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Anti-HCV effect of *Lentinula edodes* mycelia solid culture extracts and low-molecular-weight lignin

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ABSTRACT

Lentinula edodes mycelia solid culture extract (MSCE) contains several bioactive molecules, including some polyphenolic compounds, which exert immunomodulatory, antitumor, and hepatoprotective effects. In this study, we examined the anti-hepatitis C virus (HCV) activity of MSCE and low-molecular-weight lignin (LM-lignin), which is the active component responsible for the hepatoprotective effect of MSCE. Both MSCE and LM-lignin inhibited the entry of two HCV pseudovirus (HCVpv) types into Huh7.5.1 cells. LM-lignin inhibited HCVpv entry at a lower concentration than MSCE and inhibited the entry of HCV particles in cell culture (HCVcc). MSCE also inhibited HCV subgenome replication. LM-lignin had no effect on HCV replication, suggesting that MSCE contains additional active substances. We demonstrate here for the first time the anti-HCV effects of plant-derived LM-lignin and MSCE. The hepatoprotective effect of LM-lignin suggests that lignin derivatives, which can be produced in abundance from existing plant resources, may be effective in the treatment of HCV-related diseases.

1. Introduction

Mushrooms reportedly exhibit immunomodulatory, antitumor, antiviral, antibacterial, and antiparasitic effects [1]. *Lentinula edodes* is a common edible mushroom in Japan and China. Hot-water extracts of *L. edodes* mycelia cultured on solid medium are sold commercially as nutritional supplements. The main components of *L. edodes* mycelia solid culture extract (MSCE) are sugars, proteins, and polyphenolic compounds that exhibit a variety of pharmacologic activities, such as hepatoprotective [2–5], anti-tumor [6] and immunomodulatory [7] effects. In a previous study, we fractionated MSCE to determine the active component responsible for the hepatoprotective effect. The activity was analyzed by measuring the protective effect of MSCE on carbon tetrachloride-induced injury in cultured primary rat hepatocytes; the strongest effect was observed in a fraction consisting primarily of lignin hydrolysate [8].

Lignin is a polymeric natural product found in abundance in the plant kingdom and is a highly heterogeneous polymer of phenylpropanoid monomers. Culture of *L. edodes* mycelia on solid lignocellulose-containing medium results in secretion of a lignin-degrading peroxidase that produces low-molecular-weight lignin hydrolysate [9]. Various physiologic activities of lignin derivatives have been reported [10], including anti-HIV [11], immunostimulatory [12], and antioxidant activities [8]. Thus lignin, which is present in abundance in the environment, might be a promising source for producing pharmaceuticals.

Clinical reports have indicated that MSCE effectively alleviates the symptoms of chronic hepatitis C infection. Progression of hepatitis C virus (HCV) infection may cause liver fibrosis and chronic hepatitis, in turn leading to cirrhosis and ultimately to hepatocellular carcinoma. Standard therapy consists of pegylated interferon-α and ribavirin, and the combined use of these agents with an HCV nonstructural protein inhibitor has been recognized as an effective hepatitis C therapy. However, the treatment has numerous drawbacks such as side effects, high cost, and the high possibility of resistant strain emergence [13]. Recently, direct antiviral agents (DAAs) have been used clinically, with very high sustained virologic response and fewer side effects than interferon-based treatments. Unfortunately, the high cost of DAA-based treatments imposes a heavy burden on national healthcare systems. Therefore, alternative antiviral treatments that are available worldwide to patients with

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HCV are needed. Recent studies have shown that plant-derived flavonoids and polyphenols exhibit anti-HCV activity through various mechanisms, including inhibition of virus entry and genome replication [14–17]. These plant-derived compounds could become effective, safe, and low-cost anti-HCV pharmaceuticals.

In this study, we demonstrate for the first time the anti-HCV effect of MSCE. Our results indicate that among the various components in MSCE, low-molecular-weight lignin functions primarily in inhibiting HCV entry into cells.

2. Materials and methods

2.1. Cell culture

Huh7.5.1 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% FCS. Huh7.5.1 1bFeo cells, kindly provided by Dr. N. Sakamoto (Hokkaido University, Japan), were cultured in Dulbecco's modified Eagle's medium supplemented with 10% FCS and 500 μg/ml of G418. The cells were maintained in a 5% CO_2 atmosphere at 37 °C.

2.2. Preparation of viruses

Cell-culture-derived HCV (HCVcc) was produced from Huh7.5.1 cells expressing MT miR-122 according to the method of a previous report [18], with minor modifications. Infectious titer was determined using a focus-forming assay and expressed in focus-forming units (FFUs) [19]. Pseudotype vesicular stomatitis virus (VSV) bearing HCV envelope glycoproteins (HCVpv) and VSV envelope glycoproteins were prepared as described previously [20].

2.3. Assays of inhibition of HCVpv and VSVpv infection

Huh7.5.1 cells were treated with pseudotype virus or mixtures of pseudotype virus and MSCE (Kobayashi Pharmaceutical Co. Ltd., Ibaraki, Osaka, Japan) or alkaline hydrolysate of lignin ($lignin_{AH}$; Sigma–Aldrich, St. Louis, MO, USA) at the indicated concentrations. After an additional 24 h of culture, luciferase activity was measured using a commercially available kit (PicaGene, Toyo Ink, Tokyo, Japan) and luminometer (Tristar LB941; Berthold Technologies, Bad Wildbad, Germany) according to the manufacturer's instructions. Cell viability was assessed with a WST-8 kit (Nacalai Tesque) according to the manufacturer's instructions.

2.4. Assay of inhibition of HCVcc infection

Huh7.5.1 cells were treated for 2 h with HCVcc or mixtures of HCVcc and $lignin_{AH}$ preincubated for 30 min at the indicated concentrations. The cells were then washed and cultured for an additional 24 h, after which HCV RNA was extracted and analyzed by qRT-PCR as follows. Total RNA was isolated from cells using an RNeasy mini kit (QIAGEN, Valencia, CA). HCV genomic RNA was reverse transcribed and amplified using a Taqman RNA-to-Ct 1-step kit (Life Technologies, Gaithersburg, MD) with sense (5′- GAG TGT CGT GCA GCC TCC A - 3′) and antisense (5′- CAC TCG CAA GCA CCC TAT CA -3′) primers. The kinetics of cDNA amplification were monitored using a ViiA 7 Real-Time PCR System (Life Technologies) with a reporter probe corresponding to nucleotides 238 to 267 of the 5'-conserved region of the HCV genotype (5′-GCC CGC AAG ACT GCT AGC CGA GTA GTG TTG G -3′) conjugated with 6-carboxyfluorescein and 6-carboxytetramethylrhodamine at the 5′ and 3′ termini, respectively (Integrated DNA Technologies, Coralville, IA, USA). Intracellular GAPDH mRNA was also amplified using a Taqman RNA-to-Ct 1-step kit (Life Technologies) with sense (5′- TGT AGT TGA GGT CAA TGA AGG G -3′) and antisense (5′- ACA TCG CTC AGA CAC CAT G -3′) primers and a reporter probe (5′- AAG GTC GGA GTC AAC GGA TTT GGT C -3′) conjugated with 6-carboxyfluorescein and IowaBlackFQ and ZEN quencher at the 5′ and 3′ termini and internal position, respectively (Integrated DNA Technologies). Values for the HCV genomic RNA were normalized to those for GAPDH mRNA.

2.5. Assay of inhibition of HCV subgenome replication

Huh7.5.1 1bFeo cells were treated with MSCE or $lignin_{AH}$ for 72 h, after which RNA was isolated from cells and prepared using TRIzol reagent (Life Technologies, Carlsbad, CA, USA). First-strand cDNA was synthesized using AMV reverse transcriptase (TaKaRa Bio, Otsu, Japan) and a random 6mer oligonucleotides primer (Life Technologies) and S100 Thermal cycler (BioRad Laboratories Inc., Hercules, CA, USA). The expression of each cDNA was estimated using SYBR Premix Ex TaqII (Tli RNaseH Plus) (TaKaRa Bio) according to the manufacturer's protocol. Fluorescent signals were analyzed using a StepOne plus system (Applied Biosystems). Genes were amplified using the following primer pairs: HCV subgenome, 5′- CGT AAC ACC AAC GGG CGC GCC ATG -3′ and 5′- CTC GTC CTG CAG TTC ATT CAG GGC -3'; GAPDH, 5′- GGT CTC TGA CTT CAA CA -3′ and 5′- GTG GTC GTT GAG GGC AAT G - 3'. Expression of the HCV subgenome was normalized to that of GAPDH.

2.6. Statistical analysis

Values are expressed as the mean ± SD. Dunnett's test was used for statistical analyses. A P value of less than 0.05 was considered indicative of statistical significance.

3. Results

3.1. Anti-HCV effect of MSCE

We examined the anti-HCV effects of MSCE *in vitro* using HCV pseudotype virus (HCVpv). Fig. 1 shows the inhibitory effect of MSCE on entry of HCVpv into Huh7.5.1 cells. MSCE significantly inhibited the entry of HCVpv in a dose-dependent manner, and the inhibitory effect was observed with two types of HCVpv with different envelope proteins derived from genotype 1a (H77 strain) and 1b (Con1 strain). Cell viability did not change at the indicated doses.

3.2. Inhibition of HCV entry by low-molecular-weight lignin

A previous study showed that low-molecular-weight lignin (LM-lignin) is the hepatoprotective component in MSCE ($lignin_{MSCE}$) [8]. As it is very difficult to purify LM-lignin from MSCE, we examined whether a lignin derivative has a direct inhibitory effect on HCV using a commercially available alkaline hydrolysate of lignin ($lignin_{AH}$). Fig. 2 shows that $lignin_{AH}$ inhibited the entry of two types of HCVpv in a dose-dependent manner. Compared with inhibition by MSCE, only one-tenth of the concentration of $lignin_{AH}$ was needed to demonstrate inhibitory activity. Cell viability did not change at the indicated doses. These results suggest that $lignin_{MSCE}$ contributes to the anti-HCV effect primarily through inhibition of HCV entry.

The effect of MSCE and $lignin_{AH}$ on VSV entry into Huh7.5.1 cells was examined to determine the specificity of the viral entry inhibition activity (Supplimental Fig. S1). Although MSCE inhibited VSV entry, $lignin_{AH}$ had no effect, suggesting that MSCE contains other components that inhibit VSV entry.

We next examined the inhibition of virus entry by $lignin_{AH}$ using actual HCV particles, HCVcc [19]. Huh7.5.1 cells were incubated in the presence of HCVcc and $lignin_{AH}$ for 2 h, after which the

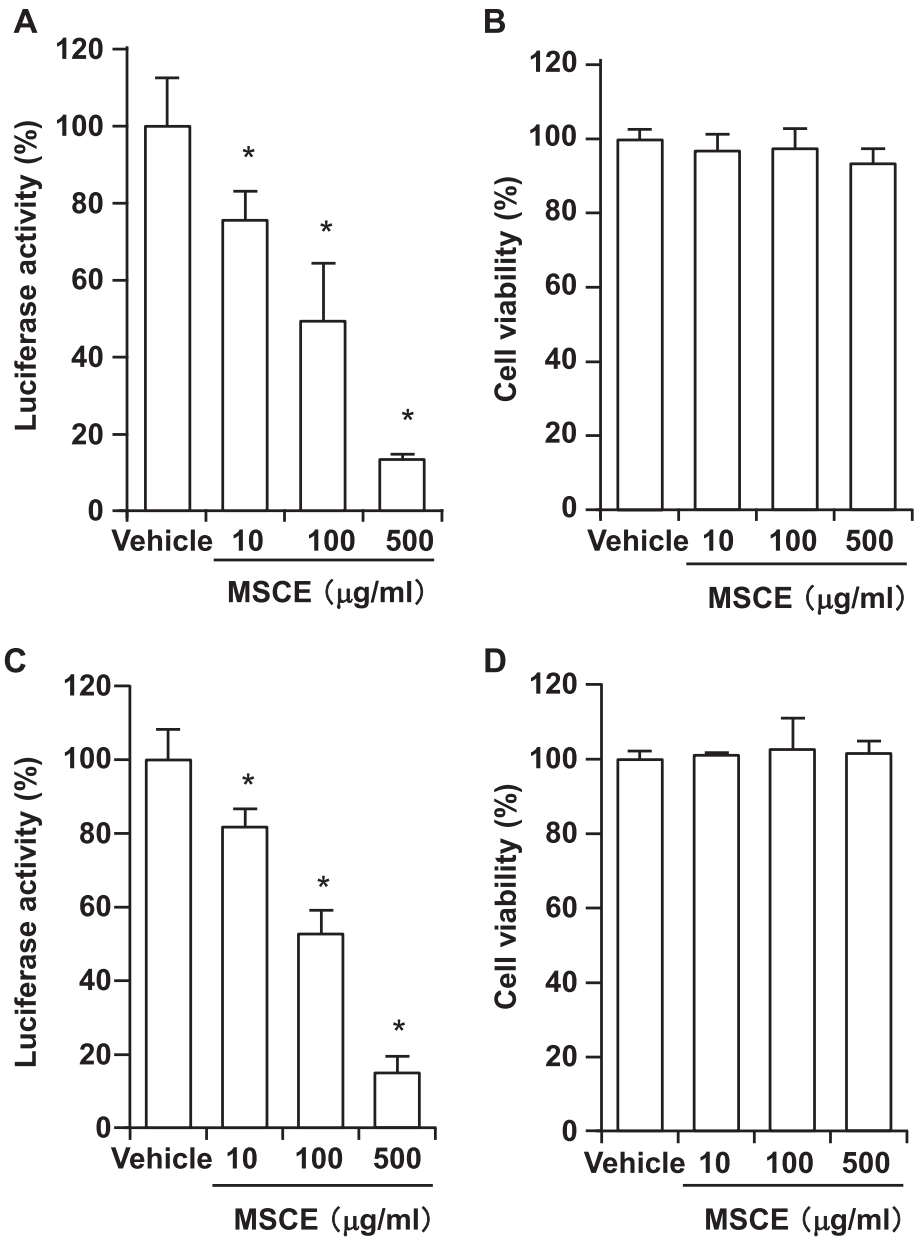

Fig. 1. Inhibition of HCV entry by MSCE. Huh7.5.1 cells were infected with HCVpv (A, B: H77; C, D: Con1) and simultaneously treated with MSCE. Twenty-four hours after HCVpv infection, luciferase activity (A, C) and cell viability (B, D) were assayed. All data represent the mean ± SD (n = 3, $^*P < 0.05$ vs. vehicle-treated group, as determined using Dunnett's test).

additive-containing medium was replaced with fresh medium. Cells were examined for the presence of HCV genomic RNA 72 h later. The results showed that the level of HCV genomic RNA decreased with $lignin_{AH}$ treatment in a dose-dependent manner (Fig. 3).

Fig. 4 shows the effects of MSCE and $lignin_{AH}$ on HCV replication. MSCE significantly inhibited replication of the HCV subgenome, whereas $lignin_{AH}$ had no effect. Thus, the anti-HCV effect of $lignin_{AH}$ appears to be limited to inhibition of virus entry. $Lignin_{AH}$ was not cytotoxic to Huh7.5.1 cells up to a concentration of 1 mg/ml (data not shown).

4. Discussion

Shiitake mushroom fruiting bodies are commonly used for human consumption in Asia. In this study, we examined components isolated by hot-water extraction from the mycelia of *Lentinula edodes* cultivated on solid medium. MSCE have been shown to suppress the secretion of inflammatory cytokines in mice with ConA-induced liver injury (unpublished data). In a previous study, we showed that MSCE inhibits liver fibrosis in chronic hepatitis induced by dimethylnitrosamine [2]. As the hepatoprotective effect was observed following oral administration of MSCE [3], the component(s) responsible for the effect must be absorbed in the intestine and then transported to the liver. We showed for the first time in this study that MSCE inhibits HCV entry *in vitro* using a human hepatic cancer cell line. Our results suggest that MSCE treatment may retard the progression of HCV-related diseases.

MSCE is composed primarily of sugars, proteins, and polyphenols. In our previous study, a lignin-rich digestion fraction exhibited hepatoprotective activity [8]. Undegraded lignin is a high-molecular-weight phenolic polymer that consists of a

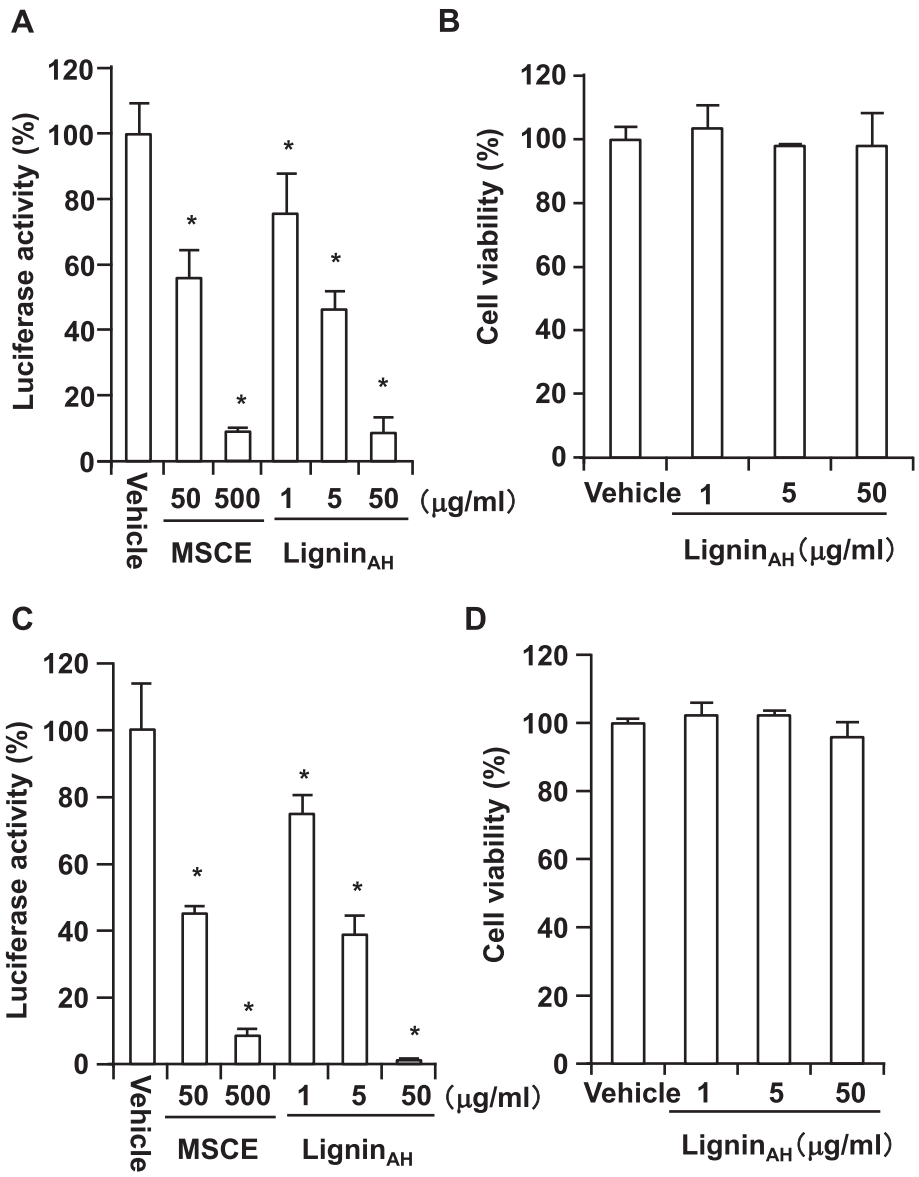

Fig. 2. Inhibition of HCVpv entry by lignin$_{AH}$. Huh7.5.1 cells were infected with HCVpv (A, B: H77; C, D: Con1) and simultaneously treated with MSCE or lignin$_{AH}$. Twenty-four hours after virus infection, luciferase activity (A, C) and cell viability (B, D) were assayed. All data represent the mean ± SD (n = 3, $^{*}P < 0.05$ vs. vehicle treated group, as determined using Dunnett's test).

phenylpropane carbon skeleton with 3 carbons in the benzene ring. Although undegraded lignin does not exhibit antioxidant activity, the LM-lignin-rich fraction of the MSCE does exhibit superoxide dismutase (SOD)-like activity. It has been reported that lignin exerts antioxidant activity following degradation to the low-molecular-weight form [21]. A mycelium-derived lignin-degrading peroxidase appears to produce hepatoprotective LM-lignin. Commercially available LM-lignin also exhibits both SOD-like and hepatoprotective activities [8]. Low-molecular-weight lignin derivatives produced from abundant natural plant resources thus hold promise as potential agents for treating various liver diseases.

In this study, lignin$_{AH}$ strongly inhibited the entry of HCV into cultured cells. Lignin$_{AH}$ was as effective as a ten-fold amount of MSCE. The IC_{50} of lignin$_{AH}$ was approximately 5 μg/ml, and lignin$_{AH}$ was found to be non-toxic to Huh7.5.1 cells at least to a concentration of 1 mg/ml. The effects of lignin$_{AH}$ and MSCE on another virus, VSV, differed from the effects on HCV. MSCE inhibited the entry of VSV in a dose-dependent manner, whereas lignin$_{AH}$ had no effect on VSV entry at the same concentration that almost completely inhibited HCV entry. These results suggest that an MSCE component(s) other than LM-lignin is responsible for inhibiting VSV entry. Alternatively, it is possible that the lignin formed by enzymatic digestion of the mycelia has different virus specificity than the lignin produced by alkaline hydrolysis.

The mechanism through which LM-lignin inhibits HCV entry is unclear. Several cell surface receptors for HCV entry have been reported to date, including CD81, claudin-1, low-density lipoprotein receptor, occludin, and SR-BI [22]. Because lignin$_{AH}$ demonstrated inhibitory activity soon after addition, it is not likely that the gene expression of cell surface receptors was affected. It has been shown that prior to binding to the receptors, HCV attaches to cells through interaction between viral apolipoprotein E (apoE) and heparan sulfate on the cell surface [23]. Raghuraman reported that heparan sulfate and the lignin sulfate formed by chemical sulfation of natural lignin are structurally similar [24]. Thus, LM-lignin might inhibit the attachment step by binding to viral apoE before the

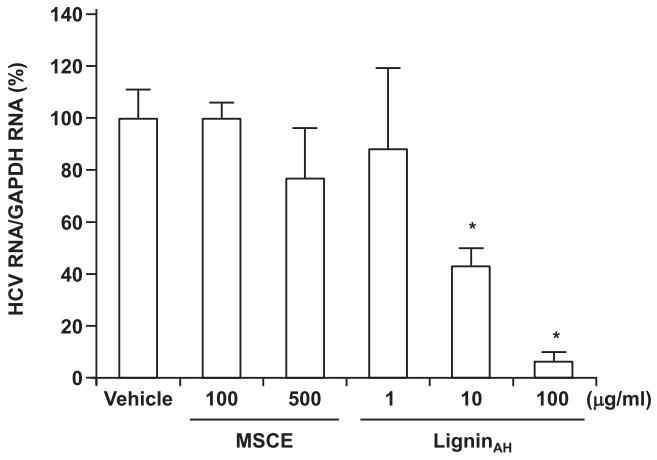

Fig. 3. Inhibition of HCVcc entry by lignin$_{AH}$. Huh7.5.1 cells were infected with HCVcc and simultaneously treated with lignin$_{AH}$. Two hours after infection, HCVcc particles were washed out. Seventy-two hours after HCVcc infection, HCV genomic RNA and GAPDH mRNA were isolated from the cells and analyzed by qRT-PCR. Data represent the mean ± SD (n = 3, $^*P < 0.05$ vs. vehicle treated group, as determined using Dunnett's test).

interaction with cell surface heparan sulfate. Further analyses using various lignin derivatives are needed to clarify the mechanism of HCV entry inhibition.

Biological activity has been reported for various lignin derivatives and hydrolysates, including sulfonated lignin [25], lignophenols [26], and lignin$_{MSCE}$ [8]. As for antiviral activity, sulfated lignin is known to inhibit the entry of human immunodeficiency virus (HIV) and herpes simplex virus, which infect host cells through heparan sulfate [12,25]. Worldwide, many patients are co-infected with HCV and HIV. Antiviral agents that are effective against both HCV and HIV would therefore be tremendously beneficial. Our research suggests that lignin derivatives may be effective in treating HCV and HIV co-infection. In this study, we found that LM-lignin is not only minimally cytotoxic, it also strongly inhibits HCV entry into hepatocytes; thus, it might also prove to be suitable as an anti-HIV agent. The results of our study indicate that lignin derivatives, which can be produced from abundant existing plant resources, are promising antiviral agents.

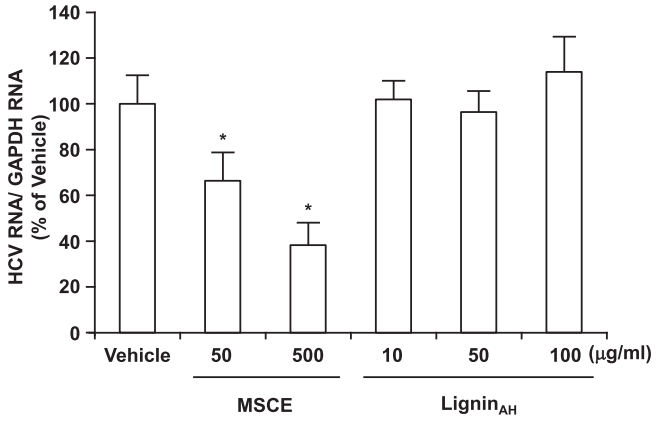

Fig. 4. Inhibition of HCV subgenome replication by MSCE. Huh7.5.1 1bFeo cells containing an HCV subgenome replicon were treated with MSCE or lignin$_{AH}$ at the indicated concentrations for 72 h, after which HCV subgenome RNA and GAPDH mRNA were isolated from the cells and analyzed by qRT-PCR. Data represent the mean ± SD (n = 3, $^*P < 0.05$ vs. vehicle treated group, as determined using Dunnett's test).

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.04.104.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.04.104.

References

[1] S.P. Wasser, A.L. Weis, Therapeutic effects of substances occurring in higher basidiomycetes mushrooms: a modern perspective, Crit. Rev. Immunol. 19 (1999) 65–96.
[2] S. Akamatsu, A. Watanabe, M. Tamesada, R. Nakamura, S. Hayashi, D. Kodama, M. Kawase, K. Yagi, Hepatoprotective effect of extracts from Lentinus edodes mycelia on dimethylnitrosamine-induced liver injury, Biol. Pharm. Bull. 27 (2004) 1957–1960.
[3] A. Watanabe, M. Kobayashi, S. Hayashi, D. Kodama, K. Isoda, M. Kondoh, M. Kawase, M. Tamesada, K. Yagi, Protection against D-galactosamine-induced acute liver injury by oral administration of extracts from Lentinus edodes mycelia, Biol. Pharm. Bull. 29 (2006) 1651–1654.
[4] A. Itoh, K. Isoda, M. Kondoh, M. Kawase, M. Kobayashi, M. Tamesada, K. Yagi, Hepatoprotective effect of syringic acid and vanillic acid on concanavalin a-induced liver injury, Biol Pharm. Bull. 32 (2009) 1215–1219.
[5] A. Itoh, K. Isoda, M. Kondoh, M. Kawase, A. Watari, M. Tamesada, M. Kobayashi, K. Yagi, Hepatoprotective effect of syringic acid and vanillic acid on CCl4 -induced liver injury, Biol. Pharm. Bull. 33 (2010) 983–987.
[6] K. Okuno, K. Uno, Efficacy of orally administered Lentinula edodes mycelia extract for advanced gastrointestinal cancer patients undergoing cancer chemotherapy: a pilot study, Asian Pac J. Cancer Prev. 12 (2011) 1671–1674.
[7] K. Tanaka, S. Ishikawa, Y. Matsui, M. Tamesada, N. Harashima, M. Harada, Oral ingestion of Lentinula edodes mycelia extract inhibits B16 melanoma growth via mitigation of regulatory T cell-mediated immunosuppression, Cancer Sci. 102 (2011) 516–521.
[8] Y. Yoshioka, H. Kojima, T. S., K. Tsuji, M. Tamesada, K. Yagi, N. Murakami, Low-molecular-weight lignin-rich fraction in the extract of cultured Lentinula edodes mycelia attenuates carbon tetrachloride -induced toxicity in primary cultures of rat hepatocytes, J. Nat. Med. 66 (2012) 185–191.
[9] I.T. Forrester, A.C. Grabski, C. Mishra, B.D. Kelley, W.N. Strickland, G.F. Leatham, R.R. Burgess, Characteristics and N-terminal amino acid sequence of a manganese peroxidase purified from Lentinula edodes cultures grown on a commercial wood substrate, Appl. Microbiol. Biotechnol. 33 (1990) 359–365.
[10] H. Sakagami, T. Kushida, T. Oizumi, H. Nakashima, T. Makino, Distribution of lignin-carbohydrate complex in plant kingdom and its functionality as alternative medicine, Pharmacol. Ther. 128 (2010) 91–105.
[11] H. Takayama, G. Bradley, P.K. Lai, Y. Tamura, H. Sakagami, A. Tanaka, M. Nonoyama, Inhibition of human immunodeficiency virus forward and reverse transcription by PC6, a natural product from cones of pine trees, AIDS Res. Hum. Retroviruses 7 (1991) 349–357.
[12] J.B. Lee, C. Yamagishi, K. Hayashi, T. Hayashi, Antiviral and immunostimulating effects of lignin-carbohydrate-protein complexes from Pimpinella anisum, Biosci. Biotechnol. Biochem. 75 (2011) 459–465.
[13] J. Vermehren, C. Sarrazin, New hepatitis C therapies in clinical development, Eur. J. Med. Res. 16 (2011) 303–314.
[14] S. Haid, A. Novodomska, J. Gentzsch, C. Grethe, S. Geuenich, D. Bankwitz, P. Chhatwal, B. Jannack, T. Hennebelle, F. Bailleul, O.T. Keppler, M. Poenisch, R. Bartenschlager, C. Hernandez, M. Lemasson, A.R. Rosenberg, F. Wong-Staal, E. Davioud-Charvet, T. Pietschmann, A plant-derived flavonoid inhibits entry of all HCV genotypes into human hepatocytes, Gastroenterology 143 (2012) 213–222 e5.
[15] S. Ciesek, T. von Hahn, C.C. Colpitts, L.M. Schang, M. Friesland, J. Steinmann, M.P. Manns, M. Ott, H. Wedemeyer, P. Meuleman, T. Pietschmann, E. Steinmann, The green tea polyphenol, epigallocatechin-3-gallate, inhibits hepatitis C virus entry, Hepatology 54 (2011) 1947–1955.

[16] R. Khachatoorian, V. Arumugaswami, S. Raychaudhuri, G.K. Yeh, E.M. Maloney, J. Wang, A. Dasgupta, S.W. French, Divergent antiviral effects of bioflavonoids on the hepatitis C virus life cycle, Virology 433 (2012) 346–355.
[17] L. Bachmetov, M. Gal-Tanamy, A. Shapira, M. Vorobeychik, T. Giterman-Galam, P. Sathiyamoorthy, A. Golan-Goldhirsh, I. Benhar, R. Tur-Kaspa, R. Zemel, Suppression of hepatitis C virus by the flavonoid quercetin is mediated by inhibition of NS3 protease activity, J. Viral Hepat. 19 (2012) e81–8.
[18] R.K. Jangra, M. Yi, S.M. Lemon, Regulation of hepatitis C virus translation and infectious virus production by the microRNA miR-122, J. Virol. 84 (2010) 6615–6625.
[19] T. Wakita, T. Pietschmann, T. Kato, T. Date, M. Miyamoto, Z. Zhao, K. Murthy, A. Habermann, H.G. Krausslich, M. Mizokami, R. Bartenschlager, T.J. Liang, Production of infectious hepatitis C virus in tissue culture from a cloned viral genome, Nat. Med. 11 (2005) 791–796.
[20] H. Tani, Y. Komoda, E. Matsuo, K. Suzuki, I. Hamamoto, T. Yamashita, K. Moriishi, K. Fujiyama, T. Kanto, N. Hayashi, A. Owsianka, A.H. Patel, M.A. Whitt, Y. Matsuura, Replication-competent recombinant vesicular stomatitis virus encoding hepatitis C virus envelope proteins, J. Virol. 81 (2007) 8601–8612.
[21] W.N. Setzer, Lignin-derived oak phenolics: a theoretical examination of additional potential health benefits of red wine, J. Mol. Model 17 (2011) 1841–1845.
[22] L.W. Meredith, G.K. Wilson, N.F. Fletcher, J.A. McKeating, Hepatitis C virus entry: beyond receptors, Rev. Med. Virol. 22 (2012) 182–193.
[23] J. Jiang, W. Cun, X. Wu, Q. Shi, H. Tang, G. Luo, Hepatitis C virus attachment mediated by apolipoprotein E binding to cell surface heparan sulfate, J. Virol. 86 (2012) 7256–7267.
[24] A. Raghuraman, V. Tiwari, Q. Zhao, D. Shukla, A.K. Debnath, U.R. Desai, Viral inhibition studies on sulfated lignin, a chemically modified biopolymer and a potential mimic of heparan sulfate, Biomacromolecules 8 (2007) 1759–1763.
[25] M. Qiu, Q. Wang, Y. Chu, Z. Yuan, H. Song, Z. Chen, Z. Wu, Lignosulfonic acid exhibits broadly anti-HIV-1 activity–potential as a microbicide candidate for the prevention of HIV-1 sexual transmission, PLoS One 7 (2012) e35906.
[26] S. Sato, Y. Mukai, J. Yamate, T. Norikura, Y. Morinaga, K. Mikame, M. Funaoka, S. Fujita, Lignin-derived lignophenols attenuate oxidative and inflammatory damage to the kidney in streptozotocin-induced diabetic rats, Free Radic. Res. 43 (2009) 1205–1213.