RESEARCH ARTICLE

Efficacy of Multiple Low-dose Photodynamic TMPYP4 Therapy on Cervical Cancer Tumour Growth in Nude Mice

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Abstract

Objective: Photodynamic therapy (PDT) is an emerging therapeutic procedure suitable for the treatment of cervical cancer. However, the side effects of PDT are severe, including skin ulceration, so we designed an experiment to examine the effects of multiple low- dose photodynamic therapy of 5, 10, 15, 20-tetrakis(1-methylpyridinium-4-yl) porphyrin (Tmpyp4) on tumour growth by utilizing a model in nude mice implanted with Hela cervical cancer cells. Materials and Methods: Female BALB/c nude mice (aged 5–6 weeks, weighing 18–20 g) were used. Hela cervical cancer cells were injected subcutaneously (1×10^7 cells/200 μL). Ten days after injection, the mice were divided into three groups (n=6), the A group of controls without any treatment, the B group receiving a single-treatment with Tmpyp4 (10 mg/kg, intratumor injection) and irradiation (blue laser, 108 J/cm^2), and the C group given three-treatments with Tmpyp4 (10 mg/ kg, intratumor injection) and irradiation at intervals of two days. After starting treatment, tumours were measured every two days, to assess growth. At 2 weeks after the last treatment of C group, tumour tissue and organs were collected from each mouse to evaluate tumor histology and organ damage. Results: Tumour growth in C group was significantly inhibited compared with A and B groups (P <0.05), without any injury to the skin and internal organs. Conclusion: Our novel findings demonstrated that multiple low-dose photodynamic therapy of Tmpyp4 could inhibit cervical cancer growth significantly with no apparent side effects.

Keywords: Photodynamic therapy - Tmpyp4 - cervical cancer - nude mice

Asian Pac J Cancer Prev, **14 (9)**, 5371-5374

Introduction

In recent years, although the widespread screening of cervical cancer, especially the systemic use of the Papanicolaou (Pap) smear (Cronje, 2005), cervical cancer remains the second most common cause of death from cancer in women worldwide (Sreejata et al., 2012), China accounts for 29% of the 51,000,000 new cases of cervical cancer each year (Kim et al., 2009). But due to cervical cancer screening, cervical cancer can be detected early and is younger trend, expecially early cervical cancer and cervical intraepithelial neoplasia (CIN), some young patients with no children had a strong desire for fertility. So in addition to traditional treatments including operation, chemotherapy and radiotherapy, photodynamic therapy may become an alternative treatment.

Photodynamic therapy (PDT) is an emerging tumor treatment approach in recent years. Since 20 years ago, Clinical study of Dougherty on photodynamic therapy of treating malignant tumor was successful for the first time (Thomas et al., 1978), which caused a lot of clinical departments concerned. At present, photodynamic therapy has been widely used in the Department of dermatology, respiration, gastroenterology, and gynaecology and et al (O'Connor et al., 2009). Photodynamic therapy (PDT) consists of an interaction between a photosensitizer and absorbed light (Ewelina et al., 2012).

The mechanism of killing tumor cell by PDT depended on the photosensitizer concentration and the light fluence (Mathews et al., 2009). But the side effects of PDT were severe, even beyond 6 months out, they still had skin ulceration, seriously required multiple skin grafts (Gary et al., 2012). We have used 5, 10, 15, 20-tetrakis (1-methylpyridinium-4-yl)porphyrin (Tmpyp4, 20 mg/ kg) for peritumoral injection of cervical cancer xenografts in nude mice, found that the high dose photosensitizer can cause skin ulceration, and longer duration (four weeks) ,even without laser irradiation or illumination (Figure 1). In order to reduce the side effects of PDT, we designed low-dose PDT schemes to observe the efficacy of photodynamic therapy of Tmpyp4 for cervical cancer xenografts in nude mice.

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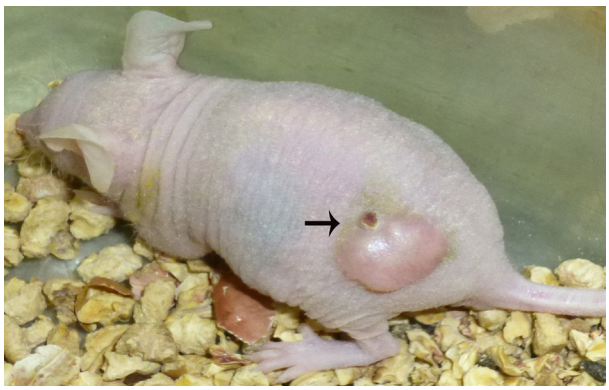

Figure 1. Skin Ulcer Occurred after use of High Doses of Tmpyp4 in Peritumoral Injection

Materials and Methods

HeLa human cervical cancer cell line was obtained from Gynecologic Cancer laboratory of Qilu Hospital of Shandong University (Jinan, China). The cell lines were cultured in Dubelcco's modified Eagles's medium (DMEM) supplemented with 10% fetal calf serum (FCS) (Hyclone, USA), penicillin (100 units/ml) and streptomycin (100 units/ml) (Sigma-Aldrich st. Louis, MO, USA) in a humidified atmosphere containing 5%CO_2 at 37°C.

Photosensitizer Tmpyp4 was purchased from Merck Drugs & Biotechnology (Germany). Tmpyp4 were dissolved in 0.9% sodium chloride solution, its concentration was 1mg/ml and was kept in 4°C refrigerator.

The PDT was carried out using Osram laser generator apparatus LD435 (Shandong Teacher's University Bio-Pharmaceutical, Jinan, China). The wavelength was set at 435 nm .

Female Balb/C nu/nu mice (5-6 weeks of age, weighing 18-20 g) used in this experiment were obtained from Slack laboratory animal (Shanghai, China). The mice were housed in a specific pathogen- and germ-free environment where the temperature was maintained at 25°C ± 3°C with 60%-70% air humidity and a 12-hour light-dark cycle.

Nude mice were subcutaneous injection (s.c.) inoculated with 1×10^7 of HeLa cells, suspended in phosphate-buffered saline (PBS), in the back of left rear legs. After inoculation, tumors were observed, on day 10 when tumors reached 5-6 mm in average diameter in two perpendicular diameters using a caliper, tumor-bearing mice were randomly allocated into 3 groups (n = 6). A group was the control group without any treatment, B group was the single-treatment group with Tmpyp4 (10mg/kg, intratumor injection) and irradiation, C group was the three-treatment group with Tmpyp4 (10 mg/kg, intratumor injection)and irradiation at an interval of two days (on day 10 and day 13 and day 16), that was B group of mice were treated only once,but C group of mice were treated three times. Six hours after intratumor injection, mice in group B and C were irradiated with blue laser (wavelength 435nm, output power 180mw, spot diameter 1.5 cm, irradiation distance 2 cm) for 15 minutes (energy density 108 J/cm^2). All the mice were anesthesia with

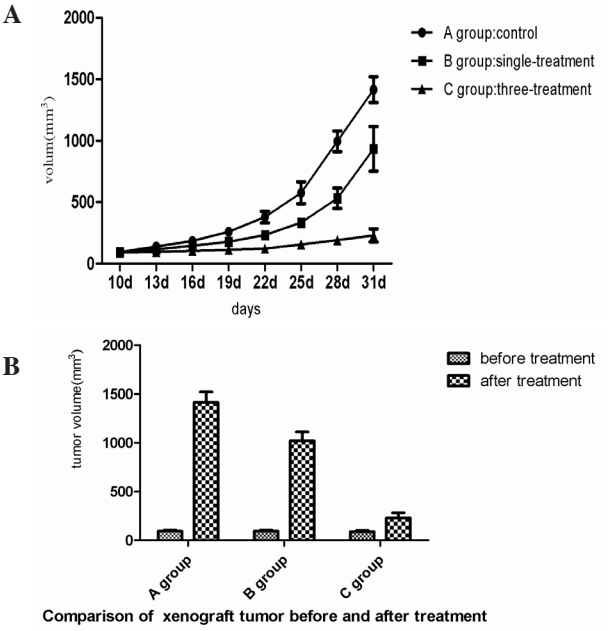

Figure 2. A: Tumor Growth Curve Diagram. B: Comparion of Tumor Volume of the Nude Mice Before and after Treatment

5% Chloral hydrate (10 ml/kg) intraperitoneal injection during laser irradiation. All operations were performed in the dark conditions.

After starting treatment, tumors were measured every two days, Tumor volume was calculated using the volume formula: volume (mm^3) = $\pi/6 \times$ (large diameter × [short diameter]^2) (Luis-Alberto et al., 2008), to draw the tumor growth-curve of tumor volume according time in every groups. At the same time, we monitored the weight and observed the life of mice.

When 2 weeks after the lasted treatment of C group, all the mice were killed and the tumor masses and internal organs (include the heart, lung, liver, spleen and kidney) were excised carefully and were then fixed with 4% paraformaldehyde for further evaluation of histopathological changes and organ damages. paraffin-embedded tissue samples were cut into 4μm sections (Leica histotome Slicer, Germany). The sliced tissue was fixed onto a slide and stained with hematoxylin and eosin.

Statistical analysis

All results were expressed as the mean ± standard deviation of mean. Statistical analyses were performed by one-way analysis of variance using SPSS version 17.0. The differences between means was considered statistically significant when the probability level (*P*-value) was less than 0.05 ($P<0.05$).

Results

Determination of tumor volume

The C group that received three-PDT treatment with Tmpyp4 were compared to the B group that received single-treatment and also to control group A, we drawed the tumor growth-curve of tumor volume according time in every groups (Figure 2 A), and we compared the tumor volume of mice the beginning and end of treatment

Figure 3. Observation on Side-effects and Tumor Inhibition of Three Groups

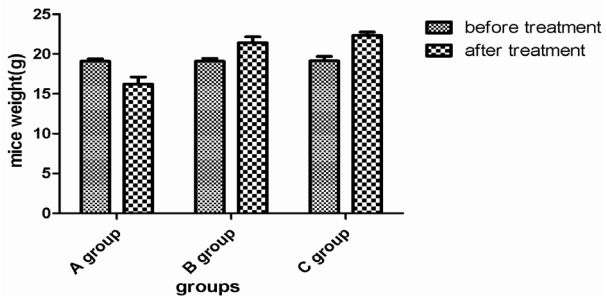

Figure 4. Comparision of Body Weights in Three Groups Before and after Treatment

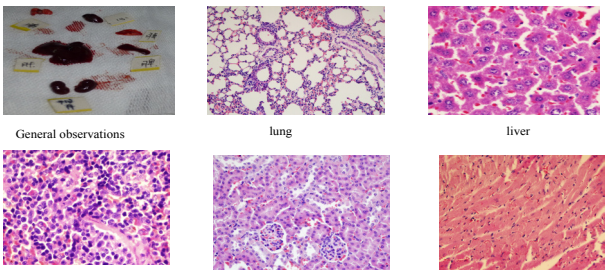

Figure 5. The General and Microscopic Observation on Viseral Organs of C Group

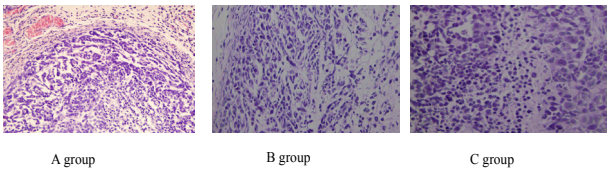

Figure 6. HE Staining of Tumour Tissue of the Three-treatment Group

(Figure 2 B). The tumor volume of each group had no significant difference before treatment (P>0.05), the tumor volume of A group was 93.45±12.0 (mm^3), that of B group was 96.44±10.06 (mm^3), that of C group was 89.89±11.96 (mm^3). At the end of treatment, the tumor volume of C group (229.84±53.30 mm^3) was significantly smaller than A (1415.98±105.47 mm^3) and B groups (1023.08±87.22 mm^3), P values were less than 0.05. The tumor growth was inhibited obviously in three-treatment group of PDT. But the tumor of group B continued to grow after short-term inhibition.

Observation on side-effects of multiple low-dose photodynamic therapy of Tmpyp4

There was no significant difference in body weight of mice among three groups before treatment (p>0.05). At the end of the experiment, body weight of A group decreased, there was a significant difference between before and after treatment (p<0.05). After treatment than before the mice weight of B group and C group increased, and there was a statistically significant difference (p<0.05) (Figure 4). So we concluded that weight of B and C group had not weight loss ,on the countrary weight increased, however nomal control group of mice appeared thin and hypoergia with tumor volume increased (Figure 3 A group). The mice of B and C group were increased in weight, living in good conditions (Figure 3 B group and C group). The mice skin of B and C group had no apparent damage, even in cases that were not protected from light (After the laser treatment ,the mice had not been protected from light). The internal organs of three-treatment group showed no pathological damage (Figure 5). HE staining section of tumor tissue in the three-treatment group showed cell size smaller, dense cytoplasm,eosinophilic dyeing enhanced and the formation of apoptotic bodies,which tipped cell apoptosis increased (Figure 6).

Discussion

Photodynamic therapy (PDT) is painless, minimally invasive, repeatable and can be completed in the clinic, it can also be combined with surgical operation, radiotherapy or chemotherapy application. So PDT is one of the most promising and non-invasive methods for treating malignant or premalignant tissue. PDT device is simple, composed of photosensitizer and laser light and easy to carry, moreover it's treatment price is cheaper than some chemotherapy ,so it may be accepted by patient. Principles of PDT treatment of disease is that the specific wavelength of non-ionizing light triggers oxidative photodamage and subsequent death of the targeted cells (Shirasu et al., 2013). Some research suggested that the extent of photocytotoxicity after PDT is dependent on the photosensitizer type, the medication dose, the type of tumor, the light fluence rate and the total light exposure dose (Dolmans et al., 2003; Xu and Leung et al., 2006). other research suggested that different adminstration of a photosensitizer resulted in different effects (Shirasu et al., 2013) .

Some photosensitizer excretion in the skin is slow, retention time is long, so photosensitizer is easy to produce skin toxicity. During treatment, avoid light time is longer, and intravenous administration of the photosensitizer can reach the body, affect the body metabolism, so sometimes the photodynamic therapy were undesirable adverse effects. Meyer-Betz performed the first human PDT experiment using hematoporphyrin on himself, photosensitivity, the edema and hy-perpigmentation persisted for 2 months after the injection of hematoporphyrin and exposed himself to sunlight (Meyer-Betz, 1913).

5, 10, 15, 20-tetrakis (1-methylpyridinium-4-yl) porphyrin (TMPyP4) that was used in the study is one of the porphyrin derivatives, and is a quadruplex ligand ,which could bind to and stabilize the G-quadruplex formation in human telomere, to inhibit the telomerase function, and to cause cellular senescence (De Cian et al., 2008). To date, telomerase activity has been detected in some normal cells, including peripheral blood, cord blood and bone marrow lymphocytes, the basal layer of the skin (Harle-Bachor and Boukamp, 1996; Hsiao et al., 1997; Yui et al., 1998). So the porphyrin class of

telomere and telomerase interactive agents may affect normal cells (Rha et al., 2000). Although the telomerase activity in those normal tissues is weaker than tumor tissues, telomerase-targeting agents could induce side effects in those normal tissues. Moreover ,Tmpyp4 is an photosensitizer and can be excited by optima blue light to generate singlet oxygen in cancer lesions to rapidly react with any nearby biomolecules and to promote cell death. So the photosensitizer lack sufficient tumor selectivity and are taken up in the neighboring normal tissues, resulting in some adverse effects. For the above reasons, we selected the topical administration of the photosensitizer but not systemic administration. We have used the high dose of tmpyp4 on tumor xenografts in nude mice in peritumor injection, found that drug gathered one side of the tumor and skin ulcer appeared on drug accumulation without light irradiation, therefore we use the intratumor injection method to study the effect of multiple low-dose PDT of tmpyp4 on cervical cancer in nude mice.

Our experiment found that PDT of tmpyp4 did not completely eliminate tumours by the way of intratumor injection, but it could inhibite the tumor growth. The tumor volume of single-dose group continued to grow rapidly in the short-term inhibition, there were certain antitumor activity compared with the normal control group, but with the prolongation of time, the growth trend to accelerate, long-term effects were poor. However tumor growth inhibition of the three-treatment group was obvious and continued for long time, long-term effects were good, and the side effects of the three-treatment group were not be found. Yoo JO et al. reported that low-dose PDT triggers apoptotic cell death, whereas high-dose PDT predominantly causes necrotic cell death (Yoo et al., 2011). Our study also found multiple low-dose PDT treatment promotes the apoptosis of tumor cells. The study provided the theoretical basis for clinical safe and effective application of Tmpyp4 to treat cervical cancer.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (No. 81072122).

References

Castro-Morales (2008). Use of an orthovoltage X-ray treatment unit as a radiation research system in a small-animal cancer model. J Exp Clin Cancer Res, 27, 57.

Cronje HS (2005). Screening for cervical cancer in the developing world. Best Pract Res Clin Obstet Gynaecol, 19, 517-29.

De Cian A, Lacroix L, Douarre C, et al (2008). Targeting telomeres and telomerase. Biochimie, 90, 131-55.

Dolmans DE, Fukumura D, Jain RK (2003). Photodynamic therapy for cancer. Nat Rev Cancer, 3, 380-7.

Dougherty TJ, Kaufman JE, Goldfarb A, et al (1978). Photoradiation Therapy for the Treatment of Malignant Tumors. Cancer Res, 38, 2628-35.

Harle-Bachor C, Boukamp P (1996). Telomerase activity in the regenerative basal layer of the epidermis in human skin and in immortal and carcinoma-derived skin keratinocytes. Proc Natl Acad Sci USA, 93, 6476-81.

Hsiao R, Sharma HW, Ramakrishnan S, Keith E, Narayanan R (1997). Telomerase activity in normal human endothelial cells. Anticancer Res, 17, 827-32.

Kim K, Zang R, Choi SC, Ryu SY, Kim JW (2009). Current status of gynecological cancer in China. J Gynecol Oncol, 20, 72-6.

Mathews MS, Angell-Petersen E, Sanchez R, et al (2009). The effects of ultra low fluence rate single and repetitive photodynamic therapy on glioma spheroids. Lasers Surg Med, 41, 578-84.

Meyer-Betz F (1913). Untersuchung uber die biologische (photodynamische) Wirkung des Hamatoporphyrins und anderer Derivate des Blut- und Gallenfarbstoffs (in German). Dtsch Arch Klin Med, 112, 476-503.

O'Connor AE, Gallagher WM, Byrne AT (2009). Porphyrin and nonporphyrin photosensitizers in oncology: preclinical and clinical advances in photodynamic therapy. Photochem Photobiol, 85, 1053-74.

Raychaudhuri S, Mandal S (2012). Current status of knowledge, attitude and practice (KAP) and screening for cervical cancer in countries at different levels of development. Asian Pac J Cancer Prev, 13, 4221-7.

Rha SY, Izbicka E, Lawrence R, et al (2000). Effect of telomere and telomerase interactive agents on human tumor and normal cell lines. Clin Cancer Res, 6, 987-93.

Rogers GS (2012). Continuous low-irradiance photodynamic therapy: a new therapeutic paradigm. J Natl Compr Canc Netw, 10, S14-7.

Shirasu N, Nam SO, Kuroki M (2013). Tumor-targeted Photodynamic Therapy. Anticancer Res, 33, 2823-31.

Szliszka E, Czuba ZP, Kawczyk-Krupka A, et al (2012). Chlorin-based photodynamic therapy enhances the effect of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) in bladder cancer cells. Med Sci Monit, 18, BR47-53.

Xu CS, Leung AW (2006). Photodynamic effects of pyropheophorbide-a methyl ester in nasopharyngeal carcinoma cells. Med Sci Monit, 12, BR257-62.

Yoo JO, Lim YC, Kim YM, Ha KS (2011). Differential cytotoxic responses to low- and high-dose photodynamic therapy in human gastric and bladder cancer cells. J Cell Biochem, 112, 3061-71.

Yui J, Chiu CP, Lansdorp P (1998). Telomerase activity in candidate stem cells from fetal liver and adult bone marrow. Blood, 91, 3255-62.