Evaluation of Topical Photodynamic Therapy of Mammary Carcinoma with an Experimental Gel Containing Liposomal Hydroxyl-aluminium Phthalocyanine

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Abstract. Background: Photodynamic therapy (PDT) is a clinically-accepted approach for the therapy of many types of cancer. This study focused on the treatment of mammary carcinoma by topical administration of hydroxyl-aluminium phthalocyanine (AlOH-PC), compared to a clinically-approved photosensitizer (Metvix, Galderma & PhotoCure ASA, Inc., Oslo, Norway). Materials and Methods: MDA-MB 231 cells were subcutaneously injected into the right flank of athymic nude mice. Mice with grown tumours were used for in vivo efficacy studies. Different doses of liposomal AlOH-PC were applied to determine the most effective dose. In later studies, Metvix or our liposomal-AlOH-PC gel formula were used. Topical application of photosensitizers was followed by the PDT irradiation at 600-700 nm (635 nm peak). Tumour growth was measured three times weekly. Results: Therapeutic studies revealed that AlOH-PC treatment led to complete tumour remission in 90% (9/10) of experimental animals, whereas usage of the commercially available Metvix only postponed the tumour growth. Moreover, usage of liposomal AlOH-PC shortened the time allowed between the application of the photosensitizer and light exposure: for Metvix, hours are usually needed, while the tested liposomal AlOH-PC showed remarkable outcomes after only 10 min. Conclusion: Liposomal AlOH-PC gel appears to be potentially suitable for PDT of mammary carcinoma.

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Moreover, they demonstrate a high coefficient of singlet oxygen production, as well as a high extinction coefficient (over 100,000/M/cm) (14). Comparison of metal-free and metallated phthalocyanines revealed the better sensitizing properties of the metallated ones (14). Among those, a hydroxyl-aluminium phthalocyanine (AlOH-PC) (Figure 1B) represents a potential candidate for PDT because its synthesis is inexpensive and uncomplicated, while it also possesses a strong photodynamic activity (5).

In this study we focused on the treatment of mammarian carcinoma by the topical, liposomal formulation of AlOH-PC alone, and in comparison with the clinically-approved topical photosensitizer Metvix (8, 15).

Materials and Methods

Preparation of gel containing phthalocyanine liposomes. The detailed preparation of AlOH-PC-containing liposomes is a patented procedure (16). Briefly, sterile lecithin solution of concentration 1-40 mg/ml underwent microfluidization, using a semi-industrial microfluidizer M-110L (Micrifluidics, Inc., Newton, MA, USA), to produce particles smaller than 1 μm. Lyophilized AlOH-PC powder (Synthesis, Inc., Rybitvi, Czech Republic) was added to the suspension, and the mixture underwent further microfluidization in a smaller chamber leading to the formation of liposomes of size less than 500 nm. Such preparation leads to production of organic solvent-free liposomes. Final liposomes containing AlOH-PC were then mixed with translucent gel Gel 2 (magistral gel on the basis of carboxymethylcellulose, Pharmgest, Ltd., Pribram, Czech Republic) in 1:1 ratio.

Experimental cell line. A Caucasian human mammary adenocarcinoma cell line MDA-MB-231 was purchased from the European Collection of Cell Cultures (ECACC, Salisbury, UK – distributed by Sigma-Aldrich, Ltd.). Before application, cells had been cultivated in RPMI-1640 medium supplemented with 10% fetal bovine serum, 2% penicillin/streptomycin, 1.25% L-glutamine, and 1% sodium pyruvate.

Experimental animal models. The anticancer effect of both photosensitizers used in our experiment was tested in immunodeficient athymic nude CD-1 strain mice – Crl:CD1-Foxn1nu outbred homozygous nu/nu Mus musculus var. alba (obtained from AnLab, Ltd. and Charles River Laboratories International, Inc., Prague, Czech Republic). A total of 70 animals were 4-weeks old males and at the time of transplantation they weighed 18-22 g. The animals were kept in an air laminar flow box for small laboratory animals under aseptic conditions with radiation-sterilized bedding SAWI – Research Bedding (Jelu-Werk, Ltd., Rosenberg, Germany), were fed by irradiated Ssniff diet (Ssniff Spezialdiäten, Ltd., Soest, Germany) and had unlimited access to autoclaved water. All mice referred to here were treated in accordance with the Act on Experimental Work with Animals (Decrees No. 311/97; 117/87 and Act No. 246/96) of the Czech Republic, which is fully compatible with the corresponding European Union directives.

Experimental groups. Mice were divided into groups of 10 animals. 7 groups were as follows: 1 control (untreated) group without any compound and without irradiation and 6 experimental (treated) groups – 4 experimental groups treated with liposomal AlOH-PC gel, three with and one without irradiation; and 2 experimental groups treated with Metvix gel, one with and one without irradiation. Each of these three experimental groups treated with
AlOH-PC gel with irradiation, was a group with different AlOH-PC-concentration (2, 3, 4 mg/ml).

**Experiment – photodynamic therapy.** Harvested MDA-MB-231 (1×10⁶) cells were administered subcutaneously as a mixture with BD Matrigel™ (I.T.A.-Intertact, Ltd., Prague, Czech Republic) into the abdominal right flank of athymic nu/nu mice. When the tumours reached a size of about 6×6×6 mm (surface area of approx. 2 cm²), mice were randomly divided into control and experimental groups, as described above. At the start of PDT itself, all mice were subjected to thiopental narcosis: the simultaneous intramuscular application of ketamine (concentration of 100 mg/kg) and xylazine (concentration of 16 mg/kg), a total volume of 5 ml/kg was used. To determine the most appropriate dose of ALOH-PC in the liposomal gel, three different concentrations, 2, 3, and 4 mg/ml, of ALOH-PC were tested. Each gel was applied topically to the tumour (0.2 ml per tumour) and after 10 min (according to (5)) was irradiated by a xenon lamp (ONL 051; Preciosa Crytur, Trutnov, Czech Republic). In comparison to the commercial Metvix, ALOH-PC gel with a concentration of 4 mg/ml (ALOH-PC) was tested. As indicated by the manufacturer, Metvix contained 160 mg of methyl-aminolevulinate (as hydrochloride) per 1 g of this product. Metvix cream was also topically applied to the tumour (0.4 mg cream per tumour) but irradiated by the same xenon lamp after 3 h after application (as recommended by the European SMPC). In all experimental cases, the contingent irradiation lasted exactly 7 min and tumours were irradiated at 0.97 W with a total energy of 100 J/cm² from the distance of 1 cm.

Tumour growth was recorded twice weekly for 30 days. Tumour volumes and visible surface necrotic areas were measured as follows: tumour volume $V = \frac{l \times w^2}{2}$ and necrotic area $S = l \times w$, where $l$ was the length and $w$ was the width of this area. All mice were observed regularly because of the risk of tumour regrowth and were sacrificed 30 days after PDT. The macroscopic tumour disappearance was histologically supported on the 31st day after PDT.

**Statistical analysis of data.** Evaluation of the results was performed by comparing experimental and control tumour growth curves in the fixed time intervals set up before the respective trial (day 1, 4, 7, 11, 14, 18, 21, 25, 28 and 30). At the endpoint of the experiment, on day 30, the tumour growth inhibition (TGI%) was calculated by the formula: $1 - \left( \frac{\text{average tumour volume in experimental group}}{\text{average tumour volume in control group}} \right) \times 100$. The results were statistically evaluated by the unpaired two-sided Student’s $t$-test.

**Results**

In vivo determination of the most appropriate dose of phthalocyanine liposomes in the gel for further experiments. We tested three different ALOH-PC gel preparations to determine the most effective concentration of photosesitizer for treatment of xenotransplanted mammary carcinoma (MDA-MB-231 cell line). Gels with liposomal ALOH-PC concentrations of 2 and 3 mg/ml, both effectively reduced the tumour growth rate and the effect of such treatment over the untreated control group was significant (Figure 2). At the endpoint of the experiment (day 30), the tumor size in the
animals treated with 2 and 3 mg/ml liposomal AlOH-PC was half the size of the untreated control tumours.

Moreover treatment with the liposomal AlOH-PC gel at 4 mg/ml led to shrinkage of tumours from the very beginning, after the photodynamic therapy (Figure 2). Within a 7-day period, tumour size decreased to 1/10 of the size before the treatment. Without any other treatment, no additional significant growth was documented. At day 30 of the experiment, tumour in AlOH-PC-treated animals was almost 20-times smaller than in untreated control animals.

Comparison of phthalocyanine liposomal gel and Metvix in vivo. We also tested the possible effect of photosensitizers themselves (without irradiation) on the growth of
experimental mammary carcinoma in mice. These results are representative of four independent experiments (Figure 3). As can be seen in Figure 3B-D, tumour cell number growth in all three experimental groups performed very similar growth rates, which documents that without irradiation the photosensitizers have no effect on tumour growth.

On the other hand, first signs of tumour necrosis were found in both experimental groups with subsequent irradiation 24-48 h after irradiation. In successful cases, the final histological result of PDT was a scar covered by neoepithelial tissue. Treatment with Metvix delayed tumour growth (Figure 3E); growth was diminished for two weeks, but after that time, rapid growth was documented in all tested animals within the Metvix-treated group. At the endpoint of the experiment, tumour size was slightly smaller than in both experimental groups without irradiation or in untreated control group.

As shown in Figure 3E, liposomal AIOH-PC gel showed a superior efficacy in the treatment of experimental mammary carcinoma (MDA-MB-231 cell line). We documented a complete remission in 9/10 of the tested mice. Recurrent slow tumour growth was documented only in one tested mouse from day 11. The rest of the tumours in the remaining mice did not grow for the rest of the experiment (30 days). Tumour growth inhibition (TGI%) for mice treated with AIOH-PC liposomal gel with subsequent irradiation was 98.40%, whereas only 11.63% for mice treated with Metvix with subsequent irradiation. The obtained results were statistically significant at the 1% level of significance (α=0.01).

Discussion

Topical administration of photosensitizers adds several benefits over traditional systemic administration, such as reduced systemic toxicity, avoiding first-pass metabolism in the liver, and minimization of photosensitivity induction. Moreover, previous studies by our group have revealed the unsurpassed drug-to-light time interval of 10 min for liposomal AIOH-PC (5). As this is much shorter than that of many commercially available photosensitizers [e.g. Metvix drug-to-light interval is typically 4-6 h (5)], this feature should be useful in wider clinical usage. Patients would not have to wait around for hours between gel application and irradiation, and would also reduce cost for hospitals, insurance companies, and ultimately the public treasuries.

There is a significant potential for PDT to be utilized for new indications other than those currently available (17, 18). Since modern endoscopy techniques combined with fiberoptic systems are able to deliver light to almost any part of the body, the PDT option is no longer limited to the area of superficial tumours. As a brief example of applications, Roche and colleagues (19) constructed a suitable light delivery system for PDT on gastrointestinal cancer. Such uses are also supported by the development of new-generation photosensitizers that are activated after irradiation by light of longer wavelengths (e.g. porphyrins ~630 nm; texafrins ~734 nm; bacteriochlorins ~740 nm). It is well-known that the light of longer wavelength penetrates tissue more easily than light of shorter wavelengths. Biological responses can therefore be documented at two to three times greater tissue depth. These facts open up the possibility for topical administration of photosensitizers for indications other than skin cancer.

In this pilot study, the efficacy of topical application of a gel containing liposomal AIOH-PC on xenotransplanted mammary carcinoma was examined. The documented proof-of-principle results for liposomal AIOH-PC, especially in contrast to those of Metvix treatment, highlights the need for further studies of this compound. Interactions between the tumour microenvironment and the drug will need to be intensively studied, employing fluorescently-labelled tumour cells (20, 21), and more physiological conditions should be studied using, for example, an orthotopic model described by Hoffman (22).

Conclusion

Microfluidization leads to production of vesicles containing AIOH-PC predominantly with a size distribution of 180-480 nm (mean size=200-300 nm). Liposomal gel seems to be potentially suitable for PDT of mammary carcinoma. In comparison with Metvix, there was no need for a long interval between gel application and irradiation (only 10 min). Moreover, the most effective dose of 4 mg/ml showed a good therapeutic effect against mammary carcinoma, with 9 out of 10 mice cured.

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References


