Administration of oral tranexamic acid is equally effective with intradermal injections in reducing the amount of melanin in female marmots (*Cavia porcellus*) exposed to ultraviolet-b

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**ABSTRACT**

**Introduction:** Oral studies of tranexamic acid significantly reduce the MASI (Melasma Area and Severity Index) value but have not evaluated a decrease in the amount of melanin. The purpose of this study is to prove that oral tranexamic acid can reduce the amount of melanin and have the same effectiveness as intradermal injections in female guinea pigs exposed to ultraviolet B.

**Method:** A posttest only control group design study was conducted using 32 female mice, aged 6-8 weeks, weight 250-280 grams, which were randomly divided into 2 groups. Group 1 was given an intradermal injection of tranexamic acid 5 mg/ml, 0.5 ml/cm and exposure to UVB. Group 2 was given oral treatment of tranexamic acid 250 mg twice a day and exposure to UVB. The treatment was given for 4 weeks. Total UVB exposure was 390mj/sec. The study was conducted in the animal laboratory of the Udayana medical faculty.

**Results:** The statistic result showed that the data were normally distributed and homogenous. The comparative test with the independent sample t-test indicated that there was no significant difference between intradermal injections with oral tranexamic acid administration in reducing the amount of melanin in female guinea pigs exposed to UVB with p> 0.05.

**Conclusion:** Oral tranexamic acid significantly reduced the amount of melanin and had the same effectiveness as intradermal injections in reducing the amount of melanin in female guinea pigs exposed to UVB.

**Keywords:** tranexamic acid, melanin, ultraviolet b, guinea pigs

**INTRODUCTION**

The aging process is a natural process characterized by a decrease or change in physical, psychological and social conditions in interacting with other people. The aesthetic aspect is one of another aspect of aging. Such as facial appearance in the form of spots or black spots, dull, wrinkle, dry, sagging skin, thin, and benign tumors. Environmental factors that play a major role in the aging process are ultraviolet (UV) radiation. Continuous exposure to UV light can cause a state of damage to the structure and function of the skin which are accelerating aging of the skin. It is also called photoaging. One sign of aging that interferes with appearance is hyperpigmentation, namely the change in skin color on the face in the form of brown or black stains such as melasma. Melasma is a pigmentation disorder characterized by macules or symmetrical hyperpigmented patches, often on the right and left face, with uneven borders. The most prevalence occurs in women young to mid-age, with Fitzpatrick IV-V skin type.

Tranexamic acid is a synthetic derivative of the lysine amino acid which works by inhibiting plasmin activity synthesized in response to UV light on keratinocytes. It works by blocking plasminogen binding to keratinocytes, which ultimately decreases free arachidonic acid and reduces prostaglandin products which are known to be stimulators of tyrosinase enzyme activity. Tranexamic acid decreases the production of promelanogenic factors and reduces erythema and vascularity. The dose used is much lower than the dose for antifibrinolytics.

Several studies have been conducted, such as oral tranexamic acid which gives subjective clinical improvement results by calculating melasma scores (MASI). Lee et al. evaluate the administration of tranexamic acid 250 mg twice a day for 4 months. The use of intradermal injection tranexamic acid provides significant results in improving clinical outcomes as measured by a decrease in MASI score was conducted by Lee et al. for 12 weeks. Previous studies on tranexamic acid therapy against melasma hyperpigmentation used only subjective evaluations, whereas the oral administration of tranexamic acid has not yet been evaluated. Hyperpigmentation objective evaluation
The research obtained that tranexamic acid can overcome UV pigmentation in guinea pigs because it has a structure that resembles humans. The study was carried out by means of intradermal injections which showed that the decrease in the number of melanocyte cells was not significant but the amount of melanin decreased.

Therapy with intradermal injections of tranexamic acid gives significant results in the treatment of melasma, but has the disadvantage of the pain effects such as burning, swelling, and erythema which resulted in patient uncomfortable. With the same purpose and effectiveness, oral therapy of tranexamic acid does not cause irritating effects such as those found in the treatment of intradermal injections, although epigastric discomfort sometimes occurs, but this generally does not disturb the patient.

The purpose of this study is to prove that oral tranexamic acid can reduce the amount of melanin and have the same effectiveness as intradermal injections in female guinea pigs exposed to ultraviolet B.

METHODS

An experimental study with randomized posttest only control group design was conducted to evaluate the effectiveness of oral tranexamic acid compared to intradermal injections. The study consisted of two groups with a sample of 16 female guinea pigs per group. Group 1 is treatment group 1 (P1), given UVB exposure and intradermal injection of tranexamic acid 5 mg / mL, dose 0.5 ml / cm once a week. Group 2 (P2) was given exposure to UVB and oral tranexamic acid 250 mg twice a day dose conversion to 0.027 mg/gBB. The total dose of UVB is 390 mJ / cm2 given for 2 weeks. Histopathological and skin tissue examination was done with Masson-Fontana staining after 4 weeks of treatment. The amount of melanin is calculated by the percentage of the pixel area of melanin compared to the pixel of the entire epidermal tissue.

RESULTS

On the 45th day, forty-eight hours after exposure, the guinea pigs were euthanized using excess doses of ketamine (100 mg / kg bb) intra-peritoneally. The skin of the back area was cleaned from the fur and then histopathological preparations were made using Masson-Fontana staining which gives black color to melanin. The amount is calculated by the percentage of the pixel area of melanin compared to the pixel of the entire epidermal tissue.

This study did not use a control group, because it used reference data from previous year study that had used guinea pigs as the control group experimental animals in exposing them with UVB 65 mJ / sec, for 65 seconds, every Monday, Wednesday and Friday in two weeks. The histopathological picture of melanin in guinea pigs skin epidermal tissue is presented in Figure 1 (A)

After four weeks of treatment, the back skin tissue of the guinea pig was biopsied for histopathological examination. Melanin stained black on Masson-Fontana staining. The histopathological picture of melanin in the epidermal tissue of guinea pigs is shown in Figure 1 (B, C)

The results of this study showed that administration of oral tranexamic acid was as effective as intradermal injections in reducing the amount of melanin in female guinea pigs exposed to UVB. It indicating the amount of melanin group 1 (P1) was 1.22% ± 0.37 and the melanin in group 2 (P2) was 1.41% ± 0.40. No significant difference found between the average number of melanin intradermal injections and oral tranexamic acid (p> 0.05) by independent sample t-test.

Table 1, shows that with the independent sample t-test, there was no significant difference between the average number of melanin intradermal injections and oral tranexamic acid (p> 0.05)
Table 1  Average number of melanin between groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Average (%) ± SD</th>
<th>average difference</th>
<th>t</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>P1 (Injection)</td>
<td>1,22±0,37</td>
<td>0,19</td>
<td>1,37</td>
<td>0,178</td>
</tr>
<tr>
<td>P2 (Oral)</td>
<td>1,41±0,40</td>
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DISCUSSION

Guinea pigs were chose as a subject because they have many biological similarities with humans and guinea pigs are widely used in research. Guinea pig’s skin color is varies because guinea pigs have melanin, both from eumelanin and pheomelanin, but some are albino. The skin characteristics of guinea pigs indicate that the guinea pig epidermis has the same thickness as the thickness of the human epidermis. The distribution of melanin in guinea pigs is more similar to the distribution of melanin in humans. The selected guinea pigs were colored guinea pigs. The skin of guinea pigs if exposed to UV B will become darker which is similar as human skin.

UV radiation is a source of free radicals and one of the external factors that cause skin aging. UV light will damage the sulfhidryl group, a tyrosinase enzyme inhibitor, so that in the presence of UV light, the tyrosinase enzyme works optimally and triggers the process of melanogenesis.

UV light exposure stimulates the synthesis of Plaminogen activator and increases plasmin activity in keratinocytes. Plasmin is a protease that increases the release of arachidonic acid (AA) through phospholipase A2. Arachidonic acid is free to stimulate melanogenesis through its metabolite product; prostaglandin E2. UV light exposure causes prostaglandins to activate signaling pathways in the growth, differentiation and apoptosis of melanocytes. PGE2 is released by keratinocytes due to UV exposure, which stimulates the formation of melanocyte dendrites and melanocyte tyrosinase activity. Furthermore, AA release increases plasmin in endothelial cells and in turn increases the levels of the hormone α-MSH which activates melanin synthesis in melanocytes. Plasmin also releases bFGF which is a potential melanocyte growth factor, all of which causes increased production of melanin. Single chain urokinase Plasminogen Activator (sc-uPA) in keratinocytes affects the increase in the number of melanocytes, tyrosinase activity, cell perimeter, area, and increase in dendrite. Plasmin significantly increases the amount of Sc-uPA. Sc-uPA further increases keratinocyte growth, differentiation and migration, then increases melanocyte activity in-vitro.

Tranexamic acid prevents pigmentation by exposure to UV light by interfering with the structure of plasminogen in the basal layer of the epidermis and inhibits activator plasminogen activity converting plasminogen to plasin in keratinocytes. This process causes a reduction in free arachidonic acid which decreases prostaglandin production and tyrosinase enzyme activity in melanocytes and melanogenesis. TRP-1 and TRP-2 enzyme are very important in Raper Mason melanogenesis. Here tranexamic acid works by decreasing the TRP-1 and TRP-2 levels. Activation of the extracellular signal-regulated kinase (ERK) signaling pathway causes MITF degradation which will reduce melanogenesis. Tranexamic acid stimulates ERK and lowers regulation of MITF protein levels which reduce inflammation of melanogenesis by decreasing the expression of protein tyrosinase.

The dosage used in this study is 250 mg twice a day, because at this dose an effective minimum dose of therapy is reached: 10-16 µg / ml so that it produces the same effective intradermal injection of 5 mg / ml 0.5ml dose / cm. Achieving a minimum dose of effective oral tranexamic acid in this study influences the results of its similarity in effectiveness with intradermal injections. The intradermal injections of tranexamic acid in this study carried out once a week because in intradermal injections, the drugs will accumulate on the skin and will be released slowly to the target cell, while oral tranexamic acid is absorbed up to 40%, bioavailability 30-50% and systemic metabolism <5% to reach the target cell.

CONCLUSION

Oral tranexamic acid therapy 250 mg a day and intradermal injection of tranexamic acid 5 mg / mL every 1 week for 4 weeks showed no significant difference between the test groups with p > 0.05. These data indicate that oral tranexamic acid and intradermal injections have the same effectiveness in reducing the amount of melanin in the guinea pig skin epidermis. According to this finding, it is known that the treatment of skin hyperpigmentation by intradermal injections and oral tranexamic acid is equally effective in providing improvements with objective assessment.
REFERENCE