High-magnesium concentration and cytokine production in human whole blood model

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Abstract. The potential influence of magnesium (Mg) on inflammatory responses was assessed using an ex vivo model – human whole blood incubated with and without lipopolysaccharide (LPS). Addition of LPS leads to higher levels of cytokines including TNF-α and IL-6. No significant effect of Mg was observed following LPS stimulation whereas high concentration of Mg inhibited the baseline level (without LPS) of TNF-α and IL-6 production. This observation contrasts with that of a previous one on Mg-deficient animals. Therefore, the weak efficiency of increasing Mg concentration in this study on the whole blood from healthy volunteers suggests that the efficiency of Mg supplementation on cytokine production induced by endotoxin challenge depends on Mg status.

Key words: magnesium, cytokine, endotoxin, inflammation, blood

There are several papers showing epidemiological, clinical and experimental evidence on the relationship between magnesium status and the inflammatory response [1-3]. It is well established that Mg deficiency in experimental animals leads to the increased leukocyte and macrophage activation, release of inflammatory cytokines and acute phase proteins and excessive production of free radicals [4]. In vitro studies have shown that a reduction in extracellular Mg resulted in phagocyte and vascular cell activation [4-8]. On the other hand, an increase in extracellular Mg in these models reduced the inflammatory response [7, 9]. As shown by Bussière et al. [7], increasing extracellular Mg concentration in the incubation medium decreased the superoxide anion production by polymorphonuclear leukocytes following activation by opsonized zymozan. An anti-inflammatory effect of Mg has also been shown in endothelial cells [10-13]. This relationship between extracellular Mg and the inflammatory response was mainly ascribed to its calcium antagonist properties [5]. However, despite large available data from ex vivo studies on cells from experimental animals there are no studies performed on human circulating cells.

In the present work we examined the effect of increasing Mg2+ concentration on the inflammatory response to septic shock using an ex vivo human whole blood model.

Material and methods

The protocol of the whole blood stimulation with lipopolysaccharide (LPS) was similar to that previously described [14, 15]. Blood from 13 healthy volunteers (5 men and 8 women) was collected into heparinised tubes (Vacutainer®, lithium heparin, Becton Dickinson, Le Pont-De-Claix, France). The study was approved by the local ethics committee. 20 μL of whole blood was diluted in 170 μL of Hank’s Buffered Salt Solution (HBSSS) supplemented with penicillin and streptomycin (100 U/100 μg per
mL) containing various concentrations of magnesium, as MgSO₄. Ten μL of LPS (E. coli O111B4) in HBSS or 10 μL of HBSS alone were then added to the blood samples to give a final concentration of 500 ng of LPS/mL. The resulting final dilution of blood was ten fold and final concentrations of magnesium were 1, 3 and 10 mM. All samples were prepared in triplicate in 96-well cell culture plates. Plates were incubated at 37°C in a 5% CO₂ atmosphere. Cell viability (white blood cells) was assessed using the trypan blue exclusion method and gave viability results of more than 90%. After 18 hrs incubation, whole blood samples were centrifuged (250 g) and supernatants were stored at -80°C until cytokine measurements. TNF-α, IL-6 and IL 8 were the cytokines measured, using commercial kits following manufacturer’s instructions (Genzyme, Cambridge, MA, USA). Measurements

**Figure 1.** Effect of Mg²⁺ concentrations on the cytokine production by human whole blood. Magnesium was added as MgSO₄. Values are expressed as mean ± SEM (n = 13). The results were analyzed by one-way ANOVA with Student-Newman Keuls post hoc test. Differences with p-values of < 0.05 were considered significant. Means not sharing the same letter are significantly different. LPS(-) and LPS(+) means without or with lipopolysaccharide (LPS) added, respectively.
were made in duplicate. Results are expressed as means ± SEM. The results were analyzed by one-way ANOVA with Student-Newman-Keuls post hoc test. Differences with p-values of < 0.05 were considered significant.

Results and discussion

The results show that under basal (without LPS) conditions, only the high (10 mM) MgSO$_4$ concentration inhibited the spontaneous production of the proinflammatory cytokines studied by incubated whole blood (figure 1). In the LPS-stimulated conditions there was only a tendency for an inhibitory effect of 10 mM MgSO$_4$ for IL-6 and IL-8 production observed (figure 1).

The whole blood model has been extensively used and is considered as a useful tool for investigating immunomodulating effects on a mixed white blood cell population [16]. This test was also proposed as a prognostic indicator for patients at high risk for developing a sepsis syndrome [17]. Several laboratories have used this experimental approach but it is difficult to compare the results between these studies because of a lack of standardization with regard to LPS concentration, incubation time, blood dilution, cytokine studied and anticoagulant used. For this reason in the present work we extrapolated previously published approaches to select our own experimental conditions. Within the conditions studied, our results show that, in the conditions studied, the cytoprotective and anti-inflammatory action of MgSO$_4$ appears relatively weak and is only observed with the highest Mg$^{2+}$ concentration. Other more extended studies with more appropriate experimental conditions are needed to precise this action with regard to the intensity of septic shock and inflammatory response. However, the results of the present experiment are supported by previously performed in vivo studies, consisting of assessing the influence of plasma Mg concentration on cytokine production in rats after endotoxin challenge [18]. A significant increase in TNF-α plasma levels was observed in Mg-deficient rats compared to rats fed the control diet. Mg-deficient rats that received Mg replacement therapy before endotoxin challenge had significantly lower TNF-α plasma values than those receiving saline before endotoxin. These data clearly indicate that increasing plasma Mg of Mg-deficient rats by Mg supplementation prior to endotoxin challenge results in lower TNF-α production. This in vivo performed experiment also indicates that there were no significant differences in plasma TNF-α values in control animals that received Mg or saline before endotoxin challenge, despite a marked increase in plasma Mg levels. These results suggest that the efficiency of Mg supplementation on cytokine production depends on the Mg status and might explain the weak efficiency of increasing Mg concentration in human blood from healthy non Mg-deficient volunteers.

References


