Identification of new chemosensitizer agents against multidrug resistant cancer cells

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Multidrug resistance (MDR) occurs when cancer cells develop cross-resistance to structurally and functionally unrelated cytostatic drugs and represents a major cause of chemotherapy failure (Baguley et al., 2010). The enhanced activity of ATP-binding cassette proteins (ABC), among them P-glycoprotein, seems to be involved in MDR. At present, the use of ABC transporter inhibitors (also known as chemosensitizers) in association with cytotoxic drugs represents a new approach to overcome MDR (Wink et al., 2012). In this context, the present study was aimed at evaluating the potential ability of a synthetic and two natural compounds, α-hexyl cinnamaldehyde (HCA), β-caryophyllene (CRY), and β-caryophyllene oxide (CRYO), to act as chemosensitizers.

Initially, the cytotoxic effects of test compounds in drug resistant human cancer cells (Caco-2 and CEM/ADR5000) and sensitive cells (HeLa and CCRF-CEM) was evaluated by MTT assay (Mosmann, 1983). Furthermore, the potential additive, synergistic or antagonistic effects between non toxic concentration (IC_{10} and IC_{20}) of the test compounds and doxorubicin, or the other substances studied, have been evaluated in Caco-2, CCRF-CEM, and CEM/ADR5000 cells. The nature and extent of the interaction were evaluated by the combination index method (CI) and isobologram analysis respectively (Zhao et al., 2004), while the potential enhancement of drug effectiveness was quantified by the cytotoxicity enhancement ratio (RR) (El-Readi et al., 2010). Finally, the interaction of CRY, CRYO and HCA with ABC transporters was studied by the rhodamine 123 assay in Caco-2 and CEM/ADR5000 cells, which overexpress these proteins (El-Readi et al., 2013). The substances did not inhibit the cell growth both in resistant and in sensitive cell lines (200 μM 50< 1000 μM). However, HCA was the most cytotoxic substance, especially in the sensitive CCRF-CEM cells (IC_{50}= 212.95 μM). All the compounds increased the cytotoxicity of doxorubicin, but HCA was the most active. In fact, in the presence of the IC_{20} concentration of HCA, the potency of doxorubicin was 6.39-fold higher in Caco-2 (IC_{50} from 5.24 to 0.82 μM), of 7.37-fold higher in CEM/ADR5000 (IC_{50} from 74.28 to 10.08 μM), and of 52.5-fold higher in CCRF-CEM (IC_{50} from 0.42 to 0.008 μM). In presence of the IC_{20} concentration of CRY and CRYO, the cytotoxicity enhancement ratio for doxorubicin was 4.56 in Caco-2 cells and 7 in CCRF-CEM cells, respectively. HCA was the most effective reversal agent also in combination with CRY and CRYO. The IC_{20} concentration of HCA increased the cytotoxicity of CRY 1.74-fold in Caco-2 (IC_{50} from 1103.34 to 633.34 μM), 7.22 fold in CEM/ADR5000 (IC_{50} from 368.48 to 51.04 μM), and 9.90-fold in CCRF-CEM (IC_{50} from 311.59 to 31.47 μM). The cytotoxicity of CRYO was increased 2.72 fold in Caco-2 cells (IC_{50} from 332.30 to 122.03 μM), 7.91 fold in ADR (from 297.98 to 37.67 μM), and by 11-fold in CCRF-CEM (IC_{50} from 235.18 to 21.38 μM). In the rhodamine 123 assay and in both cell lines used, HCA (20 μM) produced a 1.34-fold higher retention of the fluorescent probe with respect to the same concentration of verapamil (used as a standard ABC transporter inhibitor). Also CRY and CRYO inhibited the ABC transporters but with lower potency than verapamil.

In conclusion, our present results showed that HCA is effective as a reversal agent, increasing the cytotoxicity of both doxorubicin and the other substances tested in cancer cells. Furthermore, it is active as inhibitor of ABC-transporters. HCA could be useful in MDR reversal, by favouring the efficacy of chemotherapeutic drugs in human P-gp expressing cells.

References

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