Development of Disulfiram-Loaded Poly(Lactic-co-Glycolic Acid) Wafers for the Localised Treatment of Glioblastoma Multiforme: A Comparison of Manufacturing Techniques

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ABSTRACT: Glioblastoma multiforme (GBM) is the most common primary malignant brain tumour in adults with a very poor prognosis. This paper describes the development of disulfiram (DSF)-loaded biodegradable wafers manufactured using three standard techniques: compression, solvent casting and heat compression moulding. The paper demonstrates that neither technique has an adverse effect on the stability of the DSF within the wafers. However, the solvent casting technique results in an interaction between the poly(lactic-co-glycolic acid) (PLGA) and the DSF. The physical state of the DSF within the wafers was dependent on the manufacturing technique, with the DSF in the wafers manufactured by compression or solvent casting retaining between 40% and 98% crystallinity, whereas the DSF in the wafers manufactured using heat compression moulding was completely amorphous. Release of DSF from the wafers is dependent on the degradation of the PLGA, the manufacturing technique used, and the DSF loading. DSF in the compressed and heat compression moulded wafers had a similar cytotoxicity against a GBM cell line compared with the unprocessed DSF control. However, the cytotoxicity of the DSF in the solvent-casted wafers was significantly lower than the unprocessed DSF. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci

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INTRODUCTION

Glioblastoma multiforme (GBM) is the most common primary malignant brain tumour in adults with a very poor prognosis. Even after surgery, radiotherapy and chemotherapy, the overall survival rate for patients with GBM is 42.4% at 6 months, 17.7% at 12 months and 3.3% at 2 years.[1] The current treatment for GBM is surgical resection of accessible tumour, which is often limited if the tumour is located near to critical regions of the brain, followed by radiotherapy and adjuvant chemotherapy. However, this has not been very successful, with a standard course of radiotherapy, following surgical removal of the tumour, extending a patients' life from 6 to only 9 months, whereas an increased dose of radiation is not possible, because of undesirable side effects.[2,3]

Systemic delivery of chemotherapeutic drugs into the neurons and glial tissues of the brain is challenging because of the presence of the blood–brain barrier (BBB), which consists of tight junctions between the endothelial cells lining the cerebral capillaries.[4] The BBB is very selective and consequently only low-molecular-weight, electrically neutral, hydrophobic molecules are able to freely cross this barrier.[5–7] Many chemotherapeutic drugs, which tend to be large, ionically charged and hydrophilic, cannot cross the BBB from the bloodstream into the brain at levels needed for therapeutic effect, which means that an intolerably high systemic drug levels are required in order to achieve the therapeutic levels required in the brain.[3,6,8,9] Furthermore, if the drug does manage to diffuse across the BBB, it can very quickly diffuse back making it difficult to obtain a constant therapeutic concentration after systemic administration. One option to overcome this issue is the use of implantable devices to deliver the chemotherapeutic drug directly to the tumour, which offers a number of advantages over systemic administration, including increased drug stability as it remains in the delivery device until released, direct delivery to the site of action, lower dose of drug required and reduced side effects because of the avoidance of systemic circulation.[10] Furthermore, local drug delivery may be suitable for the treatment of GBM as approximately 80%–90% return within 2 cm of the resection site.[3]

The Gliadel® wafer is an example of one such device, which was approved by the Food and Drug Administration in 1996 for the treatment of recurring GBM.[11,12] It is a disc-shaped, 200 mg biodegradable wafer 14 mm in diameter and 1 mm thick manufactured using a copolymer 1,3-bis-(p-carboxyphenoxy) propane and sebacic acid (in a molar ratio of 80:20) containing 3.85% (w/w) of the chemotherapeutic agent Carmustine.[9,10] The polymer and active ingredient are dissolved in dichloromethane, before they are spray dried into microspheres varying from 1 to 20 μm, which are then compressed into wafers. Following the surgical removal of a primary brain tumour, up to

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eight wafers are implanted in the resection cavity and the Carmustine is released from the wafers over a 5-day period, whereas the polymer matrix degrades over a period of 6–8 weeks.[11] Gliadel® wafers enable the delivery of a chemotherapeutic agent directly into the resection cavity and thus overcome the issues associated with BBB. A small randomised, double-blind, placebo-controlled clinical trial involving 32 patients demonstrated that the Gliadel® wafer increased the median survival rate, after surgery, for patients with grade IV tumours from 39.9 weeks to 58.1 weeks.[12] A much larger trial involving 240 participants demonstrated that the Gliadel® wafer (in addition to surgery and radiation) increased the median survival rate of patients with newly diagnosed high-grade gliomas from 11.6 months (placebo) to 13.9 months (Gliadel®).[13] A randomised placebo-controlled clinical trial in 222 patients with recurrent malignant gliomas demonstrated that Gliadel® increased the survival rate 23 weeks to 31 weeks, whereas the 6-month survival rate was 50% greater in those patients treated with Glidael® compared with the placebo group.[14] However, a Cochrane Review stated that Gliadel® results in a prolongation of survival without an increased incidence of adverse events when used as primary therapy, whereas in recurrent disease, it does not appear to confer any added benefit.[15] In addition, only a third of patients suffering from GBM respond to treatment with Carmustine,[16] whereas in some patients, cerebral oedema was reported as one of the major adverse effects associated with Gliadel®.[17] Other types of implantable devices, such as millirods,[18,19] disks/wafers,[20,21] foams[22] and gels[23] as well a range of micro and nanoparticle formulations[24–29] are currently being developed for the localised delivery of chemotherapeutic drugs to the brain.

Poly(lactic-co-glycolic acid) (PLGA) is a biodegradable and biocompatible copolymer that has been used in the manufacture of a range of drug delivery devices such as disc/wafers, rods, scaffolds, films, foams micro and nanoparticles to deliver a range of drugs from peptides and proteins to chemotherapeutic and analgesic drugs as well as antibiotics and vaccines in a sustained and controlled manner.[22,30–40] The drug release from a PLGA drug delivery device can be controlled by the ratio of lactic and glycolic acid in the polymer, the molecular weight of the PLGA used as well as the physiochemical properties of the active and the shape of the drug delivery device.[31,41–44] Furthermore, blending the PLGA with another polymer can influence the mechanical properties as well as the drug release properties of the device.[45]

Disulfiram (DSF), which is an antialcoholic drug, has been shown to have an anticancer effect against GBM,[46–48] which is copper (Cu) dependent[49,50] as Cu plays a crucial role in redox reactions and triggers the generation of reactive oxygen species (ROS) which induce apoptosis in human cells.[51] DSF can chelate Cu(II) forming a DSF/Cu complex which improves the transport of Cu into cancer cells and is a much stronger ROS inducer than Cu alone.[52,53] Drug-induced ROS accumulation is usually counterbalanced by the activation of NFκB, an antiapoptotic factor inhibiting ROS and ROS-induced cytotoxicity.[54] However, DSF is also capable of inhibiting activity of NFκB.[55] Furthermore, it has been demonstrated that DSF can potentiate the cytotoxic effect of other anticancer drugs and ionising radiation. These characteristics and its low toxicity make DSF an attractive candidate for the treatment of GBM.

MATERIALS AND METHODS

Materials

Poly(D,L-lactide-co-glycolide) with a 50:50 lactide–glycolide ratio and varying degradation rates ranging from days (DLG 1A), weeks (DLG 4A) to months (DLG 4E) were purchased from Evonik Industries (Birmingham, Alaska). DSF, dichloromethane copper (II) chloride ($CuCl_2$) and sodium dodecyl sulphate (SDS) were purchased from Sigma–Aldrich (Dorset, England) and all materials were used as supplied. The brain cancer cell line U373 was purchased from ATCC (Middlesex, UK).

Manufacture of 10% and 20% (w/w) DSF-Loaded PLGA Wafers Using the Direct Compression Method

The required amount of DSF and each PLGA were weighed out and then mixed together using a mortar and pestle. Fourhundred milligram of each DSF/PLGA blend was weighed out and placed into a 14-mm diameter die of a KBr press and compressed under 15 tonnes of pressure to produce either a 400-mg 10% or 20% (w/w) DSF-loaded PLGA wafer 1 mm thick and 14 mm in diameter.

Manufacture of 10 and 20% (w/w) DSF-Loaded PLGA Wafers Using the Solvent Casting Methods

The required amount of DSF and each PLGA were weighed out, then mixed together and dissolved in 50 mL of Dichloromethane (DCM) using a mortar and pestle. The DSF/PLGA solutions were then poured into a petri dish left for 2 weeks to allow the DCM to evaporate off leaving either a 10% or 20% (w/w) DSF-loaded PLGA disc. To ensure no residual solvent remained, the disc was placed into a vacuum oven for 3 days. Individual wafers (1 mm thick and 14 mm in diameter) weighing approximately 400 mg were subsequently cut out from the larger disc.

Manufacture of 10% and 20% (w/w) DSF-Loaded PLGA Wafers Using the Heat Compression Method

The required amount of DSF and each PLGA were weighed out and then mixed together using a mortar and pestle and subsequently placed onto a class plate heated to 80°C and allowed to soften for 2 min. The DSF/PLGA blend was compressed under 0.5 tonne of pressure to form a large DSF/PLGA disc 1 mm thick. Individual wafers (1 mm thick and 14 mm in diameter) weighing approximately 400 mg were subsequently cut out from the larger disc.

Content Uniformity and Drug Stability of 10% and 20% (w/w) DSF-Loaded PLGA Wafers

Each DSF-loaded PLGA wafer ($n = 4$) was cut into small pieces placed into 50 mL of DCM and left over night until completely dissolved. The DCM was subsequently evaporated offer and the DSF/PLGA residue was resuspended in 40 mL of ethanol causing the PLGA to precipitate out while the DSF remained in solution. The sample was then placed into an orbital shaking incubator (Unitron HT infors) at 37°C and 60 rpm overnight to ensure all of the DSF went into solution. The ethanol was then analysed using the DSF stability indicating HPLC method.

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