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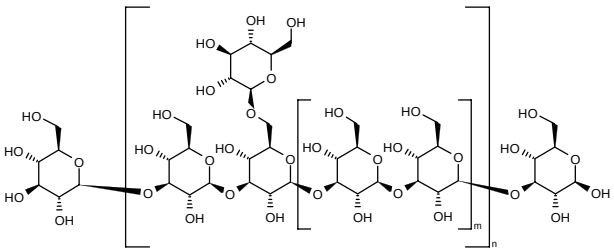
G009

Hepatoprotectant
Immunostimulant

β-(1-3)-Glucan with β-(1-6) side chain isolated from the Basidiomycetes *Ganoderma lucidum*



Mol wt: 12 kDa

EN: 246402

Culture and Purification

Ganoderma lucidum IY009 was cultured at 28 °C for 7 days in a large fermentor containing culture media. The culture mycelia were harvested, extracted and placed in an alkali solution neutralized with acetic acid at room temperature for 24 h. After the residue containing the mycelial fragments was discarded by centrifugation, the supernatant was difiltrated by an ultrafiltration system. The high molecule fraction obtained by freeze drying was named G009.

Description

Brown or grayish brown powder, soluble in water.

Introduction

G009 was originally isolated from *Ganoderma lucidum*, a fungi belonging to the Basidiomycetes class that has been used for many years as a treatment for various diseases in China, Korea and Japan, and is regarded as a very effective herbal medicine (1). Ganoderma lucidum is thought to have antineoplastic activity and has been used for treating patients with immunodeficiency resulting from cancer, aging, infectious disease, *etc.* (2-4). Recently, it was found that polysaccharides from some microorganisms have immunostimulatory activity. As a result, many research groups, especially in Japan, Korea and China, have actively studied polysaccharides from fungi and have reported that *Ganoderma lucidum* is a very strong immunostimulant (Table I).

Our research group began testing immunologically effective species of the *Ganoderma* genus about 10 years ago and finally identified and characterized the most effective strain, *Ganoderma lucidum* IY009. To elucidate the active fraction of *Ganoderma lucidum* IY009, the whole culture was purified and the protein bound polysaccharide, G009, was found to have potent immunostimulatory and hepatoprotective activity. In preclinical studies, G009 was shown to be safe and effective as an immunostimulatory and hepatoprotective agent and was also effective in preventing cirrhosis of the liver in bile duct-ligated rats. The compound also displayed antitumor activity as well as anticarcinogenic effects.

Pharmacological Actions

Hepatoprotective effects

G009 exhibited obvious antifibrotic effects in animals with experimentally induced cirrhosis of the liver. G009 administered after bile duct ligation/scission dose-dependently decreased hydroxyproline levels in liver tissue, reduced necrosis of liver cells, suppressed blood stasis and inflammation and improved bile duct proliferation. These results suggest that the compound would be effective in the prevention and treatment of liver diseases, including cirrhosis (3).

Elevated levels of GOT and GPT induced by acetaminophen were reduced by 80.5 and 79.6%, respectively, in animals treated with G009 at a dose of 10 mg/kg and by 90.8 and 86.6%, respectively, in animals given 20 mg/kg. G009 treatment also normalized histology of liver tissue from acetaminophen-treated animals (3).

Effects on CCl_4-induced injury and lipid peroxidation

G009 not only recovered fatty liver, but significantly lowered blood levels of GOT and GPT in rats with CCl_4-induced hepatitis. G009 also normalized blood levels of

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Table I: Polysaccharides with immunostimulatory properties produced from Basidiomycetes.

Product	Origin	Action	Phase
Lentinan	*Lentinus edodes*	Anticancer	Launched
Schizophyllan	*Shizophyllum commune*	Anticancer	Launched
Krestin (PSK)	*Coriolus versicolor*	Anticancer	Launched
Coporang	*Coriolus versicolor*	Anticancer	Launched
Meshima-ex	*Phelinus linteus*	Anticancer	Launched
Licovek	*Coriolus versicolor*	Hepatic	Launched
Livax	*Coriolus versicolor*	Hepatic	Launched
Sizofiran	*Shizophyllum commune*	Lung cancer	Pending preregistration
		Hepatitis B	Filing phase III
Acylfulvene	*Omphalotus illudens*	Cancer	Phase I

GOT and GPT in thioacetamide-induced acute hepatitis, further demonstrating the protective effects of G009 against liver damage (5).

In rats with CCl_4-induced liver toxicity, G009 decreased GOT and GPT levels in a dose-dependent manner and was also responsible for significantly reducing MDA synthesis in liver homogenates and blood. A dose of 25 mg/kg was particularly effective in inhibiting lipid peroxidation by as much as 59.3%. In animals with galactosamine-induced toxicity, a model similar to viral hepatitis, G009 (100 mg/kg) showed protective effects by decreasing GOT and GPT levels by 50.0 and 54.2%, respectively (5).

G009 exhibited inhibitory effects against lipid peroxidation, as well as protective effects against ascorbic acid-induced liver toxicity in rats. Since a close relationship between lipid peroxidation and liver damage was observed in this study, G009 is now expected to play a major role in treating liver disease (5).

Effects on nitric oxide production

In studies using cultured Raw 264.7 cell line, G009 stimulated macrophage production of nitric oxide (NO) by inducing expression of the NO synthase gene; NO production was shown to be completely suppressed by NG-MMA. G009 also was shown to stimulate the synthesis of reactive oxygen intermediates in human neutrophils. These effects of G009 may be due to its ability to strengthen the immune system through stimulation of the immune cells and alteration of cellular physiology (6, 7).

Antitumor and immunostimulatory activity

G009 appeared to have potent antitumor activity *in vivo*. It did not show any direct cytotoxicity on sarcoma 180 cells, but did stimulate antibody production, opsonization of macrophages in the ICR mouse and superoxide ion production from isolated macrophages. G009 also activated complement C3 in human serum; EDTA, a chelator of cation-related complementary activation, was shown to inhibit this antitumor activity. Treatment with G009 resulted in prolonged life span and inhibition of tumor growth in mice transplanted with P388 and L-1210 leukemia cell lines (2, 8).

Anticarcinogenic effects

In an *in vivo* model using benzo(a)pyrene to evaluate anticarcinogenicity, the incidence and number of lung tumors in G009-treated animals were significantly inhibited by 32.4 and 29.6% with concentrations of 10 and 2 mg/ml, respectively. Therefore, G009 may be a potential candidate as a chemopreventive agent (8, 9).

Pharmacokinetics and Metabolism

Up to 75% (relative percentage to radioactivity measured in urine, breath and carcass) of orally administered [^{14}C]-G009 was found in the stomach. When the AUC_{24h} values of plasma radioactivity were compared in animals administered the agent intravenously and orally, systemic bioavailability was 18%. Considering that this study was performed for 24 h, the bioavailability should have been higher. In addition, [^{14}C]-G009 accounted for 25% of the total radioactivity in plasma after oral administration. Therefore, systemic bioavailability of unchanged compound should have been lower than that of the total radioactivity. Metabolism of [^{14}C]-G009 was very rapid and complete, with most metabolites being catalyzed by normal enzymes involved in glycolysis. Thus, the radioactivities detected in organs or tissues most likely resulted from incorporation of radioactive carbon into the cellular carbon pool. A slow increase in plasma radioactivity implies that the gastric intestinal microflora is involved in the initial hydrolysis of these polymers. This hypothesis is supported by the observations that almost all of the administered radioactivity remained in the stomach. At 4 h after administration, radioactivity in the caecum decreased and very little absorption of radioactivity was observed in the stomach and small intestine (10, 11).

Table II: Stability of G009.

Tests	Methods or standard conditions	Results
Physical characteristics	Determine if physical characteristics initially observed are maintained throughout the storage period	OK
Qualitative analysis	Standard procedure of KFDA (TLC)	OK
Dissolubility test	General methods listed in Koren Pharmacopeia	OK
Carbohydrate content	100 mg of G009 should contain 50-80 mg of carbohydrates	OK
Weight deviation	General methods listed in Korean Pharmacopeia	OK
Heavy metal content	Heavy metal content lower than 100 ppm	OK

Toxicology

Results from acute toxicity studies in rats given single oral doses of G009 (500-2100 mg/kg) for 14 days did not show any significant change in weight or abnormal clinical signs. No specific changes associated with the drug were found on autopsy.

Studies in which rats were administered single i.p. doses of G009 (500-2100 mg/kg) for 7 days concluded that the LD_{50} values for males and females were 876.16 and 975.62 mg/kg, respectively.

Acute single-dose oral toxicity studies of G009 (312.5-5000 mg/ml for 14 days) in ICR mice showed no abnormal clinical symptoms, changes in weight or specific changes in internal organs associated with treatment. Results indicated that the LD_{50} value in both males and females was over 5000 mg/kg.

Acute toxicity tests in mice after single i.p. administration of G009 (640-2700 mg/kg) for 7 days showed that the LD_{50} values for male and female animals were 1821.9 and 1691.5 mg/kg, respectively.

In a 30-day repeated dose toxicity study in rats, the optimum dose of orally administered G009 for 1 month was 2000 mg/kg/day, since no significant clinical changes associated with the drug were observed.

A 28-day repeated dose toxicity study in Beagle dogs administered oral (gavage) doses of G009 indicated that a maximum dose of 1000 mg/kg was safe for oral administration (12).

Mutagenicity

In a micronucleus test in rodents, polychromatic erythrocytes containing micronucleus were rarely observed with any of the G009 dose groups. According to naked eye evaluation, no symptoms indicating toxicity of the drug were observed in any of the animals tested. Moreover, the proportion of polychromatic erythrocytes to whole erythrocytes for each dose group was not significantly different from controls (13).

A chromosomal aberration test in rodents demonstrated that 1% of negative control cells, with or without metabolic defects, had chromosomal aberrations as compared to less than 3% of G009-treated cells at all drug concentrations tested.

In a reverse mutation test using 4 strains of *Salmonella typhimurium* subjected to metabolic activation using S-9 mix exhibited the same frequency of the revertant as the control with all G009 concentrations evaluated (14).

Taken together, these results indicate that G009 has no mutagenicity.

Antigenicity

G009 showed no antigenicity in a passive cutaneous anaphylaxis (PCA) response test in rat using sera from sensitized mice given doses of G009 1-10 times higher than the clinical doses expected to elicit sensitization. In addition, G009 showed no antigenicity according to the PCA response in guinea pigs. When compared to the response of control animals given ovalbumin, G009 showed almost no antigenicity at all dose levels (15, 16).

Stability

Test samples from 3 different lots of G009 were packaged into HDPE bottles and stored at 40 °C, 75% RHC *versus* room temperature. The samples were then tested for stability on the first day of storage and then periodically every 2 months. The results of 6 tests used to assess the stability of G009 are summarized in Table II.

Clinical Studies

No clinically significant abnormal symptoms were reported in a phase I single- and multiple-dose escalation study. When compared to placebo, no statistically significant changes in pharmacokinetic parameters were observed with any of the doses tested and no dose-dependent changes were observed. During a specific period of time, blood levels of MDA, collagen and NO were significantly altered in the single-dose group and NO concentrations were increased in the multiple-dose group as compared to baseline levels before administration. However, similar variations were observed in the placebo group, indicating that these changes were not drug-related.

G009 did not cause any side effects even at the maximum dose. The maximum dose is expected to be 2400 mg for once-daily dosing and 1200 mg for twice-daily administration. The recommended dose for phase II trials is 1200 mg/day.

Phase II clinical studies are being conducted at Hanyang University Hospital in Korea.

Manufacturer

Il Yang Pharmaceutical Co. Ltd. (KR).

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