WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Effect of Intradermal Injection of Tranexamic Acid on Tyrosinase Levels and Melanin Content in Guinea Pigs (*Cavia Porcellus*) Exposed to Ultraviolet B

Diah Kartika[1], I.G.A.A. Praharsini[2], Agung Wiwiek Indrayani[3]

[1]Biomedical Anti-Aging Medicine Magister Program, Faculty of Medicine, Universitas Udayana, Denpasar, Bali, Indonesia.
[2]Department of Dermatology and Venereology, Faculty of Medicine, Universitas Udayana, Prof. dr. I.G.N.G. Ngoerah Hospital, Denpasar, Bali, Indonesia.
[3]Department of Pharmacology and Therapy, Faculty of Medicine, Universitas Udayana, Denpasar, Bali, Indonesia.
Email: kartikachndraa@gmail.com

ABSTRACT

Background: Ultraviolet B (UVB) is one of the causes of aging due to high levels of free radicals. Tyrosinase can increase due to exposure to UVB rays, thereby increasing the amount of melanin. Tranexamic acid can inhibit tyrosinase so it can reduce the amount of melanin. This study aimed to prove the effect of intradermal injection of tranexamic acid with various concentrations on tyrosinase and melanin in hyperpigmentation disorders due to exposure to ultraviolet B.

Methods: This research used a posttest-only control group design method. Subjects were guinea pigs (*Cavia porcellus*), male, healthy, local strain, 3-4 months old, weighing 300-350 grams, totaling 28 animals divided into seven groups, each with 4 animals. Group K- was the normal group, group K1 was given exposure to UVB, group K2 was given exposure to UVB and placebo injection, group P1 was given intradermal injection of tranexamic acid with a concentration of 10 mg/ml, group P2 was given intradermal injection of tranexamic acid with a concentration of 20 mg/ml, group P3 was given an intradermal injection of tranexamic acid at a concentration of 50 mg/ml, and group P4 was given an intradermal injection of tranexamic acid at a concentration of 100 mg/ml. Tyrosinase levels were determined using ELISA, while the amount of melanin was determined by histopathological examination with Masson Fontana staining.

Results: The results showed that the mean tyrosinase levels in the control group (54,28 ± 3,25 [K1] and 52,22 ± 0,85 [K2]) were significantly higher compared to the treatment group (33,14 ± 1,09 [P1]; 27,20 ± 0,91 [P2]; 24,30 ± 0,83 [P3]; 22,70 ± 0,80 [P4]) ($p < 0.001$). The mean amount of melanin in the control group (20,63 ± 1,41 [K1] and 19,22 ± 1,05 [K2]) was significantly higher than the treatment group (11,04 ± 1,10 [P1]; 7,98 ± 1,65 [P2]; 4,28 ± 1,20 [P3]; 2,10 ± 0,52 [P4]) ($p < 0,001$).

Conclusion: It can be concluded that intradermal injection of tranexamic acid can reduce tyrosinase levels and the amount of melanin in male guinea pigs (*Cavia porcellus*) exposed to ultraviolet B.

Keywords: intradermal, melanin, tranexamic acid, tyrosinase, ultraviolet B

Introduction

Ultraviolet (UV) originating from sunlight has an important role in various physiological processes such as cell growth, vitamin D production, immune system regulation, and melanin formation. However, due to the depletion of the stratospheric ozone layer, excessive exposure to UV can cause negative impacts on human health. Among all types of UV radiation, UVB radiation in particular has the property of suppressing the immune system, which can cause a decrease in the defense system of the skin.[1]

Melanin is a natural pigment found in many organisms and is formed by melanocytes in the skin. The process of forming melanin involves several enzymes and protein structures, one of which is the tyrosinase enzyme. Melanin is formed by the phenolic acid precursor L-tyrosine via the Raper-Mason pathway. This precursor will undergo hydroxylation to become L-DOPA after being catalyzed by the enzyme tyrosinase, a melanosome-containing cuprum. Disturbances in melanin formation can lead to pathological conditions, including hyperpigmentation.[2,3]

Tranexamic acid, which was originally used as a fibrinolytic agent, is now starting to be used as an antihyperpigmentation agent. This modality can work by competitively inhibiting plasminogen binding to keratinocyte cells so that the activation of plasminogen into plasmin, and the stabilization of blood clotting is hampered. In addition, tranexamic acid will reduce the production of free arachidonic acid in plasma resulting in a decrease in prostaglandin production and this modality becomes an anti-inflammatory agent. Inflammation can cause a hyperpigmentation process so the administration of

tranexamic acid can inhibit the melanogenesis process which is preceded by a decrease in the activity of the tyrosinase enzyme in cases of skin pigmentation disorders due to inflammation.[4,5] Thus, administering tranexamic acid can potentially treat abnormalities in the formation of melanin in the skin. Because the concentration of tranexamic acid used in several studies is still limited to 4 mg/ml to 100 mg/ml, this study aimed to determine the effect of different concentrations on tyrosinase and melanin parameters.

Methods

This research was an experimental study using animals with a randomized posttest-only control group design. This research was conducted at the Animal Unit of the Pharmacology Laboratory of the Faculty of Medicine Universitas Udayana and the Integrated Biomedical Laboratory of the Faculty of Medicine Universitas Udayana. The study was conducted for 14 weeks, from November 2023 to February 2024.

The subjects used were healthy male guinea pigs (*Cavia porcellus*), aged three to four months, weighing 300-350 grams, and local strain. The dropout criteria was dead during the study. Based on the Arifin formula, we used 28 samples divided into seven groups using simple random sampling. Group A was the normal control group without substance administration or UVB irradiation. Group B was the group where subjects were given only UVB irradiation. Group C was the negative control group where subjects were given UVB irradiation and intradermal injections of normal saline. Group D was given UVB irradiation and intradermal injection of tranexamic acid with a concentration of 10 mg/ml. Group E was given UVB irradiation and intradermal injection of botulinum toxin A with a concentration of 20 mg/ml. Group F was given UVB irradiation and intradermal injection of botulinum toxin A with a concentration of 50 mg/ml. Group G was given UVB irradiation and intradermal injection of botulinum toxin A with a concentration of 100 mg/ml.

Intradermal injection of tranexamic acid was given on day 7 and day 21 with a volume of 0,1 ml per 1 cm^2 of UVB exposed area. Tyrosinase level and melanin content were examined from UVB irradiated skin and collected through punch biopsy. Tyrosinase level was examined using the ELISA method. Melanin content was observed from histopathological examination with picrosirius red staining at 400 times magnification. Ultraviolet B irradiation was given using a UVB lamp PL-S9W/01/2P with a wavelength of 311 nm. Ultraviolet B irradiation was given in a dose of 65 mJ/cm^2 with a duration of 65 seconds and the distance to skin was 1 cm for four weeks. Ultraviolet B irradiation was given three times per week (Tuesday, Thursday, and Saturday) at 2 PM local time.

The data was analyzed using IBM SPSS version 26.0. The Shapiro-Wilk test was done to assess the distribution of the data. Levene's test was done to assess the variance of the data. One-way ANOVA test was performed to evaluate the difference between groups and the least significance difference (LSD) test was performed as the posthoc analysis. P value of less than 0.05 was considered significant.

Results

Tyrosinase level and melanin content in all groups were found to be normally distributed and homogenous ($p > 0,05$). In **Table 1**, it can be seen that based on one-way ANOVA test, there was a difference in tyrosine level in at least between two groups. The graphic that compares tyrosinase levels between groups can be seen in **Fig. 1**. In **Table 2**, based on posthoc analysis, it can be seen that there was a significant difference in tyrosine level between groups ($p < 0,001$).

Table 1. Comparative one-way ANOVA test of tyrosine level

Groups	N	Mean ± SD (pg/mL)	p-value
Group A	4	27.47 ± 0.23	
Group B	4	54.28 ± 3.25	
Group C	4	52.22 ± 0.85	
Group D	4	33.14 ± 1.09	< 0.001
Group E	4	27.20 ± 0.91	
Group F	4	24.30 ± 0.83	
Group G	4	22.70 ± 0.80	

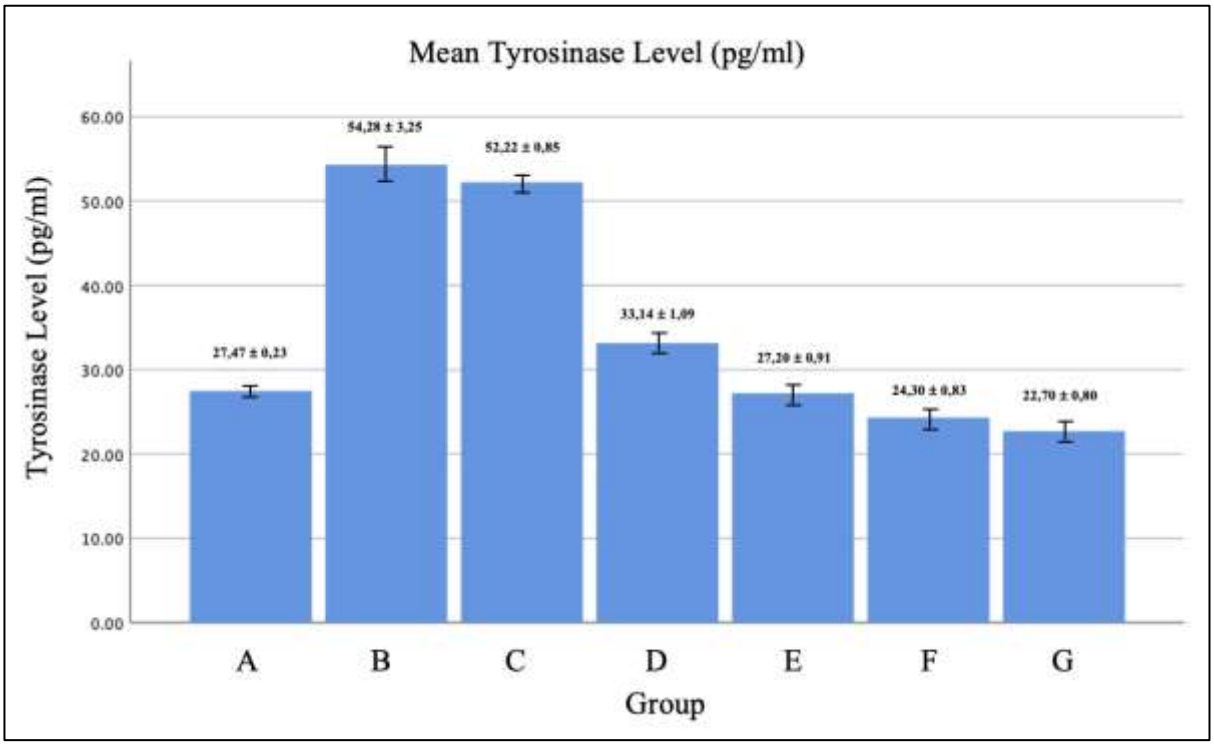

Figure 1. Comparison of tyrosinase levels between groups

Table 2. Post-hoc analysis of tyrosine level

Groups	Comparator	Mean difference	p-value
Group A	Group B	-26.81	< 0.001
Group A	Group C	-24.75	< 0.001
Group A	Group D	-5.67	< 0.001
Group A	Group E	-0.27	0.636
Group A	Group F	3.17	< 0.001
Group A	Group G	4.77	< 0.001
Group B	Group C	0.06	0.245
Group B	Group D	21.14	< 0.001
Group B	Group E	27.08	< 0.001
Group B	Group F	29.98	< 0.001
Group B	Group G	31.58	< 0.001
Group C	Group D	19.08	< 0.001
Group C	Group E	25.02	< 0.001
Group C	Group F	27.92	< 0.001
Group C	Group G	29.52	< 0.001
Group D	Group E	5.94	< 0.001
Group D	Group F	8.84	< 0.001
Group D	Group G	10.44	< 0.001
Group E	Group F	2.90	< 0.001

Group E	Group G	4.50	< 0.001
Group F	Group G	1.60	0.003

In **Table 3**, it can be seen that based on one-way ANOVA test, there was a difference in melanin content in at least between two groups. The graphic that compares melanin content between groups can be seen in **Fig. 2**. In **Table 4**, based on posthoc analysis, it can be seen that there was a significant difference in melanin content between groups ($p < 0{,}001$).

Table 3. Comparative one-way ANOVA test of melanin content

Groups	N	Mean ± SD (% pixel)	p-value
Group A	4	7.93 ± 1.25	
Group B	4	20.63 ± 1.41	
Group C	4	19.22 ± 1.05	
Group D	4	11.04 ± 1.10	< 0.001
Group E	4	7.98 ± 1.65	
Group F	4	4.28 ± 1.20	
Group G	4	2.10 ± 0.52	

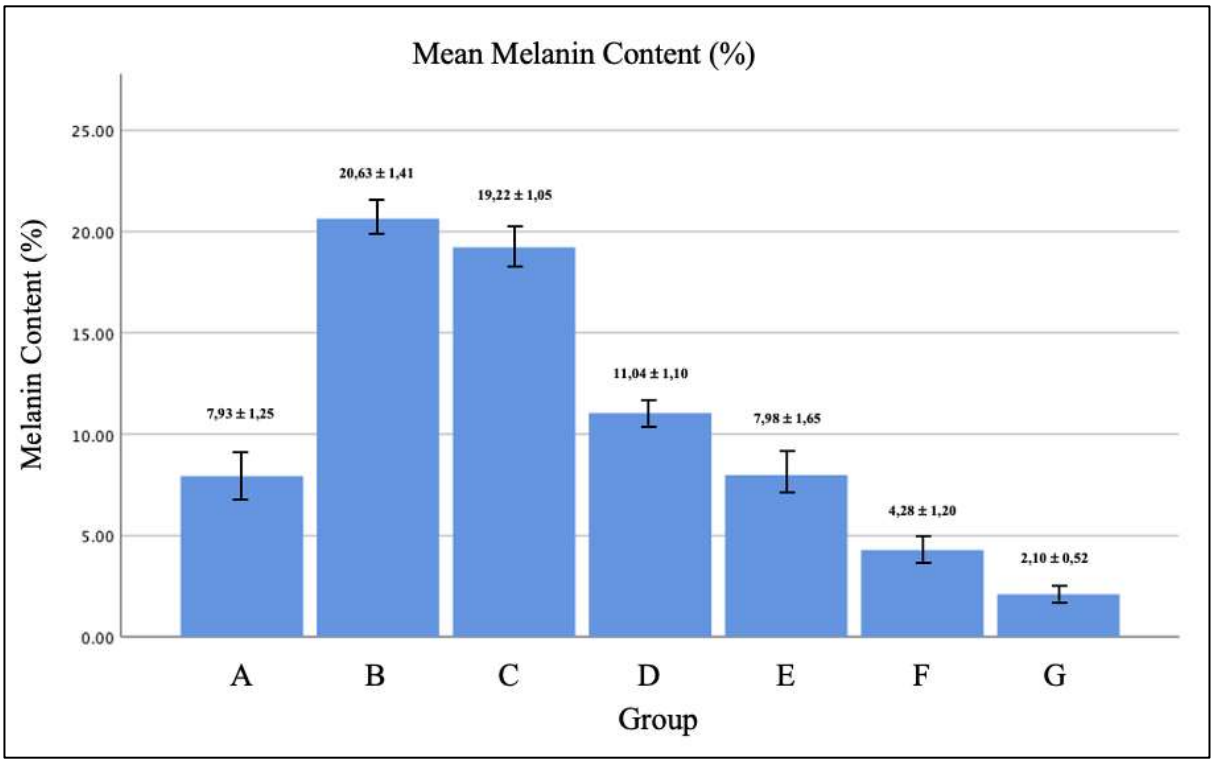

Figure 2. Comparison of melanin content between groups

Table 4. Post-hoc analysis of melanin content

Groups	Comparator	Mean difference	p-value
Group A	Group B	-12.705	< 0.001
Group A	Group C	-11.290	< 0.001
Group A	Group D	-3.115	0.002
Group A	Group E	-0.055	0.949
Group A	Group F	3.650	< 0.001

Group A	Group G	5.830	< 0.001
Group B	Group C	1.415	0.114
Group B	Group D	9.590	< 0.001
Group B	Group E	12.650	< 0.001
Group B	Group F	16.355	< 0.001
Group B	Group G	18.535	< 0.001
Group C	Group D	8.175	< 0.001
Group C	Group E	11.235	< 0.001
Group C	Group F	14.940	< 0.001
Group C	Group G	17.120	< 0.001
Group D	Group E	3.060	0.002
Group D	Group F	6.765	< 0.001
Group D	Group G	8.945	< 0.001
Group E	Group F	3.705	< 0.001
Group E	Group G	5.885	< 0.001
Group F	Group G	2.180	0.019

In **Fig. 3**, we can see the histology examination of the skin with Masson Fontana staining and 400 times magnification.

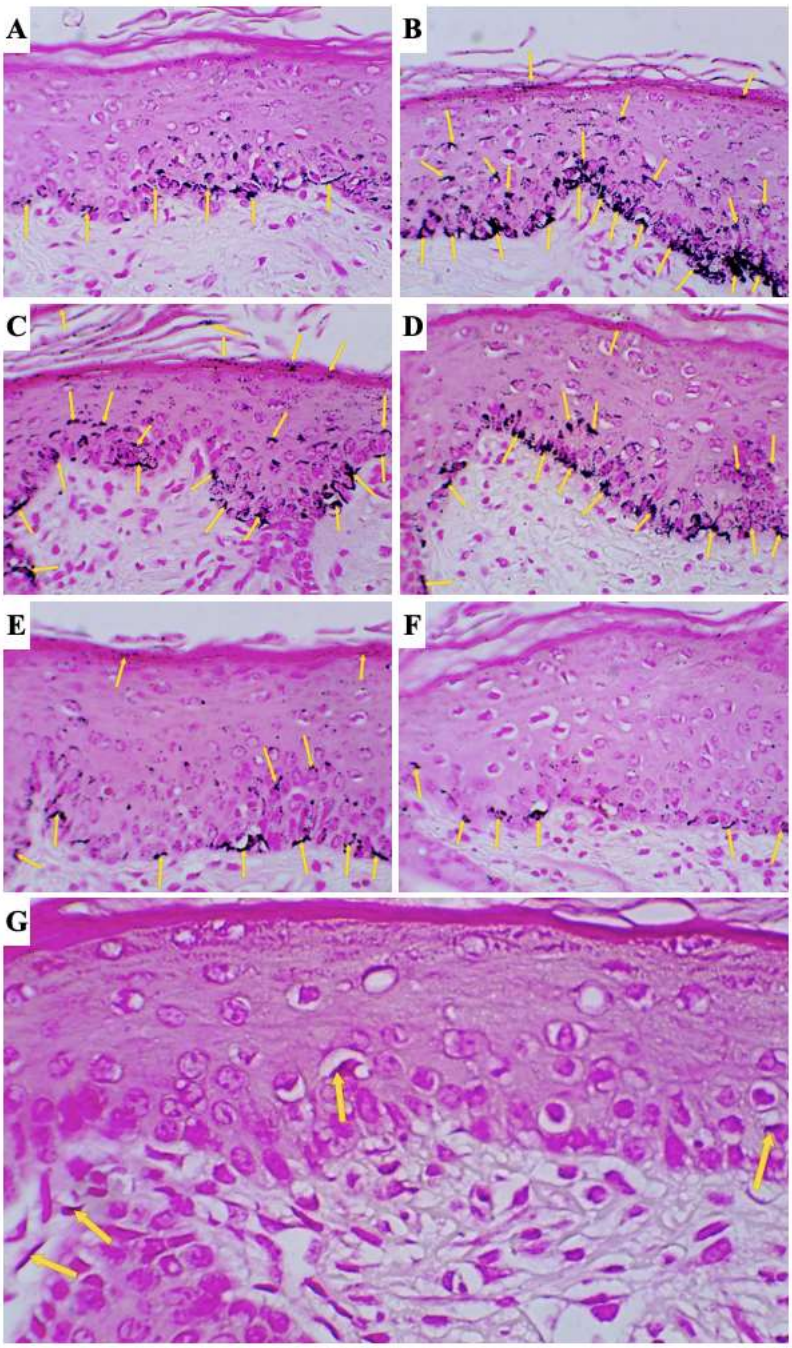

Figure 3. Histology result of the skin of guinea pig. Melanin pigments are black and are shown with yellow arrow **A:** Melanin content in group A, occupying 6.34-9.38% of the epidermal area. **B:** Melanin content in group B, occupying 19.1-22.38% of the epidermal area. **C:** Melanin content in group C, occupying 18.32-20.58% of the epidermal area. **D:** Melanin content in group D, occupying 9.78-12.08% of the epidermal area. **E:** Melanin content in group E, occupying 5.86-9.78% of the epidermal area. **F:** Melanin content in group F, occupying 2.96-5.76% of the epidermal area. **G:** Melanin content in group F, occupying 1.5-2.74% of the epidermal area.

Discussion

The results obtained from this study showed that the tyrosinase levels in the control group were the highest among the groups given exposure to UVB radiation. Meanwhile, the treatment group showed lower tyrosinase levels along with the high concentration of tranexamic acid given. From these statistical results, it can be concluded that exposure to UVB radiation significantly increased tyrosinase levels compared to the group that was not exposed to UVB radiation. In addition, it can also be concluded that the administration of tranexamic acid had the effect of reducing tyrosinase levels after exposure to UVB radiation. The concentration of tranexamic acid also affects tyrosinase levels, where higher concentrations of tranexamic acid have significantly lower tyrosinase levels.

Zhu *et al*.[6] conducted in vitro research to determine the effect of tranexamic acid on tyrosinase activity in human umbilical vein endothelial cells (HUVEC) by targeting the vascular endothelial growth factor (VEGF) receptor. The study reported that VEGF165 increased the expression of VEGF receptors, including VEGFR-1, VEGFR-2, and NRP-1 in HUVECs. However, tranexamic acid can reduce the expression of the VEGF receptor. The study also reported that VEGF165 increased tyrosinase phosphorylation of VEGFR-1 and VEGFR-2 in HUVECs. However, tranexamic acid also inhibits the increase in tyrosinase phosphorylation.[6]

Liu *et al*.[7] conducted research to determine the effect of administering tranexamic acid with hyaluronic acid-coated liposome nanogels for topical treatment of hyperpigmentation. The study reported that administering tranexamic acid could significantly inhibit tyrosinase activity compared to the control group. Administration of tranexamic acid together with hyaluronic acid-coated liposome nanogels was also reported to provide better results when administered topically.[7]

Kim *et al*.[4] conducted in vitro research to determine the inhibitory effect of tranexamic acid on post-inflammatory hyperpigmentation. Post-inflammatory hyperpigmentation was induced by laser exposure to human melan-a cells and melanocytes. This study reported that tranexamic acid had an inhibitory effect on tyrosinase activity as the dose increased. Tranexamic acid has also been reported to reduce levels of tyrosinase, TRP-1, and TRP-2.[4]

Tyrosinase is a rate-limiting enzyme in melanin biosynthesis. Tranexamic acid causes downregulation of melanocyte tyrosinase activity or tyrosinase mRNA transcription. This likely occurs because tranexamic acid can also reduce TRP-1 and TRP-2 levels in addition to tyrosinase levels. These melanogenic enzymes are regulated by the same transcription factor, namely microphthalmia transcription factor (MITF). Tranexamic acid stimulates the ERK signaling pathway, which causes phosphorylation and degradation of MITF.[4]

The results obtained from this study showed that the melanin content in the control group was the highest among the groups given exposure to UVB radiation. Meanwhile, the treatment group showed a lower melanin content along with the high concentration of tranexamic acid given. From these statistical results, it can be concluded that exposure to UVB radiation causes an increase in the melanin content that is exposed to UVB radiation compared to groups that are not exposed to UVB radiation. Apart from that, it can also be concluded that the administration of tranexamic acid has the effect of reducing the melanin content after being exposed to UVB radiation. The concentration of tranexamic acid also affects the melanin content, where a higher concentration of tranexamic acid has a significantly lower melanin content.

Zhu *et al*.[6] reported that the administration of tranexamic acid could reduce melanin synthesis induced by the administration of VEGF165 by reducing melanocyte cell proliferation and decreasing tyrosinase activity. This may occur because tranexamic acid can neutralize VEGFR-1 and VEGFR-2.[6] Liu *et al*.[7] also reported that administering tranexamic acid could reduce the melanin content significantly. Administration of tranexamic acid together with hyaluronic acid-coated liposome nanogels is also reported to provide better results when administered topically because it can deliver tranexamic acid well to melanocytes.[7] Kim *et al*.[4] reported that tranexamic acid had an inhibitory effect on melanin synthesis as the dose increased in post-inflammatory hyperpigmentation that was induced by laser exposure to human melan-a cells and melanocytes.[4]

Melanin is produced in two different forms, namely eumelanin and pheomelanin. Tyrosinase and tyrosine-related protein 1/2 (TRP1/2) are the main factors involved in melanin synthesis. In melanogenesis, tyrosinase is a rate-limiting enzyme that catalyzes the conversion of tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA) and subsequently oxidizes this molecule to form dopaquinone. Eumelanin is synthesized via TRP-2, which functions as a DOPA chrome tautomerase and catalyzes the rearrangement of DOPA chrome to 5,6-dihydroxyindole-2-carboxylic acid (DHICA). Next, TRP-1 oxidizes DHICA to its carboxylated form, indole-quinone.[8] Pheomelanin is synthesized through a reaction involving dopaquinone and cysteine. In addition, tyrosinase and TRP1/2 are transcriptionally regulated by microphthalmia-associated transcription factor (MITF) in melanocytes.[9] Phosphorylation of MITF by extracellular signal-regulated kinase (ERK) 2 results in degradation of MITF via the proteasome pathway.[10]

The cellular autophagy system is known to play an important role in removing waste proteins and eliminating non-functional organelles, including mitochondria, endoplasmic reticulum, and peroxisomes.[8,11] Recent studies have shown that autophagy is also involved in melanin biogenesis and the degradation of melanosomes, the lysosome-associated organelles where melanin is synthesized, suggesting that its activation is associated with a decrease in melanin pigment production.[12] Research shows that autophagy regulators have an important role in the early stages of melanosome formation. Beclin-1, which is an autophagic modulator WIPI1, and microtubule-associated protein light chain 3 (LC3) are potent regulators of melanogenesis.[5] Mechanistic target of rapamycin (mTOR) is a known inducer of autophagy because its positive regulation suppresses this process (via Akt and mitogen-activated protein kinase [MAPK] signaling). However, its negative regulation (by AMPK and p53 signaling pathway) promotes autophagy. The ERK1/2 pathway plays an important role in regulating autophagy.[5,10]

Tranexamic acid is a plasmin inhibitor that has been used to treat heavy bleeding due to trauma, surgery, and menstruation.[13] Tranexamic acid is a synthetic derivative of the amino acid lysine and exerts its effects by reversibly blocking the lysine binding site on the plasminogen molecule.[14] Tranexamic acid has also emerged as a potential drug to treat melasma because it inhibits melanin synthesis by inhibiting the plasminogen/plasmin pathway, thereby blocking the interaction between melanocytes and keratinocytes.[15] Because plasminogen is also present in cultured human keratinocytes, which are known to produce plasminogen activators, tranexamic acid affects keratinocyte function.[4]

Badran *et al*. showed that intradermal injection of tranexamic acid shows better clinical improvement in melasma patients compared to the topical route. Topical administration of tranexamic acid at the maximum dose provides improvement that is not as good as intradermal injection with 62.7% improvement with a dose of 10 mg/mL and 39.1% improvement with a dose of 4 mg/mL.[16] Pazyar *et al.* reported that intradermal injection of tranexamic acid with concentration of 4 mg/ml and 10 mg/ml were found to be effective in reducing the hyperpigmentation index in melasma without significant side effects. Administering 0.5 ml tranexamic acid injection (concentration 50 mg/ml) or the equivalent of a dose of 0.1 ml at each injection point, given

every two weeks for 12 weeks effectively reduced hyperpigmentation index but often caused a burning sensation in the area of injected skin without other significant side effects.[17] Another study found that a 0.1 ml injection with a concentration of 100 mg/ml still provided maximum therapeutic effects with minimal side effects.[18] However, currently there are no conclusive studies regarding toxic doses in intradermal tissue, but it can be concluded that with administration of 0.1 ml at each injection site, doses of 4 mg/ml to 100 mg/ml still have a good effect on melasma hyperpigmentation with minimal side effects.

In conjunction with tyrosinase and melanin, tranexamic acid has the effects of activating the autophagy system by upregulating autophagy-related proteins, including Beclin-1, Atg12, and LC3, through inhibiting mTOR expression. Tranexamic acid can activate the ERK signaling pathway, resulting in MITF degradation and downregulating the expression of melanogenesis-related proteins such as tyrosinase and TRP1/2. Tranexamic acid also directly suppresses the production of proteins and enzymes related to melanogenesis.[5]

The limitation of this study was the short research period so it cannot observe long-term side effects that may occur. It is hoped that in the future, intradermal injection of tranexamic acid can be used as an alternative ingredient to prevent aging due to exposure to UVB radiation. This research will benefit knowledge in the science of anti-aging medicine, where intradermal injection of tranexamic acid has been proven to prevent an increase in tyrosine and melanin after exposure to UVB radiation. In the end, it is hoped that this research can also be applied to humans, but further research is needed, including safety indices and clinical trials in humans, so that the results of this research are truly useful.

Conclusion

Intradermal injection of tranexamic acid can reduce tyrosinase levels and the melanin content in male guinea pigs (*Cavia porcellus*) exposed to UVB radiation. It is necessary to carry out research over a longer period to determine the side effects of intradermal injection of tranexamic acid. Furthermore, research on the toxic effects of administering tranexamic acid, research on side effects, research to determine deeper working mechanisms of tranexamic acid, and research regarding the use of topical preparations as a minimally invasive approach to reduce pain should be conducted.

Conflict of interest

The authors declare no conflict of interest regarding the preparation of this manuscript.

Funding

The authors received no specific funding in the preparation of this manuscript.

Ethical clearance

This study had clearance from the Animals Ethics Committee Faculty of Veterinary Universitas Udayana with registration number B/240/UN14.2.9/PT.01.04/2023.

Author contribution

Authors contributed equally during the preparation process of this manuscript.

References:

1. Sipahutar YH, Albaar N, Purnamasari HB, Kristiany MG, Prabowo DHG. Seaweed extract (*Sargassum polycystum*) as a preservative on sunscreen cream with the addition of seaweed porridge. *IOP Conference Series: Earth and Environmental Science*. 2019;278(12072):1-8.
2. Fan Y-F, Zhu S-X, Hou F-B, Zhao D-F, Pan Q-S, Xiang Y-W, *et al*. Spectrophotometric assays for sensing tyrosinase activity and their applications. *Biosensors*. 2021;11(8):290-312.
3. Li C, Kuai L, Cui R, Miao X. Melanogenesis and the Targeted Therapy of Melanoma. *Biomolecules*. 2022;12(12):1874.
4. Kim MS, Bang SH, Kim J-H, Shin H-J, Choi J-H, Chang SE. Tranexamic acid diminishes laser-induced melanogenesis. *Annals of Dermatology*. 2015;27(3):250-256.
5. Cho YH, Park JE, Lim DS, Lee JS. Tranexamic acid inhibits melanogenesis by activating the autophagy system in cultured melanoma cells. *J Dermatol Sci*. 2017;88(1):96-102.
6. Zhu JW, Ni YJ, Tong XY, Guo X, Wu XP, Lu ZF. Tranexamic acid inhibits angiogenesis and melanogenesis in vitro by targeting VEGF receptors. *International Journal of Medical Sciences*. 2020;17(7):903.
7. Liu Y, Han Y, Zhu T, Wu X, Yu W, Zhu J, *et al*. Targeting delivery and minimizing epidermal diffusion of tranexamic acid by hyaluronic acid-coated liposome nanogels for topical hyperpigmentation treatment. *Drug Deliv*. 2021;28(1):2100-2107.
8. Navone F, Genevini P, Borgese N. Autophagy and Neurodegeneration: Insights from a Cultured Cell Model of ALS. *Cells*. 2015;4(3):354-86.

9. Jeong M-H, Yang K-M, Kim J-K, Nam B-H, Kim G-Y, Lee S-W, *et al*. Inhibitory effects of *Asterina pectinifera* extracts on melanin biosynthesis through tyrosinase activity. *Int J Mol Med*. 2013;31(1):205-12.
10. Kim ES, Shin JH, Seok SH, Kim JB, Chang H, Park SJ, *et al.* Autophagy mediates anti-melanogenic activity of 3'-ODI in B16F1 melanoma cells. *Biochem Biophys Res Commun*. 2013;442(3-4):165-70.
11. Zhou Y-Y, Li Y, Jiang W-Q, Zhou L-F. MAPK/JNK signalling: a potential autophagy regulation pathway. *Biosci Rep*. 2015;35(3):e00199.
12. Murase D, Hachiya A, Takano K, Hicks R, Visscher MO, Kitahara T, *et al*. Autophagy has a significant role in determining skin color by regulating melanosome degradation in keratinocytes. *J Invest Dermatol*. 2013;33(10):2416-2424.
13. Vel R, Udupi BP, Prakash MVSS, Adinarayanan S, Mishra S, Babu L. Effect of low dose tranexamic acid on intra-operative blood loss in neurosurgical patients. *Saudi J Anaesth*. 2015;9(1):42-48.
14. Shahid A, Khan A. Tranexamic acid in decreasing blood loss during and after caesarean section. *J Coll Physicians Surg Pak*. 2013;23(7):459-62.
15. Tse TW, Hui E. Tranexamic acid: an important adjuvant in the treatment of melasma. *J Cosmet Dermatol*. 2013;12(1):57-66.
16. Badran AY, Ali AU, Gomaa AS. Efficacy of topical versus intradermal injection of tranexamic acid in Egyptian melasma patients: A randomised clinical trial. *Australasian Journal of Dermatology*. 2021;62(3):e373-e379.
17. Pazyar N, Dezfuly MB, Hadibarhaghtalab M, Parvar SY, Molavi SN, Mapar MA, Zeinali M. Intradermal injection of 100 mg tranexamic acid versus topical 4% hydroquinone for the treatment of melasma: A randomized, controlled trial. *The Journal of Clinical and Aesthetic Dermatology*. 2023;16(1):35-40.
18. Pazyar N, Molavi SN, Hosseinpour P, Hadibarhaghtalab M, Parvar SY, Dezfuly MB. Efficacy of intradermal injection of tranexamic acid and ascorbic acid versus tranexamic acid and placebo in the treatment of melasma: A split-face comparative trial. *Health Science Reports*. 2022;5(2).