Addressing the PEG problem in nanomedicine: Optimising the biodistribution and pharmacokinetics of polysarcosine-based star dendrimer drug delivery systems by PET/CT

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Background: Polyethylene glycol (PEG) is the gold standard stealth polymer in nanomedicine however concern around anti-PEG immunity has sparked the search for less immunogenic alternatives, e.g. the polypeptoid polysarcosine (pSar). We investigate the stealth properties of pSar *in vivo* via a novel drug delivery platform, a pSar stealth coated dendrimer (SDend). By PET/CT we compare the biodistribution and pharmacokinetics of three drug-free SDends with small, medium and large pSar stealth layers in healthy mice and compare their tumour uptake in an immunocompetent 4T1 mouse model.

Methods: Three pSar SDends (SD28/SD56/SD100) were synthesised via the ring-opening polymerisation of sarcosine N-carboxyanhydride from a generation-5 poly(L-lysine) dendrimer macroinitiator, and functionalised with deferoxamine (*p*-NCS-Bz-DFO) for radiolabelling with zirconium-89, and Alexafluor647 (AF647-NHS) for *ex vivo* fluorescence. Radiolabelling was performed by mixing functionalised SDends with neutralised [^{89}Zr]Zr-oxalate at RT for 1h and purified by size-exclusion chromatography. To study differences in bioD/PK and tumour uptake, [^{89}Zr]Zr/AF647-SD28/56/100 were administered intravenously to healthy or immunocompetent 4T1 tumour bearing female BALB/c mice (~2.5 MBq, ca. 2.5 x10^{15} particles) and PET/CT images acquired 1.5, 24, 48, 72 and 96h post injection (p.i.).

Results: Studies in healthy mice revealed a positive correlation between pSar chain length and blood $t_{1/2}$ emphasising the stealth ability of pSar. All SDends remain in circulation 96h p.i. (10.9 ± 1.6, 16.6 ± 0.5 & 18.2 ± 2.1 %IA/g for SD28/56/100 respectively. SD100, with a blood $t_{1/2}$ of 44.8h (vs 25.9 & 28.2h for SD28/56 respectively) was deemed the best candidate for potential passive tumour targeting and its tumour uptake was compared with that of SD28. We observed high uptake of both SD28 and SD100 in 4T1 tumours, achieving 15.6 ± 2.7 %IA/g for and 18.5 ± 1.6 %IA/g in the tumour 96h p.i. for SD28 and SD100 respectively. Remarkably, SD100 displayed significantly decreased liver and spleen uptake vs. in healthy mice (9.0 ± 0.3, 10.4 ± 0.9 %IA/g in liver and 16.3 ± 3.3, 29.5 ± 2.0 in spleen 96h p.i. for tumour and healthy mice respectively). Results suggest the immunogenicity of pSar SDends is not impacted by the disease state and highlights that SDends preferentially accumulate in the tumour over immune organs and are therefore promising passive drug delivery systems.

Conclusions: By comparing the *in vivo* behaviour of three pSar SDends in healthy mice we reveal the stealth capability of pSar by its ability to significantly reduce immune recognition while prolonging blood circulation. We demonstrate the potential utility of pSar SDends as a novel drug delivery platform due to their high passive tumour uptake, resulting in a drug delivery system with high therapeutic potential.