Hemocompatibility of Liposomes Loaded with Diglyceride Esters of Methotrexate and Melphalan

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INTRODUCTION: A variety of nanoscale drug delivery systems, including liposomes, offer promising approaches for disease diagnosis, treatment, and prevention [1]. Since majority of the systems is to be administered systemically, there is no doubt hemocompatibility studies of the formulations are obligatory. Here we report the results of hemocompatibility studies of antitumor liposomes carrying diglyceride esters of well-known anticancers, methotrexate and melphalan (**MTX-DG** and **Mlph-DG**, respectively), in the lipid bilayer. Grafting of carbohydrates of sialyl Lewis (**SiaLe**) family onto the surface of liposomes was used to allow for their active targeting to sites of inflammation and neovascularization [2].

METHODS: Liposomes composed of egg phosphatidylcholine (**PC**) – phosphatidylinositol (**PI**) of baker's yeast – **MTX-DG**/**Mlph-DG**, 8:1:1 (by mol), either targeted with 2 mol % of $SiaLe^X$-PEG-DG or not, were prepared by extrusion through polycarbonate membrane filters with mean pore diameter of 100 nm. Liposome size was controlled by methods of dynamic light scattering (**DLS**) and electron microscopy (negative staining and freeze-fracture techniques). Liposome composition was verified with gel chromatography on a Sepharose CL-4B column. Zeta potential was evaluated using a Nano ZS Malvern Zetasizer.

The following panel of hemocompatibility tests was performed according to ISO standards (10993-4). Hemolysis of red blood cells (**RBC**) was assessed by a colorimetric procedure employing Drabkin's reagent. RBC and platelet morphology, counting, and size distribution were analyzed on a Coulter II Multisizer. Beckton Dikinson kit (Human C3a ELISA for quantification of Human C3a-desArg) was used to estimate complement activation in the presence of liposomes. Coagulation cascade functioning was controlled on a Behring Coagulation Timer (Dade Behring).

RESULTS: According to DLS, electron microscopy, gel filtration, spectrophotometry, and elemental analysis of phosphorus concentration data, the prodrugs incorporate completely into lipid bilayer of monolamellar liposomes less than 100 nm in diameter [3]. Drug-loaded liposomes were found to be negatively charged, mean zeta potential ranging from –33.9 ± 1.8 through –45.6 ± 0.8 to –52.7 ± 2.5 mV for formulations containing 10 mol % Mlph-DG, 2.5 mol % MTX-DG, and 10 mol % MTX respectively. Liposomes loaded with Mlph-DG, either targeted or not, didn't exhibit any significant hemoreactivity. On the opposite, liposomes containing MTX-DG induced elevated C3a levels and abnormal coagulation times in a concentration dependent manner. The reactivity of liposome surface wasn't influenced by the structure of carbohydrate ligand attached ($SiaLe^X$ versus $SiaLe^A$) or by the presence or absence of PI, an anti-opsonizing component of the formulation. Important is the fact that decrease of liposome loading with MTX-DG from 10 to 2.5% resulted in lower surface charge density, smaller liposome size and thus considerably reduced impact on the complement activation and coagulation cascades.

DISCUSSION & CONCLUSIONS: Interference of MTX-liposomes with the processes of blood coagulation and complement activation may be ascribed to physical adsorption of at least one of the protein components involved in the interrelated cascades on the surface of MTX-liposomes.

The hemocompatibility tests are of screening nature, meaning they are designed to distinguish between tolerated and unsustainable candidates for drug delivery systems. The data obtained so far proved the relatively good hemotolerance of the liposomes loaded with lipophilic prodrugs despite of some undesirable yet manageable effects on coagulation and complement activation linked to the nature of the drug.

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