IN VITRO CYTOTOXICITY OF DEXTRAN-METHOTREXATE CONJUGATES IS DEPENDENT ON THE MOLECULAR WEIGHT OF THE CARRIER USED

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Introduction: Methotrexate (MTX) is one of the drugs widely utilized in the treatment of oncological and hematological diseases. There were numerous attempts to amend this agent by chemical modifications or conjugations with different carriers. The conjugations of the MTX could prolong the time of the drug activity, therefore improving its antitumor effect. Our aim was to investigate *in vitro* cytotoxicity of MTX coupled with different dextran macromolecules in comparison with free drug. We were also interested in establishing the possible dependency of cytotoxicity on the molecular weight of the carrier.

Materials and methods: Five dextran-MTX conjugates were synthesized in our laboratory using dextrans with different molecular weights as the carriers (T_{10}, T_{40}, T_{70}, T_{110} and T_{500} respectively). Conjugation was performed by adding active form of MTX to dextran-containing solution. The stability of prepared conjugates was assessed at different conditions and compounds were stored at -20ºC after lyophilization. All conjugates had approximately the same level of substitution. Free MTX was used as the reference compound. A549 (human non-small cell lung carcinoma), SW707 (human rectal adenocarcinoma) and P388 (murine leukemia) cell lines were used for *in vitro* cytotoxicity assay. Compounds were tested at four consequent concentrations and all tests were repeated three times. The *in vitro* cytotoxic effect of all agents was examined after 72-hour exposure of the cultured cells to varying concentrations of the tested compounds, using the SRB assay and computing average 50% inhibitory concentration (IC_{50}) dose as an endpoint.

Results: Cytotoxicity studies *in vitro* revealed that all dextran-MTX conjugates had approximately 10-fold higher IC_{50} values in comparison with free drug, therefore had lower cytotoxicity, and this difference was statistically significant. There was also difference in the cell line sensitivity to the free MTX and all conjugates. The P388 cell line was the most sensitive and had approximately 10-fold lower IC_{50} in comparison with respective IC_{50} in A549 and SW707 cell lines. The sensitivity of SW707 was just a slight higher than of A549, though still statistically significant. Further analysis established that there was negative correlation between molecular mass of the dextran used as a carrier and the cytotoxicity of dextran-MTX conjugates *in vitro*.

Conclusions: Data of *in vitro* experiments revealed that dextran-MTX conjugates have lower cytotoxicity in comparison with free MTX. The results also showed that there is dependency of the *in vitro* cytotoxicity of dextran-MTX conjugates on the molecular weight of the dextran macromolecule. This fact probably could be explained by different *in vitro* bioavailability of the compounds tested and deserve further investigation. We also consider that *in vivo* tumor models have to be applied to determine whether or not this decreased cytotoxicity *in vitro* would be accompanied by a decrease of an overall toxicity and antitumor activity *in vivo*. These studies are actually undertaken.