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# The pH-Dependent and Enzymatic Release of Cytarabine From Hydrophilic Polymer Conjugates

**R. POLA[1], O. JANOUŠKOVÁ[1], T. ETRYCH[1]**

[1]Institute of Macromolecular Chemistry of the Czech Academy of Sciences, Prague, Czech Republic

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**Summary**

Cytarabine is one of the most efficient drugs in the treatment of hematological malignancies. In this work, we describe the synthesis and characterization of two different polymer conjugates of cytarabine that were designed for the controlled release of cytarabine within the leukemia cells. Reactive copolymers of N-(2-hydroxypropyl)methacrylamide (HPMA) and 3-(3-methacrylamidopropanoyl)thiazolidine-2-thione) or 3-(N-methacryloylglycyl-phenylalanylleucylglycyl)thiazolidine-2-thione were used in the study as reactive polymer precursors for reaction with cytarabine. The enzymatic release of cytarabine from the conjugate containing a GFLG spacer utilizing cathepsin B was verified. In addition to enzymolysis, the pH-dependent hydrolysis of cytarabine from both copolymers was also confirmed. Approximately 40 % and 20 % of the drug was released by spontaneous hydrolysis at pH 7.4 within 72 h from the polymer conjugates with the GFLG and β-Ala spacers, respectively. At pH 6.0, the spontaneous hydrolysis slowed down, and less than 10 % of the drug was liberated within 72 h. The results of the cytotoxicity evaluation of the polymer conjugates in vitro against various cell lines showed that the cytotoxicity of the polymer conjugates is approximately three times lower in comparison to free cytarabine.

**Key words**

HPMA copolymers • Drug delivery system • Nucleoside analogues • Cytarabine

**Corresponding author**

R. Pola, Department of Biomedicinal Polymers, Institute of Macromolecular Chemistry of the Czech Academy of Sciences, Heyrovského nám. 2, 162 06 Prague 6, Czech Republic. E-mail: pola@imc.cas.cz

## Introduction

Nucleotide and nucleoside analogues have served as cytostatic and antiviral drugs for more than thirty years (Alexander and Holy 1994). The first antiviral drug developed based on the acyclic guanosine analogue acyclovir was found to be a selective anti-herpes simplex virus agent in 1978 (Schaeffer *et al.* 1978). The problems of using these analogues as cytostatics or antivirals are due to their toxic side effects in healthy tissues and also their poor cell penetration. These drugs are usually negatively charged; consequently, they are repelled by negatively charged cell membranes. To overcome these problems, scientists began to develop “prodrugs," which are chemically modified substances that lack pharmacological activity and must be enzymatically, or by other means, transformed into the target metabolite with required biological effects by the biological system it enters (Kratz *et al.* 2008). Even more complicated is the cellular uptake of nucleotides. Because of these limitations, we recently developed polymer therapeutics (Pola *et al.* 2013, Pechar *et al.* 2011, Etrych *et al.* 2012) using a polymer carrier as specific delivery systems for nucleoside analogues to minimize the side effects of toxicity or poor cellular uptake. In principle, any drug with a suitable functional group, including nucleoside analogues, can be covalently attached to a polymer carrier. Polymer therapeutics based on copolymers of *N*-(2-hydroxypropyl)methacrylamide (HPMA) have been developed and are water-soluble, non-toxic, non-immunogenic and biocompatible drug carriers (Kopecek *et al.* 2000). The superior biological activity of HPMA-based conjugates with drugs has been

verified *in vivo*. It has been shown that the covalent attachment of a low-molecular-weight drug to a polymer carrier provides prolonged blood circulation, lower toxicity, higher tumor-specificity and a reduction or even elimination of the undesired side effects compared with the parent drug. Moreover, it was shown that the covalent attachment of a drug to a polymer carrier *via* a specific spacer inactivated the drug, which remained inactive during its transport in blood circulation. The drug is reactivated upon its release from the carrier inside the target tissue or inside the target cells. In the case of antivirals, they should be activated inside the infected cells. The controlled intracellular release of nucleoside analogues could be achieved by a pH-sensitive hydrazone bond or *via* an enzymatically degradable oligopeptide spacer, which would both stable in the blood stream and cleavable inside the cells.

Some low-molecular-weight nucleoside analogues, such as 1-β-D-arabinofuranosylcytosine (cytarabine, cytosine arabinoside) and 2′,2′-difluoro-2′-deoxycytidine (gemcitabine, dFdC), have been used as cytostatics (Prakasha Gowda *et al.* 2010). Cytarabine is a chemotherapeutic agent that interferes with the synthesis of DNA. Cytarabine in the body is rapidly converted into cytosine arabinoside triphosphate, which damages DNA during synthesis. The main advantage of the application of cytarabine for neoplastic treatment is its preferential activity against rapidly dividing cells. Cytarabine also inhibits both DNA and RNA polymerases and the nucleotide reductase enzymes needed for DNA synthesis. This agent has been the most effective chemotherapeutic agent used in the treatment of acute myeloid leukemia (AML) since the 1960s (Krance *et al.* 2001). In addition to AML, it is also used in the treatment of other hematological malignancies, such as acute lymphoblastic leukemia, chronic myelocytic leukemia, erythroleukemia and mantle-cell lymphoma. Despite the fact that cytarabine is one of the most frequently used chemotherapeutics for the treatment of hematological malignancies, it has side effects, such as neurotoxicity, myelosuppression (Herzig *et al.* 1983) and the development of resistance against cytarabine (Lamba 2009). Although treatment with cytarabine induces a response in 65-80 % of newly diagnosed AML patients, the clinical outcome is suboptimal because most of the patients relapse with a resistant form and have a poor response to other therapies. Hence, polymer-cytarabine conjugates may markedly overcome these serious limitations and help increase the efficacy of the therapy.

In this paper, we describe synthesis of *N*-(2-hydroxypropyl)methacrylamide (HPMA)-based polymer conjugates with cytarabine bound *via* an enzymatically degradable tetrapeptide spacer, Gly-Phe-Leu-Gly and a control conjugate with cytarabine bound *via* an enzymatically non-degradable spacer, β-Ala. The results of these drug release experiments and the cytotoxic activity of the conjugates are shown and discussed. Three cell lines derived from patients with treatment-refractory diffuse large B-cell lymphoma (DLBCL) and mantle cell lymphoma (MCL) and two cell lines, SU-DHL-5 (large B-cell lymphoma, LBCL) and Jeko-1 (mantle cell lymphoma, MCL), were involved into the *in vitro* study.

## Methods

*Chemicals*

Methacryloyl chloride, 1-aminopropan-2-ol, 3-aminopropanoic acid, 4,5-dihydrothiazole-2-thiol, dimethylaminopyridine, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, azobisisobutyronitrile (AIBN), 4-cyano-4-thiobenzoylsulfanylpentanoic acid, tert-butyl alcohol and pyridine were purchased from Sigma-Aldrich. Acetonitrile, dichloromethane, methanol and other common solvents and chemicals were purchased from Merck, s.r.o. 2-chlorotrityl chloride resin and protected amino acid derivatives were purchased from Iris Biotech GmbH, Germany. Cytarabine was purchased from Abcam Biochemicals, United Kingdom. All chemicals and solvents were of analytical grade.

*Analysis*

The synthesis and purity of monomers were monitored by reversed-phase HPLC using Chromolith Performance RP-18e columns (100×4.6 mm, Merck, Germany) with a linear gradient of water-acetonitrile (0-100 % acetonitrile) in the presence of 0.1 % TFA with a UV-VIS diode array detector (Shimadzu, Japan). The molecular mass of the monomers was determined using mass spectrometry performed on an LCQ Fleet mass analyzer with electrospray ionization (ESI-MS) (Thermo Fisher Scientific, Inc., MA, USA). The determinations of the molecular weights and polydispersity of the copolymers and the release of the drug from the polymer conjugate were carried out by size exclusion chromatography (SEC) on a HPLC system (Shimadzu, Japan) equipped with UV, differential refractive index, and multi-angle light scattering (LS) DAWN Helleos II (Wyatt Technology Corp., USA) detectors. For the

analysis, a TSK 3000 SWXL column (Tosoh Bioscience, Japan) (80 % methanol, 20 % phosphate buffer pH 6.5) at a flow rate of 0.5 ml/min was used. The content of the thiazolidine-2-thione (TT) groups and cytarabine was determined spectrophotometrically on a Helios Alpha UV/VIS spectrophotometer (Thermospectronic, UK) using the absorption coefficients for TT in methanol ($\varepsilon_{305}$=10,800 l·mol$^{-1}$·cm$^{-1}$) and for cytarabine in methanol ($\varepsilon_{301}$=5,065 l·mol$^{-1}$·cm$^{-1}$).

*Synthesis of the monomers*

*N*-(2-Hydroxypropyl)methacrylamide (HPMA) was prepared by the reaction of methacryloyl chloride with 1-aminopropan-2-ol (AMP) in dichloromethane (Ulbrich *et al.* 2000). Methacrylamidopropanoic acid (Ma-β-Ala-OH) was prepared by the reaction of methacryloyl chloride with 3-aminopropanoic acid in aqueous alkaline medium (Rejmanova *et al.* 1977). *N*-Methacryloylglycylphenylalanylleucylglycine (Ma-GFLG-OH) was prepared using automatic solid phase peptide synthesis using 2-chlorotrityl chloride resin (Pola *et al.* 2013). 3-(3-Methacrylamidopropanoyl)thiazolidine-2-thione (Ma-β-Ala-TT) was synthesized by the reaction of Ma-AP-OH with 4,5-dihydrothiazole-2-thiol in the presence of dimethylaminopyridine. The synthesis was performed similarly as previously described (Subr and Ulbrich 2006) using 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide hydrochloride instead of *N,N′*-dicyclohexylcarbodiimide, allowing for the removal of the water-soluble urea derivative by extracting the organic solution with water. 3-(*N*-Methacryloyl-glycylphenylalanylleucylglycyl)thiazolidine-2-thione (Ma-GFLG-TT) was prepared from Ma-GFLG-OH by the same procedure as described for Ma-β-Ala-TT. The monomers were characterized using HPLC (single peak) and ESI-MS (Ma-β-Ala-TT: calculated 258.3, found 259.1 [M+H]; Ma-GFLG-TT: calculated 561.7, found 562.9 [M+H]).

*Synthesis of the polymer precursors*

Copolymer **1,** poly(HPMA-*co*-Ma-β-Ala-TT), was prepared by reversible addition−fragmentation chain transfer (RAFT) polymerization of HPMA (92 mol %, 3 g) and Ma-β-Ala-TT (8 mol %, 471 mg) using AIBN (9.35 mg) as an initiator and 4-cyano-4-thiobenzoylsulfanylpentanoic acid (25.20 mg) as a chain transfer agent. The polymerization mixture was dissolved in tert-butyl alcohol (25.302 ml, 0.9 M solution of monomers) and transferred into a glass ampule. The mixture was bubbled with Ar and sealed. After 16 h at 70°C, the product was isolated by precipitation with acetone; the precipitate was then washed with diethyl ether and dried under vacuum. Copolymer **1** was then reacted with AIBN (10 molar excess) in DMSO (15 % w/w solution of polymer) under Ar for 3 h at 70 °C in a sealed ampule to remove the dithiobenzoate (DTB) ω-end groups (Perrier *et al.* 2004). The reaction mixture was isolated by precipitation with acetone. The precipitate was washed with diethyl ether and dried under vacuum to yield copolymer **1**. Copolymer **2,** poly(HPMA-*co*-Ma-GFLG-TT), was prepared by the same procedure mentioned above using RAFT polymerization. The molar ratio of monomers:CTA:initiator=400:2:1, was used. The molar ratio of HPMA:Ma-GFLG-TT in the reaction mixture was 90:10.

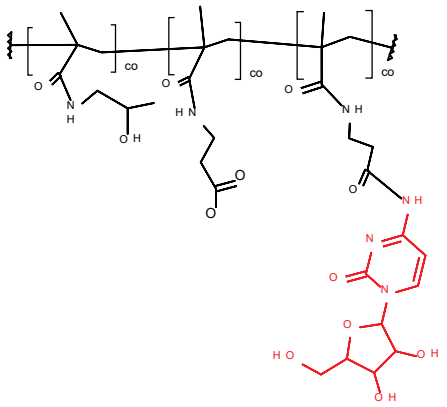
*Polymer conjugates with cytarabine*

Polymer precursor 1 (50 mg, 22.55 μmol TT) was dissolved in pyridine (0.5 ml) with cytarabine (5.5 mg, 22.55 μmol) and heated for 48 hours at 50 °C. The yellow solution became colorless, the reaction was ended by the addition of AMP (22.55 μmol). The reaction mixture was precipitated in diethyl ether and dried. The final conjugate was dissolved in water, purified by chromatography on Sephadex G 25 resin in water (PD 10 column, Pharmacia), and freeze-dried, which yielded 38 mg (76 %) of conjugate 3 as a white powder. Conjugate 4 was prepared similarly, starting with polymer precursor 2 (Fig. 1).

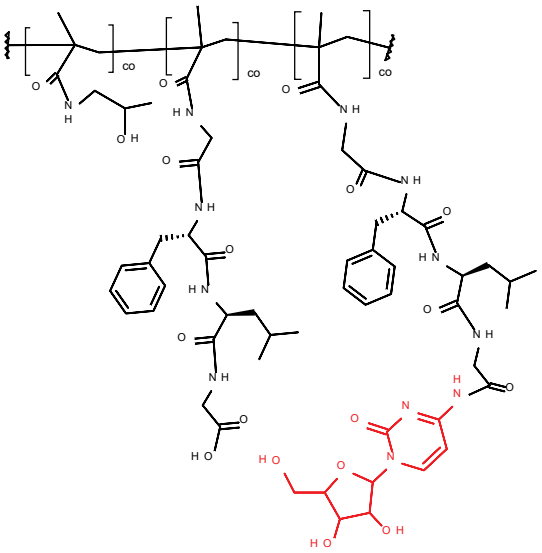
*In vitro release of cytarabine from the polymer conjugates*

The enzymatic activity of cathepsin B was probed immediately before the release measurements using *N*-α-benzoyl-DL-arginine p-nitroanilide hydrochloride (Bz-Arg-Nap) as a substrate (Pechar *et al.* 1997). The cathepsin B-catalyzed hydrolysis of cytarabine from the polymer conjugates was performed at polymer concentrations of 0.5 mg/ml (potential sites of the cleavage at a concentration of approximately $2\times10^{-3}$ mol l$^{-1}$) and at an enzyme concentration [E]=$2\times10^{-7}$ mol l$^{-1}$ at 37 °C in 0.1 M phosphate buffer ($KH_2PO_4$, NaOH, pH=6.0, 0.001 M EDTA, and 0.01 M glutathione). The process of enzymolysis was monitored by SEC and HPLC, and the rate of cytarabine release was calculated from the area of the corresponding peaks at 301 nm (bound cytarabine) or 270 nm (free cytarabine).

The spontaneous hydrolysis and release of



Conjugate **3**



Conjugate **4**

**Fig. 1.** Chemical structure of conjugate 3 (β-Ala) and conjugate 4 (GFLG) with the drug cytarabine (red).

cytarabine was measured in two different PBS buffers to mimic the blood stream, pH 7.4 and pH 6.0, which serves as a model of endosomes in tumor cells, at 37 °C. Conjugates (0.5 mg/ml) were incubated in these buffers; the mixture was measured using HPLC to observe the decrease in the polymer amount at 301 nm.

*Cell lines*

The cell lines UPF4D and UPF1H were kindly provided by Dr Klener's group at the Institute of Pathological Physiology of Charles University in Prague. The cell lines were derived from patients with treatment-refractory diffuse large B-cell lymphoma (DLBCL) and mantle cell lymphoma (MCL), respectively. Both cell lines were established after rituximab-based treatments. UPF4D CD20$^-$ cells were obtained by the long-term culture (3 months) of the original UPF4D cell line. This cell line spontaneously lost CD20 expression during long- term cultivation *in vitro*. The cell lines SU-DHL-5 (large B-cell lymphoma, LBCL) and Jeko-1 (mantle cell lymphoma, MCL) were obtained from the DSMZ (German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany).

The cells were cultivated in RPMI-1640 medium (Thermo Scientific, Prague, Czech Republic) supplemented with penicillin (100 U/ml), streptomycin (100 µg/ml), and heat-inactivated 10 % FBS for SU-DHL-5 and Jeko-1 cells or 15 % FBS for UPF1H and UPF4D cells.

*In Vitro Cell Viability Assay*

The cells ($7\times10^3$) were seeded in 100 µl of media per well in 96-well flat-bottom plates (TPP, Sigma-Aldrich, Prague, Czech Republic) 24 h before adding the polymer conjugates bound cytarabine or free cytarabine. In the cytotoxicity testing, the concentrations of conjugates **3** and **4** ranged from 0.01-10 µM of the cytarabine equivalent. The concentration of free cytarabine ranged from 0.005-5 µM for all cell lines, except Jeko-1. Because of cell sensitivity, a lower range, 0.001-1 µM, was used.

The cells were incubated with polymer conjugates or free drug for 72 h. Then, 10 µl of the Alamar Blue® cell viability reagent (Life Technologies, Prague, Czech Republic) was added to each well and incubated for 4 h at 37 °C. The active component of the Alamar Blue reagent, resazurin, was reduced to the highly fluorescent compound resorufin by viable cells. The fluorescence of resorufin was detected on a Synergy Neo plate reader (Bio-Tek, Prague, Czech Republic) at an excitation wavelength of 570 nm and at an emission wavelength of 600 nm. Cells cultivated in medium without the conjugates were used as controls. Three wells were used for each concentration. The assay was repeated three times.

## Results

The polymer precursors were synthesized using RAFT polymerization, and both precursors had a narrow distribution of molecular weights (Table 1). Cytarabine

was attached to reactive polymer precursors by an aminolytic reaction of the amino group of cytarabine and the TT groups of the respective polymer precursor. The molecular characteristics of the reactive polymer precursors and polymer conjugates with cytarabine are shown in Table 1.

**Table 1.** Characterization of prepared polymer precursors and polymer conjugates.

| Sample | Structure | $M_w$[a] | $M_w/M_n$[a] | TT (mol %)[b] | Cytarabine (wt %)[c] |
|---|---|---|---|---|---|
| **1** | *p(HPMA-co-Ma-β-Ala-TT)* | 44,800 | 1.12 | 6.5 | - |
| **2** | *p(HPMA-co-Ma-GFLG-TT)* | 35,600 | 1.18 | 8.15 | - |
| **3** | *p(HPMA-co-Ma-β-Ala-Cytarabine)* | 56,300 | 1.23 | - | 10.5 |
| **4** | *p(HPMA-co-Ma-GFLG-Cytarabine)* | 58,700 | 1.26 | - | 12.6 |

[a]Molecular weights were determined by SEC using RI and LS detection. [b]TT determined by UV/VIS spectrophotometry in methanol ($\varepsilon_{306}$=10,280 l·mol$^{-1}$·cm$^{-1}$). [c]Cytarabine determined by UV/VIS spectrophotometry in methanol ($\varepsilon_{301}$=5,065 l·mol$^{-1}$·cm$^{-1}$).

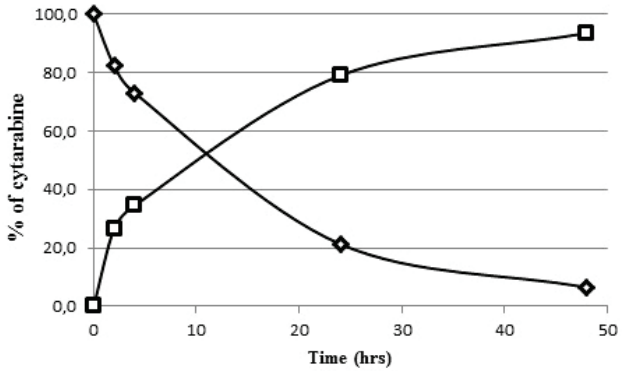



**Fig. 2.** Enzymatic release of cytarabine from conjugate 4. The increasing amount of released cytarabine was measured at 270 nm (□), and the decreasing amount of cytarabine corresponding to the polymer-bound drug was measured at 301 nm (◇) using HPLC.

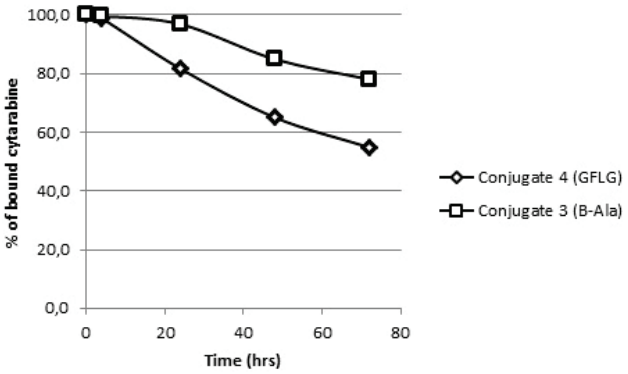



**Fig. 3.** Hydrolytic release of cytarabine from conjugate 3 and 4 at pH 7.4. The decrease in the peak corresponding to polymer-bound cytarabine was monitored at 301 nm using HPLC.

The enzymatic release (Fig. 1) of cytarabine from conjugates **3** and **4** was monitored by HPLC. A significant amount of released cytarabine was found with conjugate **4**, which contained an enzymatically degradable GFLG spacer. There was no enzymatic cleavage of cytarabine observed with conjugate **3**, which contained a β-Ala spacer.

Because conjugate **3** is also cytotoxic we thought that besides the enzymatic cleavage of cytarabine a chemical hydrolysis also helps to the drug release. So the spontaneous hydrolytic release of cytarabine from conjugates **3** and **4** was determined by HPLC at pH 6.0 (data not shown) and pH 7.4 (Fig. 2). The hydrolysis was faster at pH 7.4 than at pH 6.0 for both conjugates. At pH 6.0, the hydrolysis was very slow for both conjugates. After 72 hours, only 10 % of free cytarabine was detected for each conjugate. At pH 7.4, the hydrolytic release of cytarabine from conjugate **3** was slower than from conjugate **4**. After 72 hours, more than 40 % of free cytarabine was released from conjugate **4**, and only approximately 25 % of free cytarabine was released from conjugate **3**.

The cytotoxicity of the polymer conjugates was compared with the cytotoxicity of free cytarabine using two commercially available cell lines, Jeko-1 and SU-DHL-5, and three cell lines derived from patients with diffuse large B-cell lymphoma (UPF4D CD20$^-$ and UPF4D CD20$^+$) and mantle cell lymphoma (UPF1H) (Table 2). The cytostatic effect of free cytarabine and of both conjugates was dependent upon the cell line. The cell line most sensitive to treatment with cytarabine and its polymer conjugates was the mantle cell lymphoma cell line Jeko-1, followed by mantle lymphoma cell line –

UPF1H. Using these cell lines, we also found the highest differences between the cytotoxicity of the free drug and the polymer conjugates (7- and 3.4 fold respectively higher cytotoxicity of the free drug compared with the polymer conjugate **4**). All other cell lines exhibited only approximately 2-3 times higher sensitivity to free cytarabine than to the polymer conjugates. We found that the sensitivity of the CD20 positive cell line, UPF 4D $CD20^+$, was slightly higher than that of the corresponding CD20 negative cells (UPF 4D $CD20^-$) to both the polymer conjugates and to the free drug. The most resistant cell line to treatment with both the polymer conjugates and the free drug was the large B cell lymphoma cell line SU-DHL-5. The cytotoxicity of free cytarabine was 2.2 - 2.6 higher than that of conjugate **4**, except for the cell line Jeko-1 and UPF1H. Conjugate **4** was 2-4 times more cytotoxic in comparison with conjugate **3** in all tested cell lines.

**Table 2.** The $IC_{50}$ (μM) values of the 5 different cell lines and the ratios of the $IC_{50}$ values.

| SAMPLE | 4D/$CD20^+$ | 4D/$CD20^-$ | SU-DHL-5 | Jeko-1 | 1H |
|---|---|---|---|---|---|
| *Conjugate 3 (β-Ala)* | 0.637±0.01 | 1.170±0.55 | 1.358±3.13 | 0.273±0,02 | 0.313±0.052 |
| *Conjugate 4 (GFLG)* | 0.271±0.09 | 0.348±0.11 | 0.497±0.03 | 0.084±0,01 | 0.072±0.02 |
| *Cytarabine* | 0.113±0.07 | 0.156±0.04 | 0.191±0.04 | 0.012±0.002 | 0.021±0,01 |
| **RATIO** | | | | | |
| *Conjugate 3/4* | 2.4 | 3.4 | 2.7 | 3.3 | 4.3 |
| *Conjugate 4/Cytarabine* | 2.4 | 2.2 | 2.6 | 7.0 | 3.4 |

## Discussion

The major aim of this work was the synthesis and subsequent *in vitro* verification of the cytotoxicity of polymer-cytarabine conjugates containing both enzymetically degradable and non-degradable bonds between the drug and the polymer carrier. A comparison of the cytotoxicity of polymer conjugates with that of unmodified free cytarabine was also reported.

Surprisingly, the cytotoxicity of control polymer conjugate **3** containing an enzymatically non-cleavable spacer, β-Ala, was only 2.4 to 4.3-fold lower than that of the lysosomotropic polymer conjugate **4**. This finding indicates that besides the enzymatic cleavage of cytarabine a pH-dependent chemical hydrolysis also contributes to the drug release. Therefore, we subjected both polymer conjugates to hydrolysis at pH 7.4 and 6.0, which corresponds to the pH of blood and the intracellular environment, respectively.

The enzymatic release of cytarabine from conjugate **4** by the action of a lysosomal protease, cathepsin B, was measured using a HPLC system. We have observed the increasing peak of free cytarabine at a retention time of 0.5 min at 270 nm and a decreasing polymer peak at 2.4 min (301 nm), which corresponded to the remaining polymer-bound cytarabine. The data after the subtraction of the amount of cytarabine released at pH 6.0 by spontaneous non-enzymatic hydrolysis are shown. Figure 1 shows the relatively fast release of the drug from conjugate **4**; more than 90 % of the total cytarabine was released within two days. The incubation of the control, conjugate **3**, which contained a β-Ala spacer, with cathepsin B resulted in a very low amount (10 %) of the released drug. This corresponds to the amount of drug released by spontaneous hydrolysis at pH 6.0.

The spontaneous hydrolytic release of cytarabine from conjugate **3** and **4** was determined at pH 6.0 to model the endosomes and lysosomes of tumor cells and at pH 7.4. Both were tested at 37 °C. The pH value 7.4 was chosen because it is the physiological pH of the blood and also the pH of cell culture media used for the cytotoxicity evaluation. Figure 2 reveals that the rate of chemical hydrolysis of the amide bond between the drug and the polymer carrier in conjugate **4** at pH 7.4 was almost 1.5-fold faster than that in conjugate **3**. This is probably due to the different chemical structures of the spacers GFLG or β-Ala.

At pH 6.0, the non-enzymatic hydrolysis of both polymer conjugates was much slower than at pH 7.4, leading to the release of less than 10 % of cytarabine from the polymer conjugates **3** and **4**.

The evaluation of the cytotoxicity of the polymer cytarabine conjugates showed significant differences between conjugates with different spacers. While the spacer in conjugate **3** is enzymatically non-cleavable, cytarabine hydrolyzed slowly from the polymeric backbone at pH 7.4 and very slowly after penetration of the cell. The spacer in conjugate **4** also slowly hydrolyzed at pH 7.4, but an efficient and more rapid release occurs in the presence of lysosomal proteases after penetration of the cell. Thus, the rate of drug release and, consequently, the cytotoxicity of conjugate **4** is higher than that of conjugate **3,** from which cytarabine is cleaved only by slow, pH-dependent hydrolysis. In all the experiments, the parent drug, free cytarabine, showed the highest cytotoxicity. However, a comparison of the lower cytotoxicities of the polymeric drug conjugates with the free drug *in vitro* has already been published (Kunath *et al.* 2000, Etrych *et al.* 2002, Vicent *et al.* 2004). The advantage of polymer therapeutics becomes clear *in vivo*. The application of polymer conjugates *in vivo* can minimize the side effects of the free drug. The polymer conjugates circulate for a longer time in the body, and they can target specific types of cells. It is worth mentioning that the difference between the cytotoxicity of the free cytarabine and conjugate **4** is smaller than that of doxorubicin and polymer-doxorubicin conjugates, where the difference in cytotoxicity is usually more than 10-fold (Etrych *et al.* 2002). This effect can be caused by the lower ability of free cytarabine to cross the cell membrane. The high sensitivity of Jeko-1 cell line may reflect the difference in the diffusion of free cytarabine across the cell membrane and the metabolism of the nucleoside in this cell line.

Significant differences in the $IC_{50}$ values in the cell line derived from one patient (UPF 4D, which lost its expression of CD20 during cultivation *in vitro*) were observed. This cell line showed lower sensitivity to cytarabine and its conjugates than UPF 4D $CD20^{+}$.

## Conclusions

We designed, synthesized and evaluated the preliminary physico-chemical and biological properties of two polymer conjugates of the anti-cancer drug cytarabine. The polymer conjugates were able to release the free cytarabine through spontaneous pH-dependent hydrolysis and also by highly specific enzymatic degradation. The polymer conjugate containing a GFLG spacer between cytarabine and the polymer carrier was found to be a suitable candidate for further biological studies. This polymer conjugate combined pH-sensitive hydrolysis with the enzymatic activity of the lysosomes of tumor cells. Moreover, we demonstrated that the cytotoxic effect of the polymer drug conjugate with a GFLG spacer was only slightly lower than that of free cytarabine and higher than that of the polymer conjugate with an enzymatically non-degradable spacer. We conclude that the tested polymer conjugate with a GFLG spacer has significant potential for *in vivo* application, and an evaluation is currently under way.

## Conflict of Interest

There is no conflict of interest.

## Acknowledgements

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