Influence of oral calcium medication on nasal resistance in the nasal allergen provocation test

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Although calcium has been used for several decades to treat allergic diseases of the skin and respiratory tract, controlled studies demonstrating the action of oral preparations in allergic rhinitis are lacking. This placebo-controlled, double-blind, crossover study shows that 1000 mg calcium administered orally significantly inhibits the allergen-induced swelling of the nasal mucosa in the allergen provocation test. Sneezing and secretion, which are allergic symptoms, were not reduced. This study is the first to confirm the positive effect of oral calcium on a symptom of allergic rhinitis. (J ALLERGY CLIN IMMUNOL 1993;91:599-604.)

Key words: *Oral calcium medication, nasal allergen challenge, airway resistance*

Oral calcium medication has been used for many decades in the treatment of allergic diseases of the respiratory tract and skin.[1-3] The use of calcium is based on reports of clinical experience and empirical findings, and few clinical studies have been conducted. In view of this situation, allergy as an indication for calcium therapy has increasingly been the subject of controversial discussion in recent years.

Calcium is of central importance in allergic reactions. The transmembrane influx of calcium is an important step in the activation and degranulation of basophils and mast cells after cross-linking of IgE molecules on the cell membrane.[4-6] In a number of in vitro studies on human basophils and rat mast cells it was possible to reduce the release of histamine by means of calcium channel blocking drugs.[7-9] Recent studies suggest that altered calcium mobilization in respiratory tract muscle is directly implicated in the induction of respiratory tract hyperreactivity,[10, 11] possibly caused by toxic oxygen products.[12] Finally, capillary permeability ("vascular leakage") is a function of the partially calcium-dependent adhesion and contraction of endothelial cells.[13, 14]

In a double-blind, placebo-controlled, crossover study in patients with grass pollen allergy we were able to show that 9 mmol calcium administered intravenously significantly inhibits the increase in airway resistance versus placebo after nasal allergen provocation.[15] We also observed a trend toward reduction of secretion and urge to sneeze, although this was not statistically significant. After intravenous injection of calcium, the serum calcium concentration increased by 0.4 ± 0.05 mmol at the time of provocation. We repeated the study with oral administration of 1000 mg calcium to verify whether a less marked increase in the serum calcium level of the kind expected to occur after oral dosing of calcium can also reduce the airway obstruction on allergen provocation. This report shows that oral treatment with calcium also significantly inhibits the increase in nasal airway resistance after allergen provocation. The mean increase in the serum calcium level was 0.11 ± 0.08 mmol; sneezing and secretion symptoms were not significantly reduced.

MATERIAL AND METHODS

Thirty patients (20 men and 10 women) with a history of grass pollen allergy confirmed in a skin test were enrolled in the study outside the pollen season. The study was designed as a placebo-controlled, randomized, double-blind trial with a crossover arrangement. It was conducted in accordance with the rules of the Declaration of Helsinki and

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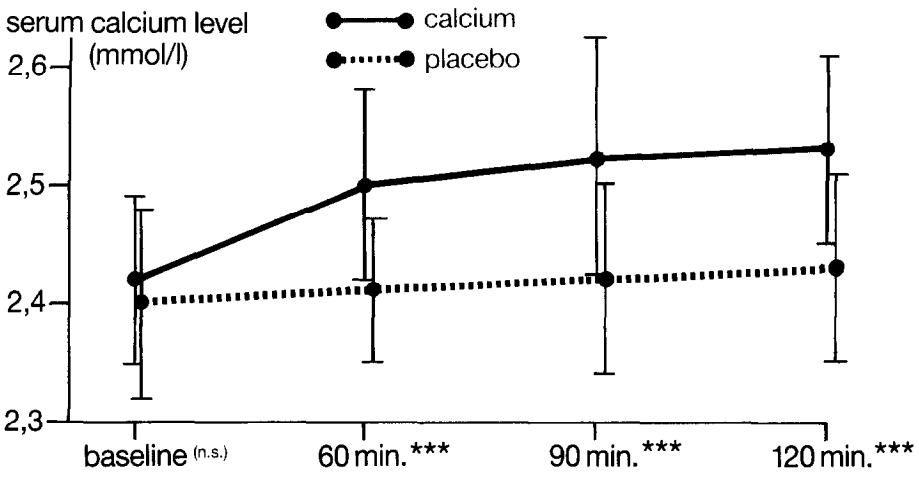

FIG. 1. Serum calcium levels after oral application of 1000 mg calcium or placebo in 27 patients (crossover design). (***$p < 0.0001$).

was approved by the Ethical Committee of Mannheim University; the patients gave their informed written consent to participate. Pregnant women and patients with acute symptoms of rhinitis and bronchial asthma, diseases of the gastrointestinal tract, disorders of calcium and phosphate metabolism, and diseases of the kidneys or cardiovascular system were excluded. The intake of any antiallergic medication and drugs affecting the calcium metabolism was excluded for at least 1 week before the study, with the exception of astemizole (excluded 6 weeks before the study).

Nasal provocation was performed at 1-week intervals, with patients being given a low-calcium diet 3 days before each test. The tests were carried out after a standardized, low-calcium breakfast after a 12-hour fast. The participants received a single dose of 1000 mg calcium (4.954 gm calcium lactogluconate + 0.9 gm calcium carbonate) or placebo as an effervescent tablet in 200 ml of water, and blood samples were taken to determine the serum calcium level before and 60, 90, and 120 minutes after the medication. Ninety minutes after intake the patients were challenged with increasing concentrations of a highly purified, freeze-dried, six-grass extract (Allergopharma, Reinbek, Hamburg, Germany): 3125, 6250, 12,500, 25,000, 50,000, and 100,000 BU/ml (biological units per ml). The provocation solution was applied to the head of the inferior turbinate on the side of the nose, with the lower initial airway resistance measured by means of a metered pump spray under speculum observation (two metered doses of 0.04 ml each). The nasal airway resistance was measured by active anterior rhinomanometry before and 10 minutes after provocation by means of an MP 441 Rhinomanometer (Allergopharma, Reinbek) following the guidelines of the International Standardization Committee for Rhinomanometry.[16] The study target variable was a 40% reduction of the nasal flow value at 150 Pa, representing a 66% increase of unilateral airway resistance. At the same time, the symptoms of allergic rhinitis were quantified by means of a symptom score (maximum 11 points, 0 to 3 points each for sneezing, secretion, and obstruction, 1 point for accompanying conjunctival or pharyngeal reaction).

Serum calcium was determined on a Hitachi 717 Autoanalyser (Hitachi Medical Corp., Tokyo, Japan) following the manufacturer's operating instructions.

The study was analyzed statistically in accordance with the crossover model of Lehmacher.[17]

In addition to confirmatory analysis of the main end point "allergen concentration," an exploratory analysis was performed for the effect on the symptoms of rhinitis.

For clearer presentation of the results, the calculations were performed with the decimal logarithm of the allergen concentration. To eliminate intraindividual differences between the two examinations and similar period effects, the difference examination 1 − examination 2 was calculated for both sequences and compared by means of the two-sample t test at $\alpha = 0.05$. To test residual effects, the sum of examination 1 + examination 2 was assessed for both sequences by means of the two-sample t test at a two-sided $\alpha = 0.05$. In a similar manner the secondary target variable "symptoms of rhinitis" was subjected to exploratory analysis by means of the two-sample Wilcoxon U test for tied samples.

RESULTS

Patients. Twenty-seven of the 30 patients recruited were included in the final analysis. The reasons for classification as dropouts were the following: lacking compliance, absence of flow reduction after provocation, and incomplete documentation. The three dropouts are included in the sample description but were eliminated from the analysis of efficacy. Fourteen subjects received calcium, and 13 received placebo treatment first.

The patients were between 18 and 31 years of age (mean, 24.63 ± 3.26 years) and had had allergic rhinitis for a mean 10.8 ± 0.6 years. In the 2 years before the study allergic symptoms occurred on av-

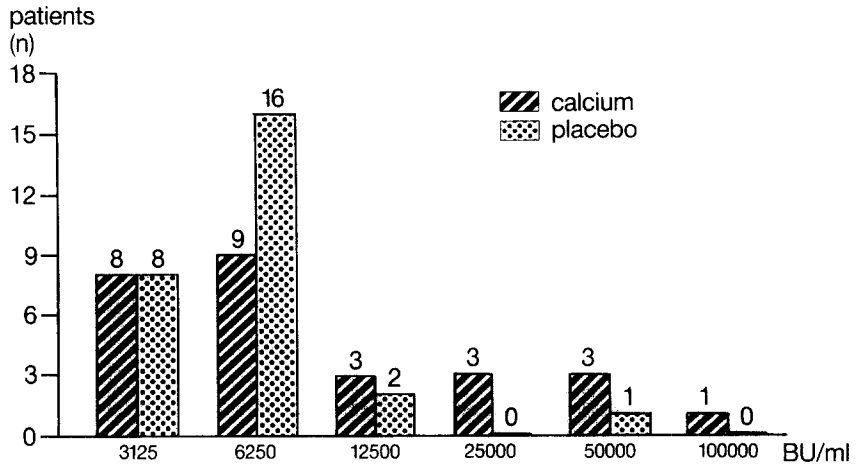

FIG. 2. Allergen concentrations (BU/ml) needed for 40% reduction of nasal flow after oral application of calcium or placebo in 27 patients (crossover design). Difference between both groups is statistically significant ($p < 0.05$).

erage during 3.2 ± 0.9 months, and two thirds of the patients had further allergic disorders. No significant differences were observed between the treatment groups for any of the variables mentioned.

Serum calcium concentration. The baseline calcium values were 2.42 ± 0.08 mmol before administration of calcium and 2.40 ± 0.08 mmol before treatment with placebo (NS).

All three measurements (60, 90, and 120 minutes) revealed that oral administration of calcium had led to a significant increase in the serum calcium concentration to a peak level of 2.53 ± 0.08 mmol after 120 minutes, whereas after placebo no significant changes occurred in serum calcium (Fig. 1).

Therefore at the time nasal provocation was performed, increased serum calcium concentrations after calcium medication were present, compared with placebo.

Flow reduction. The criterion of a 40% reduction of nasal flow was already reached in 24 of 27 subjects, with placebo medication at 3125 ($n = 8$) or 6250 ($n = 16$) BU/ml, whereas with calcium treatment these concentrations led to a positive provocation test only 17 times (eight and nine persons, respectively). Each of the concentration steps 12,500, 25,000, and 50,000 BU/ml was reached by three subjects, and one subject did not achieve a sufficient flow reduction even with 100,000 BU/ml (Fig. 2). The exact values for the allergen thresholds in nasal provocation for 40% reduction of flow for each of the 27 subjects after placebo and after calcium treatment are given in Table I.

An intraindividual comparison revealed that 13 subjects reached the same allergen concentration with calcium and placebo, and that three subjects taking

TABLE I. Allergen concentration thresholds (BU/ml) for 40% reduction of flow after placebo and after calcium treatment

Subject	Sequence	Allergen concentration threshold	
		Calcium	Placebo
1	Cal/Pla	9375	9375
3	Cal/Pla	9375	9375
4	Cal/Pla	9375	21875
9	Cal/Pla	3125	3125
10	Cal/Pla	9375	9375
11	Cal/Pla	3125	3125
14	Cal/Pla	21875	9375
15	Cal/Pla	46875	21875
17	Cal/Pla	196875	96875
21	Cal/Pla	9375	3125
22	Cal/Pla	9375	3125
26	Cal/Pla	46875	9375
27	Cal/Pla	21875	3125
30	Cal/Pla	46875	9375
2	Pla/Cal	9375	9375
5	Pla/Cal	3125	3125
6	Pla/Cal	3125	3125
7	Pla/Cal	9375	9375
8	Pla/Cal	96875	9375
13	Pla/Cal	3125	3125
16	Pla/Cal	96875	9375
18	Pla/Cal	21875	9375
19	Pla/Cal	9375	9375
23	Pla/Cal	3125	9375
25	Pla/Cal	96875	9375
28	Pla/Cal	9375	9375
29	Pla/Cal	3125	3125

Cal, Calcium; *Pla*, placebo.

placebo and 11 subjects taking calcium required a higher allergen concentration. A statistically significant difference ($p < 0.05$) in favor of calcium was found between the allergen concentrations reached with calcium and concentrations reached with placebo (Fig. 2). With calcium therapy the allergen concentration required for flow reduction was 0.22 log units, that is, 66% higher. Assuming a flow reduction of 50% as criterion, an even greater difference was evident at the $p < 0.01$ level, where the superiority of calcium over placebo represented an increase in the allergen concentration of 74%.

In contrast, no significant differences between the treatment and placebo group were detected for any of the rhinitis symptoms, either singly or collectively.

Adverse effects

Four patients reported adverse effects such as tiredness, nausea, sensation of warmth on physical effort, or diarrhea, although a relationship between the symptom (diarrhea) and intake of calcium was assumed to be present in only one case. The study was not discontinued in any of the patients as a result of the documented side effects. Therefore with one inconclusive exception the tolerability of the calcium medication was very good.

DISCUSSION

In this placebo-controlled, double-blind, crossover study in patients with grass pollen allergy we were able to show for the first time that the oral administration of calcium significantly reduces the swelling of the nasal mucosa after nasal allergen challenge. This project therefore confirms a recently published study of similar structure performed with intravenous dosing of calcium.[15] Administered orally, calcium acts only on the airway obstruction monitored by rhinomanometry, but not on the other nasal allergic symptoms.

Nasal provocation testing was performed according to the guidelines of the German Society of Allergy and Immunology Research,[18] with a 40% reduction of the unilateral nasal flow value at 150 Pa as study target variable. In this challenge model unilateral provocation is preferred to bilateral to reduce patients' impairment, increase safety, and keep the unexposed side open for active anterior rhinomanometry of the challenged nostril. However, we have to bear in mind that nasal cycling may lead to misinterpretation of a nonspecific reaction as allergic. In this study reductions of nasal flow were paralleled by increased symptoms, indicating the specificity of these reactions. Furthermore, with the crossover design of the study the possible effects of nasal cycling would affect both groups the same way.

Although calcium belonged to the standard therapeutic repertoire for allergic diseases in the 1920s and 1930s and is still widely used in the German speaking countries, only a small number of controlled studies, some of them contradictory, have been devoted to examining the effect of calcium on the skin. For example, a double-blind, crossover study with the antihistamine drug clemastine versus clemastine and 500 mg calcium orally showed that calcium additionally reduces histamine-induced erythema of the skin up to 9 hours after medication.[3] A controlled study on the effect of oral calcium in allergic rhinitis has so far not been described.

The degranulation of human mast cells and basophils is associated with an increase of the intracellular calcium concentration, with intracellular calcium stores being depleted in a first phase, and extracellular calcium ions flowing through membrane channels into the cell in a second phase.[4] From this it has been concluded that preventing the influx of calcium into the mediator cell is an essential therapeutic goal of antiallergic medication.[7] In past years it has also been reported that calcium channel blockers can exert protective effects in the presence of allergic reactions in animals and human beings.[7-9] Two different types of calcium channels have been described, and the receptor-controlled calcium channel has been claimed to be implicated in the release of histamine from the human basophil granulocyte.[5] Cromoglycate, a compound of recognized antiallergic efficacy, has also been claimed to prevent the inward movement of calcium through binding to specific membrane proteins. From these findings it would follow that administration of calcium and the consequent increase of extracellular calcium levels would increase the release of histamine from basophils and mast cells in response to various stimuli. However, it has been reported that both the reduced and the supramaximal concentration of extracellular calcium reduces the secretory response of peritoneal rat mast cells.[19-21] Pearce[20] assumes that superficial calcium stores in the membrane regulate the influx of the cation into the cytosol of the cell. With high extracellular calcium levels, the cell membrane may be stabilized and the exocytosis of the cell granules prevented.

Extracellular calcium is capable of reducing the release of histamine induced by compound 48/80 in the rat mast cell.[6] It has also been shown that dextran-induced release of histamine in peritoneal rat cells can be suppressed by high extracellular calcium levels.[22] An effect of this kind has also been described for human peripheral leucocytes: peripheral leukocytes of

patients with urticaria, pollinosis, and psoriasis were stimulated with anti-IgE and the calcium ionophore A23187, after which the release of histamine was reduced in a dose-dependent manner by the increase in the extracellular calcium level.[23] When extrapolating these results to our study, however, two points should be borne in mind: First, the increase of the serum calcium level by 0.1 mmol after oral intake remains within the physiologic limits for serum calcium concentration (which is an important criterion for safety of therapy). It has been shown that this increase influences hormonal regulation (parathyroid hormone secretion) in a remarkable manner and so has more implications than just adding to the body calcium,[24] but still the increase of extracellular calcium in the tissue is expected to be low.

Second, stabilization of the mast cells and an associated reduction of histamine release should also lead to a reduction of sneezing and secretion after nasal allergen provocation.

However, we could demonstrate only a marked reduction of allergen-induced swelling of the nasal mucosa. Therefore it seems unlikely that the antiallergic effect of calcium primarily derives from stabilization of the mediator cells.

Finally, calcium has a nonspecific effect on the membrane permeability of endothelial cells and on capillary permeability, the latter depending on the contraction of the endothelial cells and the function of the occluding junctions.[25, 26] In addition to a calcium-independent mechanism, there is also a calcium-dependent mechanism for the intercellular adhesion of endothelial cells, which is specifically inhibited by monoclonal antibodies.[27] Perfusion of corneal endothelium with a calcium-free or low-calcium medium leads to a reversible breakdown of apical junctional complexes resulting in corneal swelling.[28] An increase of extracellular calcium lowers the permeability of the vascular endothelium in the main capillaries of the rat and also reduces the response to histamine, serotonin, and bradykinin.[14] Furthermore, the addition of low doses of calcium inhibits the formation of dextran-induced anaphylactoid edema in the rat.[22] This effect of calcium on capillary permeability should at least partially explain the reduction of the swelling of the tissue of the nasal concha after allergen provocation, since the transudation of serum proteins and fluid into the mucosal tissue is reduced, and the bradykinin and histamine-induced vasodilatation may also be partially antagonized.

In the nasal provocation model we were able to demonstrate a clinically relevant reduction of allergen-induced swelling. The other allergic symptoms measured by means of symptom scores such as sneezing, secretion, conjunctivitis, and throat irritation were not significantly reduced. These findings are consistent with those of the previous study with intravenous administration of calcium. Evidently intraindividual differences exist in the effect of calcium, and some patients therefore benefitted exceptionally well from the oral calcium treatment. On the other hand, oral calcium was found to be well tolerated in this study with a very low rate of side effects, and a similarly favorable tolerability profile is also to be expected on long-term use of the preparation.[29] This could explain the positive effect of calcium on the symptoms of patients with allergic diseases of the respiratory tract and skin frequently observed in clinical trials. It may also be assumed that long-term therapy with oral calcium preparations over several weeks, for example, in patients with seasonal allergic rhinitis, could permanently reduce nasal obstruction. Since antihistamines in particular do not adequately act on this symptom, a combination with an oral calcium preparation would appear suitable. As might be expected, this combination causes fewer side effects than the combination of an antihistamine with α-sympathomimetics, use of which is not recommended for longer than 2 weeks. A controlled study on the effect of long-term oral calcium medication on symptoms of allergic rhinitis has not yet been conducted.

In this double-blind, placebo-controlled, crossover study we were able to demonstrate that oral calcium reduces a major symptom of the allergic nasal reaction. Further studies are needed to define the nature of the pharmacodynamic effect and explore the action of long-term oral medication in seasonal and perennial symptoms of rhinitis.

REFERENCES

1. Sollmann T. A manual of pharmacology and its applications to therapeutics and toxicology. Philadelphia: WB Saunders, 1948:787-891.
2. Debelic M, Haeseler K. Zur Objektivierung der antiallergischen Wirksamkeit eines oralen Kalziumpräparates. Med Welt 1977;25:346-9.
3. Petersen KG, Kerp L. Zum Einfluß von Calcium auf die Antihistaminwirkung von Clemastin. Allergologie 1985;8:270-1.
4. Takei M, Uraschima H, Endo K, Muramatu M. Role of calcium in histamine release from rat mast cells activated by various secretagogues; intracellular calcium mobilization correlates with histamine release. Biol Chem Hoppe Seyler 1989;370: 1-10.
5. Tedeschi A, Miadonna A, Lorini M, Arquati M, Zanussi C. Receptor-operated, but not voltage-operated, calcium channels are involved in basophil leucocyte activation and histamine release. Int Arch Allergy Appl Immunol 1989;90:109-11.
6. Chakravarty N, Wei-Jun Yu. Regulatory role of calcium on histamine secretion. Agents Actions 1986;18:1-2.
7. West GB. Further thoughts on mast cells, calcium channels

and histamine release. Int Arch Allergy Appl Immunol 1990;91:214-6.
8. Gianella E, Masini E, Palmerani B, Pistelli A, Mannaioni PF. Modulation of anaphylactic histamine release by calcium channel agonists and antagonists. Agents Actions 1988;23:3-4.
9. Bindslev Jensen C, Clementsen P, Stahl Skov P, et al. Comparison of the action of calcium antagonists on basophil histamine release. Allergy 1987;42:441-6.
10. Ahmed T, D'Brot J, Abraham W. The role of calcium antagonists in bronchial reactivity. J ALLERGY CLIN IMMUNOL 1988;81:133-43.
11. Black JL, Marthan R, Armour CL, Johnson PR. Sensitization alters contractile responses and calcium influx in human airway smooth muscle. J ALLERGY CLIN IMMUNOL 1989;84:440-7.
12. Weiss EB, Bellino JR. Leukotriene-associated toxic oxygen metabolites induce airway reactivity. Chest 1986;89:709-16.
13. Martinez-Palomo A, Meza J, Platy G, Cereijids M. Occluding junctions in cultures. J Cell Biol 1980;87:736-45.
14. Busch L, Tessler J, Bazerque PM. Effects of calcium and EDTA on rat skin capillary permeability and on its response to histamine, serotonin and bradykinin. Acta Physiol Pharmacol Latinoam 1989;39:227-34.
15. Bachert C, Drechsler S, Keilmann A, Seifert E, Schmidt R, Welzel D. Verminderung der Reaktivität bei Rhinitis allergica durch intravenöse Applikation von Kalzium. Arzneimittelforschung/Drug Research 1990;40:984-7.
16. Clement PAR. Committee report on standardization of rhinomanometry. Rhinology 1984;22:151-5.
17. Lehmacher W. Analysis of the cross-over design in the presence of residual effects. Stat Med 1991;10:891-9.
18. Gonsior E, Bachert C, Berdel D, et al. Richtlinien für die Durchführung von nasalen Provokationstests mit Allergenen bei Erkrankungen der oberen Luftwege Allergologie 1990;13:53-5.
19. Truneh A, Pearce FL. Characteristics of and calcium requirements for histamine release from rat peritoneal mast cells treated with Concanavalin A. Int Arch Allergy Appl Immunol 1981;66:68-75.
20. Pearce FL. Die Stimulus-Sekretions-Kopplung in der Mastzelle. Allergologie 1984;7:27-9.
21. Day RO, Wade DN. Influence of flufenamic acid and calcium ion concentration on the histamine release from rat mast cells induced by compound 48/80 and the calcium ionophore A23187. Biochem Pharmacol 1978;27:1385-7.
22. Hanahoe THP. Effects of calcium on dextran anaphylactoid oedema in the rat. Br J Pharmacol 1972;44:314-6.
23. König W, Baron M, Ehring F. Effect of calcium mineral solutions on the histamine release from human peripheral leukocytes. Arzneimitelforschung/Drug Research 1984;34:52-4.
24. Herfarth K, Drechsler S, Imhoff W, et al. Calcium regulating hormones after oral and intravenous calcium application. (Submitted for publication.)
25. Galdal KS, Stein AE. Effects of divalent cations and various vasoactive and haemostatically active agents on the integrity of monolayers of cultured human endothelial cells. Thromb Res 1981;21:273-84.
26. Baetscher M, Brune K. An in vitro system for measuring endothelial permeability under hydrostatic pressure. Exp Cell Res 1983;148:541-7.
27. Heimark RL, Degner M, Schwartz StM. Identification of a Ca^{2+}-dependent cell-cell adhesion molecule in endothelial cells. J Cell Biol 1990;110:1745-56.
28. Araie M, Hamano K, Eguchi S, Matsumoto S. Effect of calcium ion concentration on the permeability of the corneal endothelium. Invest Ophthalmol Vis Sci 1990;31:2191-3.
29. Kochersberger G, Bales C, Lobaugh B, Lyles KW. Calcium supplementation lowers serum parathyroid hormone levels in elderly subjects. J Gerontol 1990;45:159-62.