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Antiherpetic activities of various protein bound polysaccharides isolated from *Ganoderma lucidum*

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Abstract

To investigate antiherpetic substances from *Ganoderma lucidum*, various protein bound polysaccharides, GLhw, GLhw-01, GLhw-02, GLhw-03, were isolated by activity-guided isolation from water soluble substances of the carpophores. These substances were examined for their antiviral activities against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) by plaque reduction assay in vitro. Among them, the acidic protein bound polysaccharide, GLhw-02 of a brownish substance, exhibited the most potent antherpetic activity with 50% effective concentrations (EC_{50}) of 300 ~ 520 μg/ml in Vero and HEp-2 cells, and its selectivity indices (SI) were more than 20. GLhw-02 was identified to consist mainly of polysaccharide (approximately 40.6%) and protein (approximately 7.80%) by anthrone test and Lowry–Folin test, and showed the usual molar ratio (C:H:O = 1:2:1) of carbohydrates by elemental analysis. These results suggest that GLhw-02 possesses the possibility of being developed from a new antiherpetic agent.

Keywords: *Ganoderma lucidum*; Protein bound polysaccharide; Activity-guided isolation; Antiherpetic activity; Herpes simplex viruses; Selectivity index (SI)

1. Introduction

Ganoderma lucidum (Fr.) Karst. (Ganodermataceae) in basidiomycetous fungi has been used to treat various human diseases such as hepatitis, hypertension, arthritis, bronchitis and tumorigenic diseases in oriental folk medicine (Kim and Kim, 1990).

Investigations on biologically active components of the carpophores and the cultured mycelia of *G. lucidum* have shown that this mushroom has various biological activities. *G. lucidum* was reported to contain some intensely bitter components including lucidenic acid A, B, C, D, E, lucidone A, and ganoderic acid B and C (Nishitoba et al., 1985a,b), which were known to inhibit histamine release from mast cells (Kohda et al., 1985; Nogami et al., 1986), an angiotensin converting enzyme that is responsible for hypertension (Komoda et al., 1985) and growth of liver cancer cells (Toth et al., 1983).

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G. lucidum has also been reported to contain polysaccharides and protein bound polysaccharides which have antitumor (Kim et al., 1980; Miyazaki and Nishijima, 1981) and antihypertension activities (Park et al., 1987), and decrease the blood glucose level (Hikino et al., 1985).

Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) are responsible for a broad range of human infectious diseases. Moreover, HSV infections were reported to be recognized as a risk factor for human immunodeficiency virus (HIV) infection (Hook et al., 1992). HSV-2 is also known as oncogenic virus which have the ability to convert cells into tumour cells (Lapucci et al., 1993). Acyclovir (ACV) is a well-known antiherpetic agent which exhibits selective toxicity against infected cells by phosphorylating HSV-derived thymidine kinase. However, prolonged therapies with ACV, the most successful antiherpetic drug, have resulted in some undesirable complications (Richman et al., 1987) and also induced the emergence of drug-resistant viruses (Larder et al., 1989). Therefore, it is necessary to develop new antiherpetic agents with different targets from the standard therapy.

In a previous study on antiviral activities of various water and methanol soluble substances isolated from *G. lucidum*, HSV-1 and HSV-2 were the most sensitive viruses to water soluble substances. The antiherpetic activity of water soluble substances isolated by activity-guided isolation were examined in more detail, and showed that the carpophores of *G. lucidum* contain protein bound polysaccharides that can inhibit the HSV multiplication in vitro.

2. Materials and methods

2.1. Materials and reagents

Artificially grown carpophores of *Ganoderma lucidum* (Fr.) Karst (Ganodermataceae) were purchased from a local herbal drug store and authenticated by Dr Byung Kak Kim, Departmant of Microbial Chemistry, Seoul National University. A voucher specimen (No. Cpm 605) has been deposited at the Medicinal Plants Herbarium of the college. Eagle's minimum essential medium (MEM), trypsin, penicillin, streptomycin and amphotericin B were purchased from Gibco BRL (Gaithersburg, MD). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and crystal violet were purchased from Sigma (St. Louis, MO). Vero cell (African green monkey kidney cell, ATCC CCL 81), HEp-2 cell (Human epidermoid carcinoma cell of the larynx, ATCC CCL 23), HSV-1 F strain ATCC VR-733 and HSV-2 G strain ATCC VR-734 were obtained from the American Type Culture Collection (Rockville, MD).

2.2. Activity-guided isolation of various protein bound polysaccharides

The carpophores of *G. lucidum* (500 g) were extracted with hot water for 8 h. The extract was concentrated to a tenth of the original volume, and added with three volumes of ice cold EtOH to precipitate high molecular weight components. After standing out overnight at 4°C, it was centrifuged and the precipitates were lyophilized, and GLhw (3.88 g) of a brownish substance was obtained. The supernatants were also concentrated and lyophilized, and GLlw (10.98 g) of a dark brownish substance was obtained.

GLhw (3 g), which is a high molecular weight component of water soluble substances, was applied to the DEAE-cellulose (Cl^- form, Sigma) column (bed volume = 50 ml). It was eluted with H_2O (pH 7.2) and the anthrone test (620 nm) and Lowry–Folin test (540 nm) were performed against each fraction. The positive fraction as shown by the anthrone and Lowry–Folin test was designated GLhw-01 (550 mg). The remaining substances on DEAE-cellulose column were eluted by 2 M NaCl. The positive fraction as shown by the anthrone and Lowry–Folin test was designated GLhw-02 (850 mg).

A solution of GLhw-02 (250 mg) in 10 mM sodium phosphate buffer (pH 6.8) was applied to the Sepharose CL-4B (Pharmacia, Sweden) column (bed volume = 85 ml). It was eluted with the same buffer and the anthrone test and Lowry–Folin test were performed against each fraction, and the positive fraction were designated

GLhw-03 (165 mg) and GLhw-04 (21 mg), respectively.

2.3. Cells and viruses

Vero and HEp-2 cells were cultured with MEM supplemented with 10% (v/v) heat inactivated fetal bovine serum (FBS), 100 IU/ml penicillin, 100 μg/ml streptomycin and 0.25 μg/ml amphotericin B. The cells were maintained at 37°C in a humidified atmosphere with 5% CO_2 and were subcultured twice a week. Virus stocks were prepared in Vero cell cultures and stored at −70°C. Virus titer was determined using plaque assay.

2.4. Cytotoxicity assay

For cytotoxicity assay, the cells were seeded in 96 well plates (Falcon, NJ) at an initial density of 3.5×10^4 cells per well. After the cells had been incubated for 16–18 h at 37°C, various concentrations of substances were added, and the incubation was continued for 48 h. Viable cell yield was determined by MTT reduction assay which was previously described (Scubiero et al., 1988). The cytotoxicity was expressed as the 50% cytotoxic concentration (CC_{50}) which is the concentration of substances to inhibit the growth of cells up to 50% by regression analysis.

2.5. Antiherpetic activity assay

Antiherpetic activity was evaluated by plaque reduction assay previously described (Shigeta et al., 1992). Host cell monolayers grown in 24 well plates (Falcon, NJ) were infected with about 150 plaque forming units (pfu) of virus per well in the absence or presence of various concentrations of substances. After 1 h adsorption, agar overlay medium containing substances of various concentrations was overlaid. After 1–3 days of incubation at 37°C, virus plaques were counted. The degree of inhibition was expressed as the 50% effective concentration (EC_{50}) which was calculated as the concentration of substances required to reduce virus plaque by 50% using regression analysis. Antiherpetic activity for each substance was evaluated by selectivity index (SI) which was calculated by dividing the CC_{50} by EC_{50}. ACV (Sigma), which is clinically used for the treatment of herpetic disease, was used as a positive control under this assay system.

2.6. Chemical analysis

Total polysaccharide contents of various protein bound polysaccharides were analysed by the anthrone test using glucose as a standard. After the anthrone test was performed, the optical density (OD) of each sample was measured at 620 nm, and contents of polysaccharide were calculated by regression analysis. Also, protein contents were analysed by the Lowry–Folin test. Bovine serum albumin (BSA) was used as a standard, and the OD of each sample was measured at 540 nm. The elemental composition of each substance was determined by Carlo Erba elemental analyzer.

3. Results and discussion

In a previous study on antiviral activities of various water and methanol soluble substances from *G. lucidum*, it was found that the water soluble substances especially inhibited the cytopathic effects of HSV-1 and HSV-2. Therefore, in order to find antiherpetic substances from *G. lucidum*, the carpophores (500 g) were extracted with H_2O, and GLhw (3.88 g) of a brownish substance was isolated from the precipitates of H_2O fraction by EtOH precipitation. GLlw (10.98 g) of a dark brownish substance was also obtained by concentration and lyophilization from the supernatants. The antiherpetic activities of the two water soluble substances, GLhw and GLlw, were evaluated by plaque reduction assay. The inhibitory effects of them on plaque formation of HSV-1 and HSV-2 in Vero and HEp-2 cells are shown Table 1. GLhw inhibited plaque formation of HSV-1 and HSV-2 in Vero cells with EC_{50} of 880 and 590 μg/ml and in HEp-2 cells with EC_{50} of 1230 and 580 μg/ml, respectively, and had the cytotoxicity on Vero and HEp-2 cells with CC_{50} of 7860 and 9430 μg/ml. Therefore, GLhw exhibited a potent antiherpetic activity with SI of 8.93

and 7.67, and 13.32 and 16.26 on HSV-1 and HSV-2, but GLlw did not show a significant antiherpetic activity.

As most of the mushrooms of basidiomycetes including *G. lucidum* have very hard carpophores, the extraction yields were very low in the experiment, yielding 0.77 and 2.19% as GLhw and GLlw, respectively. In order to precipitate high molecular weight components from a water extracted solution, ethanol is frequently used. And many of the biologically active polysaccharides were isolated by ethanol precipitation method (Sone et al., 1985; Kim et al., 1993). Therefore, the major components of GLhw might be high molecular weight components such as polysaccharides and proteins.

The GLhw (3 g) was applied to the DEAE-cellulose column and eluted with H_2O followed by 2 M NaCl solution to obtain GLhw-01 (550 mg) of a white substance from a neutral fraction and GLhw-02 (850 mg) of a brownish substance from an acidic fraction, and their antiherpetic activities were compared with GLhw by plaque reduction assay. The acidic GLhw-02 showed more potent activity than the neutral GLhw-01 and its EC_{50} on HSV-1 and HSV-2 was 300 and 440 μg/ml in Vero cells, and 330 and 520 μg/ml in HEp-2 cells respectively, and SIs of GLhw-02 were more than 20 (Table 2, Figs. 1 and 2). The acidic GLhw-02 (250 mg) was applied to Sepharose CL-4B column to obtain GLhw-03 (165 mg) of a brownish substance and its antiherpetic activity was compared with GLhw-02 by plaque reduction assay. GLhw-03 exhibited a potent antiherpetic activity as like GLhw-02, and had no cytotoxicity at a concentration of 10 mg/ml (Table 2). However, GLhw-03 did not show further increase in antiherpetic activity compared to GLhw-02. This result may reflect a difference of purity or loss of active substances during purification.

The contents of polysaccharide and protein of four protein bound polysaccharides, GLhw, GLhw-01, GLhw-02 and GLhw-03, are shown in Table 3. The polysaccharide contents were determined by the anthron test, and average content of polysaccharide was 51.62%. Also, the protein contents were determined by Lowry–Folin test, and average content of protein was 6.67%. The elemental composition of each substance determined by elemental analyzer was carbon, hydrogen and oxygen. And their molar ratios are shown in Table 4, indicating the usual molar ratios (C:H:O = 1:2:1) of carbohydrates.

Most of the antiviral or antitumor polysaccharides isolated from the hot water extract of *G. lucidum* were reported to be a branched β-glucans in (1→3)-β-, (1→4)-ß- and (1→6)-β-linkages and the average molecular weight was 1 050 000 (Mizuno et al., 1984). Therefore, protein bound polysaccharide represented the inhibitory effect on the infection of HSV-1 and HSV-2 may be these β-glucans. It appears that the protein and polysaccharide were bound since the protein moiety was not completely removed during the purifi-

Table 1
Evaluation of antiherpetic activities of GLhw and GLlw on herpes simplex viruses (HSV) by plaque reduction assay

Antiherpetic substances	Host cells	CC_{50} (μg/ml)	EC_{50} (μg/ml)		SI[a]	
			HSV-1[b]	HSV-2[c]	HSV-1	HSV-2
GLhw[d]	Vero	7860	880	590	8.93	13.32
	HEp-2	9430	1230	580	7.67	16.26
GLlw[e]	Vero	2100	940	940	2.23	2.27
	Hep-2	2070	1310	980	1.58	2.11

[a] Selectivity index (SI) = CC_{50}/EC_{50}.
[b] HSV-1, herpes simplex virus type 1 F strain.
[c] HSV-2, Herpes simplex virus type 2 G strain.
[d] GLhw: high molecular weight components isolated from water soluble substances of *Ganoderma lucidum*.
[e] GLlw, low molecular weight components isolated from water soluble substances of *G. lucidum*.

Table 2
Evaluation of antiherpetic activities of GLhw-01, GLlw-02 and GLhw-03 on herpes simplex viruses (HSV) by plaque reduction assay

Antiherpetic substances	Host cells	CC_{50} (μg/ml)	EC_{50} (μg/ml)		SI[a]	
			HSV-1[b]	HSV-2[c]	HSV-1	HSV-2
GLhw-01[d]	Vero	>10 000	1290	1890	>7.75	>5.29
	HEp-2	>10 000	450	1110	>22.22	>9.01
GLhw-02[e]	Vero	>10 000	300	440	>33.33	>22.73
	Hep-2	>10 000	330	530	>30.30	>19.23
GLhw-03[f]	Vero	>10 000	660	560	>15.15	>17.86
	Hep-2	>10 000	530	960	>18.27	>10.42
Acyclovir	Vero	913	0.6	0.9	1521.67	1014.44

[a] Selectivity index (SI) = CC_{50}/EC_{50}.
[b] HSV-1, herpes simplex virus type 1 F strain.
[c] HSV-2, herpes simplex virus type 2 G strain.
[d] GLhw-01, neutral protein bound polysaccharide isolated from GLhw.
[e] GLhw-02, acidic protein bound polysaccharide isolated from GLhw.
[f] GLhw-03, protein bound polysaccharide purified from GLhw-02.

cation, and the entity of binding is uncertain. Also, these results suggest that the antiherpetic activities of protein bound polysaccharide is related to the net of electric charge.

Comparing the effects of synthetic antiherpetic agent for clinical use, the SI value of GLhw-02 seems to be much smaller than that of ACV. When one considers that most of the known compounds of the *G. lucidum* belong to polysaccharides whose average molecular weight is approximately above 50 000 (Mizuno et al., 1984), the SI value of GLhw-02 is not too small. By using the average molecular weight, EC_{50} of GLhw-02 is $6 \times 10^{-3} \sim 1 \times 10^{-2}$ μM in Vero and HEp-2 cells, and that of ACV is $2.67 \sim 4 \times 10^{-3}$ μM in Vero cells. Therefore, EC_{50} of GLhw-02 is comparable to this antiherpetic agent. The fact that the absence of toxicities in GLhw-02 has great significance, since the CC_{50} of GLhw-02 was above 10 mg/ml, and this mushroom has been used as folk medicine for treating several diseases in the Orient. *G. lucidum* appears to be very safe because oral administration of the extract did not display any toxicities (Sugiura and Ito, 1977; Kim et al., 1986).

It is known that the antiviral activities of polysaccharides are linked to the anionic features of the molecules and they inhibit the very early stages of viral infection such as attachment and penetration (Shannon, 1984), and increase with the molecular weight or the degree of sulfation (Witvrouw et al., 1994). Therefore, the antiherpetic activity of GLhw-02 would be expected to increase further by sulfating or partial digestion,

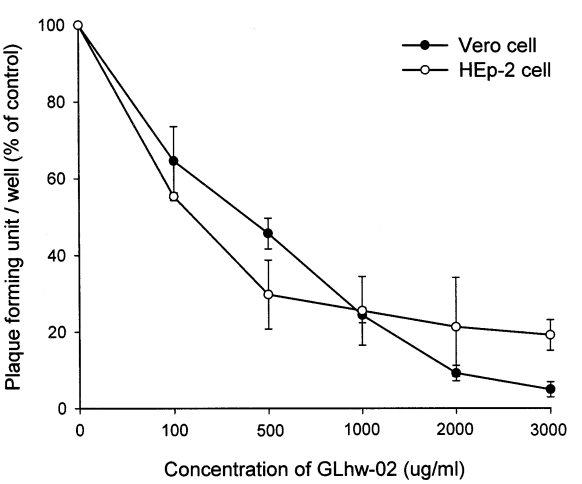

Fig. 1. Inhibitory effects of GLhw-02 on the plaque formation of herpes simplex virus type 1 (HSV-1) in Vero and HEp-2 cells. Results are expressed as percent with respect to virus control group. Each value is the mean ± S.D. of quadruplicate determinations.

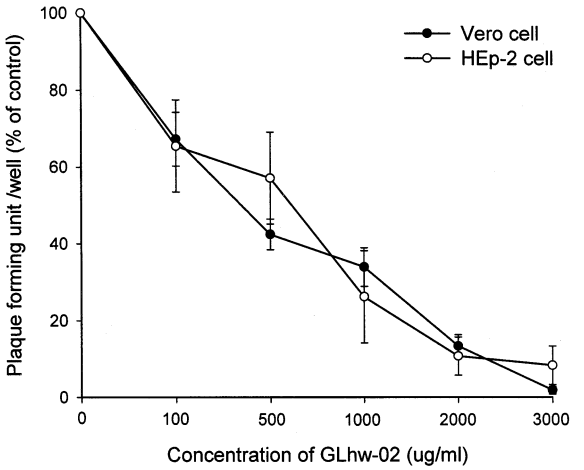

Fig. 2. Inhibitory effects of GLhw-02 on the plaque formation of herpes simplex virus type 2 (HSV-2) in Vero and HEp-2 cells. Results are expressed as percent with respect to virus control group. Each value is the mean ± S.D. of quadruplicate determinations.

and identification of the possible mode of antiherpetic activity and the molecular entity of GLhw-02 is currently in progress.

Table 3
Contents of polysaccharide and protein in each protein bound polysaccharide isolated from water soluble substances of *Ganoderma lucidum*

Protein bound polysaccharides	Contents (%)[a]	
	Polysaccharide	Protein
GLhw[b]	53.50 ± 4.16	12.14 ± 0.38
GLhw-01[c]	53.13 ± 0.99	2.94 ± 0.82
GLhw-02[d]	40.62 ± 4.68	7.80 ± 0.28
GLhw-03[e]	59.24 ± 2.59	3.78 ± 0.18

[a] Expressed in mean ± S.D. as wt.%.
[b] GLhw, high molecular weight components isolated from water soluble substances of *G. lucidum*.
[c] GLhw-01, neutral protein bound polysaccharide isolated from GLhw.
[d] GLhw-02, acidic protein bound polysaccharide isolated from GLhw.
[e] GLhw-03, protein bound polysaccharide purified from GLhw-02.

Table 4
Elemental compositions of each protein bound polysaccharide isolated from water soluble substances of *Ganoderma lucidum*

Protein bound polysaccharides	C	H	O
GLhw[b]	6.00[a]	11.24	5.29
GLhw-01[c]	6.00	12.15	5.39
GLhw-02[d]	6.00	10.38	5.22
GLhw-03[e]	6.00	10.99	4.67

[a] Expressed in mean ± S.D. as wt.%.
[b] GLhw, high molecular weight components isolated from water soluble substances of *G. lucidum*.
[c] GLhw-01, neutral protein bound polysaccharide isolated from GLhw.
[d] GLhw-02, acidic protein bound polysaccharide isolated from GLhw.
[e] GLhw-03, protein bound polysaccharide purified from GLhw-02.

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References

Hikino, H., Konno, C., Mirin, Y., Hayashi, T., 1985. Isolation and hypoglycemic activity of ganoderans A and B, glycans of *Ganoderma lucidum* fruit bodies. Planta Medica 51, 339–340.

Hook III, E.W., Cannon, R.O., Nahmias, A.J., 1992. Herpes simplex virus infection as a risk factor for human immunodeficiency virus infection in heterosexuals. Journal of Infectious Diseases 165, 251–255.

Kim, S.S., Kim, Y.S., 1990. Korean Mushrooms. Yupoong Pub, Seoul, pp. 298–299.

Kim, B.K., Chung, H.S., Chung, K.S., Yang, M.S., 1980. Studies on the antineoplastic components of Korean basidiomycetes. Korean Journal of Mycology 8, 107–113.

Kim, M.J., Kim, H.W., Lee, Y.S., Shim, M.J., Choi, E.C., Kim, B.K., 1986. Studies on safety of *Ganoderma lucidum*. Korean Journal of Mycology 14 (1), 49–59.

Kim, S.H., Kim, H.W., Choi, E.C., Kim, B.K., 1993. Immunological studies on *Collybia confluens*. Journal of Korean Cancer Association 25, 288–298.

Kohda, H., Tokumoto, W., Sakamoto, K., Fujii, M., Hirai, Y., Yamasaki, K., Komoda, Y., Nakamura, H., Ishihara, S., Uchida, M., 1985. The biologically active constituents of *Ganoderma lucidum* (Fr.) Karst. Histamine release inibitory triterpenes. Chemical and Pharmaceutical Bulletin 33 (4), 1367–1374.

Komoda, Y., Nakamura, H., Ishihara, S., Uchida, M., Kohda, H., Yamasaki, K., 1985. Structures of new terpenoid constituents of *Ganoderma lucidum*. Chemical and Pharmaceutical Bulletin 33, 4829–4835.

Lapucci, A., Macchia, M., Parkin, A., 1993. Antiherpes virus agents: a review. IL Farmaco 48 (7), 871–895.

Larder, B.A., Darby, G., Richman, D.D., 1989. HIV with reduced sensitivity to zidovudine (AZT) isolated during prolonged therapy. Science 243, 1731–1734.

Miyazaki, T., Nishijima, M., 1981. Studies on fungal polysaccharides. XXVII. Structural examination of a water-soluble, antitumor polysaccharide of *Ganoderma lucidum*. Chemical and Pharmaceutical Bulletin 29, 3611–3616.

Mizuno, T., Nato, N., Totsuka, A., Takenaka, K., Shinkai, K., Shimizu, M., 1984. Fractionation, structural features and antitumor activity of water soluble polysaccharide from 'Reishi', the fruit body of *Ganoderma lucidum*. Nippon Nogeikagaku Kaishi 58, 871–880.

Nishitoba, T., Sato, H., Sakamura, S., 1985a. New terpenoids from *Ganoderma lucidum* and their bitterness. Agricural Biology and Chemistry 49, 1547–1549.

Nishitoba, T., Sato, H., Kasai, T., Kawagishi, H., Sakamura, S., 1985b. New bitter C_{27} and C_{30} terpenoids from the fungus *Ganoderma lucidum* (Reishi). Agricural Biology and Chemistry 49, 1793–1798.

Nogami, M., Tsuji, Y., Kubo, M., Takahashi, M., Kimura, H., Matsuike, Y., 1986. Studies on *Ganoderma lucidum* VI. Anti-allergic effect (1). Yakugaku Zasshi 106, 594–599.

Park, J.H., Kim, H.W., Kim, Y.C., Choi, E.C., Kim, B.K., 1987. Studies on antihypertensive components of *Ganoderma lucidum* in Korea. Korean Journal of Food Hygiene 2, 57–65.

Richman, D.D., Fischl, M.A., Grieco, M.H., Gottlieb, M.S., Volberdings, P.A., Laskin, O.L., Leedom, J.M., Groopman, J.E., Mildvan, D., Hirsch, M.S., Jackson, G.G., Durack, D.T., Nusinoff, L.S., 1987. The AZT Collaborative Working Group. The toxicity of azidothymidine(AZT) in the treatment of patients with AIDS and AIDS-related complex. New England Journal of Medicine 317, 192–197.

Scubiero, D.A., Shoemaker, R.H., Paull, K.D., Monks, A., Tierney, S., Nofziger, T.H., Currens, M.J., Seniff, D., Boyd, M.R., 1988. Evaluation of a soluble tetrazolium/formazan assay for cell growth and drug sensitivity in culture using human and other tumor cell lines. Cancer Research 48, 4827–4833.

Shannon, W.M., 1984. Mechanism of action and pharmacology: chemical agents. In: Galasso, G.J., Merigan, T.C., Buchanon, R.A. (Eds.), Antiviral Agents and Viral Diseases of Man. Raven Press, New York, pp. 55–121.

Shigeta, S., Mori, S., Baba, M., Ito, M., Honzumi, K., Nakamara, K., Oshitani, H., Numazaki, Y., Matsuda, A., Obara, T., Shuto, S., De Clercq, E., 1992. Antiviral activities of ribavirin, 5-ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamide, and 6′-(R)-6′-C-methylneplanocin A against several ortho- and paramyxoviruses. Antimicrobial Agents and Chemotherapy 36 (2), 435–439.

Sone, Y., Okuda, R., Wada, N., Kishida, E., Misaki, A., 1985. Structures and antitumor activities of the polysaccharides isolated from fruiting body and the growing culture of mycelium of *Ganoderma lucidum*. Agricultural Biology and Chemistry 49, 2541–2653.

Sugiura, M., Ito, H., 1977. Toxicological studies of *Ganoderma lucidum*. Tokyo Yakka Daigaku Kenkyu Nenpo 27, 722–733.

Toth, J.O., Luu, B., Ourisson, G., 1983. Ganoderic acid T and Z: cytotoxic triterpenes from *Ganoderma lucidum*. Tetrahedron Letter 24, 1081–1084.

Witvrouw, M., Desmyter, J., De Clercq, E., 1994. Antiviral Chemistry and Chemotherapy 5, 345–359.