



Effect of garlic on liver phosphatidate phosphohydrolase and plasma lipid levels in hyperlipidemic rats

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ABSTRACT

Studies on the effects of garlic (*Allium sativum*) on hyperlipidemia have demonstrated somewhat controversial results and there have been few studies on its enzymatic mechanism. The purpose of this study was to assess the effect of garlic on the liver phosphatidate phosphohydrolase (PAP) activity, plasma lipid levels, malondialdehyde (MDA) and plasma antioxidant in rats fed either by normal or high-lipogenic diet with or without garlic. Male Wistar rats were fed by standard pellet diet (group I), standard diet supplemented with 4% garlic (group II), lipogenic diet (containing sunflower oil, cholesterol and ethanol) plus 4% garlic (group III) and only lipogenic diet (group IV). Results showed that garlic significantly reduced total cholesterol (TC), plasma triglyceride (TG), LDL-C, VLDL-C, liver triglyceride, plasma malondialdehyde (MDA) and elevated plasma antioxidant in garlic treated rats (groups II and III) compared to group IV (lipogenic diet group). Also, liver PAP activity was decreased in group II than group I whereas, the decrease in its activity in groups III and IV was due to the accumulation of triglyceride in liver. Therefore, the results are clearly indicative of the beneficial effects of garlic in reducing lateral side effects of hyperlipidemia.

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1. Introduction

Garlic (*Allium sativum* L.) and its compounds have been reported to have diverse biological activities such as regulating plasma lipid levels, anticarcinogenic, lead and mercury detoxification, anti-thrombotic, antibacterial, antioxidant, antihypertensive, antidiabetic, and various other biological actions (Abdalla et al., 2009; Agarwal, 1996; Azuma et al., 2007; Lau, 2006; Sharma et al., 2010).

Phosphatidate phosphohydrolase (PAP, EC 3.1.3.4) catalyzes the dephosphorylation of phosphatidic acid to yield inorganic phosphate (Pi) and 1,2 diacylglycerol (Carman and Han, 2006). This enzyme plays a critical role in controlling the synthesis of glycerophospholipids and triacylglycerols (Roberts et al., 1998). The produced diacylglycerol serves as a precursor for the synthesis of major glycerolipids in animal cells (Kanoh et al., 1992). This reaction is a regulatory step in the synthesis of triacylglycerol and phospholipids (Fleming and Yeaman, 1995). Additionally, triglyceride (TG) serves as the major energy storage molecule that allows organisms to survive periods of food deprivation. Also, the regulation of TG storage is very important in human diseases because both excessive and inadequate fat storage are accompanied with dyslipidemia, insulin resistance, and diabetes (Hegele

and Pollex, 2005; Petersen and Shulman, 2006; Reue and Phan, 2006). In rat hepatocyte, two different forms of PAP have been reported based on N-ethylmaleimide (NEM) sensitivity (Heidarian and Haghghi, 2008; Nanjundan and Possmayer, 2003; Fleming and Yeaman, 1995). The NEM-sensitive form (PAP₁), located in cytosolic and microsomal fractions, requires Mg²⁺ for its activity and is responsible for the synthesis of phospholipids and triacylglycerols (Dillon et al., 1997). The other form of phosphatidate phosphohydrolase (PAP₂) is primarily involved in lipid signaling pathways by modulating the second messengers of diacylglycerol and phosphatidic acid (Brindley, 2004; Sciorra and Morris, 2002).

The incidence of hyperlipidemia as well as its complications is increasing in the world. Moreover, alterations in serum lipid and lipoprotein levels result in a variety of chronic diseases such as coronary heart diseases and atherosclerosis (McKenney, 2001). Recent studies have directed their efforts toward the protective effects of plants such as garlic on hyperlipidemia (Ortega et al., 2007; Mahmoodi et al., 2006; Gorinstein et al., 2006). Most of the previous studies on garlic have shown that garlic has cholesterol and triglyceride-lowering effects (Singh and Porter, 2006; Sumioka et al., 2006). Some researches have shown the reducing effect of garlic on HMG-CoA reductase in the cholesterol biosynthesis pathway (Gebhardt, 1991; Gebhardt and Beck, 1996). Also another study indicated that garlic reduced incorporation of label glycerol into the phospholipids biosynthesis (Yeh and Yeh, 1994). Nevertheless,

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most of the previous studies on garlic focus less on the enzymes involving in triglyceride metabolism, especially PAP enzyme, in details. Garlic has many organosulfur compounds (Iciek et al., 2009) which may work synergistically whereas a single component of the garlic may not possess these properties. Therefore, the aim of this study was to examine the medicinal uses of garlic on the liver phosphatidate phosphohydrolase activity, plasma lipid levels, and liver triglyceride in rats fed on high lipogenic and normal diet.

2. Materials and methods

2.1. Chemicals

Phosphatidic acid (sodium salts), 2,4,6-tripryridyl-s-triazine (TPTZ), phenylmethylsulfanyl fluoride (PMSF) and dithiothreitol (DTT) were purchased from Sigma (Sigma Chemical Co., USA). Sodium tetraborate, bovine serum albumin, Tris-HCl, sodium acetate, ferrous sulphate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$), glacial acetic acid, ferric chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), ethylenediaminetetra acetic acid (EDTA) and ethyleneglycol-bis (beta-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) were purchased from Merck (Germany). All other chemicals were of the highest quality available.

2.2. Preparation of garlic

Cloves of fresh garlic were purchased from the local supermarket. The garlic was identified by Dr. Zainali in Isfahan Agricultural Research Center (Iran) as a local variety. Every day peeled garlic cloves were crushed mechanically in a mortar-pestle for 1 min. Then, the 4% garlic pellets were made by mixing 4 g of fresh crushed garlic with 96 g of powdered standard rat pellet diet.

2.3. Animals and experimental design

Male Wistar rats (150–200 g) were housed in the colony room on a cycle of 12 h light/12 h darkness at $21 \pm 2^\circ\text{C}$ and had free access to water and food. They were randomly divided into 4 diet groups ($n = 6/\text{group}$) as below:

Group I, normal control rats, received standard pellet diet.

Group II, animal rats were fed with a standard pellet diet supplemented with 4% garlic.

Groups III and IV, the rats were fed with a lipogenic diet containing standard pellet diet supplemented with 0.5% (w/w) cholic acid, 20% (w/w) sunflower oil and 2% (w/w) cholesterol for at least two weeks to produce hyperlipidemia. Additionally, groups III and IV drank water containing 3% (v/v) ethanol (Yanardag et al., 2005). In group III, after 2 weeks, 4% (w/w) garlic was added into lipogenic regime for 45 days, whereas the rats in group IV were maintained on lipogenic diet. On d 60 of the experiment, fasted animals anesthetized with chloroform. Blood samples were collected into test tubes containing EDTA through cardiac puncture. The plasma samples were separated by low speed centrifugation (2000g) for 10 min and were stored at -80°C until they were analyzed. All animal procedures were performed with regard to Iranian animal ethics society and local university rules.

2.4. Analytical procedures

Plasma levels of total cholesterol (TC), triglyceride (TG) and HDL-C were determined by enzymatic method (Pars Azmun kit, Iran) with JENWAY spectrophotometer (model 6105, England). LDL-C and VLDL-C concentrations were calculated with Friedewald formula (Friedewald et al., 1972). Finally, liver triglyceride was extracted from liver tissue by Folch-altered method invented by Norman (1969).

2.5. Preparation of rat liver

The rat liver was perfused through the inferior vena cava with ice-cold saline (0.9%) to remove blood and inorganic phosphate from it to evaluate liver PAP activity and liver triglyceride. A part of perfused liver was homogenized in 4 volumes of ice-cold buffer A (0.25 M sucrose, 0.1 mM EDTA, 1 mM PMSF, pH 7.4) (Haghighi and Honarjou, 1987) using homogenizer (Heidolph, Silentcrusher M model, Germany) at 8000 rpm at 4°C for 6 min. The homogenate was then initially centrifuged at 4500 rpm at 4°C for 10 min, resulting in a nuclear pellet and then, the supernatant kept for the enzyme assay.

2.6. Enzyme assay

PAP activity was measured in the assay buffer (250 μl) containing 50 mM Tris HCl buffer pH 7.4, 1 mM DTT, 1 mM EGTA, 2 mM MgCl_2 , 1 mM EDTA, 0.35 mM phosphatidate and appropriate amount of the enzyme solution. The assay mixture incubated for 30 min at 37°C . The reaction stopped by adding 0.5 ml trichloroacetic acid (10%). Hence, the released Pi measured (Haghighi and Honarjou, 1987). All as-

says were linear in relation to the incubation time and the protein concentrations used in them. One unit (U) of PAP activity defined as the amount of enzyme that catalyzes the release of 1 μmol of Pi per min under the standard assay conditions. Specific activity was considered as units per mg protein. Protein concentration was determined by the method of Bradford (1976), with bovine serum albumin as the standard.

2.7. Measurement of malondialdehyde

The plasma malondialdehyde (MDA) level was measured by the thiobarbituric acid reactive substances (TBARS) method as described by Ohkawa et al. (1979). The measurements were done in duplicates and the results were expressed in μM . MDA standards were prepared from 1,1,3,3-tetraethoxypropane (TEP).

2.8. Antioxidant power assay

The antioxidant power of plasma was determined by measuring its ability to reduce Fe^{3+} to Fe^{2+} with FRAP (ferric reducing/antioxidant power) test (Benzie and Strain, 1996). FeSO_4 was used as a standard of FRAP assay at a concentration range between 100 up to 1000 μM . The results are expressed as μM .

2.9. Statistical analysis

The data were expressed as mean \pm S.D. They were analyzed by SPSS software (version 11.5). For statistical analysis of the data, group means were compared by one-way analysis of variance (ANOVA) followed by Tukey post hoc test for multiple comparison. Differences were considered significant at $P < 0.05$ level.

3. Results

3.1. Effect of garlic on the liver triglyceride and PAP activity

Table 1 summarizes the effect of garlic on the liver triglyceride and PAP activity. Group II had a significant decrease ($P < 0.001$) in the liver PAP activity compared to group I whereas, no remarkable change was observed in liver PAP activity between group II and group IV ($P > 0.05$). Group IV showed a significant increase ($P < 0.001$) in the liver triglyceride compared with other groups. Also, the liver triglyceride in group II was slightly lowered compared with group I (not significant, $P > 0.05$). The liver triglyceride in group III was significantly declined ($P < 0.001$) with respect to group IV.

3.2. Effect of garlic on plasma level of hyperlipidemia

Table 2 shows the mean plasma levels of TG, TC, VLDL-C, LDL-C, HDL-C and atherogenic index in control and treated animals with garlic in each group. The levels of plasma TG, TC, VLDL-C, and LDL-C in group IV (consuming oil and cholesterol diet) were significantly elevated ($P < 0.05$) compared to other groups. Additionally, the level of plasma TG in group III showed no salient difference compared to group I ($P > 0.05$). Plasma TG showed a noticeable difference ($P < 0.001$) between groups I and II. In group IV, the plasma

Table 1

The specific activity of PAP (nmol Pi/min/mg protein) and liver triglyceride content in experimental groups.

Groups	PAP activity (nmol Pi/min/mg protein)	Liver triglyceride (mg/g tissue)
I (control)	9.41 \pm 0.39	3.80 \pm 0.39
II	6.46 \pm 0.62*	3.01 \pm 0.65
III	8.52 \pm 0.90**	6.31 \pm 0.44**
IV	6.73 \pm 0.27	7.38 \pm 0.52

The data were expressed as mean \pm S.D, $n = 6$ in each group. Normal diet (I); normal diet supplemented with 4% garlic (II); hyperlipidemic rats treated with 4% garlic (III); hyperlipidemic rats without treatment (IV) groups.

* $P < 0.001$ compared with the corresponding value for group I (normal control animals).

** $P < 0.001$ compared with the corresponding value for group IV (lipogenic regime).

Table 2
Effect of garlic on TC, TG, LDL-C, HDL-C, VLDL-C levels and atherogenic index in hyperlipidemic rats.

Groups	TC (mg/dl)	TG (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)	VLDL-C (mg/dl)	Atherogenic index (units)	
						TC/HDL-C	LDL/HDL-C
I	87.71 ± 13.61	55.98 ± 9.22	23.52 ± 2.07	51.67 ± 5.39	10.50 ± 2.43	1.70 ± 0.16	0.47 ± 0.13
II	79.17 ± 4.62	40.82 ± 8.65*	24.06 ± 1.02	47.10 ± 5.22	8.17 ± 0.91*	1.73 ± 0.12	0.58 ± 0.09
III	119.40 ± 15.67**	56.01 ± 6.31**	54.77 ± 5.62**	57.96 ± 7.18	11.24 ± 1.25**	2.27 ± 0.07	1.01 ± 0.11
IV	142.25 ± 29.93#	75.56 ± 4.77#	70.67 ± 10.81#	58.41 ± 8.72#	15.11 ± 0.95#	2.42 ± 0.26#	1.21 ± 0.23#

The data are expressed in mean ± S.D, $n = 6$ in each group. Normal diet (I); normal diet supplemented with 4% garlic (II); hyperlipidemic rats treated with 4% garlic (III); hyperlipidemic rats without treatment (IV) groups.

* $P < 0.001$ compared with the corresponding value for group I (normal control animals).

** $P < 0.001$ compared with the corresponding value for group IV (lipogenic regime).

$P < 0.001$ compared with the corresponding value for groups I and II.

level of cholesterol significantly increased ($P < 0.001$) compared to groups I, II and III. On the other hand, the plasma level of TC in the rats which consume standard diet supplemented with garlic (group II) was lower than group I (control group) but not significant ($P > 0.05$). In group III, LDL-C were remarkably ($P < 0.05$) higher than groups I, II and IV. Moreover, no significant difference ($P > 0.05$) was seen for LDL-C between groups I and II. HDL-C reduce slightly in group II compared to group I (not significant, $P > 0.05$). VLDL-C significantly decreased ($P < 0.001$) in groups II and III compared to groups I (control group) and IV, respectively. There was a reduction (not significant, $P > 0.05$) in atherogenic index in group III compared with group IV (Table 2). In added, no salient change was seen in atherogenic index between groups I and II.

3.3. Effect of garlic on the plasma level of malondialdehyde

Fig. 1 shows that after the consumption of lipogenic diet, a significant ($P < 0.001$) increase in plasma malondialdehyde was observed in group IV (the group consumed oil and cholesterol diet) when compared with other groups. On the other hand, in group II the consumption of garlic led to an important ($P < 0.05$) reduction of plasma malondialdehyde in comparison with group I (control group). Also, a significant ($P < 0.001$) reduction of plasma malondialdehyde was seen in group III, fed with a lipogenic diet supplemented with garlic, as compared with group IV.

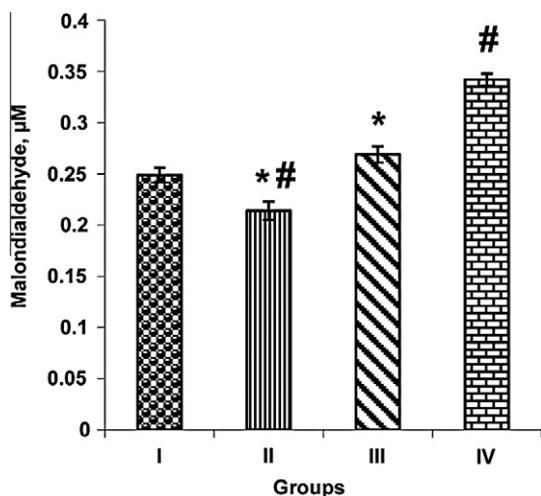


Fig. 1. Plasma malondialdehyde level in normal diet (I); normal diet supplemented with 4% garlic (II); hyperlipidemic rats treated with 4% garlic (III); hyperlipidemic rats without treatment (IV) groups. The data are expressed as mean ± S.D, $n = 6$ in each group. * $P < 0.001$ compared with the corresponding value for normal control animals. # $P < 0.001$ compared with the corresponding value for hyperlipidemic rats without treatment.

3.4. Effect of garlic on the plasma level of antioxidant power

Fig. 2 shows the plasma level antioxidant power in each experimental animal group. Garlic treatment resulted in a significant ($P < 0.001$) elevation of plasma level antioxidant power in group II as compared to the other groups. There was a significant ($P < 0.001$) reduction in plasma level antioxidant power in groups III and IV. No significant change was observed in plasma level antioxidant power between groups III and I ($P > 0.05$).

4. Discussion

The consumption of excessive calories by human, leads to high serum TG. On the other hand, increased levels of serum low-density lipoprotein cholesterol (LDL-C) and TG show that the diet containing fatty acids and cholesterol leads to hypercholesterolemia (Hegsted et al., 1965). Fruit and vegetables have chemopreventive and flavoring agents which act against various diseases especially hyperlipidemia (Ortega et al., 2007). Experimental studies in recent years have indicated that garlic has cholesterol and triglyceride-lowering, antibacterial, hypoglycemic, hypotensive potential and antiaggregatory properties (Agarwal, 1996; Aviello et al., 2009; Azuma et al., 2007; Gorinstein et al., 2007). The effects of garlic on serum lipid levels have been investigated in human and animal models and indicate inconsistency in the reported results

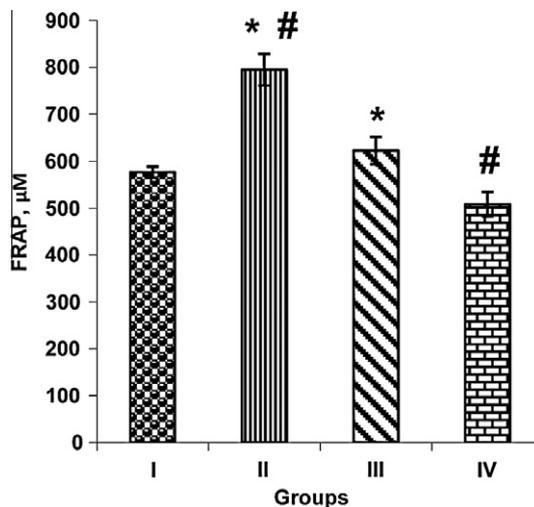


Fig. 2. Plasma antioxidant capacity (FRAP) in normal diet (I); normal diet supplemented with 4% garlic (II); hyperlipidemic rats treated with 4% garlic (III); hyperlipidemic rats without treatment (IV) groups. The data are expressed as mean ± S.D, $n = 6$ in each group. * $P < 0.001$ compared with the corresponding value for normal control animals. # $P < 0.001$ compared with the corresponding value for hyperlipidemic rats without treatment.

(Ali et al., 2000). In this study to elucidate the mechanism of the triacylglycerol lowering action of garlic, we measured liver triglyceride content and the liver PAP enzyme activity involving in TG biosynthesis. Our data have shown that 4% garlic supplementation in hyperlipidemic rats was highly effective in reducing serum cholesterol and TG levels as compared to the high cholesterol diet and control diet group of animals (Table 2). The same results were obtained by other investigators (Singh and Porter, 2006; Sumioka et al., 2006). Also, it has been reported that methanol and water extractable fractions of fresh garlic prevented the 1-¹⁴C-acetate incorporation into the biosynthesis of cholesterol and depressed the rate of [²⁻³H] glycerol incorporation into the biosynthesis of triacylglycerol, diacylglycerol and phospholipids (Yeh and Yeh, 1994). The above published works do not assess the effect of garlic on PAP activity. In our study however, the garlic supplementation resulted in higher reduction of PAP activity in group II than group I (control group) (Table 1). Therefore, the serum and liver triglyceride reduction in group II are due to decline of the liver PAP activity (Tables 1 and 2). Allicin (diallyl disulfide), an active constituent of garlic, has been reported to lower the serum lipid profile in hyperlipidemic rabbits (Eilat et al., 1995). In another study cycloalliin, a sulfur imino acid compound with a cyclic structure found in onions, markedly reduced serum TG concentration in rats without significant alteration on liver PAP activity and hepatic triglyceride content (Yanagita et al., 2003). This result in PAP activity contradicts our study which can be due to the composition of garlic in this study. Garlic contains more than 30 biological organosulfur agents such as diallyl trisulfide, diallyl disulfide and diallyl sulfide (Amagase et al., 2001; Iciek et al., 2009). These agents may work synergistically to reduce liver PAP activity and exhibit various biological activities which a single component such as cycloalliin may not be able to do. On the other hand, in lipidemic groups liver PAP activity also declined with respect to control group but, their liver triglyceride concentration increased in this study (Table 1). It has been reported that excessive intake of fatty acids leads to an accumulation of TG in many tissues, particularly in fat tissue and non-adipose tissues such as liver (Lebovitz, 2001). Also, it was shown that fatty acid esters promote the inactivation of PAP (Elabbadi et al., 2005). Fatty acids and their acyl-CoA esters regulate PAP by a negative allosteric interaction, leading to the formation of inactive PAP fatty acids (or -acyl-CoA esters) complex (Elabbadi et al., 2005). Therefore, the reduction of liver PAP activity in this study in groups fed with high lipid regime (groups III and IV) is due to the accumulation of triglyceride (Table 1), fatty acids or acyl-CoA esters in the liver. On the other hand, liver PAP reduction activity in groups fed with high lipid regime (groups II and III) can probably be due to the defense mechanism of liver in lowering the production of endogenous TG. Therefore, serum and liver TG will accordingly decline. Besides, in this study liver fat content significantly increased in animal groups fed by lipogenic regime (groups III and IV) with respect to the group I (control group). Garlic resulted in slight decrease in liver triglyceride in group fed by garlic (group II) compared with the control animals (group I). These results were consistent with the reduction of PAP activity in group II. In contrast, groups fed by lipogenic regime (groups III and IV) had high TG content in the liver as compared to the normal control animals (group I).

Studies on cell lines and animal models have shown that allicin also inhibit the HMG-CoA reductase level in the cholesterol biosynthesis pathway (Gebhardt, 1991; Gebhardt and Beck, 1996). In this study, garlic supplementation resulted in significant reduction of cholesterol in group III when compared to group IV (lipemic regime group). However, garlic supplementation reduced TC in group II as opposed to group I but the difference was not significant (Table 2). Our findings were in line with other investigators who reported that garlic consumption decreased serum total

cholesterol and TG but increased HDL cholesterol in hyperlipidemic individuals and animals (Gorinstein et al., 2006; Mahmoodi et al., 2006; Thomson et al., 2006).

Previous studies have reported the antioxidant effect of garlic (Durak et al., 2004; Gorinstein et al., 2006). Oxidative stress disrupts the equilibrium between prooxidants and antioxidants in biological systems and leads to lipid peroxidation (LPO) and free radical generation (Romero et al., 1998). The level of malondialdehyde (MDA) is considered as a biomarker of LPO (Lykkesfeldt, 2007). Garlic extracts increase superoxide dismutase (SOD) and catalase (CAT) activities in vascular cultured cells. S-allylcysteine sulfoxide (alliin), a bioactive compound of garlic, prevents the reduction of hepatic SOD and CAT activities in diabetic rats (Gorinstein et al., 2006). Also, it has been reported that garlic significantly lowers plasma and erythrocyte MDA levels while increasing antioxidant enzyme activity in elderly subjects (Avci et al., 2008). In the present study, plasma MDA significantly increased in hyperlipidemic animals whereas, antioxidant power of plasma declined. Garlic supplementation potentially resulted in significant elevation of plasma antioxidant power and decreased plasma MDA level in treated rats (Figs. 1 and 2), suggesting that garlic might have antioxidant activities to produce such responses. In this respect, there are published reports concordant with our results (Durak et al., 2004; Gorinstein et al., 2006). The antioxidant potential of garlic is due to its organosulfur, alliin, and nonsulfur components such as N-fructosyl-arginine and N-fructosyl-glutamate which were shown to have antioxidant activity (Amagase et al., 2001; Ryu et al., 2001). Therefore, the garlic supplementation used in our study appears to lower oxidative stress related to hyperlipidemic regime and to decrease lipid oxidation.

The findings of this work shows garlic has beneficial effects in reducing liver triglyceride, plasma MDA and liver PAP activity. Also, garlic is useful in the control of hyperlipidemia, abnormalities in lipid profiles and oxidative stress in hyperlipidemic regime conditions.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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