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Kinetics of a nucleoside release from lactide-caprolactone and lactide-glycolide polymers *in vitro*✪★

Tomasz Kryczka[1], Paweł Grieb[1], Maciej Bero[2], Janusz Kasperczyk[2] and Piotr Dobrzynski[2]

[1]*Medical Research Center, Polish Academy of Sciences, 02-106,Warszawa, Poland;*
[2]*Institute of Polymer Chemistry, Polish Academy of Sciences, 41-800, Zabrze, Poland*

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We assessed the rate of release of a model nucleoside (adenosine, 5%, w/w) from nine different lactide-glycolide or lactide-caprolactone polymers. The polymer discs were eluted every second day with an artificial cerebrospinal fluid at the elution rate roughly approximating the brain extracellular fluid formation rate. Adenosine in eluate samples was assayed by HPLC. Three polymers exhibited a relatively constant release of adenosine for over four weeks, resulting in micromolar concentrations of nucleoside in the eluate. This points to the neccessity of further development of polymers of this types as intracerebral nucleoside delivery systems for local treatment of brain tumors.

Routine treatment for both primary and metastatic malignant brain tumors, which usually consists of surgery and post-surgical radiation therapy, is frequently ineffective [1, 2]. Cytostatic drugs are of limited value, mainly because of their inability to effectively cross the blood-brain barrier and their systemic toxicity. In particular, some nucleoside analogs display cytotoxic or radiosensitizing activity towards malignant tumors *in vitro* but, when administered peripherally, are toxic at doses far below those required for evoking clinical response in patients with brain neoplasms. For example, there is some evidence that cladribine (2-chlorodeoxyadenosine) in micromolar concentrations is cytotoxic towards human malignant gliomas *in vitro* [3, 4], but the maximal tolerated dose

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✉Author for correspondence: Tomasz Kryczka, Laboratory of Experimental Pharmacology, Medical Research Center, Polish Academy of Sciences, A. Pawińskiego 5, 02-106 Warszawa, Poland; phone: (48 22) 608 6474; fax: (48 22) 608 6527; e-mail: tkryczka@medprakt.pl

of this compound following systemic administration corresponds to plasma concentration of 40–100 nM whereas cerebrospinal fluid concentration is only 25% that in plasma [5]. It is, therefore, not surprising that a recent clinical study showed ineffectivity of cladribine on gliomas [6].

Intracranial implantation of biodegradable polymers for the local delivery of chemotherapy or for tumor tissue radiosensitization is a promising strategy in the treatment of brain tumors because it allows to establish a high and sustained concentration of a cytotoxic compound or a radiosensitizer at the tumor site while peripheral cytotoxicity is avoided [7, 8]. In the present study we evaluated kinetics of release of adenosine (used as a model nucleoside) from newly-synthesized lactide-caprolactone and lactide-glycolide polymers. Our aim was to select polymers capable, in conditions roughly approximating the intracerebral environment, to release adenosine at a sustained micromolar concentration for at least 4 weeks.

MATERIAL AND METHODS

Nine lactide-caprolactone or lactide-glycolide polymers containing adenosine (approx. 5%, w/w) have been synthesized (Table 1).

Synthesis of copolymers. The syntheses of the copolymers were performed in bulk in sealed glass ampoules using zirconium(IV) acetylacetonate or dibutylmagnesium as non toxic initiators. The obtained copolymers were precipitated with methanol and dried at 50°C under vacuum [9, 10]. Composition of copolymers were defined by ^{1}H and ^{13}C NMR spectroscopy. The average molecular mass (Mn) of the prepared copolymers was about 30 000–40 000 Da.

Preparation of the films from L,L-lactide/glycolide and glycolide/ε-caprolactone copolymers. A 0.1 mm thick film was prepared from a solution of the copolymer in methylene chloride containing adenosine (5%, w/w). The solution was cast by means of a standard device for casting on a glass plate and the solvent was evaporated at ambient temperature. The resultant film was then dried under reduced pressure. The polymer films were stored at room temperature in anhydrous environment.

In vitro *studies of adenosine release.* About 2.0 mg of a polymer was placed in a column (10 cm length and 10 mm diameter) and immersed in 800 mg of 150–212 μm glass beads (Sigma). The columns were kept at 37°C and eluted every second day with 1 ml of artificial cerebrospinal fluid (Fig. 1). Eluates were collected for adenosine assay. A 250 mm × 4.6 mm 5 μm Supelcosil C18 column, 0.05 M phosphate buffer, pH 3.0, + 3% acetonitrile mobile phase, and Merck Hitachi LaChrom System with Autosampler L-7250, Programmable Detector L-7420, Pump L-7100, and Integrator D-7500 were used. The flow rate was 1 ml/min and UV detection was performed at

Table 1. Polymers assessed in the study

Sample No.	Polymer composition
#1	18%-glycolide 82%-LL-lactide
#2	75%-DL-lactide 25%-caprolactone
#3	10%-glycolide 90%-caprolactone
#4	60%-caprolactone 40%-LL-lactide
#5	10%-glycolide 90%-caprolactone
#6	40%-DL-lactide 60%-caprolactone
#7	50%-caprolactone 50%-LL-lactide
#8	15%-glycolide 85%-LL-lactide
#9	80%-DL-lactide 20%[10%-glycolide + 90%-caprolactone]

254 nm. Under these conditions, the retention time of adenosine was 6.4 min.

RESULTS

The adenosine concentration *vs* time curves of the release of adenosine from newly-synthesized polymers are shown on Figs. 2A–C. The polymers assessed in the present study may be divided into three groups. Those belonging to the first group were characterized by a rapid but short-lasting and variable release of adenosine (Fig. 2A). The second group consisted of polymers which released adenosine continuously but adenosine concentration in the eluates was high at the beginning and fell down sharply, reaching undetectable levels after approximately two weeks (Fig. 2B). Polymers belonging to the third group produced a sustained >10 μM concentration of adenosine for a period of three to five weeks (Fig. 2C).

DISCUSSION

Lactide-caprolactone or lactide-glycolide polymers are good candidates for intracranial delivery of drugs because they are biocompatible with brain tissues [11]. We have recently developed several methods of their polymerization and loading with nucleosides (which will be described elsewhere). However, properties of these polymers cannot be predicted on theoretical grounds. In particular, the rate of disintegration of a polymer can be not uniform and it can differ from the rate of

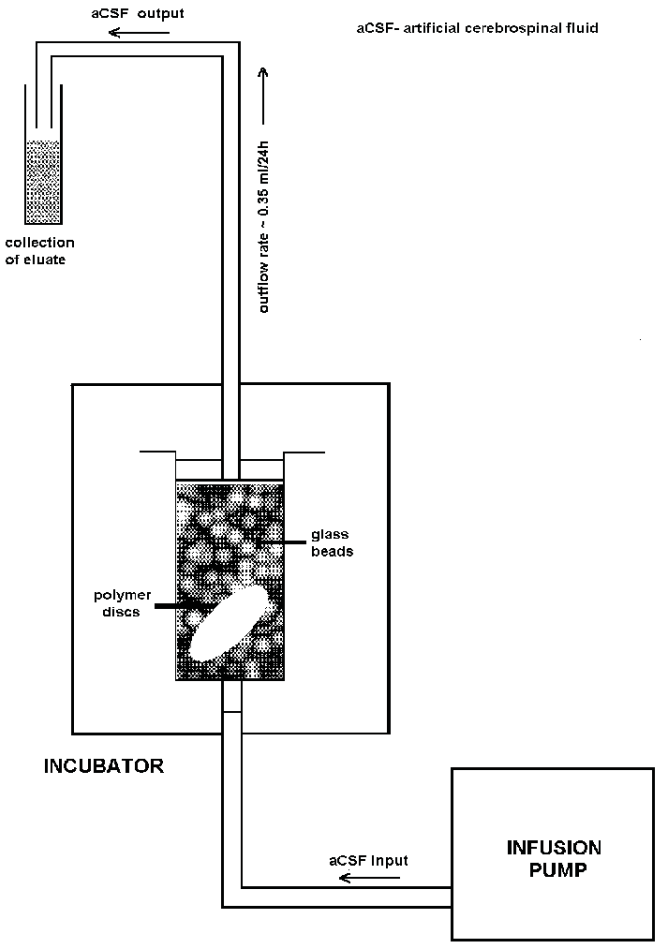

Figure 1. Schematic diagram of the experimental system.

nucleoside unloading, because the dispersion of nucleoside across the polymeric matrix is not always uniform. Also, a thermodynamic disequilibrium may exist between nucleoside molecules and the matrix, in which case the unloading of a nucleoside will be faster than disintegration of the polymeric matrix.

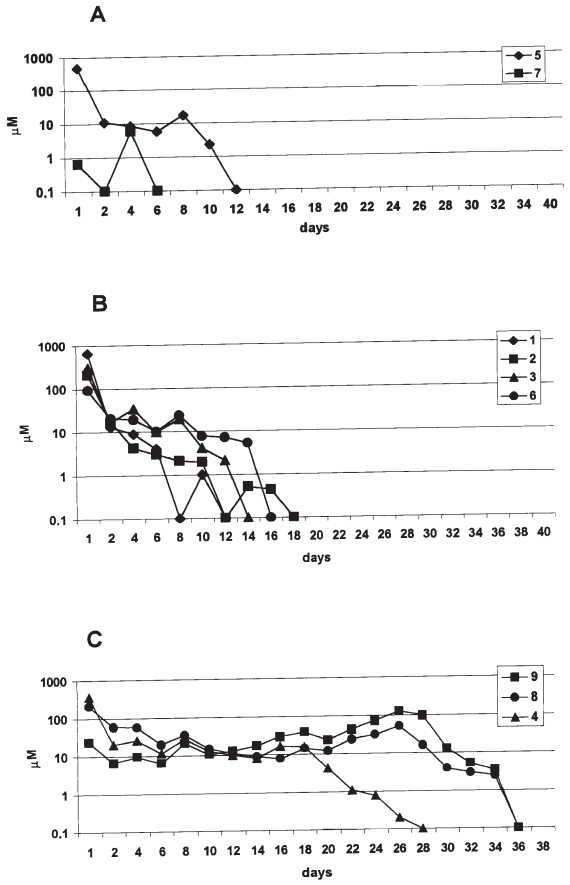

Figure 2. Adenosine concentration in eluates *vs* time: A – first group of polymers; B – second group of polymers; C – third group of polymers.

For numbering of polymers see Table 1.

In order to select polymers suitable for further development as carriers capable of delivering high and sustained concentrations of cytostatic or radiosensitizing nucleosides it was, therefore, necessary to assessment the rate of release of a nucleoside in appropriate conditions. As far, as the rate of unloading of a drug from a biodegradable polymer implanted to the brain is concerned, two factors should be considered decisive: the rate of polymer degradation and unloading of a nucleoside, and the rate of convective and diffusive transport of a drug liberated from polymeric matrix. While the first of these factors is probably an intrinsic property of a given polymer/nucleoside structure, the second depends on the rate of extracellular fluid formation and flow in the brain.

In the whole Rhesus monkey brain the extracellular brain fluid flow has been determined as approx. 11.6 μl/min [12]. The extracellular brain fluid flow rate in humans is probably of the same order (no data are available), and of that flow only a small fraction will get in contact with an implanted polymer. It may, therefore, be assumed that the rate of elution of polymer samples used in the present study, averaging 0.34 μl/min, may roughly approximate conditions of convective transport which would occur during polymer disintegration after its implantation into animal or human brain.

For practical reasons (considerable cost of cytostatic or radiosensitizing nucleoside analogs) in the present experiments we have used adenosine as the model nucleoside, and identified a few polymers which display favorable kinetics of its release. Since nucleosides introduced into the polymers do not chemically bind to the polymeric matrix, it is rather unlikely that the kinetics of release of drugs such as 2-chloro-2′-deoxyadenosine, or 5-bromodeoxyuridine from these polymers will be vastly different, but this remains to be tested experimentally.

A few groups of workers reported recently on the newly-synthesized biodegradable polymers for intracerebral delivery of nucleosides. Williams *et al.* [13] described a poly(bis(*p*-carbophenoxy)propane):sebacic acid polymers containing 10–50% of a radiosensitizing nucleoside 5-iododeoxyuridine, which upon intracranial implantation to mice xenografted with human glioblastoma, produced 35–40% labeling of glioma cells after four or eight days, holding promise for efficient radiosensitization of human brain tumors. Doiron

et al. [14] described polymers of similar composition loaded with 5-bromodeoxyuridine, which produced similar tumor cell labeling efficacy upon intratumoral implantation in a mouse.

RIF-1 tumor model, but displayed an initial rapid loss of the nucleoside over 24 h followed by a slower release extending over the next five days. Probably the most advanced polymer for delivery of a nucleoside is poly-(D,L-lactide-glycolide) microspheres loaded with 5-fluorouracil, developed by Menei *et al.* [15]. Following implantation of these microspheres into the wall of surgical bed after dissection of glioblastoma in patients, the authors detected sustained concentrations of 5-fluorouracil in the cerebrospinal fluid for at least one month.

As far as the kinetics of release of a nucleoside is concerned, our selected polymers compare favorably with those described recently by the others. Their further development as carriers for intracerebral delivery of cytotoxic or radiosensitizing nucleoside analogs for human tumor chemotherapy and/or radiosensitization *in situ* is, therefore, indicated.

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