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Assessment of safety of 5-aminolevulinic acid–mediated photodynamic therapy in rat brain

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ABSTRACT

Background: Oral 5-aminolevulinic acid (ALA) induces biosynthesis/accumulation of the natural photo-sensitizer protoporphyrin IX (PpIX) in cancer cells. ALA is used widely in photodynamic diagnosis (PDD) and therapy (PDT) during malignant glioma surgery, but few studies have examined the effects of photodynamics plus ALA on normal brain tissue in vivo. We investigated the effects of ALA-mediated PDD and PDT on normal brain tissue.
Methods: We established a rat model in which the brain surface was irradiated through the skull by light-emitting diode (635 nm) after ALA administration. Using this model, we investigated the effects of various amounts of light irradiation with various ALA doses on brain tissue.
Results: Neurological symptoms developed with administration of ALA at 240 or 120 mg/kg accompanied by irradiation at 100 or 400 J/cm^2, respectively. Dye leakage occurred due to disruption of the blood–brain barrier (BBB) at 90 mg/kg and 100 J/cm^2, respectively. Thickness of the cortex increased significantly at 240 mg/kg and 400 J/cm^2, respectively. The number of neurons appeared to decrease at 200 mg/kg plus 400 J/cm^2, respectively, and there was an increase in the number of cells that were positive for terminal deoxynucleotidyl transferase deoxyuridine triphosphate nick-end labeling (TUNEL) staining.
Conclusions: ALA-mediated PDT is safe at doses of 90 mg/kg or less followed by light irradiation of 100 J/cm^2 in rat brains. At doses above this threshold, ALA-PDT led to irreversible BBB and brain damage in rats.

1. Introduction

Photodynamic diagnosis (PDD) is a practical tool used during surgical operations on aggressive brain tumors, such as malignant gliomas [1–3] and meningiomas [4,5]. As a second-generation photosensitizer, 5-aminolevulinic acid (ALA) has been used for brain tumors frequently in recent years, as it can be administered orally and causes few adverse reactions. In their randomized controlled study, Stummer et al. [2] reported the practical application of intraoperative PDD and ALA in the diagnosis of malignant glioma. In addition, a recent report evaluated the cost-effectiveness of ALA-mediated fluorescence image guided surgery (FIGS) in newly diagnosed high-grade gliomas compared to white-light surgery [6,7]. Consequently, ALA-mediated FIGS has become an indispensable surgical technique and standard for care of malignant gliomas worldwide [8]. Orally administered ALA induces biosynthesis and accumulation of protoporphyrin IX (PpIX), a natural photo-sensitizer, in cancer cells.

ALA is incorporated into photodynamic therapy (PDT) for certain cancer treatments. PDT holds considerable promise for many solid tumors. Theoretically, ALA-PDD and ALA-PDT act through a photon-induced physicochemical reaction that is induced by excitation of PpIX exposed to light. However, few reports exist on the use of ALA-PDT in the treatment of malignant glioma [9–12]. Additionally, some reports indicated that brain edema is an adverse effect resulting from normal tissue injury and blood–brain barrier (BBB) disruption [13–16].

ALA, a natural substance, is metabolized in different organs, including the liver, to form PpIX [17], and PpIX is released from living cells into the systemic blood circulation by the action of an adenosine-triphosphate-binding cassette transporter known as ABCG2 [18]. Thus, the level of PpIX can become elevated in the blood after ALA administration. At a high dose of light irradiation, PpIX in the blood might induce damage in the endothelial cells of normal blood vessels, which could lead to disruption of the BBB, thereby causing brain edema and brain injury.

We established a rat in vivo model to assess the safety of ALA-PDD/PDT in the normal brain. We examined the possibility that strong light

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irradiation following administration of high doses of ALA may cause BBB disruption or brain injury. Our findings contributed to evaluations of the safety of ALA-PDD/PDT in normal human brain tissue.

2. Materials and methods

2.1. Development of a non-craniotomy ALA-PDT model

All experimental procedures planned in this study were approved by the animal review committee of our institution and performed in accordance with the institution's guidelines for animal experiments. Fisher rats (male, 10 weeks old, 190–210 g body weight) were anesthetized with an intraperitoneal injection of a mixture of medetomidine 0.15 mg, midazolam 2 mg, and butorphanol 2.5 mg/kg. All animals, except sacrificed rats, were given atipamezole hydrochloride 0.15 mg/kg post-operation.

To prevent disruption of the BBB through vibration or frictional heat generated by drilling during the craniotomy, we developed a non-craniotomy experimental model in this study. To measure the attenuation rate of light extinction accurately through the cranial bone, the rats were sacrificed and a craniotomy was performed. A photosensor was placed on the front surface of a cranial bone fragment, which was irradiated from a 3-cm distance with a light-emitting diode (LED) light source with a wavelength of 635 nm (optical power meter #3664; Hioki, Nagano, Japan; Fig. 1). The intensity of the LED light was adjusted to 100 mW/cm^2 on the front surface of the cranial bone. The irradiated field was covered with a rubber shield in a window (7 mm × 7 mm) to prevent the surrounding area from being exposed to the light (Fig. 1, right). A photosensor was placed on the back surface of the cranial bone fragment (Fig. 1, left). The light intensity at the back surface was measured as 51.2 ± 5.32 mW/cm^2, and the rate of light extinction through the bone was calculated (Fig. 1, left). Experiments were performed with this model, referred to as the “non-craniotomy light irradiation model,” based on the assumption that the rate of light extinction was approximately 50%.

2.2. Fluctuations in the plasma concentrations of PpIX after intraperitoneal administration of ALA in rats

Rats were administered ALA intraperitoneally at doses of 60, 120, and 240 mg/kg. Then, 500 μL blood was collected from the tail vein 2, 4, and 6 h after ALA administration. The blood samples were centrifuged (10,000*g* for three min), and the resulting upper layers containing plasma components were stored at −80 °C until they were used. The concentration of PpIX in the plasma samples was measured by high-performance liquid chromatography [19]. The plasma PpIX concentrations reached their maximal levels at 4 h after ALA administration. Thus, all subsequent experiments were performed 4 h after ALA administration.

2.3. Effects of 635-nm light irradiation following ALA administration on the normal rat brain

Our preliminary study before the experiment demonstrated slight BBB disruption with intraperitoneal administration of 90 mg/kg ALA + 400 J/cm^2 light irradiation, and histological change with 160 mg/kg ALA + 400 J/cm^2 light irradiation. To clarify the safety threshold for BBB disruption, neurological deficit, and histological change, we set the ALA and irradiated light doses as described below.

After induction of anesthesia, 0, 30, 60, 90, 120, 160, 200, or 240 mg/kg ALA was dissolved in 1 mL physiological saline and administered intraperitoneally to Fischer rats (male, 10 weeks old, 190–210 g). They were anesthetized four hours after ALA administration, when the PpIX concentrations peaked, as described above. The rat head was fixed with a stereotactic frame, and an incision was made in the skin of the head to expose the cranial bone in the 3.5-mm^2 area from the midline to the right side. Subsequently, a rubber shield with a window (7 × 7 mm) was placed directly on the right lateral skull. The right lateral skull then was irradiated with an LED light (635 nm) at 0 (control), 100, or 400 J/cm^2 (100 mW/cm^2) from a distance of 3 cm above the skull. After light irradiation was completed, the wound was sutured, and the rat was released from the fixation frame.

2.4. Neurological assessment

Neurological severity scores [20] were modified for classification into the following categories: muscle (muscle status and abnormal movement), sensory (visual, tactile, and proprioceptive), reflex, and balance. The neurological symptoms under each condition were assessed on an 18-point scale (modified neurological severity score: minimum, 0 [normal state]; maximum, 18).

2.5. Macroscopic and histological assessment

The rats were anesthetized 48 h after light irradiation, and 1 mL 2% Evans blue dissolved in physiological saline was injected via the penile vein. The rats were perfused, fixed with 10% formalin, and rinsed with 0.9% physiological saline at 30 min post-injection of Evans blue. Next, their brains were removed, and the Evans blue staining in the brain was confirmed macroscopically. Each brain was sectioned into six 2-mm slices, and the area stained with Evans blue on each slice was traced by hand and measured using the open-source image processing program

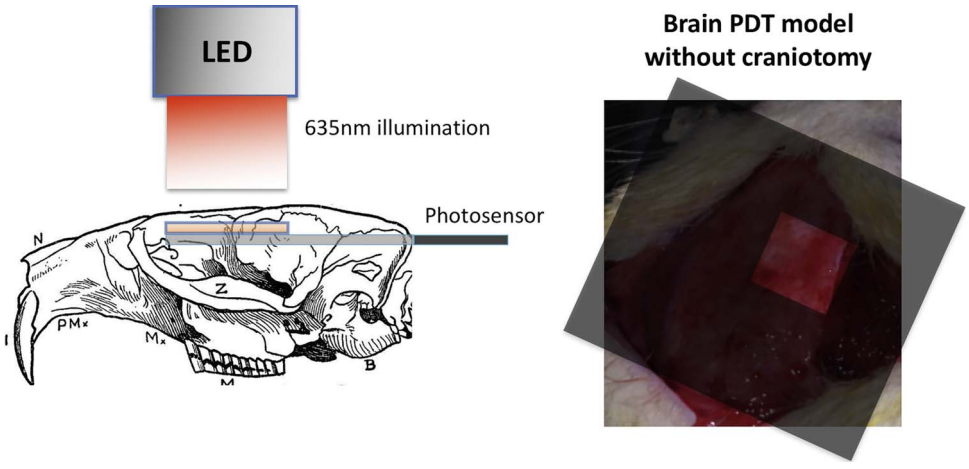

Fig. 1. A photosensor was placed beneath the rat frontal bone, and the transcranial luminous energy was measured. The average quantity of light was 51.2 ± 5.32 mW/cm^2, when 100 mW/cm^2 excitation light was illuminated through the cranium (n = 12). The mean extinction ratio of the light intensity was 48.8%.