Original Article

Ameliorating Role of Pomegranate on Minocycline induced Pigmentation in the Epidermis of Guinae Pig

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ABSTRACT

Objective: To investigate the ameliorating role of pomegranate on minocycline induced pigmentation in the epidermis of guinea pig.

Study Design: An experimental observational study.

Place and Duration of Study: This study was conducted at the Anatomy Department, BMSI, J PMC, Karachi.

Materials and Methods: 30 adult guinea pigs were divided into 3 groups A B and C. In this study, A served as control, B was given Minocycline, while C was given Minocycline with Pomegranate for 8 weeks, after which their skin was processed for histological examination of morphology of melanocytes and pigmentation in Dopa Oxidase stained sections under light microscope.

Results: The melanin pigmentation deposition in Minocycline treated group B was distributed densely and extended till stratum corneum as compared to the control group A, while in the Pomegranate treated group C along with Minocycline, the melanin pigmentation was considerably reduced and was observed to be distributed sparsely extended till stratum basale. The morphology and number of melanocytes in both treated groups remain same as compared to control group A

Conclusion: Based on the present study it is concluded that pigmentation induced by Minocycline can be reduced and may be prevented by taking pomegranate simultaneously.

Keywords: Pomegranate, Minocycline, Epidermal Pigmentation, Melanocytes, Guinea pigs.

INTRODUCTION

Skin the largest human body organ provides a major interface between the environment and the body and is constantly exposed to an array of chemical and physical environmental pollutants. In addition a large number of dietary contaminants and drugs can manifest their toxicity in skin[1]

Drugs may cause exanthemas, utricaria, hypersensitivity syndromes, cutaneous necrosis and hyperpigmentation of the skin[2]

Numerous common drugs can stimulate human skin pigmentation such as certain antibiotics (sulfonamides and tetracycline's), diuretics, nonsteroidal anti-inflammatory drugs, pain relievers, and some psychoactive medications.[3] These pigmentary changes caused by drugs usually result in a limited degree of morbidity,[2] but unwanted pigmentation can produce a significant Psychological stress.[4] The pathogenesis of these pigmentary disorders can be categorized into several mechanisms out of which one is enhanced melanin production with or without an increase in the number of active melanocytes.[2]

Minocycline, a semi synthetic, broad spectrum antimicrobial tetracycline that was introduced in 1967,[5] to be used in the treatment of acne, [6,7] is extensively being used as an anti-inflammatory, antiapoptotic, antichemotactic, collagenase inhibitory, and immunomodulatory agent.[8,9,10] It also acts as an inhibitor of hydrogen peroxide-induced oxidative stress.[11] A well- documented and cosmetically displeasing side effect is skin pigmentation in its long term therapy.[12,13,14]. Burns et al[15] (2011) had quoted multiple histological and electron microscopic studies that have demonstrated increased melanin, haemosiderin, and either Minocycline or a metabolite in the skin.

Pomegranate is native from the Himalayas.[16] It is used in the traditional medicines of different Asian cultures for the treatment of a variety of ailments.[17] The fruit is a rich source of polyphenol compounds, [18] which are: anthocyanins such as cyaniding, delphinidin and hydrolysable tannins such as ellagic acid, gallic acid accounting for 92% of the antioxidant activity.[19] Its main constituent, ellagic acid, is an antioxidant,[20] cytoprotective agent against oxidative stress induced by alcohol[21] and drug-induced liver injury.[22] It is mostly abundant in berries, walnuts, pecans, pomegranate, cranberries and other plant food in the form of hydrolysable tannins called ellagitanins.[21] Ellagic Acid has been reported to prevent pigmentation caused by sunburn.[23] Also, in a topical application study on guinea pig and human skin, inhibitory effect of ellagic acid on UV –induced pigmentation in the skin was observed.[24,25]

As the most essential enzyme in the melanin biosynthetic pathway is tyrosinase and it is the only enzyme absolutely required for melanin production

which is known to be a metaloenzyme, containing copper at an active site, and ellagic acid suppresses tyrosinase activity.[23] Zho and Goa[26] (2008) also reported the copper chelating role of ellagic acid resulting in decrease proliferation of melanocytes.

In the perception of above mentioned literature and studies, it is clear that although various studies being done to explore the protective effects of pomegranate against pigmentation induced by UV-irradiation. The present experimental study was designed to explore the protective role of pomegranate in Minocycline induced pigmentation of skin.

MATERIALS AND METHODS

In this experimental study,30 adult male guinea pigs weighing between 400 – 600 grams were taken and divided into 3 groups A, B and C. Group A animals served as control, group B received Minocycline 0.0003mg/G body weight/ day orally, and group C received Minocycline 0.0003mg/G body weight of animal/day orally along with pomegranate 0.4mg/G body weight of animal/day orally for 8 weeks. All the guinea pigs were sacrificed under ether anesthesia in a glass container and one skin fragment of two inches size, from abdomen was taken from each animal. It was fixed in 10% formalin in the pH 7.4 buffer (v/v) plus 0.44 M sucrose for DOPA-OXIDASE TISSUE BLOCK METHOD. A 2 mm thin vertical section was cut from the fragment and paraffin blocks were made, 4 to 5 micron thick sections were obtained were counter stained with Meyer's Haematoxylin and observed under light microscope. The results were considered statistically significant if the P-value was < 0.05.

RESULTS

In control group A, the shape of DOPA positive melanocytes was observed and found to be ovoid to round in shape with sparsely stained dendrites spread within the keratinocytes of stratum spinosum. The nuclei were ovoid to round in shape while the cytoplasm was pale staining with varying dendritic processes (black brown) scattered between keratinocytes of stratum basale and spinosum. Melanin pigment was deposited in scattered pattern within the stratum basale and graded as "+" (Figure-1 & table-1). The mean number of melanocytes in abdomen was 1.52 ±0.05 (table-2).

Table No. 1: Distribution and Extension of Melanin Pigment Deposition in Epidermis of Guinea Pig

Group	Treatment given	Dose & duration	Distribution and extension of melanin pigment deposition		
			Sparse*	Moderate**	Dense***
A (n=10)	Control	Laboratory diet ad labitum for 8 weeks	+		
B (n=10)	Minocycline	0.0003mg/G body weight/day(Orally) for 8 weeks			+++
C (n=10)	Minocycline + Pomegranate	0.0003mg/G body weight/ day + 0.4mg/G body weight/ day(Orally) for 8 weeks	+		

*Sparse: melanin pigment deposited in scattered pattern up to stratum basale
** Moderate: melanin pigment deposited in patches up to stratum spinosum
*** Dense: melanin pigment deposited uniformly up to stratum corneum

Table No.2: Mean number of melanocytes in the abdominal epidermis of various groups in guinea pig

Group	Treatment given	Dose & duration	Mean number of melanocytes
A (n=10)	Control	Laboratory diet ad labitum for 8 weeks	1.52± 0.05*
B (n=10)	Minocycline treated	0.0003mg/G body weight/day(Orally) for 8 weeks	1.51± 0.01*
C (n=10)	Minocycline + Pomegranate	0.0003mg/G body weight/ day + 0.4mg/G body weight/ day(Orally) for 8 weeks	1.50± 0.003*

*P-value >0.05 (Insignificant); **P-value < 0.05 (Significant); ***P-value < 0.01 (Moderately significant); ****P- value < 0.001 (Highly significant)

In group B, the morphology of melanocytes appeared to be similar but the intensity of melanin pigment was dark when compared with the group A animals. Melanin pigment deposition was dense in uniform pattern within all the layers of epidermis i.e. from stratum basale up to stratum corneum and graded as "+++" (Figure-2 & table-1). The mean number of melanocytes in abdomen was 1.51 ± 0.05 which was insignificant (P- value>0.05) compared to control group A animals (table- 2).

In group C, not only the morphology of melanocytes but also the intensity of melanin pigment was appeared to be similar to that in group A animals. Melanin

pigment deposition was sparse in scattered pattern within the stratum basale and graded as "+" (Figure-3 & table-1). The mean number of melanocytes in abdomen was 1.50 ± 0.003 which was insignificant (P-value > 0.05) when compared to the both groups B and A animals (table-2).

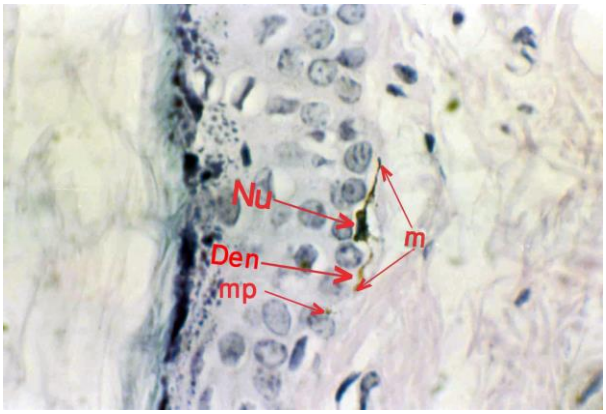

Figure-1:DOPA-OXIDASE & Mayer's Haematoxylin stained section, from abdominal epidermis of control guinea pig animal, showing the morphology of normal melanocyte(m) with ovoid nucleus(Nu) and brown black pigmented dendrites(Den) scaterred among the adjacent keratinocytes and melanin pigment deposition(mp) sparsely distributed and extended within the stratum basale. Photomicrograph X100.

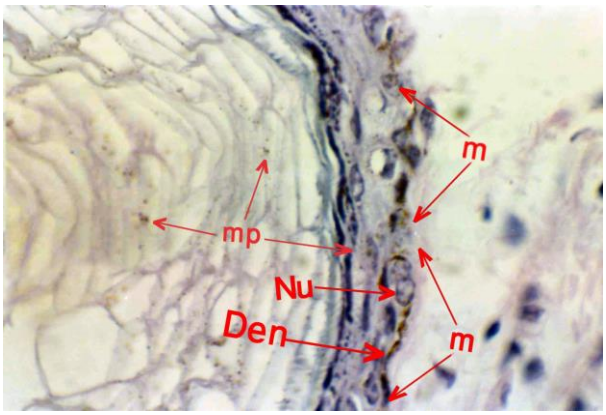

Figure 2: DOPA-OXIDASE & Mayer's Haematoxylin stained 5 µm thick section, from abdominal epidermis of Minocycline treated guinea pig, showing the morphology of melanocyte (m) with ovoid nucleus (Nu) and brown black pigmented dendrites (Den) scaterred among the adjacent keratinocytes and melanin pigment(mp) deposition is densely distributed and extended up to stratum corneum. Photomicrograph X100

DISCUSSION

Human skin exists in a wide range of different colors and gradations, ranging from white to brown to black.[3] Differences in skin color are related to the number, size, shape distribution and degradation of melanin - laden organelles called melanosomes.[15] Thus, melanin synthesis within melanosomes and their distribution to keratinocytes within the epidermal melanin unit determines skin pigmentation, [3] which also protects the skin from the harmful effects of sunlight.[4] Melanin is synthesized in the melanocyte, with tyrosinase playing an important role in the process. As a result of tyrosinase activity, tyrosinase is transformed first into 3, 4-dihydroxyphenylalanine (dopa) and then into dopa quinone, which is converted, after a series of transformations, into melanin. [3,15]

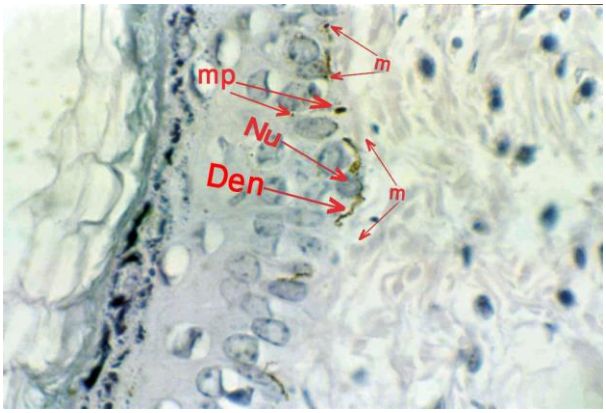

Figure 3: DOPA-OXIDASE & Mayer's Haematoxylin stained 5 µm thick section, from abdominal epidermis of Minocycline treated guinea pig protected with pomegranate, showing the morphology of normal melanocyte (m) with ovoid nucleus (Nu) and brown black pigmented dendrites (Den) scaterred among the adjacent keratinocytes and melanin pigment(mp) deposition sparsely distributed and extended within the stratum basale. Photomicrograph X100.

Minocycline, a tetracycline derivative antibiotic, is commonly used to treat infections of various systems such as integumentary, genital and urinary systems.[9] It is also recognized as an effective, well tolerated therapy in rheumatoid arthritis, induces pigmentation. Thus, cutaneous pigmentation is a well recognized complication of Minocycline therapy.[13,14]

Pomegranate (Punica granatum) is used as a medicine for a variety of ailments. It is used in the form of an aqueous decoction for dysentery and diarrhea; [20] The fruit has been developed into dry and liquid forms to provide an alternative convenient source form of ellagic acid.[18] The Ellagic Acid has been proved to be highly effective in suppressing the melanogenesis by reacting with activated melanocytes and without causing any cellular injury.[23]

In Group B, the morphology of melanocytes was similar to the group A animals but melanin deposition was dense and deposited till the stratum corneum due to the inhibition of hydrogen peroxide induced selective destruction of melanocytes by the minocycline that acts as an inhibitor of free radical production and lipid per oxidation capable of attenuating the oxidative stress-induced toxicity.[11] Prasad and Kanwar[10] (2010) explained that 90% of the vitiligo patients showed the progression of the disease was arrested and 22% showed repigmenttation of the diseased lessons due to

direct radical Scavenging activity of minocycline. The increased pigmentation was due to the activation of melanocytes through its surface receptors through α-MSH that is being modulated by Minocycline activated Keratinocytes in vitro.[6] Mouton, Jordan and Schneider[7] in 2004 reported that the pigmentation that started after 4-6 weeks of Minocycline treatment in 36% acne vulgaris patients.

In group C, the morphology of melanocytes and the melanin pigmentation was similar to control group A animals of melanin because of inhibitory effects of Ellagic Acid on the tyrosinase enzyme resulting in decreased pigmentation.[24] Yoshimura et al (2005) demonstrated that the Ellagic Acid rich pomegranate extract inhibited the mushroom tyrosinase activity and exhibited its inhibitory effects in U-V induced pigmentation of skin in rats when given orally by showing the decrease in number of DOPA positive melanocytes quantitatively. Kasai et al (2006)[25] also suggested that Ellagic Acid rich pomegranate extract when given in its lowest dose for 4 weeks shows protective effect on sunburn caused by UV irradiation.

CONCLUSION

It is concluded that Pomegranate is very potent in providing protection against Minocycline induced pigmentation due to its main constituent, ellagic acid, which directly inhibits melanogenesis. This suggests that the concomitant use of pomegranate with Minocycline can reduce pigmentation in patients on long term Minocycline therapy. The present work is under progress for further extended studies in exploring the role of pomegranate.

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