Controlled delivery of protoporphyrin IX precursors for photodynamic therapy of tumors using water soluble polymer

Kevin Kotalík[1], Vladimír Šubr[1], Marcela Filipová[1], Tereza Běláková[1], Tomáš Křížek[2], Alice Šimonová[2], Jun Fang[3], and Tomáš Etrych[1]

[1] Institute of Macromolecular Chemistry, Czech Academy of Sciences, Prague, Czech Republic
[2] Department of Analytical Chemistry, Faculty of Science, Charles University, Prague, Czech Republic
[3] Faculty of Pharmaceutical Sciences, Sojo University, Kumamoto, Japan

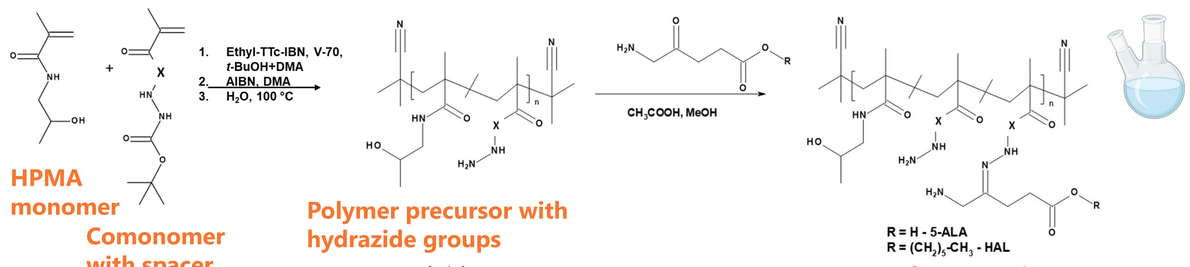
1. INTRODUCTION

Among current anticancer treatment strategies, photodynamic therapy (PDT) gains the interest of many researchers, offering especially non-invasiveness and low side effects. Three main components - photosensitizer, light and oxygen - are necessary for this strategy with the basic principle laying on light irradiation of the tumor tissue after photosensitizer administration causing singlet oxygen and other reactive oxygen species production resulting in tumor death. Nevertheless, basic photosensitizers are not delivered into the tumor in a controlled way and are distributed among the whole body, which can cause skin photosensitivity after the therapy. An interesting approach in PDT is the use of endogenous photosensitizer, protoporphyrin IX (PPIX). In this case, the photosensitizer is administered in form of a prodrug, 5-aminolevulinic acid (5-ALA), which is metabolized to PPIX intracellularly(1). 5-ALA itself is a non-toxic compound, however, still lacks tumor targeting properties and suffers from poor pharmacokinetics.

To improve 5-ALA pharmacokinetics, we used a water soluble polymer-based nanosized drug delivery system. Such nanotherapeutics are known to be accumulated in the tumor tissue due to so-called enhanced permeability and retention (EPR) effect, based on leaky tumor vasculature and absence of lymphatic drainage. As carriers for such nanotherapeutics, various polymers are used with advantage, mostly due to their biocompatibility. A promising material is poly[*N*-(2-hydroxypropyl)methacrylamide] (PHPMA), showing excellent biocompatibility and versatility for nanotherapeutics design(2).

Herein, we present the synthesis of water soluble PHPMA conjugates with 5-ALA or its ester (HAL) as well as physico-chemical characterization. Moreover, the attachment of 5-ALA/HAL via pH sensitive hydrazone bond enabling selective release of the prodrug in tumor tissue was performed verifying also the pH sensitive behavior. Biological evaluation of the polymer conjugates was performed *in vitro* and *in vivo* showing enhanced PDT effect (tumor growth inhibition) with no side effects.

2. SYNTHESIS AND CHARACTERIZATION



HPMA monomer + **Comonomer with spacer X**

Polymer precursor with hydrazide groups

- Water soluble
- Molar mass under renal filtration limit
- Narrow molar mass distribution (RAFT)

Polymer conjugate bearing 5-ALA/HAL

- pH sensitive hydrazone bond

Polymer precursors

Polymer	M_n [g mol^{-1}]	M_w [g mol^{-1}]	$Đ$	$D_{h,i}$ [nm]	$NHNH_2$ group content [mol. %]
P-Acap	39000	44000	1.13	9.0	22.0
P-βAla	39000	46000	1.18	8.6	24.4
P--	41000	46000	1.12	9.9	14.0

Polymer conjugates

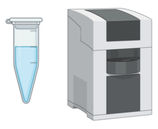
Polymer	M_n [g mol^{-1}]	M_w [g mol^{-1}]	$Đ$	$D_{h,i}$ [nm]	Prodrug content [wt. %]
P-Acap-5-ALA	49000	60000	1.22	13.0	17.5
P-βAla-5-ALA	N.D.*	N.D.*	N.D.*	10.2	18.9
P-5-ALA	51000	57000	1.12	8.9	10.2
P-Acap-HAL	52000	62000	1.19	10.8	10.5
P-βAla-HAL	50000	61000	1.22	10.6	11.9
P-HAL	54000	74000	1.37	8.5	5.8

Methods: M_n, M_w and $Đ$ was determined using SEC (DMF+LiBr), D_h was determined by DLS, $NHNH_2$ content was determined by NMR and 5-ALA/HAL content was determined using HPLC with precolumn derivatization (acetylacetone, formaldehyde) and fluorescent detection after hydrazone bond total hydrolysis. *N.D. = Not determined due to sample insolubility in mobile phase.

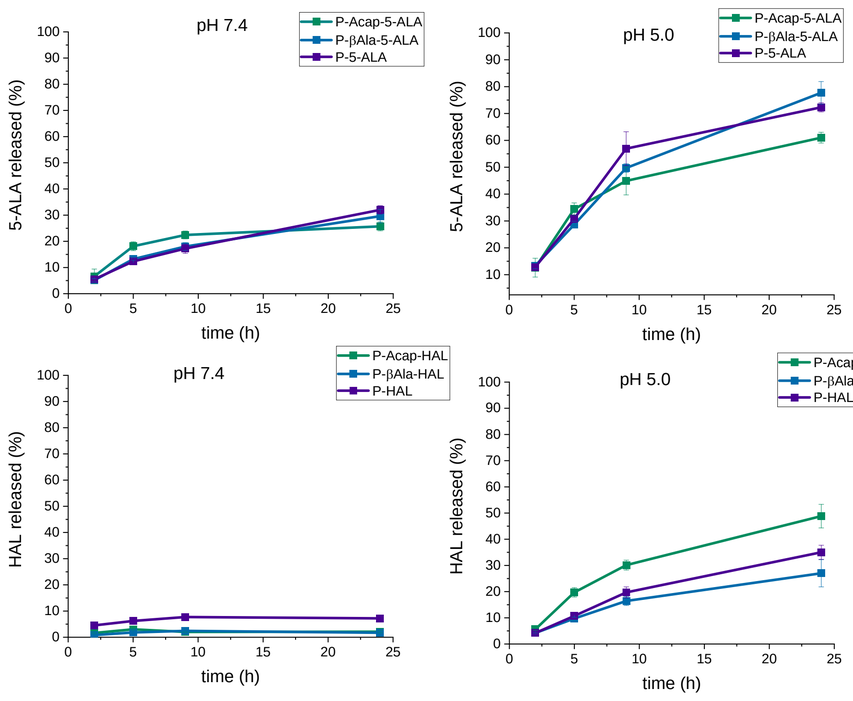
3. *IN VITRO* PRODRUG RELEASE AND CYTOTOXICITY

Prodrug release

- Aqueous buffers – pH sensitivity of hydrazone bond – pH 7.4 (blood) vs pH 5.0 (tumor microenvironment)
- pH sensitive behavior of hydrazone bond with low effect of spacer **X** (Acap vs βAla vs no spacer)
- Significant effect of ketone (prodrug) – HAL slower than 5-ALA
- Detection using capillary electrophoresis with C^4D detection



5-ALA

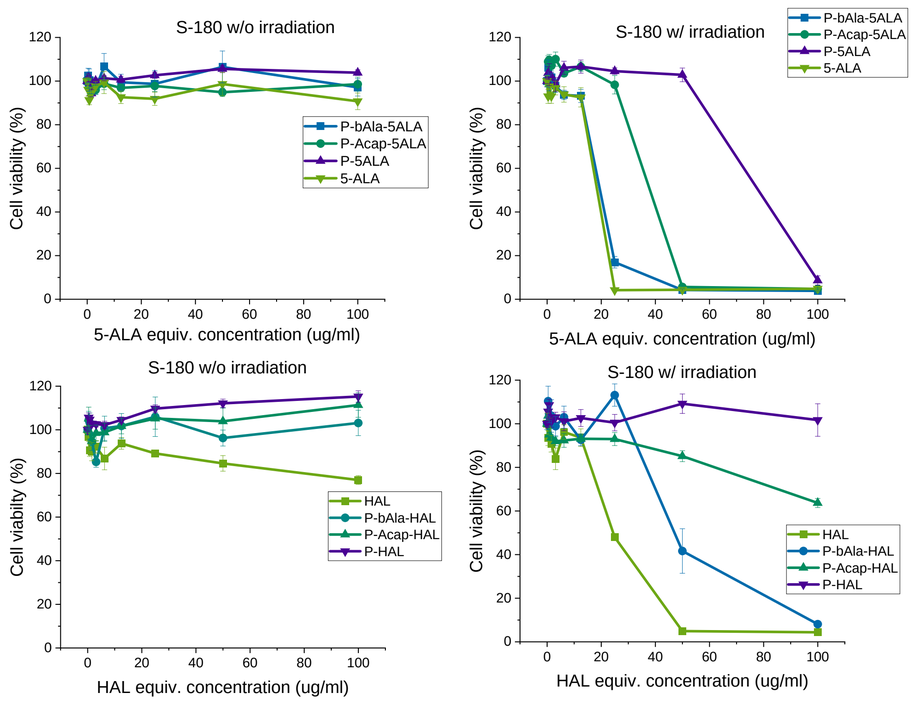


HAL

***In vitro* cytotoxicity**

- S-180 mouse sarcoma, MTT assay
- No dark cytotoxicity (w/o irradiation)
- Significant PDT effect (irradiation w/ λ = 420 nm (1 J cm^{-2})) ✔
- Lower effect of HAL conjugates (compared to 5-ALA conjugates) – due to slower release of HAL

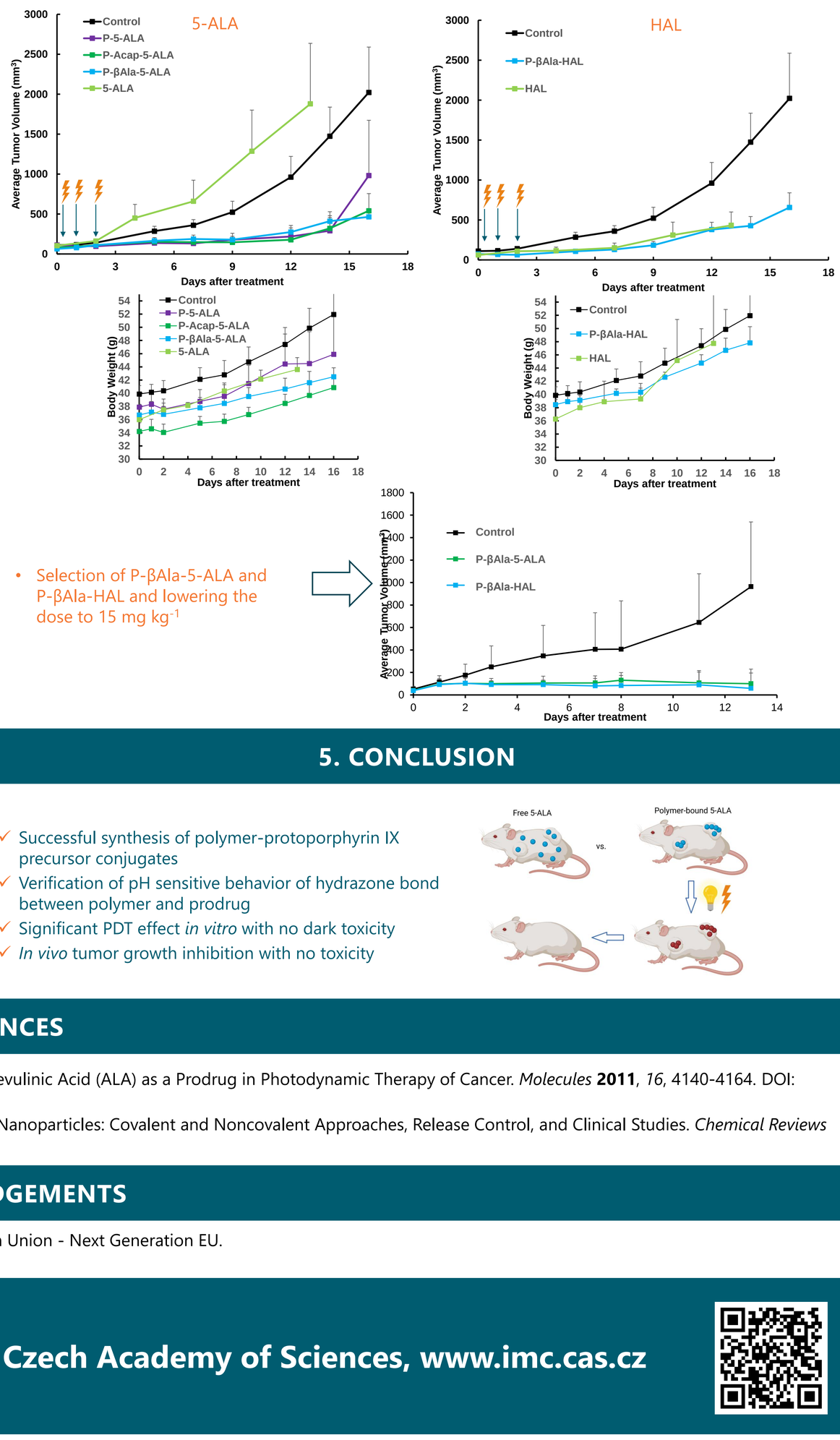
5-ALA



HAL

4. *IN VIVO* THERAPEUTIC EFFICACY

- Mouse sarcoma S-180
- 30 mg kg^{-1} i.v., n = 3
- Irradiation at 6, 24 and 48 hours – Xe lamp (400-700 nm, 27 J cm^{-2})
- Ability to suppress tumor growth compared to free 5-ALA ✔ no toxicity according to body weight ✔



- Selection of P-βAla-5-ALA and P-βAla-HAL and lowering the dose to 15 mg kg^{-1}

5. CONCLUSION

- ✓ Successful synthesis of polymer-protoporphyrin IX precursor conjugates
- ✓ Verification of pH sensitive behavior of hydrazone bond between polymer and prodrug
- ✓ Significant PDT effect *in vitro* with no dark toxicity
- ✓ *In vivo* tumor growth inhibition with no toxicity

6. REFERENCES

(1) Wachowska, M.; Muchowicz, A.; Firczuk, M.; Gabrysiak, M.; Winiarska, M.; Wańczyk, M.; Bojarczuk, K.; Golab, J. Aminolevulinic Acid (ALA) as a Prodrug in Photodynamic Therapy of Cancer. *Molecules* **2011**, *16*, 4140-4164. DOI: 10.3390/molecules16054140

(2) Ulbrich, K.; Holá, K.; Šubr, V.; Bakandritsos, A.; Tuček, J.; Zbořil, R. Targeted Drug Delivery with Polymers and Magnetic Nanoparticles: Covalent and Noncovalent Approaches, Release Control, and Clinical Studies. *Chemical Reviews* **2016** *116* (9), 5338-5431. DOI: 10.1021/acs.chemrev.5b00589

7. ACKNOWLEDGEMENTS

This work was supported by the project National Institute for Cancer Research (LX22NPO5102)- Funded by the European Union - Next Generation EU.