COMPARISON OF THE IN VITRO EFFECTS OF NAMI-A AND ANTICANCER DRUGS ON CELL VIABILITY, INVASION AND MMPS RELEASE IN B16 MURINE MELANOMA CELLS

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The development of metastasis is one of the main problems related to the pharmacological treatment of tumors, leading to the failure of antitumour chemotherapy, and it is the major cause of death in tumor bearing patients. Specific *in vitro* test (*Auerbach R et al., 2000; Auerbach R et al., 1991; Springer TA, 1990; Simon N et al., 1992; Repesh LA, 1989)* and experimental procedures are widely used in order to evaluate the mechanism of action of new compounds able to interfere with the metastatic process. Considering that large-scale pre-clinical studies need a great number of samples, *in vitro* systems show the advantage, over *in vivo* models, to spare money and time (*White RJ, 1982*). A preliminary sequence of test based on the study of cytotoxic effects, cell cycle analysis and an invasion assay was settled out in our laboratories focused on the selective antimetastatic ruthenium compound NAMI-A (imidazolium *trans*-dimethylsulfoxideimidazoletetrachlororuthenate) (*Zorzet S et al., 2000*).
My studies are therefore aimed at evaluating the efficiency of this sequence of test for the identification of selective antimetastatic properties in experimental compounds. For this purpose I used two murine lines, B16F1 and B16F10 melanoma cell lines, which have been widely used as model systems in studying the metastasis behaviour. NAMI-A is used as reference antimetastatic drug whereas common anticancer drugs such as *cis*-platin (CDDP), doxorubicin, paclitaxel (PTX) and 5-fluorouracil (5-FU) are submitted to the above test to evaluate their response to cytotoxic unspecific metastasis inhibitors. Cytotoxicity was evaluated by the MTT assay after a 24h and 72h treatment with compounds at doses ranging from 1 nM to 100 µM, depending of the compound used. Even though drug treatment lasted 72 h, no cytotoxicity was found with NAMI-A up to 100 µM. As expected, CDDP, doxorubicin, PTX and 5-FU gave a statistical decrease in cell viability at doses three to four logs lower than the maximum dose used for NAMI-A. Flow cytometry analysis following propidium iodide staining, of cell cycle phases, performed 24h and 48h after 1h drug exposure of B16F10 cells to 100 µM NAMI-A, opposite to the effects on B16F1, showed the increase of cells in S phase and the decrease of cells in G1 phase. This effect is transient as it is abolished 48h after the end of the treatment. NAMI-A induced an irreversible modification of cell cycle distribution was observed only at the elevated and cytotoxic 1mM concentration and it was limited to the B16F1 subline. Conversely, cytotoxic drugs gave a permanent modification of the cell cycle distribution consistent with their mechanism of action and related to a decreased cell viability. Invasion assay was carried out on transwell cell culture chambers coated with fibronectin and matrigel, and with the lower compartment filled with conditioned *medium* derived from 3T3 mouse fibroblast. Quantification of invading cells was performed 96h after cell plating and was achieved by counting the cells that had completely invaded the lower compartment: cell counts were standardized over ten fields under a light microscope. In these experimental conditions cytotoxic NAMI-A didn't reduce the number of invading cells and anticancer drugs were able to inhibit invasion only at doses that decreased cell growth (according to MTT data). On the other hand, 1h pretreatment of cells with NAMI-

A 100 µM gave a statistical decrease in cell invasiveness 96h after sowing as compare to controls. Gelatin zymography test, performed to evaluate the effect of 1h treatment on MMP-2 and MMP-9 proteases release in *medium*, showed 100 µM NAMI-A, to which cells were exposed for 1h, to consistently inhibit the release of these matrix metallo-proteases. The *in vivo* action of these compounds showed NAMI-A is able to inhibit lung metastasis formation much better than PTX and doxorubicin, in mice bearing i.m. implants of MCa mammary carcinoma.
These data show the capacity of the selected *in vitro* test to identify compounds active on metastases with a mechanism unrelated to direct and unspecific cell cytotoxicity.

References

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