ELSEVIER

Journal of Magnetism and Magnetic Materials 252 (2002) 396–398

journal of magnetism and magnetic materials

www.elsevier.com/locate/jmmm

A double-coated magnetite-based magnetic fluid evaluation by cytometry and genetic tests

M.L.L. Freitas[a], L.P. Silva[a], R.B. Azevedo[a], V.A.P. Garcia[a], L.M. Lacava[a], C.K. Grisólia[a], C.M. Lucci[a], P.C. Morais[b], M.F. Da Silva[b], N. Buske[c], R. Curi[d], Z.G.M. Lacava[a,*]

[a] *Dept. de Genetica e Morfologia, Instituto de Ciências Biológicas, Universidade de Brasília, 70910-900 Brasília-DF, Brazil*
[b] *Instituto de Física, Universidade de Brasília, C.P. 04455, 70919-970 Brasília-DF, Brazil*
[c] *Berlin Heart AG, Wiesenweg 10, D-12247 Berlin, Germany*
[d] *Instituto de Ciências Biológicas, Universidade de São Paulo, 05508-900 São Paulo-SP, Brazil*

Abstract

Magnetite nanoparticles pre-coated with dodecanoic acid and ethoxylated alcohol (DE) were used to obtain a physiologically stable magnetic fluid (DE–MF) sample. Three different doses of DE–MF were intraperitoneally applied to mice. Blood and peritoneum cytometry and micronucleus test were performed for 1–21 days after injection to investigate the DE–MF toxicity. Changes in cell population, peritoneum inflammation, and potential DE–MF genotoxic action were all time and dose dependent. At the lowest dose (5×10^{15} particles/kg), DE–MF seems to be useful as a drug precursor with both diagnostic and therapeutic values.

Keywords: Biocompatibility; Cytometry; Magnetic fluid; Magnetite; Micronucleus

Magnetic fluids (MFs) are stable colloidal suspensions composed of magnetic nanoparticles (MNPs) dispersed in organic or inorganic liquid carriers. In order to be used for biomedical purposes, MNPs must be pre-coated with substances that let them stable, biodegradable, and non-toxic in physiological medium. After pre-coating, biological effectors can be adsorbed at the MNP surface to produce biocompatible materials with specific biomedical applications. However, the drawback that mostly concerns the wide use of the biocompatible MF technology is the possibility of biological effector desorption from the MNP surface and the biological effects of the pre-coated MNP alone.

The present study reports on several in vivo biological tests carried out with a water-based MF containing magnetite nanoparticles obtained by chemical co-precipitation of Fe (II) and Fe (III) ions in alkaline medium. After precipitation the nanoparticles were pre-coated with dodecanoic acid followed by an ethoxylated polyalcohol (DE) to obtain a stable DE–MF sample developed as a precursor of anticancer drug [1]. Previous results showed an average core particle diameter of 9.4 nm [2] and grafting coefficients of 10^{-11} and 10^{-12} mol/cm^3 for the inner and outer shell, respectively [3]. Six different groups of female Swiss mice ($n = 5$) were studied: control animals were not treated (Group 1) or treated either with 0.002 M ethoxylated alcohol (Group 2) or with 0.002 M ethoxylated alcohol plus 0.002 M dodecanoic acid (Group 3). Experimental groups were intraperitoneally treated with a bolus dose of the DE–MF sample containing about 5×10^{15} (Group 4), 5×10^{16} (Group 5) or 5×10^{17} (Group 6) particles/kg. Blood and peritoneum cytometry experiments were carried out 1, 7, 14, and 21 days after DE-MF application using the methodology previously described [4]. The results were analyzed by the statistical Scheffe test (ANOVA, $p < 0.05$). The micronucleus (MN) test was performed 24 h after MF application to evaluate the DE–MF genotoxic and

*Corresponding author. Fax: +55-61-3072963.
E-mail address: zulmira@unb.br (Z.G.M. Lacava).

0304-8853/02/$ - see front matter
PII: S0304-8853(02)00655-8

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 the bone marrow of mammals. Anucleated polychromatophilic erythrocytes (PCEs) are normally less than 30 h old and stain differently than normochromatophilic erythrocytes (NCEs). Cytotoxicity is revealed when the %PCE obtained by PCE/(PCE + NCE) is far from the normal expected value (50%). Differences of both MN rates and %PCE between spontaneous and DE–MF 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 MF treatments.

It was observed that ethoxylated alcohol (Group 2) or ethoxylated alcohol plus dodecanoic acid (Group 3) treatments did not change significantly the control MN (Table 1) or cytometry data during all the experiments. At the lowest concentration (Group 4), DE–MF sample showed no genotoxic or cytotoxic effects (Table 1) on bone marrow cells, no alterations in blood cell populations, though causing an increase in the peritoneal neutrophil population (data not shown). This slight inflammation was not detected on peritoneum 7 days after injection. For the intermediate dose (Group 5), the DE–MF induced micronucleus in the PCE (Table 1), suggesting potential genotoxic action. Under DE–MF influence a marked decrease in bone marrow proliferation, evidenced by the reduced proportion of PCE was observed (Table 1). Further, an increase in neutrophil population simultaneous to a decrease in mononuclear phagocytes was induced by the intermediate dose in the animal peritoneum 1 day after injection. The cell population changes were not observed 7 days after the DE–MF injection. Nevertheless, a significant increase in the lymphocyte population was observed from 14 to 21 days after the DE–MF injection, a signal of inflammation persistence or a possible opportunistic infection [7]. On the other side, this DE–MF sample had no effect on the blood cell populations at any time (not shown). For the highest dose (Group 6) the DE–MF showed the most severe effects. An increase in the MN induction was observed in the PCE (Table 1). Although there is a lower MN induction in bone marrow erythrocytes than observed in Group 5, such reduction is probably related to the higher cytotoxic effect observed in Group 6 (21.1%). Anyway, further investigation is required for a better assessment of the genotoxic potential of DE–MF. In the peritoneum of Group 6, a huge increase in neutrophil population concomitant to a decrease in the mononuclear phagocyte population was observed, 1 day after the injection (Fig. 1). Population changes were also observed 7 (decrease of lymphocytes) and 21 days (decrease of mononuclear phagocytes) after the DE–MF injection. Furthermore, in Group 6 the differential blood cell counts showed an increase in neutrophils and a decrease in lymphocyte population during all the experiments. Nevertheless, 21 days after DE-MF injection the differences in the frequency of blood cell populations were not significant. All the observed DE-MF effects were time and dose dependent and can be related to macrophage activity; macrophages are able to produce substances such as interleukins, enzymes, and reactive oxygen species that are involved in changes in the peritoneum cell population, cytotoxicity, and genotoxicity [8,9].

Once at the lowest dose (5×10^{15} particles/kg) the DE-MF sample induced no morphological alterations in the peritoneal organs (data not shown), no genotoxicity, but only a slight and temporary inflammatory effect; we

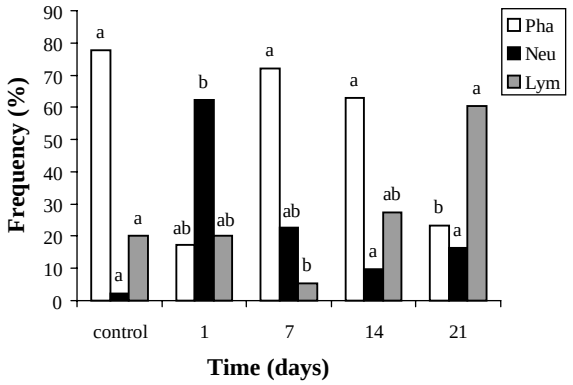

Fig. 1. Effects of DE-MF (5×10^{17} particles/kg) on the peritoneal cytometry of mice. Pha, mononuclear phagocytes; Neu, neutrophil; Lym, lymphocyte; *b*, statistically different from the same cell type indicated by *a* ($p < 0.05$).

Table 1
Effects of DE-MF on micronucleus induction and percentage of polychromatophilic cells

Group	MN PCE	%PCE
1—Control	2.0	46.72
2—0.002 M ethoxylated alcohol	4.6	49.54
3—0.002 M ethoxylated alcohol plus 0.002 M dodecanoic acid	5.0	49.4
4—DE-MF, 5×10^{15} particles/kg	7.4	40.2
5—DE-MF, 5×10^{16} particles/kg	10.0[a]	33.58[b]
6—DE-MF, 5×10^{17} particles/kg	7.6[a]	21.1[c]

MN, micronucleus (%); PCE, polychromatophilic cell; %PCE = PCE/(PCE + NCE).

[a] Statistically different from group 1 ($p < 0.05$).

[b] Statistically different from group 3 ($p < 0.05$).

[c] Statistically different from groups 3 and 4 ($p < 0.05$).

conclude that at this sample concentration the DE-MF could be considered as a potential precursor of anticancer drug. We also conclude that cytometry and micronucleus tests are very useful to investigate MF biological effects.

This work was partially supported by the Brazilian agencies FAP-DF, CNPq/PIBIC, FINATEC, and CAPES.

References

[1] D. Günther, N. Buske, DE patents No. 4325386, 1993 and 4372826, 1993.
[2] B.M. Lacava, R.B. Azevedo, L.P. Silva, Z.G.M. Lacava, K. Skeff Neto, N. Buske, A.F. Bakuzis, P.C. Morais, Appl. Phys. Lett. 77 (2000) 1876.
[3] P.C. Morais, S.W. Silva, M.A.G. Soler, N. Buske, Gen. Anal. Biomol. Eng. 17 (2001) 41.
[4] Z.G.M. Lacava, R.B. Azevedo, L.M. Lacava, E.V. Martins, V.A.P. Garcia, C.A. Rébula, A.P.C. Lemos, M.H. Sousa, F.A. Tourinho, P.C. Morais, M.F. Da Silva, J. Magn. Magn. Mater. 194 (1999) 90.
[5] W. Schmid, Mutation Res. 31 (1975) 9.
[6] C.K. Grisólia, I. Ferrari, Braz. J. Genet. 20 (1997) 411.
[7] W. Guo, R. Willen, R. Andersson, H. Parsson, X. Liu, K. Johansson, S. Bengmark, Int. J. Artif. Organs 16 (1993) 276.
[8] S.H. Khan, I. Emerit, J. Feingold, Free Rad. Biol. Med. 8 (1990) 339.
[9] K. Wyj, Biomaterials 20 (1999) 2213.