Peptides specifically target bacteriophage MS2, filled with an apoptosis-inducing agent, to tumour

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Summary

We employed bacteriophage MS2 for the targeted delivery of an apoptosis-inducing agent into tumour tissue. The targeted delivery was provided by iRGD peptides, ligands of integrins presumably located on the surface of tumour tissue novel vasculature endotheliocytes. Peptides were synthesized and conjugated to MS2 capsid proteins. Tl^+ ions as $TlNO_3$ were used as apoptosis-induced agents, which penetrated into phage particles and tightly bond to the phage RNA. Peptide-modified MS2 preparations filled with Tl^+ caused cell death in several tested solid tumour cell cultures and tumour mass loss in xenograft mice with inoculated with MCF7 and DA-MB-231 cells, while no effect was observed with control preparations (MS2 + peptide, MS2 + Tl^+). Overall concentration of $TlNO_3$ in peptide-modified MS2 filled with Tl^+ was 500000 times lower than LD_{50} , and therapeutic index of the phage-based preparation was about 15000. The results show the perspective of the use of bacteriophage MS2 filled with Tl^+ and targeted by iRGD peptides as a lead substance for solid tumour therapy.

Introduction

A target-delivering drug system (fig. 1) composed of: 1) a ligand - iRGD peptide (fig. 2), known to bind to $\alpha V\beta 3$ and $\alpha V\beta 5$ integrins that are expressed on endothelial cells of the newly formed tumour vasculature and certain tumour cells, and to activate an endocytic/exocytic transport pathway *via* the interaction with neuropilin-1 [1]; peptide ligands contained Gly3 spacers for their conjugation with phage particles; 2) a vehicle - bacteriophage MS2 with a single-stranded genomic RNA, replicating only in F-pilus-bearing enteric bacteria that are neither symbiotic nor pathogenic for human organisms. MS2 capsid contains pores permeable for metal ions (Tl^+). MS2 serves an impermeable vehicle for Tl^+ , excluding its toxic action on tissues except tumour and its vasculature; 3) an active substance - Tl^+ , a toxic metal ion known to cause cell apoptosis. Tl^+ tightly binds to viral RNA and hence does not leak from MS2 particles [2]. It is not removed from cancer cells *via* multidrug resistance-responsible systems [3].

Figure 1: A schematic image of bacteriophage MS2 conjugated with iRGD peptide and filled with Tl^+ .

Figure 2: Structure of iRGD peptides

Experimental

iRGD peptides were prepared by automated SPPS (433A peptide synthesizer, Applied Biosystems) on 2-Cl-Trityl resin from Fmoc-amino acids *via* FastMoc procedure (activators - HBTU with Oxyma Pure); cysteine derivatives (Trt- and AcM-protected) were coupled with DIC/Oxyma Pure. Cyclization *via* formation of S-S bridges was achieved on totally protected peptides in solution by I_2 . After the cyclization the peptides were deprotected, purified by reverse-phase HPLC and characterized by LC-MS for the conformity of their structure and purity. Peptides were conjugated to MS2 phage particles by using a bifunctional reagent dimethyl adipimidate through protein and peptide free NH_2 groups [4]. Filling of peptide-MS2 particles with Tl^+ was achieved by incubating peptide-conjugated MS2 (3.3×10^{10} pfu (plaque forming units)/mL) with 0.5 mM $TlNO_3$ with further precipitation by polyethylene glycol 6000 and dialysis. Tl^+ dosage was 5×10^{-7} g-equiv per 1 pfu (determined fluorimetrically and mass-spectrometrically). Four different preparations of peptide-conjugated MS2 filled with Tl^+ containing four different iRGD peptides were mixed in about equimolar ratios for further testing. Testing of the drug delivery system was performed on cultivated MCF-7 (hormone-dependent breast cancer) and DA-MB-231 (hormone-independent breast cancer) cells and nude mice with inoculated MCF-7 and DA-MB-231 xenografts. Cell viability was tested in serum-free (enhanced phagocytosis) and serum-containing media by Evans Blue staining. Effect on animal xenografts was evaluated after 10 p.o. or i.p. injections of 10^8 pfu/kg/day by comparing tumour area in experimental and control animal groups. Peptide-MS2 without Tl^+ and MS2+ Tl^+ without peptides were used as controls.

Results and Discussion

iRGD peptides were prepared in 95% purity. Lys^8 -containing iRGD peptides formed intermolecular rather than intramolecular S-S bridges by air oxidation of deprotected peptides and did not readily form S-S bridges while on resin, and we could successfully cyclize these peptides only in a totally protected state. Arg^8 -containing peptides were prepared by the same scheme as well. Incubation of peptide-conjugated MS2 particles in $TlNO_3$ solution at neutral pH resulted in the drop in Tl^+ concentration in the media up to 30 times compared to the initial one, thus representing the filling of the phage particles with Tl^+ , which was tightly bound to the phage RNA. Free Tl^+ content of the preparation was $<10\%$, while Tl^+ content inside phage particles was about 5×10^{-11} g-equiv./pfu. An equivalent of 10^8 pfu peptide-modified MS2 filled with Tl^+ added to cell cultures resulted in 80-85% both MCF7 and DA-MB-231 cell death in enhanced phagocytosis conditions, with much lower apoptosis in serum-containing media. No cell death was observed in control experiments. When peptide-modified MS2 filled with Tl^+ was injected to MCF7- and DA-MB-231-xenograft nude mice, it caused tumour mass loss up to 2.5 times compared to control (fig. 3).

Before injections

After injections

Figure 3: Tumour necrosis areas visualized on photographs and determined by ScanScope CS2

LD₅₀ for iRGD-MS2-Tl was estimated as 1.5×10^8 pfu/kg in Wistar-Kyoto rats. Overall concentration of TlNO₃ in peptide-modified MS2 filled with Tl⁺ was 500000 times lower than TlNO₃ LD₅₀, and therapeutic index of the phage-based preparation was about 15000. The results show the perspective of the use of bacteriophage MS2 filled with Tl⁺ and containing a target delivery system as a lead substance for solid tumour therapy. No Tl⁺ leakage was observed during iRGD-MS2-Tl storage. Hence iRGD-MS2-Tl was recommended for preclinical investigations as an anticancer drug for the treatment of breast cancer.

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