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Selective reduction of the interaction of magnetic nanoparticles with leukocytes and tumor cells by human plasma

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Abstract

Carboxymethyl-dextran coated magnetic nanoparticles can interact with viable human cells. The interaction of the nanoparticles is cell-type specific. The addition of human plasma led to a dramatic reduction of magnetically separable leukocytes in comparison to tumor cells. We conclude that low plasma concentrations might support an efficient enrichment of circulating epithelial cells from the peripheral blood of tumor patients.

Keywords: Nanoparticles; Carboxymethyl-dextran coating; Leukocytes; Tumor cells; Human plasma; Epithelial cell enrichment

1. Introduction

It has been shown that dextran coated magnetic nanoparticles can interact with living cells [1–4]. This interaction implies the attachment of the particles to the cell surface and the intake of the particles into the cell. Ingested particles are surrounded by a membrane and form phagosomes, which fuse with lysosomes due to intracellular transport. These particles are not only ingested by specialized cells—so-called professional phagocytes [3]. They can also enter into other cell types, e.g. epithelial cells [1,2,5]. We have shown recently that magnetic nanoparticles interact with living cells in a cell-type specific manner [6]. When isolated leukocytes or cells from the breast cancer cell line MCF-7 were inoculated with magnetic nanoparticles in short-term incubation, significantly more tumor cells were magnetically labeled than leukocytes. Others have shown that the interaction can be modulated by selected parameters, e.g. the nature of the nanoparticles [7] or the specific coating of the magnetic cores [8]. We have also shown that the composition of the incubation buffer and the conditions of incubation are important. After

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establishing the optimal incubation parameters we tried to optimize the differential labeling of tumor cells and leukocytes by magnetic nanoparticles.

The aim of this study was to examine the modulation of interaction of magnetic nanoparticles with leukocytes and epithelial-derived tumor cells in vitro and in vivo in more detail. We and other investigators had shown that the incubation of whole peripheral blood samples with magnetic nanoparticles failed to label any cells sufficiently for magnetic separation. In contrast, under plasma free conditions peripheral blood leukocytes and tumor cell lines as well as primary tumor cells could be efficiently labeled with magnetic nanoparticles. Thus, we tested whether human plasma might be a potent modulator of nanoparticle–cell interaction.

2. Materials and methods

2.1. Cell lines and peripheral blood leukocytes

The breast carcinoma cell line MCF-7 and the chronic myeloic leukemia (CML) cell line K-562 were obtained from DSMZ (Braunschweig, Germany) or ATCC (Manassas, VA, USA). Both cell lines were cultivated under standard conditions with DMEM or RPMI1640 with 10% fetal calf serum. The adherent cell line MCF-7 was treated with trypsin-EDTA in order to detach the cells from the plastic surface of the cell culture flask. Peripheral blood leukocytes were prepared by erythrocyte lysis (Qiagen, Hilden, Germany) from whole blood samples of healthy volunteers or patients with informed consent. The plasma fraction was removed by centrifugation (300*g*, 5 min, 14 °C) before erythrocyte lysis and the whole supernatant was defined as plasma. The leukocyte pellet was washed with erythrocyte lysis buffer twice and then resuspended in PE buffer (phosphate-buffered saline (PBS) and 2 mmol EDTA).

2.2. Magnetic nanoparticles

The nanoparticles produced by one of us (N.B.) consisted of a superpara-(ferro)-magnetic core from magnetite/maghemite. The TEM-size diameter of the core varied between 3 and 15 nm. The average diameter was 5 nm. The nanoparticles were coated with carboxymethyl (CM)-dextran to yield a hydrodynamic diameter of the nanoparticle clusters of 200–300 nm. The saturation magnetization ranged from 4.5 to 6.2 mT.

2.3. Incubation of cells with magnetic nanoparticles

MCF-7 or K-562 cells (1×10^6 cells per 500 µl reaction vial) and/or leukocytes (2.5×10^6 cells per 500 µl reaction vial) were incubated in a short-term incubation (4 to 12 min) with magnetic nanoparticles in PE at 37 °C. Thereafter, magnetically labeled cells were separated using a SuperMACS and MS columns (Miltenyi-Biotech, Bergisch-Gladbach, Germany). The separated cells were designated as the positive fraction (retained in column) and the negative fraction (effluent). Cells from both fractions were quantified (Particle Count & Size Analyzer Z2, Beckman-Coulter, Krefeld). Cell-mixture experiments were additionally analysed by flow cytometry (FACS Calibur, Becton–Dickinson, Heidelberg) using human epithelial-antigen (HEA)-specific staining or autofluorescence.

The plasma experiments were performed as follows: (i) Isolated leukocytes or tumor cells were pre-incubated in a plasma/PE solution containing different concentrations of plasma (0, 1, 5, 10, 25%) for 30 min at room temperature. Before incubation with magnetic nanoparticles the plasma/PE solution was removed and replaced by PE. (ii) Isolated leukocytes or tumor cells or a mixture of both were incubated in a plasma/PE mixture supplemented with different amounts of plasma (0, 1, 5, 25%) for 12 min. (iii) Different amounts of plasma (final concentrations 0, 5, 10, 25%) were added to magnetic nanoparticles/cell mixtures after 4 min of plasma free incubation. The incubation of the cells was then continued for additional time periods (0, 4, 8, 26 min).

3. Results and discussion

3.1. Plasma during incubation

CM-dextran coated magnetic nanoparticles interact with human viable cells without any further

specific groups, e.g. antibodies, attached to the cell surface and are incorporated into vesicles via endocytotic mechanisms. Most of the nanoparticles are retained in the cells within vesicles, mainly phagosomes [9]. The interaction of the magnetic nanoparticles with cells depends on various parameters, e.g. cell type, duration of incubation or presence of inhibiting or supporting agents [10]. In whole blood samples, we failed to label cells efficiently for magnetic separation. In contrast, enriched leukocyte fractions in PE buffer were labeled efficiently within a short time. Therefore, we tested the influence of different plasma concentrations by incubating tumor cells and leukocytes with CM-dextran coated magnetic nanoparticles for 12 min with PE and different amounts of plasma (0, 1, 5, 25%). We could demonstrate a clear reduction of magnetically separable cells with increasing plasma concentrations (Fig. 1).

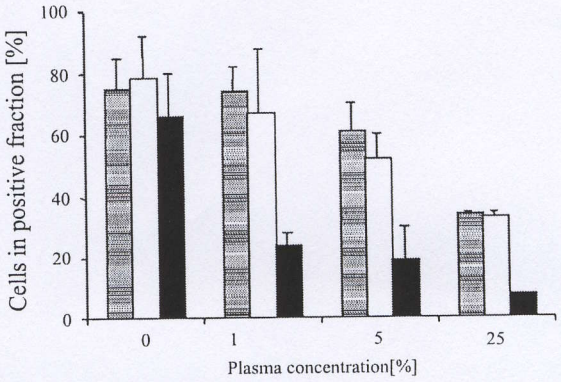

Fig. 1. Human plasma reduces the interaction of magnetic nanoparticles with viable cells. 1×10^6 cells from the breast carcinoma cell line MCF-7 (□) or the chronic-myeloic leukemia (CML) cell line K-562 (▒) and 2.5×10^6 enriched human peripheral blood leukocytes (■) were incubated with 0, 1, 5, and 25% human plasma. After an incubation time of 12 min cells were separated with MACS and the total cell number of the positive and negative fraction were estimated with a Coulter Counter Z2. Cells in the positive fraction are presented as % of the initially applied cell number. Each bar represents 4 experiments ± SD.

Leukocytes showed the most pronounced decrease of separable cells. Even the presence of 1% plasma in the incubation buffer caused a dramatic decrease of more than 60% in comparison to incubation without plasma. Higher concentrations of plasma led to a further decrease in separable cells. MCF-7 and K-562 did not respond as sensitively as the leukocytes to the addition of plasma. PE buffer with 1% plasma caused a reduction of 10% of the total cell amount in the positive fraction. Further increase of the plasma concentration resulted in a 40% loss of MACS-separable cells at a plasma concentration of 5% and a 65% reduction in the positive fraction at a plasma concentration of 25% compared to cell cultures incubated in plasma free PE buffer. Ninety six percent of the cells of the positive fraction as well as the negative fraction were viable and the tumor cells could be recultivated without any significant loss of cells. These results were obtained with cultures of tumor cells or leukocytes only.

Next, we mixed 5% tumor cells and 95% leukocytes and incubated the cell suspension with magnetic nanoparticles in the presence of plasma. The tumor cells were labeled with HEA and the absolute amount of cells in the positive and negative fraction was determined at several time points (Fig. 2). In order to analyse the dramatic decrease of leukocytes between 0% and 1% plasma in more detail, we skipped the addition of 25% plasma and introduced a setting with 0.5% plasma. The vast majority of tumor cells (breast cancer cell line MCF-7) was labeled within seconds. Longer incubation resulted in a continuous increase of separable cells up to 95% after 20 min. Similar results were obtained in the presence of 0.5% and 1% plasma. The addition of 5% plasma during the incubation led to a reduction of about 5% in the total number of separable cells. In contrast, the leukocytes showed again a dramatic reduction in binding and/or incorporation of nanoparticles in the presence of plasma. Under plasma free conditions, initially 10% of the leukocytes were separable with MACS. During the next 20 min the positive fraction rose to 80% magnetically labeled leukocytes. Already the addition of 0.5% plasma reduced the amount of separable cells to less than 40% of the initially applied cells. A further increase in plasma concentration within the incubation medium up to 5% led to a nearly complete inhibition of magnetic labeling of leukocytes. Thus, we could confirm the

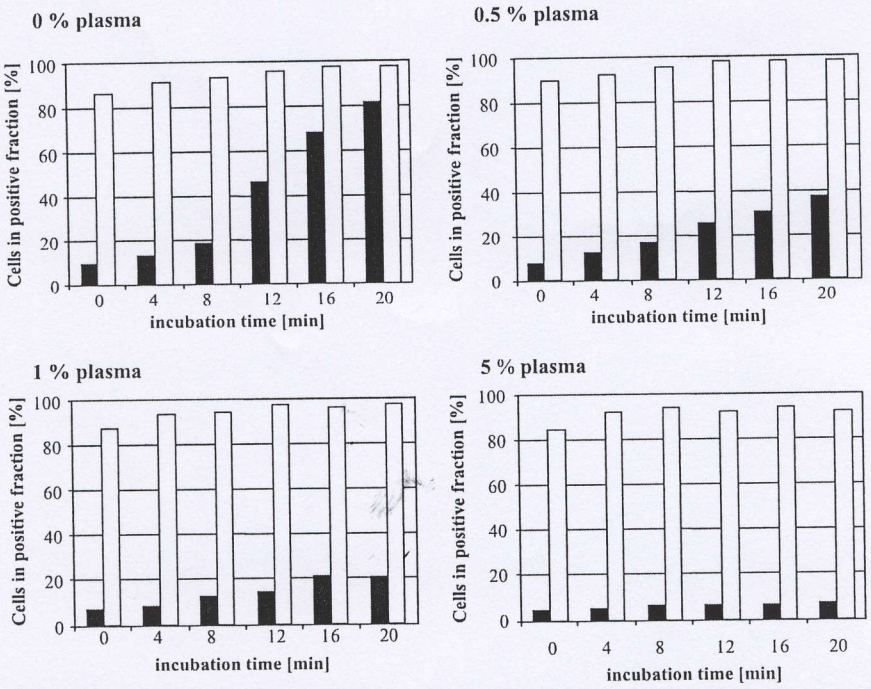

Fig. 2. In a cell suspension leukocytes were dramatically affected by the addition of plasma, but not the tumor cells. 2×10^6 cells (ratio leukocytes:MCF-7 95:5) were incubated with magnetic nanoparticles in the presence of 0, 0.5, 1 and 5% plasma. After 0, 4, 8, 12, 16, 20 min, samples were labeled with HEA (for visualization of MCF-7) and separated with MACS. The total cell number of the positive and negative fractions were estimated with a Coulter Counter Z2. The amount of HEA-positive cells was determined with FACS. MCF-7 □; leukocytes ■.

results which were obtained with cultures of tumor cells or leukocytes. The inhibitory effect of plasma on leukocytes is not restricted to the plasma of the leukocyte donor. Plasma of unrelated volunteers led to the same decrease in the numbers of magnetically separable leukocytes as the donor plasma, which points to common plasma components or properties causing the inhibitory effect.

3.2. Pre-incubation of nanoparticles or cells with plasma

In order to further clarify the mode of action of plasma, we pre-incubated the tumor cells and leukocytes with different concentrations of human plasma. Subsequently, a plasma free incubation with the nanoparticles followed for 12 min. The pre-incubation of MCF-7 with plasma did not inhibit the interaction with the nanoparticles. The same holds true for pre-incubation of leukocytes. Thus we conclude that the plasma-effect was not conservable and plasma, magnetic nanoparticles and cells need to be present at the same time.

3.3. Addition of plasma during the incubation blocked the interaction of magnetic nanoparticles with leukocytes

After 4 min of plasma free incubation, plasma of different concentrations was added and incubation continued. The plasma arrested the interaction of the leukocytes with the nanoparticles (see Fig. 3). Even continued incubation for an additional period of 26 min did not change this result. In the presence of 5% plasma we observed a slight increase with a final amount of 20% labeled leukocytes, whereas in the presence of 10% and 25% plasma the level of separable cells was stable at 15% of the initially applied number of leukocytes. Compared to the control setting with-

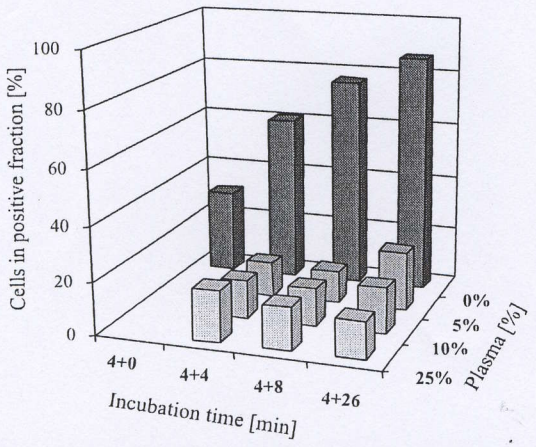

Fig. 3. Human plasma attenuates the labeling of leukocytes after application during incubation with magnetic nanoparticles. 2×10^6 enriched human peripheral blood leukocytes were incubated with 0, 5, 10 and 25% plasma after an initial 4 min incubation of the cells with magnetic nanoparticles. The cells were separated after additional 4, 8 and 26 min of incubation with MACS. Cells in the positive fraction were quantitated with a Coulter counter Z2.

out plasma nearly all cells were labeled magnetically after 30 min of incubation. In contrast to these findings with peripheral blood leukocytes the human breast cancer cell line MCF-7 was not affected by this regime.

Having established these conditions in an in vitro artificial system, using tumor cells lines with peripheral blood leukocytes, we studied, whether the same conditions held true for circulating tumor cells in vivo. We had shown that we can detect circulating epithelial cells in peripheral blood of the vast majority of patients with solid tumors using fluorochrome-labeled HEA antibodies. If such cells could be isolated or enriched by our magnetic beads, this would be a valuable tool for different analytical approaches. Therefore, we incubated leukocyte fractions from tumor patients in the presence of human plasma. First analyses showed that isolated leukocytes from the tumor patients exhibited the same dramatic reduction in magnetic labeling as the leukocytes from healthy volunteers. The circulating epithelial cells within the leukocyte fraction from the tumor patient, in contrast, showed a delayed response to increasing plasma concentrations. Thus, we have first evidence that low plasma concentrations will support an efficient enrichment of circulating epithelial cells from the peripheral blood of tumor patients.

In conclusion, our approach using CM dextran coated magnetic nanoparticles may not only allow efficient recovery of circulating tumor cells from peripheral blood, but also enable deselection of normal leukocytes using appropriate plasma concentrations and thus enrichment of tumor cells from peripheral blood.

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