly in the patients. Patients given D.S.C.G. (preserved and unpreserved) showed a significantly greater mean reduction in scores for sneezing (-17,8) than patients not given D.S.C.G. (-4,4) ($p<0,05$*). A similar tendency was found for blocking and running, but the differences were not significant.

With regard to blocking an unexpected significant difference was discovered. Patients given D.S.C.G. with preservative showed a substantial mean improvement (-13,5) while patients given D.S.C.G. without preservative showed a mean worsening (+ 2,8) ($p < 0,05$*). For sneezing and running D.S.C.G. plus preservative was again better than D.S.C.G. alone, but the differences were not significant.

In the assessment of irritancy made at the end of the trial no differences were demonstrated between volunteers/patients or between preservative/no preservative. On the other hand there was a significant difference between D.S.C.G./no D.S.C.G., ($P = 0.018$**) only one person in the former group complaining of slight irritation whereas there were 8 in the latter group of whom 2 had moderate and 6 slight irritation.

Changes of histological measures

Although numerous significance tests were carried out no highly significant ($P \leqslant 0.01$) changes were shown throughout the treatment concerning the 18 histological measurements in relation to volunteer/patient, D.S.C.G./no D.S.C.G. and preservative/no preservative.

In the analysis of the histological measurements three differences were significant at the 5% level. The percentage of uneven epithelium increased on average in subjects given D.S.C.G., but decreased on average in subjects not given D.S.C.G. The mean number of cells with cilia increased in volunteers, but decreased in patients. The number of mononuclear roundcells in the submucosa increased on average in subjects given preserved solutions and decreased on average in subjects given non-preserved solutions.

DISCUSSION

The analysis of symptoms pre and post treatment present some interesting results. With respect to blocking it is apparent that those paients who had D.S.C.G. only, showed no clinical improvement whereas those who received D.S.C.G.

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*) Two tailed t-tests

**) Fisher "exact" test

and preservative had a marked improvement; although for sneezing and running the same interaction was not found to be significant, it is interesting to note that for these symptoms there was a similar trend. These findings suggest that a combination of D.S.C.G. and preservative is clinically more effective than D.S.C.G. alone; this is contrary to the idea that the preservatives could act as a chemical irritant. A possible explanation is that there is a synergistic action between D.S.C.G. and preservatives; this appears to be unlikely from the knowledge of pharmacology of D.S.C.G. and the fact that it does not potentiate the action of a number of other therapeutic substances such as anti-histamines and bronchodilators.

The pathogenesis of perennial rhinitis cannot totally be ascribed to an allergic reaction. It is possible that other factors are involved such as irritation, infection and psyche. In those patients who have a long history of the disease low grade infection is undoubtedly present so that both the allergic reaction and the infection will produce a final tissue reaction which is inflammatory in nature. If, therefore, an infective element is a contributory factor in this disease, then it is possible that the frequent application of bacteriocidal agents in low concentrations could be beneficial by reducing pathogenic organisms and thus reduce the inflammation caused by infection. Thus a combination of D.S.C.G. and preservative may have a two-fold action; the former acts by stabilizing the mast cell membrane and prevents the release of histamine, the latter has an antiseptic action, and both therefore will reduce the final pathological change of inflammation. A third possible explanation is that the preservatives protect D.S.C.G. against bacterial decomposition. However, when obtaining an unexpected result which is not highly significant, with a limited number of persons, one should be cautious in drawing definite conclusions, especially when multiple significance tests are carried out as in this study.

The incidence of side-effects reported was minimal; nasal irritation was recorded by six patients, four of these received a solution of preservative only and two the diluent; no patient receiving D.S.C.G. reported any side effects. These figures suggest that nasal irritancy is probably part of the symptomatology of the disease rather than a true side-effect.

ZUSAMMENFASSUNG

Die Absicht der Untersuchung war zu entscheiden ob Konservierungsmitteln Benzalkonium Chloride und Phenylethanol in einer 2% Dinatrium Cromoglycate Nasen-spray ein chemisches Rhinitis hervorrufen könnte.

16 Patienten mit vasomotorische Rhinitis und 16 Kontrolpersonen nahmen teil in der Untersuchung. Die Untersuchungsparameter waren sowohl klinische als histologische. Es wurde gefunden, dass die Konservierungsmitteln in den untersuchten Konzentrationen die Nasenschleimhaut nicht beschädigten.

Ausserdem fanden wir das Dinatrium Cromoglycate mit diesen Konservierungsmitteln klinisch mehr effektiv als Dinatrium Cromoglycate allein war.

Die letzte wiederspricht den ursprünglichen Annahme dass diese Konservierungsmitteln chemisch irriterend wirken könne.

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Otopathological Laboratory,
University ENT Department,
Rigshospitalet, Blegdamsvej 9,
2100 Copenhagen, Denmark.