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STUDIES ON THE INTERACTION OF SELF-ASSEMBLED DEXTRIN NANOPARTICLES WITH MURINE MACROPHAGES AND BLOOD CLEARANCE

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KEYWORDS

Dextrin, nanoparticles, macrophages, phagocytose

INTRODUCTION

The rapid removal of intravenously administered colloidal drug carrier systems by the mononuclear phagocytic system (MPS) is an obstacle to the efficient targeting of solid tumours and inflammatory tissues[1,2]. Knowledge on the interaction between macrophages and drug carriers is essential in the design of more effective therapeutic strategies using nanobiotechnological devices. Macrophages may be the therapeutic target, for the treatment of macrophage-associated pathologies or vaccination purposes, given their activity as antigen-presenting cells[3,4]. Otherwise, when other targets are envisaged, phagocytic activity should ideally be avoided, providing the drug carrier time enough to reach the target.

In this work, the interaction between dextrin nanoparticles (with two populations: 25 and 150 nm) - recently developed in our laboratory - and murine bone marrow-derived macrophages (BMDM) was evaluated (cytotoxicity and nitric oxide production). Fluorescein-labelled nanoparticles were used to assess the phagocytic uptake (*in vitro* by Confocal Laser Scanning Microscopy) and blood clearance after intravenous injection.

MATERIALS AND METHODS

Cytotoxicity and Nitric Oxide Production

The cytotoxicity of the dextrin nanoparticles was evaluated, *in vitro*, using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay. The tetrazolium salt is widely used to quantify cytotoxicity, by colorimetry, that is metabolically reduced to highly coloured end products, formazans[5]. The nitric oxide production was evaluated by quantifying the nitrite accumulation in cell culture supernatants using the Griess method[6].

Confocal observation of the macrophages

In order to evaluate the phagocytic activity, macrophages were seeded on coverslips and stimulated with LPS (100 ng/mL) and IFN-γ (1 ng/mL). Then, the cell culture was incubated with or without fluorescein-containing nanoparticles (1.0 or 0.1 mg/mL) for 6 h. The cover glasses were washed twice with PBS and cells were fixed with methanol absolute and labelled with DAPI (staining nucleous-blue).

Blood Clearance

A solution of fluorescein-labelled nanoparticles (100 µL, 0.3 mg/mL) was injected in the tail vein. After pre-defined periods following nanoparticles administration, blood was collected through tail cut. Blood was centrifuged (14000 rpm, 40 min, 4ºC) and the fluorescence intensity of supernatant was analysed. The samples corresponding to the beginning of the experiment (0 h) were collected immediately after injection.

RESULTS AND DISCUSSION

The presence of nanoparticles, in the range of concentrations studied, does not affect cell viability as measured by the MTT assay (Figure 1a). Cells treated with nanoparticles showed a MTT optical density similar to the control (untreated cells). The nanoparticles effect on the nitric oxide production by cultured macrophages was analysed (Figure 1b). Cells were incubated for 48 h with nanoparticles alone and the production of nitric oxide was not detected in the conditions of the assay. Moreover, as expected, the addition of LPS and IFN-γ to the culture medium induced nitric oxide production. The effect of combining LPS and IFN-γ with different nanoparticles concentration revealed that the release of nitric oxide was similar, irrespective of the presence of nanoparticles. The presence of the nanoparticles seems not to promote any

inflammatory action or elicit a reactive response when in contact with macrophages.

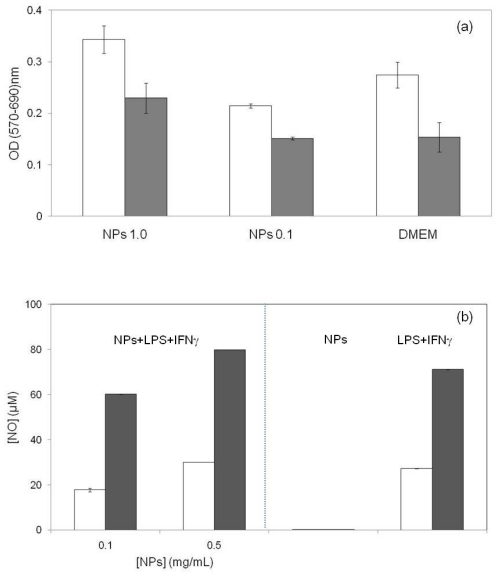

Figure 1 – BMDM viability (a) with different nanoparticles concentrations (1.0 or 0.1 mg/mL) and nitric oxide production (b) using 0.5 or 0.1 mg/mL nanoparticles, after 24 (□) or 48 h (▩) of incubation. For nitric oxide assay BMDM were stimulated with LPS (100 ng/mL) and IFN-γ (1 ng/mL). The error bar corresponds to the standard deviation.

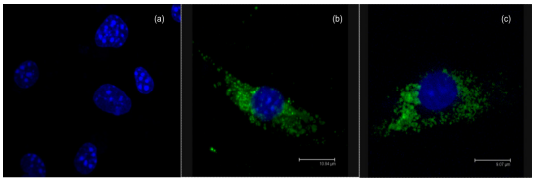

Figure 2 Fluorescence images of BMDM obtained by confocal microscopy, without nanoparticles contact (a), and with 6 h of incubation with fluorescein-containing nanoparticles, 0.1 mg/mL (b) or 1.0 mg/mL (c).

Confocal microscopy was used to examine the uptake of fluorescein-labelled nanoparticles by macrophages. In the control culture (Figure 2a) only nucleus stained with DAPI are visible. Cells incubated with nanoparticles show small green spots (Figure 2b, c), in the cytoplasm, that can be attributed to the fluorescein in the internalised nanoparticles. Fluorescent material is concentrated in cellular organelles, irrespective of the concentration used. The cell membrane is not visible, suggesting that the nanoparticles are not adsorbed on the cell surface.

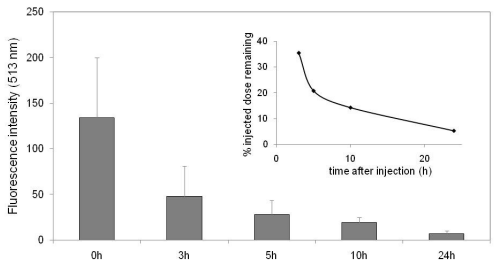

Figure 3 - Blood clearance profile of fluorescein-labelled nanoparticles. Inset shows the percentage of the initial (0 h) fluorescence, remaining in the bloodstream at different time. The error bar corresponds to the standard deviation.

The blood clearance profile for fluorescein-labelled nanoparticles is shown in figure 3. The elimination of nanoparticles from systemic circulation is relatively fast in the first 3 hours, then proceeding at lower rate. In the first period studied, 3 h, about 65% of the nanoparticles were removed from the bloodstream and in the next 2 h another 15% fade away. At 24 h, only 5% of the injected dose is circulating in the bloodstream.

CONCLUSIONS

In vitro studies with BMDM revealed that nanoparticles are non cytotoxic and do not elicit a reactive response when in contact with macrophages. Confocal observation demonstrated that BMDM internalize the nanoparticles. The blood clearance profile reveal a moderately long circulation time, compatible with drug delivery or diagnostic applications. Dextrin nanoparticles may be used to address phagocytic cells.

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