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HYPERICIN – BASED PHOTODYNAMIC THERAPY: COMPARATIVE ANTITUMOR ACTIVITY AND UPTAKE STUDIES IN MURINE EHRLICH ASCITE CARCINOMA.

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Hypericin was found to exhibit the highest antitumoral activity in treating EAT by photodynamic therapy (PDT): Hypericin>HPde>PII>$TPPS_4$>ALA. Moreover, 25% of mice after Hyp-based PDT survived 4 months, if compare with control group. Antitumor activity of these photosensitizers was in rather clear correlation with accumulation potential.

Key words: photodynamic therapy (PDT), hypericin (Hyp), Ehrlich ascite carcinoma.

INTRODUCTION

Hypericin is photoactive natural pigment which mostly presents in *Hypericum perforatum* [1]. It has been convincingly shown, that hypericin has comparatively high singlet oxygen generation and a high fluorescence yield. Several lines of evidence indicate, that the compound binds strongly to plasma proteins [2]. It's important to note, that hypericin has never exhibit toxic or genotoxic effects *in vitro* or *in vivo* [3-4].

So far no reports have been published reflecting comparative antitumor efficiency of hypericin. So, the aim of this study was to compare the PDT effects of hypericin with the effects of other well-known photosensitizers.

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MATERIALS AND METHODS

Chemicals. Hematoporphyrin dimethyl ether, 5-amino-levulinic acid (ALA), Meso-tetra-(para- sulfophenyl) porphin ($TPPS_4$), Hypericin (Hyp) Photofrin II (PII) were used.

Tumors. The experiments were carried out using mice with Ehrlich ascite carcinoma.

Irradiation sources. The light source used for

irradiation consisted of tungsten lamp (500 W), optical system for light focusing and optical filter for UV and infrared light elimination (370 nm<λ<680 nm). Light intensity at the position of the cells was 50 mW/cm^2. The irradiation time - 90 s.

Measurements of intracellular concentration of photosensitizer. Tumor cells were suspended in phosphate-buffer solution (PBS) to an optical density OD=0,6 (3.7 mln/ml). The fluorescent spectra of the suspension were measured with a unique spectrofluorimeter СФР-1 (Moscow, Russia).

RESULTS

1. EAT growth delay after hypericin based photosensitization was used as one of the parameters to evaluate PDT efficiency. The used drug concentration in all cases was 40 mg/kg body weight, as optimal for ascite tumor. Incubation time was picked up 3 h. Hence, the results obtained are depicted in Fig. 1.

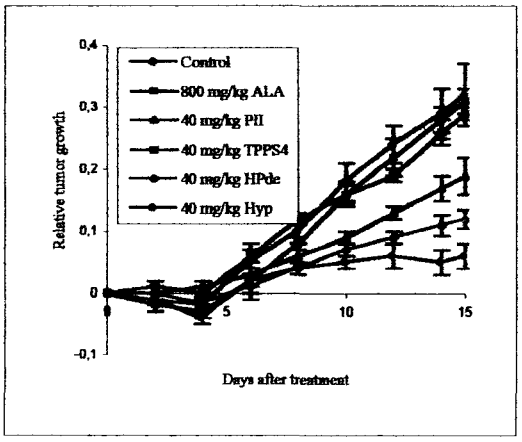

Fig. 1. Relative EAT growth after PDT with different photosensitizers

The data, however, obtained with different well-known photosensitizers, including PII, ALA, HPde, $TPPS_4$, suggested, that there is a great difference in the antitumoral efficiency of these drugs. For instance, ALA, being clinically established agent, is absolutely in effective in delaying the tumor growth. The similar results were obtained with $TPPS_4$, while PII and HPde established much more significant growth inhibition following 15 days after PDT treatment. Surprisingly, Hyp exerts the highest antitumor activity, if compare with all photosensitizers under investigation.

2. The broad spectrum of different antitumoral activities, which have been found in EAT cells, using ALA, PII, $TPPS_4$, HPde and Hyp, had prompted us to examine the accumulation potential of these drugs. Thus, data obtained, are presented in Fig.2.

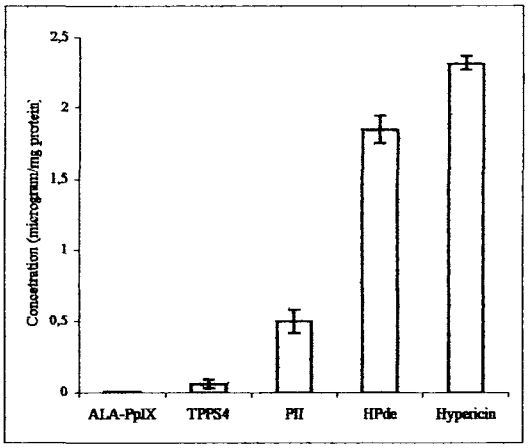

Fig. 2. The intracellular concentration of different photosensitizers in Ehrlich ascite tumor cells (40 mg/kg i.p. 3 hour incubation)

It's evident, that different first- and second generation photosensitizers exhibit significant differences in the relative concentration, normalized on protein amount in

EAT cells. The most interesting is, that such well-known photosensitizers as PII, PpIX (when ALA as precursor is used) or $TPPS_4$ exhibit very poor accumulation under these experimental conditions. On the contrary, HPde and most of all Hyp showed very high and notable accumulation potential in these cells.

3. In order to clear up hypericin's phototoxic potential, to ascertain it's antitumoral efficiency, and therapeutic outcome, survival of mice, treated with hypericin based PDT was observed. Data, presented in Fig.3.

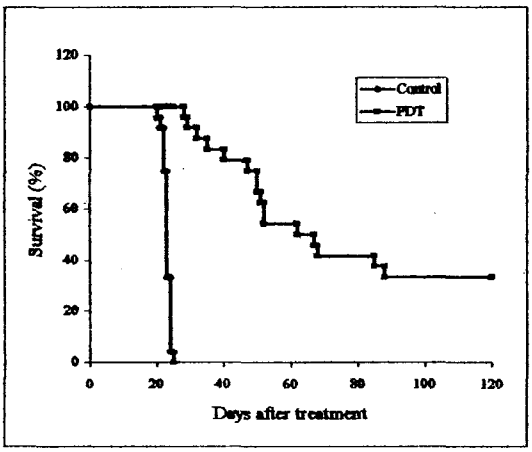

Fig. 3. Survival of mice, bearing EAT: ♦ - control group; ■ - PDT treated group (40 mg/kg hypericin, i.p. injected, 3 hours incubated).

In 25 % of survived mice no signs of EAT were observed - tumors were impalpable within the all life and no recurence was observed.

CONCLUSIONS

It is evident, that hypericin is potent and very effective photosensitizer in EAT model, when compared to other commonly used compounds ALA, PII, $TPPS_4$ and HPde, i.e. it might be very effective for treating of those tumors in which this molecule seems to easily accumulate.

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