Effects of the metronomic treatment of β-caryophyllene in combination with low-dose doxorubicin in liver cancer cells

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Standard chemotherapy scheduling requires the administration of maximum tolerated doses of anticancer drugs to reach a good therapeutic efficacy, and is often responsible for various side effects and chemoresistance development. A metronomic chemotherapy, based on the repeated administration and/or continuous infusion of low doses of the anticancer drug, has been proposed as an alternative strategy to the standard protocol, due to its more favourable pharmacokinetic and pharmacodynamic profiles [1]. At moment, although this approach seems useful to treat chemoresistant cancer types, its efficacy remains still undefined [2]. An additional strategy, based on the use of chemosensitizing agents, has been recently proposed to increase the standard chemotherapy effectiveness and reduce the high-dose related toxicity [3]. A lot of natural compounds are reported to possess in vitro chemosensitizing properties; among them, the natural sesquiterpene β-caryophyllene (CRY), known to possess different beneficial properties [4], was found able to increase the cytotoxicity of low dose doxorubicin (DOXO), by likely inhibiting the efflux pumps, in leukemic cells [5]. On the basis of these evidences and taking into account that metronomic scheduling seems to represent the next generation of multitarget cancer therapy, in the present study the ability of CRY to increase the efficacy of low-dose doxorubicin in liver cancer cells was evaluated by applying a metronomic protocol. To this end, human liver HepG2 and cholangiocarcinoma CCA cancer cells have been used as models of hepatocellular carcinoma. Cytotoxicity of both CRY and doxorubicin were evaluated by MTT assay [3]. The metronomic protocol was based on a 2 h low-time exposition to the test substances, followed by a cell washing and 72 h incubation for restoring: this scheduling has been applied one, two and three times. In addition, a 24 h prolonged exposition protocol was assayed. These protocols were used for both the substances alone (CRY, 1, 5, 10, 50, 75 and 100 µg/ml; DOXO,1-1-10-25-50-100 and 500 µg/ml) and the combination of DOXO and a nontoxic concentration of CRY (10 µg/ml). Under our experimental conditions, DOXO reached the maximum inhibition (about 90 %) at 50 µg/ml; at the same concentration, a weak cytotoxicity (about 40 %) was found for CRY. In combination with CRY, the effects of DOXO resulted significantly increased with a higher sensitivity of HepG2 cells. In the 24 h prolonged exposition, a 20-30 % potentiation of DOXO was found at higher concentrations (50-100 µg/ml). In the metronomic scheduling, the weak DOXO cytotoxicity (about 35 % inhibition), found at 50 µg/ml after 2 h exposition, resulted significantly increase after three repeated treatments (reaching a 60 % inhibition). The combination with CRY produced an additional 30 % potentiation of DOXO after two repeated treatments. Particularly, two-repeated metronomic treatment with 10 µg/ml DOXO (which was ineffective when assayed alone and in the standard protocol) produced a 45 % cytotoxicity in combination with CRY. Under the same conditions, 50 µg/ml DOXO reached a 70 % inhibition in respect to the 35 % inhibition of the substance alone. A similar behaviour was found when the CRY and DOXO combination was

assayed metronomically in CCA cells, although repeated treatments induced no additional increases.

These results highlight a possible role of CRY as a chemosensitizing agent for doxorubicin-based chemotherapy of liver cancer and suggest further investigation of the metronomic scheduling as alternative strategy to improve the efficacy of low-dose anticancer drugs.

References

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