

INTRATUMORAL ACCUMULATION OF FOLATE RECEPTOR TARGETED USPIO

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Purpose : The folate receptor (FR) is an overexpressed membrane protein in many human tumors like ovary, uterus, kidney and brain. We have grafted folate molecules on USPIO in the aim of a specific internalisation of the magnetics particles into tumors cells via receptor-mediated endocytosis [1]. The use of folate molecules like ligand [2] is enhanced by its high affinity for the FR ($K_D \sim 10^{-10}$ M), low immunogenicity, ease of modification and small size (Mr 441.4).

Subject and Methods : Following cell lines were be used, HeLa (that naturally over-expresses FR), CHO-K1 (Chinese Hamster Ovary), and FRGPI-16 which are CHO-K1 stably transfected with the FR. Internalisation of folate receptor-targeted nanoparticles was studied by confocal microscopy and fluorescence X with hard X-ray, and quantified by ICP-AES. We have studied the contrast enhancement of the signal in magnetic resonance imaging (MRI) after incubation with targeted particles. Cells were growth on RPMI 1640 medium (without folic acid) containing targeted USPIO. After 3 hours of incubation, cells were submitted to confocal microscopy, ICP-AES and MRI studies. Controls were cells incubated with non-specific USPIO.

Results : The specific internalisation of folate grafted USPIO (USPIO-G-folate) was first demonstrated by confocal microscopy after 3 hours incubation (figure 1). Fluorescence has been detected into the cytoplasm of over-expressing-FR cells (HeLa and FRGPI-16) while it is weakly present into CHO-K1 (non expressing FR). The same experiment has been reproduced but with 1 mM folic acid into the medium. In this case, fluorescence was away from all the cells. This observation tend to demonstrate the specific internalisation of our particles by FR. Non-specific USPIO have a very weak internalisation into cells. Single-cell mapping with hard x-ray generated by synchrotron confirm these observations (figure 2) (in collaboration with Dr S. Bohic, ESRF-Grenoble) and by ICP analysis (figure 3) which shows increased internalisation of specific particles compared with non-targeted particles. MRI studies show increased modifications of the contrast with T_2 weighted spin-echo sequences on cultured cells (figure 4).

Conclusion : We have reach a specific vectorisation and internalisation of targeted-USPIO into tumoral cells by the folate receptor pathway. Current NMR imagery of cultured cells tend to show the same results that confocal microscopy. This internalisation is sufficient to produce a significant increasing of the NMR signal to observe tumoral cells.

[1] Antony AC [1992] Blood 79:2807-2820

[2] Gruner BA, Weitman SD [1998] Invest. New Drugs 16:205-219