

MAGNETOLIPOSOME EVALUATION USING CYTOMETRY AND MICRONUCLEUS TEST

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INTRODUCTION: A large number of applications of magnetic nanoparticles (MNPs) in the biotechnology and biomedicine field still remain to be devised. In particular, MNPs covered with bio-mimetic materials, as for instance magnetoliposomes (MLs) [1], do offer a wide range of new opportunities. MLs consist of magnetic nanoparticles wrapped by a phospholipid bilayer, probably presenting lipid molecules oriented in a similar way to that observed in biological membranes [2]. These biocolloidal structures can be successfully applied for several purposes, as for instance in drug-delivery systems, magnetic resonance imaging markers for cancer diagnosis, and thermal cancer therapy [3]. The present study reports on several *in vivo* biological tests carried out with a ML sample developed as a precursor of more complex thermal cancer therapy systems.

MATERIALS AND METHODS: DMPG (dimyristoylphosphatidyl-glycerol) and DMPC (dimyristoylphosphatidyl-choline) were obtained from Avanti Polar Lipid Products (Birmingham, Alabama, USA). Lipid dispersions were prepared by ultrasonication of DMPG (10%) and DMPC (90%) at 37 °C. Magnetite (Fe₃O₄) nanoparticles with a diameter of approximately 14 nm were prepared by co-precipitation of FeCl₂ and FeCl₃ in the presence of an excess of ammonia, and subsequently coated with lauric acid to obtain a stable dispersion. Upon co-incubation and dialysis of this so-called water-adapted magnetic fluid in the presence of DMPG-DMPC vesicles, the laurate coat is replaced by phospholipid molecules. During incubation and dialysis for 2-3 days of this water-based magnetic fluid in the presence of preformed sonicated phospholipid vesicles, ML (named DMPC-ML) are formed and subsequently captured from solution with high-gradient magnetophoresis [1,2]. Female Swiss mice were studied: control animals (n = 5) were not treated. In the experimental group adult female Swiss mice were endovenously treated with a bolus dose of 100 µL of DMPC-ML (1.8x10¹⁵ particles/mL). Blood cytometry experiments [3,4] were carried out 1, 6, 12, 24, and 48 hours and also 14 and 28 days after DMPC-ML administration. Imprints of peripheral blood cells were made in glass slides, stained by Wright-Giemsa and five hundred cells per each animal were scored for the cytometry. The data were analyzed by the statistical Scheffe test (ANOVA, p < 0.05). The micronucleus (MN) test [4] was performed 12, 24, and 48 hours after DMPC-ML administration to evaluate ML genotoxic and cytotoxic effects [5,6]. Clastogenic drugs induce MN, a chromosome fragment left in the cell after the expulsion of the main nucleus during maturation of erythroblasts to erythrocytes in bone marrow of mammals. Anucleated polychromatophilic erythrocytes (PCEs) are normally less than 30 hours old and stain differently from normochromatophilic erythrocytes (NCEs). Cytotoxicity is

revealed when the %PCE obtained by $[\text{PCE}/(\text{PCE} + \text{NCE})] \times 100$ is far from the normal expected value (50%). Differences of MN rates and %PCE between spontaneous and DMPC-ML treated cells were tested for significance using the Mann-Whitney test with p < 0.05. As far as we know this study reports for the first time MN tests performed after ML treatments.

RESULTS: Our characterization data showed that DMPC-ML has an average bilayered structure diameter of 20 nm containing 13.27 mg magnetite/mL and 10.24 µmol phospholipid/mL, and thus a phospholipid/magnetite ratio of 0.77 µmol/mg. The DMPC-ML treatment caused no animal death during the 28 days of experiment. Cytometry analysis provided the differential counting of lymphocyte, neutrophil, monocyte, and eosinophil populations. As far as the monocyte and eosinophil populations are concerned, data from the DMPC-ML-treated animals were not different from those of the control group (data not shown). Neutrophil population starts to increase 1 hour after DMPC-ML treatment (Fig. 1), reaching its maximum value 6 hours after ML administration. However, 12 hours after DMPC-ML treatment, the neutrophil population had already decreased in the peripheral blood. From 24 hours until 28 days after administration the cell frequencies are the same as in the control animals. Simultaneously to the neutrophil population increase the lymphocyte population presented a maximum decrease 6 hours after DMPC-ML treatment. Further, 24 hours after DMPC-ML treatment the proportion of lymphocytes in blood returned to control values (see Fig. 1). At the tested concentration, DMPC-ML treatment presented no significant increase in the MN frequency on PCE (Table 1) or NCE (data not shown) on bone marrow along the experiment. There was observed a tendency of MN induction only after 24 hours of ML treatment. The percentage of polychromatophilic cells (see Table 1) presented a significant decrease 24 hours after the DMPC-ML treatment.

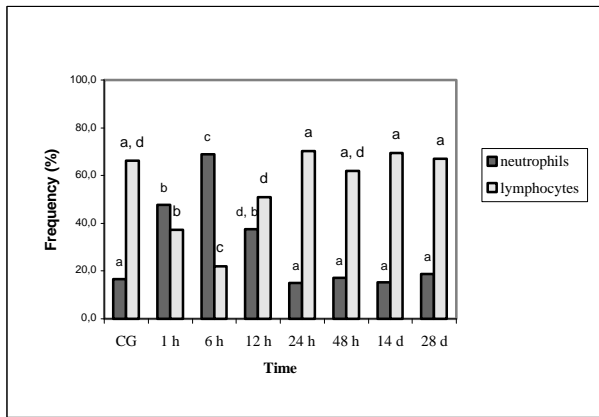


Fig. 1: Effects of DMPC-ML (100 μL of 1.82×10^{15} particle/mL) on the blood cytometry of mice; a-d different letters show statistical differences found in the same cell type frequency ($p < 0.05$); CG = control group.

Table 1. Effects of DMPC-ML on micronucleus induction and percentage of polychromatophilic cells as a function of time treatment.

Group	MNPCE	% PCE
Control	2.9	39.8
ML-12h	2.2	30.3
ML-24h	3.7	21.1*
ML-48h	2.4	26.7

MN, micronucleus (%); PCE, polychromatophilic cell; %PCE = $[\text{PCE}/(\text{PCE} + \text{NCE})] \times 100$; *, statistically different from control group ($p < 0.05$).

DISCUSSION & CONCLUSIONS: The cell population changes detected by blood cytometry analysis revealed an inflammation process that may be considered as a normal host response to the presence of foreign species [4]. The observation of no blood cell population changes 24 hours after the DMPC-ML treatment suggests that a slight instead of a severe inflammation process took place. The absence of MN induction in bone marrow cells revealed that DMPC-ML has no genotoxic effect. Nevertheless, the ML treatment presented a slight cytotoxic effect revealed by the decrease of %PCE on bone marrow cells 24 hours after the ML treatment. This cytotoxic effect is probably related to the slight increase in the MN induction observed after the same period of time. Based on these data we conclude that DMPC-ML is reasonably biocompatible and may be considered as a precursor for magnetothermolysis purposes. Further evidence for this conclusion is supported by the fact that no morphological alterations were observed in several organs examined, even when ML is detected by magnetic resonance experiments, after DMPC-ML administration.

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