Noncompetitive inhibition of phospholipase A_2 activity by magnesium

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Zusammenfassung

Magnesium (Mg^{2+}) erfüllt vielfältige Funktionen im Organismus. In der Therapie des akuten Asthmas kann es intravenös und/oder als Aerosol aufgrund seiner bronchodilatatorischen Wirkung eingesetzt werden. Klinisch ist Asthma u.a. durch eine entzündliche Komponente, die mit einer Aktivitätserhöhung der Phospholipase A_2 einhergeht, geprägt. Ziel der Studie war es, die Kinetik der Hemmung der PLA_2 durch Mg^{2+} zu bestimmen. Die PLA_2 wurde mit verschiedenen Substratkonzentrationen und entweder Puffer oder Mg^{2+} inkubiert. Anschließend wurde die Enzymaktivität in einem radioaktiven Assay bestimmt. Die Daten wurden zur Ermittlung der Hemmungsart nach Lineweaver-Burk ausgewertet. Mg^{2-} hemmt die PLA_2 mit statistischer Signifikanz ($p \leq 0.01$). Eine Abnahme des V_{max} Wertes bei gleichbleibendem K_M nach Mg^{2-} Einfluß spricht für eine nichtkompetitive Hemmung. $K_I = 3.25$ mM.

Summary

Magnesium (Mg^{2-}) has a multitude of physiological effects. A new approach in the therapy of acute asthma is the use of intravenous and/or aerosolized Mg^{2-} as bronchodilatator. Asthma has also an inflammatory component, dependent among other things on the phospholipase A_2 (PLA_2) activity. Purpose of this study was to quantify the inhibitory effect of Mg^{2-} on the PLA_2 activity in an in vitro model and to describe the mechanism of inhibition. PLA_2 was incubated with different substrate concentrations and either buffer or Mg^{2-}. The activity was measured in a radioactive assay. Lineweaver-Burk representation of the data was used to determine the type of inhibition. Mg^{2-} inhibits statistically significantly the PLA_2 activity ($p \leq 0.01$). After Mg^{2-}-exposure Vmax is decreased. The inhibition is noncompetitive. $K_I = 3.25$ mM.

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Introduction

Asthma is a disease of the airways with bronchospastic and inflammatory components. Reports in the recent literature suggest an increase in asthma-related morbidity and mortality [7]. This fuels the search for new drugs and application routes in the therapy for acute asthma i.a. phospholipase A_2 (PLA_2) inhibitors [1, 12]. Magnesium (Mg^{2-}) is a potent PLA_2 inhibitor [20, 10]. The use of Mg^{2+} in the therapy of asthma has been recently extensively reviewed [7] and was also the topic of editorials [5]. PLA_2 (EC: 3.1.1.4) is a key enzyme in the metabolism of phospholipids. PLA_2 catalyzes the hydrolysis of membrane phospholipids in the sn-2-position to release fatty acid – mainly arachidonic acid – and cytptoxic products such as lysophosphatidylcholine (LPC) [9]. The extracellular PLA_2 (type I) is secreted by serous glands, while the intracellular PLA_2 type II is found in all cells investigated so far [16]. PLA_2 plays a major role in inflammatory reactions. Increased serum levels of PLA_2 (up to 30.000 U/ml) are described during inflammatory disease [16]. Type I initiates and propagates inflammation, modulates chemotaxis, phagocytosis and superoxide generation. It also enhances the vascular permeability [16]. The cytosolic type II releases fatty acids for the eicosanoid pathway. The release of PLA_2 may potentiate the inflammation in asthma and also causes a contraction of the airways [24]. A large number of activated megakaryocytes and platelets can be found in the airways in status asthmaticus [21]. The purpose of this study was to quantify the inhibitory effect of Mg^{2+} on platelet membrane PLA_2 activity in an *in vitro* model and describe the mechanism of inhibition.

Abbreviations:

- AA: arachidonic acid
- α: factor linking the true V_{max} determined in the absence of the inhibitor to the apparent V_{max} determined in the presence of an inhibitor: apparent $V_{max} = (1/\alpha)\, V_{max}$
- BL: baseline
- Ca^{2+}: calcium
- FEV_1: forced expiratory volume in 1 sec
- K_I: inhibition constant
- K_M: Michaelis-Menten constant
- LPC: lysophosphatidylcholine
- Mg^{2+}: magnesium
- MV: mean value
- p: probability value
- PC: phosphatidylcholine
- PLA_2: phospholipase A_2
- SD: standard deviation
- V_{max}: maximal velocity

Materials and methods

All determinations of PLA_2 were performed using commercially available chemicals.

Preparation of human platelet membranes

Ten millitres of blood were taken from three female and four male healthy volunteers with normal body weight and no medications. Three were

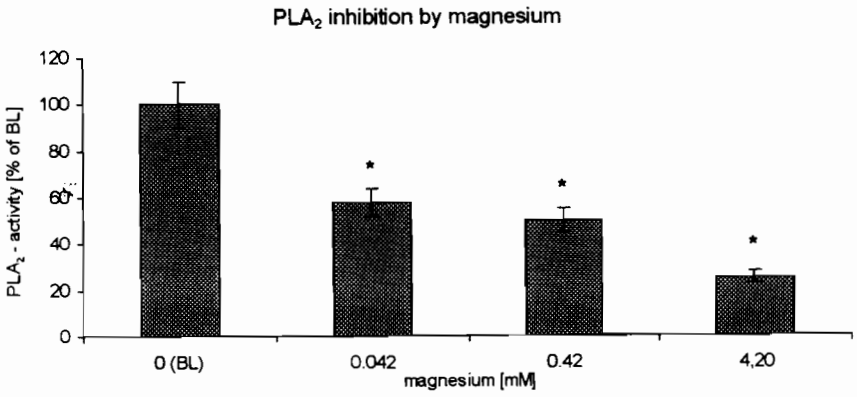

Fig. 1: PLA_2 activity [% of BL] (y-axis) after incubation with different concentrations of Mg^{2+} (0 – 0.042 – 0.42 – 4.2 mM) (x-axis) for 30 min. (n = 9) * = $p \leq 0.01$.

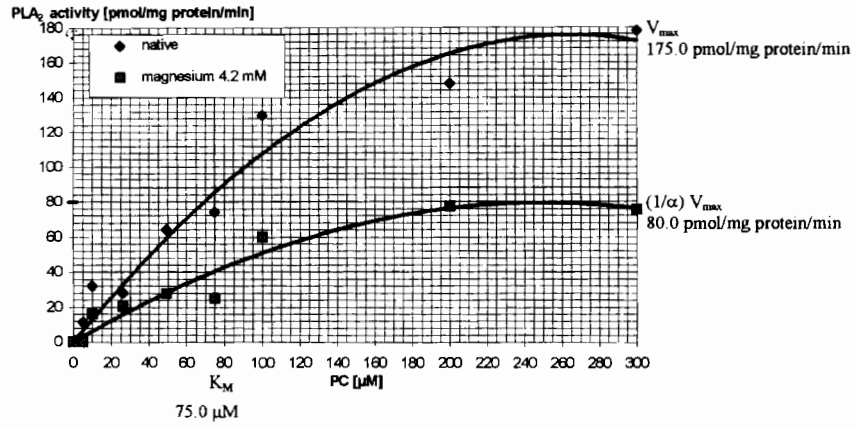

Fig. 2: Michaelis-Menten representation: V_{max} is apparently decreased after exposure to 4.2 mM Mg^{2+}, while K_M does not change as shown in the Michaelis-Menten representation.

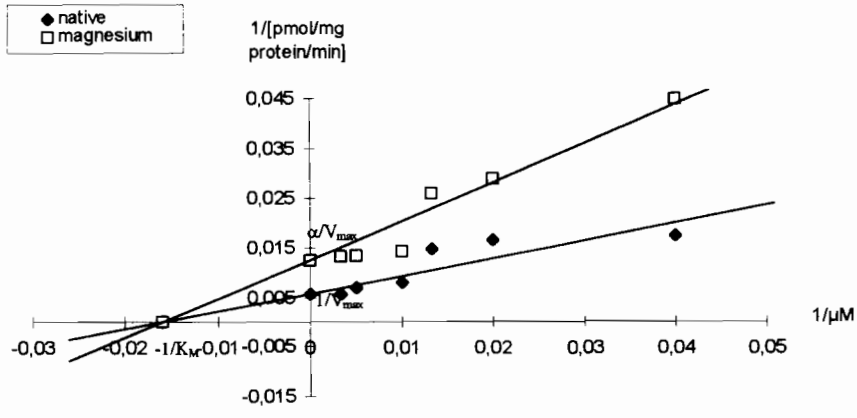

Fig. 3: Lineweaver-Burk representation: Magnesium (4.2 mM) inhibits PLA_2 activity in a noncompetitive manner. Coefficients of correlation: $r_{native} = 0.98$ $r_{Mg} = 0.95$

smokers and four nonsmokers. Platelets were sedimented by centrifugation and suspended into TRIS buffer (pH 7.4; 50 mM) containing 8.0 g of saccharose in 100 ml.

Determination of the protein concentrations

The modified Lowry method was used to measure the protein concentrations. [8, 17].

Magnesium exposure

Human platelet membranes containing active PLA_2 dissolved in TRIS buffer (pH 9.0; 1M) were incubated for 30 min with a) or b) respectively:

a. TRIS buffer = native samples
b. Mg^{2+} : 1, 10 or 100 µg/ml (0.042, 0.42 or 4.2 mM)

After the incubation time the protein concentration was diluted to 20 µg/ml by adding TRIS buffer.

Assay of PLA_2 activity

PLA_2 activity was measured by the method of *Flesch* [6] and *Sundaram* [23] as previously reported [14]. PLA_2 activities were expressed in pmol/mg protein/min. Mean values were used for statistical analysis with the Mann-Whitney rank order test (fig. 1). Baseline values (BL = native activity: incubation time of 0 min, no drugs added) were considered to be 100%. All other values were expressed as a percentage thereof.

K_M and V_{max} determinations

Commercially available purified porcine PLA_2 (Sigma, Steinheim-Germany) was incubated with different substrate concentrations (0 – 300 µM) in the absence or presence of Mg^{2+} (4.2 mM) for 30 min respectively. The PLA_2 activity was determined in a commercially available radioactive PLA_2 assay (Scintillation Proximity Assay: SPA; Amersham, Braunschweig-Germany). Mean values (MV)

	Native sample ([I] = 0)	Mg^{2+} sample ([I] = 4.2 mM)
K_M [µM]	75.0	75.0
α	1	2.19
$(1/\alpha)V_{max}$ [pmol/mg protein/min]	175.0	80.0
K_1 [mM]	N.A.	3.53

Tab. 1: The effect of increasing concentrations of Mg^{2+} on PLA_2 activity in the absence of substrate as determined 30 min after substrate addition. MV and SD (n = 9) of absolute values [pmolAA/mg protein/min] and as a percentage of BL. Compared to BL the inhibition is statistically significant: $p \leq 0.01$ (*)

Mg^{2+} [mM]	0 (BL)	0.042	0.42	4.2
[pmol/mg protein/min]	23.8 ± 3.7	13.9 ± 2.1*	11.8 ± 2.0*	5.9 ± 3.4*
[%]	100 ± 16	58 ± 9*	50 ± 8*	25 ± 14*

of the data were plotted as Michaelis-Menten (fig. 2) and Lineweaver-Burk (fig. 3) diagrams.

Results

Results are shown in table 1 and figures 1, 2 and 3 and can be summarized as follows:

Dose dependency (human platelet PLA_2):

- Magnesium inhibits PLA_2 activity in a dose dependent manner. Subphysiological Mg^{2+}-levels (0.042 mM and 0.42 mM) cause an inhibition of 50 – 60% of native activity. Concentrations in the supraphysiological range (4.2 mM) decrease the activity to 25%. Compared to baseline values the results are statistically significant ($p \leq 0.01$). K_1 = 3.25 mM (human platelet PLA_2). [table 1 and figure 1]

Kinetic determinations (porcine PLA_2):

- V_{max} is apparently decreased after exposure to 4.2 mM Mg^{2+}, while K_M does not change as shown in the Michaelis-Menten representation [figure 2].
- Lineweaver-Burk representation suggests a noncompetitive inhibition [figure 3].

Discussion

Mg^{2+} is a potent PLA_2 inhibitor [20, 10]. Its use for asthma therapy has been advocated as early as 1987 [13, 19] and little later by *McNamara* who suggested that i.v. applied Mg^{2+} could help avoid endotracheal intubation [11]. Inhaled Mg^{2+} decreases bronchoconstriction (*in vitro* and *in vivo*) and increases FEV_1 in histamine [19, 18], metacholine [4] and betanechol [22] induced bronchoconstriction. In addition, to the well known muscle relaxing effect, Mg^{2+} has anti-inflammatory properties such as stabilization of mast cell membranes [22] and attenuation of neutrophil burst reaction [3].

The physiologic magnesium blood level in humans is in the range 0.6 to 1 mmol/l. We used in vitro two subphysiologic (0.042 and 0.42 mM) and one supraphysiologic (4.2 mM) concentration. When applied i.v. Mg^{2+} levels in the blood will of course increase. This increase (depending on the total amount and application rate) will in most cases level off due to intracellular uptake at around 2 – 3 mmol/l. The supraphysiologic concentration we used is at the higher end of those achievable in vivo by i.v.-application. However when applied intratrachealy, the local magnesium concentration can easily exceede 4.2 mM. As such we conclude that the inhibitor concentrations we used in vitro have clinical significance and are easily achievable in vivo.

A marked inhibitory effect of subphysiological, physiological and supraphysiological Mg^{2+} levels on platelet membrane PLA_2 activity was demonstrated. PLA_2 activity depends on the presence of Ca^{2+}-ions. The Mg^{2+}-ion competes with Ca^{2+} to bind at the PLA_2-molecule. Other divalent cations have a similar inhibitory effect [20, 10]. These findings imply that PLA_2 activity is modulated by Mg^{2+} and that an increase in Mg^{2+} concentration could further reduce the activity of the enzyme. While the source of the enzyme is probably of little importance for the Mg^{2+} effect, we felt that platelets who have been shown to play a pivotal role in asthma, are probably a good source for the enzyme [21].

We have shown that the Mg^{2+}/PLA_2 interaction leads to a decreased maximal velocity (V_{max}) while K_M does not change. The Lineweaver-Burk representation suggests a noncompetitive (mixed) inhibition where Mg^{2+} interacts with the PLA_2-molecule and the enzyme-substrate-complex. The inhibitory effect of Mg^{2+} on PLA_2 activity is much more pronounced than that of furosemide [15], an other drug which was shown to have a positive effect when inhaled in asthma [2].

PLA_2 is involved in inflammatory processes. As such an inhibition of PLA_2 by Mg^{2+} in vitro suggests an anti-inflammatory effect. If the results are reproducible in vivo the application of Mg^{2+} in asthma-therapy, as an anti-inflammatory drug with additional smooth muscle relaxant effect, might be warranted.

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References

[1] *Barnes, P.J.:* New drugs for asthma. Clin. Exp. Allerg. **26** (1996) 738 – 745.

[2] *Biance, S.; Robuschi, M.; Vaghi, A.; Pasargiklian, M.:* Prevention of exercise-induced bronchoconstriction by inhaled frusemide. Lancet **30** (1988) 252 – 255.

[3] *Cairns, C.B.; Kraft, M.:* Magnesium attenuates the neutrophil respiratory burst in adult asthmatic patients. Acad. Emerg. Med. **3** (1996) 1093 – 1097.

[4] *Chande, V.T.; Skoner, D.P.:* A trial of nebulized magnesium sulfate to reverse bronchospasm in asthmatic patients. Ann. Emerg. Med. **21** (1992) 1111 – 1115.

[5] *Cydulka, R.K.:* Why magnesium for asthma? Acad. Emerg. Med. **3** (1996) 1084 – 1085.

[6] *Flesch, I.; Schmidt, B.; Ferber, E.:* Acylchain specificity, and kinetic properties of phospholipase A_1, and A_2 of bone marrow derived macrophages. Z. Naturforsch. **40c** (1985) 356 – 363.

[7] *Jagoda, A.; Shepherd, S.M.; Spevitz, A.; Joseph, M.:* Refractory asthma, part 1: epidemiology, pathophysiology, pharmacologic interventions. Ann. Emerg. Med. **29** (1997) 262 – 274.

[8] *Lowery, O.H.; Rosebrough, N.J.; Farr, A.L.; Randall, R.J.:* Protein measurement with the folin phenol reagent. Biol. Chem. **193** (1951) 265 – 275.

[9] *Mansbach, C.M.:* Phospholipases: old enzymes with new meaning. Gastroenterology **98** (1990) 1369 – 1382.

[10] *Märki, F.; Franson, R.:* Endogenous suppression of neutral-active and calcium-dependent phospholipase A_2 in human polymorphonuclear leukocytes. Biochem. Biophys. Acta **879** (1986) 149 – 156.

[11] *McNamara, R.M.; Spivey, W.H.; Skobeloff, E.; Jacubuwitz, S.:* Intravenous magnesium sulfate in the management of acute respiratory failure conplicating asthma. Ann. Emerg. Med. **18** (1989) 197 – 199.

[12] *Mlodzik, H.:* Agents for the treatment of asthma: patent analysis 1990 – 1995. Expert. Opin. Ther. Pat. **6** (1996) 57 – 60.

[13] *Okayama, H.; Aikawa, T.; Okayama, M.; Sasaki, H.; Mue, S.; Takishima, T.:* Bronchodilating effects of intravenous magnesium sulfate in bronchial asthma. J. Am. Med. Assoc. **257** (1987) 1076 – 1078.

[14] *Petroianu, G.; Helfrich, U.; Schmitt, A.; Bergler, W.; Ruefer, R.:* Dose-dependent inhibition of phospholipase A_2 by paraoxon in vitro: preliminary results. J. Appl. Toxicol. **17** (1997) 421 – 424.

[15] *Petroianu, G.; Helfrich, U.; Rüfer, R.:* Influence of theophylline and furosemide on the PLA_2 activity. Naunyn-Schmiedeberg's Arch. Pharmacol. **356** (Suppl. 1) (1997) R29.

[16] *Pruzanski, W.; Vadas, P.:* Soluble phospholipase A_2 in human pathology: clinical-laboratory interface. In Mukherjee, A.B. (ed): Biochemistry, Molecular Biology, and Physiology of Phospholipase A_2 and its Regulatory Factors. Plenum Press N.Y. **279** (1990) 239 – 251.

[17] *Richterich, R.:* Klinische Chemie. Akad. Verlagsges., 4th edn, Frankfurt 1978, 527 – 530.

[18] *Rolla, G.; Bucca, C.; Bugiani, M.; Arossa, W.; Spinaci, S.:* Reduction of histamine induced bronchoconstriction by magnesium in asthmatic subjects. Allergy **42** (1987) 186 – 188.

[19] *Rolla, G.; Bucca, C.; Caria, E.:* Dose-related effect of inhaled magnesium sulfate on histamine bronchial challenge in asthmatics. Exp. Clin. Res. **14** (1988) 609 – 612.

[20] *Rönnko, S.:* Purification and characterization of phospholipase A_2 from bovine prostate. Int. J. Androl. **15** (1992) 394 – 406.

[21] *Slater, D.; Martin, J.; Trowbridge, A.:* The platelet in asthma. Lancet **12** (1985) 110.

[22] *Spivey, W.H.; Skobeloff, E.M.; Levin, R.M.:* Effect of magnesium chloride on rabbit bronchial smooth muscle. Ann. Emerg. Med. **19** (1990) 1107 – 1112.

[23] *Sundaram, G.S.; Shakir, K.M.M.; Barnes, G.; Margolis, S.:* Release of phospholipase A2 and triglyceride lipase from rat liver. Biol. Chem. **253** (1978) 7703 – 7710.

[24] *Vadas, P.; Stefanski, E.; Wloch, M.; Groux, B.; Van den Bosch, H.; Kennedy, B.:* Secretory non-pancreatic phospholipase A_2 and cyclogenase-2 expression by tracheobronchial smooth muscle. Eur. J. Biochem. **235** (1996) 557–563.

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