AN INTRAVITREAL CONTROLLED-RELEASE MICRONEEDLE IMPLANT TO TREAT INTRAOCULAR LYMPHOMA WITH 2-METHOXYESTRAIOL

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INTRODUCTION

Intraocular lymphoma (IOL) refers to infiltration of the vitreous humor, retina, and choroid by malignant lymphocytes. The primary chemotherapy agent to treat IOL is methotrexate (MTX), which is the most effective drug against lymphoma cancer [1]. However, there are side effects such as bone marrow depression and hair loss since the growth of normal body cells may also be affected by MTX. In addition, the optimum dose of MTX has not been defined yet due to: 1) the dose heterogeneity in prospective trials, and 2) the fact that MTX is often associated with other drugs and/or radiotherapy. A new potential chemotherapeutic agent for IOL is 2-methoxyestradiol (2ME2). The endogenous oestrogen metabolite 2ME2 has been shown to be an antiangiogenic and antiproliferative in a variety of tumour cell lines [2]. In addition, it has shown to be non-toxic in animal studies (i.e. no evidence of hair loss, gastrointestinal disturbance or leukocyte reduction in bone marrow and thymus).

IOL is commonly treated with systemic chemotherapy. However, the blood-retinal barrier may necessitate higher dosage during intravenous drug administration to achieve therapeutic levels near the retina. The high dosage of drug increases the risk of systemic adverse effects. To avoid the systemic adverse effect and overcome the blood-retinal barrier, intravitreal therapy e.g. controlled release implants can be considered to treat the IOL.

The purpose of this study is to develop a sustained release 2ME2 implant for the local drug delivery to the eye to treat intraocular lymphoma. To establish a new treatment method using controlled release implant, 1) therapeutic range of a new test drug (2ME2) is evaluated by conducting proliferation study on lymphoma cell line; 2) biodegradable microneedle implant is fabricated to obtain sustained release of 2ME2 within the therapeutic range.

METHOD

A. Proliferation studies to evaluate therapeutic range of 2ME2

Cell culture: Diffuse large cell lymphoma; non-Hodgkin’s B cell lymphoma (Farage and Pfeiffer from ATCC) are grown in RPMI 1640 supplemented with 10% FBS, 2nM L-glutamine, 1.0 mM sodium pyruvate, 100 µ/ml penicillin and 100 mg/ml streptomycin (CMEM/10%FBS). Cells are maintained under standard conditions (37°C, 5% CO2 in a humidified atmosphere).

Proliferation assay: Tetrazolium-based colorimetric assay is used to quantify the antiproliferative effects of 2ME2 and MTX using microplate reader. Cells are resuspended in 96-well microtitre plate at varying concentrations of 2ME2 and MTX. Final concentration of 2ME2 and MTX ranges from 0 to 10 µM in CMEM/5% FBS, and 100 µL of the appropriate solution is added to each set of wells in quintuplicate. The control solution (no drugs) is concentrated 0.1 % DMSO to equal the amount of DMSO in the 10 µM solution. The cells are allowed to incubate at standard conditions for 24 hr, 48hr, and 72 hr, after which 20 µL per well CellTiter 96 Aqueous One (Promega, MTS tetrazolium compound) is added. The tetrazolium-based compound is bioreduced by metabolically active cells to a colored formazan. The absorbance of each well is determined using a microplate reader.

B. Fabrication of microneedle implant and it’s release rate

Fabrication protocol: The process for the fabrication of biodegradable polymeric microneedle of intravitreal implant is based on micromolding technique from high-aspect-ratio PDMS. It begins by laser drilling on cycloolefin copolymer (COC) to make holes. Then, steel needles are fitted into the holes to make the master structure. This array of steel needles is coated with polydimethylsiloxane (PDMS) to make an inverse mold. This PDMS mold is used to create
polymeric microneedle implant by using solvent cast method. The solution of PLA and 2ME2 (25% w.t.) dissolved in organics solvent casts in the PDMS mold. Negative pressure is applied to PDMS mold to load the solution on needle part and then the implant is dried for 3 days. To increase the mechanical strength, the polymer in the PDMS mold is heated to glass transition temperature. Then, the drug loaded implant is used to measure the release rate.

Measurement of release rate: Solid phase extraction is performed using a Vac Elut SPS-24 solid phase extraction chamber and Varian Bondelut C18 columns. The columns are conditioned with 2 ml of methanol and the equilibrated using 2 ml of water. 1 ml aliquots are applied to the column, and then rinsed with 2 ml of 5% methanol in water. Elution is performed with 2 ml of methanol, which is evaporated to dryness under a continuous stream of nitrogen at 40°C. The extracts are reconstituted in 200 µl of 50% acetonitrile in water using vortex-mixing, and 170 µl is injected into HPLC system to measure the concentration of 2ME2.

RESULT

The antiproliferative effects of 2ME2 and MTX in Diffuse large cell lymphoma; non-Hodgkin's B cell lymphoma have been evaluated in Fig. 1. Relative viable cell number in cells exposed to 2ME2 and MTX for 72 hr is expressed as a percentage of number of untreated cells. MTX and 2ME2 show antiproliferative effect on lymphoma cell lines (Fig. 1). As assessed by a tetrazolum-based colorimetric assay, concentration of 2ME2 and MTX resulting in 50% inhibition of proliferation (IC50) is shown in Table 1. Both 2ME2 and MTX (reference) have comparable antiproliferative effect on both lymphoma cell lines.

Fig. 2 shows the 2ME2 release from the microneedle implants prepared by the ratios of high and low molecular weight PLA (PLA-130,000/PLA-6,500, 80/20 w.t.). The microneedle implants (n=3) have a mean release rate of 0.31 µg/hr over a 7 week period, after reaching steady state (after 2 day). The clearance of 2ME2 from the human eye is 3.35 ml/hr [1, 2]. Assuming steady state and applying eq. 1, the vitreous concentration of 2ME2 in the human eye is estimated to be 0.0925 µg/ml (= 0.3 µM) when the implant is placed in the human eye.

Concentration [µg/ml] = Release Rate [µg/hr]/ Clearance [ml/hr]  

![Image](320x454 to 543x599)

Estimated in vivo concentration from the implant

It is expected that the mean concentration of 0.3 µM of 2ME2 could inhibit lymphoma cell proliferation up to 25 and 60% for Pfeiffer and Farage, respectively as compared with the untreated cell as shown in Fig. 1.

DISCUSSION

There is no animal model for intraocular lymphoma and 80% are diffuse large B cell type for primary intraocular lymphoma. Therefore, anti-proliferative effects of MTX and 2ME2 on diffuse large cell lymphoma; non-Hodgkin's B cell lymphoma cell lines have been investigated in vitro in this study. Comparisons between the lymphoma cell lines treated with MTX and 2ME2 give information for 2ME2 efficacy on lymphoma cell lines. Therapeutic range (IC50) varies from 0.07 to 0.27 µM for MTX, and 0.21 to 0.45 µM for 2ME2 on the lymphoma cell lines. A study of the in vitro cytotoxic activity in 63 different cell lines has demonstrated that therapeutic levels of MTX range from 0.1 to 1 µM with a mean IC50 of 0.32 µM [1]. The results are comparable to those obtained in the previous studies.

The cumulative amount of 2ME2 released from the implant is proportional to the square root of time. These facts support that 2ME2 release rate follows zero order release kinetics without any significant burst. For 7 week period, around 30% of initial amount of drug is released from microneedle implant, which means that it can release the drug over 6 month. Therefore, the designed implant is expected to sustain mean concentration of 0.3 µM for 2ME2 over 6 months, which is within the therapeutic range (0.21 to 0.45 µM) to treat intraocular lymphoma in human eye. Also the implant is made of biodegradable polymer (PLA) and has beveled microneedle tip for easy implantation in the eye. These facts may reduce the procedure of implantation surgery and removal operation when drug release is complete.

In conclusion, sustained-release of 2ME2 using intravitreal biodegradable microneedle implants, designed to deliver therapeutic levels of 2ME2 for an extended period of time, shows promise for the treatment of IOL.

![Image](324x186 to 576x339)

Table 1. 50% inhibition of proliferation (IC50) of 2ME2 and MTX for lymphoma cell lines

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<th>FAG</th>
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<td>0.27</td>
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REFERENCES