Mini Review

The effect of geranylgeranylacetone on human osteoclastogenesis and synovitis in patients with rheumatoid arthritis

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Rheumatoid arthritis (RA) is a chronic inflammatory disease associated with synovitis and bone destruction. The levels of monocyte/macrophage-derived cytokines, including TNFα, interleukin-1 (IL-1), and IL-6, and the T cell-derived cytokine, IL-17, all of which are involved in the pathogenesis of RA, are elevated in the synovial fluid of RA patients.

Geranylgeranylacetone (GGA), an acyclic polyisoprenoid known as teprenone, has been widely used as an antiulcer drug. We have reported that GGA inhibits human osteoclastgenesis, and that GGA increases the bone mineral density in ovariectomized rats and tail-suspended rats. These effects are due to inhibiting the prenylation of geranylgeranylpyrophosphate (GGPP) by GGA in the mevalonate pathway. Recently, we also demonstrated that GGA induces cell death in fibroblast-like synoviocytes from patients with RA. These findings suggest that GGA may be available as a new agent for RA and osteoporosis.

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Introduction

RA is a chronic inflammatory disease characterized by the synovitis[1] and the destruction of articular cartilage and bone. The levels of monocyte/macrophage-derived cytokines, including TNFα, interleukin-1(IL-1), and IL-6, and the T cell-derived cytokine, IL-17, all of which are involved in the induction of osteoclasts, are elevated in the synovial fluid of RA patients, suggesting that cytokine-mediated osteoclastogenesis occurs in the joints[2,3].

Statins, 3-hydroxy-3-methylglutary-CoA reductase inhibitors, are widely used to treat hyperlipidemia. In addtition to their benefical lipid-lowering effects, statins have shown to have pleiotropic effects[4,5] in various systems such as the immune system, cardiovascular system, nervous system and skeletal system[6,7]. In particular, the immunosuppressive effect of statins has been highlighted. Statins improve endothelial function, decrease oxidative stress and inflammation. *In vitro* studies have shown that statins suppress natural killer cells, regulate DNA synthesis in cycling cells and inhibit monocyte chemotaxis. Thus, statins might be used as an immunomodulator in autoimmune diseases

such as RA. In fact, atrovastatin and simvastatin have anti-inflammatory effect with patients with RA[8-11]. Many of these effects are related to the inhibition of isoprenoid synthesis, which serves as lipid attachment for small G proteins implicated in intracellular singnaling. These small G proteins, whose proper membrane localization and function depend on isoprenylation, play an important role in the pleiotropic effects of statins[12-14]. Recently, fluvastatin (Fluv) has been reported to induce apoptosis in RA synoviocytes through the blocking of protein geranylgeranylation[15] .

We recently demonstrated that geranylgeranylacetone(GGA)[16,17] potently inhibit the human osteoclastogenesis induced by soluble receptor activator of nuclear factor-κB ligand (sRANKL)[18-21]. These effects were due to inhibition of the function of geranylgeranylpyrophosphate (GGPP) in the mevalonate pathway. Thus, small G protein also plays an important role in the pleiotropic effects of GGA. Moreover, GGA prevents bone loss in ovariectomized (OVX) rats and tail-suspended rats *in vivo*[18]. In addition, we also reported that GGA induces cell death in RA synoviocytes.

In this mini review, we describe the effect of GGA on osteoclastogenesis and the anti-inflammatory effect on RA, as well as introducing some other pleiotropic effects.

Chemical structure of GGA

As shown in Figure 1, GGA has almost the same chemical structure as the side chain of menatetrenone, vitamine K_2. GGA, an acyclic polyisoprenoid, has been widely used as an antiulcer drug since 1984. GGA increases the synthesis and secretion of gastric mucin as well as the components of high molecular-weight glycoproteins and surface-active phospholipids[22,23] .

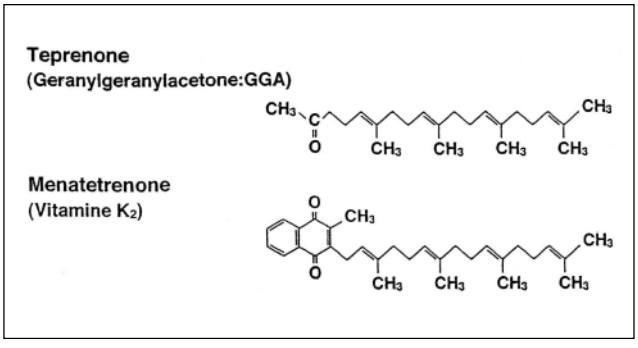

Fig.1 Chemical structures of GGA and menatetrenone
GGA has almost the same chemical structure as the side chain of menatetrenone. (Modified from Nanke et al: Calcif Tissue Int, 77: 376-385, 2005.)

The role of GGA in human osteoclastogenesis *in vitro*

GGA (500-1000 ng/mL) dose-dependently inhibited the formation of osteoclasts from human monocytes induced by sRANKL[18]. GGA induced degradation of actin rings in mature osteoclasts at pharmacological concentrations.

GGA blocks the function of GGPP in the mevalonate pathway

The degradation of actin rings of osteoclasts induced by GGA (1000 ng/mL) was reversed by GGPP (10 μM) but not by farnesylpyrophosphate (FFP)[18]. Thus, GGA blocked the function of GGPP by competitive inhibition of the mevalonate pathway (Fig.2).

Effect of GGA on ovariectomized (OVX) and tail-suspended rats

GGA increased the bone mineral density (BMD) of the total femur, proximal metaphysis and diaphysis of the femur in OVX rats. GGA also prevents bone loss induced by hindlimb unloading in tail-suspended rats. GGA increased histological bone volume in both OVX rats and tail-suspended rats. These findings suggest that GGA is available as a new agent for osteoporosis.

The effect of GGA in fibroblast-like synoviocytes from patients with RA

Since Fluv induces apoptosis in RA synoviocytes by inhibiting protein geranylgeranylation in the mevalonate pathway, we

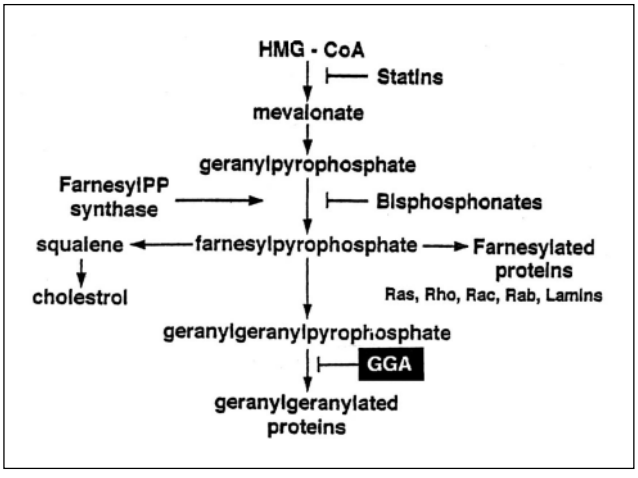

Fig.2 Putative effect of GGA on the mevalonate pathway
GGA plays a role in inhibiting the prenylation of GGPP. (Modified from Nanke et al: Calcif Tissue Int, 77: 376-385, 2005.)

Table 1a Pleiotropic effect of GGA (1)

GGA induces overexpression of the heat shock protein in various organs	
Human gastric mucosa	Yanaka et al 24)
Guinea pig gastric mucosal cells	Takano et al 25), Hirakawa et al 26)
Rat gastric mucosa	Hirakawa et al 26)
Heart	Shinohara et al 27), Yamanaka et al 28)
Kidney	Suzuki et al 29)
Liver	Ochikawa et al 30), Fudaba et al 31)
Retina	Kitanei et al 32)
GGA protects against ischemic/reperfusion injury	Hirakawa et al 26), Ooie et al 33) Harada et al 34)

Table 1b Pleiotropic effect of GGA (2)

GGA induces protective porteins	
Neuronal nitric oxide(NO) synthase	Nishida et al 35), Fujiki et al 36)
Apoptosis	Endo et al 37)
protein kinase C	
Central nervous system	Uchida et al 38)
Gastric mucosa	Rokutan et al 39)
Myocardium	Yamamoto et al 40), Yamanaka et al 28)
Liver	Hirota et al 41)
Cochlea	Sone et al 42)
Brain	Yenari et al 43)

Table 1c Pleiotropic effect of GGA (3)

GGA inhibits cancer invasion	Hashimoto et al 44)
GGA induces COX-2 expression and incresed PGE(2) production via activation of the nuclear factor-kappaB sites of COX-2 gene promotes	Yenari et al 43), Nishida et al 45), Wojcik et al 46)
GGA is a novel therapy for artial fibrillation	Brundel et al 47)
GGA has an effective on gentamycin ototoxity in rat cochler culture	Sano et al 48)
GGA has antiviral effect that enhances MxA expression and phyoshorylation of PKR during influenza virus infection	Unoshima et al 49)

hypothesized that GGA also induces cell death in fibroblast-like synoviocytes (FLS) from patients with RA by inhibiting protein geranylgeranylation. Synovial tissues were obtained from patients with RA at the time of total knee arthroplasty. FLS in 3 passages were cultured with various concentrations of GGA (0.1-4.0 mg/ml) and 0.1 and 0.5 μM of Fluv for 48 hours. We also examined the effect of GGA and Fluv in human fibloblasts from skin (CCD-25SK). The number of cells demonstrating cell death was counted by trypan blue staining. In the absence of GGA, there was no apparent cell death on trypan blue staining. The concentration of 0.1-4.0 mg/ml GGA induced cell death in RA synoviocytes. The number of synoviocytes demonstrating cell

death induced by 0.1 and 0.5 μM of Fluv was significantly higher compared with that by medium alone. Neither GGA (0.1-4.0 μ g /ml) nor Fluv induces cell death of fibroblasts from skin (data not shown).

We demonstrated that GGA induced cell death in FLS from patients with RA, but not in skin fibroblasts. We showed a marked reduction in RA synovial FLS survival though the induction of cell death when the cells were cultured with GGA (100-4000 ng/ml). As reported recently, Fluv also induced cell death[15]. We have previously demonstrated that GGA potently inhibits human osteoclastogenesis induced by sRANKL and induces degradation of actin rings in mature osteoclasts *in vitro* as well as preventing bone loss in both ovariectomized rats and tail-suspended rats *in vivo*[18-22]. GGPP reversed the GGA-induced degeneration of the actin rings of osteoclasts. Thus, GGA blocked the functions of GGPP by competitive inhibition of the mevalonate pathway.

Pleiotropic effect of GGA and statins

Klinderler et al reported that statin-induced expression of CD56 on vascular endothelium under hypoxia is a potent mechanism for the anti-inflammatory action of statin in RA[50]. Thus, these finding suggests that statins would be a useful immunomodulator in autoimmune diseases such as RA. In fact, atrovastatin and simvastatin have shown anti-inflammatory effects in patients with RA[8-11].

Many of these effects are related to the inhibition of isoprenoid synthesis, which serves as a lipid attachment for small G proteins implicated in intracellular singnaling. These small G proteins, whose proper membrane localization and function depend on isoprenylation, play an important role in the pleiotropic effects of statins[7,12-14]. In fact, Fluv has been reported to induce apoptosis in RA synoviocytes through the blocking of protein geranylgeranylation[15]. RhoA plays a role as the key regulator of TNFα-induced NF-κB activation, which ultimately results in the secretion of proinflammatory cytokines in RA synoviocytes[51]. This small G protein also plays an important role in the anti-inflammatory effect of GGA in RA. Table 1 shows other pleiotropic effects of GGA.

In summary, the recent our study clearly demonstrated that GGA induced cell death in RA synoviocytes and had an anti-imflammatory effect. GGA is known to induce HSP, COX-2 and protein C and protect cell from ischemia and reperfusion injury. We previously reported that GGA inhibits human osteoclastogenesis[18]. Thus, GGA has therapeutic implications in RA by reducing synovitis, inflammation, bone destruction and osteoporosis.

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