

Conferone from *Ferula schtschurowskiana* Enhances Vinblastine Cytotoxicity in MDCK-MDR1 Cells by Competitively Inhibiting P-Glycoprotein Transport

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Abstract

Overexpression of the protein transporter P-glycoprotein (Pgp, MDR1) at the cell surface is a major cause of multidrug resistance (MDR) and poor response to treatment in cancer chemotherapy and therapy for leishmaniasis. The present study shows that conferone, a sesquiterpene coumarin ether isolated for the first time from *Ferula schtschurowskiana*, endemic in Uzbekistan, enhances the cell toxicity of vinblastine (VBL) in MDR1-transfected Madin-Darby canine kidney (MDCK-MDR1) cells. Conferone presents the advantage to mediate this effect at safe concentrations. At 10 μ M, it efficiently competes with the photoactivatable cyclosporin A analogue (SDZ 212-122) for the binding to Pgp and accumulates [³H]-VBL to a higher extent than cyclosporin A or cniadiadin. [³H]-VBL accumulation is dose-dependent and correlates with the inhibition of Pgp photolabeling affinity, supporting the hypothesis that conferone sensitizes MDCK-MDR1 cells to VBL by competitively inhibiting drug efflux. In MDCK-MDR1 cells, [³H]-VBL accumulation appears to be almost completely

dependent on inhibition of Pgp transport. However, the strict specificity of conferone to this efflux pump has to be demonstrated in cell lines expressing other protein transporters. Collectively, our findings identify conferone as a powerful modulator of Pgp transport and a promising molecule for the treatment of MDR malignancies and leishmaniasis. Complementary *in vitro* and *in vivo* studies are, however, needed to assess the value of conferone as a reversal drug in human therapy. Considering its high affinity for Pgp, conferone may have an additional usefulness as a tool for the design or the (hemi)synthesis of agents probing Pgp. To our knowledge, this is the first report identifying sesquiterpene coumarins from *Ferula* as possible drug candidates for the reversion of MDR encoded by the MDR1 gene or the synthesis of agents probing Pgp.

Key words

Cancer · conferone · sesquiterpene coumarin · multidrug resistance · P-glycoprotein (Pgp, MDR1) · reversal activity · photolabeling · *Ferula schtschurowskiana* · Apiaceae

Introduction

P-glycoprotein (Pgp, ABCB1) is an adenosine triphosphate (ATP)-binding cassette transporter which acts as a drug efflux pump. This membrane protein is physiologically expressed at high lev-

els in certain non-cancerous cells such as liver, kidney, colon and small intestine apical cells, in which it contributes to the excretion of xenobiotics and cell detoxification [1]. Pgp also contributes to the blood-brain barrier that protects the brain by extruding hydrophobic compounds that are potentially neurotoxic

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out of capillary endothelial cells [2]. Pgp, thus protects the cells against xenobiotics and, thereby, has beneficial effects. However, by reducing the intracellular accumulation of many usual chemotherapeutic drugs (including anthracyclines, mitoxantrone, vinblastine, vincristine, paclitaxel, docetaxel) into cancer cells, Pgp also contributes to multidrug resistance (MDR) and poor responses to chemotherapy [1], [3], [4], [5]. The overexpression at the cancer cell surface of Pgp encoded by the MDR1 gene correlates with a bad prognosis [1], [5]. Cells exhibiting the MDR1 phenotype are commonly observed at relapse in certain tumors initially sensitive to treatment, such as small cell lung carcinoma, neuroblastoma [6] and advanced ovarian or breast carcinoma [1], [5]. This phenotype is also frequently observed in kidney and colon cancers because these cells physiologically express high amounts of Pgp [1]. Restoring the susceptibility of multidrug-resistant cancer cells overexpressing Pgp to chemotherapy has thereby clinical significance.

The demonstration that verapamil (a calcium blocker) and cyclosporin A (an immunosuppressive agent) may sensitize resistant cells overexpressing Pgp to *Vinca* alkaloids by competitively blocking drug efflux has focused interest on chemosensitizers, also termed Pgp modulators or reversal agents [1], [5]. Recent randomized trials have confirmed the value of this approach in oncology, but have also demonstrated that clinically applicable drugs remain to be discovered [1], [5]. To have clinical value, drug candidates need to be safe at concentrations overcoming MDR. Although their cytotoxicity remains largely unknown, recent data have identified certain sesquiterpenes from *Celestraceae* and *Euphorbiaceae* as potent reversal agents [7], [8]. In addition to their clinical interest in the field of oncology, reversal agents have potential applications in the treatment of leishmaniasis, a parasitic disease identified as the second most common cause of death after malaria [9]. Most conventional mammalian Pgp inhibitors are ineffective in resistance related to overexpression of Pgp in the protozoan parasite *Leishmania*. However, natural and semi-synthetic dihydro-beta-agarofuran sesquiterpenes from *Celestraceae* have demonstrated *in vitro* activity in *Leishmania* lines overexpressing Pgp [9].

Collectively, these data suggest that it would be of interest to screen sesquiterpene derivatives for the identification of clinically active reversal agents. On the basis of a previous report showing that certain complex coumarins may block Pgp transport and reverse MDR in human multidrug-resistant cancer cells [10], we have tested a natural coumarin ether with a drimane-derived nucleus termed conferone (see structure in Fig. 1). Sesquiterpene coumarins are quite rare in higher plants except for plants of the genus *Ferula* (*Apiaceae*) distributed from the Mediterranean Sea to Central Asia [11], [12], [13], [14], [15]. Conferone was first isolated from *Ferula conocaula* [15]. In this study, it was isolated for the first time from *F. schtschurowskiana* Rgl. et Schmalh, endemic in Uzbekistan. Collectively, the present data demonstrate that conferone binds with high affinity to Pgp and enhances the toxicity of vinblastine in Madin-Darby canine kidney (MDCK) cells transfected with a human MDR1 cDNA through a competitive inhibition of Pgp transport function. To the best of our knowledge, this is the first time that a sesquiterpene coumarin from the genus *Ferula* is reported to exhibit reversal activity.

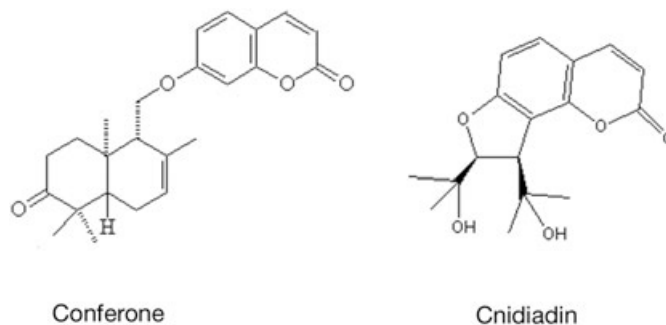


Fig. 1 Structure of conferone.

Material and Methods

Cells and chemicals

Parent sensitive (MDCK-WT) and MDR1-transfected Madin-Darby canine kidney (MDCK-MDR1) cells were a gift from Amanda Yancy (AstraZeneca Pharmaceuticals). Both were cultured in DMEM supplemented with 10% heat-inactivated fetal bovine serum. Stock cell cultures were maintained as monolayers in Glutamax Eagle's minimum essential medium (MEM) with Earle's salts supplemented with 10% fetal calf serum, 5 mL of 100 mM solution of vitamins, 5 mL of 100 mM sodium pyruvate, 5 mL of a solution of non-essential amino acids and 2 mg of gentamicin base. Cells were maintained at 37 °C under a humidified atmosphere containing 5% CO₂.

[³H]-VBL (11.3 Ci/mmol) was purchased from Amersham Pharmacia Biotech (Oakville, Canada). CsA, SDZ 212-122, a diazirine-CsA (dz-CsA) derivative and the monoclonal antibody (mAb) directed against CsA were gifts from Novartis Pharma, Canada. All medium components were from Gibco (Gaithersburg, MD, USA). Other chemicals were from Sigma-Aldrich (Oakville, Canada).

Plant material

Roots of *Ferula schtschurowskiana* Rgl. et Schmalh. (*Apiaceae*) were collected during flowering in the valley of the Zeravshan river (Uzbekistan) and identified by Dr. A. M. Nigmatullaev (Laboratory of Medicinal Plants, S. Yu. Yunusov Institute of the Chemistry of Plant Substances, Tashkent, Uzbekistan). A voucher specimen has been deposited in the Herbarium of the Institute.

Extraction and isolation of conferone

Dried ground roots (1 kg) of *F. schtschurowskiana* were extracted five times with EtOH (5 L). The residue (52 g) was submitted to low pressure column chromatography on silica gel and elution by an increasing gradient of *n*-hexane in EtOAc adapted from a previous report [15]. In brief: separation was monitored at 254 nm after TLC on silica gel and migration in *n*-hexane-EtOAc (3:1). Active fractions eluted with *n*-hexane-EtOAc (70:10) were combined and crystallized from *n*-hexane-EtOAc (50:10) to give conferone with a yield of 3.29% (0.33% of the dry roots). The isolated compound was formally identified as conferone by means of [α]_D²⁰, UV, IR, EI-MS and ¹H-NMR (400 MHz with TMS as internal standard and CDCl₃ as solvent) spectroscopy. All data (see below) were in agreement with those reported in the literature for authentic conferone [11].

Cell survival assay

MDCK-MDR1 cells were seeded at 5000 cells per well in 96-well plates. VBL was used at its IC_{50} value, in the presence or the absence of cnicidin or conferone. The cells were grown for 72 h at 37 °C under an atmosphere containing 5% CO_2 and the cell survival was determined with a standard WST-1 assay as previously described [16]. The respective cytotoxicity in MDCK-MDR1 and MDCK-WT cells was assayed with crystal violet according to [17].

[3H]-VBL accumulation in MDCK-MDR1 cells

MDCK-MDR1 cells were seeded at 150,000 cells per well in 24-well plates and cultured for 4 days at 37 °C in an atmosphere containing 5% CO_2 . At confluence, the cells were washed twice with HBSS (1.3 mM $CaCl_2$, 5.4 mM KCl, 0.44 mM KH_2PO_4 , 0.5 mM $MgCl_2$, 0.83 mM $MgSO_4$, 137 mM NaCl, 4.2 mM $NaHCO_3$, 0.34 mM Na_2HPO_4 and 25 mM D -glucose, pH 6.5) at 37 °C. Cells were then pre-incubated for 30 min at 37 °C with HBSS containing DMSO (0.1%, v/v) with or without cnicidin (0–100 μM) or CsA (0–10 μM) or conferone (0–66 μM) in the presence or absence of indomethacin. [3H]-VBL (0.23 μCi) was then added at a concentration of 20 nM to each well and the cells were further incubated for 2 h at 37 °C. [3H]-VBL accumulation was stopped by washing the cells five times with cold PBS (150 mM NaCl, 2.7 mM KCl, 1.3 mM KH_2PO_4 , 8.1 mM Na_2HPO_4 , pH 7.4). The cells were then lysed with 0.1% Triton X-100 at room temperature. The cell lysates were placed into scintillation vials and the radioactivity was counted.

Photoaffinity labeling of Pgp with SDZ 212–122

Pgp affinity photolabeling was assessed as previously described [10] with the photoactivatable CsA analogue SDZ 212–122 that possesses a diazirine (dz) group at position 8. In brief, MDCK-MDR1 membranes (50 μg) isolated as described in [17] were incubated for 1 h in the dark at 25 °C in 10 mM Tris-HCl, pH 7.5, containing SDZ 212–122 (0.1 μM) and coumarins at the indicated concentrations, or SDZ 212–122 (0.1 μM) alone (100% photolabeling). After treatment, the membranes were cross-linked at 254 nm for 5 min at 4 °C with a Stratalinker UV 2400 lamp (Stratagene; La Jolla, CA, USA). Laemmli electrophoresis buffer (62.5 mM Tris-HCl, pH 6.8, 10% glycerol, 2% SDS, 5% mercaptoethanol, 0.01% bromophenol blue) was added to the membrane samples, and the proteins were resolved by SDS-PAGE on 6.25% acrylamide-bisacrylamide (29.2:0.8) gels with a Mini-Protein II apparatus. The proportion of dz-CsA linked to Pgp was immunodetected by Western blotting with an mAb directed against CsA. All experiments were performed at least three times. The intensities of the bands obtained from each photolabeling experiment were calculated with a personal densitometer SI (Molecular Dynamics; Sunnyvale, CA, USA).

Results

Conferone enhances the susceptibility of MDCK-MDR1 cells to vinblastine. To evaluate its potentiality to reverse MDR, we first tested conferone for its capacity to enhance the cytotoxicity of vinblastine (VBL) in MDCK-MDR1 cells. We previously identified the IC_{50} of VBL as 0.56 μM in MDCK-MDR1 cells and as 0.56 μM and the resistance of this mammal cell-line to VBL as 14-fold that of the parental MDCK line [10]. In a first series of experiences,

cnidiadin, a furanocoumarin previously identified as a chemomodulator [10], was used as positive control. The cytotoxicity of VBL/conferone and VBL/cnicidin combinations was determined and compared to that of each compound, singly. Conferone or cnicidin were tested at 10 μM and, vinblastine (VBL) at 0.6 μM , a concentration close to its IC_{50} value in MDCK-MDR1 cells. Data in Fig. 2A show the survival of MDCK-MDR1 cell after a 72-h continuous exposure to the different chemicals, singly or combined. Singly, VBL decreased MDCK-MDR1 cell survival by 57%. The cytotoxicity was increased to 87% when VBL was combined with 10 μM of conferone, and to 91% in combination with 10 μM cnicidin. However, in single use, conferone at 10 μM was not toxic for MDCK-MDR1 cells while cnicidin decreased the cell survival by about 50%. This indicates that, although the two coumarins enhanced the cytotoxicity of VBL in MDCK-MDR1 cells, conferone was the only compound effective at a non-toxic concentration.

Multiresistant MDCK-MDR1 cells are more susceptible to conferone than sensitive MDCK-WT cells. Complementary experiments done to compare the cytotoxicity of conferone (0–50 μM) in MDR1 transfected-MDCK (MDCK-MDR1) and in sensitive parental MDCK-WT cells showed a higher susceptibility of the sensitive MDCK-WT line. Conferone was cytotoxic in both cell lines,

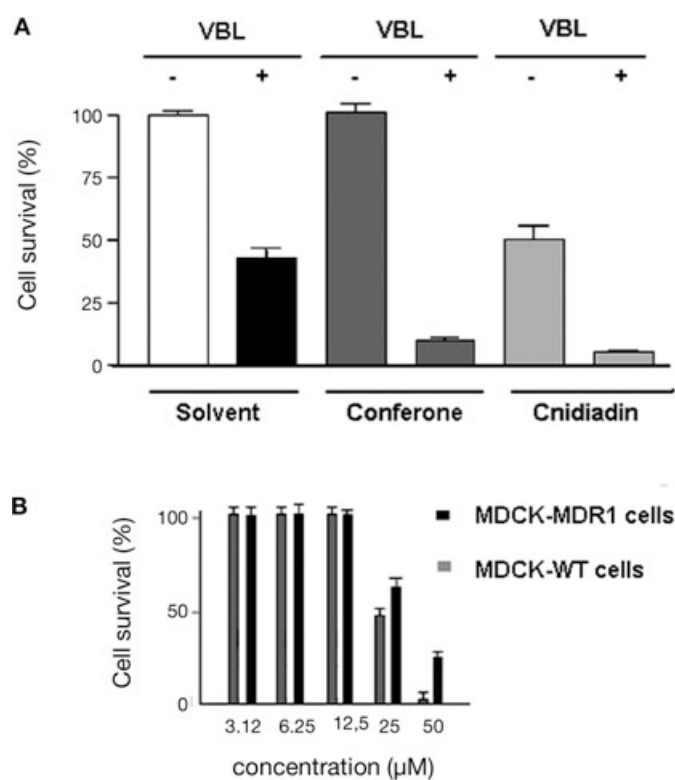


Fig. 2 Cell toxicity of conferone in resistant MDCK-MDR1 and sensitive MDCK-WT cells. **A** Cell toxicity of conferone (10 μM) used singly or combined with vinblastine (VBL, 0.6 μM) in MDCK-MDR1 cells. The cell survival was evaluated with the WST-1 assay after a 72 h-exposure to vinblastine as described in Materials and Methods. Cnicidin (10 μM) was used as positive control and DMSO (0.1%) as negative control. The results are expressed as the percentage (mean \pm SD) of cell survival vs. negative control (100% survival). Values are the result of three independent experiments. **B** Comparison of the cell toxicity of conferone in MDCK-WT and MDCK-MDR1 cells. The cell survival was evaluated colorimetrically with crystal violet. Values are the result of four independent experiments.

at concentrations higher than 12.5 μM , but induced no toxicity below this concentration (Fig. 2B).

Conferone dose-dependently enhances $[^3\text{H}]$ -VBL uptake in MDCK-MDR1 cells. Because the different cytotoxicities of conferone in the sensitive and the MDR1-transfected MDCK cells suggested that this coumarin was a Pgp substrate, we evaluated its capacity to modulate cancer drug efflux, by measuring the uptake of $[^3\text{H}]$ -vinblastine ($[^3\text{H}]$ -VBL) in MDCK-MDR1 cells exposed during 120 min to an increased concentration of conferone. Cnidiadin and the clinically active drug CsA were used as positive controls. The solvent (DMSO 0.1%) served as negative control. The dose-response curves of $[^3\text{H}]$ -VBL accumulation presented in Fig. 3 show that conferone at 0–66 μM (Fig. 3A), cnidiadin at 0–100 μM (Fig. 3B) and CsA at 0–10 μM (Fig. 3C) dose-dependently

accumulated $[^3\text{H}]$ -VBL in MDCK-MDR1 cells. The 3 profiles of drug accumulation were, however, completely different. The concentration required to reach 50% of the maximal accumulation (AC_{50}) was established as 1.8 μM for CsA instead of 6.5 μM for conferone and 26.4 μM for cnidiadin. Nevertheless, at the highest tested concentration, the maximal uptake decreased in the order: conferone > cnidiadin > CsA (Fig. 3). When each chemical was tested at 10 μM , conferone was the most effective compound. It increased $[^3\text{H}]$ -VBL uptake by 8.7-fold instead of 6.1- for CsA and 3.4-fold for cnidiadin.

In MDCK-MDR1 cells, the accumulation of $[^3\text{H}]$ -VBL uptake by conferone is almost completely dependent on Pgp. Pgp is not the unique efflux pump conferring MDR. Other transporters belonging to the ATP binding cassette (ABC) including multidrug resistance protein (MRP) and breast cancer resistance protein (BCRP/ABCG2) are also implicated. Proteins from the MRP family (MRP 1–9), especially MRP1 (ABCC1) that transport glutathione-, glucuronate- and sulfate-conjugated drugs as well as BCRP, that transport mitoxanthrone and camptothecine derivatives (topotecan, irinotecan), are frequently expressed in resistant cancer cells [1], [5]. Most multiresistant cells co-express different multidrug transporters [1]. The co-expression of MDR1 and MRP1 correlates with a poor response to treatment and a bad prognosis [18]. Certain chemicals have the capacity to block drug efflux by different transporters. A good example is quercetin which reverses VBL transport by both MRP1 and Pgp [19], [20], [21], [22]. As a first approach to evaluate if conferone may affect $[^3\text{H}]$ -VBL transport by MRP proteins, we examined, in MDCK-MDR1 cells, if $[^3\text{H}]$ -VBL uptake was differently affected when conferone was used in the presence or the absence of indomethacin. Indomethacin modulates MRP transport in cells overexpressing this family of transporters. As a consequence, indomethacin analogues have recently been used to reverse multidrug resistance due to MRP overexpression [23]. The finding that, in MDCK-MDR1, the increase in $[^3\text{H}]$ -VBL uptake induced by conferone (10 μM) was not significantly affected when conferone was used in combination with indomethacin (Fig. 4) indicates that, in this cell line, the accumulation of $[^3\text{H}]$ -VBL induced by conferone is

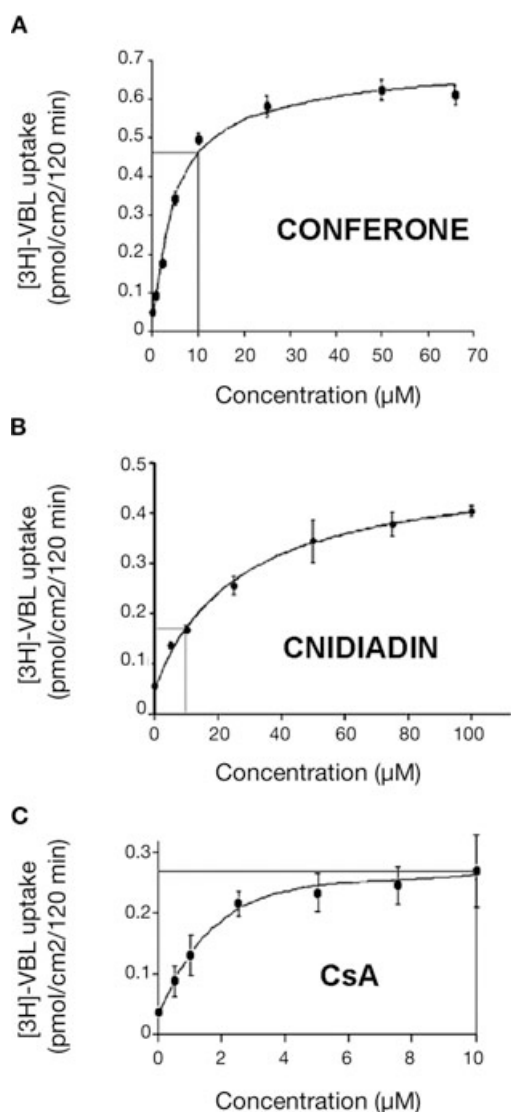


Fig. 3 Enhancement of $[^3\text{H}]$ -VBL uptake in MDCK-MDR1 cells exposed to conferone, cyclosporine A or cnidiadin. The $[^3\text{H}]$ -VBL uptake was determined by measurement of the radioactivity in cell lysates after a 120-min exposure to conferone (0–66 μM , A), cnidiadin (0–100 μM , B), or CsA (0–10 μM , C) as described in Materials and Methods. The solvent (DMSO 0.1%) served as negative control. Results are expressed as the amount of drug accumulated (pmol/cm²/120 min) in treated and control cells. Each datapoint is the result (mean \pm SD) of values obtained from three independent experiments performed in duplicate.

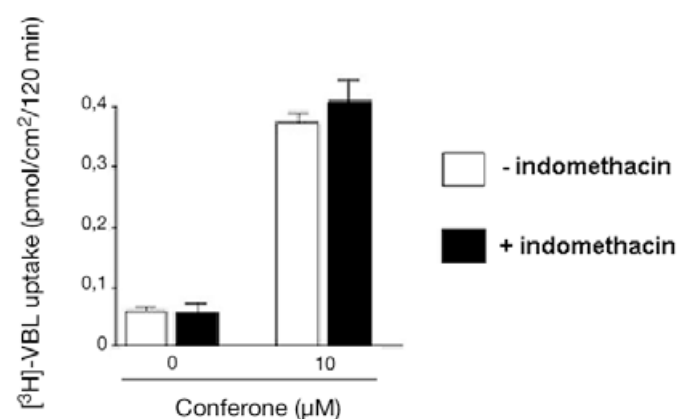


Fig. 4 Modulation of $[^3\text{H}]$ -VBL uptake by indomethacin. $[^3\text{H}]$ -VBL uptake was measured in MDCK-MDR1 cells after a 120-min exposure to conferone (0 and 10 μM) in the presence (white bars) or the absence (black bars) of indomethacin. Each datapoint is the mean \pm SD of values obtained from two independent experiments performed in triplicate.

almost completely dependent on Pgp transport. However, as [^3H]-VBL uptake was not enhanced when indomethacin was used alone, it is likely that MDCK-MDR1 do not express MRP protein or that the activity of these proteins is very low. Thereby, it cannot be ascertained from the present experiences that conferone does not affect drug efflux by protein transporters other than Pgp. Studies in cell lines expressing specifically these efflux pumps are needed to verify this hypothesis.

Conferone competitively inhibits the photolabeling of Pgp by a CsA analogue. To verify that [^3H]-VBL accumulation by conferone was the result of a competitive inhibition of Pgp transport and not of an alteration of membrane integrity, we then tested conferone for its ability to inhibit Pgp affinity photolabeling. In this assay, MDCK-MDR1 cell membranes were co-incubated with conferone (0–66 μM) and SDZ 212–122, a photoactivatable CsA analogue exhibiting high affinity for Pgp [17]. Then, the amount of photolabeled Pgp (Pgp linked to the CsA derivative: SDZ 212–122) was determined by blotting with a monoclonal antibody directed against CsA, then quantified by densitometry according to [10]. Both cnidiadin (0–100 μM) and conferone dose-dependently decreased the photolabeling of Pgp by SDZ 212–122. This indicates that both have the capacity to compete with this CsA derivative for the binding to Pgp (Fig. 5). However, at low concentrations, conferone inhibits SDZ 212–122 photolabeling much more efficiently than cnidiadin. Exposure to 5 μM conferone was sufficient to decrease Pgp photolabeling by 67.5% while 25 μM cnidiadin were required to obtain a similar inhibition. It is of interest to observe that the curve of [^3H]-VBL accumulation by each coumarin was inversely correlated with the level of photolabeling affinity inhibition by cell membranes.

Discussion

The treatment of multiresistant tumors represents an important challenge in the field of oncology. Meanwhile, the main cause of failure of cancer therapy is related to inherent or acquired overexpression of efflux pumps by tumor cells, many efforts have been undertaken to identify effective Pgp and MRP modulators.

We previously demonstrated that cnidiadin, a furanocoumarin found in certain traditional Chinese medications and in *Tordylium apulum*, a herb commonly used in Greek cuisine, inhibited Pgp transport and reversed the resistance of multiresistant cells overexpressing Pgp to *Vinca* alkaloids [11]. Unfortunately, cnidiadin is not a good candidate for human therapy because it is poorly effective at low concentrations and is toxic at concentrations needed to overcome drug resistance [11]. Studies are underway for identifying effective drug candidates. The present data show that conferone, a coumarin ether with a drimane-derived nucleus, is a powerful reversal agent with therapeutic potentiality. This is the first report identifying natural sesquiterpene coumarins from *Ferula* as promising drug candidates for reversion of MDR encoded by the MDR1 gene.

Conferone has first been isolated from the fruits of *F. conocaula* [13]. This first isolation from the roots of *F. schtschurowskiana* Rgl. et Schmalh confirms that this coumarin is synthesized by various species of the genus *Ferula* (Apiaceae) and, is thereby commonly available. It was first observed that 10 μM of conferone enhanced by 53% the cytotoxicity of VBL in MDCK-MDR1 cells, used as model for expression of the human MDR1 phenotype. As this concentration of conferone was not toxic for MDCK-MDR1 cells, the data show that, *in vitro*, a safe concentration of conferone may enhance vinblastine toxicity in multiresistant cells. Although this needs to be verified by complementary experiments, the lack of toxicity in the sensitive MDCK-WT line also suggests that conferone may have low toxicity *in vivo*. Experiments done to establish how conferone enhances the cell toxicity of VBL have shown the higher susceptibility of the parent sensitive MDCK-WT cells, which suggests that conferone is a Pgp substrate. This hypothesis is supported by the dose-dependent increase of [^3H]-VBL uptake in MDCK-MDR1 cells exposed to increased concentrations of conferone. That conferone blocks Pgp transport through a competition for the binding to Pgp is demonstrated by the inverse correlation between Pgp affinity photolabeling inhibition by MDCK-MDR1 cell membranes and VBL accumulation in MDCK-MDR1 cells. Analysis of [^3H]-VBL dose-response curves by cnidiadin, CsA and conferone has finally shown that conferone at 10 μM has a higher capacity to accumulate VBL

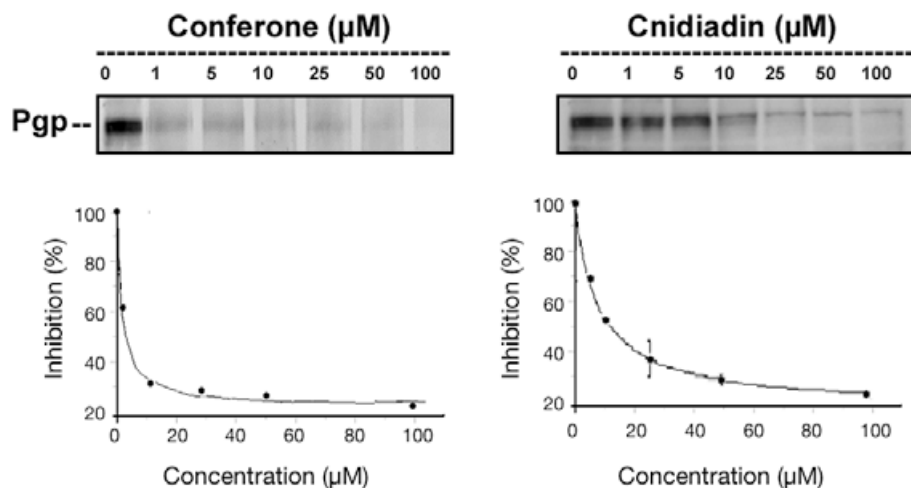


Fig. 5 Dose-dependent inhibition of Pgp photolabeling by conferone. Membranes from MDCK-MDR1 cells (50 μg) were co-incubated with the photoactivatable CsA derivative SDZ 212–122 (0.1 μM) and with conferone (0–66 μM) or cnidiadin (0–100 μM , positive control), then cross-linked with UV light. Similar amounts of proteins were resolved by SDS-PAGE on 6.25% polyacrylamide gels. The residual amount of photolabeled Pgp (Pgp linked to SDZ 212–122) was determined by blotting with a monoclonal antibody directed against CsA, as described in Materials and Methods. The corresponding band was analyzed by laser densitometry. SDZ 212–122 in DMSO 0.1 μM was used as the control of 100% photolabeling. The results are the mean \pm SD percentage ($n = 3$) of Pgp photolabeling inhibition by the tested chemical as compared to the control.

than the clinically active drug CsA or the furanocoumarin cnicidin. Collectively, these data demonstrate the high affinity of conferone for Pgp and support the hypothesis that the enhanced susceptibility of conferone-treated MDCK-MDR1 cells to VBL is the result of a reversal activity.

The capacity of conferone to bind Pgp with high affinity is in agreement with the recent observation that a binding site for sesquiterpenes exists within the transmembrane domain of Pgp [8]. Further studies are needed to identify the structural motif recognized at the binding site. However, the dose-response curve of photolabeling inhibition, unambiguously demonstrates the high affinity of conferone for Pgp. The treatment of MDCK-MDR1 cells by conferone/indomethacin combination vs. conferone or indomethacin singly shows that, in this cell line, [³H]-VBL accumulation is largely specific to Pgp. However, as MDCK-MDR1 cells express essentially this transmembrane protein, an interaction with other efflux pumps cannot be discarded and further studies in cell lines overexpressing protein transporters other than Pgp are needed to determine if, like sesquiterpenes from Celastraceae [8], conferone inhibits specifically Pgp transport.

In summary, the present data demonstrate that conferone has very high affinity for Pgp and the capacity to reverse Pgp-induced MDR by competitively blocking Pgp transport. This suggests a possible usefulness of this coumarin as a tool for the (hemi)synthesis of chemical Pgp probes for guided therapy or, for exploration of MDR1 resistance or transport activity. Data in MDR1-transfected MDCK cells identify conferone as a promising candidate for the treatment of MDR malignancies or resistant leishmaniasis. However, complementary studies in human cancer and/or in *Leishmania* lines overexpressing Pgp are needed to determine its value as a drug candidate for human therapy. To have a predictive value, these tests have to be conducted vs. effective reversal agents and vs. other sesquiterpenes inhibiting Pgp transport, such as the coumarin-ether driportlandin from *Euphorbia portlandica* L. (Euphorbiaceae) [7], the prenyl-furocoumarin-type sesquiterpenoids from *F. ferulaeoides* [12], the sesquiterpenes of Celastraceae [8], [9] or the sesquiterpenes from *Zinowiewia costaricensis* [24].

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