

# Complex function of magnesium in blood clot formation and lysis

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## Abstract

*Magnesium salts alone or mixed with other minerals and vitamins are commonly used as dietary supplements. Also, magnesium is infused intra-operatively in a variety of surgeries. Magnesium is one of the most plentiful intracellular divalent cation and is a cofactor in approximately 300 enzymatic reactions. Magnesium is considered safe; however, there are some reports on its health risk when overdosed. It has been reported that Mg<sup>2+</sup> ions play a role in blood clotting competing with Ca<sup>2+</sup> ions. Moreover, magnesium has a different and conflicting role on proteins of coagulation cascade and its net action on coagulation is difficult to predict. For this reason we have investigated whether magnesium can change clotting characteristics of plasma and blood. We have found that magnesium increases the clotting time in plasma and in whole blood in a concentration-dependent fashion. Also, blood clotted in the presence of increased concentration of magnesium, has progressively shortened the lysis time of whole blood clots. We postulated that Mg<sup>2+</sup> exerts its anticoagulant influence by acting at the stage of prothrombin activation in the blood coagulation cascade competing with calcium needed for thrombin activation. Shortening lysis of whole blood clots can utilize an alternative mechanism. PAI-1 in the presence of thrombin, vitronectin and Ca<sup>2+</sup> or Mg<sup>2+</sup> is inhibited, causing larger amounts of tPA to remain in the free form. Excessive amounts of tPA activate plasminogen that lyses clots faster. However, further investigations are required to determine the clinical relevance of these observations.*

**Key words:** magnesium, anticoagulants.

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## Introduction

Magnesium is one of the most plentiful intracellular cations and is a cofactor in approximately 300 enzymatic reactions. Only less than 1% of the total amount of Mg is present in blood [1]. Magnesium salts alone or mixed with other minerals and vitamins are commonly used as dietary supplements. Also, magnesium is infused intra-operatively in a variety of surgeries [2]. Magnesium is considered safe and beneficial [3-6], however there are some reports on its health risk when overdosed [7, 8]. For adults over 30, the recommended daily dose is 420 mg/day for men and 320 mg/day for women. However, Niedworok *et al.* report-

ed that in a group of 949 people they found that the recommended daily dose for magnesium was exceeded by 292 mg/day (183 women and 109 men). The authors emphasized that an uncontrolled excessive magnesium intake by both men and women may constitute a health threat [9].

It has been reported that Mg<sup>2+</sup> ions play a role in blood clotting competing with Ca<sup>2+</sup> ions [3, 10-13]. Anstall *et al.* reported that small amounts of magnesium added to fresh human plasma prolong the clotting time substantially. They explain this action as a cation antagonism between Mg<sup>2+</sup> and Ca<sup>2+</sup>, both competing for clotting factors. Also, they revealed an interesting fact that in Germany during and shortly after the Second World War, magnesium sulfate was

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widely used as a muscle relaxant and the blood of patients examined post mortem after such treatment was unclottable [14]. Na *et al.* investigated intra-operative infusion of magnesium sulfate by maintaining magnesium levels at the upper limit of the normal range (1.58 mmol/l) and found that Mg<sup>2+</sup> extends the clotting time [2]. Sekiya *et al.* reported that not only Ca<sup>2+</sup> cations, but also Mg<sup>2+</sup> cations stabilize the native conformation of coagulation factor IX and at physiological concentrations significantly amplify the biological activities of this factor. Using dialyzed plasma supplemented with coagulation factors, they showed also that addition of Mg<sup>2+</sup> ions augmented the apparent coagulant activity of factor IXa but not factor Xa. Moreover, activation of factor X by factor IXa in the presence of factor VIIIa, phospholipids, and Ca<sup>2+</sup> cations was accelerated by Mg<sup>2+</sup> cations. They also observed that clotting of normal plasma induced by factor VIIa was shortened by magnesium as well [10].

In the blood coagulation pathway, calcium activates prothrombin to thrombin that converts fibrinogen to fibrin. Prothrombin is post-translationally altered in a vitamin K-dependent reaction converting glutamic acids to  $\gamma$ -carboxyglutamic acid (Gla). Gla residues promote the binding of prothrombin to phospholipid bilayers in the presence of Ca<sup>2+</sup>. Binding different cations produces a structure different from that induced by calcium, nevertheless some cations can bind to phospholipids [15, 16]. Nelsestuen *et al.* reported that calcium is required for two distinct steps in prothrombin activation (factor X and prothrombin-phospholipid binding). The first step was activated by nearly all cations tested (Mg<sup>2+</sup>, Ca<sup>2+</sup>, Sr<sup>2+</sup>, Ba<sup>2+</sup>, Mn<sup>2+</sup>, Be<sup>2+</sup>, Fe<sup>2+</sup>, <sup>3+</sup>, Zn<sup>2+</sup>). However, the calcium binding sites implicated in the protein-phospholipid complex formation, show superior selectivity for cations required for the protein transition. Only strontium and barium are observed to satisfactorily replace calcium in this role. The others will form a protein-phospholipid complex with a different structure resulting in inhibition of the coagulation reactions [15].

Magnesium has a different and conflicting role on proteins of coagulation cascade and its net action on coagulation is difficult to predict. For this reason, we have investigated whether magnesium can change clotting characteristics of human plasma and blood.

## Material and methods

*Lyophilized Specialty Assayed Reference Plasma*, Cat. No. 5185 (S.A.R.P., 10 × 1 ml) from Helena Laboratories, Beaumont, TX, was prepared from a frozen pool of citrated plasma of healthy donors. S.A.R.P. has normal PT and aPTT clotting times and can be used as reference material for the following parameters: fibrinogen\*\*, factor II\*, factor V\*\*, factor VII\*, factor VIII\*, factor IX\*, factor X\*, factor XI\*\*, ristocetin cofactor\*, vWF:Ag\*, factor XII, protein C\*, protein S – total, free (\*Standardized to WHO,

\*\*Calibrated against ISTH reference material). Plasma was stored at 4°C, reconstituted by adding 1 mL of deionized water and rest for 5 minutes.

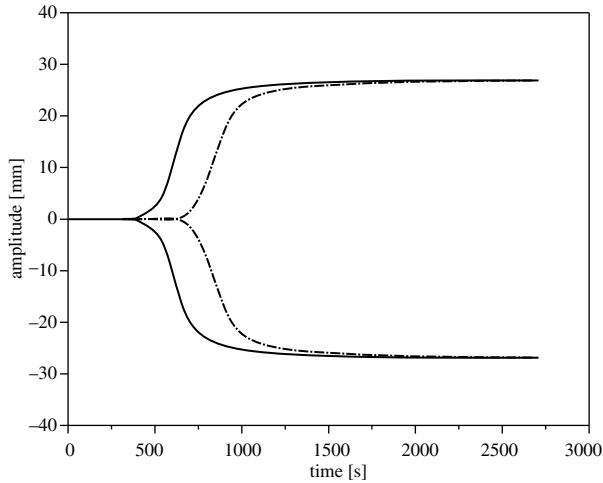
*Blood* was drawn from five healthy subjects (3 males and 2 females, aged 47-78) into plain evacuated plastic tubes and immediately placed on ice. Subsequently, 0.3 ml portions of whole blood were pipetted into glass test-tubes (10 × 70 mm) containing various concentrations of magnesium chloride (Sigma-Aldrich). Afterwards, the test-tubes were incubated at 37°C and the time of clotting of blood was recorded with a stop-watch. The clotted blood samples were then further incubated at 37°C and times of thrombolysis were observed for up to 24 hrs.

*Plasma clot formation.* In a separate experiment, human citrated plasma (pooled) was dialyzed against phosphate buffered saline (PBS), pH 7.4, and 0.2 ml portions mixed with 20  $\mu$ l of magnesium chloride solutions at various molar concentrations. Next, 20  $\mu$ l of thrombin (100 U/ml; Sigma-Aldrich) was added to each tube and the clotting time was recorded. Each experiment was done in triplicate and mean value  $\pm$  SD was calculated.

*Analysis of plasma clot formation with thromboelastography.* Thromboelastography allows for the measurement of a global coagulation profile and yields data on the kinetics and dynamics of clot formation and clot lysis in plasma and whole blood [17]. The critical part of this instrument is a pin hanging on a torsion wire and suspended in a cup holding a sample (360  $\mu$ l). This pin oscillates at 6 rpm at a 4°45' angle at 37°C. When plasma viscosity changes during clot formation, this pin motion is progressively restrained by the clot and the cup. The strength of the clot determines the degree of the force on the pin. Sodium citrated, reconstituted plasma was used for TEG assays by mixing 1 ml of plasma with 20  $\mu$ l of kaolin (Haemoscope Co., Neils, IL, USA) to which 20  $\mu$ l of CaCl<sub>2</sub> (0.2 M) or/and of MgCl<sub>2</sub> (0.2 M) was added. The critical parameters of clotting measured by TEG are as follows: R is the time from the start of the reaction until a measurable clot is detected, K is the time from the R point until a certain clot firmness is achieved, An ( $\alpha$ ) is the maximum angle that represents kinetics of clotting and LY45 (percentage) represents clot lysis 45 min after MA (maximum amplitude) [17-19].

## Results and discussion

*Thromboelastography* is an established method for measuring plasma or whole blood clotting characteristics and has been evaluated systematically for its role in the detection and management of coagulation problems. We use this method to establish the effect of magnesium on coagulation. Table 1 and Fig. 1 show that magnesium extended R time significantly in comparison with not treated plasma and differences were statistically significant. Magnesium changes the other parameters of plasma coagulation (increase K, lowers  $\alpha$ ) but no statistical significance was

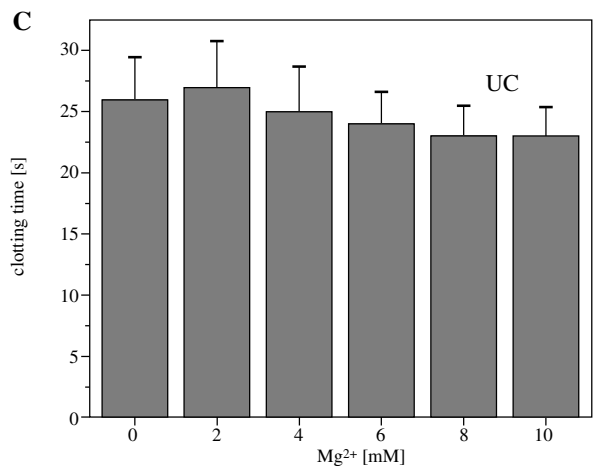
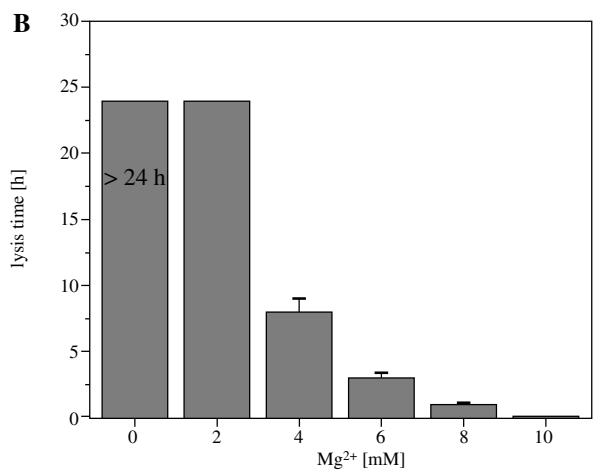
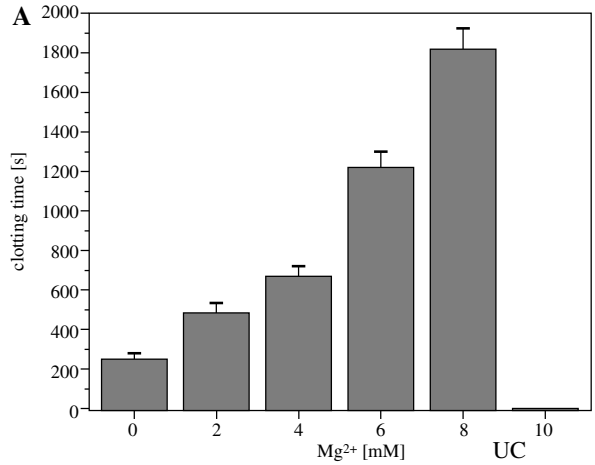


**Fig. 1.** Example of thromboelastogram of plasma: solid line – clotting was induced by addition of  $\text{CaCl}_2$ ; dotted line – clotting induced by  $\text{CaCl}_2$  in the presence of  $\text{MgCl}_2$ . Clotting parameters are practically the same except for a significant increase in R time in the presence of  $\text{Mg}^{2+}$

established. Similar observations were made for whole blood by James *et al.* [20]. It is important to notice that magnesium changes these parameters to such extent that they are out of normal range. There are no normal ranges established for plasma but by comparison with our previous experiments, R time was well out of control values (not treated) for the majority of the examined samples (> 75) [21]. Both R and K parameters are a measure of time of initiation of coagulation and these extensions suggest prolonged bleeding in patients. The  $\alpha$  angle is a measure of speed of clot formation in the initial phase and its low values could suggest extended bleeding as well. Thrombolysis, as measured by amplitude at 45 minutes, was not affected by magnesium in our study and in the report by James *et al.* [20]. These are important findings since  $\text{Mg}^{2+}$  could inhibit or potentiate the fibrinolytic activity by affecting tissue plasminogen activator activity. Stewart *et al.* investigated the effect of  $\text{Mg}^{2+}$  on tPA and they found that  $\text{MgSO}_4$  had no effect on tPA induced clot lysis (60 minutes) at up to six times of normal serum concentration [22].

Thromboelastometry is an ideal method to measure dynamics of blood clotting and clot strength. However, it cannot measure clotting parameters longer than 180 minutes [17, 23, 24]. Therefore, we have also measured plasma and blood clotting using different methods.

*Effect of magnesium chloride on spontaneous clotting whole blood and plasma.* In the freshly collected blood we have observed that magnesium increased the clotting time in a concentration-dependent fashion. In the highest concentration (10 mM) blood did not clot (Fig. 2). Since prothrombin activation requires calcium ions, it is quite pos-



**Fig. 2.** Effect of magnesium chloride on: A – spontaneous clotting of whole blood; B – thrombolysis of whole blood; C – thrombin-induced clotting of human plasma. Values expressed as means (triplicate)  $\pm$  SD; UC – unclottable

**Table 1.** Selected parameters of blood and plasma clotting by TEG

	Range in blood (TEG)	Blood control	Blood magnesium	Plasma control	Plasma Ca <sup>2+</sup> + Mg <sup>2+</sup>
R [s]	240-480	438 ±72	624 ±114	522 ±48	720 ±12
K [s]	0-240	198 ±234	306 ±42	162 ±60	222 ±60
α [°]	47-74	35.6 ±6.7	27.5 ±5.7	55.1 ±9.6	45.4 ±0.6
MA [mm]	54-72	50.2 ±4.7	46.5 ±2.4	27.0 ±3.0	24.8 ±1.0

Normal ranges were reported by: [17, 34, 35]; blood control and blood magnesium by [20].

sible that Mg competes with Ca for the binding sites at the prothrombin molecule, thus altering its ability to initiate intrinsic blood coagulation. It is also possible that magnesium may counterbalance the procoagulant effect of free iron that is known to accumulate with age [25]. By contrast, magnesium blood content is decreasing with age, and this may explain why degenerative diseases are age-related [25-29] and why hypomagnesemia potentiates the pro-thrombotic consequences of free iron [30]. In view of the fact that magnesium has no effect in dialyzed human plasma clotted with thrombin (Fig. 2C), it has to be concluded that Mg exerts its anticoagulant influence by acting at the stage of prothrombin activation in the blood coagulation cascade. Therefore, we suggest that magnesium can be used as a safe anticoagulant that does not inhibit extrinsic clotting of blood occurring at the site of vessel wall injury [32, 33].

In addition, we have found that blood clotted in the presence of increased concentration of magnesium has progressively shortened the lysis time of whole blood clots (Fig. 2B). This unexpected finding can be explained by works done by Urano *et al.* [11-13]. These investigators found that when PAI-1 enriched fibrin clots were formed by increasing concentrations of thrombin (0.1-1.0 IU/ml) in the presence of calcium, their lysis times became shorter (43.8-13.9). Times of lysis were shortened further by addition of vitronectin (17.0-0.9 h). Using fibrin autography they found that both enzymes interfered in a dose-dependent manner with complex formation between tPA and PAI-1, with large amounts of tPA remaining in the free form. They concluded that this mechanism plays a role in the enhancement of fibrinolysis [11-13]. It is plausible that magnesium activates this mechanism similarly as calcium does.

Jahnen-Dechent W and Ketteler reported that moderate hypermagnesemia (> 1.6 mmol/l in serum) is associated with hypotension, cutaneous flushing, nausea and vomiting. At higher concentrations, magnesium might lead to neuromuscular dysfunction, ranging from drowsiness to respiratory depression, hypotonia, areflexia and coma in severe cases. They also reported cardiac effects of hypermagnesemia including bradycardia, complete heart block, atrial fibrillation but not abnormalities of blood clotting or increased susceptibility to hemorrhage as a complication of magnesium therapy [31]. Contrary to that, Besselaar *et al.*

found that even a small contamination of blood collecting tubes with magnesium can significantly change blood coagulation parameters [32, 33]. It must be stressed that *in vitro* and *in vivo* findings seem to be in conflict with each other. However, hypermagnesemia is a relatively rare condition and it is possible that this coagulopathy was simply overlooked.

In conclusion, magnesium infusion or consumption can attenuate an intrinsic coagulation pathway affecting hemostatic propensity of blood. Hence, further investigations are required to determine the clinical relevance of this observation.

*The authors declare no conflict of interest.*

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