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ABSTRACT

One of the limiting factors of photodynamic therapy is cutaneous permeation of a photosensitizer or precursor. Studies report that there is a strong relationship between temperature and porphyrin synthesis in biological tissue. The use of thermogenic and/or vasodilator substances may favor both ALA/methyl-ALA permeation and protoporphyrin IX (PpIX) production in the tissue. In this study, menthol, methyl nicotinate, and ginger extract were incorporated into either the ALA or methyl-ALA cream to investigate the PpIX production in rat skin. Fluorescence spectra were collected to quantify the PpIX present in tissues. The methyl nicotinate was the one with the highest optimization effect of PpIX production after three hours of incubation of the cream. Its association with methyl-ALA caused the production to be about 50% higher than that observed for methyl-ALA alone. These results are promising as a possible strategy for decreasing the incubation time of the precursor cream in various clinical protocols and increasing the photosensitizer production in lesions.

INTRODUCTION

Vasodilator and thermogenic characteristics may contribute to the application of photodynamic therapy, since it can optimize both the production of protoporphyrin IX (PpIX) in the tissue and promote the increase of oxygen in the region. (1–3)

Menthol is known as a vasodilator in the topical application, and has an analgesic effect that would minimize the pain sensation felt by the patient during the procedure. Menthol in low concentrations (less than 3%) does not produce toxic effects and its analgesic effect is observed in concentrations of 1 to 30%. (4,5) The use of menthol in cosmetic products is permitted by Brazilian Health Surveillance Agency (ANVISA) at a maximum concentration of 1%. (6)

Methyl nicotinate has been used in topical formulations as a promoter of penetration and skin permeation of incorporated active substances. (7) The maximum concentration also permitted by ANVISA for use with professional monitoring (dermatologist) is 0.5%. (8)

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Ginger is also considered a thermogenic substance and has the advantage of being natural and without contraindications. In addition, ginger also has known benefits for the skin, oral cavity, gastrointestinal tract, lungs, liver cancer, bladder and pancreatic cancer. (9)

In this study thermogenic/vasodilator agents menthol, ginger and methyl nicotinate were incorporated into the aminulevulinic acid (ALA) and methyl minevulinate (methyl-ALA) creams to investigate possible optimization of PpIX production in the tissue using animal model.

METHODOLOGY

We used the skin of 12 male Wistar rats with about 400 g of body mass with the approval of the Ethics Committee on the Use of Animals by the Sao Carlos Institute of Physics (protocol number 9934110917). The animals were anesthetized and two areas of approximately 2 cm were trichotomized for skin exposure. The animals were anesthetized using inhalational anesthesia (Vetcase - Incotec, Serra, ES, Brazil), using mask and isoflurane (BioChimico®, Itatiaia, RJ, Brazil) between 2 and 4% for induction of the anesthetic plane, and between 1.5 and 2 for maintenance, evaluating according to respiratory and cardiac depression and the absence of response to stimuli.

Four groups were used, each group with 3 animals. In each group a distinct substance was incubated: menthol (1%), methyl nicotinate (0.5%) or ginger extract (5%), incorporated into the bases containing the precursors (ALA and methyl-ALA); in the control group, ALA and methyl-ALA creams were used without the addition of other substances. In each animal, 20% ALA cream was applied in one of the trichotomized areas, and the other was applied 20% Methyl-ALA cream. An occlusive bandage was then maintained for 3 hours. Every 60 minutes the dressing was removed, the region was cleaned and measurements of thermography and fluorescence spectroscopy were performed. After each measurement, the cream and the occlusive dressing were reapplied on the same region until the 3 hours were completed.

The thermographic camera used was FLK-Ti400 (*Fluke*®, Everett, EUA) and Fluorescence spectroscopy measurements were performed using a system composed of diode laser (emission at 408 nm) coupled to a "Y" type fiber that leads light to the tissue and collects light from the tissue, taking it to a spectrophotometer (USB2000, Ocean Optics®, USA).

Tissue biopsies were collected for the imaging using confocal microscopy to verify the production of PpIX in depth. These fragments underwent a freezing process (to minimize FS degradation) using the OCT compound (Tissue-Tek®; Sakura Finetek USA Inc.) and were kept in a freezer at -80 ° C to be cut in the direction 30 μm thick in a cryostat (Leica). The system used was an inverted fluorescence confocal microscope (Zeiss - LSM780, Zeiss, Jena, Germany) with an excitation laser at 405 nm. The acquisition was performed by two high sensitivity GaAsP photomultipliers with acquisition bandwidth varying from 490 to 600 nm; (Channel 1 - autofluorescence) and 600-700 nm (Channel 2 - FS fluorescence).

RESULTS AND DISCUSSION

Figures 1 shows the ratio between the characteristic peaks of PpIX (630nm) and skin (500nm) fluorescence from fluorescence spectroscopy throughout the 180 minutes of the incubation.

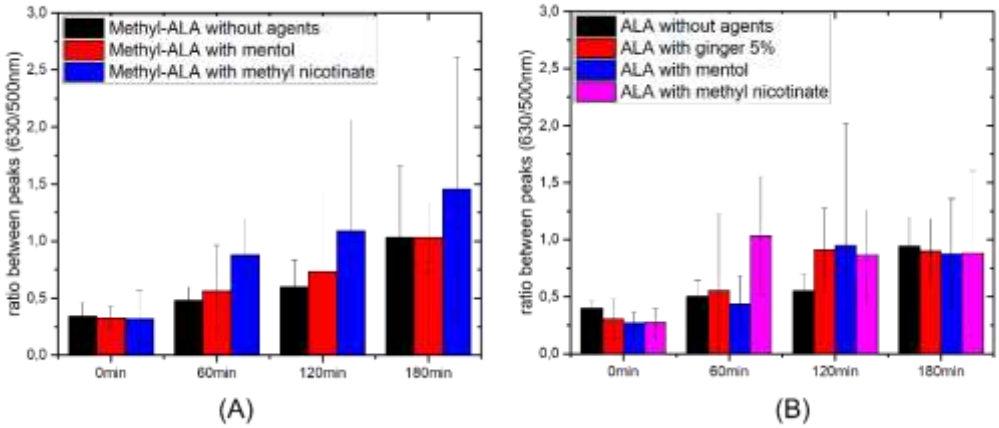

Figure 1. Variation of PpIX production during (A) methyl-ALA cream incubation without substances, with menthol and with methyl nicotinate and (B) ALA cream incubation without substances, with ginger 5%, with menthol and with methyl nicotinate.

In Figure 8A the effect of each vasodilator/thermogenic substance (menthol and methyl nicotinate) associated with methyl-ALA can be observed. Menthol causes methyl-ALA to produce more PpIX in the initial 120 minutes, but within 180 minutes that production equals the original precursor cream. However, methyl nicotinate causes the methyl-ALA to produce more PpIX in all periods and, after 180 minutes, this production is about 50% greater than that produced by methyl-ALA.

In Figure 8B all vasodilator/thermogenic substances associated with ALA cause greater PpIX production in the first 120 minutes, but this production resembles ALA without association at the end of 180 minutes.

Figure 2 shows the fluorescence of the ex vivo tissues after 180 minutes of the creams incubation using confocal microscopy.

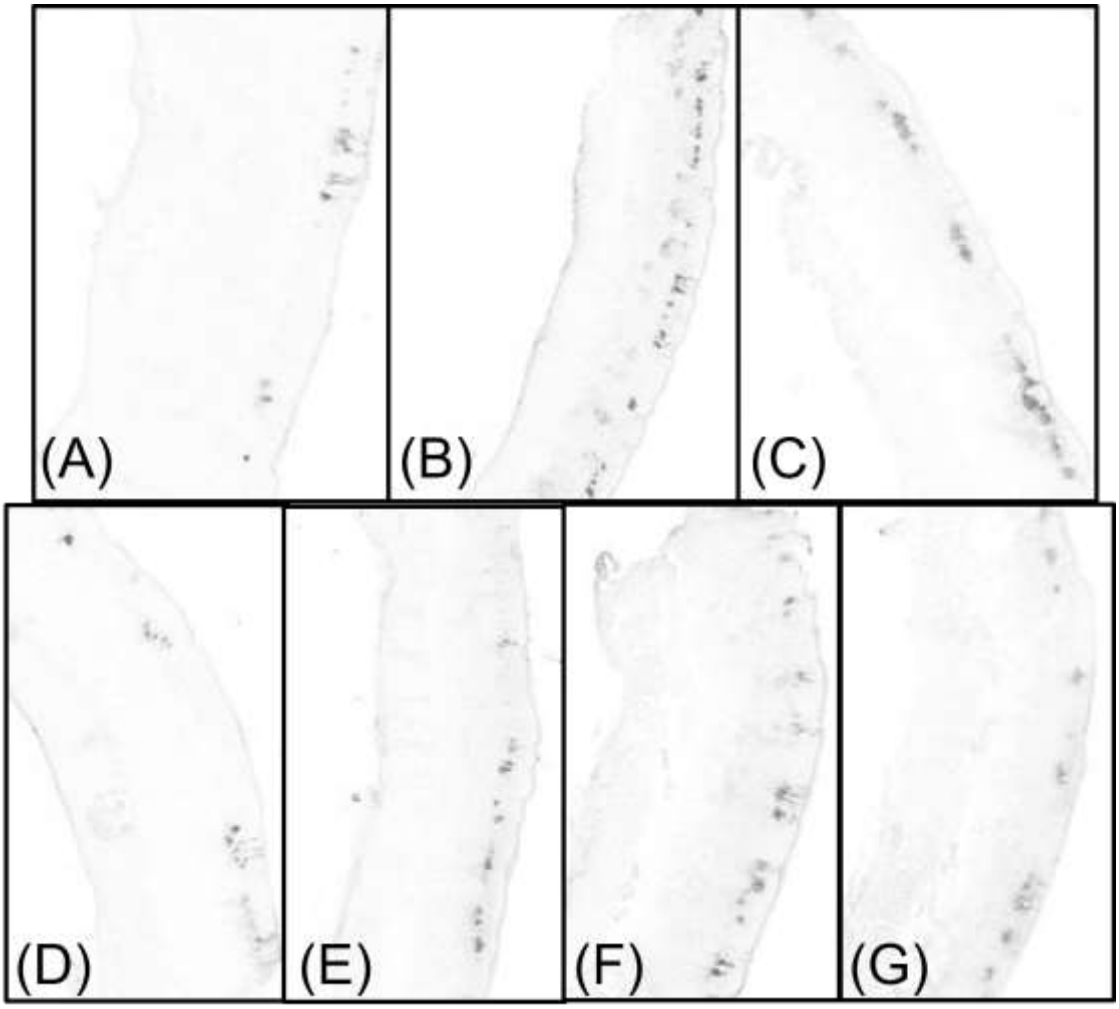

Figure 2. Confocal microscopy images of tissue pieces that received cream containing (A) only methyl-ALA; (B) methyl-ALA with menthol; (C) methyl-ALA with methyl nicotinate; (D) only ALA; (E) ALA with menthol; (F) ALA with methyl nicotinate and (G) ALA with ginger. The edges shown at the right of each image (which contains a more intense line of black pixels) are the surfaces of the tissues.

Figure 2A shows a smaller number of black pixels in the tissue that received only methyl-ALA, that is, the lower fluorescence characteristic of PpIX when compared to tissues that received methyl-ALA cream associated with menthol and methyl nicotinate substances (Figures 2B and C, respectively). Similarly, the tissue that received only ALA (Figure 2D) has fewer black pixels compared to tissues that received ALA cream associated with menthol, nicotinate, and ginger substances (Figures 2E, F, and G, respectively).

The tissue temperature variation over the 180 minutes incubation using thermography is demonstrated in Figure 3.

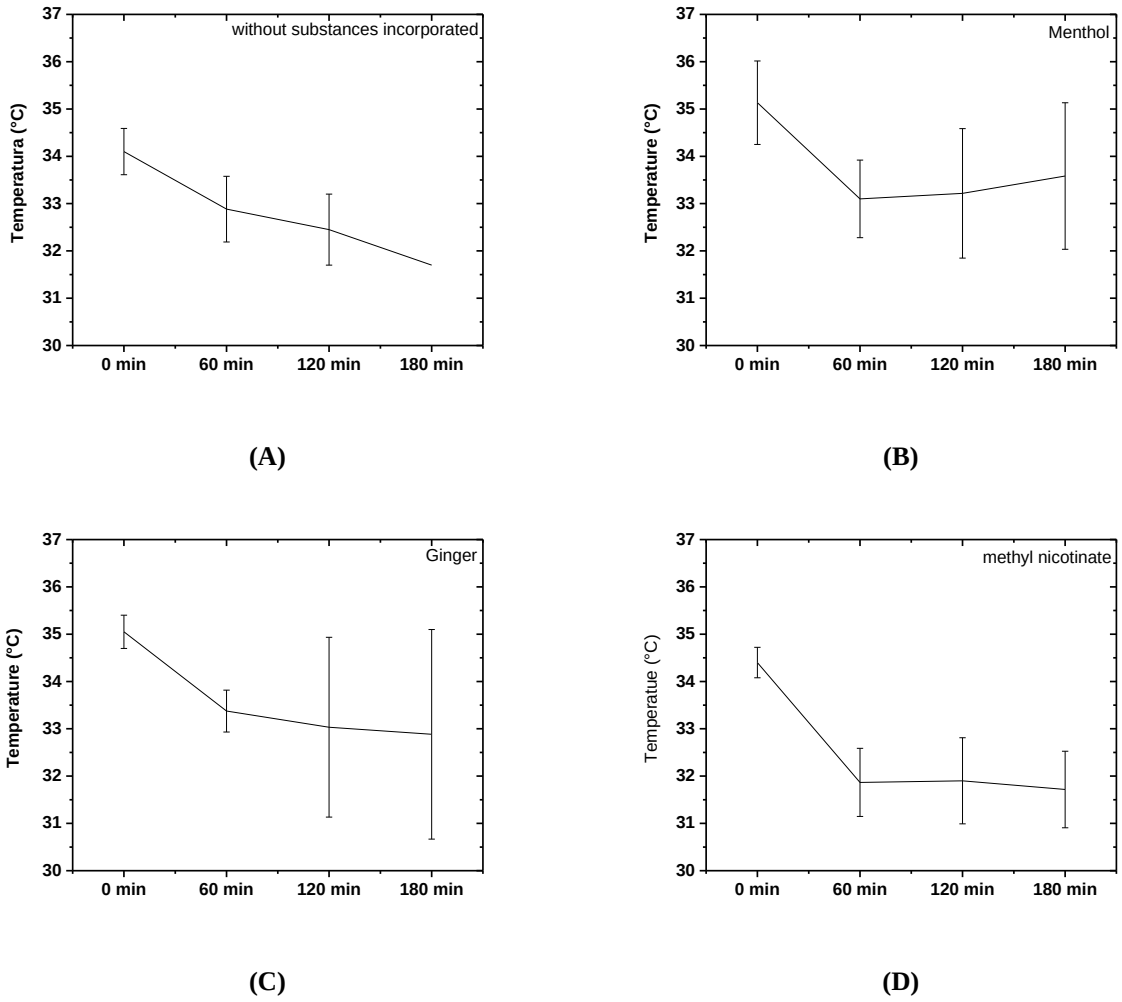

Figure 3. Temperature variation over the 180 minutes incubation of the creams (A) without incorporation of vasodilator/thermogenic substances; (B) with menthol, (C) ginger and (D) methyl nicotinate incorporated.

Figure 3A shows that creams containing only ALA or methyl-ALA caused gradual cooling of the tissue to a decrease of about 2.5 °C over the 180 minutes of incubation, because the topical cream had an emissivity value smaller than the skin, resulting in greater reflectance of the infrared radiation of the environment. (10) Figures 3B, C and D, wherein the menthol, ginger and methyl nicotinate substances are incorporated into the cream, respectively, shows that a decrease in temperature occurs only within the first 60 minutes. Over the next 120 minutes, this temperature remains constant or increases (about 0.5 ° C in the case of menthol), probably due to the time taken for the tissue to undergo vasodilator/thermogenic effect of the substances which increases the temperature of the skin. In this time interval, the thermal measurement of the surface has the two added effects: greater reflectance of the infrared radiation of the environment and increase of the temperature of the skin.

CONCLUSIONS

The methyl nicotinate was the thermogenic/vasodilator substance with the highest PpIX production after three hours of incubation of the cream. Its association with methyl-ALA caused the production to be about 50% higher than that observed for methyl-ALA.

These results are promising as a possible strategy for decreasing the incubation time of the precursor cream in various clinical protocols and increasing the photosensitizer production in lesions.

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