EFFICACY OF TRANEXAMIC ACID INTRADERMAL MICROINJECTIONS REDUCING RISK OF POSTINFLAMMATORY HYPERPIGMENTATION AFTER Q-SWITCHED ND: YAG LASER FOR THE TREATMENT OF SOLAR LENTIGINES

BY

MISS RATTIMA SRIEAKPANIT

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE (DERMATOLOGY) CHULABHORN INTERNATIONAL COLLEGE OF MEDICINE THAMMASAT UNIVERSITY ACADEMIC YEAR 2016

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Chairman	(Professor Pichit Suvanprakorn, M.D., Ph.D.)
Member and Advisor	(Punyaphat Sirithanabadeekul, M.D.)
Member and Co-advisor	(Puangpaka Jaiyao, M.D.)
Member	(Suparuj Lueangarun, M.D.)
Director, Graduate Studies	(Professor Kesara Na-Bangchang, Ph.D.)
Dean	(Associate Professor Kammal Kumar Pawa, M.D.)

Thesis Title	EFFICACY OF TRANEXAMIC ACID INTRADERMAL MICROINJECTIONS REDUCING RISK OF POSTINFLAMMATORY HYPERPIGMENTATION FOR THE TREATMENT OF SOLAR LENTIGINES
Author	Miss Rattima Srieakpanit
Degree	Master of Science
Major Field/Faculty/University	Dermatology Chulabhorn International College of Medicine Thammasat University
Thesis Advisor	Punyaphat Sirithanabadeekul, M.D.
Thesis Co-Advisor	Puangpaka Jaiyao, M.D.
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ABSTRACT

Background: Postinflammatory hyperpigmentation (PIH) after solar lentigines removal with 532-nm Q-Switched (QS) Nd: YAG laser is a major concerned cosmetic side effect especially in the patients with darker skin type. Many studies have the efforts to prevent PIH after laser treatment. But the highly effective results could not be detected.

Objectives: The purpose of this study was to evaluate the efficacy and side effects of tranexamic acid (TA) intradermal (ID) microinjections reduction risk of PIH after 532-nm QS Nd: YAG laser for the treatment of solar lentigines.

Methods: Twenty-five patients with fifty solar lentigines were received 532-nm QS Nd: YAG laser treatment. After the laser treatment, one random lesion in each patient was intradermally injected with 50 mg/mL of TA. Another lesion was intradermally injected with normal saline as control group. Pigmentation was measured

by the mexameter as melanin index (MI) and erythema index (EI) at the baseline, week 2, 4, 8 and 12. Photograph evaluation in clearing of pigmentation and severity of PIH by two independent dermatologists was evaluated at baseline, week 2, 4, 8 and 12. The patients evaluated patients' self-improvement score and overall satisfaction at week 2, 4, 8 and 12.

Results: Mean MI at the end of study was statistically significant decreased from 339.71 ± 85.57 to 312.89 ± 73.22 (P=0.009) and from 323.83 ± 67.51 to 300.12 ± 67.79 (P<0.001) in TA and control groups respectively. There was statistically significant in reduction of mean MI at week 4 compared with baseline between two groups (P=0.025). The same as mean MI, there was statistically significant in reduction of mean EI at week 4 compared with baseline between two groups (P=0.030). Overall incidence of PIH was 28%. Most difference of incidence of PIH showed at week 4 (16%). The degree of intensity and extension of PIH in TA group were less severe and widespread than control group. Although, there were no statistically significant in severity of PIH between two groups through the period of study. The side effects of TA were minimal. Two sujects from TA group reported immediate burning sensation that can resolve within an hour without any treatment.

Conclusions: A single dose of 50 mg/mL of ID TA injection can prevent PIH at week 4 after the 532-nm QS Nd: YAG laser for the treatment of solar lentigines. And this method can cause minimal side effects.

Keywords: Tranexamic acid, Solar lentigo, Postinflammatory hyperpigmentation, Q-switched 532-nm Nd: YAG laser

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Miss Rattima Srieakpanit

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LIST OF ABBREVIATIONS

Symbols/Abbreviations	Terms
AA	Arachidonic acid
ABNOMs	Acquired bilateral nevus of Ota-like macules
DOPA	Dihydroxyphenylalanine
EGF	Epidermal growth factor
EI	Erythema index
ET	Endothelin
FGF	Fibroblast growth factor
HGF	Hepatocyte growth factor
HQ	Hydroquinone
ID	Intradermal
IL	Interleukin
IPL	Intense Pulsed Light
IW	Immediate whitening
KCM	Keratinocyte conditioned medium
KGF	Keratinocyte growth factor
LT	Leukotriene
MASI	Melasma Area and Severity Index
MI	Melanin index
mMASI	Modified Melasma Area and Severity Index
MSH	Melanocyte-stimulating hormone
NO	Nitric oxide
PA	Plasminogen activator
PGE_2	Prostaglandin E_2
PIH	Postinflammatory hyperpigmentation
PMN	Polymorphonuclear

PUVA	Psoralen and ultraviolet A
Qhs	Every night at bedtime
QS	Q-switched
RA	Retinoic acid
ROS	Reactive oxygen species
SCF	Stem cell factor
Sc-uPA	Single chain urokinase plasminogen activator
SPT	Skin phototype
TA	Tranexamic acid
TCA	Trichloroacetic acid
TNF-α	Tumor necrosis factor-α
TRP	Tyrosinase-related protein
TXB2	Thromboxane B2
UVA	Ultraviolet A
UVB	Ultraviolet B
UVR	Ultraviolet radiation
VAS	Visual analog scale
VEGF	Vascular endothelial growth factor
vs.	Versus
XP	Xeroderma pigmentosum

CHAPTER 1
INTRODUCTION

1.1 Background and rationale

Solar lentigines are well-defined light to dark brown hyperpigmented macules appearing mostly on the exposed skin from natural sunlight or artificial sources of ultraviolet radiation (UVR). Following the faster improvement outcomes and less pain, laser treatment has become a popular therapeutic modality for those lesions. The 532-nm QS Nd: YAG laser is an effective laser treatment supported by high quality of evidence. The endpoint of 532-nm QS Nd: YAG laser is immediate whitening (IW) via photothermal and photomechanical reactions. Tissue could be injured from these reactions, leading to PIH. PIH is a major concerned side effect for patients with solar lentigines removal by laser treatment, especially those with darker skin type. After laser treatment, the basal cell layer is particularly destroyed. Melanin is dropped into dermis and phagocytosed by melanophage in the upper dermis, leading to dermal melanosis. PIH is composed of excess melanin production or abnormal distribution of melanin pigment in the epidermis or dermis. The injury of keratinocytes in epidermis from laser produces inflammatory mediators. Leukotrienes (LT) C4, LTD4, prostaglandin E_2 (PGE_2) and thromboxane B2 (TXB2) can increase melanin synthesis and transfer melanin to surrounding keratinocytes. From previous studies, the incidence of PIH after the 532-nm QS Nd: YAG laser treatment was approximately 6.67-53% in Asians and 24% in Thailand. Many studies have been conducted to minimize risk of PIH. Postoperatively, short- term application of topical corticosteroids can significantly reduce the risk of PIH after ablative fractional CO_2 laser resurfacing in Asians with atrophic acne scars. However, topical corticosteroids may interfere wound healing process, increase risk of acneiform eruption and infection.

1.2 Research question

TA, an antifibrinolytic agent, inhibits plasminogen activator (PA) by Reversibly blocking synthetic derivative of lysine binding sites on plasminogen molecules, so the plasminogen in the epidermal basal cells and keratinocytes cannot convert to the plasmin. As well, phospholipase A2 precursors for the membrane phospholipid secretion of arachidonic acid (AA), a precursor of PGE_2 and LT, cannot be activated. In the meantime, the keratinocyte-PA system activation by UVR induces melanogenesis process. So, TA can reduce melanogenesis and inhibit inflammatory mediators. In previous studies, various forms of TA were applied on hyperpigmented lesions, including the oral and ID injections forms of TA which found to be effective for melasma treatment. Nonetheless, the oral form of TA can cause several systemic side effects, such as gastrointestinal discomfort, myocardial infarction, pulmonary embolism and thromboembolism. Whereas, the ID TA injection may possibly yield efficacious outcomes in melanin reduction and anti-inflammation process for PIH treatment. Hence, this study aimed to compare the pigment alteration after solar lentigines removal using 532-nm QS Nd: YAG laser following by ID injection of 50 mg/mL TA versus normal saline.

1.3 Specific objective

The primary objective is to evaluate the efficacy of TA ID microinjections reduction risk of PIH after 532-nm QS Nd: YAG laser for the treatment of solar lentigines.

The secondary objectives are to evaluate the side effects of TA ID microinjections reduction risk of PIH after 532-nm QS Nd: YAG laser for the treatment of solar lentigines and to evaluate the incidence of PIH after 532-nm QS Nd: YAG laser for the treatment of solar lentigines

1.4 Hypothesis

ID injection of 50 mg/mL TA has the benefit outcome in reducing risk of PIH after solar lentigines removal with 532-nm QS Nd: YAG laser.

1.5 Keywords

Tranexamic acid
Solar lentigo
Postinflammatory hyperpigmentation
Q-switched 532-nm Nd: YAG laser

1.6 Ethic consideration

The study protocol was approved by Human Ethics Committee of Thammasat University.

1.7 Limitation

Number of sample size

1.8 Expected benefits and application

PIH is a major concerned side effect for patients with solar lentigines removal by laser treatment, especially those with darker skin type. It effects on the cosmetic outcome and patient's self-esteem. Many studies have the efforts to prevent PIH after laser treatment. But the highly effective results could not be detected. This research was to evaluate the efficacy and safety of TA ID microinjections reducing risk of PIH after 532-nm QS Nd: YAG laser for the treatment of solar lentigines. If we can prevent PIH

from the 532-nm QS Nd: YAG laser for solar lentigines removal, the patients will get the better cosmetic result and quality of life.

1.9 Obstacles and strategies to solve the problems

Diagnosis of PIH and poor clearing of pigmentation were difficultly evaluated. PIH was defined as dark lesion that was previously cleared or lighter by laser treatment or lesion without crust that darker than baseline. And poor clearing of pigmentation defined as persisted lesion that was lighter than or as dark as baseline.

Table 1.1 Administration and time schedule

	2016					2017					
	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN
Research proposal											
Ethic approval											
Data collection											
Data analysis											
Conclusion and report											
Publication											

CHAPTER 2
REVIEW OF LITERATURE

Solar lentigines are well-defined light to dark brown hyperpigmented macules varying in size from a few millimeters to several centimeters in diameter that appear mostly on natural sunlight or artificial sources of UVR exposed skin such as the dorsum hands, face and forearms (1). Solar lentigines are common in Caucasians and Asians of Mongolian extraction (2). There are various treatments of solar lentigines such as cryotherapy, laser, chemical peels, dermabrasion and topical treatment (3).

Nowadays, laser treatment becomes popular among patients because the lesions can improve faster and get less pain. From Pigmentary Disorders Academy (PDA) adapted guidelines for solar lentigines treatment from US Preventive Services Task Force (USPSTF) on health care showed the 532-nm QS Nd: YAG laser is the effective treatment that was supported with the evidence from randomized controlled trial (3). But they can develop the side effects that lead to the cosmetic problems.

PIH become a major concerned cosmetic side effects for patients with solar lentigines removal by laser especially patients with darker skin type that there are abundant melanin contents in the epidermis (4). IW is a favorable clinical endpoint that is achieved by the appropriate energy setting (5). PIH usually develops after inflammatory stimuli. Following cutaneous inflammation, melanocytes can alter the number of melanin (normal, increased or decreased). PIH composed of excess melanin production or abnormal distribution of melanin pigments in the epidermis or dermis. Two main pathogenesis of PIH are epidermal and dermal hypermelanosis. After injury, AA could be oxidized to prostaglandins or LT. Melanocytes are believed that can be stimulated by LTC4, LTD4, PGE_2 and TXB2 (6). In addition, cytokines and inflammatory mediators (interleukin-1(IL-1), IL-6, tumor necrosis factor-α (TNF-α), epidermal growth factor (EGF) and reactive oxygen species (ROS) such as nitric oxide (NO)) can also stimulate the melanocytes (7, 8). These stimulations lead to an increase melanin synthesis and melanin transfer to surrounding keratinocytes. If basal keratinocytes are destroyed, the keratinocytes that contain an increase melanin will be

phagocytozed by melanophages in the upper dermis. And they produced a blue-gray discoloration (9). From previous studies, the incidence of PIH from 532-nm QS Nd: YAG laser was 6.67-53% in Asians and average 24% in Thailand (5, 10-12). Many studies have the efforts to prevent laser-induced melanogenesis especially from ablative laser. Recently, no highly effective prevention was detected. Pretreatment topical glycolic acid and hydroquinone (HQ) combine with tretinoin cannot decrease the incidence of hyperpigmentation following ablative CO_2 laser resurfacing in the patients with skin type I-III (13). A short-term application of topical corticosteroids postoperatively can reduce risk of PIH after ablative fractional CO_2 laser resurfacing in Asian with atrophic acne scars. However postoperative topical corticosteroids may interfere wound healing, increase risk of acneiform eruption and infection (14).

TA or trans-4-aminomerthyl cyclohexane carboxylic acid is an antifibrinolytic agent. It inhibits PA by reversibly blocking synthetic derivative of lysine bonding sites on plasminogen molecules (15). Thus, the plasminogen cannot convert to the plasmin. Precursors of phospholipase A2 that assist membrane phospholipid secrete AA cannot be activated (16). AA is a precursor of PGE_2 and LT which lead to melanogenesis and inflammatory process. From the study in human keratinocytes and melanocytes, they showed plasminogen was found in the human epidermal basal cells and keratinocytes (17). The keratinocyte-PA system that activated by UVR can induce the melanogenesis process (18, 19). In vitro study showed that TA reduced melanin contents and tyrosinase activity in treated melanocytes and also decreased tyrosinase, tyrosinase-related protein-1 (TRP-1), TRP-2 protein level (20). Extracellular kinase signaling pathway and microphthalmia-associated transcription factor were also inhibited (21). In previous studies, various forms using of TA were applied on hyperpigmented lesions especially melasma. Oral TA was effective as the adjuvant in combination with IPL or Nd: YAG laser for melasma. But oral form can cause the several systemic side effects such as gastrointestinal discomfort, myocardial infarction, pulmonary embolism and thromboembolism (22). Efficacy of topical TA in the treatment of melasma is controversial (21, 23-26). A study in ID injection of TA were effective and safe for the

treatment of melasma (27). So, PIH should get the benefit from TA on melanin reduction and anti-inflammation process.

CHAPTER 3
SOLAR LENTIGO

3.1 Historical background

Hutchison defined the pigmented macules that increased with age in 1892 (28). Cawley and Curtis described a unique lesion that appeared mostly in elders in term of solar lentigo in 1950 (29). Lesion consisted of an intraepidermal melanocytes proliferation in elongated epidermal rete ridges. Hodgson assured melanocytic proliferation in solar lentigo in 1963 (30). Montagna et al. accentuated that the lesion involved both melanocytes and keratinocytes proliferation in 1980 (31).

3.2 Epidemiology

The solar lentigines are common among population with red hair and blue eyes who get easily sunburn and poorly tan. And they are common in the younger with acute or chronic sun exposure. The incidence of solar lentigines increases with advancing age. The study showed solar lentigines found in 90% of the Caucasian over 60 years of age (30). Psoralen and ultraviolet A (PUVA) lentigines are solar lentigines induced by psoralen and ultraviolet A radiation phototherapy. The incidence occurred 40-50% in patients with average of 5.7 years after starting therapy. PUVA lentigines developed in 6-8 months after single phototoxic dose of PUVA (32). Frequency and severity of lesions relate with the total number of treatment (33). Sunbed lentigines are lentigines that caused by ultraviolet A (UVA) tanning bed using. The lesions develope after 50 or more exposure (34). Reticulated or "ink-spot" lentigines are induced after single severe sunburn. These lentigines are common in patients with skin phototype (SPT) I. All lesions occur in Celtic ancestry patients who had numerous solar lentigines. They usually have only one black lentigo (35).

3.3 Etiology and pathogenesis

Solar lentigines are the marker of UVR exposures either intermittent high-intensity UVR or cumulative UVR. UVR has a major role in pathogenesis of solar lentigines as distribution on sun-exposed area after chronic PUVA phototherapy, tanning bed use and sunburn after 20 years of age (36). Increased epidermal melanocytes hyperplasia and number of keratinocytes and melanocytes after repeated UVR exposure are the primary defected of solar lentigines. The recent study demonstrates that solar lentigines are characterized by an increased vasculature (37). Chronic sun exposure induces vascular endothelial growth factor (VEGF) secretion from keratinocytes. VEGF is a major angiogenic factor from UV-irradiated skin (38). Increased vasculature in solar lentigines are thought to be the consequences of increased expression of VEGF (37). In addition, melanogenic paracrine factors such as keratinocyte growth factor (KGF), hepatocyte growth factor (HGF), stem cell factor (SCF) and endothelin 1 (ET-1) are found in the development of solar lentigines (39-42). Microarray analysis of solar lentigines showed upregulation of genes related to inflammation, fatty acid metabolism and downregulation of cornified envelope-related genes. These suggested that solar lentigines are induced by the mutation from repeated UVR exposures (43).

Melanocytes of early childhood exposed to UVR are developed melanocytic hyperplasia that lead to melanocytic neoplasia or dysplasia later (44). Some lesions are fixed cellular atypia and intraepidermal melanocytes hyperplasia that may lead to UVR induced dysplastic and neoplastic process. SPTs, ethnicity, age and burning or tanning response to sunlight are individual susceptibility for lentigo development. After chronic photochemotherapy for 1-2 years receiving, the presence of epidermal melanocytic cellular atypia can develop and persist after discontinued therapy (33). Patients with xeroderma pigmentosum (XP) increase sensitivity to UVR. They develop hyperpigmented macules on sun-exposed skin in the first 5 years of life. Those macules have the hyperplasia of atypical epidermal melanocytes. From the previous studies,

mutation in melanocytic lineage and solar lentigines are common precursor of malignant melanoma in patients with XP (45).

3.4 Clinical findings

Solar lentigines are well-circumscribed macules with smooth or irregular outlines. They are various in shape such as round, oval or irregular. And they are also various in color from light to dark brown or black. The size can be 3 mm to 2 cm in diameter. The lesions are predominant in sun-exposed area such as the face, dorsum of hands and forearms, upper chest and back. Patients with fair skinned particularly with red hair and lighter eye color easily developed solar lentigines (1). Solar lentigines are the marker of solar damage and risk factor for melanoma, basal cell cancer and squamous cell cancer (46).

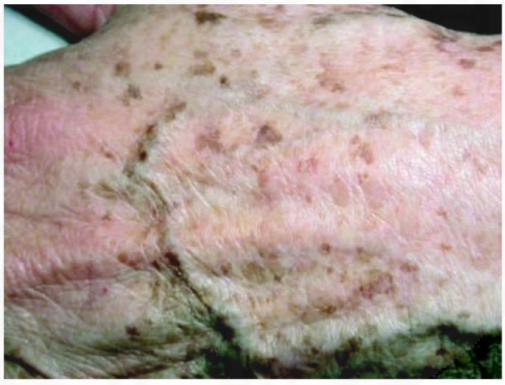

Figure 3.1 Solar lentigines of the hand (47)

Reticulated black or "ink-spot" solar lentigines are irregular border and jet-black color solar lentigines. These lesions are usually less than 5 mm in diameter. The lesions confine on sun-exposed area and look like ink spots. Only one ink-spot solar lentigo usually appears near multiple solar lentigines. Reticulated black solar lentigines are common in patients with fair-skinned or SPTs I-II.

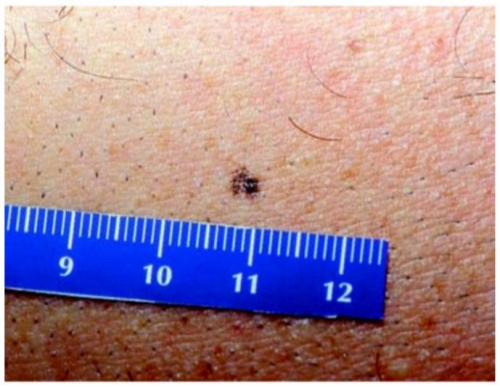

Figure 3.2 Reticulated black lentigo (47)

PUVA lentigines are well-defined hyperpigmented macules that develop after PUVA phototherapy. These lentigines develop up to 50% of patients treated with PUVA phototherapy within 5- 7 years. And the lesions can persist until discontinued phototherapy. Numbers of treatments, sex and age of starting therapy are associated with severity and frequency of lesions but not associated with SPT (33).

Solar lentigines in patients with XP are darkly and irregularly pigmented macules. The lesions are on sun-exposed skin within the first 5 years of life and persist although sun avoidance. Observation in XP population showed melanocytic hyperplasia within unstable solar lentigines. Unstable solar lentigines typically are isolated, irregular pigmented macules on solar damage background. The lesions have melanocytic hyperplasia that not extended beyond the margin of lesions. The lesions are darker, enlarger and more distinctive border during follow-up (48).

3.5 Histopathology

The histology of solar lentigines shows elongated club- shaped or budlike epidermal rete ridges that sometimes branches or fuses. There are thinned or atrophic epidermis between rete ridges. They mildly increase in numbers of epidermal melanocytes without nesting and increase melanocyte activity. Proliferation of keratinocytes and melanocytes are found. Dermis composes of solar elastosis and

moderate perivascular mononuclear cell infiltration (31). From electron microscope, the lesions show larger melanosome complexed in keratinocytes when compared with surrounding keratinocytes. Marked dihydroxyphenylalanine (DOPA) reactivity and large numbers of melanosomes suggest hyperactivity of melanocytes in solar lentigines. DOPA-reactive melanocytes in sun-exposed skin increase two-fold when compared with sun-protected skin (49).

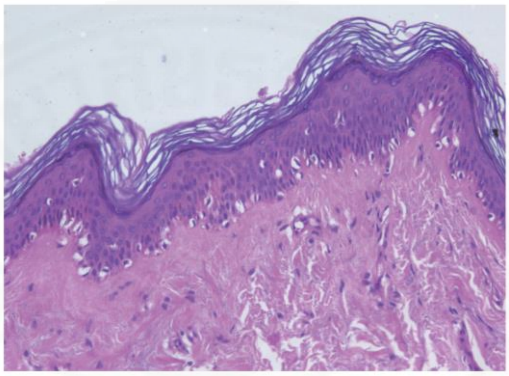

Figure 3.3 Histology of solar lentigo (50)

Upregulation of melanocyte-related gene, marked increased inflammation-related genes, downregulation of cornified envelope-related genes and moderate increased of KGF are found from immunohistochemical studies of solar lentigines (43).

The histology of PUVA lentigines reveals elongated epidermal rete ridges with increases numbers of melanocytes and large cell bodies. Atypical cellular morphologic features are found (33). Melanocytes have autophagocytosis, sharp nuclear contours invagination, double nuclei, melanin macroglobules and melanosomal alterations. Epidermal melanocytic atypia can persist for many years after treatment (51).

The histology of ink-spot lentigines shows epidermal hyperplasia, mildly increased melanocyte numbers and increased melanin at basal layer but skip areas at rete ridges (35). And upper dermis is infiltrated with melanophages (52).

3.6 Dermoscopic findings

Dermoscopic findings show homogenous pattern and diffuse light brown fingerprint-like structure with sharply, moth-eaten or jelly-like borders. Thin reticular lines and circular hypopigmented follicular openings (pseudo-network) could be found in the lesions (47).

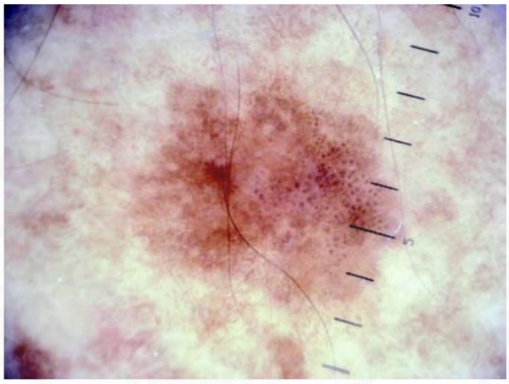

Figure 3.4 Dermoscopic image of solar lentigo (47)

3.7 Differential diagnosis

Solar lentigines should be differentiated with hyperpigmented macules lesions such as simple lentigines, ephelides, melasma and lentigo maligna (33).

Simple lentigines are well-defined, round-to-oval, homogeneous, brown or brownish-black macules. The lesions are usually less than 5 mm in diameter. The lesions can occur on sun-protected area. Simple lentigines are smaller and darker than solar lentigines. These lesions are less associated with UV exposure (53).

Ephelides or freckles are well defined and irregular light brown macules. The lesions occur on sun-exposed areas such as the face, the dorsum of hands and forearms. Freckles are common in patients with red hair and light skin. The lesions appear during childhood. They may be darker after sun exposure and fate after non-exposed UVR for a long time (54).

Melasma is symmetrical well-defined irregular border hyperpigmented patches. The lesions are common on sun-exposed area especially the face. Melasma mostly occurs in middle-aged females (55).

Lentigo maligna must be excluded especially suspected lesion in patients with XP and PUVA phototherapy. Lentigo maligna is slow-growing and usually arises in elderly on the sun-exposed area especially face and neck. It is a pre-cancerous lesion that can convert to malignancy in 5% of patients especially in the lesions larger than 4 cm. The ABCDE rule can help to diagnosis the melanoma (56).

Table 3.1 Differential diagnosis of solar lentigines by dermoscopic examination

Lesions	Dermoscopic findings
Solar lentigines (47)	Light brown fingerprint-like structure with pseudonetwork, milia-like cysts, horny pseudocysts, sharp, moth-eaten or jelly-like border, asymmetric follicular openings, hairpin vessels
Simple lentigines (47)	Brown or brownish black uniform network with thin pattern
Ephelides/ Freckles (1)	Uniform pigmentation, moth-eaten border
Melasma (57)	Light yellow to dark brown uniform patches, diffuse reticular pigmentation, jelly sign (sparing follicles and sweat gland openings that produced pseudonetwork with concave borders), dark brown or black granules

Lesions	Demoscopic findings
Lentigo maligna (56)	Annular, granular, zigzag or target-like pattern, dark or blue homogeneous areas, pseudonetwork, asymmetric follicular openings, red rhomboidal structures, gray brown streaks, white scar-like areas, circle within circle, milky red area, increased density of vascular network

3.8 Treatment of solar lentigines

There are two main categories of solar lentigines treatment including physical and topical therapies. Physical therapies compose of cryotherapy, chemical peels, intense pulsed light (IPL) and laser treatment. Topical therapies compose of HQ, tretinoin, adapalene or combination of multiple topical therapies (58). Adapted the guidelines for the treatment of solar lentigines by Pigmentary Disorders Academy shows as Table 3.4.

Table 3.2 Level of evidence (59)

Level of evidence	Definition
A	Good evidence supported this procedure
B	Fair evidence supported this procedure
C	Poor evidence supported this procedure
D	Fair evidence supported the rejection of this procedure
E	Good evidence supported the rejection of this procedure

Table 3.3 Quality of evidence (59)

Quality of evidence	Definition
I	Evidence obtained from at least one properly designed, randomized controlled trial
II-i	Evidence obtained from well designed controlled trials without randomization
II-ii	Evidence obtained from well designed cohort or case control analytic studies, preferably from more than one center or research group
II-iii	Evidence obtained from multiple time series with or without the intervention. Dramatic results in uncontrolled experiments (such as the results of the introduction of penicillin in the 1940s) could also be regarded as this type of evidence
III	Opinions of respected authorities based on clinical experience, descriptive studies, or reports of expert committees
IV	Evidence inadequate owing to problems of methodology (e.g. sample size, or length or comprehensiveness of follow-up or conflicts in evidence)

Table 3.4 Level and quality of evidence for solar lentigines therapies (3)

Therapy	Quality of evidence	Level of evidence
Cryotherapy	I	A
Laser		
• QS ruby	II-i	A
• Alexandrite	II-i	B
• 532-nm Nd: YAG	I	A
• CO_2	I-ii	B
• Argon	I-ii	B

Therapy	Quality of evidence	Level of evidence
• HMG K1 krypton	I-ii	B
• Diolite 552-nm	II-i	B
IPL	III	B
Dermabrasion	III	D
Chemical peels		
• 30% TCA	II-ii	C
Topical		
• 3% HQ	IV	C
• 0.01% RA	I	C
• 0.05% RA	I	A
• 0.1% RA	I	B
• 2% 4HA (mequinol)	I	B
• 2% Mequinol + 0.01% RA	I	A
• 0.1-0.4% RA + 5% HQ	II-iii	B
• 2% HQ/ cyclodextrin	II-i	C
• 0.1-0.3% Adapalene	I	B
• 0.1% Tazarotene	I	B

4HA, 4-Hydroxyanisole; HQ, hydroquinone; IPL, intense pulsed light; RA, tretinoin; TCA, trichloroacetic acid

From the Table 3.4, the treatments of choice are cryotherapy, 532-nm Nd: YAG laser, 0.05% RA and 2% mesquinol combined with 0.01% RA. The topical therapies may take a longer time than physical therapies to complete the results but they can be easily controlled that make the patients get less side effects (3). Laser therapies become popular choice of treatment due to they can improve lesion faster and get the less pain. But patients can get cosmetic side effects from the laser treatment such as alteration of pigmentation.

The 532-nm QS Nd: YAG laser is generally used for pigmented lesions removal. Its therapeutic endpoint is IW that caused by releasing of gas bubbles into the tissue from rupture of melanosomes (60). The QS laser destroys melanin and melanosomes with photothermal and photomechanical reaction that induce inflammation via acoustic effect (5).

CHAPTER 4
POSTINFLAMMATORY HYPERPIGMENTATION

4.1 Introduction

PIH is a common pigmentary disorder. This condition can develop in all skin types particularly darker skin types (SPTs IV-VI). It occurs at the site of inflammation. Epidermal melanosis appears as brown pigmentation. Whereas dermal melanosis appears as blue-gray pigmentation. This condition makes the patients distress and loss of self-esteem. And it is very difficult to treat (61). Then the prevention is the good choice for management.

4.2 Pathogenesis of postinflammatory hyperpigmentation

Inflammation can result in hyperpigmentation through many mechanisms. After cutaneous inflammation, alterations of melanocyte numbers are found including normal, increased or decreased. PIH is arisen from excessive melanin production or abnormal distribution of melanin in epidermis or dermis. Pathogenesis of PIH includes two mechanisms; epidermal and dermal hypermelanosis (6). After 532-nm QS Nd: YAG laser treatment, tissue is damaged with abrasion wound. And skin is preceded to healing process. Inflammatory phase composes of two essential cascades; vascular and cellular cascades. Vascular cascade is initiated with intact vessels alteration. Vasodilaion followed by vasoconstriction occurs (62). After collagen contact with plasma protein, Hageman factor or factor XII is activated to factor XIIa. Factor XIIa has a major role in complement, clotting and fibrinolysis system activation (63).

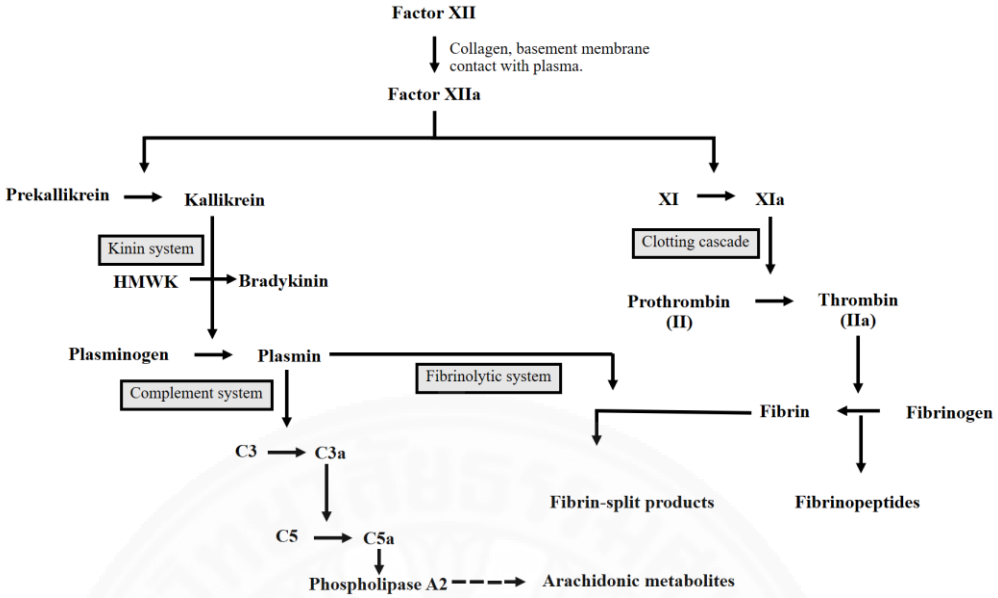

Figure 4.1 Plasma protein systems (63)

Membrane damage and C5a can activate phospholipase A2 that assist plasma membrane to release AA. AA could be oxidized to PGE_2 and LT that lead to melanogenesis and inflammatory process (63). LT stimulates melanogenesis via increasing melanocyte cell size and growth, increasing tyrosinase activity and forming new dendritic processes (6).

For cellular cascade, polymorphonuclear leukocytes (PMNs) are early migration to injury site followed by macrophages, lymphocytes, eosinophils and basophils. These inflammatory cells secrete IL-1α, IL-6, TNF-α, EGF, ET-1, SCF and ROS (7, 8). These lead to melanin transfer to surrounding keratinocytes and increase melanin synthesis. When the basal keratinocytes are destroyed, melanin will be dropped to upper dermis and phagocytzed by melanophages (9). In addition, IL-1α can stimulate KGF that release from fibroblasts. KGF binds to KGF receptors on epithelial cells lead to uptake of melanosomes. KGF receptors are varying in skin type (64). Additionally, injury can induce epidermal cell release hormone that induces pigmentation such as α-melanocyte-stimulating hormone (α-MSH) (65)

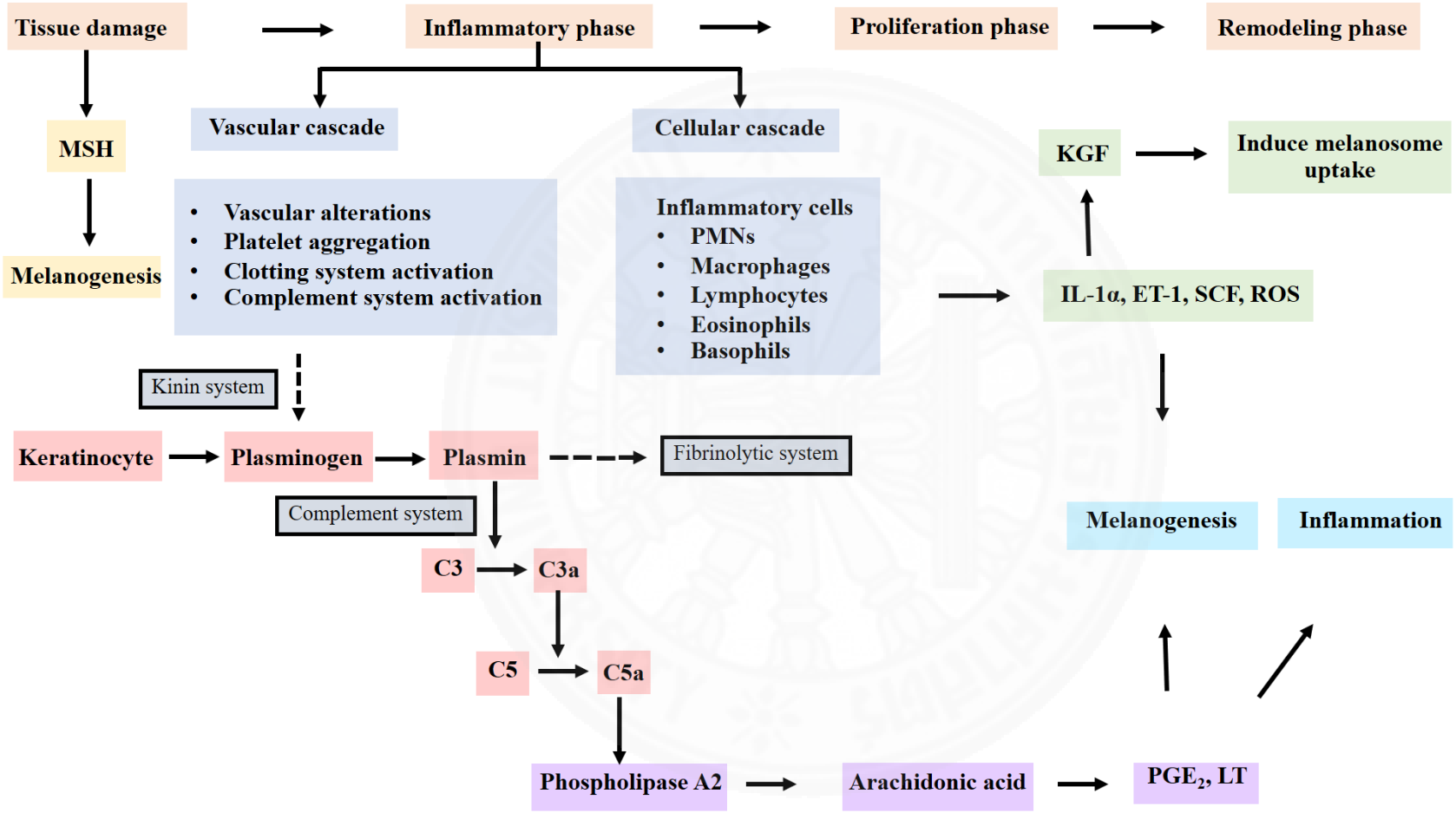

Figure 4.2 Pathogenesis of postinflammatory hyperpigmentation (7, 8, 62-66)

4.3 Incidence of PIH after 532-nm QS Nd: YAG laser in the treatment of solar lentigines

From the previous study, PIH after 532-nm QS Nd: YAG laser in the treatment of solar lentigines presented from 3 to 48 weeks after treatment (mean 4.3 weeks) and persisted for 2 to 24 weeks (mean 8.4 weeks). They showed no association between age, sex, SPTs and the incidence of PIH (67).

Table 4.1 Incidence of PIH after QS Nd: YAG laser in the treatment of solar lentigines

Year/Race	Authors	Subjects	Intervention	Outcome
2000/American	Todd *et al.* (11)	27 patients with multiple solar lentigines at the backs of both hands (SPTs I-IV)	Total of 4 treatment areas on back of each hand - Gr 1: Liquid nitrogen cryotherapy - Gr 2: Frequency-double QS Nd: YAG laser - Gr 3: HGM K1 krypton laser - Gr 4: Diolite 532-nm diode-pumped vanadate laser	Frequency-double QS Nd: YAG laser was most significant lightening ($p<0.05$), followed by krypton laser, 532-nm diode-pumped vanadate laser and liquid nitrogen **PIH incidences** at 12 weeks - QS Nd: YAG laser 1.33% (1/75) - Krypton laser 2.67% (2/75) - Others 0% (0/75)

Year/Race	Authors	Subjects	Intervention	Outcome
2013/Asian	Negishi *et al.* (5)	193 patients with 355 solar lentigines (SPTs III-V)	Divided in 4 groups - Gr 1 (n=62): 694-nm QS Ruby with aggressive irradiation (very obvious IW endpoint) - Gr 2 (n=61): 694-nm QS Ruby with mild irradiation (slightly IW endpoint) - Gr 3 (n=35): 532-nm QS Nd: YAG with aggressive irradiation - Gr 4 (n=35): 532-nm QS Nd: YAG with mild irradiation	No significant differences in degrees of clearance among 4 groups **PIH incidences**: 33.3%, 7.47%, 23.18% and 8.47% in Gr 1, 2, 3 and 4 respectively
2015/Korean	Kim *et al.* (10)	20 patients with solar lentigines on the face	Two sessions of 532-nm QS Nd: YAG laser at 4-week intervals in all solar lentigines	- Excellent response 55% - Marked response 20% **PIH incidences** 20%

Year/Race	Authors	Subjects	Intervention	Outcome
2016/Thai	Vachiramon *et al.* (12)	25 patients with at least two lesions of solar lentigines on upper extremities (SPT III-IV)	Two lesions were randomly selected for single session treatment of - Gr 1: 532-nm QS Nd: YAG laser - Gr 2: Fractional CO_2 laser	532-nm QS Nd: YAG laser showed significant improvement over fractional CO_2 laser **PIH incidences** (no significant difference from both lasers): - QS Nd: YAG laser 24% - Fractional CO_2 laser 28%

4.4 Prevention of PIH from laser treatment

Table 4.2 Prevention of PIH from laser treatment

Year/Race	Authors	Subject	Procedure	Intervention	Time	Outcome
1999/American	West and Alster (13)	100 patients (SPTs I-III)	Ablative fractional CO_2 laser resurfacing	Divided in 3 groups - Gr 1 (n=25): 10% glycolic acid, twice a day for at least 2 weeks - Gr 2 (n=25): 4% HQ Qhs and 0.025% tretinoin cream for at least 2 weeks - Gr 3 (n=50): no pretreatment	Preoperative	No significant difference between groups

Year/Race	Authors	Subject	Procedure	Intervention	Time	Outcome
2011/Japanese	Kato *et al.* (68)	32 patients with senile lentigines with or without melasma	694.5-nm QS Ruby laser	Divided in 2 groups - Gr 1 (n=15): oral TA 750 mg/day for 4 weeks - Gr 2 (n=17): control	Postoperative	Oral TA may not be effective for preventing PIH after QS Ruby laser.
2012/Thai	Unaboonkul *et al.* (69)	25 patients with ABNOMs	1,064-nm QS Nd: YAG laser	Split face Twice a day for 2 weeks of - Gr 1: Fucidic acid - Gr 2: Fucidic acid combined with betamethasome valerate cream	Postoperative	No significant difference in both groups

Year/Race	Authors	Subject	Procedure	Intervention	Time	Outcome
2013/Asian	Negishi *et al.* (5)	193 patients with 355 solar lentigines (SPTs III-V)	Divided in 4 groups Gr 1 and 2: 694-nm QS Ruby laser Gr 3 and 4: 532-nm QS Nd: YAG laser	-Gr 1 (n=62): aggressive irradiation (obvious IW endpoint) -Gr 2 (n=61): mild irradiation (slightly IW endpoint) -Gr 3 (n=35): aggressive irradiation -Gr 4 (n=35): mild irradiation	Operative	Mild irradiation reduces risk of PIH without advantage in efficacy.

Year/Race	Authors	Subject	Procedure	Intervention	Time	Outcome
2015/Asian	Cheyasak *et al.* (14)	40 patients with atrophic facial acne scar (SPTs IV-V)	Ablative fractional CO_2 laser resurfacing	Split face - Gr 1: 0.05% Clobetasol propionate ointment twice daily for the first 2 days then petrolatum jelly 4 times a day for the rest of week - Gr 2: Petrolatum jelly alone 4 times a day for 7 days	Postoperative	Significant higher incidence of PIH in petrolatum alone (75%) vs. intervention (40%)

CHAPTER 5
TRANEXAMIC ACID

5.1 Introduction

Tranexamic acid or trans-4-aminomethyl cyclohexane carboxylic acid is the synthetic derivative of amino acid lysine that usually uses as an antifibrinolytic agent (23, 26).

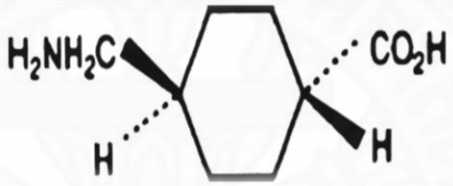

Figure 5.1 Structure of tranexamic acid (70)

5.2 Pharmacokinetics of tranexamic acid (71)

Absorption

- Onset of action: 5-15 minutes
- Duration: 3 hours

Distribution

- Protein binding: 3% at therapeutic plasma levels

Metabolism

- Half-life: 2 hours for the terminal elimination phase

Excretion

- Urine: more than 95% of the dose as unchanged drug
- Excretion: 90% at 24 hours after intravenous administration of 10 mg/kg body weight

5.3 Mechanism of tranexamic acid

TA inhibits PA as competitive inhibitor by reversibly blocking lysine binding sites on plasminogen molecules. So, the plasminogen cannot convert to the plasmin (70).

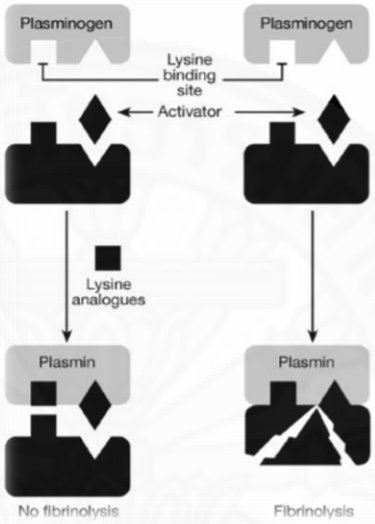

Figure 5.2 Competitive inhibitor of plasminogen activation (70)

From previous studies, plasminogen was found in the human keratinocytes and epidermal basal cells (16). In general plasmin will activate precursors of phospholipase A2 that assist membrane phospholipid to secrete AA (72). If plasminogen cannot convert to plasmin, AA will not be secreted from membrane phospholipid. AA is a precursor of PGE_2 and LT. Both lead to melanogenesis and inflammatory process. UVR can activate the keratinocyte-PA system that induce melanogenesis process. In vitro study revealed that melanin contents, tyrosinase activity, TRP-1 and TRP-2 protein levels can be reduced by TA. Single chain urokinase PA (Sc-uPA) in keratinocyte can induce tyrosinase activity, increase dendrites, cell perimeter and cell area (73). Plasmin can release basic fibroblast growth factor (FGF) which is a potent melanocyte growth factor (74).

Table 5.1 Pigmentation reducing action of tranexamic acid

Year	Authors	Subject	Outcome
1998	Maeda *et al.* (18)	UV exposed skin of Weiser-Maples guinea pigs was applied with topical TA	TA has a dose-dependent decrease in AA-induced pigmentation.
2007	Seong *et al.* (20)	Cultured normal human melanocytes of neonatal foreskin	- TA decreased viability of the melanocytes in dose dependent manner ($p<0.05$). - After UV exposure, TA significantly decreased melanin synthesis (expression of TRP-1, TRP-2 and tyrosinase) by UVB irradiation ($p<0.05$).
2007	Maeda *et al.* (73)	Human melanocytes cultured in keratinocyte conditioned medium (KCM)	- TA inhibited the tyrosinase inducing activity of human melanocytes cultured in KCM without affecting viability. - Keratinocyte-activate-melanocyte pathway can be inhibited by TA.

Year	Authors	Subject	Outcome
2010	Li *et al.* (75)	UVB exposed guinea pig skin was injected with ID TA.	- Melanin content was significantly reduced. - The number of melanocytes at the basal layer of exposed epidermis was not significantly reduced.

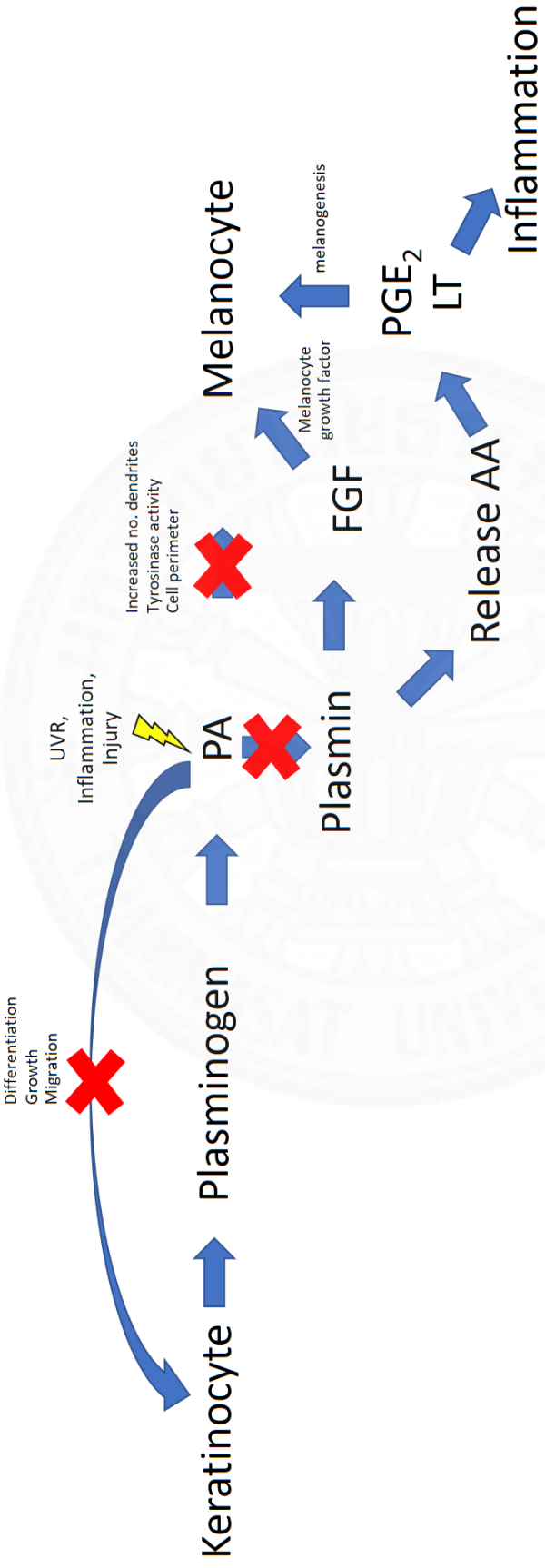

Figure 5.3 Mechanism of tranexamic acid (70)

5.4 Role of tranexamic acid for PIH treatment

In previous studies, various forms of TA were applied on hyperpigmented lesions particularly melasma. Oral TA was effective as the adjuvant in combination with IPL or Nd: YAG laser in melasma treatment. But the systemic side effects such as gastrointestinal discomfort, myocardial infarction, pulmonary embolism and thromboembolism can develop by oral form (22). Whereas, the efficacy of topical form in the treatment of melasma is controversial (21, 23-26). The studies in ID injection of TA in melasma treatment were effective and safe (27). PIH should get the benefit from TA on melanin reduction and anti-inflammatory process.

Table 5.2 Efficacy of tranexamic acid in the treatment of melasma

Year/Race	Authors	Subject	Intervention	Duration	Outcome	Side effects
Oral 2013/Korean	Cho *et al.* (22)	51 patients (SPTs III-IV)	Retrospective study All patients received IPL and 3-4 times of low fluence QS Nd: YAG laser - Gr 1 (n=24): Oral TA 500 mg/day during IPL and laser treatment - Gr 2 (n=27): only IPL and laser treatment	8 months	- Gr 1: mMASI score decreases from 11.33 ± 7.07 to 6.21 ± 5.04 (p<0.001) - Gr 2: mMASI score decreases from 11.70 ± 6.72 to 8.93 ± 5.89 (p<0.001) - Reduction of mMASI score higher in Gr 1 than Gr 2 (p=0.005)	No serious side effects

Year/Race	Authors	Subject	Intervention	Duration	Outcome	Side effects
Combination 2013/Korean	Na *et al.* (24)	25 patients 20-55 years old	2 tablets of TA 125 mg + ascorbic acid 50 mg + L-cysteine 4 mg + calcium pantothenate 4 mg + pyridoxine chloride 1 mg 3 times/day and topical TA twice daily	8 weeks	- Mean MI significantly decreased. - Histological analysis showed reduction of mast cell counts, vessel numbers and epidermal pigmentation.	No serious side effects
Topical 2012/Thai	Kanechorn Na Ayuthaya *et al.* (23)	23 patients 32-45 years old	Spilt face - Gr 1: 5% topical TA twice daily - Gr 2: Vehicle twice daily	12 weeks	- Lightening of pigmentation was showed on both sides. - The result was not significant.	More erythema at TA-treated side ($p<0.05$)

Year/Race	Authors	Subject	Intervention	Duration	Outcome	Side effects
2014/Iranian	Ebrahimi and Naeini (25)	50 patients with moderate-to-severe epidermal melasma >18 years old	Double blind split face - Gr 1: 3% topical TA - Gr 2: 3% HQ + 0.01% dexamethasone twice daily	12 weeks	- MASI score significantly decreased in both groups. - No significant difference between both groups	- Side effects of Gr 2 were significantly prominent than Gr 1 (p=0.01). - Gr 1: scale, erythema, irritation, xerosis - Gr 2: scale, erythema, irritation, dryness, hypertrichosis, inflammation

Year/Race	Authors	Subject	Intervention	Duration	Outcome	Side effects
2015/Iranian	Banihashemi *et al.*(26)	30 patients >18 years old	Split face - Gr 1: 5% topical liposomal TA twice daily - Gr 2: 4% HQ twice daily	12 weeks	- Mean MASI scores significantly decreased in both sides ($p<0.001$). - Greater decrease in liposomal TA group but not significant	Irritation 3/23 in HQ group
2016/Korean	Chung *et al.* (21)	15 patients	Split face IPL 1 time/month Total 4 sessions Followed with - Gr 1: 2%TA - Gr 2: Vehicle without TA	16 weeks	- MI and mMASI significantly decreased from baseline to 12 weeks on topical TA side but not on vehicle side. - Topical TA can prevent rebound pigmentation after IPL treatment.	None

Year/Race	Authors	Subject	Intervention	Duration	Outcome	Side effects
Injection 2006/Korean	Lee *et al.* (27)	100 patients with moderate-to-severe melasma 29-46 years old	4 mg/mL of TA was intradermally injected on lesion at 1 cm intervals once a week.	12 weeks	Significantly decrease in MASI scores from baseline to 8 and 12 weeks (13.22 ± 3.02 versus 9.02 ± 2.62 at week 8 and vs. 7.57 ± 2.54 at week12; $p<0.05$ both)	Burning and mild wheal immediately appeared at injection site and resolved within 10 minutes.

Year/Race	Authors	Subject	Intervention	Duration	Outcome	Side effects
2009 Brazilian	Steiner *et al.* (76)	18 female patients	- Gr 1 (n=9): At-home 3% topical TA twice a day - Gr 2 (n=9): 12 applications of ID TA (4 mg/mL)	2 weeks	- Significant improvement of MASI scores in each group - No significant difference between group in MASI improvement (p=0.6512) - Significant improvement on both groups by colorimetric evaluation (p=0.0008)	Minimal side effects such as erythema, local bruising, burning that well tolerated by patients

Year/Race	Authors	Subject	Intervention	Duration	Outcome	Side effects
2013/Indian	Budamakuntla *et al.* (77)	60 patients 18-50 years old	- Gr 1 (n=30): Multiple microinjections of TA (4 mg/mL) ID (1 cm interval) - Gr 2 (n=30): microneedling of 0.5-1 mL of TA (4 mg/mL) in vertical, horizontal and both diagonal directions for 4-5 times	2 months	- 35.72% MASI improvement in Gr 1 compared to 44.41% in Gr 2. - At the end of 3rd follow-up visit, 6 patients (26.09%) in Gr 1, as compared to 12 patients (41.38%) in Gr 2 showed more than 50% improvement	No major adverse events in both groups

Year/Race	Authors	Subject	Intervention	Duration	Outcome	Side effects
2015/Egyptian	Elfar and El-Maghraby (78)	60 female patients (SPTs III-V)	- Gr 1 (n=20): 0.05 mL TA (4 mg/mL) ID into lesion weekly (1 cm interval) -Gr 2 (n=20): topical silymarin cream (14 mg/mL) 1 fingertip of cream to cover affected area twice daily - Gr 3 (n=20): 50% glycolic acid peeling within period of 20-30 secs (erythema) every 2 weeks	2 weeks	- Highly statistically significant difference of change in mMASI scores after treatment among 3 groups - Gr 3 showed highest efficacy followed by Gr 2 and the least efficacy was Gr 1	- Gr 1: burning pain and wheal in all patients, erythema in 5 patients (25%) - Gr 2: no side effect - Gr 3: PIH in 6 patients (30%), highly significant difference between Gr 1 and both groups ($p<0.001$)

CHAPTER 6
RESEARCH METHODOLOGY

6.1 Subjects

Males or females with at least 2 solar lentigines on the forearms are included into the study. One lesion in each patient will be randomly injected with ID TA (N=26). Another in each patient will be injected with ID normal saline (N=26). Study protocol was approved by the Human Ethics Committee of Thammasat University.

6.1.1 Sample size

Sample size was calculated by program G* Power 3.1.7.

Effect size = 0.3

α error probability = 0.05

Power (1-β error probability) = 0.90

Number of groups = 2

Total sample size = 26

6.1.2 Inclusion criteria

(1) Male or female with multiple solar lentigines

(2) SPTs III-V

(3) Age 50-70 years old

(4) At least two similar lesions distribute on the forearms including shape, color and size

6.1.3 Exclusion criteria

(1) Skin lesion at the site of treatment

(2) Photosensitive skin

(3) Received isotretinoin, HQ, whitening agents and depigmenting treatment of ablative or non-ablative laser within preceding 3 months

(4) Topical analgesic cream or tranexamic allergy

(5) Participated in major outdoor activities

(6) Pregnancy or lactating

(7) Smoking

(8) Coagulation disorders

(9) Wound healing disorders

6.1.4 Discontinuous criteria

(1) Patients get the severe side effects during or after treatment such as burn from laser treatment, anaphylaxis during TA injection.

(2) Patients who cannot follow-up as the research protocol will be excluded.

6.2 Drugs

Transamin® (250 mg/ 5 mL or 50 mg/mL of TA) was manufactured by OLIC (Thailand) Limited and licensed by DAIICHI SANKYO CO., LTD., Tokyo, Japan.

Normal saline (0.9% NaCl) was manufactured by OTSUKA OPV JOINT STOCK COMPANY, Dong Nai, Vietnam.

EMLA® (2.5% lidocaine and 2.5% prilocaine) was manufactured by Recipharm Kariskoga AB, Kariskoga, Sweden.

6.3 Research design

(1) Subjects will be cleaned their forearms with mild cleansers before treatment.

(2) Pretreatment melanin and erythema index are measured by reflectance spectrophotometer (Mexameter MX18, Courage and Khazaka; Cologne, Germany).

(3) Pretreatment photographs are taken with Sony Cyber-Shot DSC R100.

(4) Preoperative topical analgesic cream (2.5% lidocaine and 2.5% prilocaine, EMLA®) is applied and occluded at lesions 45 to 60 minutes before treatment.

(5) All of lesions are treated by the 532-nm QS Nd: YAG laser (Lutronic Spectra XT) with spot size 1.8 mm with a fluence of 0.6 to 0.8 J/cm^2 that is determined by the clinical endpoint (slightly IW).

(6) By double-blind method, the 50 mg/mL TA is immediately injected in one lesion after laser treatment. And normal saline is injected with 30-gauge, 0.5 mL insulin syringe under sterile condition at 1 cm intervals (0.1 mL/cm^2).

(7) After the laser treatment, vaseline is immediately applied on the both lesions twice daily until the crusts peel off.

(8) Patients are instructed to apply broad-spectrum sunscreen with SPF 40 for 12 weeks, avoid sun exposure and avoid the use of any topical preparations on the lesions for the period of the study.

6.4 Outcome measurements

6.4.1 Subjective measurements

6.4.1.1 Image analysis

Series of photographs will be taken every visit. Clearing of lesion, severity of PIH will be evaluated by two independent dermatologists.

(1) Clearing of pigmentation (Quartile scale) determines improvement of lesion's color.

Worsened

Poor: <25% improvement

Fair: 26-50% improvement

Good: 51-75% improvement

Excellent: 76-100% improvement

(2) Severity of PIH determines level of hyperpigmentation after laser treatment. The evaluation is measured in 2 categories; degree of intensity and degree of extension.

Degree of intensity

None: no PIH

Minimal: 1-25% darkening

Mild: 26-50% darkening

Moderate: 51-75% darkening

Severe: 76-100% darkening

Degree of extension

None: no PIH

Minimal: involving <25% of the treated area

Mild: involving 26-50% of the treated area

Moderate: involving 51-75% of the treated area

Widespread: involving 76-100% of the treated area

6.4.1.2 Diascopic examination will be evaluated by two independent dermatologists at week 2, 4, 8 and 12. Diascopy is used to determine if erythema lesion is caused by blood within superficial vessels (inflammatory or vascular lesions), or is caused by hemorrhage (petechiae or purpura). Microscope slide is pressed against a lesion to see whether it blanches. Inflammatory and vascular lesions will blanch. Whereas hemorrhagic lesions will not blanch. In addition, diascopy can also indicate sarcoid skin lesions as turn an apple jelly color.

6.4.1.3 Patient assessment is indicator that evaluate by the patient at week 2, 4, 8 and 12. Patient assessment composes of patient's self-improvement score and patient overall satisfaction.

Patient's self-improvement score (Quartile scale)

None: no improvement

Minimal: 1-25% improvement

Mild: 26-50% improvement

Moderate: 51-75% improvement

Remarkable: >75% improvement

Patient overall satisfaction

1: Not satisfied

2: Slightly satisfied

3: Satisfied

4: Very satisfied

5: Extremely satisfied

6.4.1.4 Adverse effects determine unusual effects from the treatment. Adverse effects are evaluated at immediate post operation, week 2, 4, 8 and 12 by patients and dermatologists. Patients will be asked to report any adverse effects such as pain (be evaluated by visual analog scale or VAS), erythema, burning, itching, pigmentation change including hyperpigmentation, hypopigmentation, purpura and textural alteration.

6.4.2 Objective measurement

6.4.2.1 Mexameter will be measured for three times and used the mean. Mexameter was measured at baseline, week 2, 4, 8 and 12.

Melanin index determines a parameter from mexameter measurement that is mainly influenced by the melanin contents.

Erythema index determines a parameter from mexameter measurement that is mainly influenced by the hemoglobin contents.

6.5 Data analysis

Data will be analyzed by SPSS 21 software (SPSS, Chicago, IL, USA). P-value <0.05 is considered as statistically significance. P-value of continuous data such as MI and EI corresponds to Repeated ANOVA test. For the discrete data such as patient's self-improvement scores, patient overall satisfaction, and clearing of pigmentation, p-value corresponds with Wilcoxon Signed Ranks test. And for proportion such as incidence of PIH, p-value corresponds to Fisher's exact test.

CHAPTER 7
RESULTS

The total of 26 patients were recruited in this study. One patient was loss to follow-up.

7.1 Demographic data

The twenty-five patients with fifty solar lentigines were evaluated in this research including 18 females (72%) and 7 males (28%). The average age of subjects was 60.88 years. Most of patients were SPT IV (68%). And most of them had solar lentigines for 5-10 years (48%), more than 10 years (32%) and less than 5 years (20%). The demographic data was showed as Table 7.1. Fluence of the 532-nm QS Nd: YAG laser was 0.6-0.8 J/cm^2 (mean 0.66 $\pm$ 0.08 J/cm^2). Period of peeled-off of crust was 4 to 25 days (mean 14.6 $\pm$ 4.93 days).

Table 7.1 Demographic data

Variables	Mean ± SD
Age	60.88 ± 6.64
Sex, n(%)	
Female	18 (72%)
Male	7 (28%)
Duration	
<5 yr	5 (20%)
5-10 yr	12 (48%)
>10 yr	8 (32%)
SPT, n(%)	
III	5 (20%)
IV	17 (68%)
V	3 (12%)

7.2 Mexameter measurements

7.2.1 Melanin index

Mean MI at the end of study significantly decreased from 339.71 ± 85.57 to 312.89 ± 73.22 (P=0.009) and from 323.83 ± 67.51 to 300.12 ± 67.79 (P<0.001) in TA and control groups respectively. Mean MI in both groups have significantly decreased since week 2. Reduction of mean MI at week 4 compared with baseline between TA and control groups was statistically significant (P=0.025).

Table 7.2 Melanin index

Week	TA	Control	p-value
Baseline	339.71 ± 85.75	323.83 ± 67.51	0.501
2	336.55 ± 92.04	312.52 ± 65.63	0.329
4	301.75 ± 78.02	307.60 ± 68.97	0.794
8	311.71 ± 83.52	292.44 ± 74.22	0.425
12	312.89 ± 73.22	300.12 ± 67.79	0.562
p-value	0.009*	<0.001*	
Baseline vs. week 2	-3.16 ± 53.28	-11.30 ± 43.88	0.585
Baseline vs. week 4	-37.96 ± 30.17	-16.22 ± 31.96	0.025*
Baseline vs. week 8	-28.00 ± 40.45	-31.38 ± 21.82	0.735
Baseline vs. week 12	-29.20 ± 47.66	-24.39 ± 25.18	0.682

Values presented as mean ± SD. P-value corresponds to Repeated ANOVA test.

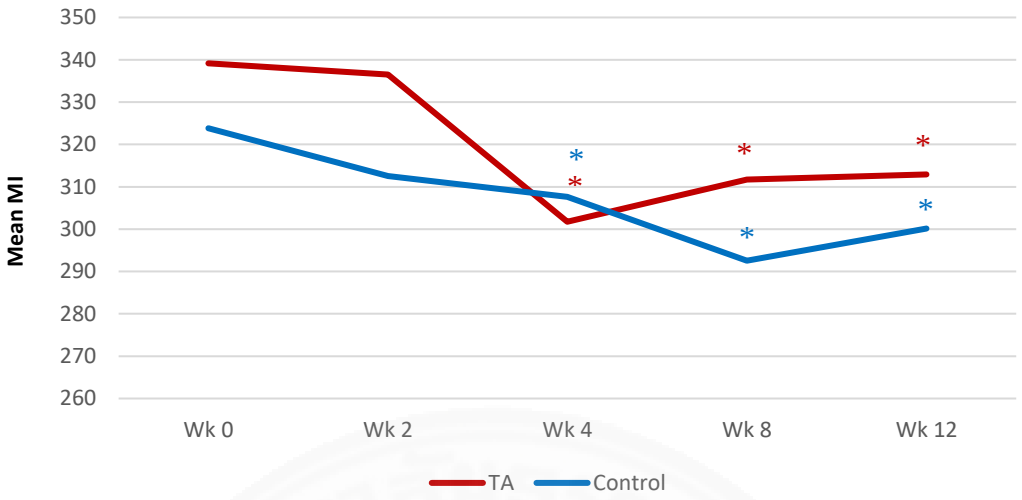

Figure 7.1 Comparison of mean MI at week 0, 2, 4, 8 and 12 between TA group and control group (*P-value<0.05)

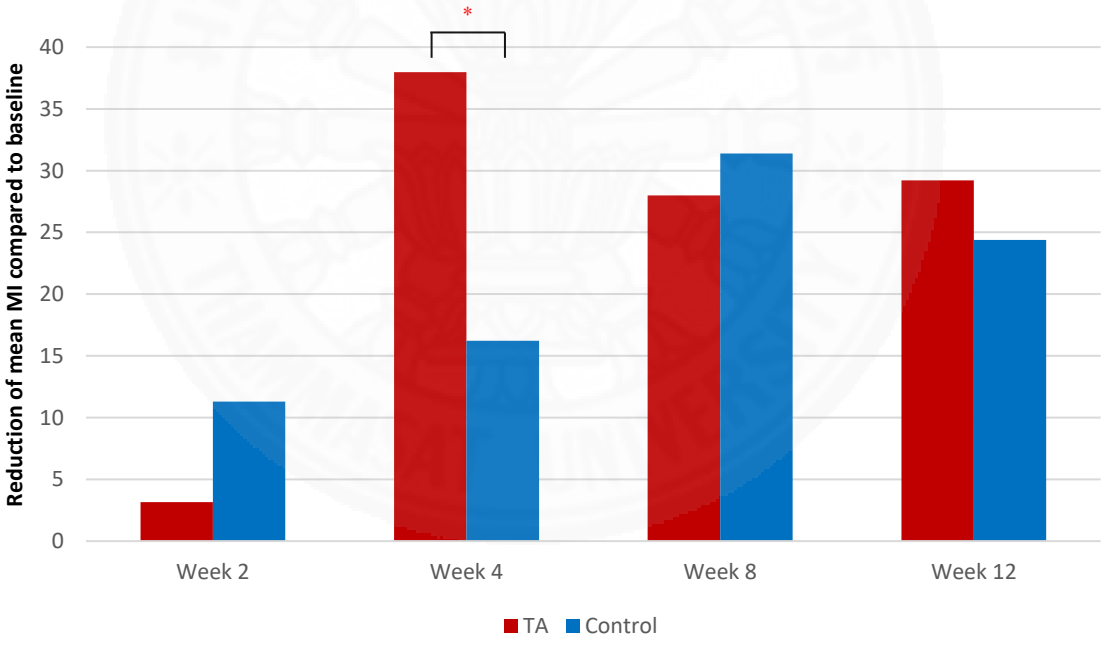

Figure 7.2 Comparison of reduction of mean MI at week 2, 4, 8 and 12 compared to baseline between TA group and control group (*P-value<0.05)

7.2.2 Erythema index

Mean EI at the end of study significantly decreased from 373.08 ± 60.31 to 339.56 ± 60.52 (P<0.001) and from 365.04 ± 55.68 to 344.93 ± 65.44 (P=0.022) in TA

and control groups respectively. Mean EI significantly decreased at week 2 and 8 in TA and control groups respectively. Difference of mean EI between TA and control groups was statistically significant at week 4 compared with baseline (P=0.030).

Table 7.3 Erythema index

Week	TA	Control	p-value
Baseline	373.08 ± 60.31	365.04 ± 55.68	0.639
2	351.12 ± 53.22	349.23 ± 53.90	0.904
4	339.77 ± 59.69	360.23 ± 55.92	0.234
8	344.37 ± 49.84	348.20 ± 52.29	0.799
12	339.56 ± 60.52	344.93 ± 65.44	0.774
p-value	<0.001*	0.022*	
Baseline vs. week 2	-21.96 ± 41.06	-15.82 ± 40.39	0.609
Baseline vs. week 4	-33.31 ± 46.78	-4.82 ± 39.57	0.030*
Baseline vs. week 8	-28.71 ± 37.54	-16.85 ± 37.22	0.284
Baseline vs. week 12	-34.04 ± 40.11	-20.12 ± 38.11	0.235

Values presented as mean ± SD. P-value corresponds to Repeated ANOVA test.

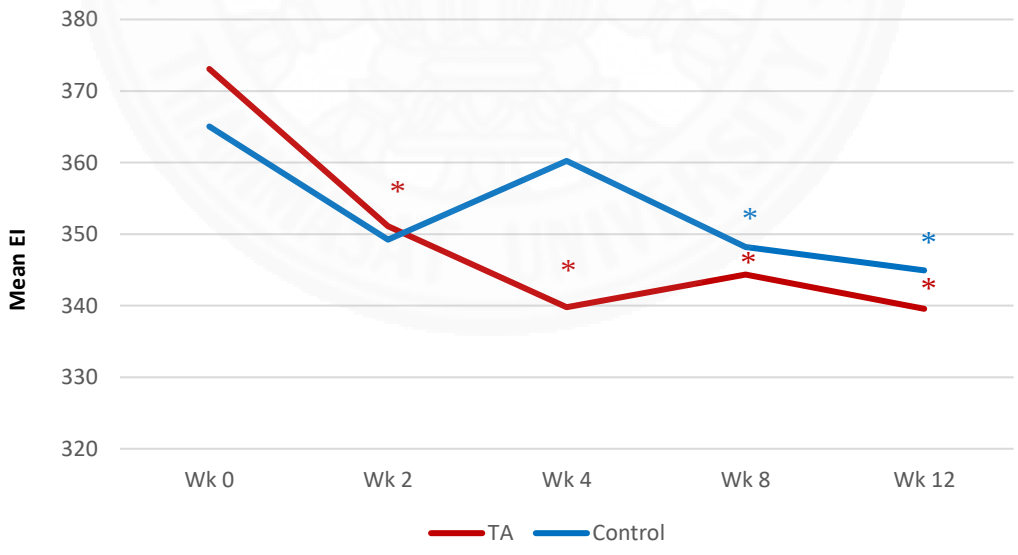

Figure 7.3 Comparison of mean EI at week 0, 2, 4, 8 and 12 between TA group and control group (*P-value<0.05)

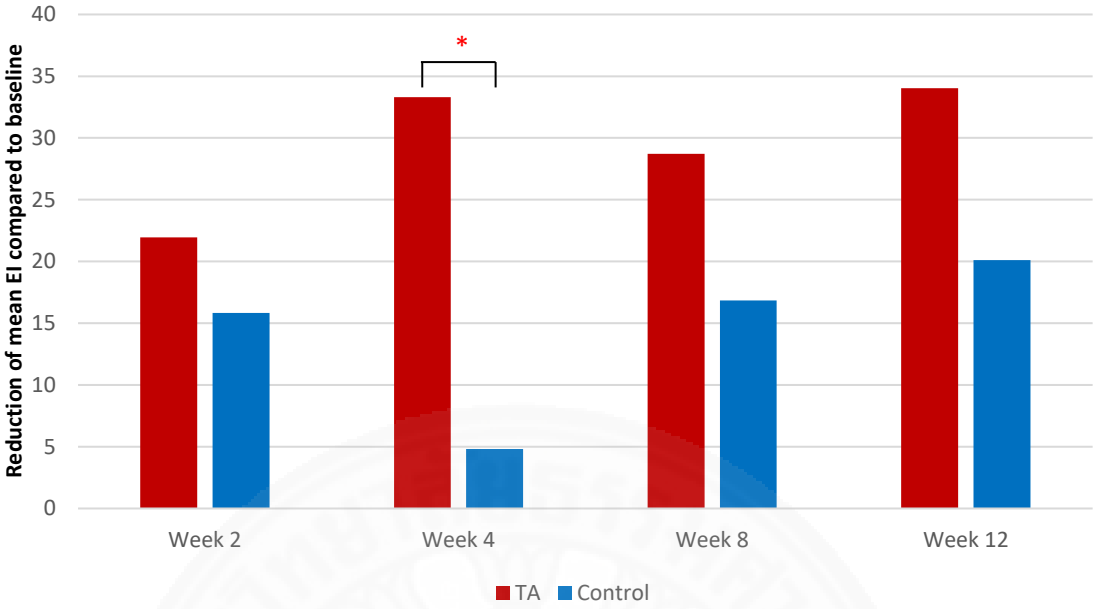

Figure 7.4 Comparison of reduction of mean EI at week 2, 4, 8 and 12 compared to baseline between TA group and control group (*P-value<0.05)

7.3 Image analysis

The photographs of all subjects were evaluated by two independent dermatologists. The photographs were taken at baseline, week 2, 4, 8 and 12.

7.3.1 Clearing of pigmentation

Clearing of pigmentation significantly improved at week 8, 12 in TA group and week 4, 8, 12 in control group. There was no statistically significant in clearing of pigmentation between both groups through the period of the study.

Table 7.4 Clearing of pigmentation

Clearing of pigmentation	TA	Control	p-value
Week 2			
Excellent	9 (36%)	8 (32%)	0.595
Good	3 (12%)	4 (16%)	
Fair	5 (20%)	3 (12%)	
Poor	8 (32%)	10 (40%)	

Clearing of pigmentation	TA	Control	p-value
Week 4			
Excellent	9 (36%)	12 (48%)	0.184
Good	9 (36%)	7 (28%)	
Fair	5 (20%)	6 (24%)	
Poor	2 (8%)	-	
Week 8			
Excellent	12 (48%)	15 (60%)	0.185
Good	6 (24%)	5 (20%)	
Fair	5 (20%)	5 (20%)	
Poor	2 (8%)	-	
Week 12			
Excellent	11 (44%)	14 (56%)	0.222
Good	5 (20%)	5 (20%)	
Fair	7 (28%)	3 (12%)	
Poor	1 (4%)	2 (8%)	
Missing	1 (4%)	1 (4%)	

Values presented as frequency (%). P-value corresponds to Wilcoxon Signed Ranks test.

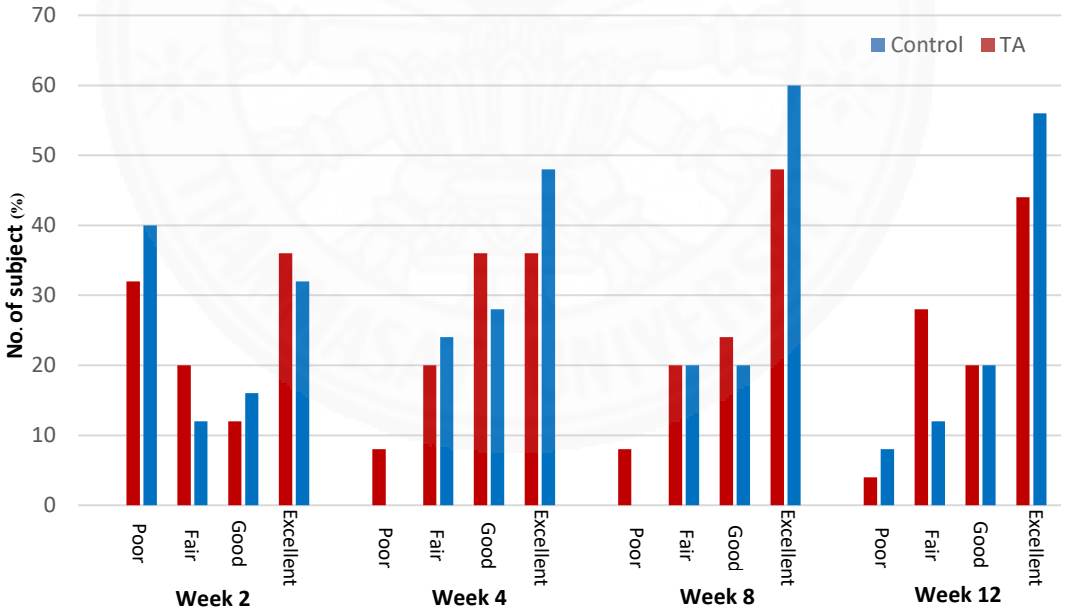

Figure 7.5 Comparison of clearing of pigmentation at week 2, 4, 8 and 12 compared to baseline between TA group and control group

7.3.2Postinflammatory hyperpigmentation

7.3.2.1 Incidence of PIH

Overall incidence of PIH through the period of study was 16% (4/25) in TA group and 28% (7/25) in control group. At week 4, incidence of PIH was lowest (12%) in TA group and highest (28%) in control group. So, the most difference of incidence of PIH showed at week 4 (16%). Although, there was no statistically significant in incidence of PIH between TA and control groups.

Table 7.5 Incidence of PIH

Incidence of PIH	TA	Control	p-value
Week 2	4 (16%)	5 (20%)	1.000
Week 4	3 (12%)	7 (28%)	0.289
Week 8	4 (16%)	6 (24%)	0.725
Week 12	4 (16%)	5 (20%)	1.000

Values presented as frequency (%). P-value corresponds to Fisher's exact test.

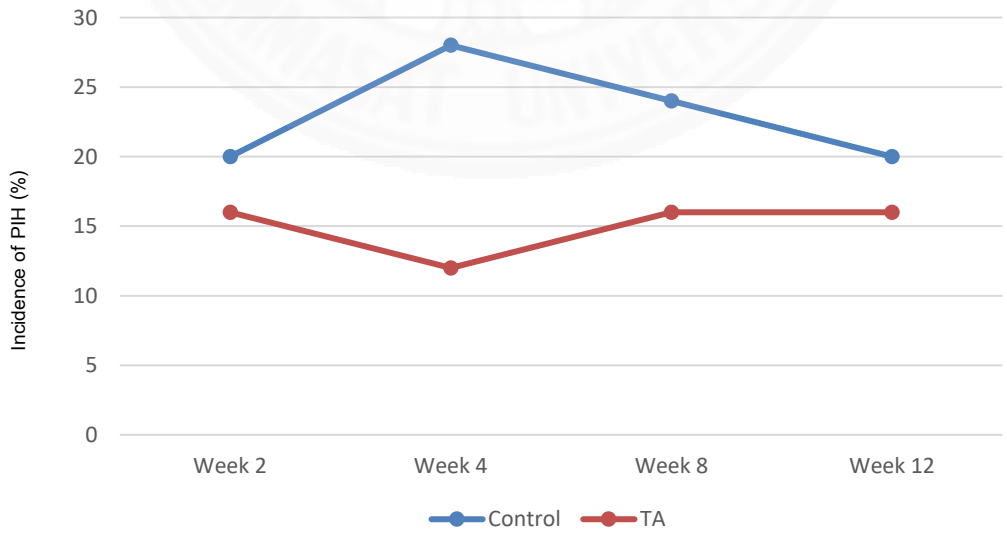

Figure 7.6 Comparison of incidence of PIH at week 2, 4, 8 and 12 between TA group and control group

7.3.2.2 Degree of intensity

Degree of intensity in TA group was less severe than control group. Although, there was no statistically significant in degree of intensity between TA and control groups.

Table 7.6 Degree of intensity

Degree of intensity	TA	Control	p-value
Week 2			
Minimal	4 (16%)	1 (4%)	0.135
Mild	-	1 (4%)	
Moderate	-	2 (8%)	
Severe	-	1 (4%)	
Week 4			
Minimal	2 (8%)	2 (8%)	0.083
Mild	1 (4%)	2 (8%)	
Moderate	-	2 (8%)	
Severe	-	1 (4%)	
Week 8			
Minimal	2 (8%)	1 (4%)	0.146
Mild	2 (8%)	3 (12%)	
Moderate	-	1 (4%)	
Severe	-	1 (4%)	
Week 12			
Minimal	4 (16%)	2 (8%)	0.121
Mild	-	1 (4%)	
Moderate	-	1 (4%)	
Severe	-	1 (4%)	

Values presented as frequency (%). P-value corresponds to Wilcoxon signed Ranks test.

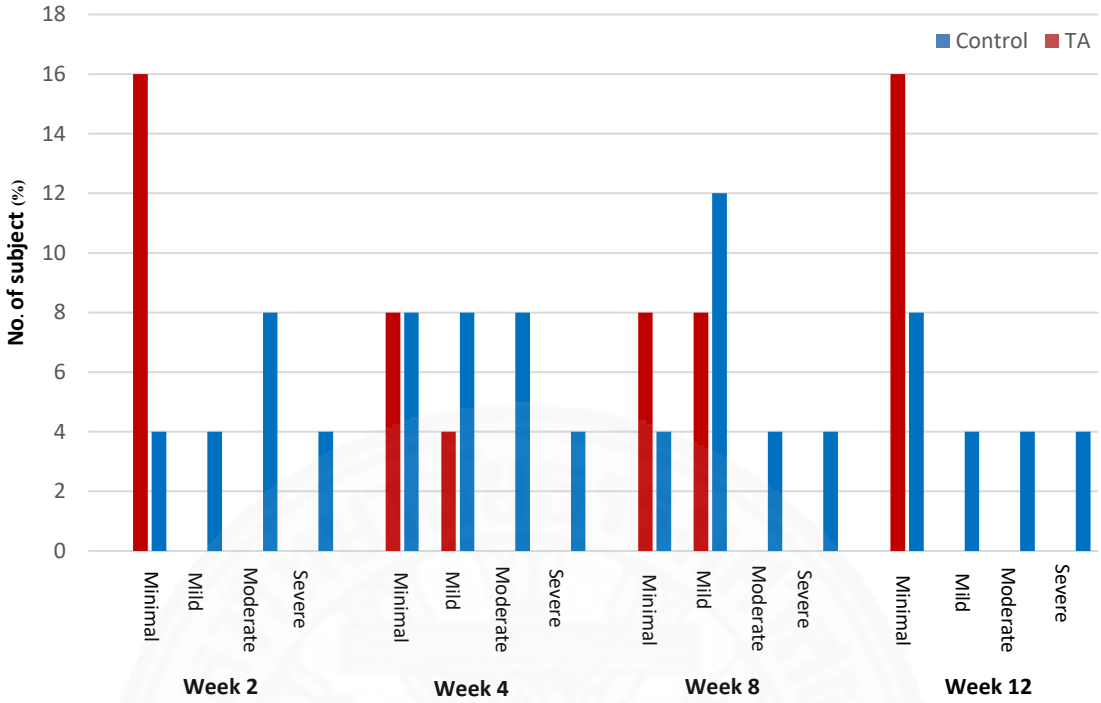

Figure 7.7 Comparison of degree of intensity of PIH at week 2, 4, 8 and 12 between TA group and control group

7.3.2.3 Degree of extension

The same as degree of intensity, degree of extension in TA group was less widespread than control group. Although, there was no statistically significant in degree of extension between TA and control groups.

Table 7.7 Degree of extension

Degree of extension	TA	Control	p-value
Week 2			
Minimal	4 (16%)	1 (4%)	0.135
Mild	-	1 (4%)	
Moderate	-	2 (8%)	
Widespread	-	1 (4%)	
Week 4			
Minimal	2 (8%)	2 (8%)	0.083
Mild	1 (4%)	2 (8%)	
Moderate	-	2 (8%)	
Widespread	-	1 (4%)	

Degree of extension	TA	Control	p-value
Week 8			
Minimal	2 (8%)	1 (4%)	0.168
Mild	2 (8%)	4 (16%)	
Moderate	-	-	
Widespread	-	1 (4%)	
Week 12			
Minimal	4 (16%)	2 (8%)	0.121
Mild	-	1 (4%)	
Moderate	-	1 (4%)	
Widespread	-	1 (4%)	

Values presented as frequency (%). P-value corresponds to Wilcoxon signed Ranks test.

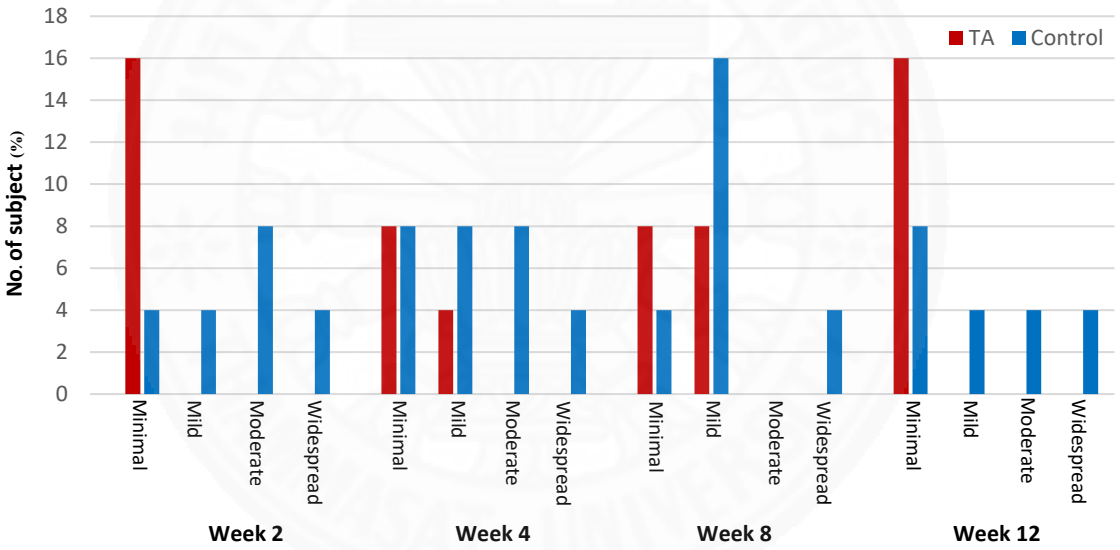

Figure 7.8 Comparison of degree of extension of PIH at week 2, 4, 8 and 12 between TA group and control group.

7.4 Diascopic examination

There was no statistically significant in diascopic examination between TA group and control group throughout the period of study.

Table 7.8 Diascopic examination

Diascopic results	TA	Control	p-value
Week 2			
Non-blanchable	2 (8%)	3 (12%)	0.257
Semi-blanchable	5 (20%)	6 (24%)	
Complete blanchable	17 (68%)	15 (60%)	
Missing	1 (4%)	1 (4%)	
Week 4			
Non-blanchable	1 (4%)	3 (12%)	0.589
Semi-blanchable	8 (32%)	6 (24%)	
Complete blanchable	16 (64%)	16 (64%)	
Week 8			
Non-blanchable	1 (4%)	1 (4%)	0.577
Semi-blanchable	8 (32%)	6 (24%)	
Complete blanchable	16 (64%)	18 (72%)	
Week 12			
Non-blanchable	1 (4%)	3 (12%)	0.679
Semi-blanchable	6 (24%)	3 (12%)	
Complete blanchable	17 (68%)	18 (72%)	
Missing	1 (4%)	1 (4%)	

Values presented as frequency (%). P-value corresponds to Wilcoxon signed Ranks test.

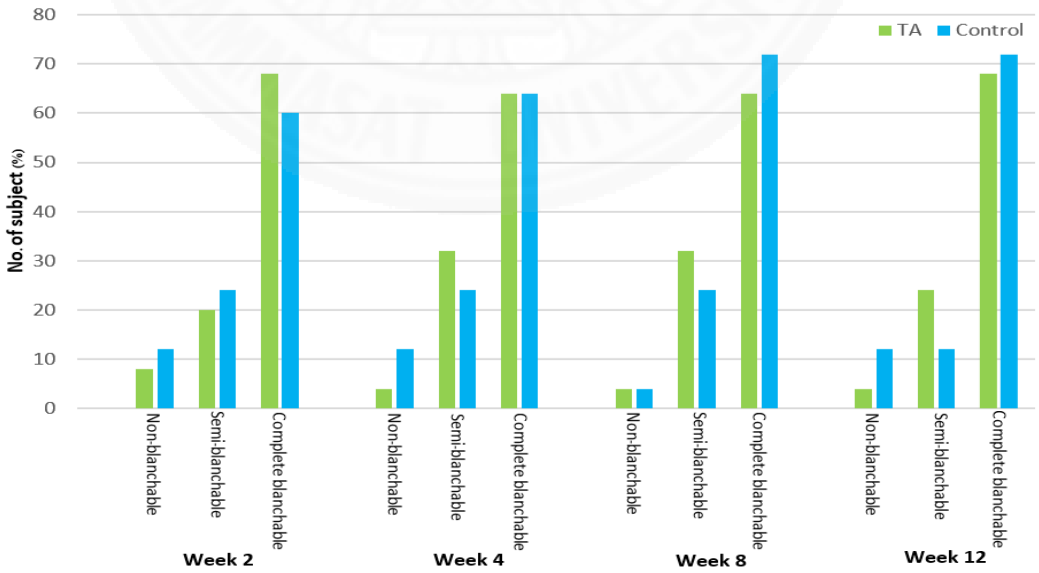

Figure 7.9 Comparison of diascopic examination at week 2, 4, 8 and 12 between TA group and control group

7.5 Patient assessment

7.5.1 Patient's self-improvement score

All of patients in TA group had moderate to remarkable improvement at the end of study. Whereas, 4% of patients in control group had mild improvement. But there was no statistically significant in patient's self-improvement score between both groups.

Table 7.9 Patient's self-improvement score

Self-improvement score	TA	Control	p-value
Week 2			
Minimal	5 (20%)	4 (16%)	1.000
Mild	10 (40%)	11 (44%)	
Moderate	3 (12%)	4 (16%)	
Remarkable	7 (28%)	6 (24%)	
Week 4			
Minimal	-	-	
Mild	4 (16%)	5 (20%)	0.854
Moderate	6 (24%)	5 (20%)	
Remarkable	15 (60%)	15 (60%)	
Week 8			
Minimal	-	-	0.792
Mild	2 (8%)	1 (4%)	
Moderate	5 (20%)	6 (24%)	
Remarkable	18 (72%)	18 (72%)	
Week 12			
Minimal	-	-	0.317
Mild	-	1 (4%)	
Moderate	2 (8%)	3 (12%)	
Remarkable	22 (88%)	20 (80%)	

Values presented as frequency (%). P-value corresponds to Wilcoxon signed Ranks test.

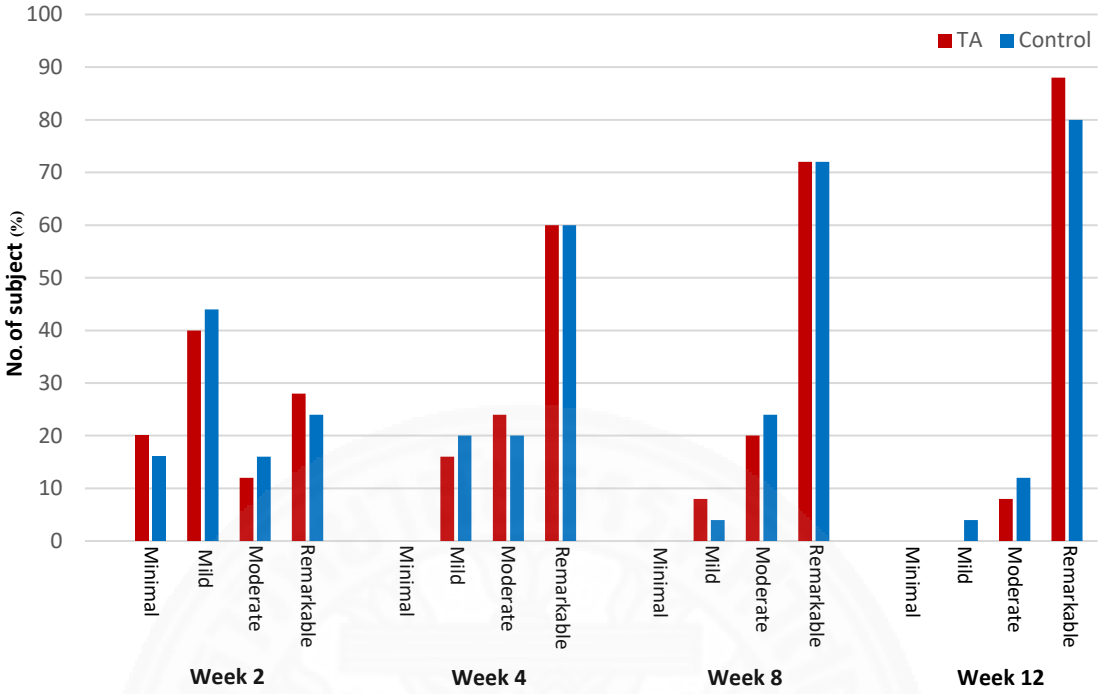

Figure 7.10 Comparison of patient's self-improvement score at week 2, 4, 8 and 12 compared to baseline between TA group and control group

7.5.2 Patient overall satisfaction

At the end of study, patient overall satisfaction was 62.5% very satisfied and 37.5% extremely satisfied. None of patient was not satisfied with the treatment. And patient overall satisfaction significantly improved throughout the period of study.

Table 7.10 Patient overall satisfaction

Patient overall satisfaction	Week 2	Week 4	Week 8	Week 12
Not satisfied	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Slightly satisfied	1 (4%)	0 (0%)	0 (0%)	0 (0%)
Satisfied	10 (40%)	5 (20%)	2 (8%)	0 (0%)
Very satisfied	8 (32%)	13 (52%)	14 (56%)	15 (62.5%)
Extremely satisfied	6 (24%)	7 (28%)	9 (36%)	9 (37.5%)
p-value	**Reference**	**0.005***	**0.001***	**0.001***

Values presented as frequency (%). P-value corresponds to Wilcoxon Signed Ranks test.

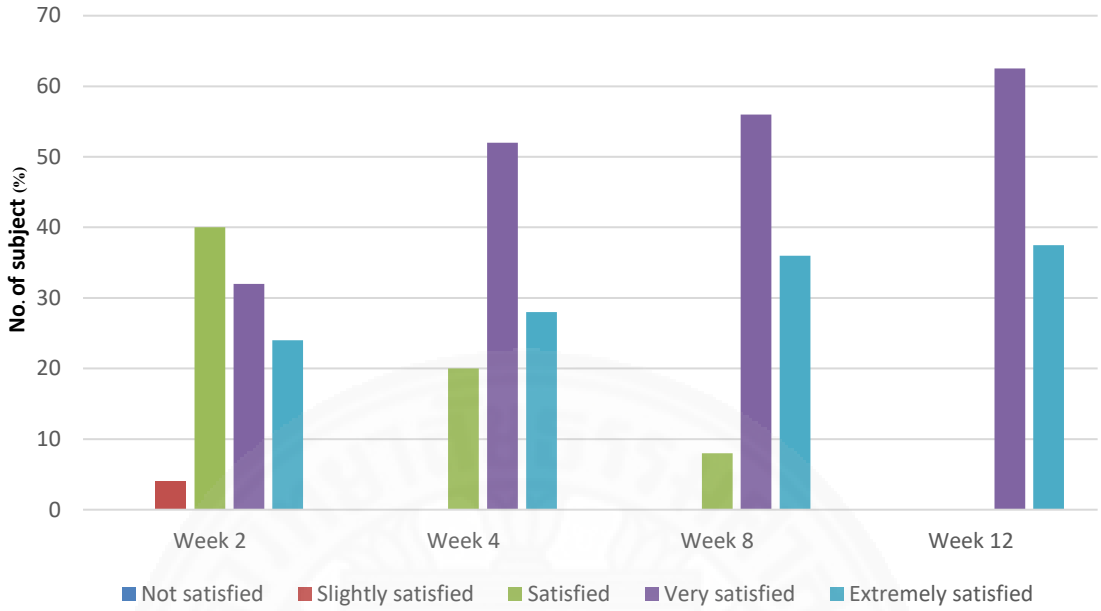

Figure 7.11 Comparison of patient overall satisfaction at week 2, 4, 8 and 12 between TA group and control group.

7.6 Adverse effects

Apart from PIH, VAS in immediate post-procedure ranged from 0 to 5 (mean 1.32 ± 1.18). Two subjects reported immediate mild burning sensation after TA injection, but the symptom can resolve within an hour without any treatment.

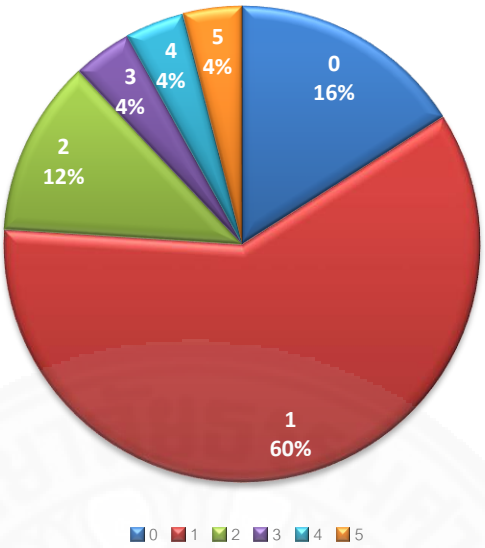

Figure 7.12 VAS in the immediate post-procedure

CHAPTER 8
DISCUSSION AND RECOMMENDATIONS

8.1 Discussion

Solar lentigines are light to dark brown hyperpigmented macules that appear mostly on natural sunlight or artificial sources of UVR exposed skin (1). This condition is common in Caucasians and Asians of Mongolian extraction especially in adults with long-term repeated sun exposure (2). The subjects recruited in this study were 50-70 years old. Less than 50 years old subjects were excluded because hormonal effects in pre-menopause period will be the confounder effects for alterations of pigmentation. And more than 70 years old were also excluded due to delayed wound healing that will affect the results of study. There were no statistically significant in baseline mean MI and EI between TA group and control group. The 532-nm QS Nd: YAG laser which is an effective treatment for solar lentigines possessing the highest level and quality of evidence for solar lentigines removal (3). The clearing of pigmentation was over 50% in all patients from previous studies (5, 79). Unfortunately, this laser could frequently induce PIH especially in patients with darker skin type (3). From previous study, the incidence of PIH after 532-nm QS Nd: YAG laser for solar lentigines removal was 24% in Thailand (12). In this study, the incidence of PIH after 532-nm QS Nd: YAG laser for solar lentigines removal was 28%. PIH after laser treatment is a major concerned cosmetic side effect that influences the self-esteem of patient. There were many efforts to prevent this important side effects such as preoperative topical glycolic acid or HQ in ablative fractional CO_2 laser resurfacing (13), postoperative topical fucidic acid combined with betamethasone valerate cream in 1,064-nm QS Nd: YAG laser treatment (69) but the effective results could not be detected. Nowadays, the most effective prevention was found from postoperative topical corticosteroids (0.05% clobetasol propionate ointment) in fractional CO_2 laser resurfacing. The result showed significant higher incidence of PIH in petrolatum alone (75%) versus intervention (40%). But long

term use of topical corticosteroid can develop many side effects such as acneiform eruption, infection or wound healing process interfering (14). We desired to discover the new effective modality for PIH prevention with less adverse effects.

Recently, TA has a major role for hyperpigmented lesions treatment especially melasma. From the literature reviews, ID injection and oral forms were effective for melasma treatment. But patient will develop severe side effects especially thromboembolism events from the oral form (22). For topical form, the effect for melasma improvement was inconclusive. Although, the topical therapies take a longer time than physical therapies to complete the results, they can be easily controlled and less side effects (3). In 2006, Lee et al. used 4 mg/mL of TA ID injection once a week on melasma. The result showed significant reducing in the MASI from baseline to 8 and 12 weeks (27). Our study used 50 mg/mL of TA ID injection to reducing risk of PIH after laser treatment because we wanted to inject a single dose of TA on the lesion to reduce poor drug compliance. Dosage of TA injection in this study ranged 0.05-2.5 mg that is less than antifibrinolytic dose. The antifibrinolytic dose of TA for intravenous route was 10 mg/kg (71). From previous studies, the side effects from ID TA injection were minimal such as burning sensation, wheal, erythema and local bruising that well tolerated by patients (27, 76, 78). From our study, the side effects from ID TA were also minimal. Two subjects from TA group reported immediate mild burning sensation at the lesions but this symptom can resolve within an hour without any treatment. In addition, VAS was ranged from 0 to 5 (mean 1.32 ± 1.18).

At the end of study, mean MI decreased from 339.71 ± 85.57 to 312.89 ± 73.22 ($P=0.009$) and from 323.83 ± 67.51 to 300.12 ± 67.79 ($P<0.001$) in TA group and control group respectively. Although, there was no statistically significant in mean MI between two groups throughout the period of study. The same as mean MI, clearing of pigmentation evaluated by dermatologists was significantly decreased in each group. And there was no statistically significant in clearing of pigmentation between two groups throughout the period of study. These suggested that TA had no positive effect in mean MI and clearing of pigmentation of solar lentigines removal with 532-nm QS

Nd: YAG laser. In addition, there was statistically significant in reduction of mean MI at week 4 compared with baseline between TA group and control group (P=0.025). And incidence of PIH was lowest (12%) in TA group and highest (28%) in control group in week 4 of the study. So, the most difference of incidence of PIH between two groups was week 4 (16%). Although, there was no statistically significant in incidence of PIH between two groups throughout the period of study. These suggested that TA had a potential to prevent PIH in week 4. Increase of sample size in further study will increase significance of result. In 2016, Kang et al. showed PIH after 532-nm QS Nd: YAG laser in the treatment of solar lentigines presented from 3 to 48 weeks after treatment (mean 4.3 weeks) and persisted for 2 to 24 weeks (mean 8.4 weeks) (67). And in 2010, Chan et al. reported PIH from fractional ablative CO_2 laser resurfacing for skin rejuvenation and acne scars in Asians was highest (55%) at week 4 (80). These suggested that TA can prevent PIH from solar lentigines removal by 532-nm QS Nd: YAG laser at week 4 that is the week of mean and peak incidence of PIH. And then PIH was slowly fade out. Degree of intensity and extension of PIH in TA group had less severe and less widespread than control group. Although, there was no statistically significant in severity of PIH between two groups. TA had a tendency in reducing severity of PIH including degree of intensity and extension. That may be investigated in futher study by increasing of number of sample size. The same as mean MI, mean EI also decreased from 373.08 ± 60.31 to 339.56 ± 60.52 ($P<0.001$) and from 365.04 ± 55.68 to 344.93 ± 65.44 ($P=0.006$) in TA group and control group at the end of study respectively. Reduction of mean EI at week 4 compared with baseline between TA and control groups was statistically significant (P=0.030). These suggested that degree of erythema may be correlated with degree of pigmentation. There were no statistically significant in diascopic results between two groups. Diascopic examination is subjective evaluation. Minimal change of the lesion may be difficult to diagnosis that can affect evaluation results. The same as mean MI and EI, patient's self-improvement score significantly improved throughout the period of study in each group. Whereas, there

was no statistically significant in patient's self-improvement score when compared between two groups. These also confirmed that difference in clearing of pigmentation between two groups did not appear in the view of patients.

The pathogenesis of PIH occur in epidermal and dermal layers of skin (6). Drug administration with ID route can be distributed to cover both layers of skin. The mechanisms of PIH occur via inflammatory process including vascular and cellular cascades (62). TA inhibits PA in vascular cascade. Then, PGE_2 and LT cannot be activated (70). Role of TA in cellular cascade and other pathogenesis of PIH was not found. So, TA can reduce PIH after 532-nm QS Nd: YAG laser treatment but it may not be the most effective method in PIH prevention.

In conclusion, single dose of 50 mg/mL of ID TA injection can prevent PIH at week 4 after the 532-nm QS Nd: YAG laser for the treatment of solar lentigines. And this method can cause minimal side effects.

8.2 Recommendations

For the further studies, increasing the numbers of sample size and longer follow-up period will increase reliable of the study and more understanding in the nature of PIH. Other concentrations of ID TA injection should be evaluated to find out truly appropriated dose for PIH prevention. Other methods that can prevent all or other parts of pathogenesis of PIH should be investigated for the most effective method in PIH prevention.

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APPENDICES

APPENDIX A

MELANIN INDEX

Melanin index												
	TA					Control						
ID	Baseline	2nd	4th	8th	12th	Baseline	2nd	4th	8th	12th
1	356.67	313.67	333.67	308.67	330.33	317.67	308.67	346.33	320.33	294.67
2	371.33	357.00	335.67	344.67	337.33	425.67	406.33	411.00	374.33	390.33
3	292.33	266.67	268.33	266.33	330.67	313.67	243.67	251.00	256.67	294.33
4	255.67	314.67	315.33	278.00	376.00	253.33	258.33	263.33	217.67	251.67
5	336.33	234.33	254.67	229.33	247.33	312.67	276.00	283.33	291.67	298.33
6	285.67	231.67	228.67	245.33	252.67	305.33	243.67	221.67	247.67	240.33
7	408.67	397.67	349.33	340.33	417.33	434.33	302.67	347.33	311.67	445.67
8	321.67	305.67	291.33	300.33	314.67	336.67	320.67	293.67	276.67	309.67
9	552.33	502.33	492.33	520.00	459.33	564.33	361.33	431.33	482.67	504.67
10	156.33	134.33	142.00	136.33	169.33	184.67	185.33	176.33	158.67	181.33
11	426.33	438.33	390.33	380.33	397.67	422.33	487.67	341.67	405.67	401.33
12	287.33	350.67	266.33	307.33	N/A	310.33	388.67	278.33	260.67	N/A
13	307.00	420.67	283.33	286.67	266.00	354.67	396.33	318.33	300.33	314.33
14	220.67	207.67	220.00	220.33	238.33	208.00	198.67	217.33	196.00	199.33
15	285.33	381.33	331.67	331.33	340.33	303.33	281.33	313.33	295.67	316.33
16	308.67	301.33	275.67	285.33	297.33	318.33	294.33	285.67	290.33	307.33
17	315.67	288.33	253.67	275.67	307.67	304.33	278.33	256.67	235.00	196.33
18	243.67	285.67	185.67	235.67	233.67	240.67	276.67	183.67	178.67	207.67
19	388.33	463.33	282.67	260.33	266.67	387.33	392.67	396.67	361.67	354.33
20	433.33	488.67	351.67	371.67	295.67	366.67	335.33	321.33	359.33	323.33
21	433.33	334.33	372.00	388.33	360.67	417.33	277.00	388.67	398.00	368.67
22	399.33	385.67	387.33	437.67	392.67	385.33	366.00	359.33	373.00	367.00
23	378.33	391.67	367.67	414.67	420.00	418.67	405.00	406.33	415.00	414.67
24	350.67	335.33	362.33	351.33	315.67	376.67	366.33	372.67	358.33	364.33
25	239.33	305.55	245.67	262.67	232.67	264.33	307.33	336.33	223.33	248.33

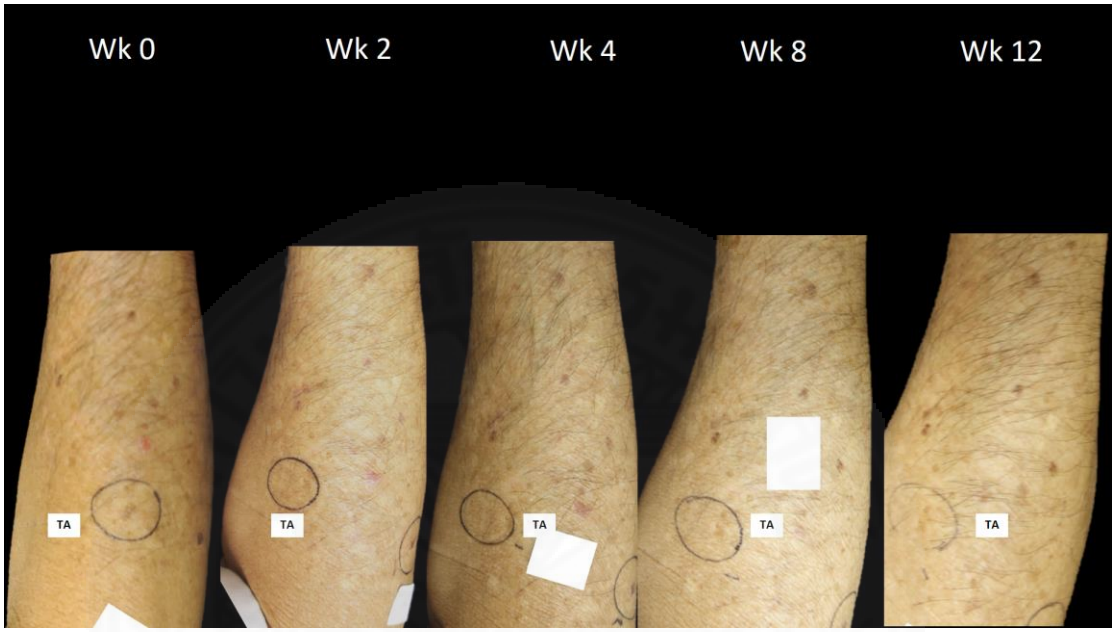
APPENDIX B

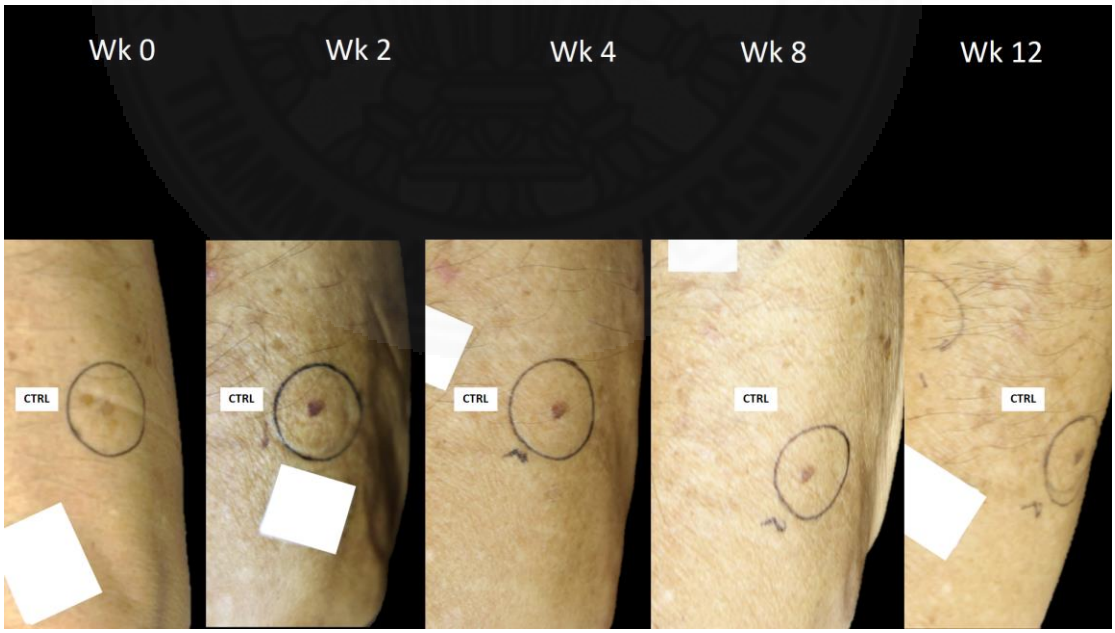
ERYTHEMA INDEX

Erythema index											
	TA					Control					
ID	Baseline	2nd	4th	8th	12th	Baseline	2nd	4th	8th	12th
1	356.00	359.67	364.33	364.00	366.67	350.33	369.67	373.33	360.33	370.33
2	386.33	355.33	353.33	364.67	350.33	373.33	350.33	454.33	361.33	366.67
3	352.00	337.00	318.00	311.67	257.33	340.67	191.33	271.33	302.33	241.33
4	358.33	343.67	360.67	359.33	398.67	369.67	326.33	317.67	280.67	284.67
5	392.33	316.33	294.67	323.33	340.00	349.00	332.00	324.67	383.33	337.67
6	310.67	250.00	281.33	297.33	277.33	326.33	274.67	261.33	318.33	290.00
7	456.33	345.33	378.67	393.33	442.33	455.67	349.33	399.33	385.33	435.33
8	472.00	383.33	354.33	384.33	378.67	369.33	386.67	379.67	394.33	409.67
9	509.00	442.67	443.67	460.33	391.33	475.33	393.33	428.33	436.67	456.67
10	302.67	255.67	271.00	243.67	261.67	286.00	292.67	285.67	301.00	255.33
11	406.33	387.67	354.33	354.33	353.67	370.33	371.33	323.33	330.67	356.67
12	360.67	322.67	209.33	285.67	N/A	398.67	344.33	330.67	282.67	N/A
13	392.33	3223.67	312.00	314.33	314.33	410.33	294.67	301.00	317.67	343.67
14	302.33	301.67	244.33	276.33	256.33	290.33	234.33	283.00	212.33	220.33
15	361.67	334.67	347.67	354.33	287.67	390.33	313.33	362.00	337.33	287.67
16	304.33	341.67	320.00	329.33	312.33	304.67	334.67	337.67	340.33	332.33
17	351.67	319.33	324.67	329.33	309.67	350.67	311.67	313.67	298.33	299.00
18	235.67	259.33	260.67	308.33	229.33	245.33	272.67	295.67	269.67	249.67
19	342.67	354.00	313.67	284.67	296.67	318.67	345.00	370.33	345.67	343.00
20	380.33	369.33	391.33	386.33	374.33	386.33	390.67	371.33	382.67	358.67
21	440.33	420.33	391.33	392.67	396.67	420.67	430.33	417.67	381.67	391.33
22	411.33	412.67	436.33	411.67	402.33	420.33	446.00	441.33	393.00	395.67
23	405.33	422.00	391.33	403.33	445.33	412.67	394.67	406.67	412.33	444.33
24	406.00	438.33	435.00	356.00	387.67	395.33	422.67	431.33	373.33	396.67
25	330.33	381.67	342.33	320.67	318.67	370.33	340.67	346.67	361.67	306.67

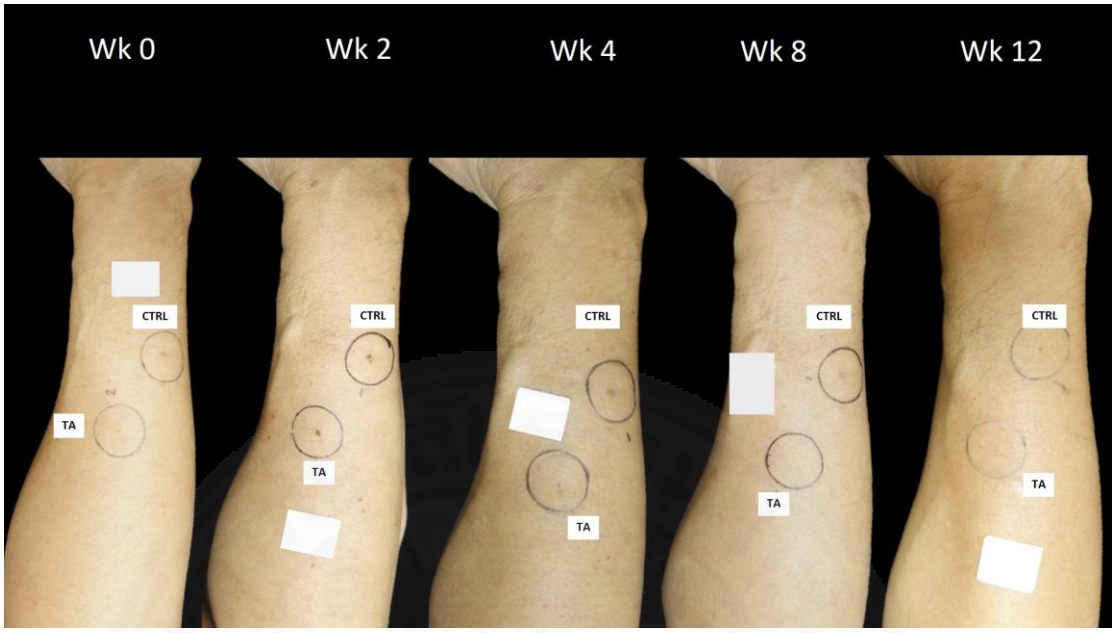
APPENDIX C
PHOTOGRAPH OF SUBJECTS

Right forearm of patient 1





Right forearm of patient 2



APPENDIX D

ACCEPTANCE LETTER FOR PROCEEDING MANUSCRIPT

The acceptance letter for proceeding manuscript for the Thai Journal of Pharmaceutical Sciences (TJPS), 2017, volume 41 (supplement issue) and JPSP NRCT Follow-Up Seminar 2017 and IAMPS 33

The JSPS-NRCT Follow-Up Seminar 2017 and 33rd International Annual Meeting in Pharmaceutical Sciences (JSPS-NRCT 2017 and IAMPS33)
Faculty of Pharmaceutical Sciences, Chulalongkorn University
254 Phayathai Road, Patumwan, Bangkok 10330 THAILAND
Tel.:+66 2218 8261 Fax.:+66 2255 8227

Date: 15 March 2017

Proceeding manuscript:
Efficacy of tranexamic acid intradermal microinjections in reducing risk of postinflammatory hyperpigmentation after Q-switched Nd: YAG laser for treatment of solar lentigines: A pilot randomized controlled trial

Authors:
Rattima Srieakpanit, Puangpaka Jaiyao, Punyaphat Sirithanabadeekul

Dear Ms. Rattima Srieakpanit,

We are pleased to inform you that your proceeding manuscript has been accepted for presentation in the JSPS-NRCT Follow-Up Seminar 2017 and 33rd International Annual Meeting in Pharmaceutical Sciences (JSPS-NRCT 2017 AND IAMPS 33), which is held on 2-3 March 2017 at The Berkeley Hotel Pratunam, Bangkok, Thailand. Your proceeding manuscript will be published The Thai Journal of Pharmaceutical Sciences (TJPS), 2017, vol.41 (Supplement Issue), page 29-32

Yours truly,

Pornchai R.

Assoc. Prof. Pornchai Rojsitthisak, Ph.D.
Chair of Scientific Program Committee

APPENDIX E

MANUSCRIPT FOR THAI JOURNAL OF PHARMACEUTICAL SCIENCES (TJPS)

TJPS Vol.41 (Supplement Issue) 2017

Thai Journal of Pharmaceutical Sciences (TJPS)

The JSPS-NRCT Follow-Up Seminar 2017 and
33rd International Annual Meeting in Pharmaceutical Sciences

Efficacy of tranexamic acid intradermal microinjections in reducing risk of postinflammatory hyperpigmentation after Q-switched Nd: YAG laser for treatment of solar lentigines: A pilot randomized controlled trial

Rattima Srieakpanit[1], Puangpaka Jaiyao[2], Punyaphat Sirithanabadeekul[1,*]

[1] Department of dermatology, Chulabhorn International College of Medicine, Thammasat University, Pathum Thani 12120, Thailand
[2] Pan Rajdhevee and Lion Supanhongsa Foundation, Bangkok 10330, Thailand

*Corresponding author Tel.+66(86)3688050; E-mail address Punyaphats.cicm@gmail.com

Keywords: Tranexamic acid, Solar lentigo, Postinflammatory hyperpigmentation, Q-switched 532-nm Nd: YAG laser

Introduction

Solar lentigines are well-defined light to dark brown hyperpigmented macules appearing mostly on the exposed skin from natural sunlight or artificial sources of ultraviolet radiation (UVR). Following the faster improvement outcomes and less pain, laser treatment has become a popular therapeutic modality for those lesions. The 532-nm Q-switched (QS) Nd: YAG laser is an effective laser treatment supported by high quality of evidence.[1] Postinflammatory hyperpigmentation (PIH) is a major concerned side effect for patients with solar lentigines removal by laser treatment, especially those with darker skin type. After laser treatment, the basal cell layer is particularly destroyed. Melanin is dropped into dermis and phagocytosed by melanophage in the upper dermis, leading to dermal melanosis.[2] PIH is composed of excess melanin production or abnormal distribution of melanin pigment in the epidermis or dermis.[3] The injury of keratinocytes in epidermis from laser produces inflammatory mediators. Leukotrienes (LT) C4, LTD4, prostaglandin E_2 (PGE_2), and thromboxane B2 can increase melanin synthesis and transfer melanin to surrounding keratinocytes.[2] From previous studies, the incidence of PIH after the 532-nm QS Nd: YAG laser treatment was approximately 6.67-53% in Asians and 24% in Thailand.[4-6] Many studies have been conducted to minimize risk of PIH. Postoperatively, a short-term application of topical corticosteroids can significantly reduce the risk of PIH after ablative fractional CO_2 laser resurfacing in Asians with atrophic acne scars.[7] However, topical corticosteroids may interfere wound healing process with an increasing risk of acneiform eruption and infection. Tranexamic acid (TA), an antifibrinolytic agent, inhibits plasminogen activator (PA) by reversibly blocking synthetic derivative of lysine binding sites on plasminogen molecules, so the plasminogen in the epidermal basal cells and keratinocytes cannot convert to the plasmin. As well, phospholipase A2 precursors for the membrane phospholipid secretion of arachinodic acid (AA), a precursor of PGE_2 and LT, cannot be activated.[8] In the meantime, the keratinocyte-PA system activation by UVR induces melanogenesis process.[9] So, TA can reduce melanogenesis and inhibit inflammatory mediators. In a previous study, various forms of TA were applied on hyperpigmented lesions, including the oral and intradermal (ID) injection forms of TA which found to be effective for melasma treatment.[10,11] Nonetheless, the oral form of TA can cause several systemic side effects, such as gastrointestinal discomfort, myocardial infarction, pulmonary embolism, and thromboembolism.[10] Whereas, the intradermal TA injection may possibly yield efficacious outcomes in melanin reduction and anti-inflammation process for PIH treatment. Hence, this study aimed to compare the pigment alteration after solar lentigines removal using 532-nm QS Nd: YAG laser following ID injection of 50 mg/mL of TA versus normal saline.

Methods

Patient selection

This study was performed in patients aged 50-70 years, with at least 2 solar lentigines on the forearms, from December 2016 to January 2017 at skin and aesthetic center, Thailand Tobacco Monopoly Hospital, Thailand. Those who were pregnant, lactating, or found with abnormal wound healing, photosensitivity disorders or any skin diseases at the treatment areas were excluded. The study protocol was approved by the Human Ethics Committee of Thammasat University.

Laser and post laser treatment:

Preoperative topical analgesic cream (2.5% lidocaine and 2.5% prilocaine; EMLA®) was applied and occluded at the lesions 45 minutes before treatment. Then, all patients received a single treatment of 532-nm QS Nd: YAG laser (Spectra-XT; Lutronic, Seoul, Korea) for their solar lentigines, with spot size 1.8 mm, fluences 0.6-0.8 J/m^2, and energy adjusted for immediate whitening clinical endpoint. Following the laser treatment, the 50 mg/mL of TA (250 mg/ 5 mL tranexamic acid; Transamin®) was intradermally injected to one random solar lentigo as TA group at 1 cm intervals (0.1 mL/cm^2), and normal saline to another as control group. After that, Vaseline was applied on all lesions twice daily until the crusts peeled off, followed by broad-spectrum sunscreen with SPF 40 for 4 weeks. Sun exposed and no topical preparations on the lesions were also suggested for all periods of the study.

Clinical evaluation

The 26 lesions of 13 cases were evaluated. As a subjective measurement, the patient's self-improvement scores in quartile scale (as: none =no improvement, minimal =1-15% improvement, mild =26-50% improvement, moderate =51-75% improvement, and remarkable =>75% improvement) were assessed at 2nd and 4th weeks after treatment. Likewise, as an objective measurement, the lesions' colour was measured by a narrow-band reflectance spectrophotometer (Mexameter MX18, Courage and Khazaka; Cologne, Germany) as melanin index (MI) and erythema index (EI) at baseline, 2nd and 4th weeks.

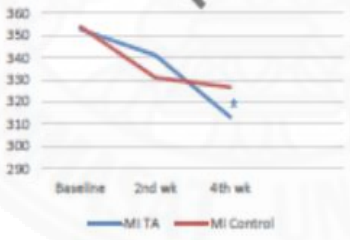
Statistical analysis

Data was analyzed using paired t-test by SPSS 21 software (SPSS, Chicago, IL, USA). P value <0.05 was considered statistically significant.

Results

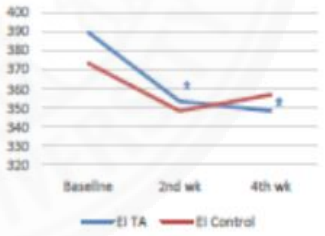
Thirteen patients with twenty-six solar lentigines were enrolled in the study. Mean age of the patients was 62.77 years (range 50-70 years), with skin types III (23.08%), IV (46.15%), and V (30.77%). Baseline data showed no statistically significant differences between the two groups in baseline MI and EI (p >0.05). Mean MI (Fig. 1) of the TA group decreased from 352.9 ± 75.13 to 341.21 ± 80.71 (2 weeks, p =0.554) and to 313.05 ± 67.69 (4 weeks, p <0.05 vs. baseline). Meanwhile, MI for the control group decreased from 354.13 ± 78.85 to 331.05 ± 57.37 (2 weeks, p =0.197) and to 326.64 ± 62.97 (4 weeks, p =0.061 vs. baseline). Significantly, the improvement of MI was observed in the TA group at the end of 4th week.

Figure 1 Melanin index



(*P < 0.05, by paired t-test)

Figure 2 Erythema index



(*P < 0.05, by paired t-test)

In addition, mean EI (Fig. 2) of the TA group decreased from 389.41 ± 56.55 to 353.49 ± 47.74 (2 weeks, p <0.05) and to 348.33 ± 43.93 (4 weeks, p <0.05 vs. baseline). While, EI of the control group decreased from 373.33 ± 46.94 to 348.33 ± 40.98 (2 weeks, p =0.133) and to 356.87 ± 53.02 (4 weeks, p =0.303 vs. baseline). The EI improvement was significantly noted in the TA group at 2nd and 4th week.

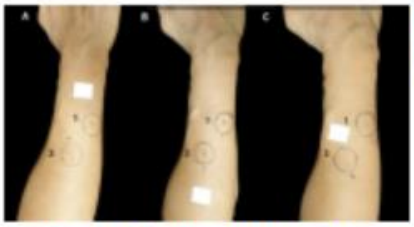


Figure 3 Photographs of right forearm (A) Baseline (B) the 2nd week after treatment (C) the 4th week after treatment (1 = control lesion, 2 =TA injected lesion)

The patients' self-improvement scores (Table 1) in the TA group were 12/13 patients (92.31%) rated as remarkable (>75% improvement) and 1/13 patients (7.69%) rated as moderate (51-75% improvement) at the end of 4th week. In the control group, 7/13 patients (53.85%) rated as remarkable (>75% improvement), 5/13 patients (38.46%) rated as moderate (51-75% improvement), and 1/13 patients (7.69%) rated as mild (26-50% improvement) at the end of 4th week. Besides, the mean patients' self-improvement scores were 90 ± 10.8 in the TA group and 75.77 ± 16.56 in the control group (4 weeks after treatment, $p<0.05$). Moreover, two patients in the TA group have burning sensation immediately after the injection, but can resolve within one hour. As well, one patient had redness on both lesions for seven days.

Table 1. Patient's self-improvement scores

Self-improvement score	TA (n =13) (%)		Control (n =13) (%)	
	2nd week	4th week	2nd week	4th week
Minimal	0 (0)	0 (0)	1 (7.69)	0 (0)
Mild	4 (30.77)	0 (0)	5 (38.46)	1 (7.69)
Moderate	4 (30.77)	1 (7.69)	3 (23.08)	5 (38.46)
Remarkable	5 (38.46)	12 (92.31)	4 (30.77)	7 (53.85)

Discussion

PIH after laser treatment is a major concerned cosmetic side effect, especially in patients with darker skin type. The Q-switched laser which is an effective treatment for epidermal lesion including solar lentigines could frequently induce PIH. Previous reports used glycolic acid, or hydroquinone preoperatively before ablative fractional CO_2 laser resurfacing[6] and lucidic acid combined with betamethasone valerate cream post 1,064-nm QS Nd: YAG laser[13], but the prevention results could not be detected. In 2015, Cheyasak et al. used postoperative topical corticosteroids (0.05% clobetasol propionate ointment) to prevent hyperpigmentation after ablative fractional CO_2 laser resurfacing. The result showed significant higher incidence of PIH in petrolatum alone (75%) vs. intervention (40%).[7] But the topical corticosteroid can make the side effects such as acneiform eruption, infection, or wound healing process interfering.

TA has a major role hyperpigmented lesions treatment especially melasma. Among various forms, ID injection and oral forms have significant result for melasma treatment. But patient will develop severe side effects especially thromboembolism events from oral TA.[10] In 2006, Lee et al. used 4 mg/mL of TA ID injection once a week on the melasma. The result showed significant decrease in the MASI from baseline to 8 and 12 weeks.[11] Our study used ID TA injected the lesion to reducing risk of PIH after laser treatment. We used 50 mg/mL of TA because TA was injected at the lesion for single time. Moreover, the doses of TA do not exceed 2.5 mg in each patient that is less than the antifibrinolytic dose. Side effect from TA injection in our study is the burning sensation in two patients immediate after injection and can resolve within one hour. The MI in TA group significantly decreased at the end of 4th week (p <0.05). In the 2nd week, MI in TA group was higher than control group because some patients still had the crust at the treatment site. The patients' self-improvement score in TA group was significantly improved when compared with control group at 2nd and 4th weeks. EI in TA group decreased at the 2nd and 4th weeks (p <0.05). But the result was not significant in control group. EI did not associate with TA injection, EI was measured at two weeks after TA injection that the effect of TA may be disappeared. The patients' self-improvement score was significantly improved in the TA group compared with the control group. In 2016, Kang et al. showed PIH from treatment of solar lentigines using 532-nm QS Nd: YAG laser presented at 4.3 weeks.[14] The further study should extend the follow-up period to observe the preventive effect of TA in the long term.

Conclusion

Intradermal TA injection can be the effective and safe therapeutic modality in reducing hyperpigmentation after solar lentigines treatment using 532-nm QS Nd: YAG laser.

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BIOGRAPHY

Name	Miss Rattima Srieakpanit
Date of Birth	September 7th, 1988
Educational Attainment	2015-2017: Master of Science (Dermatology) Chulabhorn International College of Medicine, Thammasat University
Work Position	2006-2011: Doctor of Medicine, Prince of Songkla University
Work Experiences	2012-2013: General Practice Internship Songklanagarind Hospital