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Electrokinetic dispersion of a cancer chemotherapeutic drug for the treatment of solid tumours

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ABSTRACT

Compromised tumour vascularisation presents a significant challenge to chemotherapeutic-based treatment of solid tumours. In this study we use subtle electric field characteristics to facilitate electrokinetic movement of a cancer chemotherapeutic agent in an ionically complex environment. We demonstrate that such an approach can be exploited to facilitate the electrokinetic movement of camptothecin from a peritumoural site through otherwise relatively impermeable tumour tissues. Here we demonstrate enhanced anti-tumour effects using this approach in a pre-clinical model for solid tumours and suggest one possible therapeutic application of the approach in the treatment of lung cancer.

1. Introduction

Many cancer chemotherapeutic agents are low molecular weight ($M_r < 1000$) entities and are not usually selectively delivered to tumour tissues. When administered systemically, these agents are distributed to both normal and tumour tissues by normal diffusion through capillaries. Although systemic administration of such agents may be particularly suitable for the treatment of circulation-associated cancers such as leukaemias and certain forms of lymphoma, its application as a delivery modality for the treatment of solid tumours is far from ideal because access of the chemotherapeutic agent to target cells is based on simple diffusion. Driving this diffusion in a therapeutically favourable direction necessitates saturation of normal tissues as well as the target cancer tissues and this leads to increased toxicity of normal tissues. Indeed this aspect is further exacerbated by the defective vascular architecture and deficient lymphatic drainage of solid tumours, which in turn, lead to high interstitial fluid pressure within many solid tumours [1–3]. These basic observations have provided the main impetus to develop alternative means of administering cancer chemotherapeutic agents for the treatment of solid tumours [4,5] and have also provided the impetus for the suggestion of many elaborate targeting or site-specific therapeutic strategies [6–8].

In pursuing site-specific delivery of cancer chemotherapeutic agents to resistant or relatively impermeable environments such as solid tumours the application of physical stimuli in aiding access of the therapeutic agent to target regions has been explored. In the past such approaches have given rise to the emergence of electric and ultrasonic field-aided delivery of cancer chemotherapeutic agents [8,9]. In the former, relatively high intensity electric pulses are delivered to tissues in order to facilitate transient cell membrane permeabilisation and consequential movement of the chemotherapeutic drug across the cell membrane so that it can exert its therapeutic function. In the latter, ultrasound has been applied for similar purposes. From a targeting perspective, these approaches facilitated enhanced action of the chemotherapeutic agent

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at the precise site exposed to the physical stimulus. In alternative approaches that have excluded the use of cancer chemotherapeutics others have explored the possibility of using less intense electric field strengths to facilitate tumour eradication in the absence of chemotherapeutic agents and this has become known as electrochemical therapy (EchT) [10–12]. In these studies direct current (DC) is passed through the tumour at a relatively low voltage (*circa* 5–10 V) and tumour eradication results from electrochemical effects such as the development of pH extremes at the electrodes [11]. In terms of the dose of electric current employed in these studies, values in the lower mA range have been used yielding current doses ranging from 10 to 100 C [12]. In a relatively recent and unrelated study we demonstrated that DC could be employed to facilitate net electrokinetic movement of organic cytotoxic agents in an ionically complex environment (tissue culture medium) and such an approach could be employed to facilitate detoxification of the contaminated media [13]. In viewing our results from an alternative perspective it was felt that such an approach could be exploited in addressing issues relating to the dispersion of a chemotherapeutic agent through relatively impermeable non-vascularised tissues in solid tumours.

The anticancer chemotherapeutic drug camptothecin is an alkaloid extracted from the Chinese tree *Camptotheca acuminata* and although it has been shown to exhibit potent anticancer activity, its poor solubility and toxicity compromise its use in the clinic [14]. This has led to the search for a variety of water-soluble derivatives including irinotican and topotecan and these, together with other derivatives have undergone clinical trials. Challenges such as those described for other cancer chemotherapeutic drugs in terms of toxicity to normal tissues also apply to this class of compounds. As such camptothecin, in its unmodified form, is an extremely good candidate for use in a strategy designed to effect site-specific targeting because of its adverse toxicity and reduced selectivity in terms of tumour uptake. Camptothecin itself, at pH values lower than 4.5 is uncharged and exists as a lactone, although at lower pH, the nitrogen on the B ring may be protonated, yielding a positively charged cationic molecule. At pH 7 or higher the lactone ring is hydrolysed and exists as either an uncharged weak hydroxyacid or at higher pH as the carboxylate anion [15]. Because of these complex chemical attributes, the behaviour of camptothecin in an electric field would be relatively unpredictable under physiological conditions. In this study we decided to characterise the mobility of camptothecin in an applied electric field with a view towards facilitating dispersion of that agent through solid tumour tissues. Camptothecin was also chosen for this study because it is fluorogenic and its mobility could be easily monitored during *in vivo* studies We demonstrate, using *in vitro* systems, that the application of DC can facilitate movement of the cancer chemotherapeutic drug camptothecin through target cell-containing artificial matrices towards a cathode and we further demonstrate therapeutic value associated with this approach in treating tumours *in vivo*. We suggest that such an approach may find particular application in the clinical treatment of solid tumours of the lung in which conventional systemic delivery of the therapeutic agent to the target may be severely compromised.

2. Materials and methods

2.1. Cell culture

In these studies the rat non-immunogenic acute promyelocytic leukaemic cell line, LT12, was employed as a target population. This cell line is derived from Brown Norway rats and is an extensively characterised model for human acute promyelocytic leukaemia [16]. In our studies the cell line was specifically chosen because it was cultured in suspension and as such could be evenly dispersed in artificial matrices without pre-treatment with agents such as trypsin/EDTA. The cell line was routinely cultured in RPMI 1640 medium supplemented with glutamine and 10% (v/v) foetal bovine serum (GibcoBRL, UK) at 37 °C in a humidified 5% CO_2 atmosphere. Cell viability was determined by direct counting using a trypan blue dye exclusion assay.

2.2. Cell immobilisation in agarose and determination of cell viability

LT12 cells were harvested and washed in phosphate buffered saline (PBS) by centrifugation. Cells at the required concentration were suspended in 1% (w/v) agarose (low melting point) at 35 °C. A layer of the mixture was then poured onto the surface of a Petri dish to form a gel layer with a thickness of 1.5 mm. Once solidified, the gel was sectioned to yield gel portions with dimensions of $15 \times 9 \times 1.5$ mm (length × width × height). Viability of the immobilised cells was determined using an MTT-based assay. Essentially, each portion of gel containing cells at concentrations ranging from 0 to 6×10^6 cells/ml of gel were incubated in 2 ml of MTT (2 mg/ml in growth medium) for a period of 1 h. Over this period of time, a purple colour developed in the presence of viable cells and images of each portion of gel were captured using a CMOS sensor (VGA 640. 480 24-bit CMOS sensor, Creative Labs Inc., USA). Images were analysed for colour intensity of the formazan product resulting from reduction of the MTT by viable cells using ImageTool software (The University of Texas, Health Science Center, USA). This software tool enabled the quantification of dark pixels resulting from the detection of dye reduction by viable cells in the gel portions. A linear relationship was demonstrated between the data derived from image analysis and cell concentrations up to 1×10^6 cells per ml in the gel portions indicating that this approach could be employed to conveniently and non-invasively determine cell viability in gel portions that had been exposed to specific treatments.

2.3. RIF-1 cell culture and establishing tumours

The RIF-1 cell line, a mouse radiation-induced fibrosarcoma, was routinely maintained in RPMI 1640 medium supplemented with glutamine and 10% (v/v) foetal bovine serum. Cells were grown to confluence and harvested by centrifugation after treatment with 0.05% (w/v) trypsin