antibody, 28H1, to which the PS, IRDe700DX, for tPDT is attached. Here we investigated the feasibility of using FAP-tPDT to induce cell death in murine arthritic synovium ex vivo.

Methods: After conjugation of the IRDe700DX to 28H1 (28H1-700DX), binding and specificity of the conjugate was determined. Subsequently, tPDT efficiency in vitro was established using a 3T3 fibroblast cell line stably transfected with FAP. Biodistribution using an [111In]In-DTPA-28H1 conjugate with and without IRDe700DX was performed in healthy C57BL/6N mice as well as in C57BL/6N mice with antigen induced arthritis (AIA). Finally, the potential of FAP-tPDT to induce targeted cell death in the synovial lining was determined by treating knee joints from mice with AIA ex vivo.

Results: Conjugation of IRDe700DX to the antibody did not negatively influence the immunoreactive phrase or binding capacity of the conjugate (94% for 28H1-700DX and 91% for 28H1 for both conjugates). IRDe700DX induced FAP-specific cell death in vitro. At 17.6 J/cm² radiant exposure, 89.24% ± 3.67% of fibroblasts died in the group incubated with antibody compared to control incubated with buffer only (p<0.001). Biodistribution of the compound with the PS showed increased accumulation in the liver compared to the antibody without PS (31.46 ± 2.49% injected dose per gram tissue (%ID)/g versus 5.32 ± 1.17% ID/g for the antibody with or without PS, respectively (p<0.001)). However, despite this increased clearance to the liver, accumulation in the inflamed joints was increased in the group injected with the antibody-PS construct (1.61 ± 0.08 %ID/organ vs. 11.33 ± 0.06 %ID for the antibody with or without PS (p<0.001)). Interestingly, ex vivo FAP-tPDT of knee joints of arthritic mice caused significant reduction of the PS fluorescence signal remaining versus 96.00 ± 25.08% compared to the unexposed control at baseline, p=0.047). Furthermore FAP-tPDT induced marked apoptosis as was indicated by an increased staining of the markers caspase-3 and yH2AX evident in the synovium of treated knee joints.

Conclusion: Here we demonstrated the feasibility of conjugating a PS to an antibody targeting FAP on activated SF without negatively impacting the binding capacity thereof. Furthermore we showed that this construct can then be used to deliver cell specific cytotoxicity through tPDT both in vitro and ex vivo in a mouse model of arthritis. This approach may have therapeutic potential in the treatment of RA.

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