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Studies of cell toxicity of complexes of magnetic fluids and biological macromolecules

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Abstract

In this study, we performed a comparative investigation of the binding properties of two surface-coated (carboxymethyldextran/glucuronic acid), magnetite-based biocompatible magnetic fluids with different biological macromolecules (BSA, HSA, and LDL). We also investigated the in vitro toxicity of the complex formed between the magnetic fluid and the biological macromolecule in the neoplastic cell line J774-A.

Keywords: Biocompatible magnetic fluids; Toxicity; Albumin (BSA, HSA); Low-density lipoprotein (LDL); J774 mouse macrophages; MTT assay

1. Introduction

In the last few years, there has been an increasing interest in using biocompatible magnetic fluids (BMFs) as a promising material for clinical therapy applications, as for instance in support of special treatment of neoplastic diseases [1].

Serum proteins and lipoproteins are the most abundant proteins in blood vessels. Upon administration into the blood stream, most of the drugs used for cancer treatment quickly associate with different types of serum proteins (BSA and HSA) and/or low-density lipoproteins (LDLs) [2].

The biological applications of BMFs, however, need a previous evaluation of direct in vitro cytotoxicity before being used as part of a system for drug delivery. In other words, the in vitro evaluation of the BMF toxicity is a crucial step in the analysis of its potential for in vivo procedures [3]. Therefore, the understanding of the interaction that takes place between the drug and the protein is critical in the design of new drug delivery

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systems, since most of the administered drugs are extensively and irreversibly bound to proteins and are transported mainly as a complex structure in the blood stream. As far as the complex involved in the drug delivery system is concerned, the BMF-based drug delivery system provides magnetic nanoparticles, whereas the blood stream provides different proteins (BSA, HSA, and LDL). The nature and the magnitude of the drug–protein interaction significantly influence the biological activity and release of the drug after the target tissue is reached. Among all the different types of lipoproteins, the most important in terms of drug delivery properties are the LDLs [4,5].

The binding characteristic of serum albumins and LDLs determines the drug biodistribution throughout the systemic circulation and is responsible for the pharmacological effects in the organism [6]. This is a key aspect for the development of biomacromolecules-based complexes that should direct their localization to a specific biological site. Because of the overexpression of cellular surface receptors, neoplastic tissues are easily associated with serum proteins and lipoproteins, allowing us to use this strategy as a way of increasing the uptake of specific protein/drug complexes by neoplastic tissues.

In this study, we performed a comparative toxicity investigation of the BMF/biomacromolecule complex in the J774-A cell line using different BMFs and different biological macromolecules. We also estimated the values for the binding constant (K_b) and the binding stoichiometry (n) for six complexes: two different BMFs (CMD1 and GCR) and three different biomacromolecules (BSA, HSA, and LDL). The methodology used to determine K_b and n has been previously described by Tedesco et al. [7].

2. Experimental

The cell line used in this study was the mouse macrophage carcinoma cells J774A, supplied by ATCC. J774A cells were cultured in RPMI-1640 medium (Gibco), complemented with 10% fetal bovine serum (FBS) (Gibco), 1% L-glutamine, and 1% penicillin–streptomycin (Gibco). The cells were used in the logarithmic phase of growth and cultured in a humidified incubator at 37 °C with 5% CO_2.

The methodology used to investigate BMF toxicity was the MTT classical assay. The mitochondrial reduction of 3-(4,5-dimethylthizol-2yl)-2,5-diphenyl tetrazolium bromide (MTT) to formazam was determined as an indicator of the cell viability after incubation of the cell with a BMF. The BMFs used in this study, labeled CMD1 and GCR, are based on surface-coated on magnetite (Fe_3O_4) nanoparticles, and such coating consisted of carboxymethyldextran (CMD1) or glucuronic acid (GCR). Such BMFs were coming from Berlin Heart, and were prepared and provided by Dr. Norbert Buske [8]. Both BMF samples displayed an average diameter of 9 nm. The nanoparticle concentration was set at 1.25×10^{13} particle/mL for the best performances.

The J774A cells were incubated with the BMF/biomacromolecule complex for 30 min, in cellular medium. After incubation, the cells were washed twice and the volume was completed with the addition of 200 μL RPMI-1640 in each plate, for 24 h. The 0.5 mg/mL MTT solution (100 μL per well) was added to the cells on 96-well plates, followed by incubation for 4 h, at 37 °C. After incubation, the crystals formed due to the interaction between the mitochondrial dehydrogenases and the MTT reagent were dissolved with 2-propanol, and the samples were shaken until complete dissolution of the formed product. The absorbance was measured at 560 and 690 nm, using the Molecular Devices VersaMax Tunable Microplate Reader (ELISA). The cell viability percentage was calculated with respect to the control cells incubated without the BMF/biomacromolecule complex [9].

The binding of the BMFs to serum proteins (HSA, BSA), lipoproteins (LDL), and other biological macromolecules was investigated using different spectroscopic techniques. The fluorimetric assays were chosen due to the high sensitivity of the technique in probing the intrinsic fluorescence of the tryptophan residue from the proteins, which is immediately quenched by the binding of the BMF to the protein specific sites. This provides a strategy to investigate the