

Enzyme immunoassay for the quantitative determination of ganoderic acid A from *Ganoderma lucidum*

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Abstract

For quantitative determination of ganoderic acid A (GAA, **1**), a major constituent of *Ganoderma lucidum*, a sensitive and specific enzyme immunoassay (EIA) system was developed; the side chain of GAA was extended by introducing a glycine moiety, and this compound was coupled with β -D-galactosidase (β -Gal) and bovine serum albumin (BSA) via an *N*-hydroxysuccinimide ester to give GAA- β -Gal (enzyme-labeled antigen, **5**) and GAA-BSA (immunogen, **6**), respectively. The anti-GAA antiserum, which had been elicited in rabbits by immunization with the GAA-BSA conjugate, possessed high affinity and specificity toward GAA, when the assay was carried out with a double antibody technique. A satisfactory standard curve for EIA of GAA was explored in a range of 0.1–1000 ng/tube. The antiserum of GAA had no cross-reactivity with GAA-related compounds isolated from *G. lucidum*, except for ganoderic acid γ (**11**) and ganolucidic acid A (**14**) with the cross-reactivity of 52.4 and 12.9%, respectively, due to its close similarity in structure to GAA (ganoderic acid derivatives having a carbonyl group at C-3).

The plasma concentration of GAA after its intravenous or oral administration to rats was determined by the established EIA. The AUCs after intravenous administration of GAA were 32.8 ± 9.8 and $201.5 \pm 38.7 \mu\text{g min/ml}$ at doses of 5 and 25 mg/kg, respectively. Following oral administration of GAA at doses of 5 and 50 mg/kg to rats, the plasma concentration of GAA rapidly reached a C_{max} (37 and 595 ng/ml) at 18.1 ± 2.5 and 18.0 ± 0.6 min, respectively, then decreased to 4.2 and 13.3 ng/ml at 480 min, respectively, indicating that GAA was rapidly absorbed into the body fluid from the gastrointestinal tract after the oral administration, and then decreased soon.

Key words ganoderic acid A, enzyme immunoassay, antiserum, absorption, rat plasma, *Ganoderma lucidum*.

Abbreviations BSA, bovine serum albumin; DEPC, diethyl phosphorocyanidate; EDC, 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide HCl; EIA, enzyme immunoassay; GAA, ganoderic acid A; β -Gal, β -galactosidase.

Introduction

The fruiting body of *Ganoderma (G.) lucidum* is a well known Chinese crude drug, which has been used clinically in China, Japan and Korea for a long time as a tonic and sedative, as well as for the treatment of hypertension, chronic hepatitis, hyperglycemia and chronic bronchitis.¹⁾ It was considered to promote longevity and maintain the vitality of human beings. Nowadays, this mushroom is used for leukopenia and is paid much atten-

tion to as a home remedy.²⁾

Over one hundred and thirty highly oxygenated and pharmacologically active triterpenoids have been isolated from the fruiting bodies, mycelia and spores of *G. lucidum*. Of these, ganoderic acid A (GAA, **1**), a major constituent in lanostane-type triterpenes of *G. lucidum*, was reported to have some biological activities, such as hepatoprotective activity,³⁾ inhibitory effect on farnesyl protein transferase⁴⁾ and antinociceptive activity.⁵⁾ Nishitoba *et al.* reported that GAA was an intensively bitter compound with a taste threshold (TT) value of

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1×10^{-8} M, compared with naringin (TT value, 2.5×10^{-5} M).⁶⁾ In our previous papers, we reported the biological activity of GAA such as cytotoxicity against Meth-A (Sarcoma, murine) and Lewis lung carcinoma (LLC, murine) tumor cells, and inhibitory effects on proliferation of HIV-1 and its protease enzyme.⁷⁻⁹⁾

We also reported quantitative determination of some major constituents including GAA, by high performance liquid chromatography (HPLC).⁸⁾ However, this method was insufficient in sensitivity and specificity for quantitative analysis of GAA in the biological fluids. Furthermore, pharmacokinetic studies on the bitterness principle from *G. lucidum* have not been reported hitherto.

The present paper describes the preparation of an antiserum specific to GAA, which includes the condensation of it with β -D-galactosidase (β -Gal) and bovin serum albumin (BSA). Furthermore, we performed quantitative determination of GAA in the biological fluids by using the newly developed EIA and found that an appreciable amount of GAA was detected in rat plasma after its intravenous or oral administration.

Materials and Methods

Apparatus: ¹H-NMR spectrum was measured with a JNM-LA 400 WB-FT (¹H, 400 MHz; JEOL Co., Tokyo) spectrophotometer, the chemical shifts being represented as ppm with tetramethyl saline as an internal standard. Fluorometry was taken on a Shimadzu RF-5000 spectrofluorometer (Kyoto). Preparative HPLC was carried out on a Gilson HPLC system: pump, model 305 and 306; detector, 119 UV/VIS detector (Middleton, USA). Column chromatography was carried out on Silica-gel (Kieselgel 60, 70-230 mesh, Merck, Germany). TLC was carried out on pre-coated Silica-gel 60 F₂₅₄ plates (0.25 mm, Merck) and spots were detected under a UV light and by spraying with 10 % H₂SO₄ followed by heating.

Chemicals: Ganoderic acid A (GAA, **1**) was isolated from a MeOH extract of spores of *G. lucidum* as reported previously.⁹⁾ Triethylamine, glycine methyl ester hydrochloride, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide HCl (EDC), 4-methylumbelliferyl β -D-galactoside and *N*-hydroxysuccinimide were purchased from Nacalai Tesque, Inc. (Kyoto), and diethyl phosphoroc-

anidate (DEPC) from Tokyo Kasei Kogyo Co. (Tokyo). β -D-Galactosidase (EC 3, 2, 2, 23) from *Escherichia coli* was a product of Boehringer Mannheim Co. (Mannheim, Germany). Bovine serum albumin (BSA, Cohen fraction V) and goat antiserum to rabbit IgG (H+L) IgG whole were obtained from Sigma Chemical Co. (St. Louis, MO., USA.). Complete Freund's adjuvant, incomplete Freund's adjuvant and normal rabbit serum were purchased from Wako Pure Chemical Industries, Ltd. (Osaka). Sepharose 6B was obtained from Pharmacia Biotech (Uppsala, Sweden). Ganoderic acids B (**7**), C2 (**8**), C6 (**9**), G (**10**), γ (**11**), ϵ (**12**) and η (**13**), ganolucidic acid A (**14**), ganodermanondiol (**15**), ganodermanontriol (**16**), ganoderiol F (**17**) and lucideric acid SP1 (**18**) were purified from the chloroform-soluble fraction of the MeOH extract of the spores of *G. lucidum* as reported previously.⁸⁻¹⁰⁾ Buffer A was 20 mM phosphate buffered saline (pH 7.3) containing 0.1% BSA, 0.1% NaN₃ and 0.001% MgCl₂, and buffer B, 20 mM phosphate buffered saline (pH 7.3) containing 0.1% NaN₃ and 0.001% MgCl₂.

Preparation of GAA (Methyl Glycine) Conjugate (2): Triethylamine (64.1 μ l, 0.46 mm) was added to a mixture of GAA (**1**, 99.5 mg, 0.19 mmol), glycine methyl ester hydrochloride (26.6 mg, 0.21 mm), DEPC (41.1 μ l, 0.21 mm) and dimethyl formamide (DMF, 3 ml) stirring at room temperature.¹¹⁾ After being kept overnight, water (10 ml) was added and the mixture was extracted with EtOAc (20 ml). The extract was successively washed with 1N HCl, H₂O and 10% Na₂CO₃, and dried over anhydrous Na₂SO₄, and concentrated *in vacuo*. The product was purified by chromatography on a silica gel column using 4% MeOH-CHCl₃ as an eluent to give **2** (60.6 mg, 53.5%) as colorless needles. ¹H-NMR (CDCl₃): δ 3.75 (3H, s, OCH₃), 4.00 (2H, dd, *J*= 5.3, 2.2 Hz, NHCH₂), 6.30 (1H, t, *J*= 5.2 Hz, NH).

Preparation of a GAA Glycine Conjugate (3): Compound **2** (60.6 mg) was treated with 5% KOH-MeOH (2 ml) and the mixture was refluxed on a water bath at 70 °C for 20 min.¹¹⁾ The mixture was acidified with 4N HCl and extracted with CH₂Cl₂ (100 ml). After being washed with H₂O, the CH₂Cl₂ extract was dried over anhydrous Na₂SO₄ and concentrated *in vacuo*. The residue was subjected to preparative TLC with 30% MeOH-CHCl₃ as a developing solvent. The zone with *R_f* 0.35 gave **3** (26.4 mg) in a yield of 44.5% as colorless

needles. $^1\text{H-NMR}$ (CDCl_3): δ 4.14 (2H, dd, $J= 5.8, 4.5$ Hz, NHCH_2), 7.49 (1H, dd, $J= 5.8, 3.2$ Hz, NH).

Preparation of N-Hydroxysuccinimide Ester (4) of a GAA Glycine Conjugate: EDC (9.71 mg, 0.05 mM) was added to a mixture of **3** (26.4 mg, 0.046 mM) and *N*-hydroxysuccinimide (5.83 mg, 0.05 mM) in DMF (1 ml), and the reaction mixture was stirred for 7 hr at room temperature.¹² After addition of water (10 ml), the mixture was extracted with EtOAc (100 ml). The extract was washed with H_2O , dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to afford **4** as a white amorphous powder. $^1\text{H-NMR}$ (CDCl_3): δ 2.29 (4H, s, succinimidy), 4.02 (2H, dd, $J= 5.8, 3.5$ Hz, NHCH_2), 6.78 (1H, br s, NH).

Preparation of a GAA- β -D-Gal Conjugate (5): A solution of **4** (0.05 mg, 75 nM) in dioxane (1 ml) was added to a solution of β -D-Gal (4.5 mg, 8.7 nM) in 0.05 M phosphate buffer (1 ml, pH 7.3) and the reaction mixture was stirred at 4 °C for 48 hr and then applied to a Sepharose 6B column (i.d. 1.5×35 cm) using 4 mM phosphate buffered saline (pH 7.3) containing 3 mM NaN_3 , 0.2 mM MgCl_2 and 20 mM NaCl as a eluent.¹³ Fractions eluted with the maximal enzyme activity were pooled and stored at 4 °C until use. The fractions were used as the GAA- β -D-Gal conjugate.

Preparation of a GAA-BSA Conjugate (6): A phosphate buffer (0.7 ml, pH 7.3) solution of BSA (35.0 mg, 0.51 μM) was added to **4** (10 mg, 14.9 μM) in pyridine (0.2 ml), and the mixture was stirred at 4 °C for 24 hr. The reaction mixture was successively dialyzed for 7 days against 10%, 5%, and 2.5% pyridine- H_2O followed by H_2O ,¹³ and lyophilized to give a GAA-BSA conjugate (**6**) (36.5 mg).

Preparation of an Antiserum to GAA: A GAA-BSA conjugate (**6**, 2 mg) was dissolved in sterile saline (1.5 ml) and emulsified with complete Freund's adjuvant (1.5 ml). The emulsion was subcutaneously injected into three domestic albino female rabbits at multiple sites on the back. Six boosters (2 mg each) of the immunogen, dissolved in sterile saline (2.5 ml) and emulsified with 2.5 ml of incomplete Freund's adjuvant (2.5 ml), were carried out at intervals of once every 2 weeks for 3 months and monthly thereafter. The blood was collected from the ear vein 10 to 14 days after the last booster injection. The serum was obtained by centrifugation at $2700 \times g$ for 15 min, after standing for 2 hr at room

temperature, then stored at -20 °C until use.

EIA Procedure: A sample or standard solution of GAA (40 μl , in buffer A) was added to a mixture of 10^3 -fold diluted labeled antigen GAA- β -D-Gal conjugate solution (25 μl , in buffer B) and 10^3 -fold diluted anti-GAA serum (50 μl , in buffer A). The mixture was incubated at room temperature for 2 hr and 10-fold diluted solution of goat anti-rabbit IgG (20 μl , in buffer A) and 10^2 -fold diluted normal rabbit serum (20 μl , in buffer A) were added to the reaction mixture. The mixture was then allowed to stand overnight at 4 °C. After addition of buffer A (1 ml), the resulting mixture was subjected to measuring the enzymatic activity of the immunoprecipitate. These procedures were performed to estimate the % binding (B/B_0) of the labeled antigen with antibodies in the presence of various amounts of an unlabeled antigen, GAA. B and B_0 represent the bound enzyme activities in the presence and the absence of an unlabeled antigen, respectively.

Measurement of β -D-Gal Activity: The immunoprecipitate was incubated with 0.1 mM 4-methylumbelliferyl β -D-galactoside (150 μl) at 30 °C for 30 min. The reaction was stopped by addition of 4 ml of 0.1 M glycine-NaOH buffer (pH 10.3) and the fluorescence intensity of 7-hydroxy-4-methylumbelliferone released was measured at wavelengths of 365 nm and 448 nm for excitation and emission, respectively.

Specificity of the Antiserum: The cross reaction of anti-GAA antiserum with GAA and related compounds (**7**–**18**) isolated from *G. lucidum* was examined using a GAA- β -Gal conjugate under the assay conditions described above.

Animals, Treatments and Sampling: Male Wistar rats (6 weeks old, SLC Co., Hamamatsu) were used. Animals were fed standard laboratory chow with water *ad libitum*, maintained for one week, and were fasted overnight before the experiments.

For intravenous administration, GAA dissolved in 5% DMSO-saline was injected into a tail vein of four rats at doses of 5 and 25 mg/kg, respectively. Blood samples were taken from another tail vein using a heparinized capillary micro-tube at 5, 15, 30 min, 1, 2, 4, 6, and 8 hr after the injection, and immediately separated by centrifugation. The serum sample obtained was kept at -20 °C until used for measuring the concentration of GAA by EIA.

On the other hand, GAA dissolved in 5% DMSO-H₂O was used for oral administration to rats ($n=4$ at each point) at doses of 5 and 50 mg/kg, respectively. Blood samples were obtained from a tail vein at 10, 20, 40 min, 1, 2, 4 and 8 hr, then treated as described above.

Results and Discussion

EIA for GAA

For the purpose of establishing an EIA method for quantitative analysis of GAA, BSA- and β -Gal-conjugates with GAA were prepared as an immunogen and a labeled antigen, respectively. GAA was condensed with 2-aminoacetate by using DEPC as a coupling reagent to give **2**, which was further hydrolyzed with potassium hydroxide to afford **3**. Compound **3** was treated with *N*-hydroxysuccinimide in the presence of EDC to give an *N*-hydroxysuccinimide ester (**4**), which was subsequently coupled with β -Gal and BSA to afford GAA- β -Gal (**5**) and GAA-BSA (**6**) conjugates, respectively (Chart 1).

The anti-GAA antiserum was elicited in three rabbits by immunization with the GAA-BSA conjugate (**6**).

The EIA for GAA was performed by mixing the antiserum and the enzyme-labeled antigen (GAA- β -Gal, **5**) in the presence of a test sample or an unlabeled antigen GAA, followed by addition of a goat antiserum to rabbit IgG as the second antibody. The β -Gal activity in the immunoprecipitate was fluorometrically determined with 4-methylumbelliferyl β -D-galactosidase as a substrate. A dilution curve of an anti-GAA antiserum is shown in Fig. 1. The maximal difference in fluorescence intensity was observed for 1000-fold dilution of the antiserum in the presence and absence of 100 ng GAA per tube. A standard curve for GAA was obtained in a range of concentrations of 0.1–1000 ng/tube (Fig. 2).

The GAA was equally reactive with the antiserum in the presence of rat plasma to give a similar standard curve as in buffer solutions, indicating no appreciable interference by any components in the plasma (Fig. 3). In the presence of the plasma, the coefficients of variance at 1–1000 ng/tube were 2.3–4.7% and 10.6–30.3% for intra- and inter-day assays, respectively. The specificity of the anti-GAA antiserum was tested by cross-reaction experiments with structural and functional analogues

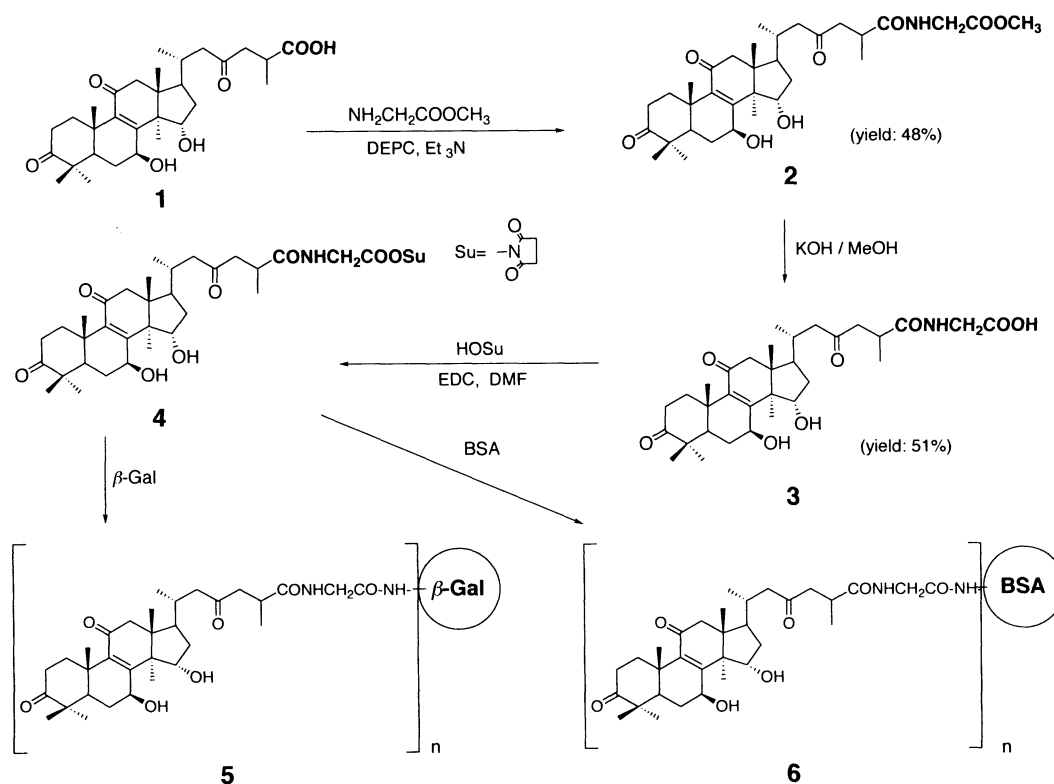


Chart 1 Preparation of an immunogen and a labeled antigen for enzyme immunoassay of GAA

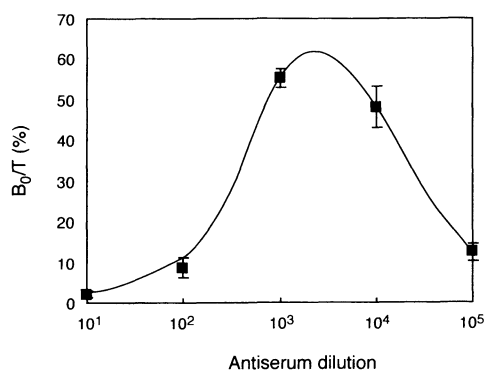


Fig. 1 Dilution curve of antisera
 B_0 = bound enzyme activity of a labeled antigen
 T = total enzyme activity of a labeled antigen ($n=3$)

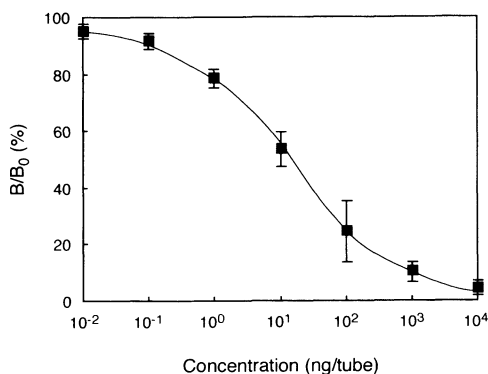


Fig. 2 Standard curve of antisera
 B = % binding in the presence of GAA
 B_0 = % binding in the absence of GAA ($n=3$)

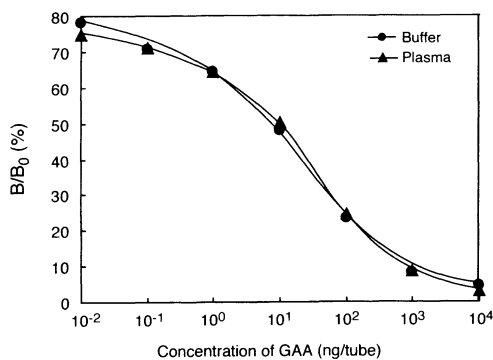


Fig. 3 Standard curve of GAA in the presence of rat plasma

isolated from *G. lucidum* (Chart 2), and the results were shown in Table 1. The anti-GAA antiserum reacted with two compounds, ganoderic acid γ (11) and ganolucidic acid A (14) with cross reactivities of 52.4 and 12.9%, respectively. The functional groups of the former were the same as those of GAA except for a double bond and a hydroxy group instead of a carbonyl group of GAA in

(1-14)

(15-17)

Lucideric acid SP1 (18)

Compound	R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇
Ganoderic acid A (1)	O			H	O	H ₂	H
Ganoderic acid B (7)			O	H	O	H ₂	H
Ganoderic acid C2 (8)				H	O	H ₂	H
Ganoderic acid C6 (9)		O	O	OH	O	H ₂	
Ganoderic acid G (10)			O	OH	O	H ₂	H
Ganoderic acid γ (11)	O			H		$\Delta^{24(25)}$	
Ganoderic acid ϵ (12)			O	H		$\Delta^{24(25)}$	
Ganoderic acid η (13)		O	O	OH		$\Delta^{24(25)}$	
Ganolucidic acid A (14)	O	H ₂		H	O	H ₂	H
Ganodermanodiol (15)	O	OH	OH	CH ₃	CH ₃		
Ganodermanotriol (16)	O	OH	OH	CH ₂ OH	CH ₃		
Ganoderiol F (17)	O	$\Delta^{24(25)}$		CH ₂ OH	CH ₂ OH		

Chart 2 Structures of ganoderic acid A (1) related compounds isolated from *G. lucidum*

the side chain. The latter was only different at C-7 position, which was not oxygenated. Since the two compounds (11 and 14) are minor constituents in *G. lucidum*,^{9,14} the antiserum is considered to be usable for determination of GAA. On the other hand, *Ganoderma* acids (7–10, 18) and alcohols (15–17) showed no appreciable cross reactivity with GAA.

Determination of Plasma GAA after Intravenous and Oral Administration of GAA

For demonstrating the applicability of the above EIA method, we examined the quantitative analysis of GAA in rat plasma after its intravenous and oral administration to rats.

Fig. 4 shows plasma concentration-time curves of GAA after the intravenous administration at doses of 5 and 25 mg/kg to rats monitored by the EIA method. The

Table I Cross reactivity of GAA-related compounds

Compound	Cross reactivity (%) ^{a)}
Ganoderic acid A (1)	100.0
Ganoderic acid B (7)	0.2
Ganoderic acid C2 (8)	0.8
Ganoderic acid C6 (9)	0.0
Ganoderic acid G (10)	0.2
Ganoderic acid γ (11)	52.2
Ganoderic acid ε (12)	0.6
Ganoderic acid η (13)	0.2
Ganolucidic acid A (14)	12.9
Ganodermanondiol (15)	0.1
Ganodermanontriol (16)	0.1
Ganoderiol F (17)	0.2
Lucideric acid SP1 (18)	0.2

a) Cross reactivity (%) represents a relative concentration of GAA (1), which is required to induce 50% inhibition of the binding with the antiserum.

Table II Pharmacokinetic parameters after intravenous administration of GAA (1) at doses of 5 and 25 mg/kg

Parameter	Dose (mg/kg)	
	5	25
A ($\mu\text{g/ml}$)	1.81 \pm 0.45	8.72 \pm 2.49
α (min^{-1})	3.34 \pm 0.38	2.97 \pm 0.24
B ($\mu\text{g/ml}$)	0.0065 \pm 0.0015	0.0175 \pm 0.0072
β (min^{-1})	0.092 \pm 0.002	0.123 \pm 0.028
$t_{1/2\alpha}$ (min)	12.4 \pm 1.6	14.0 \pm 1.1
$t_{1/2\beta}$ (min)	451.0 \pm 98.2	337.5 \pm 87.6
V_C (l/kg)	2.75 \pm 0.69	2.86 \pm 0.86
V_{dss} (l/kg)	12.37 \pm 2.21	5.64 \pm 1.82
CL_{tot} (ml/min kg)	152.43 \pm 23.07	124.07 \pm 25.08
AUC_{0-480} ($\mu\text{g min/ml}$)	32.8 \pm 9.8	201.5 \pm 8.7

Each value represents mean \pm S.D. ($n=4$)

Table III Pharmacokinetic parameters after oral administration of GAA (1) at doses of 5 and 50 mg/kg

Parameter	Dose (mg/kg)	
	5	50
C_{max} ($\mu\text{g/ml}$)	0.037 \pm 0.008	0.595 \pm 0.125
t_{max} (min)	18.1 \pm 2.5	18.0 \pm 0.6
AUC_{0-480} ($\mu\text{g min/ml}$)	3.29 \pm 0.98	27.76 \pm 0.13

Each value represents mean \pm S.D. ($n=4$)

plasma concentrations of GAA decreased rapidly in the first 1 hr and maintained for more than 8 hr. The curves were fitted well to the two-compartment model at each dose, followed by a similar elimination half lives ($t_{1/2}$; $t_{1/2\alpha}$: 12.4 and 14.0 min, $t_{1/2\beta}$: 451.0 and 337.5 min, respectively.), and the AUC_{0-480} values (201.5 and 32.8

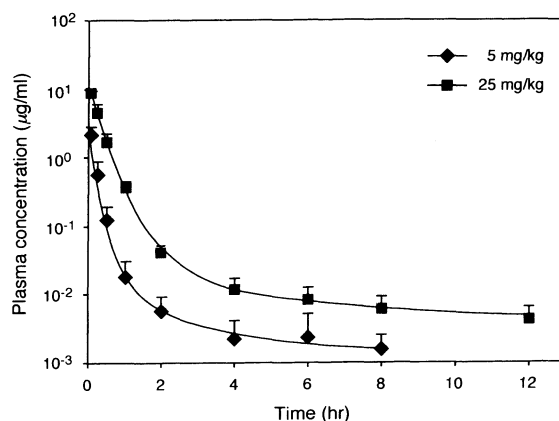


Fig. 4 Plasma concentration-time curve of GAA after intravenous administration at doses of 5 and 25 mg/kg to rats

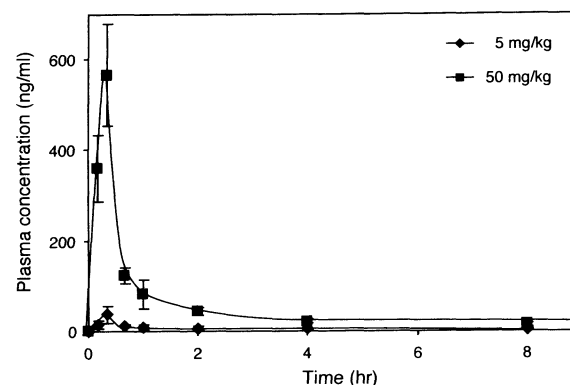


Fig. 5 Plasma concentration-time curve of GAA after oral administration at doses of 5 and 50 mg/kg to rat

$\mu\text{g min/ml}$, respectively) were dose-dependent. The pharmacokinetics of GAA were summarized in Table II.

Fig. 5 shows plasma concentration-time curves of GAA after the oral administration of GAA at doses of 5 and 50 mg/kg. GAA in the plasma appeared even 5 min after the administration, reached a maximum within a short time ($t_{max}=18$ and 18.1 min, respectively), and was eliminated rapidly (until 2 h) at each dose. The AUC_{0-480} values were 3.29 and 27.76 $\mu\text{g}\cdot\text{min/ml}$, increased proportionally to the administered dose (Table III), which was the same mode as observed after intravenous administration. The bioavailability (F) value (0.10) of GAA, obtained from AUC s after intravenous and oral administration at a dose of 5 mg/kg, suggested that about 10% of GAA was absorbed into the blood.

This is the first report on the quantitative determination and pharmacokinetic study on GAA detected in the plasma after intravenous and oral administration to rats,

using a sensitive and specific EIA method. This method will enable the determination of the concentration of GAA in the plasma and serum after administration of GAA to humans.

和文抄録

霊芝の主成分である ganoderic acid A (GAA, 1) の定量的目的で高感度かつ特異的な酵素免疫測定法を開発した。すなわち、GAA の側鎖に glycine を付加して鎖長を伸ばし、これを *N*-hydroxysuccinimide ester に導いた後、 β -D-galactosidase (β -Gal) や bovine serum albumin (BSA) と結合し GAA- β -Gal (enzyme-labeled antigen, 5) 及び GAA-BSA (immunogen, 6) 抱合体を得た。後者をウサギに繰り返し投与し anti-GAA 抗血清を得た。この抗血清は二重抗体による酵素免疫測定法で GAA に対する高感度、高特異性を示した。本酵素免疫法は 0.1–1000 ng/tube の範囲内で良好な定量曲線を示した。この抗血清は ganoderic acid γ (11) 及び ganolucidic acid A (14) (交叉率はそれぞれ 52.4% 及び 12.9%) を除いては、霊芝から単離した種々の GAA 関連化合物とは顕著な交叉反応を示さなかった。交叉率の高い 2 つの化合物は C-3 位にカルボニル基を有する構造の類似した ganoderic acid であった。

ラットに静注あるいは経口投与後の血漿中の GAA 濃度をこの新たに開発した酵素免疫測定法で定量した結果、5 及び 25 mg/kg 静注後の AUC_{0-480} は 32.8 ± 9.8 及び $201.5 \pm 38.7 \mu\text{g min/ml}$ であった。一方 5 及び 50 mg/kg 経口投与後、血漿中の GAA 濃度は 18.1 ± 2.5 及び $18.0 \pm 0.6 \text{ min}$ で最大濃度 C_{max} (37 及び 595 ng/ml) に達し、480 min では 4.2 及び 13.3 ng/ml に減少した。このことは経口投与した GAA は消化管で急速に吸収されるが、血中濃度はすぐさま減少することを示している。

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