213

EXOGENOUS MICROPARTICLES OF IRON OXIDE BIND TO ACTIVATED ENDOTHELIAL CELLS BUT, UNLIKE MONOCYTES, DO NOT TRIGGER AN ENDOTHELIAL RESPONSE

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Introduction Targeting particles to sites of inflammation is of considerable interest for molecular imaging and drug delivery. We and others have described micron-sized particles of iron oxide (MPIO) that can be directed using specific ligands to bind to mediators of vascular inflammation in vivo. Since leukocyte binding to these molecules can induce changes in the target cell, an outstanding question is whether the binding of imaging particles induces biologically significant changes in the endothelial cells, potentially initiating or propagating inflammation. We address this by looking for changes in human aortic endothelial cells (HAEC) following binding of contrast agent.

Methods 1 μm MPIO dual-targeted to E-selectin and VCAM were prepared by labelling with specific antibodies, and cellular responses to binding events assessed. Calcium flux was monitored using the calcium indicator Fluo-4 and fluorescent microscopy for analysis. Production of reactive oxygen species was investigated using a nitroblue tetrazolium blue (NBT) assay. Real-time RT-PCR was used to assess gene expression of several endothelial markers of inflammation. Antibody staining of cell surface proteins and flow cytometry analysis monitored protein levels presented by HAEC.

Results Binding of E+V-MPIO did not cause calcium flux in HAEC; normalised fluorescence intensity of Fluo-4 dropped to 0.81 (SEM ±0.02) of baseline 60 seconds post MPIO binding and to 0.64 (SEM±0.02) with vehicle only, whilst a significant increase in Fluo-4 fluorescence to 1.96 (SEM±0.48) was seen following THP-1 binding indicating endothelial calcium flux post-leukocyte binding ($P<0.001$). Assessing cells for formazan precipitate revealed minimal production of ROS in cells incubated with E+V-MPIO and ICAM-1-MPIO, and negative controls (0.3% ±0.3, 3.6% ±1.2% and 0% ±0%) ($P\leq0.001$ all comparisons). Real-time RT-PCR revealed no significant changes to gene expression when antibody-MPIO was bound ($P\geq0.05$ all comparisons); mRNA levels in arbitrary units for E-selectin were 0.93 (SEM ±0.01) compared with 0.93 (SEM ±0.006); VCAM-1 were 0.74 (SEM ±0.13) and 0.79 (SEM ±0.04); ICAM-1 were 0.68 (SEM ±0.25) and 0.6 (SEM ±0.26) for cells with E+V-MPIO bound versus controls respectively. In concordance with gene expression data, there was no change to the cell surface presentation of E-selectin in HAEC with MPIO bound compared with controls; geometric mean fluorescence intensities were 125.5 (SEM±7.5) and 157.5 (SEM±1.5) ($P=0.053$).

Conclusions Our experiments demonstrate that whilst antibody-targeted microparticles mimic the binding capability of leukocytes to inflamed endothelium, they do not trigger the same cellular responses and do not appear to initiate or compound inflammation. These properties are desirable for targeted therapeutic and diagnostic agents.