Synthesis of novel hybrid nanoparticulate-prodrug constructs for pancreatic cancer therapy

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INTRODUCTION

Pancreatic cancer is the fourth main cancer in the western world. Currently the only chemotherapy available clinically is gemcitabine. However, gemcitabine treatment only proves effective in 23.8% of patients [2].

Nano-structures (<120 nm) are capable of entering the highly permeable blood capillaries which supply the rapidly growing tumours. Once inside the capillaries they accumulate and are retained in the tumour as a result of the poor lymphatic drainage. This allows for a deeper tissue penetration which is otherwise difficult to achieve.

In this work novel prodrugs of gemcitabine have been developed which are capable of linkage on to hybrid gold-iron oxide nanoparticles (HNPs) in order to achieve deeper tissue penetration and increase drug efficacy. The linker used in this work is proposed to break down upon enzymatic hydrolysis *in vivo*, hence liberating the free drug.

MATERIALS AND METHODS

Gemcitabine was reacted with lipoic acid using established procedures to deliver prodrugs. These compounds were characterised using a combination of spectroscopic and spectrometric techniques including ^{19}F nuclear magnetic resonance spectroscopy (NMR). HNPs were synthesised and characterised using transmission electron microscopy (TEM), photon correlation spectroscopy (PCS) and inductively coupled plasma – optical emission spectroscopy (ICP-OES).

Attachment of prodrugs on to hybrid nanoparticulate surface was quantified using reverse phase high performance liquid chromatography (HPLC). *In vitro* drug release studies were carried out at varied pH: 7.8, 5.4 & 3.6 and quantified by HPLC. *In vitro* cytotoxicity of the novel formulations was carried out on BxPC-3 cells and compared with the free drug using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Drug internalisation was quantified per cell by HPLC.

RESULTS AND DISCUSSION

Novel prodrugs of gemcitabine were isolated which are substituted at the amine in the 4 position of the pyrimidine nucleus and at the 3' and 5' hydroxyl groups of the 2,2'-difluororibose moiety. The structure of these compounds was confirmed using multinuclear NMR spectroscopy.

HNPs were synthesised successfully in the magnitude of 70 nm (Fig. 1.) The dark appearance and lack of 2 nm gold seeds on the TEM indicate successful coating had occurred. Prodrug attachment to HNP was successful with as much as 5 mg mL^{-1} being detected. Drug release studies indicated that the formulation was stable over the range of pH's tested.

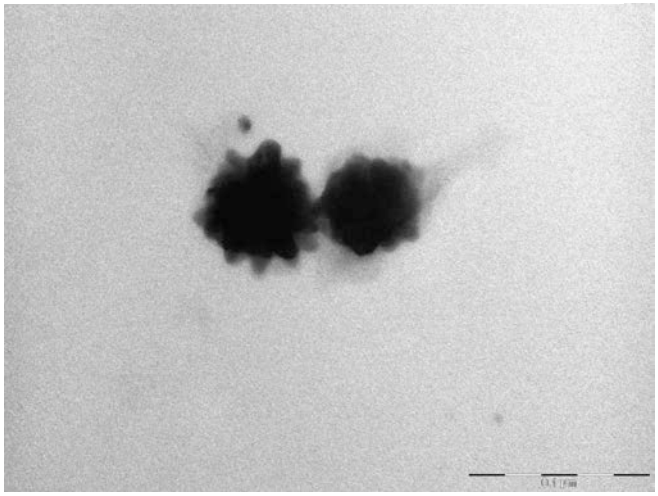

Fig. 1. Transmission electron microscopy of HNP-gemcitabine prodrug formulation showing particle size and morphology.

In vitro cytotoxicity assays showed that the novel formulation possessed a lower IC_{50} value compared with free drug (5-fold). Additionally, the intracellular concentration of drug was lower than for the formulation. It is postulated that the formulation is entering more rapidly using a different entry mechanism to the free drug resulting in raised intracellular concentrations and cytotoxic effect.

CONCLUSIONS

Further work is on-going to investigate the potential of the prodrug-nanoparticulate constructs *in vivo* for pancreatic cancer therapy.

ACKNOWLEDGMENTS

This work is funded by the Iraqi Ministry of Higher Education and Scientific Research (MOHESR)

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