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VOACAMINE, BISINDOLE ALKALOID, ACTS AS CHEMOSENSITIZING AGENT ON MULTIDRUG RESISTANT TUMOR CELLS OVEREXPRESSING P-gp

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The use of natural compounds in combination with conventional chemotherapy, now widely used in integrated cancer protocols, requires an in-depth study of the cellular and molecular effects in the combined treatment.
The aim of our work was to verify the chemosensitizing effect of the bisindole alkaloid voacamine (VOA), extracted from the *Peschiera fuchsiaefolia* plant on drug-resistant human cancer cells of different histotype (osteosarcoma, melanoma, colon carcinoma and ovarian cancer). Our goal was to analyze the target's resistance mechanisms.
We performed MTT analysis, light and electron microscopy, confocal laser scanning microscope (LSCM) and flow cytometry.
Selected cell lines overexpressed P-gp on the cell surface, which was responsible for the increased efflux of chemotherapeutic agents (such as doxorubicin), reduced drug intracellular concentration and reduced the toxic effect.
The MTT test on drug-resistant cancer cells showed that cell treatment with 1 µg/ml of VOA alone was not toxic.
When VOA was administered in combination with doxorubicin (DOX, 1 µg/ml) on osteosarcoma (U-2 OS/DX and SAOS-2-DX) and melanoma (Me30966) cells, we observed increased DOX accumulation by flow cytometry and an intracellular distribution DOX by confocal microscopy. Consequently, cell viability significantly decreased compared with DOX treatment alone. Scanning electron microscope observations confirmed the MTT data and highlighted evident morphological alterations (Meschini et al., 2003; Condello et al., 2014).
UIC2 shift assay performed by flow cytometry demonstrated that VOA was a substrate of P-gp and, as a competitive antagonist of cytotoxic drugs (such as DOX), it interfered with P-gp mediated efflux (Meschini et al., 2005).
Furthermore, LSCM observations showed that VOA depolymerized microtubules blocking DOX-mediated efflux vesicles (Condello et al., 2020).
Next, we investigated if VOA was a chemosensitizing compound when administered in combination with other P-gp substrate drugs, such as paclitaxel (PTX), or possibly with drugs that are not P-gp substrates. We analyzed the combination VOA plus PTX on tumor resistant ovarian cells (A2780 DX); as a positive control we used a colon cancer resistant cell line (LoVo DX) treated with VOA plus 5-Fluoracil (5-FU). The MTT test demonstrated that VOA has a chemosensitizing effect on resistant cells when treated in combination with P-gp substrate drugs. Optical and electron microscope observations showed irreversible cell damage induced by VOA plus PTX on A2780 DX cells. The Annexin V / IP assay and cell cycle analysis by flow cytometry and the expression of PARP cleaved by western blot showed induction apoptosis cell death.
We concluded that VOA specifically exerts its chemosensitizing function only against drugs known to be substrates of P-glycoprotein.

References

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