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Progress of Ginsenosides on Immunomodulation and Inhibition of Lung Cancer

Dongju Wei

North Cross School Shanghai, Hefei, China

zcgtcm@ahtcm.edu.cn

Abstract. Ginseng is characterized by low toxicity, multi-targeting and enhancement of body immunity in its anti-cancer effects. Ginsenosides are natural effective anti-cancer components, mainly consisting of diol-type, triol-type, and oleanolic acid-type ginsenosides. Ginsenosides are bio absorbed by intestinal flora through "Rb1-Gibberellin XVII-F2-CK-PPD", "Rb2-Rd-F2-CK-PPD", "Rg3-Rh2-PPD" and other pathways. It can be converted into saponins ck, Rh1, Rh2, Rc, PPD and other active ingredients. Metabolites of ginsenosides are used in the treatment of many types of cancers and have been shown to be effective. They function by modulating various immune cells in the body, for example, ginsenoside Rh2 significantly increases T-lymphocyte activity and significantly induces differentiation of M2 macrophages towards the M1 phenotype. Ginsenoside Rg3 can act as a cytotoxic activator of NK cells, which inhibits the growth of tumor cells and induces apoptosis and autophagy of tumor cells, and plays an inhibitory and therapeutic role in lung cancer and a variety of tumors, with certain prospects for application development. This review introduces mechanism and function of ginsenosides and discusses application in lung cancer.

Keywords: Ginsenosides; lung cancer; immune system.

1. Introduction

Lung cancer is one of the major health problems facing the world, and its incidence and fatality rates continue to be high. Currently, traditional tumour treatments include surgery, chemotherapy and radiotherapy. Surgical resection is effective for early stage solid tumours. However, there is no significant role for intermediate to advanced tumours and for non-solid tumours, such as leukaemia. Radiotherapy has a killing effect on cancers that have metastasised. However, the toxicity and side effects are very high. In addition, chemotherapy can easily make the tumour cells resistant to drugs, which is extremely damaging to the body. Therefore, compared with traditional methods, immunotherapy has the advantages of extremely low side effects, high cure rate and uninterrupted attack on tumors. As a defense network covering the whole body, the human immune system can restrict the proliferation and growth of tumor cells, and the regulation of biological response can change the host's biological response to tumor, thus playing an anti-tumor role [1]. In recent years, Chinese herbal medicines have been broadly used in many areas of cancer treatment and research due to their low toxicity, few side effects, multiple targets, and low susceptibility to drug-resistant reactions. Like lung cancer. Among these, Ginseng has shown great potential for research. Ginseng is a perennial herb in the family Pentacostaceae. It has the effect of tonifying the vital energy, rescuing the reverse and fixing the root cause, generating fluids to quench thirst, and calming the mind to help sleep. Additionally, modern pharmacological studies have shown that ginseng contains a variety of effective anti-cancer compounds. And the steroid compounds present in ginseng - ginsenosides are an important component of ginseng's anti-cancer activity, with significant anti-cancer specificity. It inhibits tumour cell infiltration, anti-tumour cell metastasis and promotes tumour cell apoptosis. Therefore, it plays an indispensable role in lung cancer treatment.

2. Classification and Biotransformation of Ginsenosides in the Intestine

Ginsenosides are continuously produced and accumulated during the growth cycle of ginseng, and their content concentration is directly proportional to its growth age. In general, high levels of

ginsenosides can be achieved with 6 years of cultivation. However,wild ginseng takes more than 10 years. Most of these ginsenosides are concentrated in the root of ginseng.

Ginsenosides into a steroidal backbone, plus different sugar groups (glucose, rhamnose, xylose and arabinose linked on C3, C6 and C20). Ginsenosides prefixed with "R" are ginsenosides isolated for the first time from the root, followed by a letter and a numerical value, which needs to be ranked according to the order of chromatographic polarity. For example, the least polar ginsenoside is Ra derived from the root, followed by Rb1, and the pharmacological activity can vary depending on the type and structure. For example, the least polar ginsenoside is Ra derived from the root, followed by Rb1, and the pharmacological activity can vary depending on the type and structure. Ginsenosides are usually classified into three groups according to the structure of their glycoside matrices: proto-ginsen-diol type, proto-ginsen-triol type and oleanolic acid type. Protopanaxadiol type is a hydrogen atom on C6, such as ginsenosides Rb1 and Rb2, which inhibit CNS, reduce intracellular calcium, antioxidant, free radical scavenging and ameliorate myocardial ischaemia-reperfusion injury. Like ginsenoside Rg1, there is a sugar side chain attached to C6, which is a proto-ginsenotriol type. Ginsenoside Rg1 has the effect of promoting the synthesis of proteins, DNA and RNA, central nervous system excitation, and increasing intelligence. As a ginsenotriol type saponin Re has antiarrhythmic active ingredients. Finally, oleanolic acid types, such as ginsenoside Ro, are mainly anti-inflammatory and anti-platelet release [1], but they have been reported less frequently. Therefore, the following will focus on the biotransformation of diol and triol ginsenosides in the presence of intestinal microbiota.

The prototype ginsenoside is a kind of saponin component that can be directly extracted from ginseng, but the skeleton contains sugar group, the molecular polarity is also large. It is not easy to be directly absorbed by human body, and its bioavailability is very low. For example, the bioavailability of ginsenosides Rc, Rb1 and Rb2 was only 0. 17%, 0. 78% and 0. 08%. The deglycosylation reactions that can occur in the human body, mediated by a cascade of gut microbes. Partially metabolised to less polar rare ginsenosides. Rare ginsenosides not only better penetrate the intestinal wall and enter the blood circulation, but also substantially increase bioavailability. Moreover, modern pharmacological studies have moreover confirmed that some of these specific metabolites usually exhibit stronger anti-tumour effects than the prototypical ginsenosides [2]. Among them, ginsenoside Rg3 has been proved to have significant physiological activities, which can reduce the viability and proliferation of lung cancer cells to some extent, and also promote the apoptosis of cancer cells [3].

For structural reasons, ginsenosides Rb1, Rb2, Rc, etc. are classified as proto-ginseng diol-type saponins. Under the action of intestinal microorganisms, it is mainly metabolised to products such as ginsenosides Rd, Rh2, CK and PPD. CK is the main form of ginseng metabolite absorbed into the blood, and glycosides are the end product PPD produced by the gradual deglycosylation of diol ginsenosides. Human intestinal flora can help in the conversion of ginsenoside components. For example, when Bae and others studied the anaerobic incubation of Rb1 with human intestinal flora, they found that Clostridium difficile K-60 could help the transformation of Rb1 in the intestinal tract, forming the metabolic transformation pathway "Rb1-Gynostemma saponin XVII-F2-CK-PPD". In addition, it was found that when Rb2 was incubated anaerobically with human intestinal flora, ginsenoside Rb2 could be converted to products such as CK and PPD via ginsenoside Rd with the help of Eubacterium spp, Streptococcus spp and Bifidobacterium sp. This pathway is "Rb2-Rd-F2-CK-PPD". The metabolic pathway of Rb2 in the gut can be found to be similar to that of Rb1 as well. Temperature also has an effect on the conversion rate of ginsenoside Rb2 to CK in this environment of human gut flora, Bae et al. found that Rb2 can be converted up to 88. 7% after incubation and conversion for 48 hours at 37°C under anaerobic conditions. In addition, the different genera of bacteria will also lead to differences in the conversion pathway of some components, such as Rg3, Clostridium spp. can directly convert ginsenoside Rg3 to ginsenoside Rh2. However, in the case of Bacteroides sp, Eubacterium sp and Bifidobacteriym sp, the ginsenoside Rg3 is converted to PPD by ginsenoside Rh2 through the pathway "Rg3-Rh2-PPD " [2].

The original ginseng triol type saponins, Re and Rg1, will be converted to ginsenosides Rh1, F1, PPT, etc. by the intestinal flora, and PPT is the final product of glycosylation of triol type ginsenosides. Bae et al. conducted a study on the conversion of ginsenoside Re. It was found that the bacterium Pseudomonas aeruginosa JY-6 had a catalytic effect on the conversion of ginsenoside Re, with which human faeces also showed good metabolic activity. However, what still needs to be explored and confirmed is that after oral administration of ginsenoside Re in rats, Rg2 can be detected in plasma but not in humans, and there is speculation that this is due to the lack of a transporter for the basal portion of rhamnolipids in humans. It has also been suggested that Re undergoes hydroxylation and dehydration of PPT in the gut, resulting in the generation of deoxy PPT. Regarding the metabolic process of ginsenoside Rg1 in the gut, it is carried out by a range of bacteria in the gut. For example, in the presence of Bacteriophage HJ15, Bacteroides JY6, Bifidobacterium bifidum K-525, and Fusobacterium K-60, it is transformed by the pathway Rg1-Rh1/F1-PPT [2].

3. Regulatory Effects of Ginsenosides on Human Immune Function

3.1. Regulation of Immune Cell Activity

Immunotherapy is widely used in the treatment of cancer, including the principle of active immunity to stimulate systemic anti-tumor effects. Tumour immunotherapy can be divided into two main categories: non-specific and specific. Non-specific tumor immunotherapy refers to the purpose of alleviating tumor symptoms by improving human immunity as a whole without specific immune cell targets. Specific tumour immunotherapy, on the other hand, has a well-defined target and mechanism, so as to activate or inhibit the target to achieve immune activation of the immune system against tumours. Nowadays, specific tumor therapy has achieved remarkable results for patients and has become the mainstream direction of tumor immunotherapy. Therefore, the activity of immune cells is particularly important in immunotherapy. Immune cells are participants in the body's immune response and are also the executors of immune functions. The body's immune cells include lymphocytes, dendritic cells, macrophages, NK cells, mast cells, and so on. Ginsenosides have a non-negligible regulatory effect on immune cells [4].

The regulatory function of ginsenosides on immune molecules plays an important role in a variety of diseases. Immune molecules are mainly produced by T lymphocytes, B lymphocytes and macrophages after being stimulated by antigens. The number of immune molecules involved in the immune response is quite large and includes cytokines, adhesion factors, CD molecules, complement and Ig. Cytokines are small molecule peptides or proteins that are secreted by cells and are highly active and multifunctional. The main ones are interleukins, tumour necrosis factor and interferon. According to the experiments on the effect of ginsenoside Rg3 on the content of IL-2 and INF-γ in ovarian cancer transplanted tumour nude mice, it can be concluded that the injection of ginsenoside Rg3 to mice can make the expression of the content of IL-2 and INF-γ in the serum of mice decrease, and it is concluded that the tumour cells are effectively controlled under the action of Rg3 [5,6].

3.2. Induction of Apoptosis and Autophagy

Apoptosis is the spontaneous programmed cell death controlled by genes, including DNA fragmentation, cell membrane remodeling and foaming, cell shrinkage, nuclear pyknosis and so on. It can also be involved in maintaining intracellular environmental homeostasis, resisting immune responses and eliminating damaged cells [7]. After tumourigenesis, apoptosis is the first process to counteract tumour cell proliferation, and in precancerous lesions, DNA damage can induce apoptosis thereby removing potentially harmful cells and thus blocking tumour growth. Ginsenosides can induce apoptosis through two main pathways, mitochondria-dependent endogenous pathway and death receptor-dependent exogenous pathway. The endogenous apoptotic pathway is mediated by intracellular signaling. In some cell stress conditions, these signals will act accordingly, such as chemotherapy, cell hypoxia, cytokine deficiency, etc.

It was found that in colorectal cancer, ginsenoside Rh2 could decrease the expression level of apoptotic gene bcl-2, while increasing the expression of caspase-3 gene, and eventually colorectal cancer cells were induced to apoptosis [4]. It is worth noting that one important mechanism by which ginsenosides induce apoptosis in tumour cells is related to the adjustment of reactive oxygen stress in tumour cells. That is ROS. ROS refers to a class of oxygen-containing free radicals and radical-prone peroxides that are associated with oxygen metabolism in living organisms. ROS promote cancer by inducing genomic instability, altering gene expression and affecting different signalling pathways [7]. Yannan Liu found that ginsenoside Rg5 induced ROS generation and activated the MAPK pathway (P38, JNK, ERK) in gastric cancer cells, leading to apoptosis and cycle blockage [4].

Autophagy is the phagocytosis of lysosomes in cells to degrade their own structures. Through autophagy, cells can clearly degrade damaged structures, senescent organelles, unwanted biomacromolecules, etc. The main function of autophagy is to remove "biological waste" in the body and promote metabolism by degrading damaged cells and mutated proteins under stress conditions such as hunger, thus realizing cell self-protection and conducive to cell survival. Lee JS et al. demonstrated that oncogenes such as BCL-2 and FLICE-like inhibitory protein can inhibit autophagy, which suggests that it is possible to inhibit the growth and multiplication of tumour cells by inducing them to undergo autophagic death, thereby achieving the goal of treating tumours. According to the study of ginsenoside Rh2 on mouse malignant melanoma cells B-16 cells, it was shown that ginsenoside Rh2 could inhibit the growth of B16 cells. The half inhibitory concentration (IC50) at 48h was about 59. 87 ±3. 75 μg/ml, with a dual concentration and time dependence. Ginsenoside Rh2-intervened B16 cells showed a significant increase in intracellular autophagic vesicles and elevated expression of autophagic death signature protein Beclin1 compared with control cells. Immunohistochemical studies demonstrated that the expression levels of Beclin1 protein and autophagy signature protein LC3 in tumour tissue sections of mice intervened with ginsenoside Rh2 were significantly higher than those of mice inoculated with tumours without ginsenoside Rh2 intervention, both when the drug was given prior to the inoculation of tumours and when the drug was given after the tumour injection. Therefore, under the intervention of ginsenoside Rh2, LC3 and Beclin1, the signature proteins of autophagy in B16 cells, are significantly increased, which may induce autophagy in B16 cells to inhibit melanoma tumors. This phenomenon may be the result of inducing autophagy of B16 cells through P13K/Akt/mTOR signaling pathway [8].

4. Inhibitory Effect of Ginsenosides on Lung Cancer

Various metabolic components of ginsenosides mentioned above have anticancer effects, and they also have this effect in lung cancer. The inhibition rate of different concentrations of ginsenoside CK on A549 cells after different time of action was detected by MTT assay,after treatment with 50, 25, 12.5, 6.25, 3.125, 1.5625 μg/ml of ginsenoside CK for 24, 48, 72, and 96 h, the growth of A549 cells was inhibited to varying degrees, and it was also found that the rate of inhibition continued to increase as the concentration of the drug increased and the duration of its action was prolonged. Studies have shown that the combination of ginsenoside CK and cisplatin is more effective against lung cancer. The effect of the combination of the two drugs on A549 cells is also concentration and time dependent. It should be noted that the combination of the two drugs in large doses should be superimposed, while the combination of the two drugs in small doses should be antagonistic [9]. Another metabolic component, ginsenoside PPD, can induce mitochondria-dependent apoptosis so that cytochrome C can be released and promote the activation of apoptotic proteins Smac, AIF, and caspase-9 to inhibit lung cancer cells. Another study found that 25-OCH3-PPD, a newly identified analogue of PPD, significantly inhibited the proliferation of A549 cells. It induces apoptosis by regulating ß-catenin, a key protein in the Wnt signalling pathway. Meanwhile, 25-OCH3-PPD also inhibited the proliferation of H358 as well as H838 lung cancer cells, reduced cell proliferation-related proteins, such as MDM2, E2F1, cyclin D1, etc., and induced cell apoptosis. PPD and CK can also inhibit multiple cancers by directly inhibiting cell survival cell pathways, such as NF-kappaB, JNK, MAPK, ERK and other signalling pathways. PPD can also inhibit the activation of the P13K-Akt pathway and thus inhibit

the proliferation of cancer cells, such as down-regulating the phosphorylation level of Akt and down-regulating the expression of GSK-3ß protein in A549 lung cancer cells. CK inhibits the level of phosphorylation of STAT3 as well as its upstream JAK1 kinase, down-regulates STAT3 target genes bcl-xL, bcl-2, survivin, cyclin E, and D1, and increases the expression of the phosphatase SHP-1. It was also mentioned above that the metabolic efficiency of ginsenosides is closely related to intestinal flora, so it is necessary to pay attention to and improve the methods of optimizing intestinal flora to improve the availability of ginsenosides biological components [10].

DNA damage caused by exposure to environmental stress is one of the key factors in the transformation of normal cells into cancer cells. When DNA damage occurs, checkpoints that are activated in the cell cycle activate cycle stasis, thereby preserving DNA integrity and repairing the damage before the cell divides. Cells can also induce apoptosis to prevent daughter cells from inheriting mutations when repair fails. This process is a continuous reaction initiated by specific proteins and regulated by histones.

The VRK1/P53BP1 signalling pathway is involved in the DNA damage response, and VRK1 plays a key role in initiating the DNA repair process and apoptosis in response to DNA damage. VRK1 can recognize local chromatin aberrations by phosphorylating specific proteins such as P53BP1, triggering downstream cascade signals, and can promote non-homologous end linking by regulating the amount and volume of P53BP1 focus formation. And it was found that ginsenoside Rg3 could treat A549 and HCC827 cells for 96d, and these two cells showed obvious short tails and round nuclei, so it can be concluded that ginsenoside Rg3 plays a role in maintaining DNA integrity. In this experiment, the researchers used immunofluorescence staining to study the expression and localization of VRK1 and P53BP1 proteins in cells, and found that the phosphorylated expression of P53BP1 proteins in the experimental group was significantly higher than that in the control group, and more concentrated in the nucleus after ginsenoside Rg3. Therefore, ginsenoside Rg3 also protects DNA integrity by activating the VRK1/P53BP1 pathway, thereby inhibiting the occurrence and activity of lung cancer cells [11]. In the study of transplanted C57BL6 mice with Lewis lung cancer, the authors pointed out that immunotherapy drugs are a hot field today, which can induce the activation of T cell co-stimulatory molecules and their ligand pathways, which can lead to the expansion and activation of anti-tumor T cells, thus playing an anticancer role. Treatment of the ICOS/ICOSL pathway relies on antagonist and agonist monoclonal antibodies currently being tested in clinical trials. Monoclonal antibodies to ICOS agonists are currently being developed for use in a range of solid tumors. This mouse study showed that Rg3 or Apatinib alone or Rg3 combined with Apatinib without the use of ICOS agonist monoclonal antibodies could inhibit tumor growth, but had no significant effect on immune response. In contrast, when the ICOS agonist monoclonal antibody is used alone without Rg3 or apatinib as an adjuvant, there is some inhibition, but not as much as when the adjuvant is added. If ICOS agonists monoclonal antibody, Rg3 and Apatinib are used together, the results of inhibiting tumor growth have been shown to be very significant, and it can also promote the cellular immune response in lung cancer model animals [12,13].

5. Conclusions

Ginsenosides are characterised by low toxicity, multi-targeting and enhancement of body immunity in their anti-cancer effects. Ginsenosides are mainly composed of ginsenoside diol type (Rb1, Rb2, RC, Rd, Rh2, etc.), ginsenoside triol type (Re, Rf, Rg1, Rg2, Rhr, etc.) and oleanolic acid type (Ro, Rh3, Ri, F4, etc.). The absorption and utilization of these active ingredients in the human body is quite low, and their absorption and transformation mainly depend on the beneficial bacteria in the human gut. Numerous studies have shown that ginsenosides can enhance the killing and phagocytosis of tumour cells by positively regulating the cytokine production of macrophages; ginsenoside Rh2 can significantly increase the activity of T-lymphocytes, significantly inducing the differentiation of M2 macrophages to the M1 phenotype, and ginsenoside Rg3 can act as a cytotoxic activator of NK cells, thus inhibiting the growth of tumour cells. Ginsenosides can induce apoptosis and autophagy. After acting on cancer cells, some ginsenosides Rh2, Rg3, PPD, etc. can up-regulate the expression

of pro-apoptotic proteins of Bcl-2 family members, and down-regulate the expression of anti-apoptotic proteins Bcl-XL and Bcl-2, leading to mitochondrial dysfunction and decreased mitochondrial transmembrane potential. The release of cytochrome C activates Caspase-9, thus initiating cell apoptosis. Ginsenoside Rh2 can increase LC3, the signature protein of autophagy in B16 cells, and inhibit melanoma by inducing autophagy in B16 cells. Ginsenosides can inhibit the proliferation of lung cancer cells, induce apoptosis and autophagy, and inhibit the invasion and migration of lung cancer cells, so as to play a certain therapeutic role in non-small cell lung cancer. However, there are still some areas of ginsenosides that need further development and research. For example, the molecular mechanism of ginsenosides in anti-tumour effects has not been fully clarified, and how they can be used in combination with other drugs is still being explored. In addition, the bioavailability of ginsenosides also still needs to be improved, as mentioned above by changing the chemical structure of ginsenosides in addition to improving the biological flora of the human intestinal tract, among other things. The study of ginsenosides is very promising, and with the concerted efforts of many researchers, ginsenosides will surely play an important role in the treatment of cancer and benefit mankind.

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