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The mechanism of reversal of multidrug resistance in cancer by a Chinese medicine monomer named Zhebeimu

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Abstract. Chemotherapy is one of the commonly used means of tumour treatment, but the occurrence of tumour multidrug resistance (MDR) has added great difficulties to tumour treatment and caused serious physical and psychological harm to patients. Modern pharmacological studies have shown that Zhebeimu (***Fritillaria thunbergii Miq***, ZBM) has the effect of reversing the drug resistance of tumour cells, and it is commonly used in the study of various malignant tumours, such as leukaemia, breast cancer and hepatocellular carcinoma. By analysing and summarizing the chemical constituents of ZBM and their related studies on reversal of tumour cell resistance in recent years, the article concludes that the chemical constituents in ZBM can achieve their reversal of drug resistance in a variety of tumour cells by inhibiting the expression of P-glycoprotein (P-gp), decreasing the exocytosis of drugs, increasing the concentration of intracellular drugs, modulating the ROS, and other mechanisms. At present, there is a relative lack of research and treatment of tumour MDR in clinical practice, and the search for reversal agents and reversal strategies for tumour MDR has become an urgent problem, and this paper provides an in-depth study of ZBM, which provides a reliable basis for subsequent application in clinical practice.

Keywords: Tumour, multidrug resistance, Zhebeimu, Chinese medicine monomer.

1. Introduction

According to the 2020 statistical report on global cancer by the International Agency for Research on Cancer (IARC) under the World Health Organisation, in 2020 there will be 19.3 million new cancer cases and nearly 10 million deaths from cancer, with 4.57 million new cancer cases and 3 million deaths in China. It is predicted that in 2050, there will be more than 27 million new patients with malignant tumours and 17.1 million deaths [1]. The frequent occurrence of malignant tumours affects people's lives, and for tumours, chemotherapy is the most important treatment today [2]. The most common problem during treatment is multidrug resistance (MDR) of tumours, which is one of the most significant problems leading to poor efficacy in the later stages of treatment. Multidrug resistance in tumours is an adaptive change of tumour cells against chemotherapy. The mechanism of its formation is complex and varied, including not only the high expression of transmembrane transporter proteins, apoptosis abnormality, abnormalities in the body's enzyme system, but also the microenvironment where the tumour is located, such as hypoxia, cytokines and so on, which is the result of the joint action of a variety of mechanisms. Conventional reversal drugs generally have the disadvantages of high toxicity, single

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target and high price, while more and more evidence shows that the active ingredients in Chinese medicine can significantly improve the sensitivity of chemotherapy and can be used as chemotherapy sensitisers. The active ingredients in Chinese medicines are inexpensive, highly effective, low toxicity and multi-targeted. Chinese medicines have demonstrated unique advantages in improving the efficacy of chemotherapeutic drugs, reducing the adverse effects of chemotherapy and improving the prognosis, therefore, their role in reversing the drug resistance of tumour cells is receiving more and more attention. ZBM is a perennial herbaceous plant of the lily family, named for its main production area in Zhejiang Province. Modern medical research has found that the main components of Fritillaria thunbergii include alkaloids, polysaccharides, flavonoids, saponins and volatile oils, etc. It has the functions of cough expectorant and asthma, analgesic and anti-inflammatory, anti-ulcer, antibacterial, antioxidant, anti-tumour and reversal of tumour multidrug resistance [3].

In this paper, the chemical constituents of Fructus zeylanica and their related studies on reversing the drug resistance of tumour cells will be reviewed, in order to provide a reference for further research and development of the natural active constituents of Fructus zeylanica as an effective reversal agent of tumour drug resistance for clinical application.

2. Mechanisms associated with multidrug resistance

2.1. Glycoprotein (P-gp) and MDR

In 1976, Ling et al. first discovered a P-gp encoded by MDR-1 (multidrug resistance gene) that can regulate the permeability of cell membranes in a colchicine-resistant Chinese hamster ovary cell line. Its relative molecular weight is 170 kd and it belongs to the ATP-binding family of transporters [4]. The physiological function of P-gp is to pump the toxic products out of the cell under the energy of ATP, and play a protective role for the tissues and cells. P-gp possesses 4 relatively independent structural domains, 2 nucleotide-binding domains (NBD) and 2 transmembrane structural domains (TMD), which interact with lipophilic drugs that are either neutral or positively charged, thereby stimulating the ATPase activity of P-gp,and the energy released from the hydrolysis of 2 ATP is used for the transport of 1 drug molecule. energy for the translocation of 1 drug molecule, transporting the drug directly from the lipid bilayer to the outside of the cell, decreasing the intracellular concentration of the drug and thus giving rise to tumour multidrug resistance. It has been revealed that the level of P-gp glycoprotein on the cell membrane is negatively correlated with drug sensitivity and the degree of intracellular drug accumulation, suggesting that this protein is related to the accumulation of drugs in the cell. It is now believed that P-gp is a drug pump, which can pump multiple drugs out of the cell and reduce intracellular drug accumulation, thus weakening the cytotoxicity of drugs and generating drug resistance [5].

2.2. Multidrug resistance-associated protein (MRP) and MDR

In 1992, Cole et al. discovered that the mrp gene encoded a transmembrane glycoprotein in the H69AR-resistant strain of human small cell lung cancer, called MRP, with a molecular weight of 190 kd, which also belongs to the class of ATP-dependent transmembrane transporter proteins. The relative mechanisms of the MDR of MRP are similar to and different from those of P-gp, with similarities being that they both rely on the supply of ATP for the pumping of drugs out of the cell, and the differences being that MRP either binds to or co-transports with GSH, altering the intracellular distribution of the drug to lower the content of the drug in the nucleus, thus reducing the absolute concentration of the drug at the DNA target site; altering the pH in the cytoplasm or organelle by forming CL channels or changing the activity of the channels, whereas a decrease in pH in the tumour cell will lead to large exocytosis of the protonated drug. In addition, MRP can also affect the distribution of drugs in the cell, so that the drugs are confined to perinuclear vesicles, preventing the drugs from entering the nucleus to exert cytotoxic effects [6].

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2.3. Lung cancer drug resistance protein (LRP) and MDR

In 1993, Scheffer found that small cell lung cancer SW-1573/2R120 has LRP gene encoded by LRP, and its relative molecular weight is 110 kd. Subsequently, Scheper et al. in 1995, when analysing the cDNA sequence of LRP gene, they found that the amino acid sequence of LRP and rat MVP (fornix protein) have 87.7% homology, which inferred that LRP is human MVP [7]. It was deduced that LRP is the human MVP. Most of the fornix body is located in the cytoplasm, and a small amount of it is located near the nuclear pore, which is connected with the nuclear membrane to form a nuclear pore complex, and is involved in the transport of materials between the nucleus and the cytoplasm of the cell. Vomeronasal bodies are composed of three proteins, MVP, vPARP, TEP1, and vRNA, of which MVP has the highest content and is the main component of the vomeronasal body. lRP is the main component of MVP, which prevents drugs targeting the nucleus from entering the nucleus through the nuclear pore and transports drugs entering the cytoplasm into transport vesicles, which are then discharged by cytosolic emesis, thus affecting the intracellular drug transport and distribution. This affects intracellular drug transport and distribution, resulting in a decrease in the concentration of the target drug, but is also dependent on ATP for energy.

3. Main bioactive substances of ZBM

ZBM was first listed in the 1963 edition of the Pharmacopoeia of the People's Republic of China, and its main constituents are alkaloids, sugars, flavonoids, terpenoids, volatile components and trace elements, etc. Among them, alkaloids are considered to be the main active ingredients. Among them, alkaloids are regarded as the main active ingredients of ZBM. The main medicinal part of ZBM is the dried bulb, and phytochemical studies have shown that, compared with the bulb of ZBM, the flowers, stems and leaves of ZBM also contain flavonoids, essential oils, saponins, alkaloids, and other chemical constituents [8].

3.1. Alkaloids

Alkaloids are the main active ingredients in ZBM, and their content can be used as the quality control index of ZBM. The 2020 Edition of Chinese Pharmacopoeia defines peimine and peiminine as the quality markers of ZBM, and requires that the overall amount of peimine and peiminine should greater than or equal to 0.080%.Alkaloids are one of the main chemical constituents in ZBM, which are classified into two major groups, namely, isosteroidal alkaloids and steroidal alkaloids, among which, the isosteroidal alkaloids include peimisine, peimine, peiminine, etc., and the steroidal alkaloids include Spirosol-5-en-3-yl acetate, Solasodine 3-b-D-glucopyranoside, (3beta, 12alpha)-3, 12-Dihydroxysolanid-5-en-1-one, etc [9].

3.2. Flavonoids

ZBM also contains a small amount of flavonoid components, mainly including quercetin, kaempferol and its glycosides. Cui et al. identified 16 flavonoid components in ZBM using high-resolution liquid-mass spectrometry, and identified 16 flavonoid components, such as quercetin, kaempferol, quercetin-7-O-rutinoside and kaempferol-7-O-β-D-glucose, etc [10]. The representative components are quercetin and kaempferol analogues, which have strong antioxidant effects. The representative components are kaempferol compounds and quercetin, which have strong antioxidant effects. Due to their antioxidant properties, they protect cells from oxidative stress, inflammation and DNA damage, and inhibit the growth of a wide range of tumour cells by blocking cell cycle progression and tumour cell proliferation as well as inducing apoptosiss.

3.3. Sugar components

At present, the study of sugar components in ZBM is rarely reported. It was found that the sugar components in ZBM are mainly monosaccharides, disaccharides and polysaccharides, of which the monosaccharide components are glucose, fructose, xylose and gulose, etc., and the disaccharides are fibre disaccharides, gentiobiosaccharides, sucrose, etc. The content of starch is the most in

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polysaccharides, accounting for about 80% of its total dry weight. With regard to biological activity, polysaccharides with rich structural information are involved in a wide range of physiological metabolic activities, like antiviral, anti-inflammatory, antioxidant, anti-tumour and regulation of the immune system and other biological activities. It has also been found that polysaccharides have certain targeting effects, which means that they can selectively interact with specific functional molecular targets in the body to exert their pharmacological effects [11].

4. Reversal of MDR by ZBM

4.1. Alkaloid regulation of protein expression

The alkaloids of ZBM are able to reverse drug resistance in many kinds of tumour cells by regulating the expression of P-gp, MRP, LRP and other proteins, as well as reducing the drug efflux and increasing the concentration of drug in the cell.

In leukaemia, Hu et al. found that in K562/A02, a drug-resistant cell line with elevated P-gp as the main resistance mechanism, and in HL-60/ADR, a drug-resistant cell line with elevated MRP as the main resistance mechanism, the reversal folds of bemethoate against the above two types of tumour cells with different resistance mechanisms were about 5.7 and 5.6 respectively [12].

Wang et al. studied the inhibitory effect of peiminine in reversing the MDR of gastric cancer cells and enhancing the inhibitory effect of chemotherapeutic drugs on the transplanted tumour of multidrug-resistant nude mice with gastric cancer, found that the transplanted tumour of the nude mice in the combination group grew slowly [13]. The average volume of the tumour in the group of adriamycin was (0.66±0.42) cm^3 at the end of the treatment, significantly lower than that of the model group with the average volume of the tumour of (1.96±0.48) cm^3 ($P<0.01$), and the average volume of the tumour of the group of adriamycin was (0.96±0.65) cm^3, significantly different from that of the model group ($P<0.05$). The mean volume of tumour in the adriamycin group was (0.96±0.65) cm^3, which differed greatly from that of the model group ($P<0.05$), suggesting that peiminine could enhance the reactiveness of gastric cancer drug-resistant cellular transplantation tumour to adriamycin, and significantly restrain the growth of gastric cancer drug-resistant transplantation tumour, and the mechanism of its action might be related to the decrease of P-gp expression and the elevation of CleavedCaspase-3 expression.

Another study showed that peimine and peiminine could inhibit the proliferation of gastric cancer parental cell line SGC-7901 and drug-resistant control group SGC-7901/VCR to different degrees, and peimine had more potential to reverse the drug resistance in gastric cancer cells, the researchers used flow cytometry to detect the mean fluorescence intensity of adriamycin in the cells of SGC-7901 and drug-resistant cell line SGC-7901/VCR, and found that after peimine (20 μg/mL) was combined with adriamycin, the average fluorescence intensity of adriamycin in SGC-7901/VCR cells was increased. The researchers used flow cytometry to detect the average fluorescence intensity of adriamycin in the gastric cancer parental cell line SGC-7901 and drug-resistant cell line SGC-7901/VCR, and found that the fluorescence intensity of SGC-7901/VCR cells on average increased by 2.33-fold after peiminine (20 μg/mL) and adriamycin were combined, indicating that peiminine could obviously restore the ability of the drug-resistant cell line in extracting and accumulating adriamycin, and thus showed that peiminine could improve the responsiveness of the SGC-7901/VCR cells to the antitumour drug, and the potential of SGC-7901/VCR cells to resist to the drug. This shows that peiminine can improve the sensitivity of SGC-7901/VCR cells to anti-tumour drugs, and has an obvious reversal effect on the drug resistance of tumour cells by reducing the expression of P-gp, decreasing intracellular drug efflux and inducing apoptosis [14, 15].

4.2. Alkaloid regulation of intracellular reactive oxygen species (ROS)

A large amount of intracellular reactive ROS production or a decrease in the function of the antioxidant protection system can lead to a malfunction of the oxidation-reduction state, resulting in oxidative damage and even death of cancer cells. Qi et al. uncovered that peimine (PM) significantly suppressed the proliferation of human chronic myelogenous leukaemia K562 cells in a concentration-dependent

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manner [16]. In addition, it was found that PM induced an increase in ROS levels and significantly decreased the expression of the antioxidant GSH in K562 cells as detected by DCFH2-DA fluorescent probe. Further pretreatment of K562 cells with the ROS scavenger NAC revealed that NAC hindered the inhibition of K562 cell proliferation by PM. The above results suggest that PM, an alkaloid in ZBM, is capable of inhibiting the proliferation of K562 cells and suppress tumour value-addition by up-regulating the expression of intracellular ROS and down-regulating the expressed volume of GSH, which imbalances the intracellular oxidation-reduction state. Recent studies have shown that a variety of ROS inducers can down-regulate P-gp expression. However, excessive concentration and high concentration of ROS will cause cellular stress response, which will instead lead to the upregulation of P-gp. Therefore, when ROS inducers are used as MDR reversal agents to inhibit P-gp, they should be used in lower doses as much as possible under the premise of ensuring reversal activity, so that the intracellular level of ROS will not exceed the physiological level by too much, resulting in the oxidative stress of the cells, and this will also just be able to reduce the toxicity and adverse effects of reversal agents on normal cells [17, 18].

5. Prospects of ZBM active ingredients in anti-tumour applications

Herbal monomers are the active ingredients in herbal medicines and are an important source for reversing tumour MDR. Many herbal monomer components including alkaloids, flavonoids, terpenoids, etc. are known to have been reported to have pharmacological activity in reversing tumour resistance. And compared with chemical synthetic drugs such as verapamil and cyclosporin A, they are highly efficient, low toxic and multi-targeted, which are valuable for the development of drug resistance inhibitors.

Chen et al. found that in hepatocellular carcinoma, quercetin could elevate the efficacy of the conventional chemotherapeutic agent 5-fluorouracil in the human hepatocellular carcinoma drug-resistant cell line BEL-7402 by blocking the FZD7/β-catenin signalling pathway [19]. In breast cancer, quercetin reversed resistance to tamoxifen in breast cancer cells through two mechanisms: it increased estrogen receptor expression to enhance sensitivity to tamoxifen, and it reduced P-glycoprotein expression while preventing Y-box binding protein 1 nuclear translocation, thereby countering multidrug resistance and cancer stem cell formation in breast cancer.Quercetin possesses the potential to act as an effective reversal agent of tumour drug resistance and has a promising clinical application. Not only that, quercetin is a representative component of the flavonoid constituents in ZBM, and there has been no report on the reversal of tumour cell resistance by quercetin in ZBM, and its effect on tumour drug resistance needs to be further investigated [20, 21].

6. Conclusion

In this paper, the tumour reverse drug resistance effect of ZBM was analysed, and it was concluded that peiminine and peiminine, the main active constituent alkaloids in ZBM, play a major role in reversing drug-resistance function in tumours, either through decreasing the effluence of the drugs, reducing the expression of the relevant proteins, or modulating the oxidative stress. Therefore, there are great research prospects in the treatment of tumours with TCM monomers and in the search for more appropriate reversal agents for clinical multidrug resistance.

Although the treatment of TCM has many advantages, its own limitations should not be ignored. Firstly, the active ingredients of TCM are complex, and most of them are compound preparations in clinical use, so it is difficult to elucidate the specific mechanism. Furthermore, in clinical practice, the treatment of TCM lacks clinical studies with high evidence-based medical evidence, no matter single drug or compound study, it is not possible to unify the intervention time and specific drugs of TCM, and it is not possible to reach the standard of first-line and second-line medication such as in western medicine, so most of the studies related to the therapeutic resistance of TCM to tumour treatment are only stayed in the cellular and animal experiments.

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