PEGylation of APIT

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Introduction

APIT (*Aplysia punctata* ink toxin) is a 60 kDa protein isolated from the defensive ink of the sea hare *Aplysia punctata*. In the meantime it is produced as a recombinant protein in bacteria. APIT kills tumor cells in an apoptosis independent manner. APIT possesses an L-amino acid oxidase activity that catalyzes the oxidative deamination of L-lysine and L-arginine.

Figure 1: *Aplysia punctata*

A PEGylation feasibility was performed in order to evaluate the impact of PEGylation on the pharmacological properties of APIT.

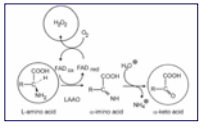

Figure 2: Amino acid depletion

Experimental

PEGylation

Free Amino groups of APIT were modified with 5kDa mPEG-NHS ester (NOF Corp., Japan) in Sodium bicarbonate buffer under alkaline conditions. The crude conjugate was purified by size exclusion chromatography on a Superdex 200pg column with PBS as eluent.

Animal experiments

Pharmacodynamics were determined in immune competent mice following a single i.v. administation. Amino acid levels in plasma were determined from Heparin-Plasma samples with an amino acid analyzer. Efficacy was tested in nude mice that were injected s.c. with GLC4 ADR (multi-drug-resistent lung carcinoma). Mice were treated i.v. every 7 days for 3 weeks. PBS and Doxorubicin were used as reference treatments. Read out was the tumor volume.

Results

PEGylation of APIT

PEGylation of APIT was performed at pH 9.0 (Figure 3). The final conjugate appeared as a homogeneous band in SDS-PAGE and contained no residual unmofied APIT. The overall protein yield was >85% and residual activity of the conjugate was >80%. PEG-APIT and APIT had similar in vitro anti-tumor activity and comparable enzyme properties regarding substrate specifity, pH- and temperature optimum. PEG-APIT contained approx. 10 mol of 5kDa mPEG per mol of APIT as deterimined by MALDI-MS.

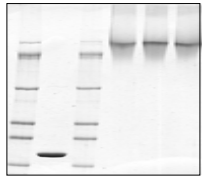

Figure 3: SDS-PAGE analylsis of APIT (lane 2) and PEG-APIT (lanes 4 to 6)

Pharmacodynamcics

Immune competent mice were treated with a single injection of 15U/kg APIT, PEG-APIT or PBS. Lysine in the control (APIT) and PBS remained at 350-400µM over the total test period.
In contrast, a treatment with PEG-APIT reduced Lysine level to zero for 96 hours.

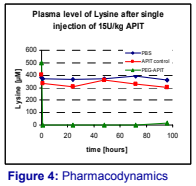

Figure 4: Pharmacodynamics of PEG-APIT after single i.v. injection

Efficacy

Mice with a multi-drug resistant lung carcinoma were treated with APIT, PEG-APIT, Doxorubicin and PBS (control). After 11 days the tumor in the PBS control had a volume of 1780%. APIT control showed a reduced tumor volume (1230%). Both, Doxorubicin and PEG-APIT showed the lowest tumor volume after 11 days (approx. 750-800%). While Doxorubicin caused a significant loss of weight in treated mice, body weight maintained stable in mice treated with PEG-APIT.

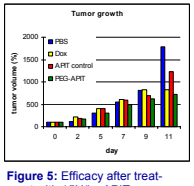

Figure 5: Efficacy after treatment with 15U/kg APIT or 3mg/kg Doxorubicin on day 0, 5 and 10, n=9 per group

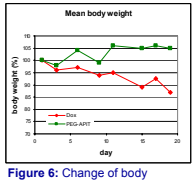

Figure 6: Change of body weight during treatment with Doxorubicin and PEG-APIT

In summary, APIT showed low tumor growth inhibition. PEG-APIT showed the best tumor growth inhibition and had no visible side effects. The Doxorubicin reference also showed good efficacy at 3mg/kg but caused significant loss of body weight.

Conclusion

PEGylation preserved the enzyme characteristics of APIT and at the same time strongly increased *in vivo* stability of the enzyme. In this way a long lasting oxidase activity was observed after single i.v. injection.
Tumor activity against multi-drug resistant lung carcinoma cell GLC4 ADR was significantly increased *in vivo*. PEG-APIT was well tolerated by mice as judged from the stable body weight during treatment.
Thus, a conjugate of APIT with linear 5 kDa mPEG could be a candidate for further development.

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