Therapeutical and Diagnostic Applications of Salicylated Polyglycerol Dendrimers for Cancer Treatment

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Abstract: In this work a salicylated polyglycerol dendrimer (PGLD-AAS) associated with gadolinium (Gd^{3+}) chelate as MRI contrast agent was synthesized and their potential effects on integrity of cellular and biochemical components of blood was studied in *in vitro* experiments. The physico-chemical properties of PGLD-AAS, such as hydrodynamic size, molecular weight and purity were analyzed by dynamic light scattering (DLS), high performance liquid chromatography (HPLC) and MaldiTof mass spectrometry. Evaluation of the blood contact properties was performed by studying the potential effects of PGLD-AAS on integrity of red blood cells (RBC), platelet aggregation, plasma coagulation, complement activation and protein binding. The potential effects of PGLD-AAS on immune cell function were performed by nitric oxide induction and CHO cells cytotoxicity *in vitro* assays. **Introduction:** During the last few decades, a number of methods have been developed to prepare bioactive dendrimers for clinical applications in oncology. Dendrimers are a class of highly branched synthetic spherical polymers consisting of a vast array of types, chemical structures and functional groups. Recent studies have shown that dendrimers are nanoparticles of significant importance for drug delivery systems due to their property of deliver the drugs into the specific sites of the body. Polyglycerol dendrimers (PGLD) are synthetic biocompatible macromolecules [1] possessing multiple free hydroxyl groups on the surface. Generation-5 (G5) PGLD dendrimers offer a carrier system having a defined branched structure capable of carrying multiple molecular entities, with a highly uniform size in a nanometer scale. In this study, we present the biocompatible and bioactivity properties of G5 PGLD dendrimer conjugated with salicylic acid (AAS) and complexed with gadolinium (Gd^{3+}).

Materials and Methods: Polyglycerol dendrimers (PGLD) with generation 5 were synthesized by a two-step process based on allylation of alcohols and catalytic dihydroxylation in according to literature [2] followed by the carbodiimide esterification with acetylsalicylic acid. A mixture of $GdCl_3$ (1 mmol) and salicylated PGLD (1 mmol) was placed in a Parr Teflon-lined stainless-steel vessel (10 cm^3), and the vessel was sealed and heated at 100 °C for 10 hours. Main FT-IR bands (KBr pellet, cm^{-1}) observed for the PGLD-AAS:Gd^{3+} are: ν 3595m, 3406m, 3402m, 1573 vs, $\nu(CO_2^-)_{asym}$, 1505s, 1403vs, 1303s, 1400s, $\nu(CO_2^-)_{sym}$, 1303s, 1264vs, 1188s, 1139m, 1076 s, 946 w, 905w, 865w, 761w, 728m, 674 s, 557s, 540s, 490m and 440w. The number of Gd^{3+} atoms containing in PGLD-AAS dendrimers was determined by the Inductively-Coupled Plasma technique (ICPM 8500, Shimadzu). Susceptibility and magnetization measurements were performed in liquid samples with a Quantum Design SQUID magnetometer model MPMS XL5, using calibrated gelatin capsules as sample holders with a small diamagnetic contribution. The magnetic susceptibility was measured in the temperature interval between 2 and 300 K, with an applied field of 25 mT. The magnetization measurements were performed at 2 K, with a maximum field of 5 T. Protein adsorption, platelet adhesion, thrombus formation and anticoagulant properties was conducted by *in vitro* experiments to examine the interaction of blood with PGLD-AAS:Gd^{3+}. The cytotoxicity of the conjugate was studied against mammalian cells (CHO).

Results and Discussion: The magnetization as a function of the applied magnetic field $Bo=\mu_o H$ at T= 2 K for $\mathbf{B_o}$ between 0 and 5 T for the PGLD-AAS:Gd^{3+} reaches almost complete saturation for a field of 5 T. It is well known that the relaxivity is one of the most important measures of the effectiveness of the contrast agent for use in magnetic resonance imaging (MRI) [3]. The relaxivity is due to a decrease of the rotational correlation time of the Gd-dendrimer complex. The relaxivity of the PGLD-AAS:Gd^{3+} measured in physiological solutions at pH 7.4, 2T and at 37 °C was $96.0 \pm 0.1\ s^{-1}\ mM^{-1}$ and appears to be convenient to be used in MRI. The PGLD-AAS did not disturb integrity of RBC, did not induce platelet aggregation and did not interfere with aggregation induced by collagen. The PGLD-AAS were not internalized via phagocytic uptake, and did not alter leukocyte proliferation, macrophage oxidative burst or macrophage chemotaxis. Important findings included no potential interference of PGLD-AAS with plasma coagulation factors or complement activation. The conjugate PGLD-AAS:Gd^{3+} exhibit specificity and bioactivity against human breast cancer derived cell line MCF-7. The drug decreased cell viability in a dose dependent manner, exhibiting an IC50 of 88.6 ± 5.3 μM with 50 μM PGLD-AAS:Gd^{3+}. The PGLD-AAS induced morphological alterations on cell line MCF-7 membrane. The biochemical and *in vitro* studies indicate that PGLD-AAS:Gd^{3+} appears to be a promising anticancer and contrast agent for MRI.

Conclusion: *In vitro* evaluation of the PGLD-AAS dendrimer associated Gd^{3+} formulation demonstrated a high degree of biocompatibility, with minimal negative effects on cell viability, immune function and blood components.

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References:

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