ImmunoTools *special* Award 2013

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Immune toxicological analyses of superparamagnetic iron oxide nanoparticles (unloaded and loaded with chemotherapeutics) for use in magnetic drug targeting in cancer therapy

In the Section of Experimental Oncology and Nanomedicine (SEON) of the Department of Otorhinolaryngology, University Hospital Erlangen, magnetic drug targeting is developed for the locoregional targeted tumour therapy. For magnetic drug targeting mitoxantrone (MTO) is loaded on superparamagnetic iron oxide nanoparticles (SPION) and these particles are applied into the tumour supplying vascular system and enriched in the tumour region employing an external magnetic field (1). Thus, high local concentrations of the chemotherapeutic agent can be achieved in the tumour region while the rest of the body is preserved from the cytotoxic effects (2). We showed, that with this cancer treatment strategy only 10% of the commonly applied systemic chemotherapeutic dose is necessary to receive tumour remission without side effects in the animal model (3).

Because of their large surface-to-volume ratio nanoparticles harbour unique properties compared with their bigger counterparts. Thus, with the same mass of applied substance the cells are confronted with a fundamentally larger particle surface and, therefore, dose-effect correlations from bulk material can`t be adopted *per se*. Since nanoparticles are highly reactive and can act catalytically, they may induce unexpected reactions.

Our future aim is to accomplish the translation from the successful animal model to the treatment of patients using $SPION^{MTO}$ in magnetic drug targeting. For this, SPION must be comprehensively toxicologically characterized to exclude any possible risk for the patients. General toxicological analyses of SPION investigating the production of reactive oxygen species, cytotoxicity, and genotoxicity are currently performed in our lab in various cell lines and primary isolates. The received data may allow us to create risk profiles of SPION and other nanoparticle systems for *in vivo* use and subsequent application for approval.

Since nanoparticles are intra-arterially applied in magnetic drug targeting approaches, the knowledge, how SPION interact with blood components, is of particular interest.

Therefore, we want to analyse the biocompatibility of SPION with erythrocytes (haemolysis, aggregation, and deformability index), platelets (coagulation, activation), complement (CH50, C3 cleavage) and leukocytes (cellular uptake, cytokine release, leukocyte proliferation, chemotaxis, oxidative burst of macrophages, and maturation of dendritic cells).

The reagents from the ImmunoTools *special* award will be used to analyse the interaction of SPION with particular blood subpopulations in human whole blood employing flow cytometry.

- The engulfment of SPION by specific cells (probably endocytosis) will be monitored by the change of the cellular side scatter in flow cytometry. In combination with spectrometry, the absolute SPION amount can be determined. For the identification of SPION engulfing cells in human blood (monocytes, neutrophils, B cells, T cells etc.) various ImmunoTools antibodies (anti-CD4, anti-CD8, anti-CD11b, anti-CD-14, anti-CD16 etc.) will be needed.

- Analysing coagulation properties of SPION: Using the ImmunoTools antibodies anti-CD41a and anti-CD62p detecting platelet activation markers will be evaluated in the absence and presence of SPION.

- Cytokine release of whole blood / isolated cells after contact with SPION will be analysed using ImmunoTools ELISA Kits IL-4, IL-6, IL-8, IL-12p40, and TNFα.

- The ability of monocytes to mature to dendritic cells (DCs) will be tested in the presence/absence of SPION. DC maturation will be induced by IL-4 and GM-CSF and the DCs will be phenotypically characterized by monitoring the following maturation markers: HLA-ABC, HLA-DR, CD40, CD11c, CD86, CD80, CD14, CD83.

References:

1. Dürr S JC, Lyer S, Tripal, P, Schwarz M, Zaloga J, Tietze R, Alexiou C. . Magnetic nanoparticles for cancer therapy. Nanotechnol Rev 2013. 2013;http://dx.doi.org/10.1515/ntrev-2013-0011.
2. Janko C, Durr S, Munoz LE, Lyer S, Chaurio R, Tietze R, et al. Magnetic drug targeting reduces the chemotherapeutic burden on circulating leukocytes. International journal of molecular sciences. 2013;14(4):7341-55.
3. Tietze R, Lyer S, Durr S, Struffert T, Engelhorn T, Schwarz M, et al. Efficient drug-delivery using magnetic nanoparticles--biodistribution and therapeutic effects in tumour bearing rabbits. Nanomedicine : nanotechnology, biology, and medicine. 2013;9(7):961-71.

ImmunoTools *special* AWARD for **Christina Janko** includes 24 reagents

FITC - conjugated anti-human CD11b, CD16, CD41a, HLA-ABC, CD40, CD86, CD56, HLA-DR,

PE - conjugated anti-human CD8, CD11c, CD80, CD19, CD14, Annexin-V,

PerCP - conjugated anti-human CD4,

APC -conjugated anti-human CD62p, Annexin-V,

recombinant human cytokines rh IL-4 and GM-CSF,

human IL-4 ELISA-set, human IL-6 ELISA-set, human IL-8 ELISA-set, human IL-12p40 ELISA-set, human TNF-alpha ELISA-set, DETAILS