The effect of magnesium on the crystal growth rate of calcium oxalate in human urine

(An in-vitro-study with regard to the potential role of Mg^{2+} in calcium oxalate urolithiasis)

By *W. Achilles* and *B. Ulshöfer*

Universitätsklinikum Marburg, Urologische Klinik (Dir.: Prof. Dr. G. Rodeck)/Klinische Forschung, Baldingerstr., D-3550 Marburg (Lahn)

Zusammenfassung

Mit dem Gelkristallisationsverfahren (GKV) wurde der Einfluß von Mg^{2+} auf die relative Kristallwachstumsrate (= V_{kr}) von Kalziumoxalat (= CaOx) im künstlichen Harn bei pH = 6 bestimmt und mit berechneten thermodynamischen Daten verglichen. Das Gelkristallisationsverfahren ist eine hochrationelle Mikromethode zur Bestimmung von Kristallisationskinetiken mittels rechnergesteuerter Scanning-Mikroskopphotometrie.

V_{kr} wird mit steigender Mg^{2+}-Konzentration signifikant erniedrigt. Der Befund läßt sich überwiegend durch die Änderung des CaOx-Aktivitätsproduktes und einen geringen kristallisationskinetischen Einfluß des Mg^{2+} deuten. Verglichen mit dem anderer Harnbestandteile (z. B. Ca^{2+}, Oxalat, Zitrat) ist der Mg^{2+}-Effekt aber relativ gering.

Das Ergebnis wird im Hinblick auf die mögliche Rolle von Magnesium für die CaOx-Urolithiasis diskutiert.

Summary

The effect of Mg^{2+} on the relative crystal growth rate of calcium oxalate (= CaOx) in artificial urine (pH = 6) has been measured by the gel crystallization method (= GCM) and compared with calculated thermodynamic data. The GCM is a highly efficient microprocedure which allows the determination of crystal growth kinetics by computer controlled scanning microphotometry.

CaOx crystal growth rate is significantly decreased by increasing Mg^{2+} concentration which may be accounted for by the alteration of the CaOx activity product and, possibly, a minor kinetic contribution. However, the effect is relatively small compared with those of other urinary constituents (i.e. calcium, oxalate, citrate).

The results are discussed with respect to the potential role of Mg^{2+} in CaOx urolithiasis.

Résumé

Les auteurs ont étudié les effets du Mg^{++} sur le taux relatif de croissance des cristaux d'oxalate de calcium (CaOx) dans de l'urine artificielle (pH 6), au moyen d'une méthode de cristallisation sur gel. Les résultats ont été comparés aux données thermodynamiques calculées. La cristallisation sur gel est une micro-méthode hautement efficace, qui permet la mesure des paramètres cinétiques de la croissance des cristaux par une microphotométrie à balayage, contrôlée par ordinateur.

L'augmentation de la concentration en ions Mg^{++} est associée à une diminution significative du taux de croissance des cristaux de CaOx. Ce phénomène est à mettre sur le compte d'une modification des produits résultants de l'activité du CaOx et, éventuellement, d'une action cinétique mineure. Cependant, cet effet est relativement faible par rapport à ceux enregistrés avec d'autres éléments urinaires (calcium, oxalates, citrates).

Les résultats sont discutés en fonction du rôle potentiel du Mg^{++} dans la formation de lithiases d'oxalate de calcium.

Introduction

The possible role of magnesium in calcium oxalate (= CaOx) urolithiasis has been discussed for about 50 years, especially since the basic solubility studies of *Greta Hammarsten* [1].

However, up to now no final answer could be given as to magnesium is really important in the genesis or efficient in prophylaxis of urinary stone formation [2, 3].

In 1979 and 1983 we reported at first time a novel optical micromethod for the determination of relative crystal growth rate of calcium oxalate (GCM: gel crystallisation method) [4]. This method has now been improved considerably by introducing computer controlled scanning microphotometry as a new, highly efficient technology for measuring crystal growth kinetics [5, 6].

In this paper we describe the effect of magnesium on CaOx crystal growth rate (= V_{cr}) in artificial urine. In contrast to solubility studies, V_{cr} reflects the total effect of thermodynamic as well as kinetic actions of Mg^{2+} on crystal growth.

In order to separate both influences, experimental data have been compared with corresponding data of CaOx supersaturation calculated from complex chemical equilibrium distribution within the system under consideration.

Materials and Methods

All reagents used were of analytical grade. Highly purified water (Ampuwa, Fresenius Inst.) was used in all experiments.

The artificial normal urine had the following composition (concentrations in mmol/l): NaCl = 110, KCl = 20, NH_4Cl = 35, KH_2PO_4 = 15, Na_2SO_4 = 20, citric acid = 2.0, $MgCl_2$ = 3, $CaCl_2$ = 4, urea = 250, NaN_3 = 0.1, HCl up to a final pH of 6.0 [6].

Artificial urines with varying metal ion concentrations were prepared by pipetting different amounts of corresponding stock solutions of $CaCl_2$ and $MgCl_2$ composing the final solutions, and HCl up to a pH of 6.0.

Gel crystallisation method (GCM): The principle of the method has been described in [4, 5]. Further improvements will be presented in a forthcoming paper [6].

Composition of the gel phase: 0.5 % agar-agar (SERVA, Heidelberg), 3 mmol/l sodium oxalate, 2 % glycerol, 0.1 mmol/l NaN_3.

Standard solution: artificial normal urine (pH = 6) as described above.

Measuring device: Automated microphotometric system (ZEISS, Oberkochen, FRG) comprising the following parts: 1. inverted microscope IM 35, 2. scanning stage adapted for microtiter plates, 3. microscope photometer SFD, 4. stabilizing power supply for the light source, 5. electronic control unit MPG 64 and 6. microcomputer HP 9816 (HEWLETT-PACKARD) for controlling the different parts of the system as well as for aquisition, processing and print of the measuring data.

Mode of measurement: dark field.

Mean unprecision of V_{cr} (mean values of 4-fold determinations): 2 %.

Results

Fig. 1 demonstrates the effect of total magnesium concentration (= Mg_T) on the relative crystal growth rate of CaOx (= V_{cr}) as measured by the GCM. For this purpose, Mg_T was varied in an artificial urine while keeping all other components practically constant. V_{cr} is shown as a function of normalized total magnesium concentration, c/c_N (where c = actual concentration and c_N = normal concentration = concentration in the normal urine = 3 mmol/l).

As may be seen from the curve, varying Mg_T from 0.25 · c_N (= 0.75 mmol/l) to 4 · c_N (= 12 mmol/l) reduces V_{cr} from 1.15 to 0.55. Lowering normal Mg_T to a half of its value (= 1.5 mmol/l) increases V_{cr} by 10 % while doubling it (to 6 mmol/l) results in a drop of V_{cr} by about 15 %.

In order to compare the experimental data directly with the degree of CaOx-supersaturation within the crystallizing system the following was done: 1. Complex-chemical equilibrium distribution in the gel phase was calculated according to [7, 8] from total concentrations of all complex-chemically active components, corresponding binding constants and pH. Because of the mixing of measuring solution and gel phase in the region of CaOx-crystallization half the original total concentrations of all components were used in the calculation. 2. The activity product of CaOx (= AP) was calculated and related to the solubility product of CaOx within the gel phase (= Kspg), which had been derived previously from the Ca_T - dependence of V_{cr} in normal artificial urine [8] and found to be about $3.6 \cdot 10^{-8}$ mol^2/l^2 [3]. The degree of supersaturation in an actual solution (= mixture of measuring solution and gel phase), expressed as $S = lg (AP/Kspg)$ then was related to the corresponding value of the normal solution, $S_N = lg (AP_N/Kspg)$, giving $S^* = S/S_N$ [8].

S*, the normalized degree of CaOx-supersaturation, may be directly compared with V_{cr} and is presented by the broken curve in Fig. 1.

In additional experiments, V_{cr} was measured as a function of total calcium at different total magnesium within the artificial urine. The results are shown in Fig. 2.

Linear regression analysis, which could be applied to the data, resulted in the different

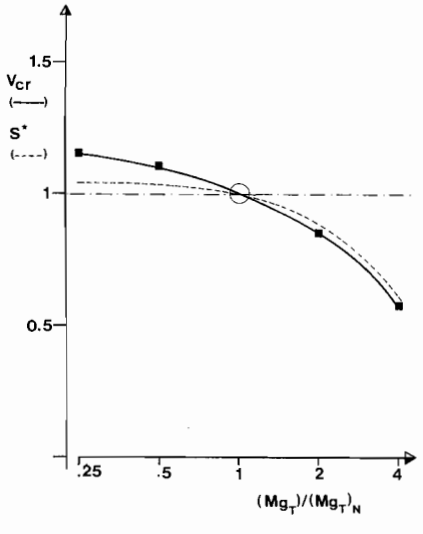

Fig. 1: Dependence of the GCM-measured relative crystal growth rate of calcium oxalate, V_{cr} (——), and corresponding normalized degree of CaOx-supersaturation within the gel phase, S* (-----), on normalized total magnesium concentration $(Mg_T)/(Mg_T)_N$ in artificial urine.

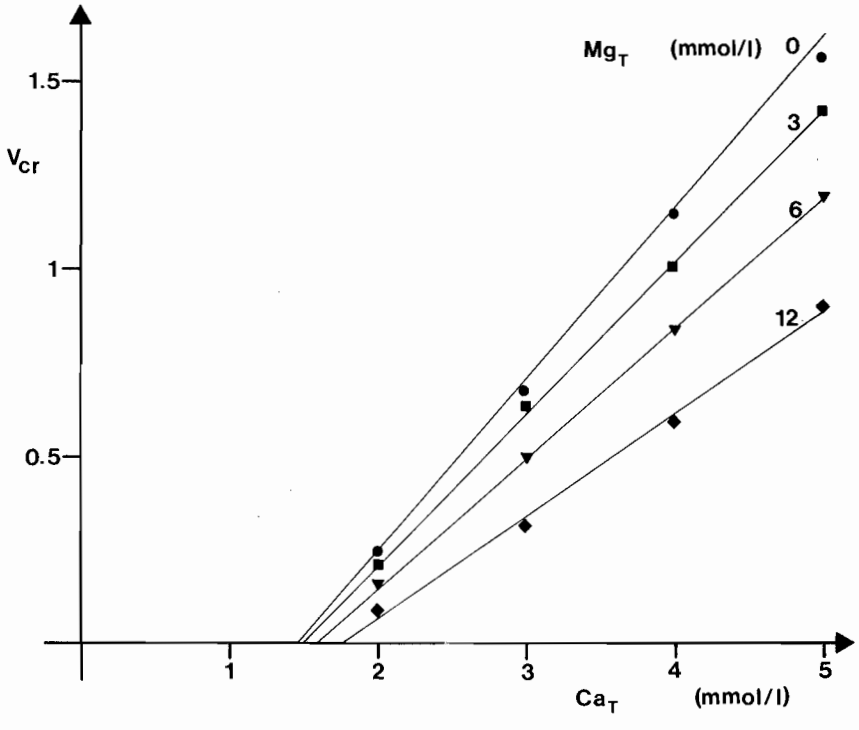

Fig. 2: Dependence of the GCM-measured relative crystal growth rate of calcium oxalate, V_{cr}, on total calcium concentration, Ca_T (abszissa), at different total magnesium, Mg_T (indicated at the curves), in solutions of artificial urine at pH = 6.

slopes of $V_{cr} = f(Ca_T)$ at different Mg_T, thus representing the extended inhibitory effect of magnesium on CaOx crystal growth rate. The section points of the lines correspond to that critical total calcium which is necessary to cause the crystallization process within the gel phase ($V_{cr} > 0$).

Conclusions and Discussion

As may be derived from Fig. 1, Mg_T shows a significant effect on CaOx crystal growth rate ($= V_{cr}$) as measured by the GCM.

Comparing the experimental data (full curves) with the calculated normalized degree of CaOx-supersaturation within the system (broken curves) shows that the reducing effect of Mg^{2+} on CaOx crystal growth can mainly be interpreted by its thermodynamic action via the alteration of the activity product of the crystal forming components.

However, especially at subnormal Mg_T, a certain kinetic effect of magnesium on V_{cr} may be seen from the deviation of calculated and experimental curves. The results of Fig. 1 are confirmed by those demonstrated in Fig. 2. Here, the thermodynamic effect of Mg_T on AP of CaOx is to be seen from the shift of the section points of the abszissa ($= Ca_T^*$). On the other side, the decreasing slope with increasing Mg_T might be interpreted as a kinetic contribution.

The total effect of Mg^{2+}, however, on CaOx crystal growth in the urinary system is relatively small compared with those of calcium, oxalate, citrate and dilution [8]. Therefore, potential effects of an oral magnesium therapy (recurrent stone prophylaxis) in order to prevent CaOx-urolithiasis, can not be expected from an increased Mg-excretion alone but rather from an indirect action on calcium and oxalate metabolism or other major crystal growth effectors within the urine.

Furthermore, as may be derived from Fig. 2, the Ca_T/Mg_T ratio, often used as a measure of CaOx stone formation risk, can not be regarded as a conclusive criterion with this respect. For instance, at the same ratio of 0.33, e.g. $Ca_T/Mg_T = 1/3$, 2/6 and 4/12, V_{cr} equals to < 0, 0.15 and 0.60, respectively.

The gel crystallization method allows the highly efficient determination of relative crystal growth rates of CaOx in undiluted samples of urine and may easily be applied to routine determinations of crystal formation risks in samples from stone formers and normals [9]. Therefore, the in-vivo-effect of crystal growth reducing therapies of CaOx-urolithiasis (possibly those including different Mg^{2+}-containing substances) can now be assessed in a short-time-test with high diagnostic relevance.

We shall report about this in forthcoming papers.

Acknowledgements

I am indebted to Mrs. *E. Kryczanek* and *H. Schmidt* for their engaged technical assistance.

This work has been supported by the Deutsche Forschungsgemeinschaft, Bonn FRG (Ac 52/1-1).

References

[1] *Hammarsten, G.:* Eine experimentelle Studie über Calciumoxalat als Steinbildner in den Harnwegen: Speziell mit Rücksicht auf die Bedeutung des Magnesiums. Leipzig: Harrasowitz 1936; S. 1–155.

[2] *Schneider, H. J.:* Die Rolle des Magnesiums in der Ätiologie und Metaphylaxe des Oxalat-Steinleidens. In: Magnesium-Stoffwechsel (*Schneider, H. J., Anke, M.*, Hrsg.). Wiss. Beiträge F.-Schiller-Universität Jena 1976.

[3] *Baltzer, G.:* Magnesium und Urologie — ein Fortschrittsbericht. Magnesium Bulletin **3**, 1a, 282–287 (1981).

[4] *Achilles, W., Mergner, Ch.* and *Simon, M.:* An Optical Micromethod for the Determination of Relative Crystallisation Rates of Calcium Oxalate in Gels: Method and Preliminary Results. Urol. Research **11**, 87–91 (1983).

[5] *Achilles, W.:* Ein optisches Mikroverfahren zur Bestimmung relativer Kristallisationsgeschwindigkeiten (Gelkristallisationsverfahren). Fortsch. Urol. Nephr. 22: Pathogenese und Klinik der Harnsteine X, p. 377–384 (*Vahlensieck, W.*, u. *Gasser, G.*, Hrsg.). Steinkopff-Verlag, Darmstadt 1984.

[6] *Achilles, W.:* Methodische Neuerungen des kinetischen Gelkristallisationsverfahrens (GKV). Automatisierte Messung des Kalzium-Oxalat-Kristallwachstums durch Scanning-Mikroskopphotometrie. Fortschr. Urol. Nephr.: Pathogenese und Klinik der Harnsteine XI; accepted for publication.

[7] *Achilles, W.* and *Ulshöfer, B.:* Calculations of complex-chemical equilibria in urine: estimate of stone formation risks and derivation of prophylactic measures. In: Urolithiasis and Related Clinical Research, p. 777–780 (*Schwille, P. O., Robertson, W. G., Smith, L. H., Vahlensieck, W.*, eds.) Plenum Press, New York 1985.

[8] *Achilles, W.* and *Ulshöfer, B.:* Der Einfluß von Harnbestandteilen auf das kinetische und thermodynamische Kristallbildungsrisiko von Kalziumoxalat. Fortschr. Urol. Nephr.: Pathogenese und Klinik der Harnsteiner XI; accepted for publication.

[9] *Achilles, W. and Ulshöfer, B.:* Die GKV-Messung relativer Kristallisationsgeschwindigkeiten von Kalziumoxalat in 24-Stunden-Sammelurinen von Normalpersonen und Steinträgern mit rezidivierender Kalzium-Urolithiasis. Fortschr. Urol. Nephr.: Pathogenese und Klinik der Harnsteine XI; accepted for publication.

For the authors: Priv. Doz. Dr. W. Achilles, Universitätsklinikum Marburg, Urologie/Klin. Forschung, Baldingerstr., D-3550 Marburg/Lahn