The Potency of Piperine as Magnesium Bioenhancer in Mice Lung

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Abstract. Magnesium (Mg) deficiency is found in many disease conditions ranging from infectious diseases to degenerative diseases. In COVID-19, magnesium deficiency is associated with susceptibility to infection and the development of severe infections. In this disease, magnesium has been suggested to play a role in the regulation of the immune system, inflammation, and bronchodilatation. Unfortunately, despite its cheap price and abundant availability in nature, magnesium deficiency is difficult to treat. Deficiency persists even after supplementation. Lack of compliance due to side effects at high doses is also another problem. This study aims to investigate the effect of piperine as a magnesium bioenhancer by measuring the bioavailability of magnesium in the lungs of healthy mice using graphite furnace atomic absorption spectrometry (GF-AAS). In addition, two different administrations were compared, ie. Repeated dose oral administrations (5 times/5 days) and single dose intraperitoneal administrations in which each of them consist of 3 testing substances, ie. Magnesium only ($MgSO_4$ 100 mg/kg), piperine only (10 mg/kg), and the combination of magnesium and piperine. One group receives no treatment and serves as a control. The results showed that piperine could increase the uptake of magnesium in the lungs. Surprisingly, this effect is independent of magnesium intake because co-administration of magnesium reduced the uptake of magnesium in the lungs. Of note, single intra peritoneal administration shows a much more prominent effect than repeated doses of oral administration. We suggest piperine could be used as a magnesium bioenhancer in the lung. Future studies using different animal models seem warranted.

Keywords: piperine · magnesium · bioenhancer · in vivo · lung

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1 Introduction

Aging and diabetes are two factors that cause severe attacks in COVID-19 patients. Interestingly, both of these conditions are associated with magnesium (Mg) deficiency. Magnesium is the most abundant intracellular cation after potassium and is involved in $>$ 600 enzymatic reactions in the body, including those that contribute to immune and inflammatory responses in COVID-19 patients [1]. The function of magnesium in the immune response is as a cofactor for the synthesis of immunoglobulins and cofactors of other processes that play a role in the function of T cells and B cells. Regulation of magnesium in immune cells involves several magnesium transport systems [1, 2].

For the treatment of respiratory disease, magnesium sulfate has been widely used in acute severe asthma. As a natural calcium antagonist, the mechanism of action of magnesium in this disease involves inhibition of airway smooth muscle contraction, dilatation of the pulmonary arteries, and reduction of pulmonary artery resistance leading to alleviation of hypoxia [3].

Despite its abundant availability in nature, unfortunately, magnesium deficiency in patients is difficult to treat. This condition occurs because an increase in magnesium intake can also be followed by an increase in its elimination [1]. Therefore, it is necessary to find a way to increase the bioavailability of magnesium, preferably without increasing its side effects, for example, by administering magnesium together with piperine as a bioenhancer.

Piperine is an alkaloid compound that is found in many Piper species plants. These plants have been used for spices and traditional medicine, eg. to treat infections. In the modern world, piperine has been known as a bioenhancer, which is a compound that can increase the bioavailability of several drug compounds and nutrients. There are also commercial preparations of piperine as a bioenhancer (Bioperine®; standardized extract with 95% piperine).

Some of the mechanism of piperine as a bioenhancer involves: 1) efflux-pump inhibition [4]; 2) inhibition of metabolizing enzymes; 3) the thermogenic properties of piperine in the intestine; 4) increase of blood supply; 5) decrease of hydrochloric acid secretion; 6) increase of active and passive nutrient transport; 7) inhibition of drug elimination; and 8) increase of free drug levels by replacing drug binding with plasma proteins [5].

Apart from being a bioenhancer, piperine also has an anti-infective therapeutic effect in vivo at the same dose as a bioenhancer (5–20 mg/kg) [6–9]. Piperine also has activity as an immunomodulator [10]. The nature of piperine as an efflux pump inhibitor indicates the potential of piperine to overcome antibiotic resistance caused by the overactivity of the efflux pump [11].

The benefits of piperine in respiratory disorders have been demonstrated in clinical and pre-clinical trials. In clinical trials, a new formulation of an anti-tuberculosis drug with piperine (Risorine®) has given promising results in the phase III clinical trial [12]. In this clinical trial, piperine 10 mg/kg can reduce rifampicin dose and reduce toxicity during therapy. In pre-clinical trials, piperine inhibited Th-2-mediated cytokine release, eosinophil infiltration, and airway hyper-responsiveness [13].

Although the effectiveness of piperine as a bioenhancer of several organic compounds is known pre-clinically and clinically, the effectiveness of piperine as a bioenhancer of essential minerals, especially magnesium, is still scarce. There is only a report on iron, zinc, and calcium in an ex-vivo study [14]. Similarly, its effect was only reported on iron bioavailability in a clinical study using athletes as the subject [15].

Two ongoing clinical trials are investigating the effects of magnesium and piperine but as separate testing substances. First, they investigate the effects of piperine as a curcumin bioenhancer for the treatment of COVID-19 patients [16], and second, they investigate the combination of magnesium with standard therapy in moderate and severe COVID-19 patients [3].

Taken together, targetting magnesium as a preventive and curative strategy in respiratory disorders seems warranted [17]. Using piperine, this study aims to investigate magnesium bioavailability in lung mice.

2 Material and Methods

2.1 Animals and Treatments

28 male Balb/C mice (The National Agency of Drug and Food Control/ BPOM) were grouped into 7 groups of 4 each, marked with permanent markers, and given food and drink ad libitum in standard mice cages. After acclimatization for 1 week, mice were treated orally (p.o) or intraperitoneally (i.p) as follows:

group without treatment, the piperine group 10 mg/kg i.p, piperine group 10 mg/kg p.o 5 days, group $MgSO_4$ 100 mg/kg i.v, group $MgSO_4$ 100 mg/kg p.o. for 5 days, piperine group dose 10 mg/kg + $MgSO_4$ 100 mg/kg i.v, piperine group dose 10 mg/kg + $MgSO_4$ 100 mg/kg p.o 5 days. Three hours after administration of the last test compound, mice were decapitated with neck dislocation. Lung samples were quickly collected and stored at − 80 ˚C until analysis. The protocol of this study has been approved by LIPI research ethics committee No. 43/klirens/VII/2021.

2.2 Magnesium Analysis

For magnesium analysis with Graphite Furnace Atomic Absorption Spectrophotometer (GF-AAS), lung samples were prepared by wet digestion in concentrated nitric acid (0.2 g samples in 3.6 mL nitric acid) at 120 °C for 30 min using a heat block (ECO COD Thermoreactors) in polypropylene tubes.

2.3 Materials

All materials were obtained from Sigma unless otherwise noted. Piperine was isolated from white pepper (Piper nigrum). $MgSO_4$ (>90%).

2.4 Statistical Analysis

Data are presented as means ± S.E.M of N experiments. GraphPad Prism® Version 5.03 was used for presenting the data and performing statistical analysis. A significance level of 0.05 was chosen for the tests. Analysis of variance (ANOVA) was used for comparing means of more than two groups followed by Tukey's Multiple Comparison Test. The unpaired-T test was used to compare the means between the 2 groups.

3 Results

Our data showed that administration of magnesium alone, both single dose intraperitoneally and multiple doses orally (5×), could not increase magnesium uptake in the lungs (5960 ng/g vs 5616 ng/g vs 5741 ng/g; $p > 0.05$) (Fig. 1a).

Interestingly, a single administration of piperine could increase magnesium uptake in the lungs significantly when given intraperitoneally compared to control (5950 ng/g vs 7672 ng/g; $p < 0.05$). However, this effect was reduced when magnesium was given with piperine concomitantly although a slight increase in magnesium uptake was still observed compared to control (5950 ng/g vs 6254 ng/g; $p > 0.05$) (Fig. 1b.).

Next, we change the dosing regimen to multiple dose oral administration. Similar patterns were observed between multiple oral administration and intraperitoneal administration. Piperine alone showed the highest magnesium uptake (6815 ng/g; $p > 0.05$). However, this effect was almost abolished to a similar value as control when magnesium was given with piperine concomitantly (5950 ng/g vs 5962 ng/g; $p > 0.05$) (Fig. 1c.).

Regarding the dosing regimen of piperine, a single intraperitoneal injection shows a better effect than a repeated dose (5×) of oral administration. However, both regimen is not statistically significant (7672 ng/g vs 6815 ng/g; $p > 0.05$) (Fig. 1d.).

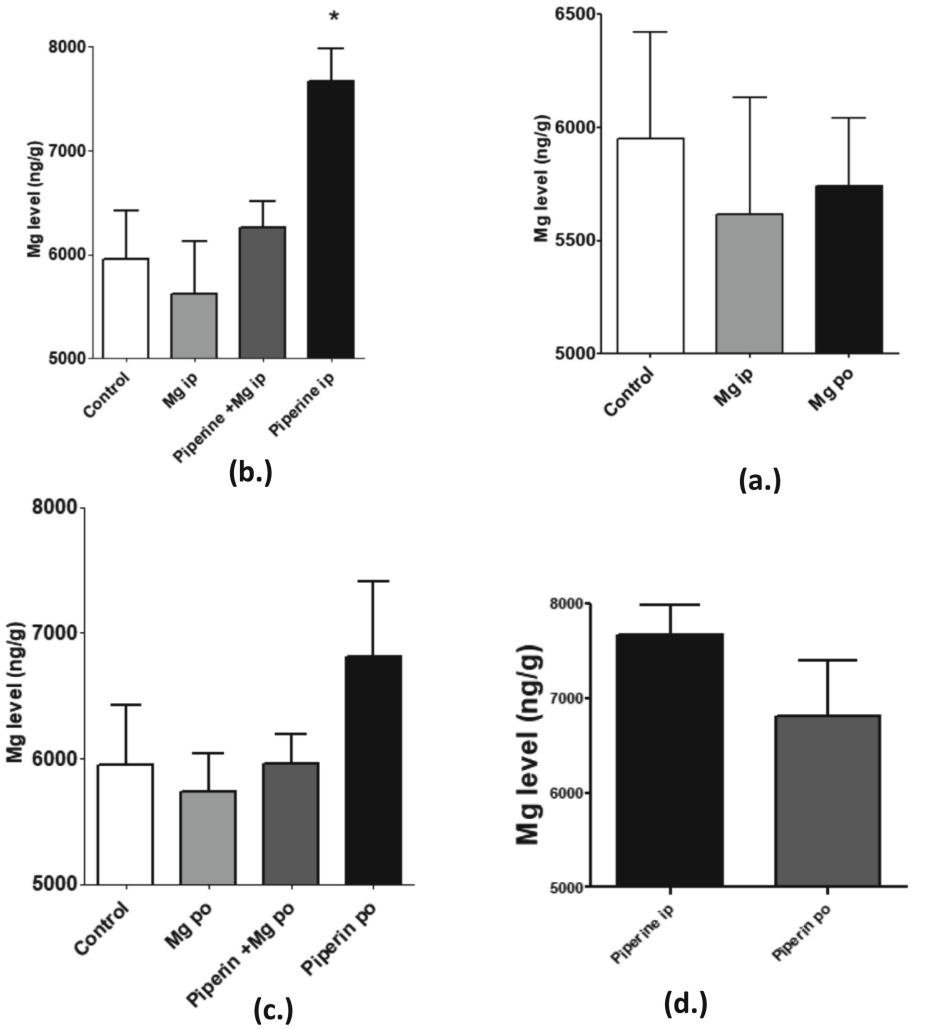

Fig. 1. **a**. Magnesium level in lung sample after oral (po) and ntraperitoneal administration (ip) compared to control. Magnesium administration could not increase magnesium uptake in the lungs. Data were analyzed by one-way ANOVA followed by Tukey's Multiple Comparison Test ($p > 0.05$ compared to control). **b.** Magnesium level in lung sample. A single dose of piperine could significantly increase magnesium uptake in the lungs when given intraperitoneally. Data were analyzed by one-way ANOVA followed by Tukey's Multiple Comparison Test (*$p < 0.05$ compared to control). **c.** Magnesium level in lung sample. Multiple doses of piperine slightly increased magnesium uptake in the lungs when given orally. Data were analyzed by one-way ANOVA followed by Tukey's Multiple Comparison Test ($p > 0.05$ compared to control). **d.** Magnesium level in lung sample. The different dosing regimen of piperine shows no statistical difference between intraperitoneal and oral administration). Data were analyzed by unpaired t-test ($p > 0.05$).

4 Discussion

Recent studies show that magnesium plays a pivotal role in the severity of COVID-19. This is because some of the risk factors that lead to severe COVID-19, eg diabetes mellitus and cardiovascular disease, were found to be related to magnesium deficiency [18, 19].

There are several ways to alleviate magnesium deficiency, one of them is the use of bioenhancers. One characteristic of bioenhancers is having a sharply strong taste or smell. In this study, we choose piperine. Besides having anti-infective properties, piperine is easily isolated. Therefore, it will be affordable in the future market. Of note, the activity of piperine as magnesium bioenhancer is rarely reported.

Our study showed that magnesium supplementation alone cannot increase magnesium uptake in the lung. This condition is possibly caused by the increase in its clearance. Surprisingly, piperine could increase the uptake of magnesium in the lungs. As predicted, this effect is independent of magnesium intake because co-administration of magnesium reduced the uptake of magnesium in the lungs. This condition is possibly caused by, again, its clearance.

Regarding the dosing regimen, we suggest that the route of administration, instead of total dosing, contributes more to the activity of piperine. The lower activity of piperine as magnesium bioenhancer, when given orally compared to intraperitoneally, is possibly caused by lower piperine blood level. Bhat [20] reported higher liver bioavailability of piperine when given intra peritoneally compared to orally.

Finally, even though oral administration of piperine showed lower magnesium bioenhancer activity, oral administration for future study seems warranted because the differences between oral and intraperitoneal are not statistically different. Of note, oral administration seems more applicable to clinical application.

The limitation of our study is that we use a normal animal. This is however not entirely incorrect because the purpose of this study is to show a clear effect between testing compounds (magnesium alone, with piperine, or piperine alone) and different dosing regimens (single intraperitoneal injection vs multiple doses oral administration). It will be more difficult to study such an effect if we directly use the animal model because so many variables will be involved. In other words, this study serves as a foundation for future studies using animal models.

The use of animal models of diseases is inevitable to study the mechanism of piperine in magnesium regulation because tissue biopsy in the patient is often unethical. In addition, animal models are needed to see the phenotype of this mechanism. Therefore, future studies using different animal models seem warranted.

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