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In vitro and *in vivo* anti-tumor activities of a gemcitabine derivative carried by nanoparticles

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ABSTRACT

Gemcitabine (Gemzar®) is the first line treatment for pancreatic cancer and often used in combination therapy for non-small cell lung, ovarian, and metastatic breast cancers. Although extremely toxic to a variety of tumor cells in culture, the clinical outcome of gemcitabine treatment still needs improvement. In the present study, a new gemcitabine nanoparticle formulation was developed by incorporating a previously reported stearic acid amide derivative of gemcitabine into nanoparticles prepared from lecithin/glyceryl monostearate-in-water emulsions. The stearoyl gemcitabine nanoparticles were cytotoxic to tumor cells in culture, although it took a longer time for the gemcitabine in the nanoparticles to kill tumor cells than for free gemcitabine. In mice with pre-established model mouse or human tumors, the stearoyl gemcitabine nanoparticles were significantly more effective than free gemcitabine in controlling the tumor growth. PEGylation of the gemcitabine nanoparticles with polyethylene glycol (2000) prolonged the circulation of the nanoparticles in blood and increased the accumulation of the nanoparticles in tumor tissues (>6-fold), but the PEGylated and un-PEGylated gemcitabine nanoparticles showed similar anti-tumor activity in mice. Nevertheless, the nanoparticle formulation was critical for the stearoyl gemcitabine to show a strong anti-tumor activity. It is concluded that for the gemcitabine derivative-containing nanoparticles, cytotoxicity data in culture may not be used to predict their *in vivo* anti-tumor activity, and this novel gemcitabine nanoparticle formulation has the potential to improve the clinical outcome of gemcitabine treatment.

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1. Introduction

Gemcitabine (2',2'-difluorodeoxycytidine, dFdC) is the active ingredient in Gemzar® (Eli Lilly & Co., Indianapolis, IN), which is the first line treatment for pancreatic cancer (Burris et al., 1997). The therapeutic efficacy of Gemzar® as a single agent is modest, and thus, Gemzar® is often used in combination therapy for non-small cell lung cancer, ovarian cancer, and metastatic breast cancer. Although extremely cytotoxic to tumor cells in culture, the clinical efficacy from gemcitabine (Gemzar®) treatment requires further improvement (Kleeff et al., 2006; Philip, 2010).

Gemcitabine is a prodrug, and its mechanism of action is based solely on intracellular phosphorylation into its active triphosphate

derivative (Bergman et al., 2002). About ninety percent of gemcitabine triphosphate (dFdCTP) is rapidly eliminated, mainly due to deamination to 2',2'-difluorodeoxyuridine (dFdU), a gemcitabine derivative with minimal anti-tumor activity (Immordino et al., 2004). The rapid metabolism of gemcitabine explains its short half-life (32–84 min for short infusions in humans) (Abbruzzese et al., 1991; Pappas et al., 2006; Reid et al., 2004) and is thought to be responsible for its modest clinical activity (Abbruzzese et al., 1991). Consequently, alternative methods were explored to improve the gemcitabine formulation such as enhancing the lipophilicity of gemcitabine by conjugating long fatty acid chains onto it. It was shown that a fatty acid ester derivative of gemcitabine (CP-4126, gemcitabine-5'-elaidic acid ester) exhibited a better anti-tumor activity than its parent compound when given orally or intraperitoneally to mice (Bergman et al., 2010), but an intravenous formulation of the CP-4126 was not reported. It was also shown that incorporation of a gemcitabine fatty acid amide derivative (4-(N)-stearoyl-gemcitabine, GemC18) into liposomes offered advantages including hindered metabolic deactivation and improved anti-tumor activity in mouse models (Brusa et al., 2007; Immordino

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