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IN VITRO MODEL FOR SCREENING THE ANTIMETASTATIC ACTIVITY OF NAMI-A: INTERACTION WITH METASTATIC AND HOST IMMUNE CELLS, AND CORRELATION WITH ITS CHEMICAL STABILITY

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NAMI-A, [H_2Im] *trans*-[$RuCl_4$(dmso)(Him)], is the first antimetastatic ruthenium based complex that has reached phase I clinical trials. The activity of NAMI-A on metastases is not due to a conventional cytotoxic mechanism, rather it seems that it promotes changes on metastatic cells to a less invasive and less malignant phenotype. To investigate its mechanism of action, a new murine metastatic cell line (metGM) has been isolated, stabilized and fully characterized during the Ph.D. fellowship.

The most important characteristics of metGM cell line, are: a) high levels of CD44 expression; b) the capability to invade matrigel membranes; c) the formation of branching invasive colonies; d) the release of MMPs; and e) the ability to give pulmonary metastases following *i.v.* inoculation into syngenic mice. These characteristics confirm the metastatic phenotype of metGM cells. Interestingly, these cells grow both in adhesion and in suspension, presenting two phenotypically different sub-populations, which show opposite response to NAMI-A. metGM cell line has therefore been used as a model for *in vitro* studying the pharmacological effects of NAMI-A on metastatic cells. 0.01 to 1 mM doses of NAMI-A cause: i) down regulation of CD44 expression; ii) reduction of matrigel invasion; iii) perturbation of mitochondrial functionality; iv) significant modifications of the invasive phenotype (reduction of membrane pseudopodes); v) G_2/M arrest of cell cycle limited to the cell population growing in adhesion; vi) no direct cytotoxicity on the cell line.

Previous *in vivo* studies in mice with solid metastasizing tumors showed the capability of NAMI-A to modify the ratio between tumor infiltrating lymphocytes (TIL) and tumor cells in favor of the former. We therefore used co-cultures of metGM cells and lymphocytes (resting or ConA activated) to investigate the role of this effect for the metastasis growth reduction by NAMI-A. The results obtained show that NAMI-A is not suppressive for the host immune system. Moreover, it induces the metabolic activation of resting lymphocytes (as determined by measuring the mitochondrial functionality) and it maintains the ConA induced activation of lymphocytes (as determined by measuring the total protein content by flow cytometry). When metGM cells and lymphocytes are concomitantly treated with NAMI-A, we measure: i) the increase of lymphocytes in S phase (+64%); ii) the increase of NO release by the immune cells; iii) the decrease of mitochondrial functionality on metastatic cells; iv) the decrease of CD44 expression in metastatic cells; v) the increase of apoptosis of metastatic cells accompanied by vi) a higher amount of lymphocytes rosetting around metastatic cells. These results suggest a selective and different mechanism of action of NAMI-A towards metastatic and host immune cells: it reduces the malignancy of tumor cells and it stimulates the natural defense activity.

Although direct tumor cytotoxicity is not expected to be responsible for its antimetastatic properties, NAMI-A has been proved to interact with cell proliferation. This effect might be due to the significant increase of tumor cells in the G_2/M phase of the cell cycle although this effect is not common for all tumor cell lines. This effect might be the result of DNA interaction of NAMI-A and of the consequent arrangements of cell cycle regulation. To elucidate if DNA is involved in the antimetastatic activity of NAMI-A we have studied by NMR spectroscopy the interactions of NAMI-A and of its *in vitro* generated metabolites with DNA model bases over a wide range of pH (3.0-7.4) and ionic strength, at 37°C. Results showed that pH and ionic strength strongly influence the pathways of *in vitro* chemical transformation of NAMI-A. In physiological solution (phosphate buffer, pH 7.4) NAMI-A disappears rapidly from the solution due to two events that occur simultaneously; a very fast, two step chloride

hydrolysis and the spontaneous release of the dmso ligand. Major molecular stability is achieved in buffer at lower pH (3-6) and in water where no chloride hydrolysis occurs and only dmso ligand is slowly released in time resulting in the formation of the $[RuCl_4(H_2O)(Him)]^-$ complex. This latter coordinates to DNA model bases, 9-methyladenine and 9-ethylguanine, whereas no coordination is observed when NAMI-A is completely hydrolyzed. DNA coordination data are in agreement with the flow cytometry analysis of the cell cycle (G_2/M arrest upon treatment). Freshly dissolved NAMI-A and NAMI-A hydrolyzed in water, i.e. $[RuCl_4(H_2O)(Him)]^-$, cause the arrest of KB cells in the G_2/M phase of cell cycle (>40%). When hydrolyzed, NAMI-A looses this ability, and atomic absorption spectroscopy data shows that cell uptake of NAMI-A is also affected by its hydrolysis, the ruthenium intracellular concentration being reduced up to 50% when NAMI-A is completely hydrolyzed in buffer before treatment.

Taken together these data bring new insights in the elucidation of the mechanism of action of NAMI-A and add new information for the structure-activity relationship for other metal-based antitumour complexes.

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