SINTESI DELLA TESI DI DOTTORATO

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LOCO-REGIONAL THERAPY OF CANCER: DISCOVERY, STABILITY AND CYTOTOXICITY OF CISPLATIN/HYALURONAN COMPLEX IN HYALCIS FILM TOWARD NEW FORMULATIONS

The "origins" of this research project were the "legacy" of Hyalcis, the invented name of a cisplatin-loaded thin film with hyaluronan (NaHA), designed for loco-regional application of cisplatin to patients with pleural mesothelioma (PM), combined to tumor resection. The efficacy and lower toxicity of the film applied intrapleurally, proved in *in vivo* studies *versus* the cisplatin (cisPt) solution, triggered the curiosity to verify the hypothesis of an existing cisPt/NaHA complex. By combining SEC-HPLC with atomic absorption spectroscopy, the complex was qualitatively confirmed, then a RP-HPLC technique was successfully developed to quantify the actual amount of drug coordinated by the polymer. The complex really existed in the film-forming mixture before layering and drying it to manufacture the film used in the *in vivo* studies.

The study of the complexation kinetic between the 2 species revealed an extensive coordination of cisPt by NaHA. A slight excess of polymer was needed compared to stoichiometric molar ratio to reach >90% of complex formed. Concerning NaHA molecular weight (MW), there was minimal difference in cisPt complexation by low or high MW hyaluronan. The interaction with cisPt cross-linked the polymer, causing closer entanglement of its chains as evidenced by rheological studies on the film-forming mixture (FFM). cisPt complexation by the polymer also determined a greater amount of free water in the dry film, which in DSC evaporated at lower T compared to the placebo film.

After demonstrating the existence of cisPt/NaHA complex in the film-forming mixture, the research moved to the films to understand whether and how the complex affected drug release from them. When the film was immersed in 0.9% NaCl solution, cisPt release started with a burst and ended in 48h, while the film completely dissolved in 6h. Independently of the degree of film dissolution, cisPt release was ruled by the complex in solution, sustaining cisPt prolonged release. It was observed that aged films (3- and 7-month-old) became insoluble in saline solution, without modifying cisPt release, that remained comparable to the fresh film. The only difference between fresh and aged films was a greater burst release.

A thorough characterization of films to understand their stability was performed at the School of Pharmacy of the St. John's University (New York, USA). The original film formulation was slightly modified, namely by removing PEG 1000 stearate from the composition. Optical microscopy

showed that the drug presence affected film morphology, which was smooth for the placebo film. Conversely, cisPt-loaded films had cavities. All films were amorphous from 1-day-old to 3-month-old according to X-ray diffraction results, confirming optimal miscibility of the components with no phase separations. DSC scans of cisPt-loaded fresh films confirmed that cisPt/NaHa complexation displaced water molecules originally bound to the polymer, thus easing their evaporation at lower temperature compared to the placebo film. Interestingly, the thermograms of aged films containing PEG 1000 stearate showed that this component had crystallized in 6 months of storage at 25 °C.

The different composition of films did not affect the release of cisPt. The hypothesis to explain the insolubility of aged films was that, during storage, cisPt partly dissociated from NaHA due to an entering group. The leaving platinum had to be replaced by a nucleophilic group, which bound covalently to hyaluronan in a tighter way. Platinum could have been the catalyst of this reaction. The new bond cross-linked the polymer chains permanently, explaining the insolubility of aged films.

The cytotoxicity of cisPt/NaHA complex was equal to that of cisplatin alone in various tumor cell lines. The greatest cytotoxic activity achieved in 2D and 3D melanoma cell models, drove the decision of revising the formulation of cisPt/NaHA complex from film to aqueous viscous solution, made of hyaluronate and cisplatin only. Obviously, the selection of other tumors in which the complex may be effective, requires further studies. Theoretically the complex is suitable for all solid tumors for which cisplatin is already the first-line therapy, but is often discontinued for its dose-limiting toxicity. Of course, the complex must be formulated in a suitable dosage form according to the tumor site.

The findings of this research and the data generated opened new knowledge for the use of the cisplatin/hyaluronan complex in cancer therapy. They will have broad implications for how this new loco-regional chemotherapy could be used for the treatment of PM, but with the potential for extrapolation to other tumors treatable with the same approach.