SOCIETÀ ITALIANA DI FARMACOLOGIA

MODELLO PER INVIO RELAZIONE DI METÀ E FINE PERIODO

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TIPOLOGIA DI BORSA RICEVUTA: borsa per soggiorno all'estero

TIPOLOGIA DI RELAZIONE (es.: metà periodo o finale): relazione di metà periodo

TITOLO DELLA RELAZIONE: Modulation of ABC-transporter activity by natural substances

RELAZIONE:

Chemotherapy with cytotoxic drugs represents the treatment of choice for patients diagnosed with locally advanced and metastatic cancer. Unfortunately, many oncological patients often became insensitive to a variety of structurally and mechanistically related and unrelated antitumor drugs, due to the development of multidrug resistance (MDR). MDR is defined as a multifactorial phenomenon in which both cellular (due to the alterations of the malignant cell biochemistry) and non-cellular mechanisms have been included (1). The first can be classified into non-classical and transport-based classical MDR phenotypes. Non-classical MDR mechanisms include interference with the activity of some enzymes (*e.g.* glutathione *S*-transferase and topoisomerase) that can reduce chemotherapy efficacy, and changes in the balance of proteins involved in apoptosis. Conversely, transport-based classical MDR mechanisms are related to the ATP-binding cassette (ABC)-transporter function, among which P-glycoprotein (MDR1 encoded by *ABCB1*), multidrug resistance protein (MRP1; encoded by *ABCC1*), and breast cancer resistance protein (BCRP; encoded by *ABCG2*) have been most extensively studied (1). These proteins are responsible for drug efflux from cells, so their overexpression in cancer cells can reduce the anticancer drug bioavailability and favour MDR development. In this context, inhibiting ABC transporter function (or their expression) by weakly cytotoxic agents (namely chemosensitizers) represents a promising approach for MDR-reversion and chemotherapy success. Furthermore, in combination with a chemosensitizer, lower doses of anticancer drugs are effective; hence the adverse effects of chemotherapy can be reduced (2). In spite of the promising properties, MDR-reversal agents at the moment known (*e.g.* verapamil) are only weakly effective

in vivo or produce too severe side-effects; therefore, searching for new bioactive compounds is still needed (3). In recent years, many researchers have turned their attention to natural products because they provide novel chemical scaffolds suitable for the development of new inhibitors. A number of natural substances have been found to resensitize resistant tumor cells by reversing MDR in vitro (i.e. curcumin, flavonoids, carotenoids, etc) (4). In particular, our recent studies have highlighted a potential chemosensitizing effect of a natural sesquiterpene, named ß-caryophyllene oxide (CRYO). This compound has shown the ability to synergistically restore the cancer cell sensitivity to the doxorubicin treatment (a common anticancer drug) in different tumor cell lines (i.e. CEM/ADR5000 cells, which over-express P-gp, and Caco-2 cells, which express P-gp, MRP1, and BCRP). Moreover, it is known to possess interesting protective activities, including genoprotective and antiproliferative ones (5, 6). Considering the above, the aim of the present research project is to evaluate the potential inhibition of ABC-transporter activity by CRYO.

During these first months, preliminary studies were carried out to establish the proper cellular model to use in the subsequent experiments. In particular, three different clones of the human hepatocellular carcinoma (HCC) PLC/PRF/5 cell line were investigated: the wild type cells (WT), and the D5- and D5+ clones, obtained by transfection in the Professor Marin's laboratory. First, the sensitivity of the different clones to common anti-cancer drugs (i.e. doxorubicin and sorafenib) was studied by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay (7). Then, the cell clones were characterized as regards the expression of the main ABC-transporter genes involved in the MDR. To this end, the total RNA from the HCC cells was extracted and used as a template to determine the MDR gene expression by RT-QPCR (7).

After establishing the cellular model, some combination experiments between doxorubicin or sorafenib and known ABC-transporter inhibitors (verapamil for MDR1, diclofenac for MRP family and fumitremorgin C for BCRP) were carried out to investigate the possible pumps involved in the resistance observed. Then, the cytotoxicity of CRYO alone and in combination with sorafenib (10 µM) was evaluated by MTT assay. A series of time-course experiments were carried out to choose the best conditions to use in the subsequent experiments. On the basis of these preliminary studies, a time based protocol (4 hours of exposure to the combined treatment) was applied to highlight only the potential effect of CRYO on ABC-transporters and not a possible increase of sorafenib effect due to a mechanism of generalized toxicity. The potential chemosensitizing properties of CRYO were investigated by using two different concentrations (50 and 100 µM, respectively), at which no toxic effects were observed. After 4h of exposure, the treatment was replaced by fresh culture medium for 72 hours; then the cell viability was measured (7).

The preliminary experiments have highlighted a different sensitivity profile of the three PLC/PRF/5 clones against the anticancer drugs doxorubicin and sorafenib. Particularly, the D5- clone showed an increase of

the resistance respect to the WT cells of about 2-fold following the treatment with doxorubicin and sorafenib. In the D5+ clone, the resistance to the doxorubicin and sorafenib was even higher, 11-fold and 3-fold, respectively. The Q-PCR analysis showed an up-regulation of MDR1, MRP1, MRP2, MRP3 and MRP5 transporter genes in the D5- and D5+ clones. The most important changes have been found for the MDR1 and MRP3 genes, for which the increase of expression was of about 249- and 533-fold, and 9- and 15-fold in the D5- and D5+ clones, respectively. As regards the combination experiments between the anticancer drugs and known inhibitors of ABC-transporters, only verapamil (10 µM) and diclofenac (100 µM) were able to increase the sensitivity of the cell clones to doxorubicin and sorafenib treatment. However, also the combination of sorafenib and CRYO significantly reduced cell viability and increased the cytotoxicity of this anticancer drug. In particular, for WT cells, the sorafenib cytotoxicity was increased of about 19% and 36% in combination with CRYO 50 and 100 µM. Likewise, for D5- clone, the cytotoxic potency of sorafenib was increased in combination with CRYO. The cell viability was reduced of about 32% and 25% in combination with CRYO concentrations of 50 and 100 µM. Viability of D5+ clone was also significantly affected by the combination with an increase of sorafenib cytotoxicity of approximately 35% and 40% in combination with both concentrations of CRYO.

Altogether these preliminary data allow us to hypothesize the involvement of MRP and MDR1 transporters in the sorafenib and doxorubicin resistance. Moreover, on the basis of the results from the combination experiments, a potential inhibition of MRP transporters by CRYO can be expected. This inhibition could explain the restore of the cell sensitivity observed.

Future experiments are planned in order to better investigate the effect of CRYO on ABC-transporter function. In particular, the efflux assay will be carried out to determine the direct inhibition of ABC-transporters by the test substance. Furthermore, the real content of sorafenib in the cells will be evaluated by high-performance liquid chromatography-dual mass spectrometry (HPLC-MS/MS). The content will be evaluated after treatment of liver cancer cells with sorafenib alone or in combination with CRYO.

References

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