Biochemical Pharmacology 92 (2014) 220–234

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Wogonin reverses multi-drug resistance of human myelogenous leukemia K562/A02 cells via downregulation of MRP1 expression by inhibiting Nrf2/ARE signaling pathway

Xuefen Xu [a,1], Yi Zhang [a,1], Wei Li [a], Hanchi Miao [a], Haiwei Zhang [a], Yuxin Zhou [a], Zhiyu Li [b], Qidong You [b], Li Zhao [a,*], Qinglong Guo [a,*]

[a] State Key Laboratory of Natural Medicines, Jiangsu Key Laboratory of Carcinogenesis and Intervention, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing 210009, People's Republic of China
[b] School of Pharmacy, China Pharmaceutical University, Nanjing 210009, People's Republic of China

ARTICLE INFO

Article history:
Received 28 July 2014
Accepted 11 September 2014
Available online 28 September 2014

Chemical compounds studied in this article:
Wogonin (PubChem CID: 5281703)
DMSO (PubChem CID: 679)
DAPI (PubChem CID: 2954)
MTT formazan(PubChem CID: 16218671)
Adriamycin (PubChem CID: 31703)
H_2O_2 (PubChem CID: 784)
tBHQ (PubChem CID: 16043)

Keywords:
Wogonin
Reversal effect
MRP1
DNA-PKcs
Nrf2/ARE

ABSTRACT

Constitutive NF-E2-related factor 2 (Nrf2) activation has been recently reported to play a pivotal role in enhancing cell survival and resistance to anticancer drugs in many tumors. Previously, much effort has been devoted to the investigation of blocking Nrf2 function in cultured cells and cancer tissues, but few researches have been undertaken to evaluate the precise mechanism of flavonoids-induced sensitivity by inhibiting Nrf2. In this study, we investigated the reversal effect of Wogonin, a flavonoid isolated from the root of *Scutellaria baicalensis* Georgi, in resistant human myelogenous leukemia. Data indicated that Wogonin had strong reversal potency by inhibiting functional activity and expression of MRP1 at both protein and mRNA in adriamycin (ADR)-induced resistant human myelogenous leukemia K562/A02 cells. Consequently, the inhibition of MRP1 by Wogonin was dependent on Nrf2 through the decreased binding ability of Nrf2 to antioxidant response element (ARE). Further research revealed Wogonin modulated Nrf2 through the reduction of Nrf2mRNA at transcriptional processes rather than RNA degradation, which is regulated by the PI3K/Akt pathway. Moreover, DNA-PKcs was found to be involved in the Wogonin-induced downregulation of Nrf2 mRNA at transcriptional levels. In summary, these results clearly demonstrated the effectiveness of using Wogonin via inhibiting Nrf2 to combat chemoresistance and suggested that Wogonin can be developed into an efficient natural sensitizer for resistant human myelogenous leukemia.

1. Introduction

The most significant cause of treatment failure in chronic myeloid leukemia is the multidrug resistance (MDR) of leukemia cells [1]. One of the most widely studied mechanisms of MDR relies on drug efflux from cancer cells mediated by ATP-binding cassette (ABC) transporters [2,3]. Multidrug-resistance-associated proteins (MRPs) belong to subfamily C of the ABC transporter superfamily (ABCC). As one of the drug transporting MRPs, the 190 kDa MRP1, which is encoded by the MRP1 gene located on chromosome 16p13, has emerged as an important contributor to chemoresistance [4]. Overexpression of MRP1 has been demonstrated to transport a broad range of organic substrates, such as glutathione (GSH) conjugates and other anionic conjugates. In cancer cells, MRP1 can even cause resistance to not only doxorubicin, but also many other widely used chemotherapeutic agents, including daunorubicin, methotrexate (MTX), vincristine and etoposide [5].

Nrf2, a Cap 'n' Collar basic leucine zipper transcription factor, plays a pivotal role in regulating cellular protective response and rapidly changes the sensitivity of the cells to oxidative or electrophilic stresses [6]. Hundreds of cytoprotective and detoxification genes regulated by Nrf2 have been identified, including xenobiotic metabolism enzymes (e.g., NAD(P)H quinone oxidoreductase 1 (NQO1) and aldoketo reductases), encoding antioxidant enzymes (e.g., glutamate cysteine ligase catalytic subunit (GCLC) and heme oxygenase-1 (HO-1)), and several ATP-dependent drug

* Corresponding authors.
E-mail addresses: zhaolicpu@126.com (L. Zhao), anticancer_drug@163.com (Q. Guo).
[1] Contributed equally to this article.

http://dx.doi.org/10.1016/j.bcp.2014.09.008
0006-2952/

efflux pumps (e.g., MRP1, MRP2 and MRP3) [7–10]. Exposure to oxidative and electrophilic stresses, such as reactive oxygen species (ROS), causes Nrf2 activation and translocation to the nucleus [11]. Then Nrf2 binds to ARE to mediate the transcriptional induction of its target genes [10]. Kelch-like ECH-associated protein 1 (Keap1), a major repressor of Nrf2, is a substrate adaptor protein for a Cul3-dependent E3 ubiquitin ligase complex.

Evidence suggested that constitutively high level of Nrf2 promotes cancer cell survival and is responsible for chemoresistance [12]. Inversely, downregulation of the Nrf2-dependent response by overexpression of Keap1 or transient transfection of Nrf2-small interfering RNA (siRNA) increases the efficacy of anticancer drugs [13]. Now Nrf2 is identified as a pharmacological target to overcome therapeutic resistance in various types of cancer with constitutive activation of Nrf2 [14]. In addition, the expression of xenobiotic metabolism genes, and drug efflux proteins are also increased in many Nrf2-activating cancer cells, providing a capacity to detoxify anticancer drugs and enhancing cell proliferation. The recent emerging reports demonstrated that constitutive activation of Nrf2 was necessary for the inducible expression of MRP1 to promote tumorigenesis and chemoresistance in several types of human cancers [15]. For example, it was found that MRP1 was regulated by Nrf2 pathway in small cell lung cancer [16]. Meanwhile, it is considered that Nrf2 activity is regulated by several upstream signaling pathways, including protein kinase C (PKC), phosphoinositol 3-kinase (PI3K), and mitogen-activated protein kinases (MAPKs, p38, ERK1/2 and JNK).

Flavonoids, a diverse family of natural polyphenolic compounds commonly occurring in plants, showed strong anti-proliferative activity against different types of human cancer in vitro and in vivo, and was able to sensitize cancer cells to anticancer drugs [17,18]. Recently, Kweon et al. [19] reported that epigallocatechin 3-gallate (EGCG), the major bioactive polyphenol found in green tea, inhibited HO-1 expression by suppressing Nrf2 in non-small-cell lung cancer (NSCLC) A549 cells. Luteolin (3′,4′,5,7-tetrahydroxyflavone), a flavonoid found in high concentrations in celery, green pepper, parsley, perilla leaf, and chamomile tea, inhibited the Nrf2/ARE signaling, reduced cellular GSH level and enhanced sensitivity of A549 cells to therapeutic drugs [20]. Brusatol inhibited the Nrf2 signaling pathway by reducing the protein level of Nrf2, and sensitized a broad spectrum of cancer cells and A549 xeno-grafts to cisplatin [21]. Although much effort has been devoted to the investigation of blocking Nrf2 function in cultured cells and cancer tissues, few research has been undertaken to evaluate the precise mechanism of flavonoids-induced sensitivity by inhibiting Nrf2.

Wogonin (5,7-dihydroxy-8-methoxyflavone), one of the major flavonoids isolated from the root of *Scutellaria baicalensis* Georgi, is the most promising anticancer candidate [22] due to its antiproliferating, apoptosis-inducing, angiogenesis-inhibiting, cell migration-inhibiting and differentiation-inducing activities. Additionally, a previous report showed that Wogonin enhanced etoposide-induced apoptosis by inhibiting P-glycoprotein [23]. We have previously found that oroxylin A, another active flavonoids isolated from the root of *S. baicalensis* Georgi, reversed multi-drug resistance of human hepatoma BEL7402/5-FU cells via downregulation of P-glycoprotein expression [24]. Recently, Yan Zhong [25] had reported that Wogonin sensitized MCF-7/DOX cells to DOX by inhibiting Nrf2 signaling. These findings indicated the possibility that Wogonin had a potent reversal effect on drug resistance and its reversal mechanism could be associated with the suppression of Nrf2 signaling pathway and drug efflux pumps. However, the precise mechanism of Wogonin on overcoming chemoresistance remains to be further elucidated. In this study, we investigated the reversal experiments of Wogonin on resistant leukemia cells, which express high levels of constitutive Nrf2, as compared with other human cancer cells [26].

Here we first showed that constitutively overexpressed MRP1 in K562/A02 cells was strongly associated with drug resistance to apoptosis. We further demonstrated that this overexpression was down-regulated by the inhibition of Nrf2/ARE signaling pathway induced by Wogonin. The findings also showed that a strong correlation existed between downregulation of the PI3K/Akt survival pathway and Nrf2/ARE inhibition in Wogonin-induced reversal effect in K562/A02 cells, and this was correlated with diminished DNA-dependent protein kinase catalytic subunit (DNA-PKcs) level, belonging to the PI3K superfamily. Our results highlight a possible mechanism by which inhibition of Nrf2/ARE activity plays a role in the Wogonin-induced downregulation of MRP1, resulting in a reversal effect in the ADR-induced resistant human myelogenous leukemia cells.

2. Materials and methods

2.1. Materials

Wogonin was isolated from *S. baicalensis* Georgi according to the previously described method [27]. Wogonin was of ≥99% or higher in all experiments, unless otherwise noted. Wogonin was dissolved in DMSO (Amresco, Amresco Inc., Solon, Ohio) as a stock solution (100 mM), stored at −20 °C, and diluted to each of the designated concentrations in the buffer solution before each experiment. The final concentration of DMSO did not exceed 0.1%. ADR and Actinomycin D (Act D) were purchased from ZheJiang HiSun Pharmacetuical Co., Ltd. (Zhejiang, China). Paclitaxel and tert-butylhydroquinone (t-BHQ) were purchased from Jiang Su Heng Rui Medicine Co., Ltd. (Jiangsu, Nanjing, China). Cisplatin was provided by QiLu Pharmaceutical Co., Ltd. (Shandong, China). 3-(4,5-Dimethylthia- zol-2-yl)-2,5- diphenyltetrazolium bromide (MTT) and Hydrogen peroxide (H2O2) solution (30%) were from Sigma Chemical Co. (St. Louis, MO). RPMI-1640 (Gibico, Carlsbad, CA) and DAPI (Invitrogen, USA) were purchased. 5(6)-carboxyfluorescein diacetate (CFDA) was obtained from Santa Cruz (Santa Cruz, CA, USA). Primary antibodies for MRP1 (1:500), Keap1 (1:500), NQO1 (1:500), HO-1 (1:500), Lamin A (1:500) and β-actin (1:2000) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Nrf2 (1:800) and DNA-PKcs (1:800) were obtained from Bioworld (OH, USA). Ubiquitin were from Cellsignaling (Danvers, MA). The IRDye^{TM} 800 conjugated secondary antibodies were the products of Rockland Inc. (Philadelphia, PA).

2.2. Cell lines

The drug-sensitive human leukemia cell line K562 and its drug-resistant variant K562/A02 were obtained from the Institute of Hematology of Chinese Academy of Medical Sciences (Tianjin, China). The cells were cultivated in RPMI-1640 media supplemented with 10% fetal bovine serum at 37 °C in a humidified atmosphere of 5% CO_2 in air. The K562/A02 cells were cultivated in the presence of 1 mM ADR. Before experiments, ADR was withdrawn from the cells for two generations.

2.3. Multidrug resistance determination

To determine the multidrug resistance of the K562/A02 cells to chemotherapeutic agents, the MTT assay was performed to determine the survival rate of cells incubated with the anticancer drugs (Adriamycin, Cisplatin or Paclitaxel) at various concentrations. After dilution in RPMI-1640 media for 24 h, 20 μL MTT dye was added to each well and incubated for an additional 4 h. The dye was solubilized with 100 μL of DMSO, and the plates were read at