Vitamin K can Suppress the Inflammation Induced by Lipopolysaccharide Administration.

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Abstract: Vitamin K (K) is essential for blood coagulation and bone metabolism in mammals. K acts as a cofactor in the posttranslational synthesis of g-carboxyglutamic acid from glutamic acid residues. In addition to liver and bone, K is found in brain, heart, kidney and gonadal tissue. However, the physiological role of K in these varied organs is not yet fully understood. It is likely that K has functions in addition to its role as a cofactor of protein g-glutamyl carboxylation. In this paper we used DNA microarray techniques to identify the effect of K status on gene expression in rat liver. Expression of genes involved in the acute inflammation response was enhanced in rats fed a K-deficient diet relative to control and K_1-supplemented diet groups. Moreover, dietary supplementation with K_1 suppressed inflammation induced by LPS administration. These results indicate that orally administrated K_1 suppresses inflammation in the rat.

Keywords: Vitamin K; DNA microarray; inflammation; IL-6

Introduction

Vitamin K (K) is essential for both blood coagulation and bone metabolism in mammals. K acts as a cofactor for microsomal γ-glutamyl carboxylase in the posttranslational synthesis of γ-carboxyglutamic acid (Gla) from glutamic acid (Glu) residues contained in K-dependent precursor proteins. There are two types of naturally occurring K: phylloquinone (K_1), synthesized in plants, and menaquinone (K_2, or MK-n), produced mainly by microorganisms. Significant amounts of menaquinones are synthesized by intestinal flora in the lower intestine and absorbed. Intestinally-synthesized menaquinones can contribute to the maintenance of K requirements in the host, but their relative contribution to K nutritional status remains a subject of debate. After absorption from the intestine, K is transported to the liver by triglyceride-rich lipoprotein chylomicrons to activate K-dependent proteins. Although a significant portion of K is redistributed to extrahepatic organs, including heart, kidney, brain, and gonadal tissue, it is not known whether this redistributed K functions as a cofactor for g-glutamyl carboxylase in these tissues.

Experiments in the present paper were intended to identify additional functions of K. We varied K nutritional status in animals and identified resultant changes in hepatic gene expression. Analysis of gene expression profiles obtained using DNA microarray revealed that a group of genes involved in the acute inflammatory response was increased in liver of K-deficient rats. Furthermore, dietary supplementation with K_1 suppressed lipopolysaccharide (LPS)-induced inflammation. These results substantiate a previously unrecognized biological function of K to suppress inflammation in animals.

Results and Discussion

K deficient (K-Def), supplemented (K-Sup), and control (Cont) diets were fed to germfree and conventional Wistar male rats. In rats fed the deficient diet, symptoms of K deficiency were observed in germfree rats after 9 days and in conventional animals after 21 days. Symptoms were observed earlier in germfree animals because they lack the intestinal flora that contribute to K nutritional status. Animals were sacrificed to obtain blood and liver samples. Because liver is the only site for synthesis of blood coagulation factors, it is a main target organ for K. To estimate the degree of K deficiency, we measured blood coagulation time (PT and APTT) and K content in liver using a fluorescent-HPLC system. PT and APTT were prolonged in both germfree and conventional K-Def diet groups. The amount of hepatic K_1 and K_2 (menaquinone-4, MK-4) was reduced in K-Def groups compared to that in K-Sup and Cont diet groups. Together, these results indicate that animals fed the K-deficient diet were in a state of severe K deficiency.

Total RNA was isolated from livers of germfree K-Def and K-Sup diet groups, and subsequently used as a template for the synthesis of radio-labeled cDNA. Labeled cDNAs were hybridized with the filter type's cDNA array. After hybridization, DNA arrays were exposed to Imaging Plate, followed by calculation of gene expression. Gene expression profiles of germfree K-Def rats differed from those of germfree K-Sup animals. In particular, expression of one gene family involved in the acute inflammatory response was enhanced in K-Def relative to K-Sup animals. To confirm the difference of expression among experimental groups, the amount of mRNA of γ_1-acid glycoprotein, fibrinogen ã-chain, and metallothionein-2 was measured by Northern blot hybridization. Expression of γ_1-acid glycoprotein, fibrinogen γ-chain, and metallothionein-2 was higher in the K-Def group than in Cont and K-Sup groups. Because animals in a K-deficient state characteristically develop internal bleeding within several organs, including liver, it is possible that such bleeding could induce expression of genes involved in inflammatory response. These results also suggest that K may be involved in anti-inflammatory reactions directly in organs.

The next experiment was conducted to clarify whether K has an anti-inflammatory effect. We asked whether K status influences the inflammatory response induced by LPS administration.

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Conventional rats fed K-Sup and Cont diets for 10 days were treated with LPS. Eighteen hours later, animals were sacrificed and blood and organs obtained. While all rats fed the K-Sup diet were alive 18 hours after LPS administration, 31% (4 out of 13) of rats fed the Cont diet died during the same time period. Plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in the K-Sup group were significantly lower than those in the Cont group. In addition, mRNA levels of liver macrophage migration inhibitory factor, a marker of inflammation was also significantly decreased in K-Sup animals. These results indicate that orally ingested K (K_1) can suppress LPS-induced inflammatory response and alleviate subsequent endotoxic shock.

To evaluate the anti-inflammatory action of vitamin K in an in vitro system, we examined the influence of vitamin K on LPS-induced gene expression in primary cultured cells prepared from mouse spleen. Cultured spleen cells were incubated with LPS added to K_1 or MK-4 for 12 h. After incubation, cells were harvested and total RNA isolated. The amount of IL-6 mRNA was measured using quantitative RT-PCR. The results indicate that K_1 had no repressive effect on LPS-induced enhancement of IL-6 expression. On the other hand, MK-4 significantly repressed IL-6 expression in LPS-treated cells.

In this paper we demonstrate enhanced expression of acute phase protein genes in animals with vitamin K deficiency. We also show in vivo suppression of LPS-induced inflammatory reaction through dietary supplementation of vitamin K. Activity of both plasma ALT and AST was markedly reduced in vitamin K-supplemented animals following LPS treatment. In addition, administration of vitamin K reduced mortality ratio following LPS treatment. Orally administered vitamin K_1 is converted to MK-4 in several organs in rat and mouse over 24 h post-administration. Liver MK-4 levels in K-Sup diet groups were much higher than those of the Cont diet groups. Intraperitoneal administration of vitamin K_1 could not protect liver cells from LPS toxicity. Therefore, it is suggested that MK-4 converted from dietary K_1 may play a role in hepatic anti-inflammatory responsiveness. This hypothesis is supported by data from the in vitro study using spleen primary cultured cells; i.e., MK-4, but K_1, significantly suppressed LPS-enhanced IL-6 expression. Thus, converted MK-4 may play a role in suppression of disease-related inflammation in extra-hepatic tissues.