Effect of tranexamic acid on melasma: a clinical trial with histological evaluation


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Abstract

Background Melasma is associated with epidermal hyperpigmentation, weak basement membrane, vascular proliferation and increased numbers of mast cell. Tranexamic acid (TXA), a plasmin inhibitor, is reported to improve melasma when injected locally. However, the effects of oral and topical TXA on melasma have not been well studied and the underlying mechanism remains unclear.

Objectives To elucidate the effects of oral and topical TXA on melasma.

Methods A clinical study was conducted with 25 women for 8 weeks from March to July 2010. Volunteers were instructed to take two TXA tablets three times a day and apply a TXA topical agent twice a day for 8 weeks. Skin pigmentation and erythema was measured using a Mexameter during each visit and skin biopsies were collected from eight subjects before and 8 weeks after treatment. Fontana-Masson, anti-CD31, antitryptase and antitype IV collagen staining was performed.

Results Twenty-two subjects completed the study and no serious adverse events occurred during the study period. The mean lesional melanin index (MI) scores decreased significantly. Interestingly, the MI scores for the perilesional skin increased. The erythema index scores of lesional and perilesional skin also showed a similar pattern. Histological analysis showed significant reduction of epidermal pigmentation, vessel numbers and mast cell counts. Type IV collagen staining was not observed in all specimens.

Conclusion TXA decreased epidermal pigmentation associated with melasma and also reversed melasma-related dermal changes, such as vessel number and increased numbers of mast cells.

Conflict of interest

This study was supported by Pacificpharma (Gyeonggi-do, Korea).

Introduction

A number of histological findings are characteristic of melasma. The amount of melanin is increased in all epidermal layers. The basement membrane of the lesional skin is often disrupted and thinner. Prominent solar elastosis, and increased numbers of blood vessels and elevated expression of vascular endothelial growth factor (VEGF) are other important features of melasma. Increased number of mast cells in lesional dermis, and increased expression of c-kit and stem cell factor have also been reported. These findings show that various dermal changes as well as increased epidermal pigmentation are associated with melasma.

Tranexamic acid (TXA) is a plasmin inhibitor. This compound is an antifibrinolytic widely used to prevent and treat haemorrhage. Localized intradermal injection of TXA has been reported to improve melasma. This study was performed to evaluate the effect of TXA oral and topical agents on melasma. We also analysed histological changes after TXA treatment to investigate the underlying mechanism of action.

Methods

Study design

Twenty-five female subjects aged 20–55 years were enrolled. This study was approved by the Institutional Review Board (IRB) of Seoul National University Bundang Hospital (IRB approval number: B-1002/093-001), and all subjects provided written informed consent. Subjects who had chronic diseases, hypercoaculative disorders, dermatitis on the face, who were pregnant, taking oral contraceptives or TXA, who had been treated for their melasma within 4 weeks prior to the study were not included. Subjects took two TXA tablets (white project tab; Pacificpharma, Gyeonggi-do, Korea) three times a day and applied a TXA topical agent (white...
project® essence, Pacificpharma) on the whole face, twice a day for 8 weeks. Each TXA tablet contained 125 mg of TXA, 50 mg of ascorbic acid, 40 mg of l-cysteine, 4 mg of calcium pantothenate and 1 mg of pyridoxine chloride. The topical agent was 2% TXA and 2% niacinamide. Subjects were asked to use standard sunscreen on the whole face during study period.

Assessment of TXA treatment efficacy

Subjects were followed up at week 4 and 8 of treatment; standardized photographs using a Robo Skin Analyzer® (Inforward, Tokyo, Japan) were taken at these times. Skin colour of the lesional and perilesional skin was measured using a Mexameter® (Courage and Khazaka, Cologne, Germany). Scores for the melanin index (MI) and erythema index (EI), which indicate the darkness and degree of erythema were measured with this apparatus. Subjects’ evaluation about improvement of pigmentation and erythema was performed at the final visit by questionnaire.

Immunohistochemistry and image analysis

To evaluate histological changes, 2 mm sized skin biopsy specimens were obtained from the lesional skin of eight subjects, before and 8 weeks after TXA treatment. Melanin was visualized with Fontana-Masson staining, and the amount of pigmentation was determined by the ratio of the pigmented area to the measured epidermal area. For immunohistochemical staining, paraffin-embedded sections were processed as previously described.2 To visualize the vascularity, a monoclonal antibody against CD31 (DakoCytomation, Glostrup, Denmark) diluted 1 : 100 was used. At 100× magnification, the number of vessels (per mm²), average vessel diameter (vessel size) and relative area occupied by blood vessels (vessel area) were determined in the dermis in an area within 400 μm from the epidermal–dermal junction. Mast cells were stained with an antitryptase antibody (Abcam, Cambridge, UK) diluted 1 : 50. At 100× magnification, mast cells (per mm²) in the dermis were counted. To assess basement membrane integrity, antitype IV collagen staining (1 : 50 dilution; Cell Marque, Rocklin, CA, USA) was performed. All the histological sections were analysed using an image analysis program (Meta Imaging series 7.7; Molecular Devices, Sunnyvale, CA, USA).

Statistics

Sequential changes of MI and EI were analysed using a paired t-test. The results of the histological analysis were analysed using the Wilcoxon test. SPSS 15.0 software (SPSS, Chicago, IL, USA) was used and P < 0.05 was considered to be significant.

Results

Evaluation of treatment efficacy by instrumental measurement

Twenty-two subjects completed the study. No serious adverse events were observed throughout the study period. The mean MI scores for the lesional skin (Fig. 1a) decreased from 191.48 (0 week) to 186.14 (4 weeks, P = 0.002), and to 184.82 (8 weeks, P = 0.017 vs. 0 week). Interestingly, MI scores for the perilesional skin (Fig. 1b) increased from 120.3 (0 week) to 126.24 (4 weeks, P = 0.024), and to 129.70 (8 weeks, P = 0.001 vs. 0 week). The darkening of perilesional skin is thought to be affected by seasonal changes, from spring to summer. EI scores also changed in similar patterns, but these were not statistically significant. EI scores for the lesional skin (Fig. 2a) decreased from 272.15 (0 week) to 255.00 (8 weeks, P = 0.112). EI scores for the perilesional skin (Fig. 2b) increased from 216.64 (0 week) to 231.14 (8 weeks, P = 0.194). However, the ΔE between the scores for lesional and perilesional skin (Fig. 2c) significantly decreased from 55.38 (4 weeks) to 23.86 (8 weeks, P = 0.013 vs. 4 weeks).
Subjective evaluation

Improvement of pigmentation (Fig. 3a) and erythema (Fig. 3b) were observed in the photographs. Furthermore, most volunteers stated that their skin became clear (90.9%), lighter in colour (95.5%) and less erythemic (90.9%).

Histological evaluation: epidermal pigmentation, vascularity, mast cell numbers and basement membrane integrity

Epidermal pigmentation significantly decreased (Fig. 4a,b, Table 1). Vessel numbers were also significantly reduced. Vessel size and area varied widely and there were no statistical significance between 0 and 8 weeks (Fig. 4c,d, Table 1). Mast cell numbers decreased significantly (Fig. 4e,f, Table 1). Type IV collagen staining was not observed in specimens collected before or after treatment (data not shown), whereas this was usually well defined in healthy skin. In summary, treatment with TXA oral and topical agents decreased epidermal pigmentation as well as vascularity and mast cell numbers.

Discussion

This study showed that lesional MI scores decreased after TXA treatment whereas non-lesional MI scores increased during the summer. These results suggest that TXA has differential effects on lesional skin and non-lesional skin, which has not been well documented in the literature. Topical TXA was reported to inhibit UV-induced pigmentation in guinea pigs by reducing UV induced...
plasmin activity and subsequent free arachidonic acid release.\textsuperscript{7,8} However, this may not be the main mechanism because it cannot explain the differential effects on lesional and non-lesional skin. Topical niacinamide, another component of a topical agent used in this study, increases skin lightness by blocking melanosome transfer.\textsuperscript{9} Ascorbic acid, one of the components of the oral agent, can also improve melasma.\textsuperscript{10} However, none of these compounds can explain the differential effects on lesional and non-lesional skin. We have also observed differential improvement of lesional melasma skin from oral TXA treatment alone. These results were obtained from a clinical trial performed independently at our hospital.\textsuperscript{11}

**Figure 4** Histological changes after 8 weeks use of tranexamic acid. (a, b) Fontana–Masson stains show reduced epidermal pigmentation (>100). (c, d) In anti-CD 31 stains, reduced vascularity is observed (>100). (e, f) Antitryptase stains show reduced mast cell numbers (>100).

**Table 1** Histological changes after tranexamic acid treatment. Skin biopsy specimens were obtained from the lesional skin of eight subjects, before and after 8 weeks of treatment. Melanin pigment was visualized with Fontana–Masson staining, and the amount of pigment was determined by the ratio of pigmented area to the measured epidermal area. Endothelium was stained with an anti-CD31 antibody. The number of vessels (per mm\textsuperscript{2}), average vessel diameter (vessel size) and relative area occupied by blood vessels (vessel area) were determined in the dermis in an area within 400 μm from the epidermal–dermal junction. Mast cells were stained with an antitryptase antibody and the number of mast cells (per mm\textsuperscript{2}) was determined in the dermis at 100× magnification. Type IV collagen was not stainable in all specimens.

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<th></th>
<th>0 week</th>
<th>8 weeks</th>
<th>(P) value</th>
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</thead>
<tbody>
<tr>
<td>Epidermal pigmentation (%)</td>
<td>28.9 ± 11.2</td>
<td>23.4 ± 11.0</td>
<td>0.025*</td>
</tr>
<tr>
<td>Vessel number (per mm\textsuperscript{2})</td>
<td>18.0 ± 4.4</td>
<td>12.5 ± 3.0</td>
<td>0.018*</td>
</tr>
<tr>
<td>Vessel size (μm)</td>
<td>31.3 ± 16.6</td>
<td>33.0 ± 23.3</td>
<td>1.000</td>
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<tr>
<td>Vessel area (%)</td>
<td>1.0 ± 0.5</td>
<td>0.8 ± 0.4</td>
<td>0.735</td>
</tr>
<tr>
<td>Mast cell number (per mm\textsuperscript{2})</td>
<td>311.9 ± 116.7</td>
<td>220.5 ± 126.5</td>
<td>0.012*</td>
</tr>
<tr>
<td>Type IV collagen staining</td>
<td>0/8</td>
<td>0/8</td>
<td>(P) value</td>
</tr>
</tbody>
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\*Wilcoxon test, statistical significance between groups: \(P < 0.05\).
Changes in EI scores showed similar pattern to those of MI: a decrease in lesional skin and an increase in non-lesional skin. These results suggest that the effects of TXA may be related to changes in erythema or related dermal changes. During our histological evaluation, we observed that decreased erythema was accompanied by reduced numbers of vessels and mast cells.

It is well known that plasmin plays an important role in angiogenesis. Plasmin converts extracellular matrix-bound VEGF into freely diffusible forms.\(^\text{12}\) TXA, a plasmin inhibitor, suppresses angiogenesis, and also inhibits neovascularization induced by basic fibroblast growth factor (bFGF).\(^\text{13,14}\) Thus, reduced erythema and vessel numbers might be the results of TXA antiangiogenic action.

In the literature, it has been reported that mast cells are increased in the dermis of melasma lesion. Mast cells are related to various histological changes associated with melasma. Repetitive UV irradiation increases the number of mast cells and mast cell tryptase.\(^\text{15}\) Tryptase degrades type IV collagen,\(^\text{15}\) thus, increased numbers of mast cells and tryptase might be the cause of weak basement membranes observed in melasma. Mast cells also play an important role in the development of solar elastosis, one of the histological features of melasma. Elastin content in UV exposed skin correlates with mast cell counts.\(^\text{16}\) Furthermore, mast cell-deficient mice do not develop solar elastosis after repetitive UV irradiation.\(^\text{17}\) Mast cells can also induce vascular proliferation by secreting various angiogenic factors, such as VEGF, FGF-2, transforming growth factor-\(\beta\).\(^\text{18}\)

Tranexamic acid can decrease the activity of mast cells, for example, mast cell activation after ischaemia and reperfusion injury is almost completely abolished by TXA treatment.\(^\text{19,20}\) In our study, the number of mast cells also decreased after TXA treatment. These findings suggest that the effect of TXA may be partly due to its inhibitory effects on mast cells which may affect vascularization and dermopathy.

Pigmentation of melasma is confined to the epidermis,\(^\text{1}\) but dermal factors are suspected to be the main culprits behind the recurrent and refractory nature of this disorder. In our study, TXA reversed melasma-related dermal changes, such as vessel proliferation and increased mast cell numbers. Although it remains unknown what dermal factors are the main causes of melasma, TXA may act by reducing dermal factors related to melasma and this might explain the differential effects of TXA on lesional skin.

In conclusion, TXA oral and topical agents improved epidermal pigmentation and erythema of melasma. These compounds also reversed melasma-related dermal changes, such as vessel proliferation and increased numbers of mast cell.

Acknowledgements
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References