



ISSN: 2377-6250 (Print)
ISSN: 2377-8318 (Online)
CODEN : OJCEB4

ARTICLE

Open Journal of Chemical Engineering (OJCE)

DOI: <http://doi.org/10.7508/ojce.01.2019.01.03>



THE PHARMACOLOGICAL EFFECTS OF TRITERPENOIDS FROM GANODERMA LUCIDUM AND THE REGULATION OF ITS BIOSYNTHESIS

Haiden Lawrence

School of Chemistry, University of Bristol, Bristol, BS8 1QU, United Kingdom
*Corresponding Author E-mail: yy3074316327@163.com

This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

ARTICLE DETAILS

Article History:

Received 12 April 2019
Accepted 20 May 2019
Available online 27 June 2019

ABSTRACT

The paper aims to focus on bioactivities and potential applications of GAs, and the elicitation strategies for GA production are also highlighted based on the fundamental role of ROS, JA, Ca²⁺ and NO, which would shed light to developing a novel approach to manipulating the biosynthesis of GA in the future. The biosynthesis of GA in *G. lucidum* is induced by stressors including various elicitors or signaling molecules, and artificially placing elicitors would enhance GA production. From the perspective of pharmacology, those secondary metabolites have many biological activities, which include antitumor, antimicrobial, anti-malaria, antihepatotoxic and blood pressure reduction activity. From the aspect from fungi, the main function of secondary metabolites is adaptive responses to biotic or abiotic stresses. Application of elicitors into in vitro cultures would mimic the biotic or abiotic attacks, resulting in the biosynthesis of secondary metabolites.

KEYWORDS

Biosynthesis, ganoderma lucidum, pharmacological effects.

1. INTRODUCTION

Ganoderma lucidum, commonly known as Lingzhi in Chinese, is a woody basidiomycetes mushroom belonging to the family of Ganodermaceae. *G. lucidum* has been used for two millennia as herbal medicine mainly in Asian areas. It had the efficacy of improving immunity, soothing the nerves, protecting the liver, detoxification and prolonging life expectancy. Over 400 bioactive compounds were found in *G. lucidum* such as polysaccharides and triterpenoids. The most important bioactive compounds in *G. lucidum* are triterpenoids which are known as ganoderic acids (GAs). The medicinal effects of GA were verified, which include anti-tumor, anti-malaria, anti-microbial, anti-inflammatory, and anti-viral effects. Due to the commercial and pharmacological value of GA, biosynthesis of GA by cell factories received more and more attention in recent years. However, the productivity and yield of GA by cell culture technology are still very low in spite of many years of efforts. Elicitors are extrinsic molecules often associated with plant pests and pathogen. Generally, elicitors are regarded as a signal molecule and could be recognized by the receptor on the surface of the plant plasma membrane or endomembrane. Subsequently, recognition events would induce a signal-transduction cascade leading to activate biosynthesis of transcription factors^[1]. Those transcription factors regulate the expression of the genes which are involved in the biosynthesis of secondary metabolisms, such as phytoalexins, flavones, triterpenoid and other defense-related compounds. An obvious positive correlation between elicitor addition and GA accumulation has been reported in the previous study. Besides, these elicitors have been well studied in *G. lucidum* within the context of mycelial cultures^[2-4]. So that elicitor would be considered as the main manner to enhance GA production in the future.

Hajjaj, Macé reported that the pharmacological effects of GAs are mainly related to the hydroxylation of their structure. For example, the ganoderic acid A (GA-A) is hydroxylated at C-3, while ganoderic acid D (GA-D) is hydroxylated at positions 7 and 15. Other triterpenes with acetoxy or hydroxyl groups at locations 3, 7 or 15, such as ganoderic acid C (GA-C), ganoderic acid B (GA-B), ganoderic acid Me (GA-Me) ganoderic acid K (GA-K) were also proven as Bioactivities metabolism. The recent research on bioactivities of *Ganoderma* are showed in Table 1 and summarized as follows.

Table 1: Bioactivities of *Ganoderma* metabolites.

Bioactivity	Active metabolite
Anti-tumor	Ganoderic acid S
	Ganoderic acid B
	Ganoderic acid F
Anti-HIV	Ganoderic acid Y
	Ganoderic acid B
	Ganoderic acid beta
Anti-malaria	Ganoderic acid F
	Schisanlactone B
Anti-hypertensive	Ganoderiol F
	Ganoderic acid K
Anti-hepatotoxic	Ganoderic acid R
Hemostasis	Ganodermic acid S

2. PHARMACOLOGICAL EFFECTS OF GAS

2.1 Antitumor activity

Ganoderic acids are well acknowledged as bioactive metabolites to improve the function of the human immune system. Toth, Bang, firstly reported antitumor activity of GA, which is triggering apoptosis in cervical carcinoma cell in vitro. Yue, Cao, found GA would inhibit the proliferation of HeLa human cervical carcinoma cells by keeping cell cycle arrest at the G2 phase. Further- more, the anti-metastasis effects of GA-Me were reported. GA-Me could inhibit cancer cell metastasis by increasing of cell adhesion and decreasing of cell motility, and it also suppresses the expression of MMP2 gene which was involved in the progression and metastasis of many forms of cancer. Therefore, GA-Me could be considered as a promising anti-metastatic compound. Also, there are many reports on the antitumor activity of another ganoderic acid. Hsu, Yu discovered that GA-B could inhibit the growth of some tumours cell lines and trigger apoptosis in human leukemia HL-60 cells. Gao, Hirakawa showed the in vivo antitumor effects of the ganoderic acid F (GA-F). This is demonstrated by cytotoxicity assay in lung carcinoma cell (LLC)-bearing mice. GA-F showed a remarkable activity in cytotoxicity and strongly inhibited the growth of the tumour without obvious side effects. Jiang, Jedinak reported that GA-Y would block the expression of the cell cycle regulatory protein CDC20, thereby proliferation and development of invasive and metastatic human breast cancer cells was also inhibited^[5].

2.2 Anti-HIV activity

Acquired immunodeficiency syndrome (AIDS), caused by HIV, is highly contagious and affect millions of people all over the world. Postponing the development of AIDS is the main goals of treatment approaches for HIV. Several articles reported that ganoderic acids could inhibit the progression of HIV including ganoderic acid beta, GA-A, and GA-B. Those ganoderic acids have been shown to have significant anti-human immunodeficiency virus protease activity, with half maximal inhibitory concentration values of 20 - 90 Millimole per milliliter. In one of the earlier research, El-Mekawy, Meselhy successfully isolated fifteen compounds from *G. lucidum* including ganoderic acid. They found that those compounds had strong inhibitory activity against HIV-1 proteases. Recently, Kang, Mutakin also indicated that ganoderic acid B possessed the highest inhibiting activity to HIV-protease of four tested triterpenoids. In addition, Zhang, Ip reported the extractive of *G. lucidum* could inhibit HIV-1 reverse transcriptase. The researches mentioned above suggest the huge potential of ganoderic acid for HIV treatment.

2.3 Anti-malaria activity

According to the estimation of Centers for Diseases Control and Prevention, there are 429,000 people died from malaria infection worldwide in 2015. Malaria is a disease caused by a Plasmodium, and it can be treated by artemisinin. However, Dondorp, Nosten reported the artemisinin resistance in Plasmodium falciparum. Lakornwong, Kanokmedhakul found the anti-malaria activity of triterpene isolated from Ganoderma. They cultured Ganoderma sp. KM01 and isolated eleven different from the mycelium, and then the antimalarial activity against *P. falciparum* of those triterpene was investigated. In vitro, *P. falciparum* assay illustrated that GA-F and schisanlactone B caused half *P. falciparum* death with the dose ranging from 6.0 to 10.0 $\mu\text{mol/L}$. Except for triterpene, based on computational molecular docking, GA-F and ganoderiol F also have the potential to restrict the growth of *P. falciparum* by inhibiting aspartic protease. Secondary metabolites such as triterpene produced by Ganoderma seem to have the potential to inhibit malaria but still require more studies^[6].

2.4 Other pharmacological effects of GAs

Since the last century, the pharmacological effects of ganoderic acids were already well studied. Hirotani, Ino reported that GA-B, GA-D, GA-F, and GA-K have antihypertensive effects by inhibiting the activity of angiotensin-converting enzymes especially GA-K, which had the highest inhibitory effect with a half maximal inhibitory concentration of 4.7 $\mu\text{mol/L}$. Kabir, Kimura also found the GA effects on reducing blood pressure and lipid levels in hypertensive rats. Furthermore, the antihepatotoxic activity of ganoderic acid R and ganoderic acid S, which was isolated from the cultured mycelia of *G. lucidum*, was proved in the galactosamine-induced cytotoxic test with primary rat hepatocytes. Besides, Wang, Chen reported that ganoderic acid S could induce the aggregation of human platelet. At a concentration of 20 $\mu\text{mol/L}$, the existing of ganoderic acid S caused platelet aggregation. Above the

threshold, the extents of cell aggregation showed a linear relationship with agent concentration.

3. THE BIOSYNTHETIC PATHWAY OF GAS

The pathway of GA biosynthesis is not fully understood yet. Earlier biogenetic research illustrates that GA is biosynthesised via the mevalonate-isoprenoid pathway from glucose until lanosterol in *G. lucidum*.

Firstly, the biosynthesis of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) catalyzed by 3-hydroxy-3-methylglutaryl-CoA synthase. Then HMG-CoA is converted to mevalonate (MVA) and further to isopentenylpyrophosphate (IPP). Squalene synthase (SQS) catalyze the enzymatic step toward squalene biosynthesis. Finally, lanosterol is formed from squalene under the catalysis of oxidosqualene cyclase (OSC) (Figure 1). Lanosterol formed by the cyclization of 2, 3-oxidosqualene is the ring skeleton of GAs. This cyclization is catalysed by lanostane synthase (LS). However, further steps from lanosterol to GA, which may include oxidation, reduction, and acylation reactions are yet unclear. In addition, it was proposed that a series of oxidation-reduction steps are likely involved in the post-lanosterol modifications. The genome analysis of *G. lucidum* reveals cytochromes P450 (CYPs), including 24 P450 clusters and 78 P450 genes might involve in catalysing the lanosterol skeletons into triterpenoids. Even though the pathway of GA biosynthesis is not fully understood, the knowledge of the genes and enzymes involved in the GA biosynthetic pathway should lead to an in-depth understanding of the regulation and physiology of GA biosynthesis.

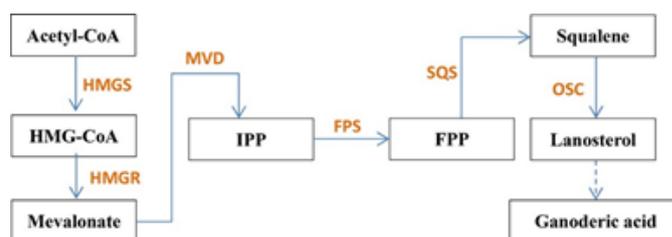


Figure 1: Biosynthetic pathway of GA. Dashed lines indicates this step is yet unclear. HMG-CoA, hydroxy-3-methylglutaryl-Coenzyme A; HMGS, HMG-CoA synthase; HMGR, HMG-CoA reductase; MVD, mevalonate-5-pyrophosphate decarboxylase; IPP, isopentenylpyrophosphate; FPS, farnesyl pyrophosphate synthase; FPP, farnesyl diphosphate; SQS, squalene synthase; OSC, oxidosqualene cyclase.

4. ELICITOR FOR REGULATING OF GANODERIC ACID BIOSYNTHESIS

4.1 Reactive oxygen species inducer

Reactive oxygen species (ROS) is a by-product of biological aerobic metabolism, including superoxide anion, hydrogen peroxide and singlet oxygen. Zhang and Zhong, reported the higher oxygen concentration would increase individual GAs production, and more spores and higher total GA content were obtained at an oxygen level of 80%. Feng, Zhang found oleic acid has an effect on promoting in GA accumulation because the oleic acid addition improved the level of dissolved oxygen in liquid submerged fermentation of *G. lucidum*. Aspirin is known as an agent which could induce oxidative stress by causing mitochondrial dysfunction. When the *G. lucidum* was cultured with 2 mmol/L aspirin, ROS production was enhanced in mycelia. And ROS was further accumulated with the aspirin concentration increasing to 4 mmol/L. You, Lee, found that GA production has increased fourfold in the submerged fermentation containing 4 mmol/L aspirin. These results illustrated that ROS contribute to the regulation and production of GA and biosynthesis in *G. lucidum*.

4.2 Metal ions

Metal ions such as calcium ion and iron ion play an essential role in the biosynthesis of metabolism in various organisms. The application of Ca^{2+} is helpful for GA biosynthesis. Xu and Zhong reported the induction of Ca^{2+} result in a threefold growth in the production of total GAs, accompanied by higher expression of three biosynthetic genes LS, HMGR

and SQS. Moreover, the binding sites of two transcription factors (CRZ1 and AreA) which are involved in nitrogen regulation both were found in the promoter regions of LS, HMGR and SQS. This phenomenon means that the biosynthesis of GAs may be regulated by the concentration of nitrogen and calcium ion synergistically. Therefore, a new integrated strategy was developed, which is adding Ca²⁺ and reducing nitrogen simultaneously to promote GA biosynthesis. Applying this strategy up-regulated transcriptional level of the biosynthetic gene (LS, HMGR and SQS) and increase the supplies of precursors, resulting in improve the production of GA by nine-fold compared with controls. In addition, the integrated strategy appeared to synergistic activity. The maximum output of GA production regulated by the integrated strategy was 1.87 g/100g in dry cell weight, which was nearly fivefold higher than the GA production regulated by either adding Ca²⁺ or reducing nitrogen.

4.3 Phytohormones

Plant hormones have been studied as elicitor for many years. The most studied one is Jasmonic acid (JA) and its derivatives such as methyl jasmonate (MeJA). They are responsible for signal transduction processes which regulate the expression of defence genes in plants. In the liquid culture of *G. lucidum* mycelium, artificially adding MeJA could induce rising of the content of endogenous MeJA. Introduction of 254 µM MeJA would improve GA content by 45.3% and up-regulated the transcriptional level of GA biosynthetic genes, such as MVD, HMGS and OSC. Ethylene (ET) is a hydrocarbon, which is involved in biochemical and morphological changes during the process of fruit ripening and senescing. In CYM medium, applying of 15 mM ethephon could enhance the growth of *G. lucidum* mycelia, resulting in GA production went up by 90%. Besides, the transcription levels of OSC and HMGR were up-regulated by threefold and fourfold compared to the control group. Salicylic acid (SA), a type of phenolic acid with an essential role in plant defence systems, is admitted to inducing systemically acquired resistance to many pathogens. The addition of SA resulted in more GA accumulation by enhancing the gene expression of HMGR and LS in the mycelium of *G. lucidum*.

There are already many reports focusing on the crosstalk among ET, JA and SA signalling pathways, but the knowledge about crosstalk among these plant hormones in *G. lucidum* is very limited. Therefore, more research about hormonal crosstalk in *G. lucidum* might open up perspectives to manipulate the biosynthesis of GA.

4.4 Nitric oxide

Nitric oxide (NO) is a gaseous signaling molecule. It is a key bioactive molecule, playing a role in many fungi physiological processes such as spore germination, hyphal growth, and the responses to environmental stresses. In *G. oregonense*, heat tolerance could be enhanced by NO through increasing the expression of HSP genes such as HSP30, HSP70, and HSP104. Introduction of the NO donor such as sodium nitroprusside (SNP) would increase GA production. In submerged fermented mycelia of *G. lucidum*, the introduction of the SNP at the concentration of 5 mmol/L could improve GA content by 40.94%. After 72 hours of introducing SNP, the expression of GA biosynthetic genes including ACAT and SE was detected to be up-regulated.

5. CONCLUSIONS AND PROSPECTS

Over the centuries, *G. lucidum* has been extensively used as a medical mu-

shroom. It produces huge amounts of bioactive secondary metabolites such as GA from its mycelia or fruiting body. From the perspective of pharmacology, those secondary metabolites have many biological activities, which include anti-tumor, antimicrobial, anti-malaria, antihepatotoxic and blood pressure reduction activity. From the aspect from fungi, the main function of secondary metabolites is adaptive responses to biotic or abiotic stresses. Application of elicitors into in vitro cultures would mimic the biotic or abiotic attacks, resulting in the biosynthesis of secondary metabolites. Introduction of elicitors such as heavy metal ion, phytohormones, hydrogen peroxide, aspirin, and NO into cultured mycelia of in *G. lucidum* have been recognized as effective ways to promote GA accumulation. Furthermore, other approaches such as genetic modification and nutrition regulation can be integrated with elicitation strategy to get a more dramatic improvement of GA productivity in *G. lucidum*. However, elicitors induce the accumulation of secondary metabolite, usually accompany with blocked cell growth, thereby lower total GA production. Therefore, dissecting the response networks of *G. lucidum* to various elicitor molecules is important for developing novel strategies for enhancing GA production.

As a whole, further research about the signal transduction induced by elicitor in *G. lucidum* will benefit in improving the yields of specific secondary metabolites. Moreover, it would also further promote the efficiency and accuracy of genetic modification by transcription factors. According to the information from metabolomics studies, manipulating metabolic pathways in *G. lucidum* with different biotechnology could offer insight for higher productivity of GAs.

CONFLICTS OF INTEREST

The author declares no conflicts of interest regarding the publication of this paper.

REFERENCES

- [1] Zhao, J., Davis, L.C. and Verpoorte, R. 2005. Elicitor signal transduction leading to production of plant secondary metabolites, *Biotechnology Advances*, 23, 283-333.
- [2] Xu, J.-W., Zhao, W. and Zhong, J.-J. 2010. Biotechnological production and application of ganoderic acids. *Applied Microbiology and Biotechnology*, 87, 457-466.
- [3] Jiang, J., et al. .2008. Ganoderic acids suppress growth and invasive behavior of breast cancer cells by modulating ap-1 and nf-kb signaling, *International Journal of Oncology*, 21, 577-584.
- [4] Gao, J.J., et al. 2006. In vivo antitumor effects of bitter principles from the antlered form of fruiting bodies of ganoderma lucidum, *Journal of Natural Medicines*, 60, 42-48.
- [5] Toth, J., et al. 1983. Cytotoxic triterpenes from ganoderma lucidum (polyporaceae): structures of ganoderic acids UZ, *Journal of Chemical Research. Synopses*, No. 12.
- [6] Paydary, K., et al. 2013. The emergence of drug resistant hiv variants and novel anti-retroviral therapy, *Asian Pacific Journal of Tropical Biomedicine*, 3, 515-522.

