FORMULATION DEVELOPMENT OF A NON-IONIC SURFACTANT VESICLES OF GEMCITABINE FOR PULMONARY DELIVERY

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Abstract— Lung cancer is a major cause of death in the world. Cancer chemotherapy is limited by adverse toxicities to normal tissues. Targeted delivery of anticancer drugs to lung cancer by inhalation would help to reduce these toxicities. Lipid based delivery systems have been shown to be effective in improving the delivery of a number of drugs and the potential of using non-ionic surfactant vesicles (NIV) to improve the delivery of Gemcitabine (Gem) was studied in this research.The NIV was used to encapsulate Gem (Gem-NIV) for delivery by the pulmonary route. The NIV was prepared using concentrations of lipid (30 and 60 mM) and characterized on the basis of size, drug entrapment efficiency and zeta potential. In vivo pulmonary delivery of Gem-NIV was evaluated using two rodent models (BALB/c mice and Sprague-Dawley rats). The drug delivery study showed that inhalation of Gem treatments (both Gem and Gem-NIV) to mice resulted in high lung concentrations of Gem. The Gem lung level was higher for Gem-NIV (60 mM) treated mice at dose 14 mg/ml (0.5ml) compared with the same dose of Gem solution. In the rats, there was a greater accumulation of the Gem in the lungs of rats in comparison with other organs when administered as a NIV formulation prepared with 60 mM lipid at a dose of Gem of 6 mg/kg in comparison with NIV prepared with 30 mM.
The results of these studies indicate that Gem-NIV show significant potential to improve delivery of Gem for the treatment of lung cancer using pulmonary administration compared with Gem solution alone.

Keywords— Pulmonary, Gemcitabine and non-ionic surfactant vesicles.

I. INTRODUCTION

Lung cancer is a major cause of death and in 2008 it was responsible for 1.38 million deaths, accounting for 18.2% of all cancer deaths in the world. There were 1.61 million new cases worldwide with the highest frequency in developed countries. In 2010, 34,859 people died because of lung cancer in the UK.
Gem is less toxic than platinum based drugs and it requires less hospitalization compared with cisplatin or etoposide as single therapies. It is one of the main drugs used to treat lung cancer. Gem is a nucleoside analogue that requires intracellular phosphorylation to produce its active diphosphate and triphosphate metabolites. Gem is rapidly deaminated by the presence of cytidine deaminase, which is present in the blood and tissues, to its inactive metabolite which is excreted in urine.
Many approaches have been attempted to extend the half-life of Gem and improve its *in vivo* cytotoxic activity. For example, conjugation of a long fatty chain (e.g. valeroyl, heptanoyl, lauroyl, stearyl and linear acyl derivatives) on to the 4-amino group of Gem protects it from chemical catabolism in plasma. Another way to increase the half-life of Gem is to use a suitable drug delivery system. Delivery systems have various structures (e.g. nanocapsules, micelles, liposomes, non-ionic surfactant vesicles, solid nanoparticles and microparticles).

The advantages of nanoscale delivery systems are their potential applications in pulmonary and cancer treatments because of their ability to penetrate tumor microvasculature, which is leaky and contains pores ranging from 100 to 780 nm in diameter.

Drug delivery systems can be used to improve drug activity, but treatment by the pulmonary administration can be used to improve targeting to the lungs. Generally, intravenous administration of drugs to treat lung cancer results in systemic distribution and oral treatment is also not ideal for lung cancer because it exposes the drugs to first pass metabolism.

II. DETAILS EXPERIMENTAL

2.1 Materials and procedure

Gem was obtained from Sequoia Research Products Ltd, Pangbourne, UK. Acetonitrile and propan-2-ol, isohexane, ethyl acetate and glacial acetic acid were obtained from Fisher Scientific (Loughborough, Leicestershire, UK). Tetra-ethylene glycol mono n-hexadecyl ether (surfactant VIII) was obtained from Nikko Chemicals Co., Ltd. through Jan Dekker UK Ltd. (Hampshire, UK). Cholesterol was obtained from Croda Chemicals Ltd. (East Yorkshire, UK). Dicetyl phosphate (DCP), deoxycytidine (2-deoxycytidine > 99%). Hypnorm® was obtained from Veta Pharma Ltd (Leeds, UK). Hypnovel® was obtained from Roche Pharmaceuticals (Nutley, USA).
AnimalsMale and female inbred BALB/c and C57BL6 mice, 8-10 weeks old, weighing 20-25g were obtained from the colonies at Strathclyde University or purchased from Harlan Olac (Bicester, UK). Male Sprague-Dawley rats weighing 200-260g bred in-house were used for tissue analysis studies. All animal experiments had University of Strathclyde ethical approval and were carried out under a UK Home project licence.
Formulation of NIV suspensions: Mono-n-hexadecyl ether tetraethylene glycol, cholesterol and dicetyl

phosphate were weighed out using a 3:3:1 molar ratio respectively, to prepare 30 and 60 mM/NIV. The lipids were melted by heating them in an oil bath at 130ºC for 5 minutes and the resultant mixture was cooled to 70ºC, and hydrated with 5 ml of preheated (70^{O}C) Gem solution to form Gem-NIV suspensions, respectively. The suspensions were homogenized at 8000 ± 100 RPM for 15 minutes at 70ºC using a Silverson mixer (Model L4R SU, Silverson Machines, UK), and fitted with a five-eighth of an inch tubular work head (Chesham, Buckinghamshire, UK).

Pulmonary delivery study: The mice bearing cancer was treated by Gem solution or Gem-NIV by the pulmonary route using an Aeroneb® Lab nebulizer (Aerogen® Inc., Galway, Ireland) and a Buxco® 10 nebulizer system (Wilmington, USA). The Aeroneb® Lab nebulizer was connected to a Volumatic™ Spacer (Allen and Hanbury, Middlesex, UK) containing 2-4 mice. Nebulisation was continued for 5 minutes. The rats used in the study were anesthetized by subcutaneous injection at a dose of 0.27 ml of an aesthetic/100g animal body weight (prepared when required using Hypnorm® and Hypnovel®, diluted 1:1 with distilled water). The rats were treated by inhalation with a Gem formulation given directly to the animal and the end of the nebulizer was connected to an 8cm acetate cylinder, which enclosed the nose and mouth area to the nebulizer head. Blood was collected into a microfuge tube and allowed to clot by leaving it at 4°C for approximately 2 hours. The blood was then centrifuged at 13000rpm for 15 min at 4°C and the resulting serum was then transferred to a fresh microfuge tube and stored at -20°C until required. The lungs were removed from the rat, weighed and stored at -20°C until required. For the Gem analysis, the organs were allowed to defrost and a sample removed/organ (0.15- 0.5 g/sample), spiked with 0.25 ml deoxycytidine (25 µg /ml saline, internal standard) and then homogenied using IKA® T10 basic homogeniser (Ultra-turrax®, Staufen, Germany) for 5 min at 30,000 rpm. The homogenized sample, defrosted serum sample was ultrafiltered at 4000 rpm for 60 min using Amicon® Ultra-4 centrifugal filter devices (30,000 MWCO, Millipore, Watford, UK). The filtrate obtained was analyzed for Gem content by HPLC.

III. RESULTS AND DISCUSSION

Current investigations showed that direct administration of Gem treatments (both Gem and Gem-NIV) to mice by inhalation resulted in high lung concentrations of Gem (Table 1). As a consequence of NIV improve Gem treatments deposition in the lungs. This agrees with a previous study in which liposomal Gem was found in high concentration in cancerous tissues of mice. up to 24 hours after treatment. Higher localization of Gem in lungs of rats can be obtained by using NIV formulated with 60 mM lipids (table 2). Gem-NIV would target the lung and even if they distribute into blood circulation, they are rapidly cleared from the blood and mainly distributed to the spleen because of the tendency of accumulation of Gem-NIV in this highly vascularized organ, leading to a decrease in the exposure of other tissues to Gem cytotoxicity.

Table 1 The concentration of Gem in the tissues of cancer bearing mice which were given the Gem solution or Gem-NIV, by inhalation. Mice (n = 12 /treatment) were treated using a nebulizer with Gem solution or Gem NIV (60mM lipid: Vesicle size, 511 ± 7.3 nm; zeta potential, -68.3 ± 7.1 and entrapment efficiency, 75.0% ± 4.1) for 5 min (0.5 ml, 14 mg/ml) and were sacrificed 30 mins after treatment. The amount of drug present in the tissues shown was determined by HPLC.* P< 0.05: Gem NIV concentration compared with the Gem concentration in tissues.

Treatments	Gemcitabine concentration (µg/gm or ml) ± SD				
	Serum	Lung	Kidney	Liver	Spleen
Gem	157.2 ± 5	45.5 ± 1	42.4 ± 6.1	15± 3.1	66.7 ± 3.1
Gem-NIV	212.2 ± 5*	124.6 ± 6*	14.05 ± 2*	4.8 ± 1.2*	80.4± 4.2*

Table 2 The concentration of Gem in the tissues of rats which were given the Gem-NIV by inhalation. Rats (n = 12 /treatment) were treated using a nebulizer with Gem-NIV (6 mg/kg, 30mM lipid Gem-NIV; Vesicle size, 255.3 ± 1.2 nm; Zeta potential, -43.3 ± 4.2 and entrapment efficiency, 55.8% ±2.09 or 60 mM lipid Gem-NIV; Vesicle size, 545.1 ± 2.8 nm; Zeta potential, -70.2 mV ± 9.4 and entrapment efficiency, 80.05% ±4.8) and were sacrificed at 30 mins after treatment. The amount of Gem present in the tissues shown was determined by HPLC. *P< 0.05: Gem-NIV (60 mM) concentration compared with Gem-NIV (30 mM).

Gem NIV	Gemcitabine concentration (µg/gm or ml) ± SD				
	Serum	Lung	Kidney	Liver	Spleen
30 mM	10 ± 0.7	192.3 ±39	9.3 ± 0.8	4.87 ± 1.1	25 ± 4.9
60 mM	1.7 ± 0.3*	477.87±25*	1.9 ± 1*	1.75 ± 0.1*	40 ± 3.9*

CONCLUSION

Gem-NIV improves delivery of Gem for the treatment of lung cancer using pulmonary administration compared with Gem solution alone.

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