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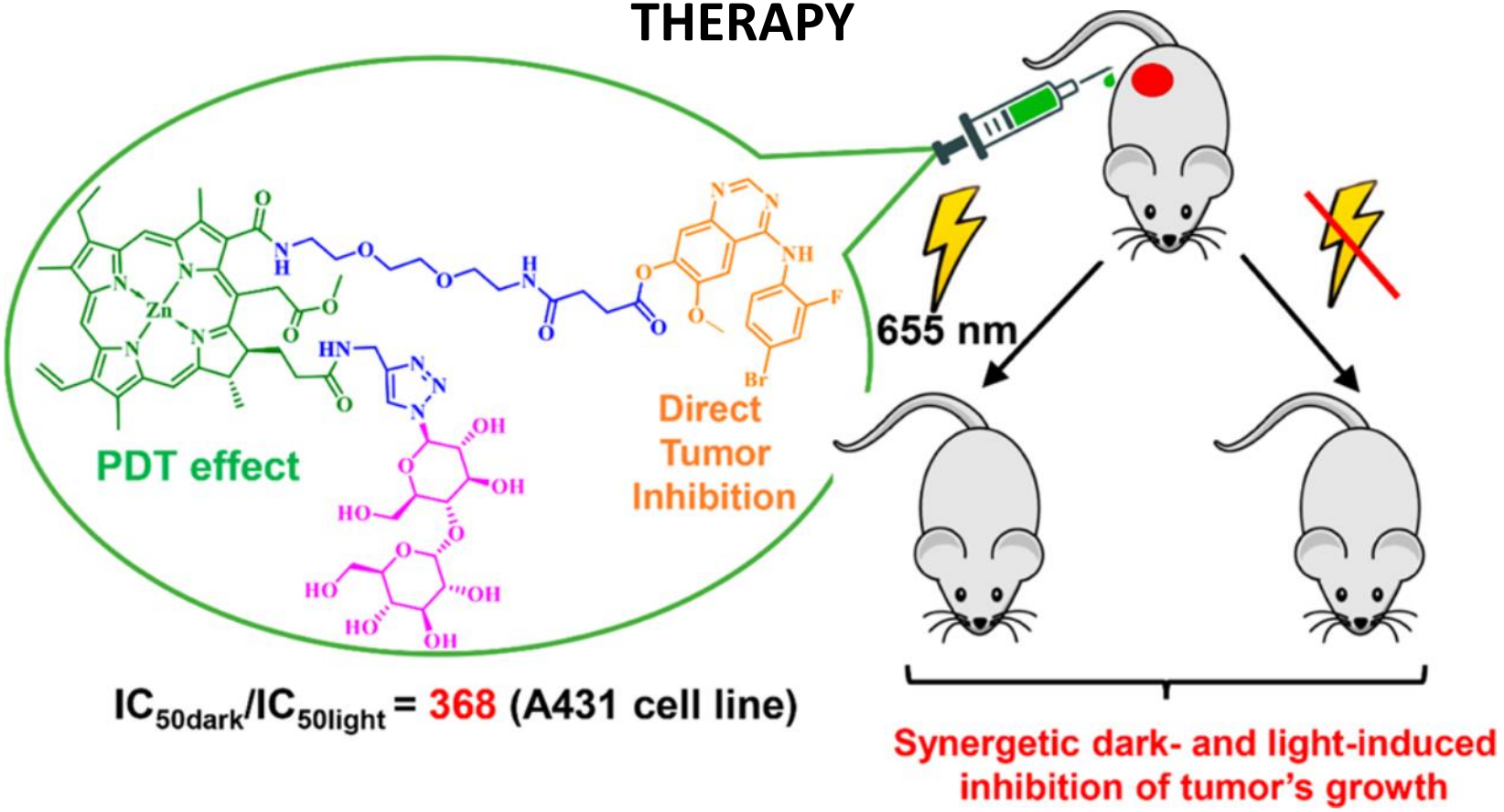
Anticancer effect of a chlorin *e6* conjugate with vandetanib for combined photodynamic and targeted therapy

Liubov V. Krylova*, Vasily F. Otvagin, Natalia S. Kuzmina, Alexander V. Nyuchev, Alexey Yu. Fedorov, Irina V. Balalaeva

Lobachevsky University, 603950, Gagarin Ave. 23, Nizhny Novgorod, Russia
* Corresponding author: lu.krylova@mail.ru

ANTICANCER EFFECT OF A CHLORIN *E6* CONJUGATE WITH VANDETANIB FOR COMBINED PHOTODYNAMIC AND TARGETED THERAPY



Abstract:

The combination of photodynamic and targeted therapeutic approaches seems to be a promising strategy for increasing the therapeutic effect of cancer treatment. The aim of this work was to evaluate the potential of a combined agent which is a conjugate of a zinc complex of photosensitizer chlorin *e6* with tyrosine kinase inhibitor vandetanib (ZnChl-Vd). It was shown that ZnChl-Vd has intense absorption and fluorescence in the red region of the spectrum with a quantum yield of fluorescence of about 2% and a singlet oxygen quantum yield of about 20%. *In vitro* experiments showed intensive accumulation of ZnChl-Vd in cells of human epidermoid carcinoma A-431 with localization in lysosomes and Golgi apparatus. The pronounced photoinduced cytotoxic effect of the conjugate at submicromolar concentrations was shown. *In vivo* studies demonstrated the selective accumulation of ZnChl-Vd in the xenograft tumors; irradiation of tumors (50 J/cm^2) led to profound tumor regression from their initial volume. In the dark, ZnChl-Vd ability to inhibit tumor growth up to 50% compared to control untreated group evidence the contribution of targeted irradiation-independent mechanism of ZnChl-Vd action. The results indicate high potential of ZnChl-Vd as an anticancer agent combining the activity of a photosensitizer and a target agent.

Keywords: photodynamic therapy; targeted therapy; chlorin e6; combined action of drugs; vandetanib

Introduction

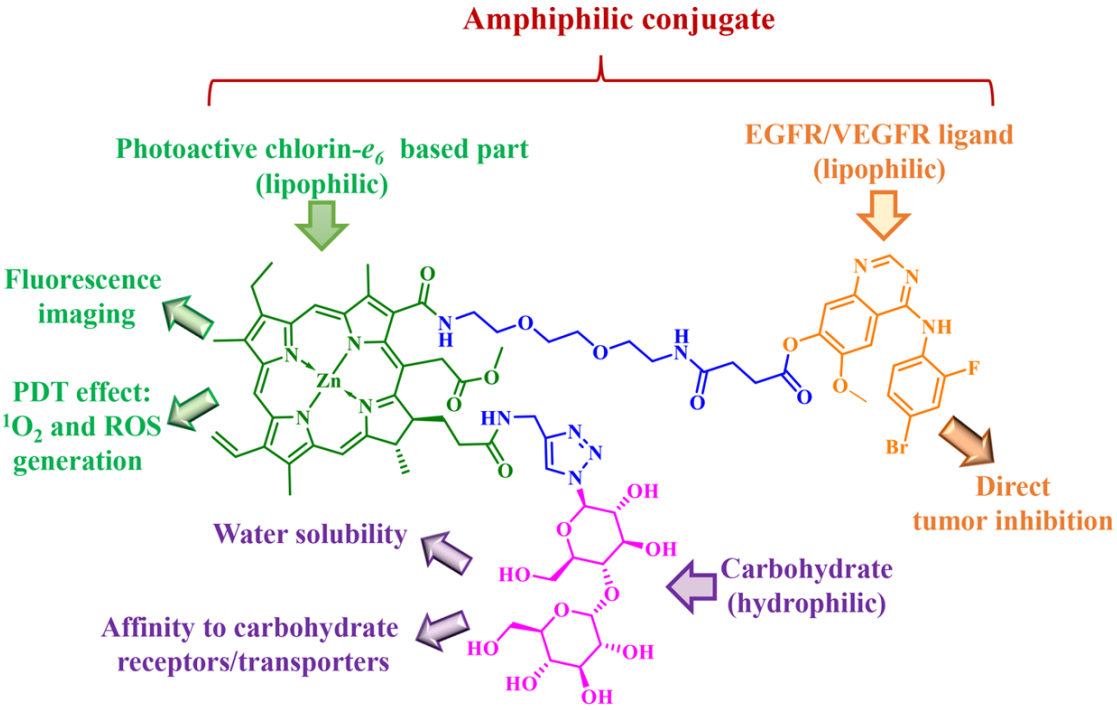
mono-therapeutic techniques → non-selectively target actively proliferating cells; toxic to the patient with multiple side effects and risks

Combination therapy are a combination of several therapeutic agents with different mechanisms of action in one drug to obtain the greatest therapeutic effect and overcome the tumor's resistance to treatment.

One of the possible directions is conjugation of a photosensitizers with cytotoxic activity molecules .

The therapeutic effect of PDT is based on the interaction of photosensitizer, light of a certain wavelength and molecular oxygen. As a result of photochemical reactions, reactive oxygen species are formed, which trigger oxidative processes in cells leading to its death.

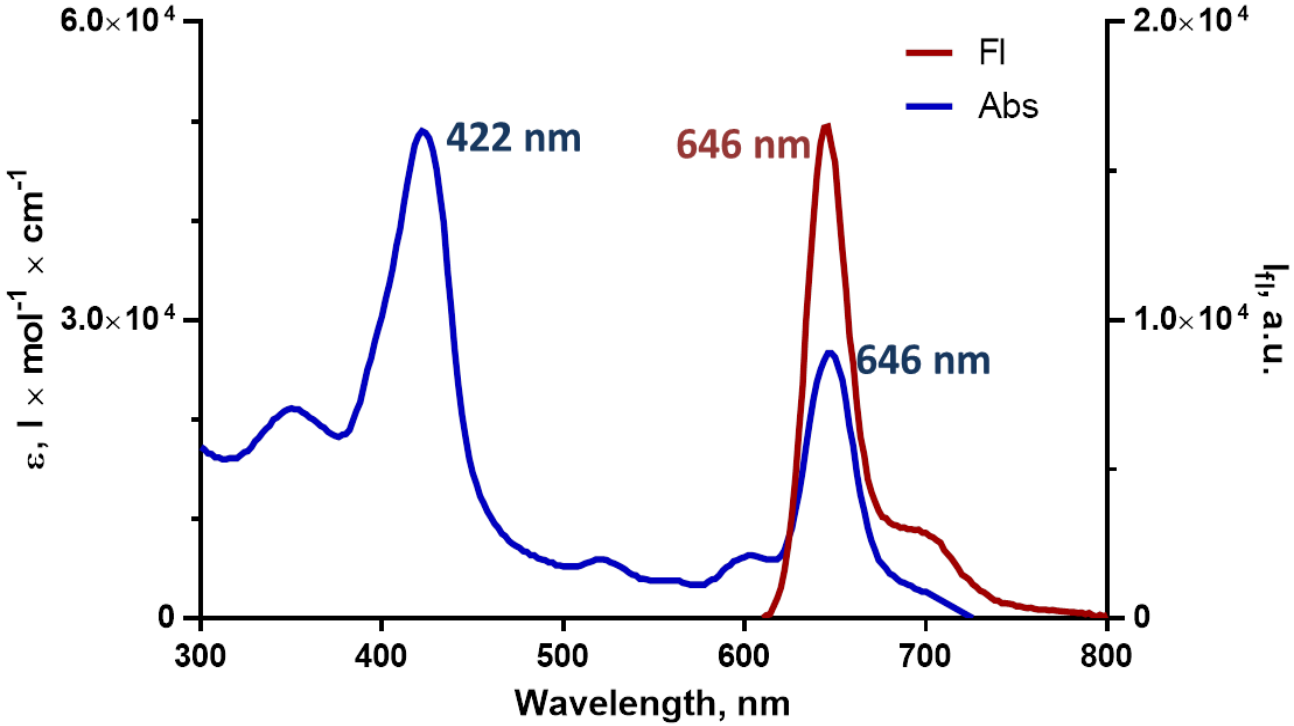
As an additional cytotoxic module, targeted agents with the directed action to molecules involved in the growth and progression of tumors are considering.



Results and discussion

Photophysical Properties

UV–visible (vis) absorption and fluorescence (λex = 410 nm) spectra of ZnChl-Vd in water (both at 5 μM)



Φ_F (%)	Φ_Δ(%)
1,2	21

Φ_F – the relative quantum yields (to rhodamine 6G) in water

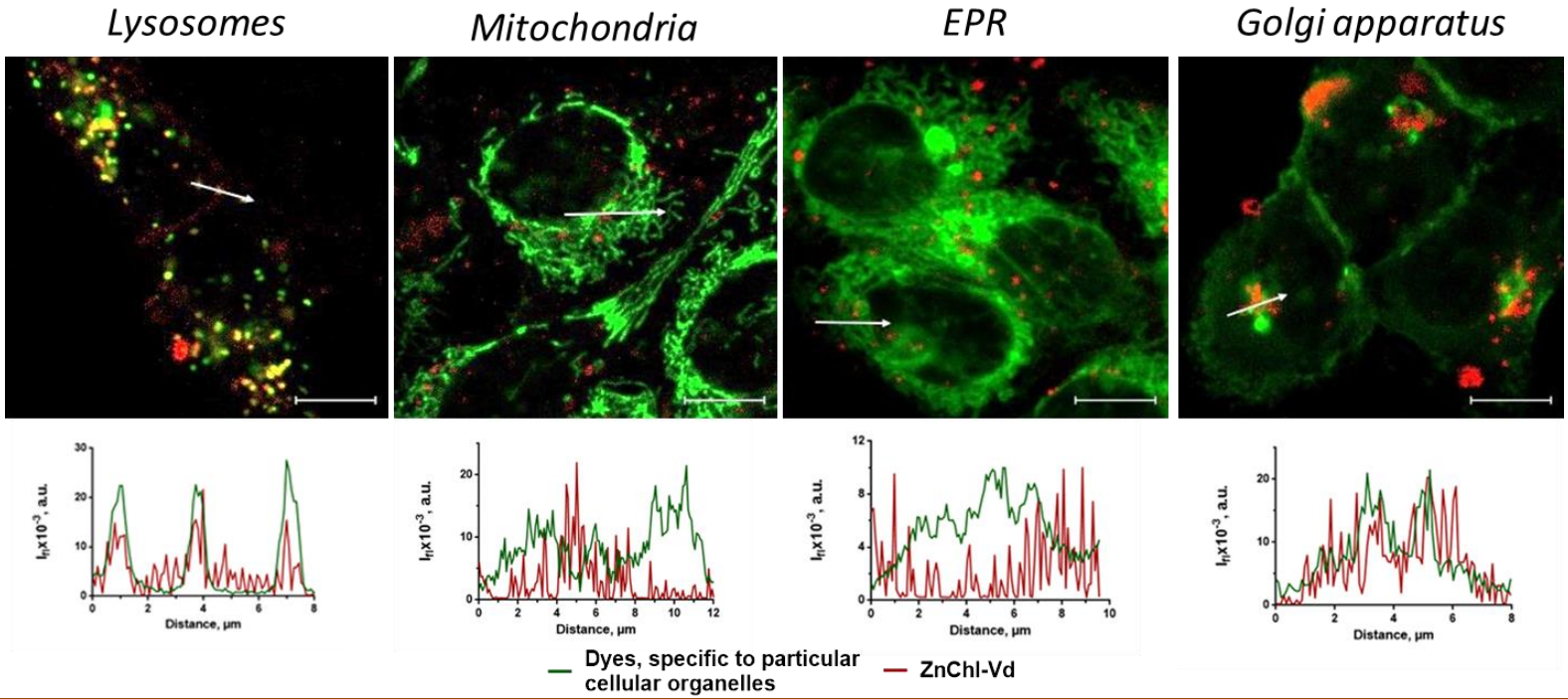
Φ_Δ – The singlet oxygen (SO) generation quantum yields were determined via chemical methods using SO sensitive traps such as 1,3-diphenylisobenzofuran (DPBF) for DMSO solution

ZnChl-Vd has intense absorption and fluorescence in the red region of the spectrum with relatively high singlet oxygen quantum yield, which provides the treatment and diagnosis of deeply localized tumors.

Results and discussion

Cellular Uptake Study

Analysis of intracellular localization of ZnChl-Vd (5 µM) in A431 cells after 4 h incubate with the compounds. The cells were stained with the dyes: LysoTracker Green for lysosomes and mitochondria; ER-Tracker for ER; and BODIPY FL C5-ceramide complexed with bovine serum albumin (BSA) for the Golgi apparatus.

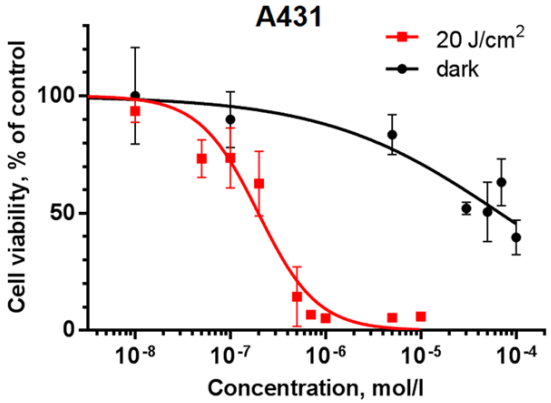


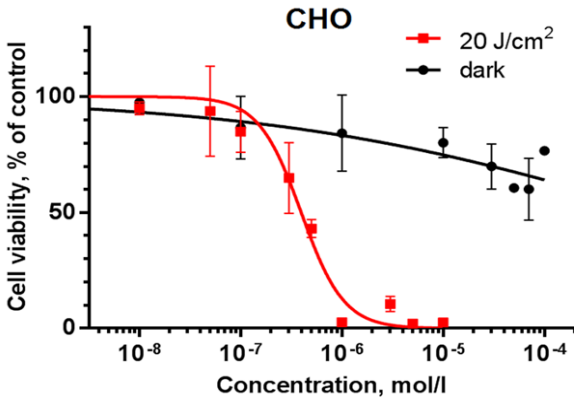
The co-localization analysis with dyes has shown that Zn-Chl-Vd in A431 cells are mainly localized in lysosomes and in the Golgi apparatus. That allows the active adenosine 5′-triphosphate (ATP)-dependent cellular uptake of conjugate to be assumed.

Results and discussion

Photodynamic Cytostatic Activity in Vitro

Cells were incubated with the compound for 4 h; then, the medium was exchanged with full fresh growth medium, and the cells were irradiated in dose 20 J/cm^2 (λ = 655−675 nm, power 32 mW/cm^2) or stayed in the dark. After the additional incubation for 24 h, cell viability was measured by the MTT assay and expressed as the percentage to untreated cells.





Relative viabilities of A431 and CHO cells treated with ZnChl-Vd in the dark or under light exposure.

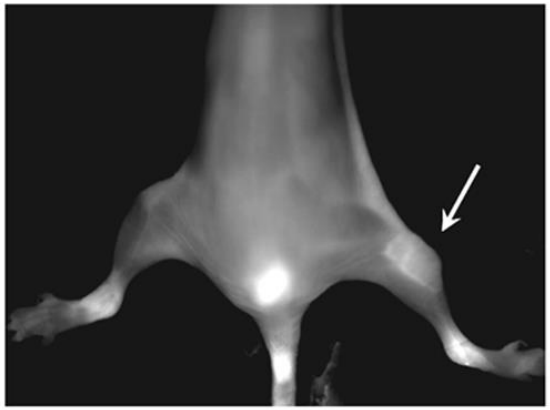
Cell line	IC_{50} *dark*, μM	IC_{50} *light*, μM
A431 ($EGFR^+$)	~70	0,19
CHO ($EGFR^-$)	>100	0,40

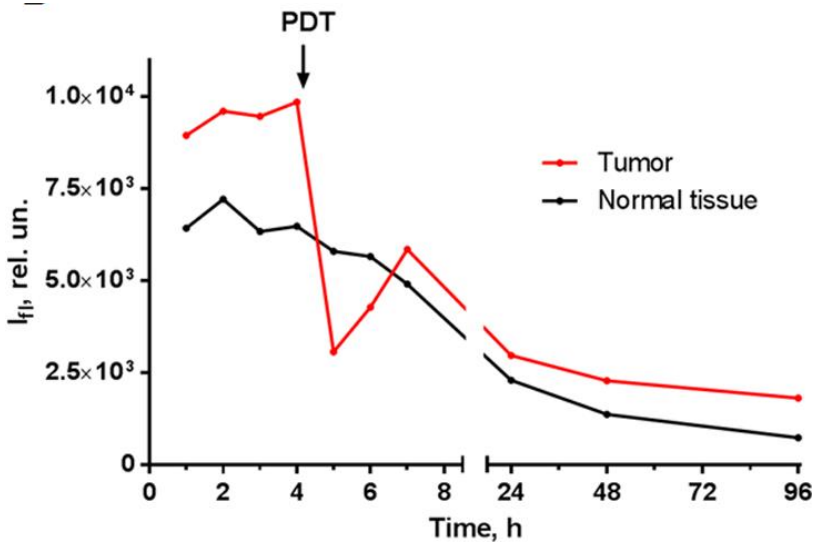
Zn-Chl-Vd is characterized by low dark toxicity for A431 cells and nontoxic for the EGFR-negative CHO line in dark conditions. It exhibited pronounced light toxicity toward EGFR-positive A431, IC50(dark)/IC50(light) ratios of ~368.

Results and discussion

In Vivo Antitumor Potency

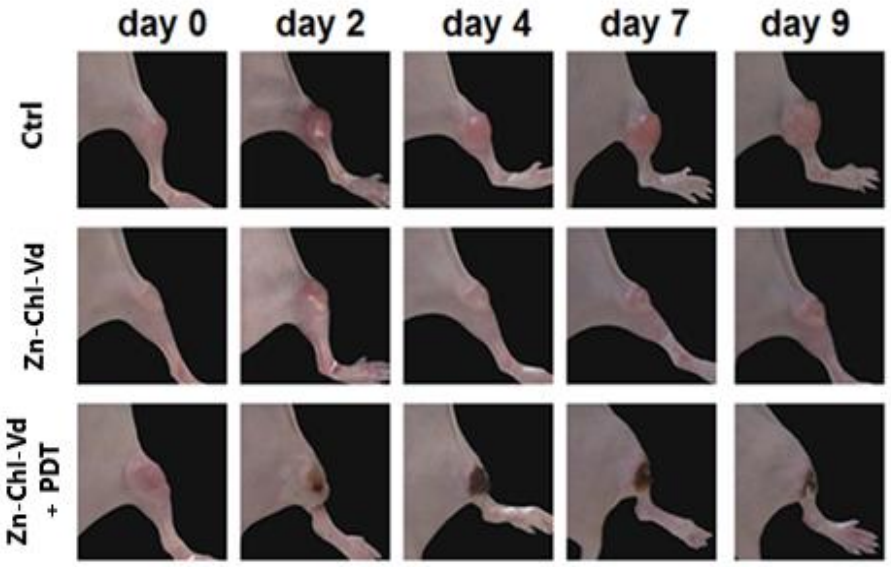
- the nude mice bearing A431 xenograft tumors
- Zn-Chl-Vd injection 15 mg/kg
- the tumor is indicated by an arrow. λex = 590 nm, λem = 600−700 nm
- photodynamic irradiation at a dose of 50 J/cm^2 (620−655 nm) was performed at 4 h after injection



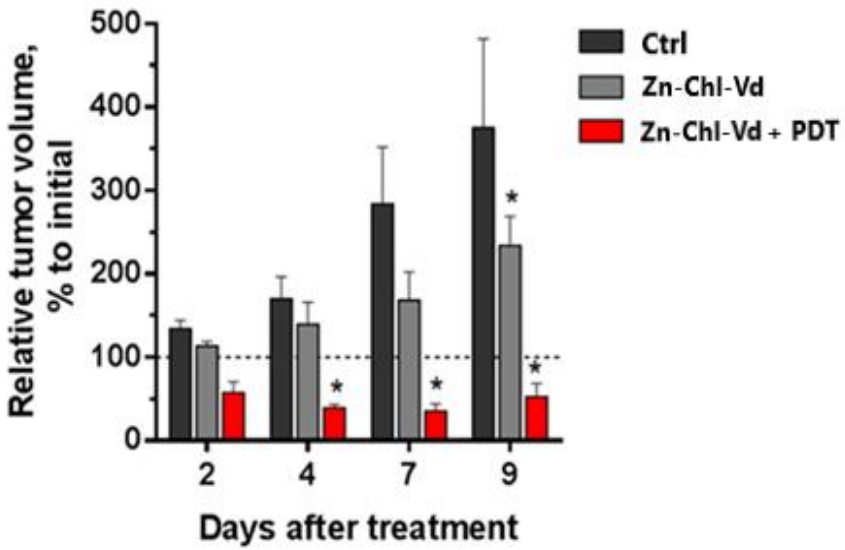


ZnChl-Vd demonstrated predominant tumor-specific accumulation. PDT was performed 4 h time point when conjugate showed peak tumor accumulation. It led to fluorescence decrease because of photobleaching, which indicates the occurrence of photodynamic reactions. A significant amount of Zn-Chl-Vd was observed both in tumor and normal tissues 24 h after injection with almost complete clearance after 4 days.

Results and discussion



In Vivo Antitumor Potency



Monitoring of tumor behavior proved high potency of performed PDT in the group of animals injected with ZnChl-Vd followed by light irradiation. The total tumor volume rapidly decreased and did not exceed ~15% of the tumor volume in the control animal group at 7–9 days after the photodynamic treatment. Zn-Chl-Vd demonstrated moderate therapeutic activity against A431 tumors also without light irradiation; the index of tumor growth inhibition was about 40%.

Conclusions

A novel water soluble conjugate ZnChl-Vd constructed on a base of PDT-active chlorin photosensitizer linked with a quinazoline EGFR/VEGFRaffine ligand demonstrated:

- intense absorption and fluorescence in the red region of the spectrum with a quantum yield of fluorescence of about 2% and a singlet oxygen quantum yield of about 20%;
- intensive accumulation in cells of human epidermoid carcinoma A-431 with localization in lysosomes and Golgi apparatus;
- demonstrated the ability to cause photoinduced cell death at a concentration of less than 1 μM when irradiated at a dose of 20 J/cm^2;
- selective accumulation in experimental tumors in *in vivo* experiments;
- the high antitumor activity during photodynamic therapy.

These results show that the EGFR-targeted quinazoline−chlorin conjugate (ZnChl-Vd) is a perspective multimodal agent for PDT.

Acknowledgments

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