Effect of Photodynamic Therapy in Melanoma Skin Cancer Cell Line A375: in vivo Study

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Background and Objectives

Aminolevulinic acid (ALA), a precursor of heme, which is converted to protoporphyrin IX, is used as an effective photosensitizer. The aim of this study is to evaluate the effect of photodynamic therapy (PDT) using 5-ALA in an in vivo experiment using melanoma skin cancer cell line A375.

Materials and Methods

Female nude mice (BALB/C/nu/nu), four weeks of age and 25-30 g (Narabio, Korea) in weight, were used in the study. When the actual tumor size reached more than 100-200 mm³, the mice were divided randomly into the PDT group (n = 7), LED (light emitting diode) group (n = 3), ALA group (n = 3), and control group (n = 7). ALA 375 mg/kg was used in the study as an intraperitoneally injected photosensitizer. The tumors were then irradiated using a 630 nm LED for 30 min after 3 hours in a dark room. Tumor dimensions were measured using a caliper at 0, 7, 14, 21, and 28 days after treatment in all groups and tumor volume was calculated.

Results

There was no significant difference in the mean tumor volumes on 0 day before treatment in each group (p>0.05). However, significant differences in the mean tumor volumes were observed between the PDT and control groups at 7, 14, 21, and 28 days after treatment (p<0.05).

Conclusion

ALA mediated PDT was found to be effective in melanoma skin cancer cell A375 induced tumor xenograft in nude mice.

Key words

Skin cancer; Melanoma; Photodynamic therapy; Aminolevulinic acid

INTRODUCTION

Photodynamic therapy (PDT) is a method used to treat cancer by irradiating laser with suitable wavelength after injection of a photosensitizer. Light activation of most photosensitizers leads to the formation of singlet oxygen and free radicals which induce necrosis or apoptosis of cancer cells.¹ And it induces destruction of tumor-associated vasculature and antitumor immunity.² PDT has localized effect and therefore can kill malignant cells without causing any damage to normal healthy cells. PDT is promising new modality to treatment cancer and less harmful side-effects than conventional chemotherapy, radiotherapy or surgery.³ 5-aminolevulinic acid (5-ALA) is converted to protoporphyrin IX (PpIX) used as effective photosensitizer in the biosynthesis of heme. The cells preloaded with PpIX are promptly killed when exposed to light.⁴ Rapidly proliferating cells such as tumor cells can produce a significant amount of PpIX from ALA,⁵ and a higher accumulation in tumor cells than in normal cells.

The purpose of the present study is to evaluate the effect of photodynamic therapy (PDT) using 5-ALA in melanoma skin cancer cell line A375 in vivo experiment.

MATERIALS AND METHODS

Photosensitizer and light irradiation

5-ALA (5-aminolevulinic acid hydrochloride; Sigma, Saint Louis, MO, USA) was diluted to 25 mg/mL with distilled water just before use. The light source used was 22 × 31 lamps 630 nm LED (light emitting diode) array (WON Technology Co., Ltd., Korea).

Cell culture

Human melanoma skin cancer cell lines A375 were used and were grown in RPMI 1,640 with 10% fetal bovine serum and 1% penicillin and streptomycin (Hyclone, Thermo Scientific, South Logan, UT, USA) at 37°C in a humidified atmosphere of 5% CO₂ in air.

Animals and tumor model

Female nude mice (BALB/C/nu/nu), 4 weeks of age and 25–30 g (Narabio, Korea) in weight, were used in the study. A375 cells (1×10^6) resuspended in 10 μ L of serum-free RPMI medium were injected using a Hamilton syringe attached to a 30-gauge standard needle in the back of mice. When the actual tumor size reached more than 100–200 mm³, the mice were divided randomly into a PDT group (n = 7), LED group (n = 3), ALA group (n = 3) and control group (n = 7). Tumors were treated as follows:

PDT group exposed to PDT, LED group receiving LED only, ALA group receiving ALA alone and control group not receiving any treatment.

Protocol of PDT

ALA 375 mg/kg was used in the study as a intraperitoneal injected photosensitizer. And the tumors were then irradiated with a 630 nm LED (400 mW/cm²) during 30 min after 3 hours in a dark room. During the PDT treatment, outer surface temperature of mice tumor was recorded in each 5 mins time interval. Tumor dimensions were measured by caliper 0, 7, 14, 21 and 28 days after treatment in all groups and tumor volume was calculated. The actual tumor size at PDT start day (0 day) was determined by a caliper in all mice. Tumor dimensions were also measured by caliper 7, 14, 21 and 28 days after treatment in all groups. Tumor volume was calculated as $\pi/6 \times a \times b \times c$, where a is the tumor length (mm), b is the tumor width (mm), and c is the tumor thickness (mm). All mice were sacrificed by the dislocation of the cervical vertebra 28 days after treatment.

Statistical analysis

Mean tumor volume 0, 7, 14, 21 and 28 days after treatment were calculated in all groups. Statistical analyses were performed using the Mann-Whitney U Test using the Statistical Package for the Social Sciences (SPSS, Chicago, USA) software for comparison of data between the control and treated groups. Differences were considered statistically significant when the p value was < 0.05.

RESULTS

During the PDT treatment, changes in temperature in mice tumor were not significant. Average temperature due to PDT treatment on mice tumor was found to be 34°C (Fig. 1). Changes in tumor volume in different treatment groups. The tumor size was increased with time in control, ALA, and LED treatment groups. But PDT group showed a complete remission at 4 weeks after PDT (Fig. 2). The mean tumor sizes were 158.75 ± 50.78 mm³ in the PDT group and 152.25 ± 89.83 mm³ in the control group on 0 day before treatment. There was no significant difference in the mean tumor volumes on 0 day before treatment in each groups ($p > 0.05$). But there were significant differences in the mean tumor volumes 7, 14, 21 and 28 days after treatment between the PDT and the other groups ($p < 0.05$) (Fig. 3).

Fig. 1. Changes in Temperature during PDT in mice tumor. During the PDT treatment, changes in temperature in mice tumor were not significant. Average temperature due to PDT treatment on mice tumor was found to be 34°C.

Fig. 3. Mean tumor volume in control, LED, ALA and PDT groups 0, 7, 14, and 21 days after PDT. There were significant differences in the mean tumor volume 7, 14, and 21 days after PDT between the PDT and the other groups (* $p < 0.05$).

Fig. 2. Changes in tumor volume in different treatment groups. The tumor size was increased with time in control, ALA, and LED treatment groups. But PDT group showed a complete remission at 4 weeks after PDT.

DISCUSSION

According to the World Health Organization (WHO) melanoma skin cancer has been increasing over the past decades with a global estimation of 132,000 melanoma-related skin cancers reported to occur each year. If melanoma is recognized and treated early, it is almost always curable, but if it is not, the cancer can advance and metastasize to other parts of the body, where it becomes hard to treat and can be fatal. High-risk melanomas may require adjuvant treatment. Because melanoma is inherently resistant to radiotherapy.⁶ Various chemotherapy, immunotherapy agents are used, but the overall success in metastatic melanoma is quite limited.⁷ So further research is needed to evaluate new treatments for this fatal disease. PDT has become a new modality for the treatment of advanced melanoma. PDT is a minimally invasive therapeutic modality which has been shown to be effective against different types of malignancies like skin and esophageal cancer.^{8,9} PDT has some advantage in cancer treatments, it has a less invasive than surgery and radiotherapy. It has no long-term side effects when used properly and PDT can be repeated many times at the same site if needed. Also can be applied in combination as several adjuvant therapeutic modalities.¹⁰ ALA-derived PpIX can be cleared from the body within 24-48 h after systemic ALA administration, and because of this rapid clearance, ALA-based PDT would reduce the risk of prolonged skin phototoxicity.¹¹ In this study, tumor volume was found to be reduced after 7 days of PDT with ALA. The study findings indicated that PDT is effective against in melanoma skin cancer cell and during the PDT treatment, changes in temperature in mice tumor were not significant. Average temperature due to PDT treatment on mice tumor was found to be 34°C. Therefore, PDT with LED irradiation has a no thermal effect in tumor tissue. However it was again started to increase slowly after 7 days in some cases but the rate of increase was not higher compared to that of control. This results suggested PDT outcome depends on the amount of photosensitizer absorbed by the tumor, light wavelength, depth of light penetration into the tumor, and light energy delivered. Adjusting tumor size or amount of photosensitizer or light energy delivered is necessary to increase anticancer effect of PDT. The present study were limited and insufficient to recommend clinical application. A further study should be carried out, researching the anticancer effects in skin melanoma.

In conclusion, ALA mediated PDT was found to be

effective against melanoma skin cancer cell A375 induced tumor xenograft in nude mice. This result suggest ALA mediated PDT may be applied to treatment option in skin melanoma.

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