



Controlled Release of Erufosine from New Cryogels Based on Polycaprolactone/Poly (Ethylene Oxide) Polymers and the Effect on Graffi Tumor-Bearing Hamsters

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Abstract

The research is focused on the novel Cryogel Polycaprolactone (PCL)/Polyethylene Oxide (PEO) used as a drug carrier in anti-tumor therapy. Cryogels were prepared *via* the combination of cryogenic treatment and photochemical cross linking. HPLC was performed in order to study the kinetics of EPC3 release from the copolymers along with tandem-mass spectroscopy. The polymer drug delivery system loaded with Erufosine was transplanted into Graffi myeloid tumor in hamsters and biometric parameters such as lethality and mean survival time were monitored. The results showed that PCL/PEO cryogel implants release therapeutic doses of Erufosine by the controlled degradation of the polymers *in vitro* and *in vivo*. *In vivo* results demonstrate an increase of mean survival time and reduction of mortality of the animals with implanted postoperatively drug-loaded cryogels. All this results indicate that the PCL/PEO cryogels represent a promising drug-release system for the treatment of cancer.

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Introduction

In the last decades, biomaterials have shown their enormous potential in medicine and therefore they have been a subject to numerous studies. For instance, they have been widely considered in the development of localized delivery systems [1]. Solid tumors can greatly benefit from localized drug delivery. This approach allows for high drug concentrations at the target site, lower systemic toxicity, and extended drug exposure. Biodegradable biopolymers have gained increased attention in cancer therapy, since surgical removal and subsequent application of the delivery system would decrease patient compliance. Macro-porous hydrogels, synthesized below the freezing point of the solvent (cryogels) have recently gained significant interest in the biomaterial field due to their unique forming properties [2]. They combine several functions including biocompatibility, controlled biodegradability with non-toxic degradation products, adequate porosity for the transportation of small molecules and optimal mechanical strength [3]. Alkyl Phosphorylcholine (APCs) are promising anti-neoplastic agents that induce cell death in tumor cells by primary interaction with the cell membrane [4,5]. In contrast to standard chemotherapeutic drugs, these synthetic phospholipid derivatives target cellular membranes and interfere with membrane lipid composition and the formation of lipid second messengers and affecting the growth, cell cycle progression and survival of tumor cells without direct interaction with cellular DNA [5,6]. As targets of APCs are PI3K/AKT pathway, the Stress Activated Protein Kinase (SAPK)/Jun-N Terminal Kinase (JNK) and the sphingolipid pathway [6-8]. Erufosine (EPC3) is APC derivative with a 22 carbon chain and reduced hemolytic toxicity [9]. Erufosine shows strong proapoptotic effects on many malignant cell lines [10] and primary tumor cells [11].

The aim of the present study was to follow *in vitro* the release of Erufosine, embedded in macro-porous PCL/PEO cryogel carriers under controlled degradation of the polymers. In addition, the *in vivo* effect of implanted cryogel-EPC3 carriers was also studied.

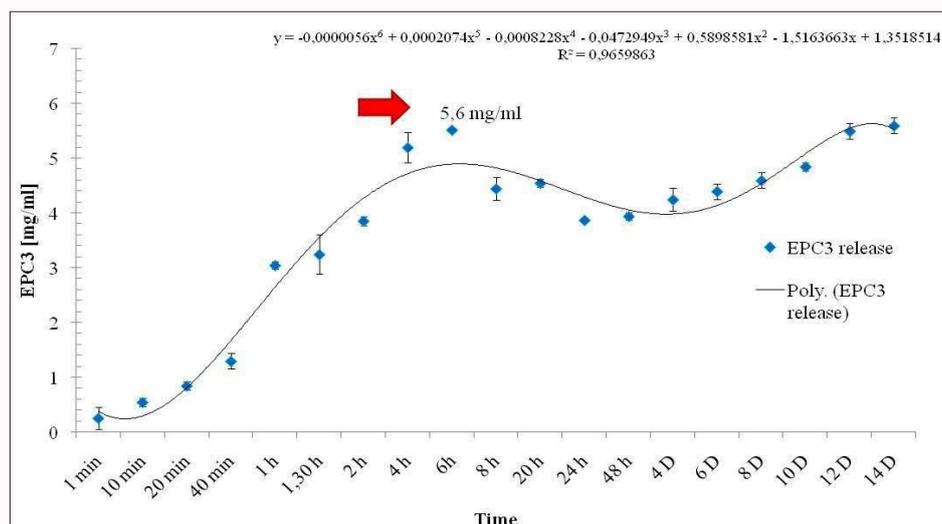


Figure 1: Controlled release of EPC3 from PCL/PEO (2:1) carriers over 14 days.

Materials and Methods

Anti-tumor agent

Erufosine (EPC3) was kindly provided by Prof. H. Eibl (MPI for Biophysical Chemistry, Göttingen, Germany).

Cells

Breast cancer MDA-MB-231 cells (ATCC collection).

Preparation of polymer implants

Cryogels were prepared via the combination of cryogenic treatment and photochemical cross linking previously described in details [2].

HPLC/MS/MS

To study the kinetics of EPC3 release from the co-polymers was used chromatographic separation followed by tandem-mass spectroscopy (triple quadrupole mass spectrometer- Thermo Electron Corporation, USA). Determination of Erufosine in culture media was performed in external standardization mode. Chromatographic separation was carried out on a HILIC analytical column with isocratic elution utilizing mobile phase consisting of methanol, water, ammonium acetate and formic acid. Detection was carried out on a tandem mass spectrometer with positive electrospray ionization and SRM-MS/MS monitoring of the protonated molecular ion of Erufosine, decomposing under controlled conditions to the most dominant respective fragment. Raw data of the mass chromatograms were collected and processed by specialized software. Concentrations were calculated in external standard mode and expressed as mg Erufosine/ml.

Induction of Graffi myeloid tumors

Male and female Golden Syrian hamsters were used. Graffi myeloid tumor was created by transplantation of Graffi viable tumor cells (2.10^4 /ml) in the interscapular area of hamsters. The tumors were developed about 11 days after implantation in all experimental hamsters. Hamsters were randomized into three groups: group 1 control (no therapy), group 2 with intratumor and group 3 with post-surgical implantation of the polymer carrier.

In vivo experimental design: Under deep anesthesia, a cut was made by scalpel in the skin around the tumor followed by

implantation of the carriers:

Group 2: Using a scalpel the implant PCL/PEO/EPC3 (2:1:1) was placed into the tumor.

Group 3: After surgical removal of the tumor the implant was inserted in the resection area. Biometric parameters such as lethality and mean survival time were monitored.

Results and Discussion

HPLC/MS/MS was used to quantify the controlled release of EPC3 in a DMEM cell medium in which the PCL/PEO polymer with embedded EPC3 was placed. Higher concentrations of 5 mg/ml to 5.5 mg/ml EPC3 were measured between the 4th and 6th hour of the experiment, and the highest measured concentration of 5.6 mg/ml EPC3 was detected at the 6th hour (Figure 1). After this, according to Figure 1 it becomes clear that the curve is entering in a plateau, lasting until day 14 of the experiment, which ensures a constant release of EPC3 in the culture.

In the conducted *in vivo* experiments a temporary protective effect was observed in hamsters with Graffi tumor reported on the parameters survival and mortality. In group two (Figure 2A), the mean survival time was 34.0 ± 2.7 days, in group three (Figure 2B) - $30.2 \pm 11,6$ days, while in control, untreated hamsters - 24.8 ± 3.5 days (Figure 2B). Twenty percentages of hamsters in group 3 survive about 50 days. A lower mortality rate was observed in hamsters with surgical intervention compared to untreated control, the effect being more pronounced in hamsters with post-surgical implantation of PCL/PEO cryogel implants loaded with Erufosine (gr.3) (Figure 2D). The observed protective effect can be explained by local therapy and the reported EPC3 release for 14 days found *in vitro* experiment by the HPLC with tandem-mass spectroscopy assays.

Conclusion

For the first time are introduced polymer carriers with embedded EPC3. *In vitro* is quantified the controlled release of EPC3 and the highest therapeutic concentration of 5.6 mg/ml EPC3 is reached at the 6th hour of the experiment. PCL/PEO/EPC3 drug-delivery system implanted into the tumor exhibited a temporarily positive effect on the biometric parameters of tumor growth - increased survival and

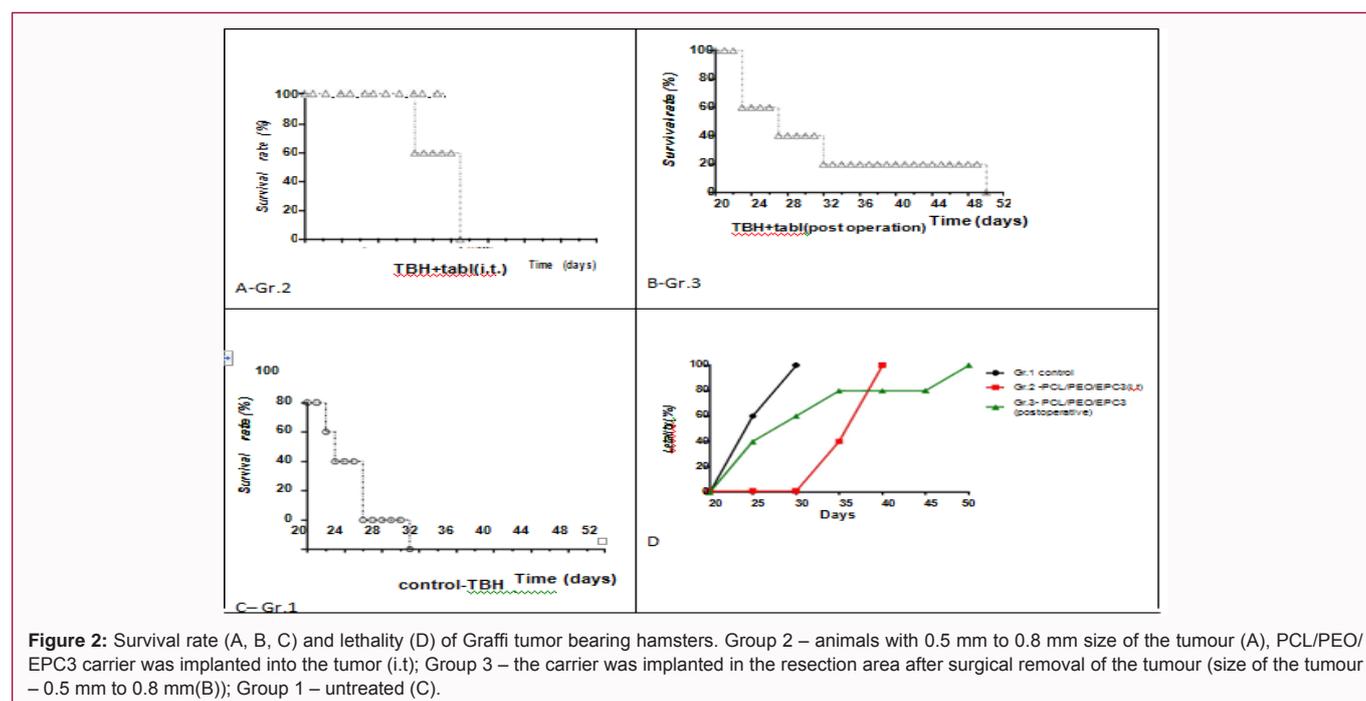


Figure 2: Survival rate (A, B, C) and lethality (D) of Graffi tumor bearing hamsters. Group 2 – animals with 0.5 mm to 0.8 mm size of the tumour (A), PCL/PEO/EPC3 carrier was implanted into the tumor (i.t.); Group 3 – the carrier was implanted in the resection area after surgical removal of the tumour (size of the tumour – 0.5 mm to 0.8 mm(B)); Group 1 – untreated (C).

decreased lethality rate. Even better results were achieved when PCL/PEO/EPC3 were implanted in the resection area after surgically removal of the tumor. All these results indicate that the PCL/PEO cryogels represent a promising drug-release system for the treatment of cancer.

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