β-Caryophyllene oxide as a new chemosensitizing agent to enhance sorafenib efficacy in liver cancer chemotherapy

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Hepatocellular carcinoma (HCC) is the fifth most frequent malignant tumor, and the third leading cause of cancer-related mortality in the world. The response of HCC to conventional chemotherapy is very poor. Currently, sorafenib is the only drug available for this condition, even if its efficacy is modest due in part to the strong multidrug resistance (MDR)1 phenotype of HCC, in which the enhanced activity of ATP-binding cassette (ABC) proteins, which actively pump out lipophilic drugs across the plasma membrane of tumor cells, play and important role2. The potential use of ABC-transporter inhibitors (chemosensitizers) in association with cytotoxic drugs represents a new approach to overcome MDR. In this context, the present study was aimed at evaluating the ability of the natural sesquiterpene ß-caryophyllene oxide (CRYO) to act as a chemosensitizer in HCC. Human HCC PLC/PRF/5 cells, both wild-type (WT) and chemoresistant (R), overexpressing ABC pumps, such as MDR1, MRP1, MRP2, MRP4 and MRP5, chemoresistant mouse hepatoma Hepa 1-6 R cells, overexpressing MRP1 and MRP2; and human lung carcinoma COR-L23 cells, overexpressing MDR1 and MRP1, have been used as in vitro models to investigate the potential chemosensitizing ability of CRYO3. As a first step, the cytotoxicity of the test substances alone or in combination was evaluated by dose-response MTT assay in all cell lines4. Then, the inhibition by CRYO of ABC transport function was measured by efflux assay using fluorescent substrates5. The actual change in sorafenib cell content, as a result of the combined treatment with CRYO, was measured by HPLC-MS/MS4. Finally, the potential chemosensitizing effect of CRYO in combination with sorafenib was investigated using the mouse xenograft model5. Tumor growth in nude (nu/nu) mice was determined after subcutaneous injection of Hepa1-6 R cells. The animals were randomly divided into three groups, which on days 1, 4, 7, 11, 14, 18, 21, 25, and 28 received vehicle alone, sorafenib 10 mg/kg or the combined treatment sorafenib 10 mg/kg plus CRYO 50 mg/kg, respectively. Cytotoxicity evaluation in vitro revealed a lack of CRYO cytotoxicity up to the concentration of 100 µM together with different sensitivity of PLC/PRF/5 (WT vs. R) cells to sorafenib. Thus, PLC/PRF/5 WT showed a 3-fold higher sensitivity to sorafenib than R cells. In combination experiments, CRYO (50 µM) significantly increased sorafenib (10 µM) cytotoxicity by +19% and +35% in the PLC/PRF/5 WT and R cells, respectively. Hepa 1-6 R and COR-L23 R cells were also affected by chemosensitizing combination: sorafenib cytotoxicity was increased by +40% and +29%, respectively. In efflux experiments, CRYO inhibited MDR1 and MRP1/2 function by -75% and -81%, respectively, while no significant effect on MRP3, MRP4, and MRP5 transport function was found. Consistently, CRYO was able to increase sorafenib content by +62% in PLC/PRF/5 R cells. Regarding in vivo experiments, the implant of Hepa1-6 R cells resulted in the formation of tumors with a final volume (FTV) of 6.6±0.3 cm3. The treatment with sorafenib moderately inhibited tumor growth (FTV=5.3±0.6 cm3), which was not statistically significant. Interestingly, the combination of sorafenib with CRYO markedly inhibited tumor growth by -58% (FTV=2.8±0.6

cm3). Altogether these results support the potential pharmacological interest of CRYO as a new chemosensitizing agent to be used in combination with sorafenib to overcome MDR phenotype in HCC. The combined treatment of CRYO with sorafenib is expected to increase the effectivity of this drug at lower doses and hence reduce the adverse effects of chemotherapy.

References

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