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.
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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 over-expression 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

interaction between BMFs and serum proteins through the evaluation of specific parameters such as the binding constant (K_b) and the stoichiometry of the complex (*n*), which clearly describe the binding process. Solutions of serum proteins, held at a constant concentration (absorbance at an excitation wavelength lower than 0.05), were titrated with small aliquots of BMF samples at three different concentrations. During the titration procedure, the total protein dilution was kept below 2%. The solution of the serum protein was excited at 290 nm, and the spectrum was recorded between 300 and 500 nm [10].

3. Results and discussion

Fig. 1 represents the double-logarithm plot of the fluorescence intensity of the HSA tryptophan residue (*F*) versus the molar concentration of the carboxymethyldextran-coated BMF ([CDM1]). The inset of Fig. 1 represents the double-logarithm plot of LDL (fluorescence intensity) against CMD1 (molar concentration). F_0 and F_∞ represent the fluorescence intensity of the protein itself and the protein saturated with the magnetic fluid, respectively. Likewise, Fig. 2 represents the double-logarithm plot of LDL (fluorescence intensity) versus GCR (molar concentration). The inset of

Fig. 2 represents the double-logarithm plot of BSA (fluorescence intensity) against GCR (molar concentration). Fig. 3 shows the fluorescence emission spectrum of HSA after addition of the GCR sample. The inset of Fig. 3 shows the double-logarithm plot of HSA versus GCR.

The analysis of the data shown in Figs. 1, 2 and 3 allows us to obtain the values of K_b and *n* (see Table 1) for the two investigated BMFs (CDM1 and GCR) in the six evaluated BMF/biomacro-molecule complexes. Similar studies based on this formalism have been successfully used to evaluate the binding properties of photosensitizers and bioluminescent compounds to biological macro-molecules [11–13].

The results of the in vitro studies using the J774A cell line as a biological model indicated that CMD1/BSA and CMD1/HSA presented about 10% toxicity, with complete absence of cell toxicity for the CMD1/LDL complex. The lower cell toxicity observed in the CMD1-based complexes, added to the values found for K_b and *n*, clearly indicates that both CMD1/BSA and CMD1/HSA present high hydrophobic properties (higher K_b and *n*) and should be indicated for hydrophobic cellular environment. This means that any drug associated with these complexes will be preferentially trapped in the double layer of the cell membrane. On the other hand, the
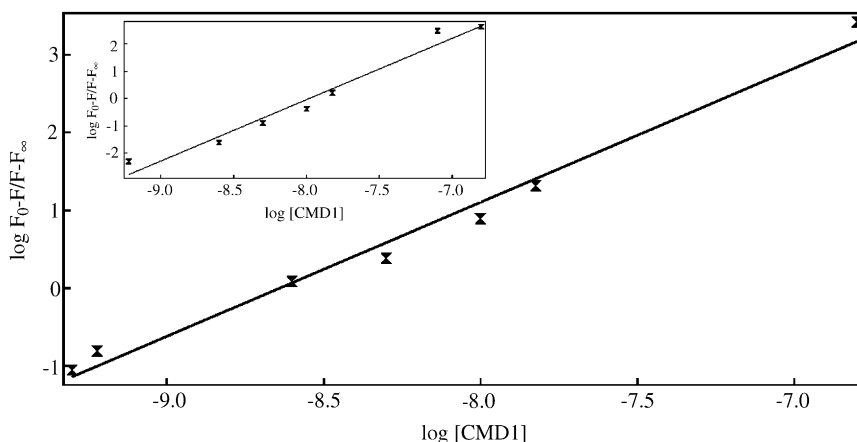

Fig. 1. Double-logarithm plot of the quenching of the HSA fluorescence related to the addition of CMD1. The inset shows the double-logarithm plot of the quenching of the LDL fluorescence related to the addition of CMD1.
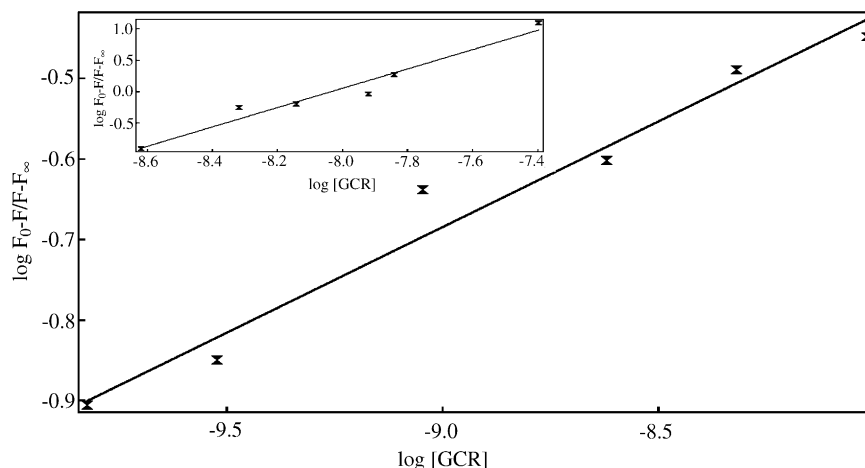
Fig. 2. Double-logarithm plot of the quenching of the LDL fluorescence related to the addition of GCR. The inset shows the double-logarithm plot of the quenching of the BSA fluorescence related to the addition of GCR.
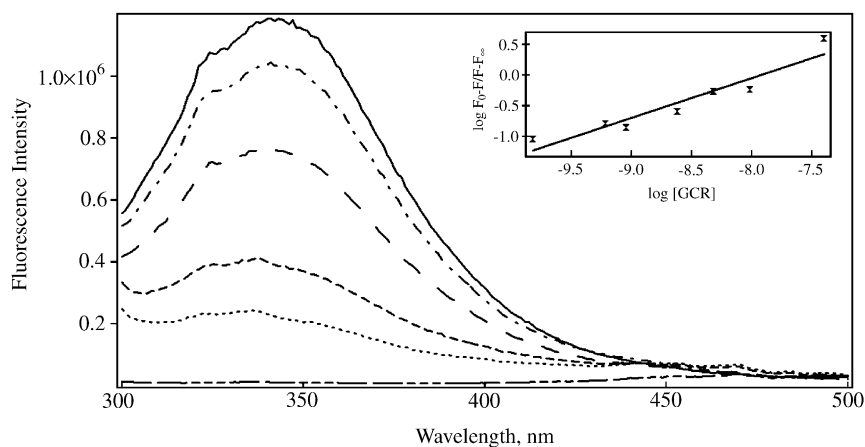

Fig. 3. Effect of GCR concentration on the fluorescence spectrum of HSA. The GCR concentration (in units of particle/mL) increases from top to bottom: 0, 2.25×10^{12}, 2.40×10^{13}, 3.60×10^{13}, 1.00×10^{14}, and 2.00×10^{14}. The inset shows the double-logarithm plot of the quenching of the HSA fluorescence related to the addition of GCR.

Table 1

Serum protein	CMD1		GCR	
	K_b $(\times 10^8)$ (M^{-1})	n	K_b $(\times 10^8)$ (M^{-1})	n
BSA	3.10	1.20	1.15	1.89
HSA	3.84	1.77	1.07	0.65
LDL	0.10	2.09	0.03	0.26

GCR/BSA and GCR/HSA complexes presented toxicity around 20%, with lower K_b and n values, when compared with the CMD1/complex. However, the GCR/LDL complex presented good stability and low cell toxicity (around 5%), indicating its choice for hydrophilic cellular environment. Our findings also indicate that the GCR/LDL complex could be used in the treatment of neoplastic tissues, considering the higher

over-expression of the LDL receptor by the neoplastic tissue.

4. Conclusion

All the data presented here clearly indicate that the choice of the MNP coating is crucial for the MC-MNP complex biodistribution, directly affecting the transport and release of the drug in the target tissue, and consequently affecting their application, biodistribution, and clinical therapy profiles. The resulting complex will present a higher effective magnetic moment and enhanced magnetic susceptibility. Both aspects are key characteristics for protein complex localization in a specific site, considering that protein systems are the most abundant and natural carriers of different kinds of drug with cellular activity. We also know that neoplastic tissues easily associate with proteins and lipoproteins, based on the over-expression of surface receptors. This fact allows us to use this strategy as a way to increase the uptake of specific protein complex drugs against neoplastic tissues. Indeed, the data presented here clearly indicate that the BMF/LDL complexes present an open frontier in drug delivery to biological medium. We are now developing an LDL-based artificial complex (named LDE) to be used as a drug delivery system for BMFs in association with other photo-active compounds for cancer treatment.

References

[1] L.M. Lacava, Z.G.M. Lacava, R.B. Azevedo, et al., J. Magn. Magn. Mater. 252 (2002) 367.
[2] M.H.A. Guedes, M.E.A. Guedes, P.C. Morais, et al., J. Magn. Magn. Mater. 272 (2004) 2406.
[3] A.M.L. Zatón, J.P. Villamor, Chemico-Biol. Interact. 124 (2000) 1.
[4] W.M. Sharman, L.E. van Lier, C.M. Allen, Adv. Drug Deliv. Rev. 56 (2004) 53.
[5] C. Sestier, Z.G.M. Lacava, L.M. Lacava, et al., J. Magn. Magn. Mater. 252 (2002) 403.
[6] B.P. Kamat, J. Seetharamappa, J. Pharm. Biom. Anal. 35 (2004) 655.
[7] A.C. Tedesco, D.M. Oliveira, Z.G.M. Lacava, et al., Appl. Phys. 93 (2003) 6704.
[8] T. Goetze, C. Gansau, N. Buske, et al., J. Magn. Magn. Mater. 152 (2002) 399.
[9] A.C. Tedesco, C.N. Lunardi, J.C.G. Rotta, J. Porphyrins Phthalocyanines 7 (2003) 493.
[10] P.P. Macaroff, D.M. Oliveira, Z.G.M. Lacava, et al., IEEE Trans. Magn. 40 (2004) 3027.
[11] C.N. Lunardi, B.S. Bonilha, A.C. Tedesco, J. Lumin. 99 (2002) 61.
[12] B.M. Aveline, T. Hasan, R.W. Redmond, J. Photoch. Photobiol. B: Biol. 30 (1995) 161.
[13] L.I. Grossweiner, J. Photoch. Photobiol. B: Biol. 58 (2000) 175.