ECRONICON OPEN ACCESS

EC PHARMACOLOGY AND TOXICOLOGY

Research Article

Permeation, Bio-Distribution, and Subcellular Localization of Azulene Photosensitizer in Normal and Inflammatory Peripheral Blood Mononuclear Cells

Teerasak Damrongrungruang[1,3,4]*, Nujjakorn Yuhun[2], Arunroong Kuntiya[2] and Patcharaporn Klaipithak[2]

[1]Division of Oral Diagnosis, Department of Oral Biomedical Sciences, Faculty of Dentistry, Khon Kaen University, Thailand

[2]Dental Student, Faculty of Dentistry, Khon Kaen University, Khon Kaen, Thailand

[3]Laser in Dentistry Research Group, Khon Kaen University, Thailand

[4]Melatonin Research Program, Khon Kaen University, Thailand

***Corresponding Author:** Teerasak Damrongrungruang, Division of Oral Diagnosis, Department of Oral Biomedical Sciences, Faculty of Dentistry, Khon Kaen University, Amphur Muang, Khon Kaen, Thailand.

Received: May 19, 2022; **Published:** June 29, 2022

Abstract

Permeation and subcellular localization of photosensitizer in photodynamic therapy leads to precisely explaining the biological effects in the clinic. We aim to study cell permeation and subcellular localization of azulene between inflamed and normal human peripheral blood mononuclear cells (hPBMCs). 1, 10, 100 and, 1,000 µM (in 1%v/v methanol+deionized water) of azulene were incubated into hPBMCs and observed under a confocal fluorescence microscope. After the optimum concentration was determined, the permeation of this concentration of azulene was observed at 5, 10, 15, 20, 25 and, 30 minutes in either inflamed or normal hPBMCs. The subcellular localization was conducted by staining with 500 nM Mitotracker Red CMXRos and Lysotracker Green DND-26 followed the manufacturer's instruction for localization determination in mitochondria and lysosome, respectively, compared with the position of azulene in hPBMCs. The result shows that the longer the incubation time, the higher the permeation. In inflamed cells, the maximum permeation was at 25 minutes which gave the emission intensity close to 30 minutes. In normal PBMCs, the maximum permeation of azulene was in 20 minutes and azulene signal could not be detected at 30 minutes. The permeation of azulene in inflamed hPBMCs is higher than in normal hPBMCs. Regarding subcellular localization, indifference between mitochondria and lysosome localization of azulene in both inflamed and normal hPBMCs was demonstrated. In conclusion, the permeation of azulene was higher in inflamed hPBMCs compared with normal hPBMCs. With the limitation of this study, azulene appears to localize at both mitochondria and lysosome.

Keywords: *Azulene; Permeation; Subcellular Localization; Peripheral Blood Mononuclear Cells; Inflammation*

Abbreviations

ALA: Amino Levulinic Acid; DLI: Drug-Light Interval; FBS: Fetal Bovine Serum; PBS: Phosphate Buffer Saline; PBMC: Peripheral Blood Mononuclear Cell; PDT: Photodynamic Therapy; pPIX: Proto Porphyrin IX; ROS: Reactive Oxygen Species

Introduction

Photodynamic therapy (PDT), a novel and target therapy, is a WHO-approved, non-invasive modality for treatment of certain diseases, for instance, superficial types of esophageal cancer and lung cancer [1]. Successful therapy relies on the specific photosensitizer in combination with a specific wavelength of light with adequate power/energy density, and oxygen availability. Generally, it is accepted that PDT is a time-consume treatment because it needs 2 main stages. Stage I-Drug-light interval (DLI) time refers to the time from an application of photosensitizer until complete absorption or saturation of photosensitizer in the target tissue or organ. Afterward, stage II-Irradiation time means the time for light to give energy and ion (especially proton) to stimulate photosensitizer to achieve adequate Reactive Oxygen Species (ROS), the other radical until exert desire effects [2].

Generally, the time used in a drug-light interval phase depends primarily on the structure of photosensitizers. The larger the structure related to the longer the DLI time. Additionally, the lipophilicity also determines the DLI time while the higher the lipophilicity associates with the shorter the DLI. Clinically, it was reported that successful treatment of early squamous cell carcinoma using a newly developed photosensitizer named tetra(m-hydroxyphenyl)chlorin or mTHPC needed DLI time of approximate 4 - 8 days [3]. Because mTHPC has a large molecular structure, thus it took quite a long time for absorption. Nonetheless, 5-amino levulinic acid (5-ALA), one of the smallest molecules that act as a precursor of an intrinsic photosensitizer Proto-porphyrin-IX (pPIX), needs a standard time of 3 hours DLI for treatment of a non-aggressive basal cell carcinoma [4]. Searching for more rapid absorbed photosensitizer and/or small lipophilic molecules is a key to minimizing DLI time.

On the contrary, the irradiation time in photodynamic therapy varies depending mainly on the power density (irradiance rate) and energy density (fluency) setting of the light sources. It is well-established knowledge that the lower power light-emitting diode (LED) requires a much longer irradiation time when compared with laser. Treatment of port-wine stains (PWS) using hematoporphyrin monomethyl ether (HMME), a vascular wall-targeted photosensitizer and using a wavelength in the range of 532 nm LED with power density 80 - 100 mW/cm^2 takes 20 - 25 minutes of irradiation time/site to control the stains [5]. While using Nd:YAG laser (half wavelength, 532 nm) at the same power density, using only 20 minutes of irradiation time [6] demonstrated effective treatment of port-wine stain. The second factor for irradiation time determination relates to the depth of the lesion. Normally, the deeply located lesions i.e., muscular layers, need more irradiation time than the lesions that are located superficially, for instance, an intramucosal layer. Treating actinic keratosis using a 630-nm diode laser with 20% methyl aminolevulinate as a photosensitizer took as long as 8 minutes to exert remission effect on the lesions [7]. On the other hand, treatment of deep internal organs e.g. lung cancer using a 630 nm diode laser with Photofrin® (porfimer sodium) as a photosensitizer took 12 minutes [8]. From these data, one of the effective means to minimize the PDT treatment time is to use the small photosensitizer molecule in order to induce rapid absorption into the target tissue.

Azulene, the simplest terpenoid compound in soothing cosmetic products, can be used as a photosensitizer in photodynamic reactions with anti-inflammatory effects [9]. Its structure is composed of only 5 and 7-carbon member aromatic structures which can effectively generate singlet oxygen only at 5 μM [10]. Wang., *et al*. used 30-minute DLI time for azulene and demonstrated that azulene is capable of trafficking into a nucleus [11]. This DLI time, however, was set without any concrete rationale or clear underlying explanation. It is generally known that azulene is a lipophilic molecule that should be rapidly absorbed into the human cell. The study to address the saturation time of azulene might have beneficial effects on shortening the clinical time of azulene-mediated photodynamic therapy.

Different types of photosensitizers have preferential organelles to exert their function. Specific subcellular localization is linked with the activity/fate of cells. Phthalocyanine, for example, usually passes through the plasma membrane and accumulates at mitochondria [12]. Chlorin-based photosensitizer, for instance, Temoporfin (Foscan®) preferentially localizes at the endoplasmic reticulum and Golgi apparatus of tumor cells [13]. Porfimer sodium or Photofrin®, one of the commonly used photosensitizers in a clinic, can retain in a plasma membrane and induce the target cell death via necrosis [14]. Aluminium phthalocyanine disulfonate induced keratinocyte differ-

entiation before apoptosis by targeting the lysosomal membrane. Collectively, whether a cell will be driven to differentiation or apoptosis depends on the dose of light and summative accumulation of reactive oxygen species products [15] as well as specific organelle localization. Interestingly, a small molecule such as methyl-amino levulinic acid (Metvex®), was demonstrated to target the phagolysosome leads to induce autophagy cell death and in turn beneficial for the malignant genotypic transformation of DOK precancerous cells [16].

To our knowledge, there is no report about permeation, subcellular localization, or bio-distribution of azulene.

Aim of the Study

The present study aims to compare the amount of azulene permeation between normal and inflamed peripheral blood mononuclear cells. Also, bio-distribution and organelle localization of azulene will be studied.

Materials and Methods

The study protocol was approved by the Ethics Committee of the Khon Kaen University, Thailand [HE631653].

Azulene preparation

Azulene (Sigma Aldrich, Singapore, Singapore) was dissolved in methanol and distilled (final concentration of methanol was less than 12.5 μl/ml or 1% v/v) and filtered using a 0.22 μm filter, kept in the aluminium-foil covered tube prior to use in a final concentration of 0.1, 1 and 10 μM. All processes were performed in dark condition.

Peripheral blood mononuclear preparation

Buffy coat leftover from anonymous whole blood was courtesy of Central Blood Bank, Srinakarin Hospital, Faculty of Medicine, Khon Kaen, Thailand. Upon arrival, a blood sample was mixed with an equal volume of 2% (v/v) of fetal bovine serum or FBS (Invitrogen, Waltham, MA, USA) in Phosphate buffer saline (Invitrogen, Waltham, MA, USA). Subsequently, 15 ml of Lymphoprep® solution (STEMCELL™ Technologies, Vancouver, BC, Canada) was mixed with the blood+PBS solution and subjected to centrifuged at 800 g, 18-20°C, for 20 minutes. The supernatant containing plasma was removed and the layer containing human peripheral blood mononuclear cells layer (hPBMCs) was carefully collected without disturbing the underlying clear Lymphoprep layer. hPBMCs were added with 40 ml RPMI-1640 (Invitrogen, Waltham, MA, USA) centrifuged at 300g for 8 ml then discard the supernatant layer. hPBMCs were then mixed with 0.4% Trypan blue (1:1 volume ratio) and counted in an automated cell counter (Biorad automated cell counter TC20TM; Biorad, California, USA). Purified PBMCs (with > 90% cell viability) were preserved in standard cryopreserve reagent and stored in liquid nitrogen until use.

Azulene permeation assay

100 μl of RPMI-1640 supplemented with 10% (v/v) FBS containing hPBMCs at a density of 10,000 cells/well were transferred into a black 96 well plate (Thermo Fisher, Waltham, MA, USA). For the inflamed group, PBMCs were treated with 50 μl of rhTNFα *(final concentration 10 ng/ml, T6674,* Alomone labs, Jerusalem, Israel) for 6 hrs. prior to azulene treatment. For the non-inflamed group, instead of TNFα the normal RPMI was added. One hundred μl of azulene (final concentration of 1, 10 100 or 1,000 μM) was then transferred to a glass slide, centrifuged at 1500 rpm for 5 minutes at room temperature, and observed of fluorescence intensity at 0, 5, 10, 15, 20, 25 and 30 minutes under fluorescence confocal microscope LSM800 inverted laser-scanning confocal fluorescence microscope (LSCFM, DM IL LE, Leica, Wetzlar, Germany) at excitation and emission wavelength 338 and 376 nm, respectively [17] and magnification of 630x. Except otherwise indicated, the experiments were performed n = 3/group and in triplicate fashion and dim condition. A single optimum concentration of azulene giving high contrast of signal was utilized in the following experiments.

Subcellular localization assay

For bio-distribution and subcellular localization of azulene, the inflamed and non-inflamed cells were prepared as mentioned earlier. Prior to azulene treatment, 400 ml of hPBMCs (1 x 10^5 cells/ml) were plated into a 12-well plate, only the inflamed group was treated with 6 hr-TNF-α (final concentration of 10 ng/ml), then 200 µl of azulene was added into each well. At 5, 10, 15, 20, 25, and 30 min, hPMBCs were collected and centrifuged at 2,500 rpm at room temperature for 5 minutes, removed supernatant, substituted with 100 µl of PBS, finally transferred to a glass slide. All cells were subjected to label with following reagent 200 *µL* of 500 nM Mitotracker®, RedCMXRos for 20 minutes and centrifuged at 2,500 rpm for 5 minutes at room temperature, removed supernatant and added 400 µl of PBS, subsequently 200 ml of 500 nM Lyso Tracker®Green DND-26 were added onto the cells for 20 minutes, at last, the cells were centrifuged at 2,500 rpm for 5 minutes at room temperature. Afterward, the supernatant was removed and substituted with 100 µl of PBS. prior to observed under an inverted laser-scanning confocal fluorescence microscope (LSCM LSM 800, Carl Zeiss, Germany) (both reagents were purchased from Cell Signalling Technology, Denver, MA, USA) incubated for 30 minutes at 37˚C, each probe was discarded thereafter sample was treated with above concentrations of azulene.

For observation, excitation and emission of each stain were *λEx* 579/*λEm* 599, *λEx* 504/*λEm* 511, and for Mitotracker and Lysotracker, respectively, under the above fluorescence confocal microscope.

Statistical analysis

Comparisons of azulene permeated through the membrane using fluorescence intensity between normal and inflamed hPBMCs at each time point (5, 10, 15, 20, 25 and 30 minutes) were undertaken. The fluorescence intensity of each stained organelle and merge pattern was descriptively conducted.

Results and Discussion

Azulene concentration selection

To determine the concentration that could be detected the fluorescence intensity, various azulene concentrations (1, 10, 100 and 1,000 µM) was incubated into hPBMCs for 30 minutes. As shown in figure 1 no fluorescence was detectable in normal hPBMCs in all azulene concentrations tested. For figure 2, the fluorescence intensity could not be detected in 1 and 10 µM, a weak signal was observed in 100 µM of azulene, in contrast, 1,000 µM azulene could emit fluorescence signal in the level which can easily be detected with high contrast by confocal fluorescence microscope. Thus 1,000 µM azulene was chosen for the subsequent experiments.

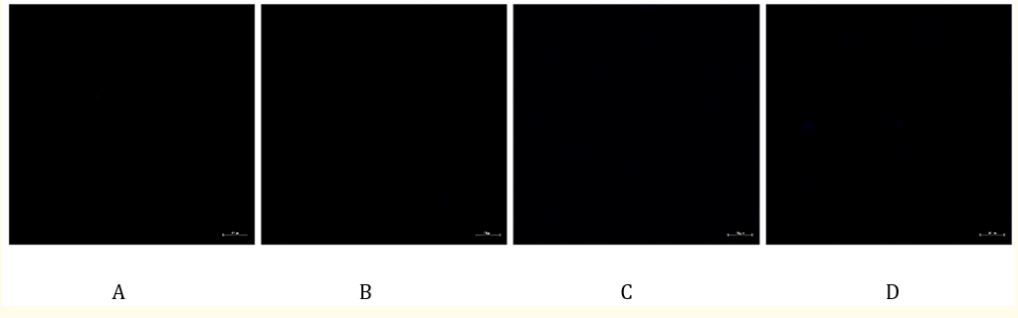


Figure 1: *Fluorescence intensity in various azulene concentrations after 30 minutes incubation A) 1 µM B) 10 µM C) 100 µM and D) 1,000 µM in normal peripheral blood mononuclear cells under laser scanning confocal fluorescence microscope at 338 nm and magnification 630x.*

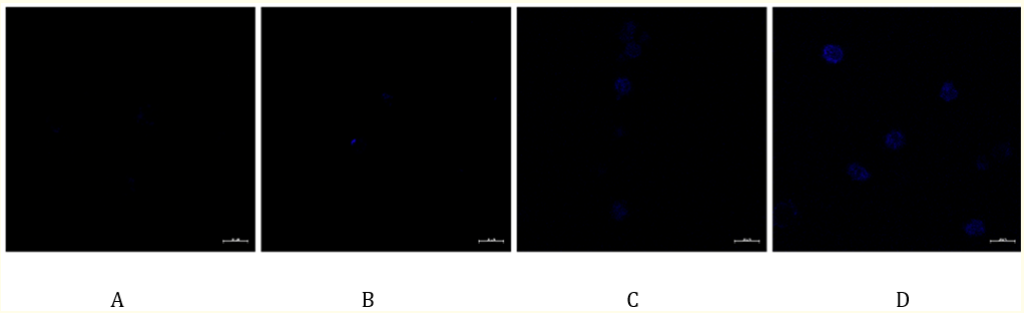
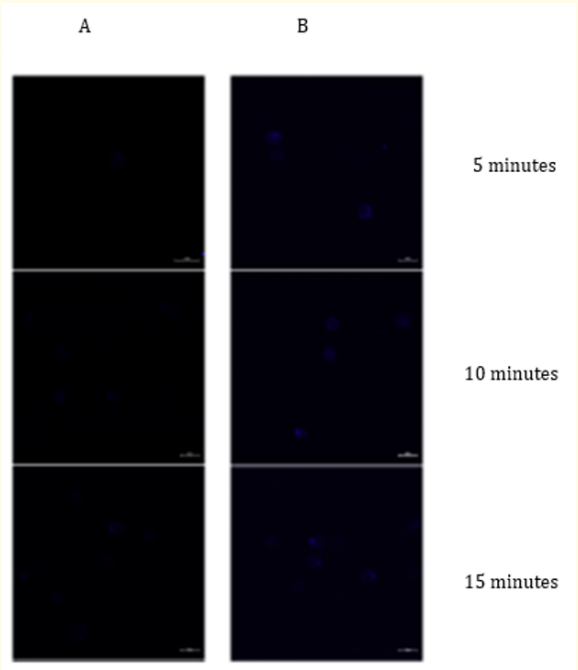


Figure 2: *Fluorescence intensity in various azulene concentrations after 30 minutes incubation A) 1 μM B) 10 μM C) 100 μM and D) 1,000 μM in 10 ng/ml TNF-α-induced inflamed peripheral blood mononuclear cells under a laser scanning confocal fluorescence microscope at 338 nm and magnification 630x.*

Comparison of azulene permeation between normal and inflamed hPBMCs

At the same incubation time, in normal hPBMCs the most prominent intracellular azulene signal was detected at 20 minutes, followed by 15 and 10 minutes, respectively. Interestingly at 30 minutes, almost azulene signal could not be detected (Figure 3A). For inflamed hPBMCs, however, the maximum fluorescence intensity was seen at 25 minutes and the signal was almost maintained until 30 minutes. Notably, we could detect the azulene signal as early as the first 5 minutes (Figure 3B).



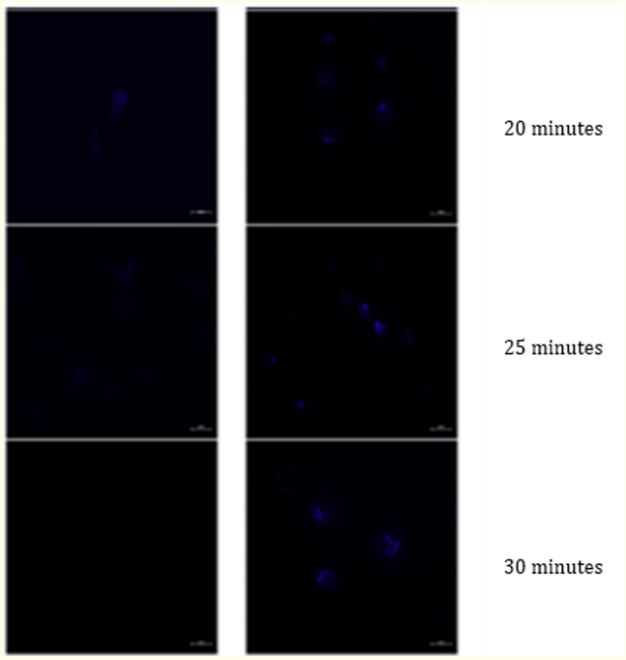
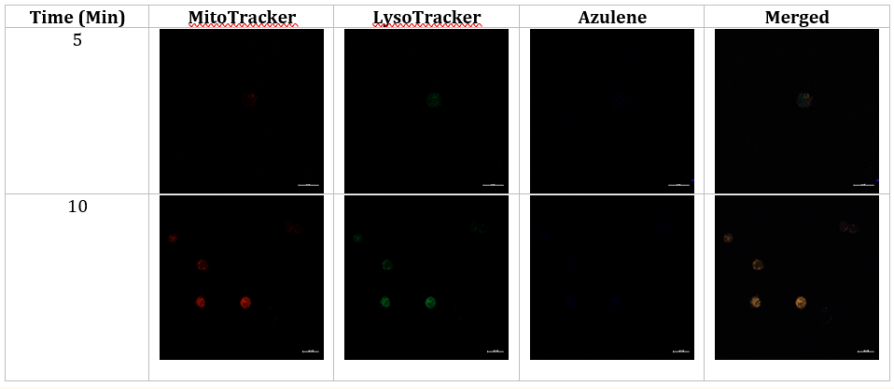


Figure 3: *Comparison of azulene fluorescence signal between A) normal and B) inflamed hPBMCs at various time courses from 5 - 30 minutes using 1000 μM azulene and observed under a laser scanning confocal fluorescence microscope at 338 nm and magnification 630x.*

Subcellular localization of azulene in hPBMCs

Under excitation using light with a wavelength of 511 and 599 nm as recommended by the manufacturer, Lysosome was stained with green color while mitochondria were stained with red color, The signal in both organelles was almost detected in the same area intracellularly (Figure 4 and 5). Notably, azulene signal was also detected in both organelles both in normal and inflamed sPBMCs but in the lesser aggregation in normal cells (Figure 4 and 5).



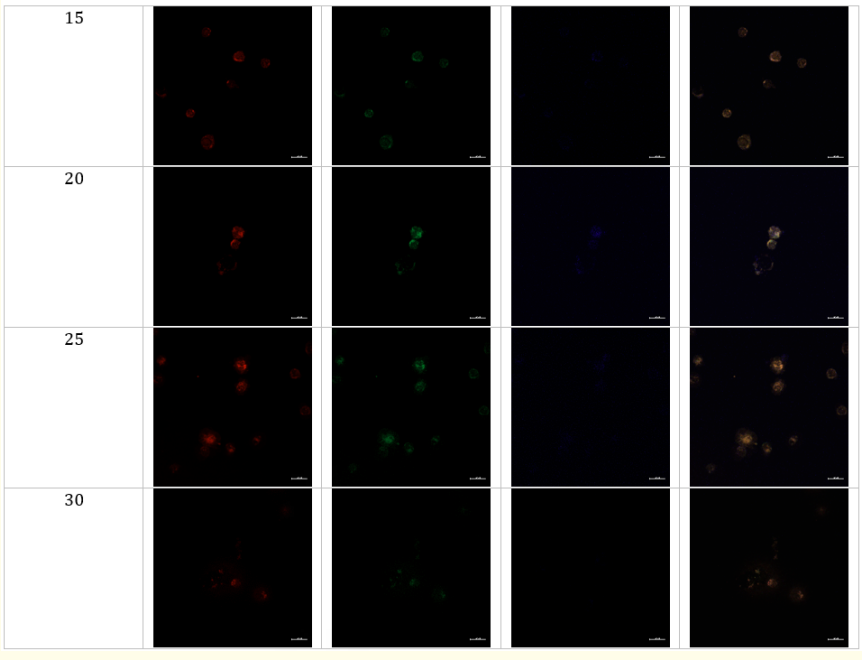
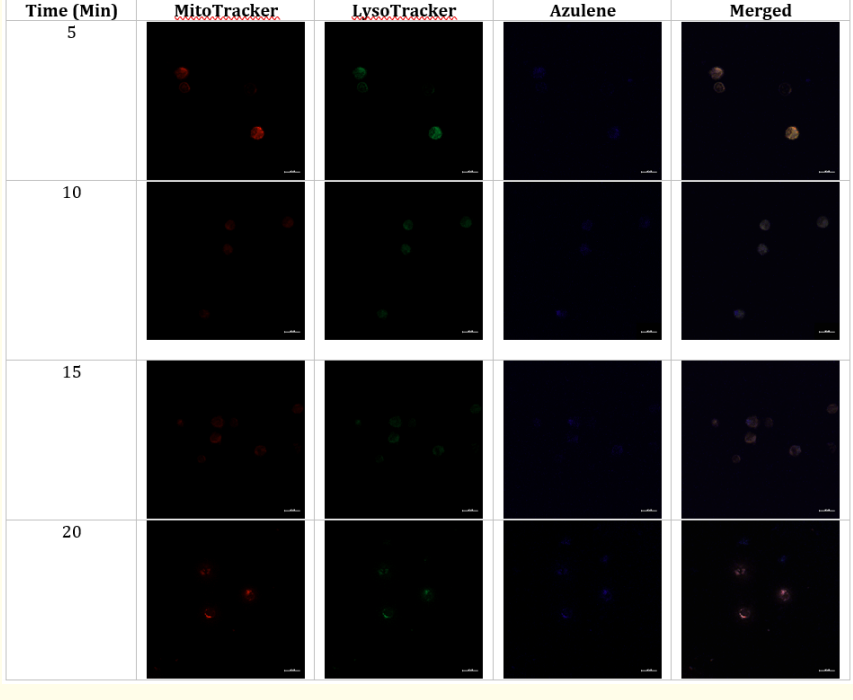


Figure 4: *Subcellular localization of Mitotracker, Lysotracker, Azulene and Merge histological photographs at 5, 10, 15, 20, 25 and 30 minutes in normal hPBMCs observed under a laser scanning confocal fluorescence microscope at 579, 504 and 338 nm for Mitotracker, Lysotracker, and azulene, respectively and magnification 630x.*



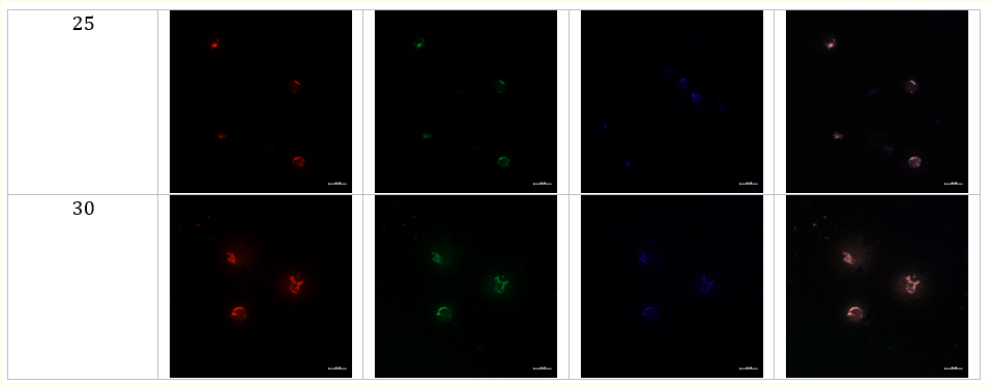


Figure 5: *Subcellular localization of Mitotracker, Lysotracker, Azulene and Merge histological photographs at 5, 10, 15, 20, 25 and 30 minutes in normal hPBMCs observed under a laser scanning confocal fluorescence microscope at 579, 504 and 338 nm for Mitotracker, Lysotracker, and azulene, respectively and magnification 630x.*

The present study successfully demonstrated temporal permeation profile as well as subcellular distribution/organelle localization of azulene and apparently observed the differences in terms of azulene permeation between normal and inflamed peripheral blood mononuclear cells between 5 - 30 minutes.

A previous study demonstrated that azulene preferentially permeated into cancer cells from squamous cell carcinoma when compared with normal epithelium cells. After permeation, azulene induced apoptosis of cancer cells via apoptosis reaction hence leading to successfully inhibited cancer including leukemic cells [18]. Wang., *et al*. proposed 30 minutes-incubation time of azulene for human Jurkat T-cells permeation and showed that within this incubation period azulene could reach the nucleus of cells [11]. This duration, however, is not practical in clinical, thus the present study aimed to observe whether, in case of using shorter pre-irradiation time, azulene could effectively permeate into human peripheral blood mononuclear cells especially inflamed ones in order to develop targeted therapy. Moreover, azulene has a small chemical structure, thus the time for the degradation process of this molecule should be reduced [19].

The present study utilized 1,000 μM of azulene because at lower concentrations, the fluorescence signal was lower than the limitation of the microscope to be detected even we plated the cells at 50,000 cells/ml. In contrast, a previous study was undertaken using hypocrellin as a photosensitizer found that the fluorescence signal from hypocrellin could be observed as low as 5 μM in the condition of cell densities at 25,000 cell/ml. This is due to the small size of azulene molecule compared with hypocrellin [20].

Azulene could permeate into the cells more when the incubation time was increased. Notably, the inflamed cells showed higher permeability that can be explained by the fact that the inflammatory process influences membrane disorganization [21]. We found that the fluorescence intensity at 25 and 30 minutes were almost equal and almost reached the maximum amount which was consistent with the results reported by Wang., *et al*. [11], Afterward, the fluorescence signal was rapidly decreased which was dramatically observed in normal hPBMCs while in an inflamed cell the signal was maintained. This phenomenon may also come from the cyclopentacycloheptene [22] which is prone to degrade easily in an intracellular environment. Further investigation at a longer time was recommended to study the exact time of maximum absorption.

Mitotracker Red CMXRos and Lysotracker Green DND-26 were utilized for tracking substances that localize in mitochondria and lysosome, respectively. Because these organs are crucial target organelles for apoptosis-induced-cell suppression [23-26]. In contrast to cell

membrane-localized photosensitizer, which generally leads to necrosis e.g. methylene blue [27]. Necrosis was used in the treatment of tumors or infections. In the case of inflammatory suppression, the goal is to inhibit inflammatory processes by using pre-apoptotic incubation time (use less time before azulene dramatic accumulation). In some circumstances, especially in severely inflamed cells, it is important to deteriorate these groups of the cell via an interstitial environment-friendly, non-toxic mechanism which is apoptosis. In the present setting, azulene is localized in mitochondria almost in the same pattern as in lysosome, this is consistent with Fan., *et al*. who demonstrated that in cholangiocarcinoma, the photosensitizer has a similar stainability to mitochondria and lysosome [28]. One limitation is our microscope has maximum magnification at 630x, in this magnification the further accurate comparison in the limited cells was difficult. It was explained that in case of azulene localization in the lysosome, this will lead to alteration of lysosomal membrane permeability, and ROS product from the photodynamic reaction will result in membrane disorganization finally inducing apoptosis autophagy and ferroptosis [18]. For mitochondria, an accumulation of azulene and ROS product usually lead to leakage of cytochrome C into cytoplasm and the cell will undergo apoptosis [29]. The localization of photosensitizer in both mitochondria and lysosome in the present study is consistent with a previous study using Pc4 photosensitizer in murine lymphoma [30].

Limitation of the Study

One limitation of the present study was the incapability of nuclear staining. First, we decided to use Hoechst 33342 staining but the nature of blue color emission from this stain [31,32] almost masked the signal of azulene. Attempting to search for nuclear staining with different emission wavelengths is crucial for studying azulene localization in the nucleus. Additionally, those stains should not emit the wavelength of Mitotracker red and Lysotracker Green

Conclusion

The present study demonstrated that azulene could penetrate to inflammatory peripheral blood mononuclear cells better than the normal counterpart. Azulene could localize in Mitochondria and Lysosome in the same fashion. s.

Acknowledgements

We would like to thank all Cytogenetic Unit Staffs of the Faculty of Dentistry, Khon Kaen University for their kind support as well as Miss Peangpen Haputhon, Faculty of Medicine, Khon Kaen University for technical guidance. This work was supported by the Research Fund of Faculty of Dentistry, Khon Kaen University [grant no DTR6403] and Research and Academic Services, Khon Kaen University. The authors thank Dr. Glenn Borlace, Faculty of Pharmaceutical Sciences, Khon Kaen University for English language assistance.

Conflict of Interest

The authors declare no competing interests.

Bibliography

1. Allison RR., *et al*. "Photodynamic therapy for the treatment of nonmelanomatous cutaneous malignancie". *Seminars in Cutaneous Medicine and Surgery* 17.2 (1998): 153-163.

2. Hamblin MR and Abrahamse A. "Factors affecting photodynamic therapy and anti-tumor immune response". *Anticancer Agents in Medical Chemistry* 21.2 (2021): 123-136.

3. Andrejevic-Blant S., *et al*. "Photodynamic therapy of early squamous cell carcinoma with tetra(m-hydroxyphenyl) chlorin: optimal drug-light interval". *British Journal of Cancer* 76.8 (1997): 1021-1028.

4. Morton CA., *et al*. "A randomized, multinational, noninferiority, phase III trial to evaluate the safety and efficacy of BF-200 aminolaevulinic acid gel vs. methyl aminolaevulinate cream in the treatment of nonaggressive basal cell carcinoma with photodynamic therapy". *British Journal of Dermatology* 179.2 (2018): 309-319.

5. Zhang Y., *et al*. "Clinical study on clinical operation and post-treatment reactions of HMME-PDT in treatment of PWS". *Photodiagnosis and Photodynamic Therapy* 20 (2017): 253-256.

6. Zhao Y., *et al*. "Hemoporfin photodynamic therapy for port-wine stain: a randomized controlled trial". *PLoS One* 11.5 (2016): e0156219.

7. Miola AC., *et al*. "Effectiveness and safety of 0.5% colchicine cream vs. photodynamic therapy with methyl aminolaevulinate in the treatment of actinic keratosis and skin field cancerization of the forearms: a randomized controlled trial". *British Journal of Dermatology* 179.5 (2018): 1081-1087.

8. Cai XJ., *et al*. "Photodynamic therapy for intractable bronchial lung cancer". *Photodiagnosis Photodynamic Therapy* 10.4 (2013): 672-676.

9. Damrongrungruang T., *et al*. "Effect of different irradiation modes of azulene-mediated photodynamic therapy on singlet oxygen and PGE2 formation". *Photochemistry and Photobiology* 97.2 (2021): 427-434.

10. Damrongrungruang T., *et al*. "Effects of photodynamic therapy with azulene on peripheral blood mononuclear cell viability and singlet oxygen formation". *Photodiagnosis and Photodynamic Therapy* 24 (2018): 318-323.

11. Wang L., *et al*. "Phototoxicity and DNA damage induced by the cosmetic ingredient chemical azulene in human Jurkat T-cells". *Mutation Research* 562.1-2 (2004): 143-150.

12. Dummin H., *et al*. "Selective photosensitization of mitochondria in HeLa cells by cationic Zn (II) phthalocyanines with lipophilic side-chains". *Journal of Photochemistry and Photobiology B: Biology* 37.3 (1997): 219-229.

13. Teiten M-H., *et al*. "Endoplasmic reticulum and Golgi apparatus are the preferential sites of Foscan® localization in tumor cells". *British Journal of Cancer* 88.1 (2003): 146-152.

14. Hsieh YJ., *et al*. "Subcellular localization of Photofrin determines the death phenotype of human epidermoid carcinoma A431 cells triggered by photodynamic therapy: when plasma membranes are the main targets". *Journal of Cellular Physiology* 194.3 (2003): 363-375.

15. Daugelaviciene N., *et al*. "Lysosome-targeted photodynamic treatment induces primary keratinocyte differentiation". *Journal of Photochemistry and Photobiology B* 218 (2021): 112183.

16. Wang YY., *et al*. "MAL-PDT inhibits oral precancerous cells and lesions via autophagic cell death". *Oral Diseases* 25.3 (2019): 758-771.

17. www.aatbio.com/fluorescence-excitation-emission-spectrum-graph-viewer/azulene

18. Tang D., *et al*. "The molecular machinery of regulated cell death". *Cell Research* 29.5 (2019): 347-364.

19. Andersen FA. "Final report on the safety assessment of azulene". *International Journal of Toxicology* 18.3 (1999): 27-32.

20. Miller GG., *et al*. "Uptake kinetics and intracellular localization of hypocrellin photosensitizers for photodynamic therapy: a confocal microscopy study". *Photochemistry and Photobiology* 61.6 (1995): 632-638.

21. Sedgwick JB., *et al*. "Effects of inflammatory cytokines on the permeability of human lung microvascular endothelial cell monolayers and differential eosinophil transmigration". *Journal of Allergy and Clinical Immunology* 110.5 (2002): 752-756.

22. Bakun P., *et al*. "*In vitro* and *in vivo* biological activities of azulene derivatives with potential applications in medicine". *Medicinal Chemistry Research* 30.4 (2021): 834-846.

23. Luo W., *et al*. "Subcellular location and photodynamic therapeutic effect of chlorin e6 in the human tongue squamous cell cancer Tca8113 cell line". *Oncology Letters* 9.2 (2015): 551-556.

24. Sandberg S., *et al*. "Porphyrin-induced photodamage as related to the subcellular localization of the porphyrins". *Acta Dermato-Venereologica Supplement (Stockhol)* 100 (1982): 75-80.

25. Tynga IM., *et al*. "The primary subcellular localization of Zinc phthalocyanine and its cellular impact on viability, proliferation and structure of breast cancer cells (MCF-7)". *Journal of Photochemistry and Photobiology B* 120 (2013): 171-176.

26. Høgset A., *et al*. "Photochemical internalisation in drug and gene delivery". *Advanced Drug Delivery Reviews* 56.1 (2004): 95-115.

27. Rodrigues GB., *et al*. "Chemical features of the photosensitizers new methylene blue N and S137 influence their subcellular localization and photoinactivation efficiency in *Candida albicans*". *Journal of Photochemistry and Photobiology B* 209 (2020): 111942.

28. Fan Z., *et al*. "ABT737 enhances cholangiocarcinoma sensitivity to cisplatin through regulation of mitochondrial dynamics". *Experimental Cell Research* 335.1 (2015): 68-81.

29. Ali-Seyed M., *et al*. "Photolon™-photosensitization induces apoptosis via ROS-mediated cross-talk between mitochondria and lysosomes". *International Journal of Oncology* 39.4 (2011): 821-831.

30. Trivedi NS., *et al*. "Quantitative analysis of Pc 4 localization in mouse lymphoma (LY-R) cells via double-label confocal fluorescence microscopy". *Photochemistry and Photobiology* 71.5 (2000): 634-639.

31. Shapiro AB and Ling V. "Effect of quercetin on Hoechst 33342 transport by purified and reconstituted P-glycoprotein". *Biochemical Pharmacology* 53.4 (1997): 587-596.

32. Ciano L., *et al*. "The dynamics of azulene S2 state characterised by femtosecond transient absorption spectroscopy". *Journal of Photochemistry and Photobiology A. Chemistry* 105.2-3 (1997): 129-134.

Volume 10 Issue 7 July 2022