Immuno-histochemical study of the expression of ICAM-1 in the skin of mice under the effect of exposure to ultra violet rays type-B, before and after topical Retinoic acid

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Abstract
The exposure to ultraviolet radiations (UVR) become a medical problem, not just cosmetics or aesthetic concern, but for their skin photoaging and photodamage.

The naturally and synthetic derivatives of vitamin A (Retinoids) may have a role in treatment and prophylaxis against skin photodamage.

The current work studied the effect of ultraviolet-B rays on the ICAM-1 expression of mice's skin, before and after topical retinoids acid.

Thirty-six mice were subjected to ultraviolet-B rays in dose of 1.4J/cm² for 15minutes every other day for 10weeks. The mice were subdivided into 3 equal groups; Radiated, Prophylactic, Treated, besides the non-exposed skin samples, which considered as control group. The prophylactic mice were subjected to topical Retinoic acid one day before UVR exposure, the mice of treated groups were subjected to topical Retinoic acid after the last UVR exposure 3times weekly for 10weeks.

Paraffin sections slides were prepared and stained with Hematoxylin and eosin stains for study the morphology.

The immuno-histochemical study for detection of ICAM-1 expression was performed using labeled streptavidin biotin technique (Dako) with the monoclonal antibody {ICAM-1 (G-5)}.
The ICAM-1 expression was evaluated as optical density, and the obtained data was statistically analyzed by using student's t-test.

The study revealed a clinical signs of photodamaged skin only in radiated group mice. Histologically epidermal thickness associated with keratinocytic atypia, and necrotic cells were observed in radiated group mice. There was a statistically significant increase in the optical density of ICAM-1 expression in radiated and prophylactic groups mice (p<0.001 and p< 0.005 respectively).

The study concluded that retinoic acid given after UVB completely reversed the morphological, histological and ICAM-1 expression changes induced by UVB exposure and retinoic acid given before UVB prevented some of the morphological, histological and ICAM-1 expression changes induced by UVB exposure.

Introduction
Repeated exposure to UVR leads to chronic changes in the appearance and function of the skin described as Photoaging and photodamage (Kaminar, 1995). The most prevalent and well-known source of UVR is the sun. Phototherapy is an additional potential source and tanning beds have become a popular source of artificial UVR (Fitzpatrick, 1997).

Photoaging and photodamage include; wrinkles (fine and coarse), roughness, laxity, mottled pigmentation, actinic (lentigines and keratosis), scaling, xerosis and telangiectasia (Leyden, 2001).
A prophylactic measures to minimize human cutaneous UVR exposures include, adsorbent, reflectant or combination sunscreen (Diffey, 1996). Treatment of photoaged skin is done by topical therapies to reverse photoaging includes the use of retinoids, alphahydroxy acid and antioxidants in addition to sunscreen.

The term retinoid includes both the naturally and synthetic derivatives of vitamin A. Retinoids have a role in regulating morphogenesis, vision, cellular proliferation, differentiation and matrix production. Many studies revealed that retinoids can be benefit in the treatment of photodamaged skin (Chen et al., 1992; DiGiovanna and Bethesda 1992; Oliver et al., 1995). Topical retinoids are also used in the treatment of acne, psoriasis, and promoting wound healing and systemic retinoids protect against cancer (Chu Hsu 1998).

Cell adhesion molecules (CAM) involved in the cell to cell adhesion required for inflammation. The members of this super-family (LFA-3 (CD58), ICAM-1-3, VCAM-1 and PECAM-1) are characterized by the presence of one or more immunoglobulin homology regions, each consisting of disulfide – bridged loop (Gearing and Newman 1994). The chief function of CAM is to bind leucocytes to cell expressing them, or to endothelium to facilitate vascular emigration or to cytokine activating fibroblasts and keratinocytes to aggregate leucocytes at sites of inflammation (Lobb, 1992). ICAM-1 is expressed lightly on normal endothelium. However, normal keratinocytes don't express ICAM-1 when examined by usual immunohistoc- hemical methods, although extremely small amounts are detectable by electron microscopy. This expression may be sufficient for normal leucocytes and Langerhans cell migration (Lonati et al., 1996).

The role of topical retinoids in the treatment of photoaging was assessed in many studies. However, no available studies were done to investigate its preventive and therapeutic role of retinoic acid on UVB induced skin using immuno-histochemical ICAM-1 expression technique.

Materials and Methods

Thirty-six adult male mice were subjected to the study. A pre-defined shaved area of 2cm x 2cm was selected in the back of each for ultraviolet exposure. Ultraviolet B rays was obtained by using F-s-40 sun-lamp produced 1.4J/cm² of wavelength 290-320nm. The lamp was placed 25cm from the back of mice for 15 minutes per session, on alternate days for 10 weeks (Chen et al 1992).

The mice were subdivided into 3 equal groups; Radiated, Prophylactic and Treated. All mice were exposed to UVB, Radiated group mice didn't subject to local retinoic acid application.

The prophylactic group mice were subjected to local retinoic acid application one day before exposure to UVB, while treated group mice were subjected UVB exposure followed by local retinoic acid administration 3 times per week for 10 weeks after last exposure.

The topical Retinoic acid was product of Ciba-Gigy (Retin A 0.05% ointment). The dose was 1/5 finger tip point (finger tip unit= amount of cream or ointment sufficient to cover 20cm² of skin) was applied to the mice shaved area. (Chaqour, et al., 1997)

Mice were sacrificed after 24hrs of last exposure to ultraviolet rays. Two skin biopsies were taken from all mice; one from the exposed area and represent experimental (Radiated, Prophylactic and Treated) samples and the other from non-exposed area and represented a control sample for each mice.

The skin biopsies were fixed in neutral buffered formal and processed for preparation of 4um thick paraffin section slides.

The paraffin sections were stained by Hematoxylin and Eosin stain for morphological change.

Immuno-histochemical technique for detection of ICAM-1 expression was performed using labeled streptavidin biotin technique (Dako) with the monoclonal antibody {ICAM-1 (G-5)}. 
Immuno-histochemical study of the expression.......

The localization of ICAM-1 was demonstrated as yellowish brown color area. (Shi et al., 1991, Taylor et al., 1994). Evaluation of ICAM-1 expression was done by the aid of Leica Image analyzer system as an optical density on 20 slide fields per biopsy (240 readings per group).

The obtained data were statistically analyzed using student t-Test for comparison between exposed and non-exposed areas for all groups.

**Results**

**Clinical Findings:**

**Exposed Skin:**

**Radiated Group**

The shaved area of the mice's skin appeared erythematous in the first 3 weeks. After 6th week, the exposed skin showed marked mottled, pigmentation, longitudinal creases (wrinkles), laxity and scales (signs of photodamage).

**Prophylactic Group**

The exposed area of mice of this group showed mild mottled pigmentation, and no creases, laxity or scales.

**Treated Group**

The shaved area of the mice's skin appeared erythematous in the first 3 weeks. After 6th week, signs of skin photodamaged were observed. After retinoic acid application, moderate clinical improvement of the photodamaged skin was noticed at the 14th week. At the end of 10 weeks administration the skin appeared nearly similar to non-exposed skin.

**Morphometric Results:** (Plate -1)

**Non-exposed Skin: (Control Group)**

The normal non-exposed skin of mice showed very thin epidermis, with ill-defined strata, stratum basale cells had clear oval nuclei (Plate1-A and Plate1-B).

**Exposed Skin: Radiated Group**

In the area exposed to ultraviolet B, skin showed an increase in the epidermal thickness (Plate1-C) associated with necrotic cells in the form of vacuolated cytoplasm and pyknotic nuclei. There was an increase of mitotic figures, compact ortho-keratosis in the stratum corneum (Plate1-D).

**Prophylactic Group**

The skin of mice that received retinoic acid before UVB exposure showed mild increase in epidermal thickness in comparison to their non-exposed skin. Most keratinocytes appeared normal. Occasional keratinocytic atypia and mild increase in mitotic figures in the stratum basale layer were seen. Also mild compact ortho-keratosis in the stratum corneum was also seen (Plate1-E and Plate1-F).

**Treated Group**

The skin of mice of this group showed normal epidermal thickness similar to that their non-exposed skin. Keratinocytes and melanocytes appeared similar to those of their non-exposed skin. Stratum corneum has basket wave appearance as that of their non-exposed skin. Both the papillary and reticular dermis was similar to their non-exposed skin (Plate1-G and Plate1-H).

**Immuno-histochemical Results:**

**Plate-2, Table -1 and Figure 1**

**Non-exposed Skin: (Control Group)**

The normal non-exposed skin of mice showed faint reaction (yellowish brown color) in the epidermis and mild diffuse cytoplasmic activity in sebaceous glands and endothelial cells of blood vessels (Plate2-A). The mean optical density of ICAM-1 in epidermis was 0.988, SD was 0.071 and SEM was 0.02.

**Exposed Skin: Radiated Group**

Expression of ICAM-1 showed marked diffuse cytoplasmic activity in the epidermis, sebaceous glands and blood endothelial cells (Plate 2-B). The mean optical density of ICAM-1 in epidermis was 1.112, SD was 0.086 and SEM was 0.025.

The statistical analysis showed highly significant increase in the mean optical density of ICAM-1 expression in the
epidermis in comparison to that of their non exposed area (p<0.001).

**Prophylactic Group**
Expression of ICAM-1 showed mild diffuse cytoplasmic activity in the epidermis, sebaceous glands and blood endothelial cells (Plate 2-C).

The mean optical density of ICAM-1 in epidermis was 1.116, SD was 0.095 and SEM was 0.027.

The statistical analysis showed mild significant increase in the mean optical density of ICAM-1 expression in the epidermis in comparison to that of their non exposed area (p<0.05).

**Treated Group**
Expression of ICAM-1 was similar to that of non-exposed skin (Plate 2-D).
The mean optical density of ICAM-1 in epidermis was 1.026, SD was 0.047 and SEM was 0.014.

The statistical analysis showed non-significant increase in the mean optical density of ICAM-1 expression in the epidermis in comparison to that of their non exposed area (p>0.05).

Table -1 Effect of exposure to ultraviolet-B rays on the ICAM-1 expression in skin of mice
Before and after topical Retinoic acid

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N.B. 1-Number of readings was (240 readings per group).

2- SD= Standard deviation  SEM= Standard error of mean  t-Test= Student’s T test

3- O.D.=Optical Density  4- p<0.05 was considered as statistically significance

Figure-1 Effect of exposure to ultraviolet-B rays on the ICAM-1 expression in skin of mice
Before and after topical Retinoic acid
Immuno-histochemical study of the expression........

Plate – 1

1-A A computerized photomicrograph of longitudinal section in the non-exposed skin of mice (Control group), showing the normal healthy epidermis, dermis and hypodermis. (Hx and E. stain, X100)

1-B A computerized photomicrograph of longitudinal section in the non-exposed skin of mice, showing very thin epidermis, and the underlying dermal collagenous fibers; fine in the dermal papillae and dense irregular in reticular dermis. (Hx and E. stain, X400)

1-C A computerized photomicrograph of longitudinal section in the skin of mice exposed to UVB (radiated group), showing acanthosis in comparison to non-exposed skin. (Hx and E. stain, X100)

1-D A computerized photomicrograph of longitudinal section in the skin of mice exposed to UVB (radiated group), showing compact ortho-keratosis in stratum corneum, mitotic figures in epidermal cells and fragmented delicate dermal collagen bundle. (Hx. and E. stain, X400)

1-E A computerized photomicrograph of longitudinal section in the skin of mice exposed to UVB (Prophylactic group), showing mild acanthosis in comparison to non-exposed skin. (Hx and E. stain, X100)

1-F A computerized photomicrograph of longitudinal section in the skin of mice exposed to UVB (Prophylactic group), showing mild acanthosis in stratum corneum, occasional
keratinocytic atypia and the dermis showing normal collagenous fibers organization in both papillary and reticular dermis. (Hx. and E. stain, X400)

1-G A computerized photomicrograph of longitudinal section in the skin of mice exposed to UVB (Treated group), showing normal epidermal thickness, dermis and hypodermis similar to non-exposed skin. (Hx and E. stain, X100)

1-H A computerized photomicrograph of longitudinal section in the skin of mice exposed to UVB (Treated group), showing normal epidermal thickness, dermis and hypodermis similar to non-exposed skin. (Hx and E. stain, X400)

Plate – 2

1-A A computerized photomicrograph of longitudinal section in the non-exposed skin of mice (Control group), showing faint brownish color areas of ICAM-1 reaction in the epidermis, mild diffuse cytoplasmic activity in sebaceous glands, hair follicles, and blood vessels (DAB Harris's Hematoxylin, X400)

1-B A computerized photomicrograph of longitudinal section in the skin of mice exposed to UVB (radiated group), showing marked diffuse brownish cytoplasmic activity of ICAM-1 in the epidermis, sebaceous glands (DAB Harris's Hematoxylin, X400)

1-C A computerized photomicrograph of longitudinal section in the skin of mice exposed to UVB (Prophylactic group), showing mild diffuse cytoplasmic activity of ICAM-1 in the epidermis, and sebaceous glands (DAB Harris's Hematoxylin, X400)

1-D A computerized photomicrograph of longitudinal section in the skin of mice exposed to UVB (Treated group), showing faint brownish color areas of ICAM-1 reaction in the epidermis similar to non-exposed skin (DAB Harris's Hematoxylin, X400)
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Discussion

Stratospheric ozone depletion leads to an increase of the potential harmful effects of UVR, reaching the earth, especially UVB, which are the most damaging UV wavelengths to the skin.

The use of retinoic acid to repair some of the tissue damage caused by chronic exposure of skin to UVR was reported in many studies, while its prevention role is still under investigation (Niles, 2000).

Many studies were done to find ways for preventing and treating photodamage. Recent evidence points to the importance of topical retinoids, particularly retinoic acid in repairing photodamaged skin at the clinical, structural and cellular levels (Matrisian, 1992 and Fisher et al., 1997).

In the present work, the epidermis of UVB groups showed ortho-keratosis in the stratum corneum and keratinocytic atypia, increase in mitotic figures and necrosis. While the epidermis of mice pre-treated with local retinoic acid before UVB exposure (prophylactic group) showed mild increase in its thickness with normal keratinocytes. In mice treated with local retinoic acid after UVB exposure, the skin was histologically similar to non-exposed skin.

Similar findings were observed in many previous studies on experimental animals and human (Grove et al., 1991; Yaar and Gilchrest, 1995 and Griffith, 1999).

Expression of ICAM-1 in the epidermis showed highly significant increase in optical density (p<0.001), which expressed as diffuse cytoplasmic activity in the epidermis, sebaceous glands and endothelial cells of blood vessels.

Krutmann et al., (1990), demonstrated an increase in ICAM-1 expression in the epidermis after UVB exposure. In contrast, Norris et al., (1991) reported that ICAM-1 expression on keratinocytes was not increased by UVB.

The difference between the present study and that of Norris et al. (1991) may be due to species difference as they used hairless mice. ICAM-1 is an inducible protein that appears at inflammatory conditions and mediates lymphocytes-accessory cell adhesion and may play a critical role in immune reactions (Nathens et al., 1998).

Mechanism by which UVB-induced increase in the expression of ICAM-1 in keratinocytes in present study may be attributed to capacity of UVB to enhance the release of some epidermal cytokines especially IL-10 which caused the increase in ICAM-1 expression detected in this study (Humphries and Newham 1999).

The study concluded that retinoic acid given after UVB exposure completely reversed the morphological and ICAM-1 expression level. While, retinoic acid is given before UVB prevented some of the morphological change and not ICAM-1 expression activity.

References


دراسة هيستوكيميائية مناعية علي مستوي أكاس-1 في جلد الجرذان تحت تأثير التعرض للأشعة فوق بنفسجية - (ب) قبل وبعد استخدام حمض الرينوتك الموضعي

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ينتمي إلى قسم الهيستولوجيا، الوراثولوجيا، والجئسية بطب قناع السؤس.

وقد أظهرت الدراسة مايلي:

- ظهور علامات أكاسينية لأصابة الحبل نتيجة التعرض المباشر للأشعة فوق بنفسجية بجرذان مجموعة المجموعة الأولى (مجموعة الضابطة). وعند زايدات ذات دلالات أحيائية في نسبة تركز أكاس-1 (باب طبقة البشرة (ظاهرة الجلد) علامةً على تغيرات اكاسينية في المجموعة الضابطة. ستستخلص من هذا الدراسة أن التعرض المباشر للأشعة فوق بنفسجية يؤدي إلى تغيرات أكاسينية. وعند فحص الجلد بعد التعرض المباشر للأشعة فوق بنفسجية يعالج هذه الأضرار.

- عند استخدام حمض الرينوتك كوقية قبل التعرض المباشر للأشعة فوق بنفسجية يقلل هذه الأضرار ويعزز الدورة بعد التعرض المباشر للأشعة فوق بنفسجية واستخدام حمض الرينوتك مятия وعلاج أضرار تعرض الجلد للأشعة الشمس. 

(ب) على جلد الجرذان قبل وبعد استخدام حمض الرينوتك الموضعي. تم تقسيم الجرذان إلى 3 مجموعات متساوية. المجموعة الأولى (مجموعة الضابطة) المعرضة للأشعة بدون وقاية أو علاج المجموعة الثانية (مجموعة عقيلة) تم استخدام حمض الرينوتك الموضعي قبل التعرض للأشعة فوق بنفسجية. المجموعة الثالثة (مجموعة معالجة) تم استخدام حمض الرينوتك الموضعي بعد نهاية مدة التعرض للأشعة فوق بنفسجية لمدة 10 أسابيع. بالأضافة إلى المجموعة الضابطة من جلد لم يعرض للأشعة فوق بنفسجية من كل الحشر. 

تم تعريض مساحة 2x2 سم في جلد من كل جرذان بعد تغير الشعر منها للأشعة فوق بنفسجية (ب) بجرعة 1.4 جول للسنتيمتر المربع لمدة 15 دقيقة كل يومين لمدة 10 أسابيع متصلة.

وتم تسجيل الجرذان وأخذ عينة من المساحة المعرضة للأشعة وأخرى لم يتم تعرضها للأشعة (المجموعة الضابطة). وتم تحضير عينات شمعية وصبغها بصبغة الهيماتوكسيلين والأبسين. تم الدراسة المناعية على مستوي أكاس-1 باستخدام التضمينات الهيستوكيميائية المناعية اللازمة.

تم قياس مستوي أكاس-1 بالكثافة الضوئية بجهاز تحليل الصور بالكمبيوتر وتحليل النتائج إحصائياً.