Novel TPGS based paclitaxel prodrug to overcome multidrug resistance of cancer cells

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ABSTRACT SUMMARY

Novel D-α-tocopherol polyethylene glycol succinate (TPGS) based redox-sensitive paclitaxel prodrug (TPGS-SS-PTX) is reported in overcoming multidrug resistance (MDR) of cancer cells. The prodrug was successfully synthesized and can self-assemble into micelles. The cleavage of disulfide bond of the prodrug, which occurs in the cancer cells, would result in rapid release of PTX and TPGS, P-glycoprotein (P-gp) inhibition by the TPGS moiety and improved cell cytotoxicity. In vitro experiments have exhibited the increased cell cytotoxicity against MDR cells of ovarian cancer (A2780/T) compared to Taxol® and the control TPGS-PTX, which the linker between TPGS and PTX was succinic anhydride (SA) instead of dithiodipropionic anhydride (DTDPA). In vivo evaluation also showed that TPGS-SS-PTX significantly delay tumor growth and substantially reduced the side effects.

INTRODUCTION

Paclitaxel (PTX) is one of the most effective broad-spectrum chemotherapeutic agents in the treatment of cancer. However, its current clinically administered dosage form has serious side effects and poor pharmacokinetic profiles and biodistribution. Another major problem in the clinical treatment with PTX is the multidrug resistance (MDR), which may greatly lower the treatment efficiency. Overexpressing of P-glycoprotein (P-gp), a kind of ATP-binding cassette, is one of the main reasons for PTX resistance. Many researchers have proved that co-delivery of a P-gp inhibitor with PTX was able to overcome MDR in cancer chemotherapy.

D-α-tocopherol polyethylene glycol succinate (TPGS), is formed by esterification of Vitamin E succinate with polyethylene glycol (PEG). TPGS has been approved by FDA as a safe pharmaceutical adjuvant in drug formulation. In recent years, it has been intensively applied in developing various drug delivery systems and also been used as a P-gp inhibitor in overcoming MDR.¹

In this study, a simple PTX prodrug based on TPGS and disulfide bond was developed and its ability to overcome MDR investigated. As we know, disulfide bonds are very stable in human blood plasma, however, they can be cleaved under intracellular reductive conditions, where the glutathione concentration is around 10 mM. This prodrug can be quickly cleaved after endocytosis by tumor cells to cause release of the active drug and consequent cancer cell cytotoxicity. Free TPGS binds with P-gp, restrains its activity and reduces the efflux of PTX, so as to reverse MDR of resistant cells (Scheme 1.). In vitro cytotoxicity against A2780 and A2780/T cells and in vivo evaluation in BALB/c-nu MCF-7 xenografted tumor of this prodrug were investigated. Taxol® and another prodrug TPGS-PTX with an insensitive conjugate bond that would release PTX slowly were chosen as the controls.

EXPERIMENTAL METHODS

Dithiodipropionic TPGS ester (TPGS-SS-COOH) was prepared by TPGS and DTDPA using DMAP as catalyst. The conjugation of PTX and TPGS-SS-COOH

![Scheme 1. schematic illustration of redox-sensitive prodrug for overcoming MDR of cancer cells](attachment://Scheme_1.png)
RESULTS AND DISCUSSION

TPGS-SS-COOH and TPGS-SA were characterized by $^1$H NMR. The signals at 2.6-2.7 ppm confirmed the successful esterification reaction between DTDPA/SA and TPGS. The typical $^1$H NMR spectra of PTX, TPGS, TPGS-SS-PTX and TPGS-PTX are shown in Fig. 1. After treatment of TPGS-SS-PTX with 10 mM GSH at pH 7.4, two peaks (2400 and 650) were showed in the GPC elution profile, while the treatment of TPGS-PTX with GSH only showed a single peak corresponding to 3035 Da. This result confirmed the redox-susceptibility of TPGS-SS-PTX. TEM and DLS showed that TPGS-SS-PTX prodrug self-assemble in micelles of about 170 nm and the colloidal aggregate is highly stable. The diameter remained unchanged more than 72h in PBS (37°C, pH=7.4).

Fig.1. $^1$H NMR spectra of TPGS, TPGS-PTX, TPGS-S-S-PTX and PTX

Cell cytotoxicity results (Fig.2) showed that for A2780/T cells, TPGS-SS-PTX exhibited significantly higher cytotoxicity than Taxol®. Due to the fast degradation property of the disulfide bond while the releasable bond is not present in the control TPGS-PTX, it was also found out that TPGS-SS-PTX did much better than TPGS-PTX in killing tumor cells. TPGS-SS-PTX exhibited comparable inhibition on tumor growth, lower systemic toxicity and longer survival duration compared to Taxol® on in vivo MCF-7 xenograft model.

Fig.2. Cell cytotoxicity of (a) A2780 and (b) A2780/T cells after 24, 48, 72h culture with PTX, Taxol®, TPGS-PTX and TPGS-SS-PTX at various equivalent PTX concentrations.

CONCLUSION

Novel TPGS based prodrug TPGS-SS-PTX was prepared successfully and it showed great potential in reversing MDR due to its redox-sensitivity and P-gp inhibition properties.

REFERENCES

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