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ORIGINAL ARTICLE

The Impact of Ultraviolet Radiations on Morphological Changes in the Skin of Albino Rats

Sangeeta Verma and Vishwakant

Department of Zoology, Agra College, Agra (Affiliated to Dr. B.R. Ambedkar University, Agra) Email: totaaram24@gmail.com

ABSTRACT

The aim of this study is to establish the show off morphological and morphometric changes in the skin of albino rats in erythemal, early post-erythemal and late post-erythemal periods after local ultraviolet irradiation. Studies was conducted on 30 albino rats weighing 350-400 g. Ultraviolet erythema was caused by exposure in 1 minimum erythemal dose. The control group included intact albino rats. After 3, 6 hours, on the 4rd, 9th, 16th, 23st, 30th day, pieces of the illuminated skin were examined using morphometric and histo-chemical methods. After 3, 6 hours after radiation, dyscirculatory alterations in the skin seen. By the 4^{th} day of the experiment a morphometric shoot of acute inflammation in the epidermis and dermis grown, apoptotic keratinocytes or sunburn cells seem together with thickening of the epidermis and an upsurge in the concentration of fibroblasts, by the 9th day dispersive-hyperplastic and degenerative alterations revealed along with dystrophy, epidermis thickness and fibroblasts density at highest level, In the long term, in the 16-30th days, dystrophic changes in the epidermis more prevalent, dys-keratosis, changes in elastic fibres, risen uneven fibrosis, collagenisation, sclerotic changes, significant thickened epidermis, raised density of fibroblasts are seen. Conclusions was made as the results obtained shown indicate noticeable morphometric changes in the skin in the area of local ultraviolet illumination observed throughout the research period.

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INTRODUCTION

UV has diversified effects on skin morpho-physiology, with some pathogenicity seen in acute and chronic ways. One most common and generalised acute effect of UV on the skin is the introduction of inflammation. UVB induces a flow of cytokines, vaso and neuro activation and effectors in the skin that combine to give rise to an inflammatory response leading to sunburn. (Slominski and Wortsman 2000; Slominski, *et al.* 2000; Slominski *et al.* 2012; Clydesdale, *et al.*, 2001; Matsumura and Ananthaswamy 2004; Skobowiat *et al.*, 2011).

Sunburn cells are apoptotic keratinocytes which can be identified by their pyknotic nuclei. If the dose of UV exceeds a threshold limit and tolerable damage response, keratinocytes stimulate apoptotic lanes and finally die (Bayerl *et al.*, 1995).

PURPOSE OF STUDY

Aim of research was to establish the structures of morphological and morphometric changes in the skin of albino rats in the erythemic, early post-erythemic and late post-erythemic periods after the local ultraviolet irradiation.

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MATERIALS AND METHODS

The studies involved 30 albino albino rats weighing 350-400 g. Erythema was seen as UV exposure of one minimum erythemic dose (1 MED) on the shaved area of skin using a mercury-quartz illuminator placed at a distance of 15 cm from the desired rat, for 3 minutes. Here, the exposed skin was shielded with a circular plate with five holes with a diameter of 5 mm. The degree of reaction was evaluated after 3, 6 hours, 4 days after radiation and until the erythema went away in points for each spot like for zero- no erythema, for 1 – clear redness, for 2 – intense erythema.

The intensity of 5 spots was investigated. The degree of the damage was assessed by the intensity and duration of the erythemia response, compaction, swelling and pain in the radiated area (Stefanov, 1998). The overall reactions (behaviour, craving, body weight, hair status, body temperature) were assessed. The control group included intact albino rats.

To study the qualitative morphological changes of the skin after local UVI exposure, rats of all groups were removed from the experiment under general anaesthesia (sodium thiopental at a dose of 50 mg kg) at different timings of the experiment (3 hours, 6 hours, 4th days, 9th days, 16th day, 23rd day, 30th day) in obedience with the desires of the European Convention for the Protection of Vertebrate Animals, using for research and other biological purposes (Strasburg, 1986). Skin pieces were fixed in 10% neutral formalin. Aftermath sections around 4 mm thick were dissected out. The piece was subjected to alcohol wiring and paraffin casting.

Survey drugs stained with haematoxylin and eosin were used for a general calculation of the condition of the tissues examined. Weigert's staining of fuchselin preparations for elastic fibres with van Gieson picro-fuchsin staining for connective tissue bio gram (Stefanov 1998; Lilli 1960; Pirs 1962).

Moreover morphometric methods were used to explore sunburnt cells, leukocyte intrusion of the epidermis and dermis, alterations in collagen and elastic fibres, epidermodermal activity, epidermis thickness and fibroblasts density in the dermis (Sevin *et al.*, 2007; Atramentova and Utevskaya *et al.*, 2008; Avtandilov 2002). The study of microscopic specimens vis a vis morphometric studies, was carried out on an Olympus microscope.

RESULTS AND DISCUSSION

The results of experiments under this study divided action of the UVI in 3 periods like early and pre erythemic stage up to 4 days, mid erythemic stage up to 9 days and late and post-erythemic (16-30 days) (Kitsyuk 2018; Myronchenko *et al.*, 2016; Myronchenko *et al.*, 2016).

Findings of local expressions of radiated skin after the fading of erythema (9-30 days) not revealed any pathological changes (tenderness, puffiness, compaction, aches of the irradiated zone).

The early and pre erythema duration was linked with peculiarities like degenerative changes with clear cut inflammation in the skin, attaining the highest severity on the 4th day after UV exposure. In the beginning UVI exposure (3 and 6 hours) dys-circulatory changes found to observe with gapping in the dermo-epidermal junctional area.

UV exposure also leads to an increase in epidermal thickness, hyperkeratosis. By causing cell injury, UV brings damage response trails in keratinocytes. Damage signals like p53 activation severely changes keratinocyte morpho-physiology, facilitating cell cycle arrest, triggering DNA repair which ultimately induces apoptosis if the damage is sufficiently big (Coelho 2009).

In accordance with data given in the table under present research, while studying morphometrically the epidermis thickening (hyperkeratosis) and the density of fibroblasts in dermis after 3 hours in radiated rats not significantly differed from intact rats (table 1). It shows that damage was not sufficient big. It is called period of no difference.

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Several hours to few days after UV contact, when damage response signals get subsided, epidermal keratinocytes proliferate vigorously intervened by a many epidermal growth factors. Increased keratinocytic division after UV radiation leads to piling of epidermal keratinocytes which increases epidermal hypertrophy as thickness (Coelho 2009). However epidermal hyperplasia defends the skin better way against UV penetration (Scott *et al.*, 2012).

In other study conducted by Veselska and Janisch (2000) the effect of UV radiation on the survival of L929 mouse fibroblasts and on the jointers of the two main cytoskeletal structures, i.e., microtubules and microfilaments was demonstrated in relation to the duration of reparation periods. A lamp was used as a source of UVA. Conclusion was made as the reparation periods went on and on, the fraction of living cells kept increasing apoptotic cells decreasing this was happened due to proliferating activity of viable cells and detachment of dead cells from the substrate (Veselska and Janisch 2020).

Some corroboration with this study was found with present one as in later hyperkeratosis kept reducing as time of radiation progresses.

Further in present study after 6 hours of UVI treatment, histological changes became prominent as leukocyte subversion along with bit changes in the collagen and elastic fibres of the dermis. Moreover apoptotically modified keratinocytes (sunburn cells), significantly upped. 6 hours after the UVI treatment, an increase in the thickness of the epidermis was revealed by a factor of 1.1 in comparison of intact animals. The fibroblasts density started to decrease, which resulted from oedema of the dermis, still significant difference not found for intact animals (table 1).

On the 4th day of the experiment, numerous sunburn cells (modified keratinocytes) were seen in the skin, indication of dermo-epidermal reactions.

Moreover marked leukocyte intrusion of the dermis, demolition of collagen and elastic fibres, as proved by morphometrical histological preparations (Myronchenko *et al.*, 2016; Myronchenko *et al.*, 2016), by the 4th day of the experiment, the thickning of the epidermis was significantly got up by two fold than intact animals. In the dermis nearby points of degenerative and caustic changes in the dermo-epidermal joint, a mild focal explosion of fibroblasts was pointed out, almost double as intact animals (table 1). In the mid erythemic period (9th day) proliferative hyperplasia along with degenerative changes like dystrophy grew morphologically in the skin.

On the 9th day after the UVI treatment, shown by the table, the thickness of the epidermis reached its zenith means it got up by 3.5 folds, mainly because of presence of the spinous, granular and horny layer. Parallel to all this density of fibroblasts on the 9th day was also observed maximum (see table I), got up to 4.00 times (table 1).

Over the time (9–16th days), epidermal and dermal leukocytes got changed in to polymorpho-nuclear leukocytes and behaved like little focal infiltrates. Moreover severity got reduced later.

In the long term, on the 16-30th day, several thickening of the epidermis, increased density of fibroblasts, dystrophic changes in epidermis became prominent, dyskeratosis, changes in the counting and organization of elastic fibres along with raised uneven fibrosis, collagenisation and sclerotic modifications were observed (Zvyagintseva *et al.*, 2013).

On the 16th day, the epidermal thickening was 2.8 times, slightly less from the 8th day. A similar trend was observed in term of fibroblasts. The density of fibroblasts was 3.7 times above than normal. On the 23rd day, the epidermal thickening further reduced as compare previous results. This day only thickness left 2.4 times and the density of fibroblasts by 2.5 times. On the 30th day, the epidermal thickening thickness and the density of fibroblasts were however still higher than normal (1.8 times and 2 times, respectively) but reduced than 16th and 23rd day findings (table 1).

Thus, even in the later periods after the UVI treatment, in the absence of local exhibitions in the skin of albino rats and the general response of the animal, at 100% of

morphological changes in the number and texture of elastic fibres, the growth of uneven fibrosis, collagenisation with next sclerotic changes were detected.

Consequently, a local single UVI exposure of the skin of albino rats in 1 MED leads to persistent histopathological conditions.

Table 1: The Epidermal Thickening in Skin (ETS) and Fibroblasts's Density in Dermis(DFD) in the focus of the local UVI in different periods of observation

Indices	Intact animals	Animals under experiment exposed to local UVI						
		3 hours	6 hours	4 th day	9th day	16 th day	23rd day	30 th day
ETS (hyperkeratosis)	37.19±	37.12±	52.56±	75.44±	152.84±	132.±11	110.10±	97.19±
mcm	1.81	1.92	2.89*	5.59*	6.13*	5.88*	32.12*	4.80*
DFD in derma, sp/mm ²	477.10±	505.48±	439.88±	1050.67±	2010.79±	1940.11±	1380.82±	1155±.87
	11.21	27.26	44.35	49.50*	88.62*	99.39*	75.74*	57.21*

Note: *significant differences in comparison with the intact animals ($p \le 0.05$)

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