Experimental Dermatology

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CONCISE COMMUNICATION

Tranexamic Acid Inhibits 17β-Estradiol-Induced Melanogenesis Through PKA-CREB-MITF Pathway

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ABSTRACT

Tranexamic acid (TXA), a well-known anti-fibrinolytic agent, has been proven effective in the treatment of hyperpigmentation, particularly melasma. Oestrogen is known as an important cause of melasma and has been reported to induce pigmentation through the oestrogen receptor or the G protein-coupled oestrogen receptor. Although various mechanisms by which TXA improves skin pigmentation have been reported, its effect on oestrogen (17β-estradiol, E2)-induced pigmentation has not yet been elucidated. In this study, we investigated the effect of TXA on melanogenesis induced by 17β-estradiol. Cell viability was assessed in primary human epidermal melanocytes treated with 17β-estradiol or TXA. The effect of TXA on pigmentation was evaluated by western blot analysis, measuring the protein levels of phosphorylated CREB (p-CREB), MITF, and tyrosinase following treatment with 17β-estradiol. First, 17β-estradiol increases melanin production through the induction of the protein expressions of melanogenesis-associated molecules, including p-CREB, MITF, and tyrosinase. Our findings demonstrate that TXA inhibits 17β-estradiol-induced melanogenesis by downregulating the cAMP–PKA pathway. Given that TXA also reduces α-MSH-induced pigmentation via decreased phospho-PKA levels, our results suggest that TXA likely inhibits E2-induced melanogenesis by modulating the cAMP–PKA–CREB–MITF axis, contributing to its depigmenting effect.

1 | Background

Hyperpigmentation is a dermatological condition characterised by excessive synthesis and accumulation of melanin in the skin. Melanin production, known as melanogenesis, is regulated by various factors, including ultraviolet (UV) radiation [1–3]. Melanogenesis is primarily regulated by several pathways, including the α-MSH/MC1R, Wnt, SCF/c-Kit, and endothelin pathways. Activation of the melanocortin 1 receptor (MC1R), G protein-coupled receptor (GPCR), by α-MSH leads to increased intracellular cyclic AMP (cAMP) levels. Elevated cAMP activates protein kinase A (PKA), which subsequently phosphorylates cAMP response element-binding protein (CREB), inducing the transcription of microphthalmia-associated transcription factor (MITF). MITF is a master regulator that upregulates the expression of melanogenic enzymes, such as tyrosinase, ultimately promoting melanin production [4]. Dysregulation of this process can result in hyperpigmentation disorders, including melasma, lentigo, and post-inflammatory hyperpigmentation (PIH). Melasma, in particular, predominantly affects women and is influenced by several factors, such as pregnancy and hormonal changes [5–7].

Oestrogen (17β-estradiol), a steroid hormone primarily involved in reproductive functions, also plays important roles in skin physiology by modulating pigmentation and preserving skin homeostasis [8–10]. Oestrogen exerts its effects through classical nuclear oestrogen receptors, ERα and ERβ, as well as the

membrane-bound G protein-coupled oestrogen receptor (GPER) [11, 12]. Previous studies have demonstrated that oestrogen promotes melanogenesis by activating the PKA–CREB–MITF signalling pathway via ERα and ERβ, with PDZ domain protein kidney 1 (PDZK1) functioning as a mediator [13]. Moreover, oestrogen has been reported to stimulate melanogenesis through GPER, which similarly regulates the PKA–CREB–MITF signalling cascade [14, 15].

Tranexamic acid (TXA), a well-known anti-fibrinolytic agent, inhibits the binding of plasminogen to fibrin, thereby preventing its conversion to plasmin and suppressing fibrinolysis. TXA is widely utilised as an effective treatment for pigmentary disorders such as melasma by inhibiting melanin synthesis [16, 17]. Several studies have reported that TXA attenuates melanogenesis by modulating autophagy and the VEGFR signalling pathway, thereby downregulating the expression of MITF and key melanogenic enzymes such as tyrosinase [18–20]. However, the mechanism of TXA in oestrogen-induced melanogenesis is still not clear. In this study, we investigated the effects of tranexamic acid on oestrogen-induced melanogenesis, focusing on the PKA–CREB–MITF signalling pathway.

2 | Questions Addressed

Tranexamic acid (TXA) is widely used to treat hyperpigmentation, but its mechanism in oestrogen-induced melanogenesis is not fully understood. This study investigates whether TXA suppresses 17β-estradiol-induced pigmentation in human melanocytes via modulation of the PKA–CREB–MITF signalling pathway.

3 | Experimental Designs

3.1 | Reagents

17β-Estradiol (E8875), tranexamic acid (857653), and α-MSH (M4135) were purchased from Sigma-Aldrich (St. Louis, MO).

3.2 | Cell Culture

Primary human epidermal melanocytes were isolated from human foreskin of three donors aged 10–13 and cultured in Melanocyte basal medium (Lonza, Basel, Switzerland) supplemented with human melanocyte growth factors. Cells were used at passage numbers between 3 and 5 and maintained at 37°C in a humidified 5% CO_2 incubator. Human foreskin samples were collected with written informed consent, in accordance with the study protocol approved by the Institutional Review Board of Severance Hospital at Yonsei University College of Medicine (Seoul, Korea) (institutional review board number 7-2016-0064).

3.3 | Cell Viability

To assess cell viability of 17β-estradiol or tranexamic acid, the MTT assay was used. Primary melanocytes in a 96-well plate were incubated with MTT stock solution at 37°C for 4 h. After labeling with MTT, the cells were incubated for 10 min in DMSO, and then the absorbance of each sample was read at 570 nm.

3.4 | Melanin Contents

To evaluate melanin production, melanocyte pellets were dissolved in 1 N NaOH at 60°C for 2 h. The absorbance at 405 nm was measured using a microplate reader. Melanin contents were calculated with a standard curve of synthetic melanin (Sigma-Aldrich) and normalised to the amount of protein, then expressed as a percentage of the control.

3.5 | Western Blot

Cell extracts were lysed in radio-immunoprecipitation (RIPA) assay buffer containing protease and phosphatase inhibitor cocktail. Proteins were separated by 10% SDS-PAGE, followed by transfer to nitrocellulose membranes. Membranes were blocked with 5% skim milk in Tris-buffered saline containing 0.1% Tween-20 and then incubated overnight (4°C) with primary antibodies against PKA, phosphorylated PKA, CREB, phosphorylated CREB (Cell Signalling Technology, Danvers, MA), MITF, gp100 (Abcam, Cambridge, United Kingdom), and tyrosinase (Santa Cruz Biotechnology, Dallas, TX). GAPDH (Santa Cruz Biotechnology) served as a control for protein loading. Finally, the membranes were washed three times with Tris-buffered saline and 0.1% Tween-20 buffer and then incubated (1 h) with 1% skim milk in Tris-buffered saline and 0.1% Tween-20 buffer containing horseradish peroxidase/secondary antibody conjugates. Signal densities of the proteins visualised were detected using chemiluminescence reagent. The intensity of each protein band was normalised to the relative expression of GAPDH. Western blot quantification was performed using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

3.6 | Statistical Analysis

Statistical analyses were conducted using GraphPad Prism version 4.03 (GraphPad Software, San Diego, CA, USA). Data are presented as means ± SD, and differences between two groups were evaluated using a two-tailed Student's *t*-test.

4 | Results

4.1 | E2-Induced Melanin Production Is Confirmed in Primary Human Epidermal Melanocytes

Primary human epidermal melanocytes (PHEMs) were treated with 17β-estradiol (E2) or tranexamic acid (TXA), and cell viability was assessed using the MTT assay (Figure 1A,B). Treatment with 0.1 μM E2 or 1 mg/mL TXA for 72 h did not show cytotoxicity. We explored the effects of E2 on melanogenesis in PHEMs. E2 treatment (0.1 μM) increased the expression level of tyrosinase protein, a key enzyme in melanogenesis, accompanied by an elevation in melanin contents (Figure 1C,D). These results indicate that E2 promotes melanin production in PHEMs without affecting cell viability.

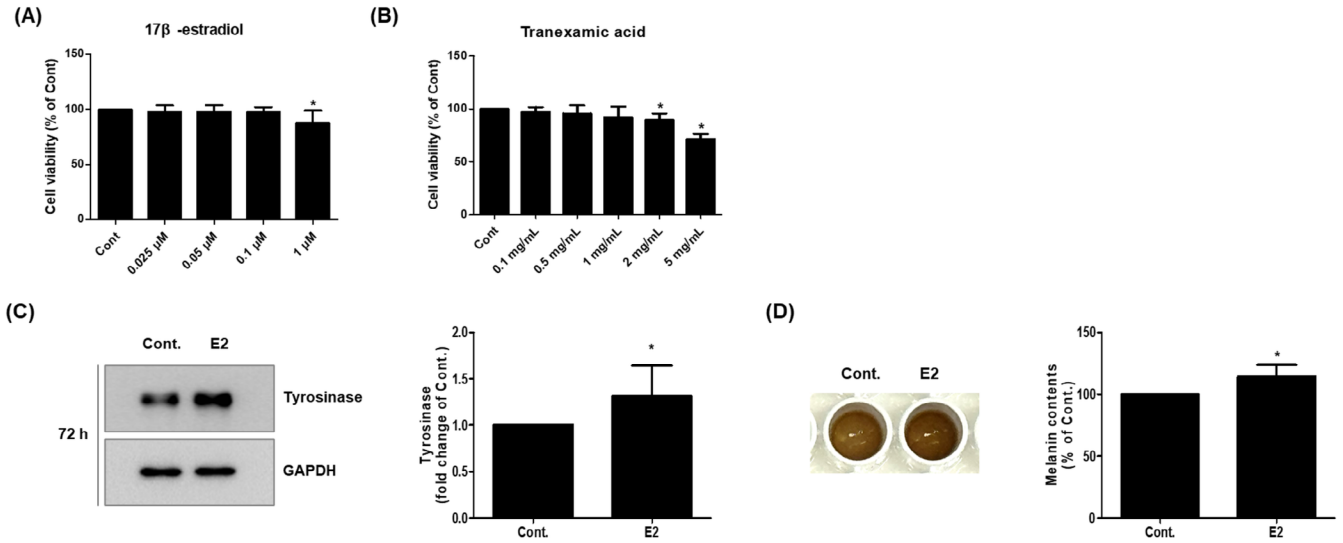

FIGURE 1 | Oestrogen induces melanogenesis in primary human epidermal melanocytes (PHEMs). (A, B) Relative cell viability of PHEMs treated with the 17β-estradiol (E2; 0.025–1 μM, $n = 5$) or Tranexamic acid (TXA; 0.1–5 mg/mL, $n = 4$) for 72 h. (C) Protein expression of tyrosinase ($n = 8$) and (D) melanin contents ($n = 5$) were measured after 72 h of incubation with E2 (0.1 μM). Results data are expressed as mean ± SD. *$p < 0.05$, two-tailed Student's *t*-test.

4.2 | TXA Inhibits E2-Induced via the CREB-MITF Pathway

To evaluate whether TXA regulates E2-induced melanogenesis, we examined melanogenesis signalling pathways by E2 treatment in the presence of TXA. E2 significantly increased the expression of melanogenesis-associated molecules, including phosphorylated CREB (p-CREB), MITF, and tyrosinase in the absence of TXA. However, pre-treatment with TXA inhibited the E2-induced upregulation of p-CREB and MITF (Figure 2A,B). Similarly, the E2-mediated increase in tyrosinase expression and melanin contents was also suppressed by TXA treatment (Figure 2C,D). These results suggest that TXA inhibits its E2-induced melanogenesis through suppressing the CREB-MITF pathway.

4.3 | E2 Activates PKA Signalling in Melanocytes, Similarly to α-MSH

To determine whether TXA truly inhibits E2-induced melanogenesis through the PKA signalling pathway, we also examined its effects on α-MSH, a representative activator of the cAMP–PKA signalling cascade. Time-dependent changes in phosphorylated protein kinase A (p-PKA) expression were assessed following treatment with E2 and α-MSH. Both treatments led to increased p-PKA expression, with notable elevations observed after 30 min of incubation (Figure 3A,B). Based on this observation, 30 min was selected as the standard incubation time for subsequent experiments.

4.4 | TXA Modulates E2-Induced Melanogenesis via the PKA Pathway

To validate whether TXA affects PKA activation in E2- or α-MSH-induced melanogenesis, PHEMs were pretreated with TXA for 1 h, followed by stimulation with E2 or α-MSH for 30 min. The upregulation of p-PKA observed in E2-treated cells was diminished by TXA pretreatment (Figure 3C). Similarly, TXA suppressed the increase in p-PKA levels induced by α-MSH (Figure 3D). These findings suggest that TXA may interfere with both E2 and α-MSH-induced melanogenesis, potentially through modulation of the PKA signalling pathway.

5 | Conclusions and Perspective

Oestrogen is known to regulate various physiological processes in the skin, including melanogenesis [8–10]. Several studies have demonstrated that oestrogen promotes skin pigmentation by upregulating melanogenesis-related molecules such as MITF and tyrosinase. These effects are mediated through both classical nuclear oestrogen receptors, ERα and ERβ, as well as the non-classical GPER [11, 12]. Although increased expression of oestrogen receptors has been observed in the skin of melasma patients, the presence of classical ERs in human melanocytes remains controversial, as some studies have reported their absence [14, 21, 22]. In contrast, GPER, a member of the G protein-coupled receptor family, is expressed in human melanocytes. GPER regulates melanin synthesis by modulating the expression of tyrosinase and MITF via activation of the cAMP–PKA signalling pathway [12, 14, 15]. Additionally, studies using GPER-specific agonists and antagonists have supported its role in regulating melanogenesis-related molecules [15]. Consistent with previous reports, our findings demonstrate that oestrogen promotes melanin synthesis in primary human melanocytes by activating the cAMP–PKA signalling pathway. Oestrogen treatment increased melanin content and tyrosinase expression, accompanied by enhanced phosphorylation of PKA, indicating that oestrogen stimulates pigmentation through CREB activation downstream of PKA. This mechanism parallels the well-characterised α-MSH–MC1R pathway, which similarly activates PKA and upregulates melanogenesis-related proteins.

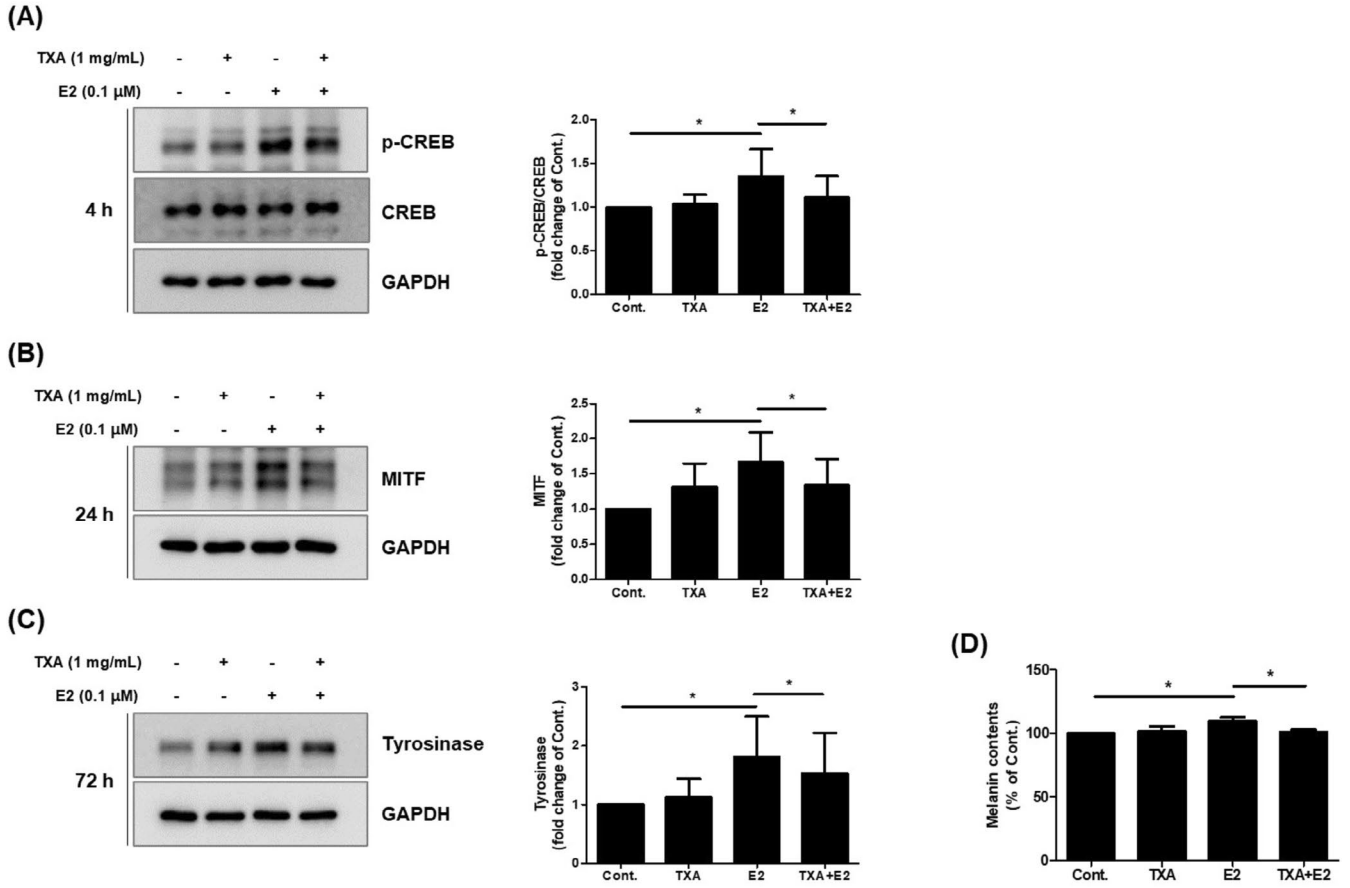

FIGURE 2 | TXA inhibits E2-induced melanogenesis via the CREB–MITF pathway. Cells were pretreated with TXA (1 mg/mL) for 1 h and then incubated with E2 (0.1 μM). The protein levels of melanogenesis-related molecules, such as (A) p-CREB (4 h, $n = 8$), (B) MITF (24 h, $n = 7$), and (C) tyrosinase (72 h, $n = 7$) were assayed using western blot. (D) Melanin contents were examined ($n = 5$). The results data are expressed as mean ± SD. *$p < 0.05$, two-tailed Student's *t*-test.

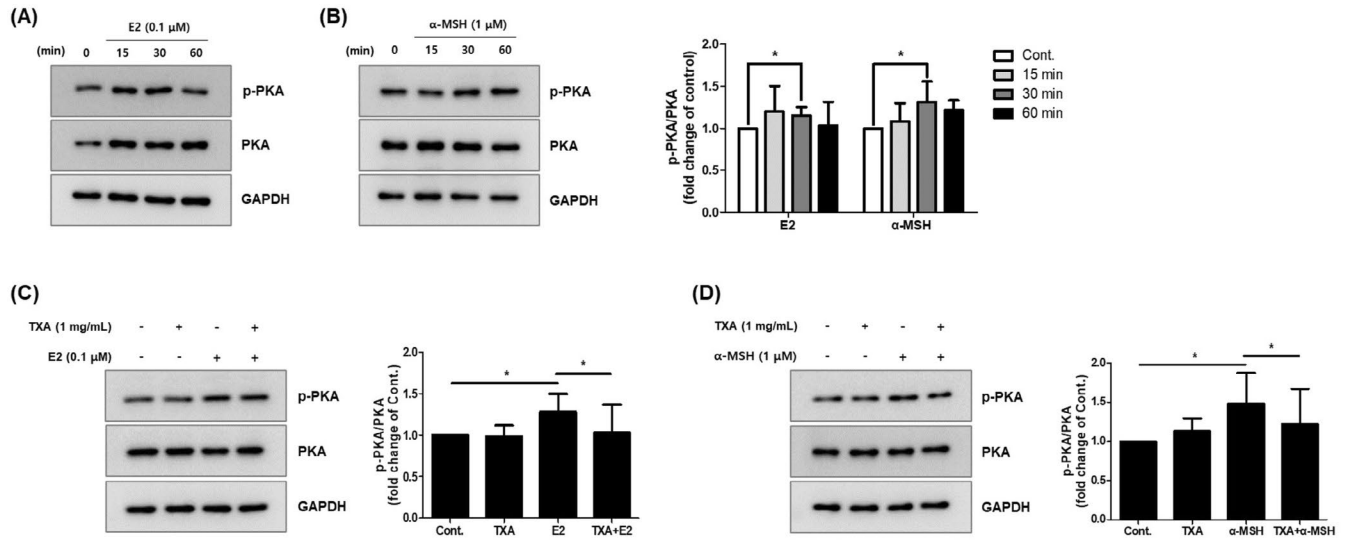

FIGURE 3 | TXA regulates E2-induced melanogenesis through the PKA pathway. (A, B) Cells were incubated with E2 (0.1 μM) and α-MSH (1 μM) for 15, 30, or 60 min, and *p*-PKA levels were assessed ($n = 6$). (C, D) Cells were pretreated with TXA (1 mg/mL) for 1 h, then incubated with E2 (0.1 μM) and α-MSH (1 μM) for 30 min. The expression of p-PKA was evaluated by western blot ($n = 6$). The results data are expressed as mean ± SD *$p < 0.05$, two-tailed Student's *t*-test.

TXA has been widely used in dermatology as an effective treatment for melasma [16, 17]. TXA exerts indirect anti-melanogenic effects by stimulating keratinocytes to secrete TGF-β1, which then suppresses melanogenesis in melanocytes via paracrine signalling [23].

Furthermore, TXA inhibits VEGF receptor activation in melanocytes and endothelial cells, reducing angiogenesis and melanogenesis [20]. It also suppresses endothelin-1 (ET-1) expression in dermal endothelial cells, thereby attenuating melanocyte activation and UVB-induced pigmentation [19, 24]. TXA has also been shown to promote autophagy in melanocytes by modulating the ERK signalling pathway and suppressing mTOR activity, which leads to downregulation of MITF and melanogenic enzymes [18].

In this study, we investigated how oestrogen modulates melanogenesis in human melanocytes and whether TXA, a well-known treatment for melasma, may inhibit E2-induced melanogenesis. However, our study was performed in vitro using primary human melanocytes, which may not fully reflect human skin under normal physiological conditions. The concentrations of E2 and TXA used in our experiments may differ from those achieved under physiological or clinical conditions, but were selected based on previous in vitro studies in human melanocytes [13, 16, 18, 20, 24]. Our data demonstrated that oestrogen enhances melanin production and upregulates the expression of key melanogenic proteins, including tyrosinase and MITF, via activation of the PKA-CREB signalling pathway. Importantly, TXA significantly reduced p-PKA levels induced by E2 or α-MSH. These findings suggest that TXA likely inhibits E2-induced melanogenesis by modulating the cAMP–PKA–CREB–MITF axis. The clinical efficacy of TXA as a treatment for melasma has been established through multiple in vivo and clinical studies [16, 24]. However, upstream signals of p-PKA in oestrogen-induced melanogenesis were not examined in this study. Collectively, this study proposes a novel mechanism underlying the melanin inhibitory effect of TXA. Our findings demonstrate that TXA effectively inhibits E2-induced melanogenesis, a key process implicated in melasma, providing mechanistic support for its therapeutic use, which is increasingly utilised in hyperpigmentation disorders, including melasma.

In summary, our study demonstrates that TXA effectively inhibits E2-induced melanogenesis in primary human melanocytes by modulating the PKA pathway, ultimately suppressing tyrosinase expression. These results provide a mechanistic basis for the clinical use of TXA in treating E2-related hyperpigmentation conditions such as melasma.

Author Contributions

Conceptualization: S.H.O., Y.J.B. Formal analysis: Y.J.B., J.Y.K. Funding acquisition: S.H.O., E.J.L. Investigation: Y.J.B. Methodology: Y.J.B., E.J.L., J.Y.K. Project administration: S.H.O. Supervision: S.H.O. Validation: Y.J.B., E.J.L., J.Y.K., S.P., S.H., I.J.w., J.M.A. Original draft preparation: Y.J.B., S.H.O. Revision and editing: Y.J.B., S.H.O.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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