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Vajiheh Neshati [a], Maryam M. Matin [a b], Ahmad Reza Bahrami [a b], Mehrdad Iranshahi [c], Fatemeh B. Rassouli [a] & Morvarid Saeinasab [a]

[a] Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran

[b] Cell and Molecular Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran

[c] Department of Pharmacognosy, Biotechnology Research Center, Faculty of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

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SHORT COMMUNICATION

Increasing the cisplatin cytotoxicity and cisplatin-induced DNA damage by conferone in 5637 cells

Vajiheh Neshati[a], Maryam M. Matin[ab]*, Ahmad Reza Bahrami[ab], Mehrdad Iranshahi[c], Fatemeh B. Rassouli[a] and Morvarid Saeinasab[a]

[a]*Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran;* [b]*Cell and Molecular Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran;* [c]*Department of Pharmacognosy, Biotechnology Research Center, Faculty of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran*

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Despite widespread application of cisplatin in treatment of transitional cell carcinomas, its efficiency is far from satisfactory due to acquired drug resistance. The present study was carried out to estimate the effects of conferone, a sesquiterpene-coumarin isolated from *Ferula badrakema*, on increasing cisplatin cytotoxicity in 5637 cells. In order to determine conferone effects, 5637 cells were cultured in the presence of different concentrations of conferone and cisplatin in combination. The cytotoxicity and DNA damaging effects were then studied using MTT and comet assays, respectively. The results revealed that 24 h after the combination of 1 μg mL^{-1} cisplatin with 32 μg mL^{-1} conferone, the cytotoxicity of cisplatin was increased by 36.76%, and comet assay analyses showed that conferone could enhance the DNA damaging effects of cisplatin by 41%.

Keywords: conferone; sesquiterpene-coumarin; cisplatin; cytotoxicity; 5637 cells

1. Introduction

Bladder cancer is the most common cancer of the urinary system. More than 90% of bladder carcinomas are transitional cell carcinomas (TCCs) derived from the uroepithelium (Lee, Smith, Hall, Waters, & Biermann, 2003). Cisplatin, which is one of the most effective agents for TCC treatment (Loehrer, Einhorn, & Elson, 1992), causes apoptosis by covalently binding to DNA strands (Pruefer, Lizarraga, Maldonado, & Melendez-Zajgla, 2008). Nevertheless, resistance to cisplatin is a problem in the chemotherapy of cancers, particularly TCCs, and it is important to find a way to overcome this problem.

Ferula badrakema (Apiaceae) is a rich source of sesquiterpene coumarins, and we have previously shown that these compounds including conferone (Neshati et al., 2009), mogoltacin (Behnam-Rassouli et al., 2009; Rassouli et al., 2011) and feselol (Mollazadeh et al., 2010) can enhance cytotoxic effects of some anti-cancer drugs. In the present study, the effects of conferone on cytotoxic and DNA damaging effects of cisplatin were assessed in 5637 cells, a TCC subline, using MTT and comet assays.

*Corresponding author. Email: matin@um.ac.ir

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2. Results and discussion

5637 cells were treated with different concentrations (2, 10, 20, 50, 100, 200 and 300 $\mu g\,mL^{-1}$) of cisplatin for 24, 48 and 72 h. The results of MTT assay revealed that IC_{50} of cisplatin was 12 $\mu g\,mL^{-1}$ after 24 h on these cells (data not shown). In order to determine the effects of conferone, which is a non-toxic agent (Neshati et al., 2009), on the cytotoxicity of cisplatin, 30 combinations of different concentrations of conferone (8, 16, 32, 64 and 128 $\mu g\,mL^{-1}$) and cisplatin (1, 5 and 10 $\mu g\,mL^{-1}$), close to its IC_{50}, were used. Since conferone was dissolved in dimethylsulfoxide (DMSO), which is a cytotoxic solution itself, DMSO controls were also used. Evaluation of cell viability in cisplatin + conferone treatments and its comparison with that of the cells treated with cisplatin + DMSO revealed that the viability of cells was greatly decreased after combination of cisplatin with conferone. By using one-way ANOVA and Tukey test, it was shown that 24, 48 and 72 h after drug administrations, there were significant differences in cell viabilities between many of conferone + cisplatin combined concentrations compared with their DMSO equivalents (Supplementary Tables S1 and S2 – online only). After calculating the increased percentage of cisplatin cytotoxicity by different concentrations of conferone, statistical analyses showed that in comparison with other combining concentrations during three consecutive days, 32 $\mu g\,mL^{-1}$ conferone significantly increased the cytotoxicity of 1 $\mu g\,mL^{-1}$ cisplatin after 24 h (36.76%, Supplementary Table S3 – online only).

According to morphological observations, the combination of 32 $\mu g\,mL^{-1}$ conferone with 1 $\mu g\,mL^{-1}$ cisplatin induced obvious morphological changes; cells were changed to spherical forms with granulated cytoplasm as shown in Figure 1(a–c). At higher combined concentrations of conferone and cisplatin, cytotoxic effects were not more prominent. This might be due to the increased percentage of DMSO that prevents the effects of conferone to be observed. To investigate the mechanism involved in cytotoxic effects of these combinations, DNA damage was analysed by comet assay. Figure 1(d–f) represents photomicrographs of DNA damage in untreated and control cells compared with cells incubated with conferone + cisplatin. The results of comet assay indicated that in cells treated with 32 $\mu g\,mL^{-1}$ conferone + 1 $\mu g\,mL^{-1}$ cisplatin, DNA damage was approximately 71%, significantly ($p < 0.001$) higher than control (1.6% DMSO + 1 $\mu g\,mL^{-1}$ cisplatin, 30%) and untreated (6%) cells (Figure 1g).

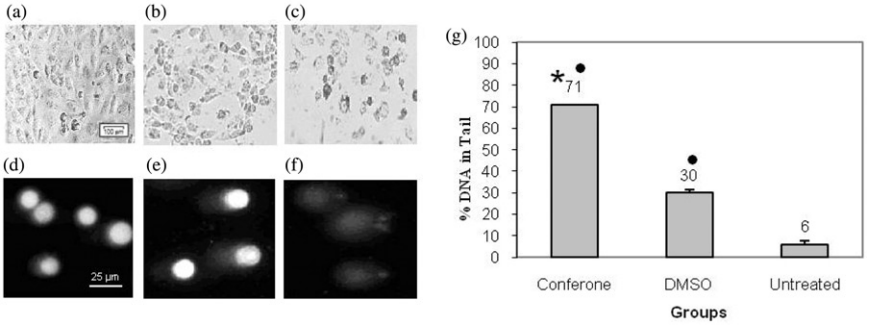

Figure 1. Representative images of morphology and DNA damages. 5637 cells cultured for 24 h: untreated 5637 cells ((a) and (d)), cells treated with 1.6% DMSO + 1 $\mu g\,mL^{-1}$ cisplatin ((b) and (e)) and cells treated with 32 $\mu g\,mL^{-1}$ conferone + 1 $\mu g\,mL^{-1}$ cisplatin ((c) and (f)). Comparison of DNA damage calculated for 5637 cells in different groups (g). Results are mean ± SEM; $*p < 0.001$, significant difference with DMSO control and untreated cells, $\bullet p < 0.05$, significant difference with untreated cells.

Resistance to cancer chemotherapeutic agents is a main barrier to successful treatment of human malignant tumours and this problem can result from several factors and various pathways. P-glycoprotein (P-gp) and multidrug resistance protein (MRP) are two of the main ATP-binding cassette (ABC) transporters, which are known to cause resistance to various cytotoxic agents (Borst, Evers, Kool, & Wijnholds, 1999). Cytotoxic drugs induce DNA damage in tumour cells and may result in apoptosis. Cisplatin is an effective drug used to treat a variety of cancers, specifically genitourinary tumours and urinary bladder TCCs (Loehrer et al., 1992), and has been shown to induce endonucleolytic DNA cleavage in tumour cells (Dabholkar et al., 1992). The accumulation of cisplatin is frequently decreased in cisplatin-resistant cell lines, and an active efflux system for cisplatin exists in some of these cells (Akiyama, Chen, Sumizawa, & Furukawa, 1999). Expression of the *MRP* gene has been demonstrated in urothelial carcinomas, and its involvement in resistance to a number of chemotherapeutic agents, currently used in the treatment of TCCs, has been shown (Kubo et al., 1996). In the present study, the effects of conferone, a sesquiterpene coumarin extracted from *F. badrakema*, was investigated on the cytotoxicity of cisplatin in 5637 cells. The results indicated that $32\,\mu g\,mL^{-1}$ conferone greatly increased the cytotoxic effects of cisplatin by 36.76%.

Our results indicated that the amount of DNA in comet tails in cells treated with $32\,\mu g\,mL^{-1}$ conferone $+ 1\,\mu g\,mL^{-1}$ cisplatin was increased by 41% compared with that in the DMSO control group ($p < 0.001$), which is in agreement with MTT results and morphological observations. However, these tests should be compared with effective reversal agents inhibiting MRP transporter to have a predictive value.

It was previously shown that conferone, the first identified natural sesquiterpene coumarin from *Ferula*, is a powerful agent with therapeutic potential for reversion of multidrug resistance encoded by the *MDR1* gene (Barthomeuf, Demeule, Grassi, Saidkhodjaev, & Beliveau, 2006). Other studies have shown that a binding site for sesquiterpenes exists within the transmembrane domain of P-gp (Munoz-Martinez et al., 2004). We have previously shown that conferone, mogoltacin and feselol could enhance the cytotoxicity of vincristine on 5637 cells by 23.6%, 32.8% and 28.3%, respectively (Behnam-Rassouli et al., 2009; Mollazadeh et al., 2010; Neshati et al., 2009). Moreover, mogoltacin showed an even greater effect on increasing the cytotoxicity of cisplatin on 5637 cells. The present study is the first report indicating that conferone can significantly increase the cytotoxicity of cisplatin. In single use, 8, 16, 32, 64 and $128\,\mu g\,mL^{-1}$ of conferone did not have any toxic effects on 5637 cells. So, conferone is considered as a compound effective at non-toxic concentrations. The probable mechanism of this effect is the inhibition of the function of MRP2 protein. Further studies on other cell lines are needed to prove this functional mechanism.

Supplementary material

Experimental details relating to this article are available online, alongside Tables S1–S3.

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