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Anti-hepatitis C virus activity of geranylgeranylacetone treatment in hepatitis C-infected patients

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Background. Geranylgeranylacetone (GGA), which is an isoprenoid compound, has been used orally as an antiulcer drug in Japan. GGA induces antiviral gene expression by stimulating the formation of interferon-stimulated gene factor 3 in human hepatoma cells. This study verified the anti-hepatitis C virus (HCV) activity of GGA in chronic hepatitis C-infected patients.
Methods. The present prospective study included 20 consecutive anti-HCV antibody-positive, HCV-genotype 1b, and chronic gastritis patients who visited Nagasaki University Hospital between January 1999 and December 1999. GGA (150 mg per day, which is the dose generally used for chronic gastritis) was taken orally for four weeks. We evaluated HCV-RNA titers and other clinical parameters at pretreatment, posttreatment, and at the endpoint of the study. Pretreatment was the beginning point of GGA treatment. Posttreatment was the termination point of GGA treatment. The endpoint was the point four weeks after the posttreatment point.
Results. All patients completed four weeks of GGA treatment and four weeks of observation. HCV-RNA titers at postpoint were not significantly diminished compared to those at pretreatment. However, HCV-RNA titers were significantly diminished at endtreatment compared to pretreatment. Unfortunately, we did not observe a case with no titer of HCV-RNA. Alanine aminotransferase values and other parameters were not affected by GGA treatment.
Conclusion. GGA has anti-HCV activities in chronic hepatitis C-infected patients. In the future, it will be necessary to examine the clinical effectiveness of the combination of treatment with both GGA and interferon in HCV patients.

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Introduction

Currently, chronic hepatitis C virus (HCV) infections are the major cause of hepatocellular carcinoma (HCC) worldwide (1). Therefore, an anti-HCV strategy is important for the prevention of carcinogenesis. The treatment of HCV with a combination of pegylated interferon (IFN) and ribavirin is effective in 80% of HCV genotype 2 or 3 cases but is less than 50% effective in genotype 1 cases. New anti-HCV agents designed to inhibit the life cycle of HCV have been developed and are used in combination with IFN-α to ameliorate the salvage rate of HCV infection (2). However, this combination therapy cannot completely eliminate chronic HCV infections. Therefore, long-term management and safety drugs for chronic hepatitis C (CHC) patients are required.

Geranylgeranylacetone (GGA) is an isoprenoid compound, which includes retinoids. GGA was developed in Japan and has been used orally as an antiulcer drug (3). GGA protects the gastric mucosa from various types of stress without affecting gastric acid secretion (4,5). Moreover, GGA suppresses cell growth and induces differentiation or apoptosis

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in several human leukemia cells (6,7). 3,7,11,15-Tetramethyl-2,4,6,-10,14-hexadecapentaenoic acid is another isoprenoid compound that was designated as an acyclic retinoid because it has the ability to interact with nuclear retinoid receptors (8) that cause apoptosis in certain human hepatoma cells (9). GGA acts as a potent inducer of antiviral gene expression, and it induces the expression by stimulating the formation of IFN-stimulated gene factor 3 (ISGF3) in human hepatoma cells (10). GGA induces the expression of antiviral proteins such as 2 5 -oligoadenylate synthetase (2 5 -OAS) and double-stranded RNA-dependent protein kinase (PKR) in hepatoma cell lines. GGA stimulates 2 5 -OAS and PKR gene expression at the transcriptional level through the formation of ISGF-3, which regulates the transcription of both genes. GGA induces the expression of signal transducers and activators of transcription 1, 2 (STAT-1, STAT-2) and p48 proteins, components of ISGF3, together with the phosphorylation of STAT1 (10). However, the anti-HCV activity of GGA has not been observed in vivo and in vitro.

At present, new treatments for CHC patients are necessary, and GGA has an IFN-like action in hepatoma cells (10). Therefore, we attempted to verify the anti-HCV activity of GGA in CHC patients.

Methods

Patients

The present prospective study included 20 consecutive anti-HCV antibody-positive, HCV-genotype 1b, and chronic gastritis patients who visited the Nagasaki University Hospital between January 1999 and December 1999. Patients were enrolled in the study after informed consent was obtained. The patients had not been previously treated with IFN therapy and were diagnosed with CHC on the basis of clinical data. The patients were evaluated with a HCV-RNA polymerase chain reaction (PCR) method (Amplicor method). The HCV-RNA high group (100,000 IU/mL or more in the serum) was identified by quantitative PCR. The criteria for HCC were assessed by abdominal imaging methods and by HCC history. The patients who were not previously diagnosed with diabetes mellitus (DM) were evaluated by the 75-g oral glucose tolerance test (OGTT). All subjects underwent OGTT with 75 g of glucose according to the recommendations of the National Diabetes Data Group of the National Institute of Health. Blood samples were taken at 0, 30, 60, 90, 120, and 180 min after administration in order to measure the plasma glucose (PG) and insulin concentrations. In this study, the DM group consisted of patients with clinically diagnosed DM or ≥110 mg/dL fasting PG and/or 140 mg/dL or high PG at 120 min.

White blood cell counts, red blood cell counts, platelet counts, hemoglobin A1c levels, alanine aminotransferase (ALT) levels, aspartate aminotransferase (AST) levels, and γ-glutamyl transpeptidase (GTP) levels were determined by hematometry and standard laboratory techniques. Clinical characteristics are shown in the Table.g

Table. Clinical characteristics at pre-GGA treatment
Characteristic mean (SD) or number

Age (years)	56 (16)
Sex (F/M)	10/10
BMI	21.0 (3.02)
Genotype 1b	20
HCV high titer	14
HCV-RNA titer	489 (378)
HCC +/-	0/20
WBC count	6004 (1585)
RBC count	447 (60)
Plt count	18.9 (7.9)
Alb level	4.46 (3.0)
AST level	49.5 (21.2)
ALT level	71 (28)
γ-GTP level	50.5 (32)
DM +/-	0/20
HbA1c level	5.05 (0.8)
FPG level	96 (13)

Data are shown as means (standard deviation) and numbers.
BMI, body mass index; HCV, Hepatitis C virus; HCC, hepatocellular carcinoma; WBC, white blood cells; RBC, red blood cells; Plt, platelets; Alb, albumin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase; DM, diabetes mellitus; HbA1c, hemoglobin A1c; FPG, fasting plasma glucose.
Normal values in laboratory tests: ALT (IU/L), 5-40; AST (IU/L), 10-40; γ-GTP (IU/L), <70 in men, <30 in women; Alb (g/dL), 4.0-5.0; WBC (cells/μL), 3500-9000; RBC ($\times 10^4$ cells/μL), 450-580 in men, 380-480 in women; Plt ($\times 10^4$ platelets/μL), 14-33; ferritin (ng/mL), 39.4-340 in men, 3.6-114 in women; FPG (mg/dL), 70-110; HbA1c (%), 4.3-5.8; BMI, body weight (kg)/height^2 (m).

Methods

The dose of 150 mg of GGA per day, which is generally used to treat chronic gastritis in Japan, was taken orally for four weeks, and it was assumed that patients took one dose a day. Pretreatment was the beginning point of GGA treatment. We evaluated HCV-RNA titers and other clinical parameters at pretreatment, posttreatment, and study endpoint. Posttreatment was the termination point of GGA treatment. Endpoint was the point four weeks after the

posttreatment of GGA. During this study, all patients were not treated with Stronger Neo-Minophagen C (Minophagen Pharmaceutical Co., Ltd., Tokyo, Japan) because of its anti-hepatitis effects or with IFN because of its anti-HCV effects.

Statistical analysis

Data were processed on a personal computer and analyzed using StatView 5.0 (SAS Institute, Inc., Cary, NC). The differences in the values of each laboratory parameter were analyzed with a t-test. P values less than 0.05 were considered statistically significant.

Results

GGA decreased the HCV-RNA titers in patients but did not affect the values of ALT

All patients completed four weeks of GGA treatment and four weeks of observation. Adverse effects were not observed in any patient. The titers of HCV-RNA (Fig. 1A) changed after the patients completed GGA treatment. Compared with HCV-RNA titers at pretreatment, titers at endpoint did not diminish significantly. However, compared to HCV-RNA titers at pretreatment, the titers were significantly diminished at posttreatment. Unfortunately, we did not observe a case with no titer of HCV-RNA. Values of ALT (Fig. 1B) and other parameters were not changed by GGA treatment. The diminished HCV-RNA titers at the posttreatment point were increased at the endpoint, which was four weeks after the posttreatment point.

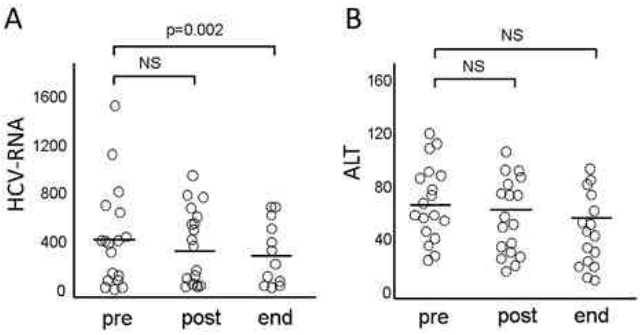

Figure 1. Titers of hepatitis C virus (HCV)-RNA at the endpoint were decreased compared to the levels at pretreatment (A), but alanine aminotransferase (ALT) levels were not changed (B).

Panel A shows serum HCV-RNA titers, and panel B shows serum ALT levels at each of the indicated points. The bar indicates the mean value. Statistical significance was accepted with p-values less than 0.05. "Pre" indicates the point of pre-geranylgeranylacetone (GGA) treatment. "Post" indicates the termination point of GGA treatment. "End" indicates the point four weeks after posttreatment of GGA. Compared to HCV-RNA titers at pretreatment, GGA treatment decreased the titer at pretreatment but not at posttreatment.

In Fig. 2, we present the case of a patient who had the most diminished HCV-RNA titers among the 20 GGA-treated patients (Fig. 2). This case had mild fluctuations of ALT levels before GGA treatment. The HCV-RNA titer was 420 K copies/mL and 380 K copies/mL at 12 weeks before treatment and at the pretreatment point, respectively. After GGA treatment, HCV-RNA titers were decreased to 2 K copies/mL and 4 K copies/mL at the endpoint and at the posttreatment point, respectively. In this case, the ALT values were also diminished in a similar manner as HCV-RNA. After the observation period, +12 weeks, HCV-RNA titers and ALT values were increased compared to those at the pretreatment point.

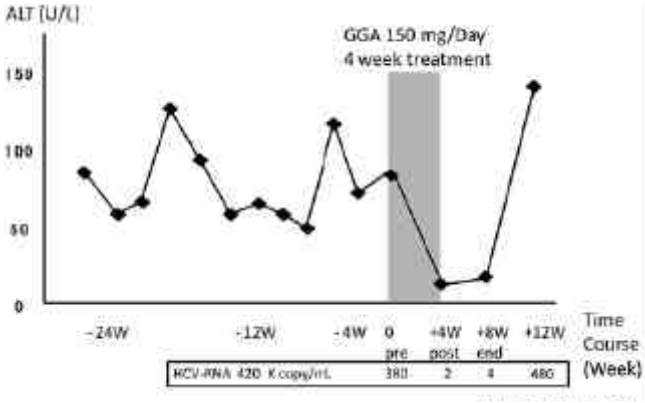

Figure 2. The clinical course of a geranylgeranylacetone (GGA)-treated chronic hepatitis C (CHC) patient.

Here, we present a case of a 53-year-old man who was an outpatient of our hospital for 5 years. He was diagnosed with chronic hepatitis on the basis of clinical data. The patient had not been previously treated with interferon (IFN). The y-axis indicates alanine aminotransferase (ALT) levels, and the x-axis indicates the time course. The duration of the GGA treatment periods is shown in the gray field. The zero point on the x-axis is the GGA treatment-starting day. HCV-RNA titers are 420, 380, 2, and 4 K copies/mL at -12 weeks, 0 weeks (pretreatment), +4 weeks (posttreatment), and +8 weeks (end of follow-up period), respectively.

Discussion

GGA demonstrated anti-HCV activity in this study. The anti-HCV effect that was due to GGA did not result in a disappearance of HCV-RNA titers in CHC patients. An adverse effect was not observed with GGA treatment.

GGA is a non-toxic heat shock protein (HSP) 70 inducer (11). Various GGA activities outside of the stomach are also related to HSP induction (12,13,14). GGA induces

thioredoxin, as well as HSP-70, in hepatocytes and other cells (15). The antiviral activity of thioredoxin is induced by AP-1 and NF-κB but not by HSP-70 (16). GGA, which has potent antiviral activities through the enhancement of antiviral factors, can clinically provide protection from influenza viral infections (17). Previously, we reported that GGA induction of antiviral proteins was dependent upon STAT-1 tyrosine phosphorylation in HuH-7 and HepG2 with which HCV was not infected (10). However, HCV products inhibit the Jak-STAT pathway in HCV-infected hepatocytes (18). The mechanism of inhibition of the Jak-STAT pathway is multifactorial and includes the expression of suppressor of cytokine signaling 3 (SOCS-3) (19), protein phosphatase 2A induction (20), STAT-3 expression (21), and IL-8 expression (22). A clarification of GGA-induced anti-HCV activity is necessary for further examination of the *in vitro* and *in vivo* effects.

The peak venous blood concentration after taking 150 mg of GGA orally is 5-7 μmol/L (23), but 50 μmol/L is the best dose for induction of PKR and 2′-5′OAS in hepatoma cell lines (10). In this study, we employed the usual dosage of GGA used to treat chronic gastritis in Japan, which is 150 mg per day. In a previous study, it was reported that portal blood concentration after taking 150 mg of GGA orally was several-fold that of the venous blood concentration (23). The usual dosage of GGA also may have a possible antiviral gene expression effect in the liver.

In conclusion, GGA, a drug that can be safely administered orally, has anti-HCV activity. Unfortunately, we did not observe a case that exhibited disappearance of HCV-RNA titers. GGA treatment is insufficient for clearance of HCV, and, therefore, it will be necessary to examine the clinical effectiveness of the combination treatment with GGA and IFN in HCV patients in the future.

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