The Effect of Psoralen on Reversing GST-\( \pi \) Mediated Multidrug Resistance

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Abstract

**Objective:** Glutathione S-transferase \( \pi \) (GST-\( \pi \)) plays a very important role in resisting to tumor chemotherapy. This study investigates the role of Psoralen in reversing GST-\( \pi \) mediated multidrug resistance (MDR), and the possible mechanism in MCF-7/ADR cells.

**Methods:** We measured the cell viability by CCK-8 assay to evaluate the cytotoxicity and multidrug resistance (MDR) reversal activity of Psoralen. To examine the alteration in targeted gene, RT-PCR was used to detect the expression of GST-\( \pi \). Western blot was used to analyze the protein level of GST-\( \pi \). Immunofluorescence method was applied to observe the activation of NF-\( \kappa \)B.

**Results:** The intracellular adriamycin drug concentration increased significantly after Psoralen treatment. Compared with those of the control group, Psoralen reduced the expression of GST-\( \pi \) at the mRNA and protein level in treatment group. The NF-\( \kappa \)B inhibitor (SN50) can significantly inhibit the expression of GST-\( \pi \) in breast cancer MCF-7/ADR cells.

**Conclusions:** Therefore, NF-\( \kappa \)B signaling pathway may be one of the mechanisms of GST-\( \pi \) mediated multidrug resistance. Our results showed that Psoralen was involved in reversing GST-\( \pi \) mediated MDR. GST-\( \pi \) mediated drug resisting mechanism may be related to the NF-\( \kappa \)B signaling pathways, and it may be the key factor of downstream in the NF-\( \kappa \)B signaling pathways.

Keywords: GST-\( \pi \); NF-\( \kappa \)B; Multidrug resistance; Breast cancer; Psoralen

Introduction

Breast cancer is considered to be a systemic disease, and is the most frequently diagnosed female malignancy in the world, which could cause serious damage to women’s health [1]. At present, the main treatments for breast cancer include chemotherapy, surgery, radiotherapy, hormonal therapy and targeted antibodies [2]. Despite advances in breast cancer treatment, the multidrug resistance (MDR) continues to be the leading cause of cancer-related deaths [3]. It is very important to explore the molecular basis of MDR and developed clinical reagents or strategies to prevent the occurrence of resistance.

There are a number of mechanisms that induce cancer cells to become nonsensitive to cytotoxic drugs, such as mutation or overexpression of the drug’s target, drug inactivation, or efflux of the drug from the cell [4]. Several evidence strongly supported that the excessive expression of GSTs is one of the primary resistance mechanisms [5]. The GST family of enzymes is divided into several classes including \( \alpha \), \( \mu \), \( \pi \), \( \delta \), etc. [6]. The effect of GST-\( \pi \) involved in detoxification has been suggested as one of the mechanisms that contribute to drug resistance [7]. Batist’s study found that the expression of GST-\( \pi \) in breast cancer drug resistant cell lines MCF-7/ADR cell was high, enzyme showed its activity as 45 folds as the original cell lines [8]. At present, studies have shown that using GST-\( \pi \) inhibitors to reverse the GST-\( \pi \) mediated MDR may be feasible for improving the efficiency of chemotherapeutic agents as well as the pharmacokinetic and pharmacodynamic profiles [9]. So far, specific reversal agents for GST-\( \pi \) are rare, ethacrynic acid emerged as one of the most accepted and studied agents in the world [10]. However, the diuresis, metabolic disorders and severe allergic reactions limit its clinic application [11]. So it is necessary to find suitable inhibitors to overcome GST-\( \pi \) mediate MDR.

Psoralen is a natural product present in several plant families [12], and it is a major active component of *Psoralea corylifolia* [13]. Studies have shown a broad spectrum of biological activities, including cytotoxicity, phytotoxicity, etc. [14]. Modern studies have shown that Psoralen has a significant inhibitory effect on tumor growth in a variety of animals and humans [15]. In the present study, we analyzed Psoralen showed potent cell cytotoxicity to MCF-7/ADR cells, Psoralen has identified that it plays an anti-tumor effect on MCF-7/ADR in vitro cell cytotoxicity [16]. And it enhances the sensitivity of MCF-7/ADR to adriamycin, which is consistent with previous studies conducted in other human cancer cell lines [17].

Previous studies in our laboratory have found that Psoralen can actually reverse breast cancer MCF-7/ADR cell [16,18]. The effect of Psoralen on reversing MDR was clear; however, the mechanism was not clear. We have found that Psoralen did not reduce the expression of MDR1, MRP and LRP, also we found that it can significantly reduce the expression of GST-\( \pi \), an obvious effect on gene and protein level. We suspected the reversal MDR mechanism may be related to GST-\( \pi \). We put forward a hypothesis that Psoralen can work as an inhibitor...
of GST-π. Then we carried out further study to determine the possible relevant regulatory pathways.

Materials and Methods

Reagents

Psoralen was supplied by Baoji Herbest Bio-Tech (Baoji, China). A cell culture reagent was supplied by Gibco Life Technology (Grand Island, NY, USA). Adriamycin was supplied by KeyGen Biotech Co. Ltd. (Nanjing, China). β-actin antibody was obtained from Proteintech (ProteinTech Group, CHI, USA). Antibodies specific for the NF-κB, and anti-rabbit IgG, HRP-linked antibody were purchased from Abcam (Cambridge, MA, USA). RevertAid First Strand cDNA Synthesis Kit and RealMaster Mix (SYBR-Green) were supplied by Thermo Scientific (Waltham, MA, USA). TRIzol were obtained from Sigma (St. Louis, MO, USA).

Cell lines

Cell lines were supplied by KeyGen Biological Technology Development (Nanjing, China).

Cell culture

We use the RPMI-1640 medium (KGM31800S-S500) to culture MCF-7 and MCF-7/ADR cells, the medium contains 10% fetal bovine serum (heat-inactivated serum), and in order to avoid bacterial contamination 100 units/mL penicillin and 100 units/mL streptomycin were contained. Cells were cultured in a humidified atmosphere contained 5% CO2 at 37°C incubator. MCF-7/ADR cells were cultured in the medium containing 1 μg/mL Adriamycin (Nanjing, China) to keep the multidrug resistance character. The culture medium was discarded, after 2 times PBS washed changed new medium for every 48 h.

Analysis of cell viability

The effect concentrations of Psoralen and SN50 were determined by CCK-8. We divided the experimental group into four groups (MCF-7/ADR, MCF-7/ADR+P, MCF-7/ADR+SN50, MCF-7/ADR+P+SN50). Our preliminary experiment was to determine the concentration of Psoralen and apply it to the later experiment, the concentration of SN50 (18 μmol/L) [19] and Psoralen (8 µg/mL) [16] had no effect on cell proliferation (P>0.05). CCK-8 results showed that the SN50 (18 μmol/L) [19] and Psoralen (8 µg/mL) [16] had no effect on cell proliferation (P>0.05). CCK-8 assay demonstrated that Psoralen can inhibit the proliferation of MCF-7/ADR cells in a dose-dependent manner, which was consistent with previous research. The MDR reversal ability of Psoralen at a concentration of 8 µg/mL and 16 µg/mL on the MCF-7/ADR cells were investigated. Adriamycin IC50 in different treatment groups has different consequences. Respectively, MCF-7/ADR=85.15 ± 2.16 µg/mL, MCF-7/ADR+P=54.55 ± 3.10 µg/mL, MCF-7/ADR+SN50=42.36 ± 1.19 µg/mL, MCF-7/ADR+P+SN50=29.55 ± 2.19 µg/mL. Psoralen and SN50 can increase the toxicity of Adriamycin on MCF-7/ADR cells (Figure 1).

Statistical analyses

Statistical Package for the Social Sciences (SPSS) software version 17.0 was used for statistical analysis. All numerical results were expressed as means ± standard error of the mean. For comparison between two groups, statistical significance was assessed using Student’s t test and P<0.05 was considered to be significant. All data were calculated from three independent experiments.

Results

Comparison of different treatment group in adriamycin IC50

CCK-8 results showed that the SN50 (18 µmol/L) [19] and Psoralen (8 µg/mL) [16] had no effect on cell proliferation (P>0.05). CCK-8 assay demonstrated that Psoralen can inhibit the proliferation of MCF-7/ADR cells in a dose-dependent manner, which was consistent with previous research. The MDR reversal ability of Psoralen at a concentration of 8 µg/mL and 16 µg/mL on the MCF-7/ADR cells were investigated. Adriamycin IC50 in different treatment groups has different consequences. Respectively, MCF-7/ADR=85.15 ± 2.16 µg/mL, MCF-7/ADR+P=54.55 ± 3.10 µg/mL, MCF-7/ADR+SN50=42.36 ± 1.19 µg/mL, MCF-7/ADR+P+SN50=29.55 ± 2.19 µg/mL. Psoralen and SN50 can increase the toxicity of Adriamycin on MCF-7/ADR cells (Figure 1).

Psoralen down regulates GST-π expression at both mRNA and protein levels

The GST-π expression was investigated by PCR and Western blot analysis.
blotting. Therefore, the concentration of 4 µg/mL, 8 µg/mL, 12 µg/mL and 16 µg/mL were chosen as the working concentration in the subsequent experiments. The GST-π mediated MDR reversal of Psoralen at different concentrations on the MCF-7/ADR cells was investigated by Western blotting both gene and protein level expression were significantly down-regulated in MCF-7/ADR+Psoralen cells.

**Immunofluorescence detecting NF-κB activation was inhibited by Psoralen**

The NF-κB activation was investigated by immunofluorescence and the data in Figure 2 demonstrated that Psoralen inhibited the nuclear translocation of NF-κB. The nucleus NF-κB expression was significantly down-regulated in MCF-7/ADR+Psoralen cells. These results suggested the MDR reversal effect of Psoralen possibly by inhibiting NF-κB activation.

**RT-PCR was used to detect the expression of GST-π**

RT-PCR analysis was performed to determine the effect of Psoralen on the gene expression of GST-π. Treatment of MCF-7/ADR cells with Psoralen or SN50 can both suppress the mRNA level of GST-π.SN50 as a specific inhibitor lead an obvious inhibitory action than Psoralen worked (Figure 3).

**Western blotting was used to detect the expression of NF-κB in the nuclear**

Protein concentration in the nuclear extracts was evaluated according to the Bradford protein assay. Nuclear extracts were stored at the following WB analysis. The expression of NF-κB in the nuclear between different treatment groups was very obvious (Figure 4). The expression of NF-κB in the nuclear in the group treated with Psoralen (8 µg/mL) was reduced indeed. The effect of Psoralen is very obvious, which was consistent with the result in the immunofluorescence.

**Discussion**

The aim of this study was to characterize the mechanism of Psoralen on reversing GST-π mediated multidrug resistance on MCF-7/ADR cells. Previous research in our laboratory found that Psoralen can reverse MDR but did not reduce the expression of MDR1, MRP and LRP, so we need to explore the reversal MDR mechanism [16,18]. In this study, we found that the expression of GST-π was inhibited after being processed for 48 hours (We found there were no difference after treated with Psoralen between 24 h and 48 h) with Psoralen. These results further suggested that the co-administration of Psoralen with anticancer drugs could enhance the intracellular concentration of chemo-therapies by inhibiting the expression of GST-π, and Psoralen can be used as an inhibitor or a reversal agent of GST-π. The effect of Psoralen on GST-π is not dose-dependent and may be influenced by other pathways; Psoralen may affect cell proliferation through the cell cycle. Further studies and discussions are needed to be done; this is also the shortcoming of our experiment.

All of the members of the highly diverse GST super family are capable of binding the tripeptide glutathione [20]. GST-π is a member of phase II detoxification enzymes families involved in metabolizing for chemotherapeutic agents [21]. High expression of GST-π and the nuclear localization of GST-π are known to correlate with the aggressiveness of tumor cells and the poor survival rate of patients [22]. Several lines of evidence indicate that the effect of GST-π in tumor cells is not on cell proliferation but the acquisition of resistance...
to anticancer drugs [23]. Kamada approached the function of GST-π under the oxidative stress condition caused by the hydrogen peroxide treatment [24]. He found that GST-π prevented apoptosis by reducing DNA damage [24]. In our experiment, we found that the expression of GST-π in experimental group treated with Psoralen was decreased significantly. There was evidence showing that Psoralen could significantly reduce the expression of NF-κB. The NF-κB activation was investigated by immunofluorescence and the data was consistent with previous research [16]. After using of NF-κB inhibitor SN50 in MCF-7/ADR cells, the expression of GST-π in the experimental group was lower than that in normal control group. We propose that GST-π may be the key factor in signaling pathway downstream of NF-κB. Subsequent results proved the hypothesis was established.

NF-κB signaling pathway is a kind of important transcription factor involved in stress response and immune cells activation, proliferation, differentiation, apoptosis and many genes which regulate the occurrence and progress of tumor [25]. NF-κB exists in almost all cell cytoplasm, only when it is activated from the cytoplasm to nucleus can it plays an important role [26]. In recent years the study found that the aberrant activation of NF-κB was closely related with the drug resistance of tumor [27]. According to a new study, using the NF-κB inhibitors can make the traditional antitumor treatment more effective [28]. It was shown by our previous experiments that Psoralen can reduce the NF-κB expression in the cell nucleus, strong evidence that Psoralen can reduce the transport of NF-κB from cytoplasm into nucleus.

In terms of anti-tumor, the role of traditional Chinese medicine which has less side effects aroused people’s attention in recent years. This study aims to develop new anti-tumor drugs to fight the resistance of tumor cells. The experiment also has many shortcomings, such as no in vivo experiments and requires further study.

Conclusion

In conclusion, our studies provided evidence that the level of GST-π mediated MDR were significantly reduced by Psoralen in MCF-7/ADR cell by suppressing the activation of NF-κB signaling pathways. This ability of Psoralen could make Psoralen act as a chemosensitizer, which reverses MDR of cancer cells induced by chemotherapy through an efficient pathway, thus provides a new opportunity for the treatment of breast tumors.

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Conflicts of Interest

We declare that we have no conflicts of interest.

References

1. Ryu EA, Lee HJ, Yoon TI, Lee ES, Lee SJ (2016) Breast cancer-specific mortality in small-sized tumor with node-positive breast cancer: a nation-wide study in Korean breast cancer society. Breast Cancer Res Treat 159: 489-498.
2. Yezhelyev M, Yacoub R, O’Regan R (2009) Inorganic nanoparticles for predictive oncotherapy of breast cancer. Nanomedicine (Lond) 4: 83-103.
3. Wu S, Wen F, Li Y, Gao X, He S, et al. (2016) PIK3CA and PIK3CB silencing by RNAi reverse MDR and inhibit tumorigenic properties in human colorectal carcinoma. Tumour Biol 37: 8799-8809.
4. Vitchez C, Jacobs WJ (2014) Resistance to Isoniazid and Ethionamide in Mycobacterium tuberculosis: Genes, Mutations, and Causalities. Microbiol Spectr 2: M2GM-0014-2013.
5. Zhu B, Liu GT, Zhao YM, Wu RS, Strada SJ (2006) Chemosensitizing multiple drug resistance of human carcinoma by Bicyclol involves attenuated p-glycoprotein, GST-P and Bip-2. Cancer Biol Ther 5: 536-543.
6. Fiocchi G, Zanni E, Cialfi S, Aurtzi C, Bicocati G, et al. (2016) Glutathione S-transferase -subunit as a phenotypic suppressor of p17Delta strain, the Kuyveromyces lactis model for Hailey-Hailey disease. Biochim Biophys Acta 1863: 2650-2657.
7. Leone G, Gelaleti GB, Jardim BV, Moschetta MG, Regiani VR, et al. (2014) Expression of glutathione, glutathione peroxidase and glutathione S-transferase pi in canine mammary tumors. BMC Vet Res 10: 49.
8. Alpert LC, Scheder RL, Berry DA, Melnychuk D, Peters WP, et al. (1997) Relation of glutathione S-transferase alpha and mu isoforms to responses to therapy in human breast cancer. Clin Cancer Res 3: 661-667.
9. Yan F, Jiang Y, Li YM, Zhen X, Cen J, et al. (2008) Reversal of P-glycoprotein and multidrug resistance-associated protein 1 mediated multidrug resistance in cancer cells by H2O2 Isomers, tetraaldehydroquinolin derivatives. Biol Pharm Bull 31: 1258-1264.
10. Awasthi S, Srivastava SK, Ahmad F, Ahmad H, Ansari GA (1993) Interactions of glutathione S-transferase-pi with ethylic acid and its glutathione conjugate. Biochim Biophys Acta 1164: 173-179.
11. Fletcher ME, Boshier PR, Wakabayashi K, Keun HC, Smolenski RT, et al. (2015) Influence of glutathione-S-transferase (GST) inhibition on lung epithelial cell injury: role of oxidative stress and metabolism. Am J Physiol Lung Cell Mol Physiol 308: L1274-L1285.
12. Zhang YT, Shen LN, Zhao JH, Feng NP (2014) Evaluation of psoralen ethosomes for topical delivery in rats by using in vivo microdialysis. Int J Nanomedicine 9: 669-678.
13. Jiang Z, Xie ZJ (2014) Induction of apoptosis in human hepatocarcinoma SMMC-7721 cells in vitro by psoralen from Psoralea corylifolia. Cell Bichem Biophys 70: 1075-1081.
14. Liu Y, Zhang L, Liao Y, Wang Y (2015) Binding characteristics of psoralen with tropsin: Insights from spectroscopic and molecular modeling studies. Spectrochim Acta A Mol Biomol Spectrosc 151: 498-505.
15. Prabha C, Maheshwari DK, Bajpai VK (2013) Diverse role of fast growing rhizobia in growth promotion and enhancement of psoralen content in Psoralea corylifolia L. Pharmacogn Mag 9: S57-565.
16. Wang X, Cheng K, Han Y, Zhang G, Dong J, et al. (2016) Effects of Psoralen as an Anti-tumor Agent in Human Breast Cancer MCF-7/ADR Cells. Biol Pharm Bull 39: 815-822.
17. Ming-Ju H, Mu-Kuan C, Ya-Yen Y, Gwo-Tarng S, Hui-Ling C(2014) Psoralen reversed doxetaxel-induced multidrug resistance in A549/D16 human lung cancer cells lines. Phytomedicine 21: 970-977.
18. Jiang J, Wang X, Cheng K, Zhao W, Hua Y, et al. (2016) Psoralen reverses the p-glycoprotein-mediated multidrug resistance in human breast cancer MCF-7/ADR cells. Mol Med Rep 13: 4745-4750.
19. Zhu BS, Xing GC, Lin F, Fan XQ, Zhao K, et al. (2011) Blocking NF-κB kappab nuclear translocation leads to p53-related autophagy activation and cell apoptosis. World J Gastroenterol 17: 478-487.
20. Ouaisii A, Ouaisii M, Sereno D (2002) Glutathione S-transferases and related proteins from pathogenic human parasites behave as immunomodulatory factors. Immunol Lett 81: 159-164.
21. Tao NN, Zhou HZ, Tang H, Cai XF, Zhang WL, et al. (2016) Sirutin3 enhanced drug sensitivity of human hepatoma cells through glutathione S-transferase pi 1/DNK signaling pathway. Oncotarget 7:50117-50130.
22. Ali-Osman F, Brunner JM, Kutuk TM, Hess K (1997) Prognostic significance of glutathione S-transferase expression and subcellular localization in human gliomas. Clin Cancer Res 3: 2253-2261.
23. Goto S, Hara Y, Uraya Y, Uzumi S, Abe K, et al. (2001) Doxorubicin-induced DNA intercalation and scavenging by nuclear glutathione S-transferase pi. FASEB J 15: 2702-2714.
24. Kamada K, Goto S, Okunaga T, Ibara Y, Tsuji K, et al. (2004) Nuclear glutathione S-transferase pi prevents apoptosis by reducing the oxidative...
stress-induced formation of exocyclic DNA products. Free Radic Biol Med 37: 1875-1884.

25. Arranz L, Caamano JH, Lord JM, De la Fuente M (2010) Preserved immune functions and controlled leukocyte oxidative stress in naturally long-lived mice: possible role of nuclear factor kappa B. J Gerontol A Biol Sci Med Sci 65: 941-950.

26. Lee JR, Seok CJ, Kim JS, Chang JM, Seo JW (2001) Expression of NF-kappaB and cytokines in chronic rejection of transplanted murine heart. J Korean Med Sci 16: 397-406.

27. Zhao N, Wang R, Zhou L, Zhu Y, Gong J, et al. (2014) MicroRNA-26b suppresses the NF-kappaB signaling and enhances the chemosensitivity of hepatocellular carcinoma cells by targeting TAK1 and TAB3. Mol Cancer 13: 35.

28. Dong X, Liu F, Li M (2016) Inhibition of nuclear factor kappa B transcription activity drives a synergistic effect of cisplatin and oridonin on HepG2 human hepatocellular carcinoma cells. Anticancer Drugs 27: 286-299.