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A suppository kit for metronomic photodynamic therapy: The elimination of rectal cancer in situ

X.F. Shi[a], W.D. Jin[a], H. Gao[b], H.J. Yin[a,*], Y.X. Li[a,*], H. Huang[a], H. Ma[c], H.J. Dong[d,e]

[a] Laboratory of Laser Medicine, Institute of Biomedical Engineering, Chinese Academy of Medical Sciences, Peking Union Medical College, Tianjin 300192, China
[b] Department of Colorectal Surgery, Tianjin Union Medical Center, Tianjin 300121, China
[c] Department of Radiology, Tianjin Medical University General Hospital, Tianjin 300052, China
[d] State Key Laboratory of Precision Measurement Technology and Instruments, Tianjin University, Tianjin 300072, China
[e] Logistics University of Chinese People's Armed Police forces, Tianjin 300309, China

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ABSTRACT

Metronomic photodynamic therapy (mPDT) was developed to improve tumor-specific responses through cell death by apoptosis. We developed an mPDT suppository kit including ALA and LED suppositories and analyzed its killing effect on rectal tumors in rabbits. Methods: The ALA (10 wt%) suppository was prepared using ALA powder, type 36 semi-synthetic fatty acid glyceride, and azone. The LED suppository was constructed by encapsulating LED units and a circuit in transparent epoxy resin. VX2 cells were injected into the rectal submucosa of rabbits to establish a carcinoma model in situ. The ALA suppository was inserted into the rectal cavity for 30 min of uptake and activated for 1 h by the LED suppository at a power density of 20 mW/cm^2. The mPDT process was repeated three times once a day. MRI was used to monitor tumor growth, histopathology and TUNEL staining were performed at 14 days after mPDT. Results: The overall response rate was 60% in the mPDT group using the kit in which the tumor size was decreased up to about 50% at 7 days post-mPDT and almost eliminated at 14 days. HE staining showed that only 6.16% of the tumor tissue remained after mPDT treatment. TUNEL detection showed that the apoptosis rate was 18.9%. Conclusion: We verified the killing effect of the mPDT suppository kit on rectal tumors in rabbits based on mPDT that induced tumor cell apoptosis.

1. Introduction

Photodynamic therapy (PDT) is a clinical treatment that combines the effects of visible light irradiation with a photosensitizing drug to cause destruction of selected cells. PDT has many advantages in cancer treatment, including less trauma, low toxicity, good specificity, and repeatability. Moreover, it can eliminate recurrent lesions and stimulate secondary immunity [1].The PDT outcome is directly related to the efficiency of singlet oxygen generation, which is a combination of photosensitizer, light characteristics (intensity and wavelength) and tissue oxygenation. Thus, photosensitizer and light play important roles in a PDT system [2–5]. Most photosensitizers are highly hydrophobic and require delivery systems, for example, Protoporphyrin IX, which is a photosensitizing agent, converted enzymatically from the prodrug 5-aminolevulinic acid (ALA), is used as a photosensitizer in PDT for cancer. However, ALA penetrates with diffculty through intact tissue; therefore, improving delivery systems for ALA will play an important role in ALA-PDT. Enhancement of ALA skin penetration can be achieved by physical methods, such as iontophoresis, laser, microneedles, ultrasound, and by adding chemical penetration enhancers, such as, dimethyl sulfoxide, oleic acid, and others [6–9]. Recent reports have described carrier nanoparticles with additional active complementary and supplementary roles and even quantum Dots in PDT and we have previously attempted to form an ALA hydrogel to enhance the permeability of ALA [10–12]. Whereas, all of these methods are more or less problematic in orthotopic treatment for colorectal cancer because of the special anatomical structure of the rectum.

In this study, we developed a specially designed ALA suppository using ALA, 36 semi-synthetic fatty acid glyceride and azone, which was considered as a good agent to enhance the absorption of ALA for tissue, focusing on rectal tumors based on metronomic photodynamic therapy (mPDT). mPDT is a specialized method to perform PDT. It involves continuous delivery of a low fluence rate of light and a photosensitizing drug over an extended period [13]. With mPDT, oxygen can be reacted sufficiently with the active photosensitizing drug to destroy the targeted tumor by inducing apoptosis, while irradiation at high fluence rates decreases the efficacy of PDT sharply due to oxygen and photosensitizer depletion, which is termed acute photodynamic therapy

* Corresponding authors.
E-mail addresses: yinzi490@163.com (H.J. Yin), yinhj@bem.cams.cn (Y.X. Li).

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(aPDT). In particular, Bisland et al. found that damage to normal tissue, especially white brain matter, can be avoided and tumor cell-specific apoptosis can be induced by mPDT in malignant brain tumors [14]. A long period of low power irradiation is essential for mPDT, however delivering light continuously to the entire tumor in a minimally invasive way has been a major technical challenge for clinical implement. Despite some implantable LED light sources for brain malignant tumors have been reported, such as LED-based backpack, LED-to-fiber-coupled source and organic light emitting diode (OLED). And even a very stable power profile ranging from 1600 to 100 uW for a 1–10 day continuous irradiation were avaible [15,16]. Unfortunately there were no reports of the LED light source suitable for rectal implantable. In this paper, we will also introduce a custom-designed LED rectal suppository light source, and was collectively referred to as a suppository kit with the ALA suppository mentioned above.

Apoptosis induces less inflammatory responses, such as edema, than necrosis. Therefore, PDT causing more apoptosis is beneficial for treatment of diseases in a fixed cavity such as the brain and rectum. Colorectal cancer (CRC) is the third most common cancer and the fourth leading cause of death from oncological reasons [17]. Surgical resection, radiation therapy, and chemotherapy are employed for CRC treatment, but they usually cause severe side effects in patients with advanced metastatic CRC [17–19]. Our previous study showed classic PDT can eliminate rectal tumors with a remarkable 60% response rate, but also causes some side effects such as rectal obstruction in rabbits [20]. In this study, we not only examined the characteristics of ALA suppository both in vitro and vivo, but also established the VX2 rabbit rectal cancer model in situ for the first time. After being treated with the mPDT based on our suppository kit, the death of tumor cells was mainly induced by apoptosis as HE staining and TUNEL immunohistochemistry results shown. In addition, complete healing rate of tumor was as high as 60%, inflammatory reactions and side effects were hardly observed. All these preliminary results demonstrated that our suppository kit tended to be an effective agent for mPDT of rectal tumors and has the potential to promote its clinical treatment.

2. Materials and Methods

2.1. Materials

5-ALA hydrochloride (4 g, powder, 99.8% purity, H20070027) was purchased from Shanghai Xiandao Chemistry Co., Ltd. (Shanghai, China). Azone (analytically pure) was purchased from Nanjing Jiangling Chemical Plant and Tianjin Commie Chemical Reagent Development Center. The suppository mold was formed by a three-dimensional printer.

2.2. Formation of the ALA Suppository

A 10 wt% ALA suppository of 0.8 g was produced by 80 mg ALA, 15 mg azone, and 635 mg type 36 semi-synthetic fatty acid glyceride. The type 36 semi-synthetic fatty acid glyceride was heated to 60 °C in a water bath, followed by addition of ALA and azone powder, and stirring to fully mix. The mixture was quickly poured into the mold that had been smeared with glycerine for lubrication. The mold was cooled at 2 °C for 1 h, any excess was scraped off, and the suppositories were lifted out of the mold. A placebo suppository was prepared without ALA by the same procedure.

2.3. Characterization of the ALA Suppository

Weight differences, melting time, and ALA content and release in vitro and in vivo were tested to characterize the formed suppository. The methods are descripted below.

(1) Weight difference. Ten suppositories were weighed and the average weight was calculated. The weight difference was calculated by the formula: $P_{Wd} = (W_i - \overline{W})/\overline{W} * 100\%$. P_{Wd} is the weight difference as a percentage, W_i is the weight of the suppository, and $\overline{W}$ is the average weight.

(2) Melting time. A suppository was transferred to a 15 mL tube containing 10 mL PBS and shaken at constant speed at 37 °C. The melting time was defined as the time at which a part of the suppository became flocculent and soft.

(3) ALA content. ALA suppositories were divided into three equal parts, and the ALA concentration of each part was detected by ethyl acetate extraction using a diaminobenzaldehyde colorimetric method. Briefly, the divided ALA suppository was dissolved in water and reacted with ethyl acetoacetate at 100 °C for 10 min at pH 4.6. Ethyl acetate was added to the cooled reaction liquid to extract the pyrrole product. The extraction liquid became red upon addition of dimethylaminobenzaldehyde. Then, the optical density was measured at 554 nm using a Multimode Reader.

(4) Release of ALA in vitro and in vivo. The release of ALA in vitro was also analyzed by ethyl acetate extraction using the diaminobenzaldehyde colorimetric method. An ALA suppository was transferring to a 50 mL tube containing 45 mL distilled water, and then the tube was placed in a shaker to shake at 100 r/min at 37 °C. Then, 1 mL of liquid was collected to measure the ALA concentration every 10 min up to 2 h, and the same volume of water was supplemented immediately afterward.

ALA release in vivo was detected by fluorescence density, because ALA is metabolized into protoporphyrin IX (PpIX) in vivo. Six rabbits were used for the experiment in which three rabbits received an ALA suppository and the other three were administered ALA intravenously. An ALA suppository was placed in the rectum of a rabbit or a solution containing the same dose of ALA as one ALA suppository was administrated via the ear vein, and then 1 mL of blood was collected from the ear vein at 0.5, 1, 2, 4, 8, 12, 24, 48, and 72 h post-administration. Then, 100 μL plasma containing PpIX derived from the blood was mixed with 1.6 mL 9% NaCl in 1 M HCl and reacted for 10 min. The mixture was centrifuged at 1000*g* for 10 min to obtain the supernatant. The supernatant was diluted 2-fold with 5% NaOH repeatedly until the concentration was within the measurable range in the linear standard curve. The fluorescence density of the samples was detected by a fluorescence spectroscope at excitation and emission of 395 and 638 nm, respectively. The detailed protocol can be found in our previous study [21].

2.4. Design and Construction of the LED Suppository

Four LED units were arrayed in an aluminum substrate that was used for cooling, and the circuit board was also integrated into the substrate. The integrated unit was encapsulated with transparent epoxy glue into the shape of the ALA suppository (a 1.5 mL Eppendorf tube was used for the mold). A wire protruded from the bottom for charging. LEDs were powered by 2.5 V at 20 mA.

2.5. Animal Model

Rabbit VX2 cells were used to establish the rectal cancer model. Japanese white rabbits were anesthetized by an intramuscular injection of xylazine hydrochloride (0.3 mL/kg). After the animal was fixed on the operating table, a hollow plastic tube (1 cm in diameter) was used to stretch the anus and rectum, and the tumor cells (1×10^7 in 0.5 mL) were seeded into the submucosa at 0.5 cm above the dentate line at an orientation of 6 o'clock. After cell seeding, the animals were maintained under standard conditions for 2 weeks. The animal experiments were approved by the Tianjin Animal Ethics Committee [SYXK(JIN)2011-0008].