

Original Article

X-Ray Induced Changes in the Epidermis of Guinea Pigs - A Morphometric Study

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ABSTRACT

Objective: Measurement of changes in the thickness of epidermis of different locations in guinea pigs after single dose X-ray irradiation.

Study Design: A prospective experimental study.

Place and Duration of Study: Department of Anatomy, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre Karachi from 2008 to 2009.

Materials and Methods: Thirty animals were taken and divided into two groups which were subdivided into three subgroups containing five animals each, according to the time of sacrifice i.e. 48 hours, 15th day and 45th day respectively. A single whole body x-radiation in a dose of 5 Gy. Animals were sacrificed under ether anaesthesia after completion of their respective period. Tissues were processed and 4 to 5 micron thick paraffin embedded sections were cut, and stained with H&E.

Results: Desquamation and decrease in thickness of epidermis was present after 48 hours which shows highly significant result when compared to control ($P < 0.001$). After wards progressively increase in hyperkeratinization was noted after 15 days which is nonsignificant ($P > 0.05$) when compared to control. After 45th day proliferation of basal cells occurred which increased the thickness of epidermis near to control.

Conclusion: The study concludes that x-radiation produces deterministic epidermal changes in all three sites i.e. face, abdomen and back of guinea pigs.

Key Words: X-ray radiation, Gy, Epidermis, hyperkeratization.

INTRODUCTION

Since the discovery of x-rays at the end of 19th century and till now their effects attract much attention. This interest is largely explained by the fact that humans (first of all skin) are exposed to this radiation during medical examination^{1,2,3}.

X- Irradiation (XRI) can affect both normal and neoplastic cells especially, rapidly growing one such as epidermal cells⁴.

X-rays are widely used for imaging and for therapeutic purposes and our knowledge about their possible injurious effects on skin is incomplete. Clinically x-rays can produce erythema, as well as dry and moist desquamation. Morphologically x-rays can produce epidermal loss, crystalolysis, cytoplasmic vacuolization, appearance of euchromatic nuclei and alter microvasculature, causes hyperkeratization, redistribution of biometals as well as basal and squamous cell carcinoma. The type and extent of these changes depends on dose, duration and frequency of x-rays⁵.

Skin injury is the deterministic effect of radiation, once the threshold dose has been exceeded the severity of radiation at any point on the skin increases with dose; threshold will vary some what among individuals⁶.

Radiation will continue to increase in importance in the diagnosis and the treatment of diseases, for this reason considerable emphasis is placed on biological basis for the use of radiation in treatment of person with malignant tumor and on the consequence to the host of radioisotopes administered therapeutically or diagnostically⁷.

Radiation induced injury is recognized for past decade as potential complication of fluoroscopically guided intervention⁸.

MATERIALS AND METHODS

This study was conducted in the department of anatomy, BMSI JPMC Karachi. Thirty adult male guinea pigs weighing 400 to 450 grams were taken from animal house of BMSI JPMC. Animals were kept under observation for one week before study to asses their behavior, and activities and were maintained on laboratory diet. Animals were divided into two groups A & B each group containing 15 animals. Group 'A' served as control. Animals were sacrificed after 48 hours, 15th and 45th day after receiving single whole body x-radiation in a dose of 5 GY at Karachi institute of radiotherapy and nuclear medicine, Pakistan atomic energy KIRAN Hospital.

All the guinea pigs were sacrificed at their respective time of treatment under ether anaesthesia. The skin of the guinea pigs was shaved and skin fragments (size of 1 cm square) from face, back and abdomen at 48 hours, 15th and 45th day were collected. Skin fragment was fixed in 10% formalin for 12-18 hours. Tissues were processed in ascending strength of alcohol and cleared in xylene and infiltrated and embedded in paraffin. 5 micron thick vertical sections were cut at rotatory microtome and floated in hot water bath and placed on glass slide. Sections were fixed in 10% formalin. Stained with H&E for morphology of skin. Statistical analysis was done on computer software SPSS, student 't' test was applied and the value $P < 0.05$ was considered significant.

RESULTS

The observation and their results on the gross and light microscopic examination were recorded. The histological observations were based on the study of 4-5 micron thick paraffin embedded sections, stained with Haematoxylin and eosin.

Table No.1: Mean Thickness of Epidermal of Face of Guinea Pigs

Groups	Subgroups	Thickness of epidermis of face in micron at the sacrificial time		
A (Control)	(n=15)	48 hours	15th day	45th day
		--	--	40.32±0.32
		--	--	
B (Treated)	B1(n=5)	21.36±0.32		
	B2(n=5)	--	39.92±0.26	
	B3(n=5)	--	--	40.28±0.47

Control group A:

In gross examination, face, abdomen and back was observed in all the animals. Skin was covered by white brown and gray hair but at the back was comparatively thick. Skin fragment were collected at 45th day after single whole body x-radiation. Mean thickness in epidermis in face was $40.32 \pm 0.32 \mu\text{m}$ as shown in table 1. The mean thickness of epidermis in Abdomen was $40.08 \pm 0.17 \mu\text{m}$ as shown in table 2. The mean thickness of epidermis was in Back was $58.04 \pm 0.29 \mu\text{m}$ as shown in table 3.

Treated group B:

Group B1: The animals of group B1 were ill looking, the skin of the face, abdomen and back appeared thin and dehydrated, patches of alopecia of varying sizes were scattered all over the skin. Skin showed varying degree of hyperemia and showed number of layers which were reduced to half and there was desquamation of surface epithelium.

Table No.2: Mean Thickness of Epidermal of Abdomen of Guinea Pigs

Groups	Subgroups	Thickness of epidermis of abdomen in micron at the sacrificial time		
A (Control)	(n=15)	48 hours	15th day	45th day
		--	--	40.08±0.17
		--	--	
B (Treated)	B1(n=5)	29.80±0.38		
	B2(n=5)	--	49.36±0.47	
	B3(n=5)	--	--	48.94±0.73

Table No. 3: Mean Thickness of Epidermal of Abdomen of Guinea Pigs

Groups	Subgroups	Thickness of epidermis of back in micron at the sacrificial time		
A (Control)	(n=15)	48 hours	15th day	45th day
		--	--	58.04±0.29
		--	--	
B (Treated)	B1(n=5)	29.80±0.38		
	B2(n=5)	--	57.84±0.41	
	B3(n=5)	--	--	57.76±0.26

The mean thickness of epidermis in, face was: $21.36 \pm 0.32 \mu\text{m}$ as shown in table 1. The mean thickness of epidermis in abdomen was: $29.80 \pm 0.38 \mu\text{m}$ as shown in table 2. The mean thickness of epidermis in back was $29.80 \pm 0.38 \mu\text{m}$ as shown in table 3. A decrease in thickness in group B1 was noted in all sites and when compared with group A it is highly significant ($P < 0.001$) result.

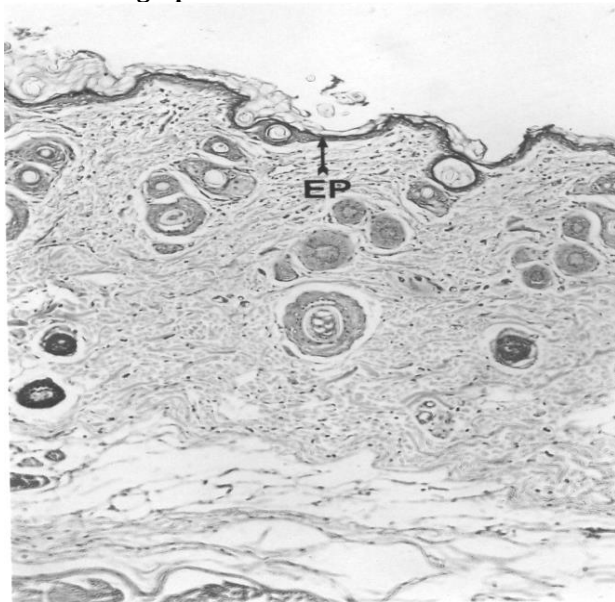
Group B2: The animals of group B2 were ill looking. The skin appeared thin and dehydrated, wide patches of alopecia were scattered all over, and showed varying

degree of hyperemia. In face abdomen and back number of cell layers were reduced to half but thickness of epidermis was near to control due to hyperkeratinization. The mean thickness of epidermis in face was: $39.92 \pm 0.26 \mu\text{m}$ as shown in table 1.

Figure No.1: H & E stained 5 μm thick longitudinal sections of face skin showing epidermal thickness (Ep) in control guinea pig. Photomicrograph x100.



Figure No.2: H & E stained, 5 μm thick longitudinal section of the face skin showing desquamation and the reduced thickness of the epidermis after 48 hours treatment with x-radiation in guinea pigs. Photomicrograph x100.



The mean thickness of epidermis in abdomen was: $49.36 \pm 0.47 \mu\text{m}$ as shown in table 2. The mean thickness of epidermis in back was $57.84 \pm 0.41 \mu\text{m}$ as shown in table 3. A slight decrease and increase in the thickness of epidermis in all three sites was noted. When compared with group A insignificant ($P > 0.05$) result was observed.

Group B3: The skin of face, abdomen and back regained its thickness, hairs were very sparse, patches of alopecia were scattered all over the skin and the hyperemia was diminished considerably.

In all three sites the epidermis showed increased number of cell layers as near to control and keratinization was also decreased. The mean thickness of epidermis in, face was: $40.28 \pm 0.47 \mu\text{m}$ as shown in table 1. The mean thickness of epidermis in abdomen was $48.94 \pm 0.73 \mu\text{m}$ as shown in table 2. The mean thickness of epidermis in back was $57.76 \pm 0.26 \mu\text{m}$ as shown in table 3. A slight decrease and increase in thickness in group B3 when compared with group A which was insignificant ($P > 0.05$).

DISCUSSION

All mammalian cells are affected by ionizing radiation; moderate variability exists among different cell types and tissue with respect to their susceptibility to specific effect such as cell death¹.

Exposure to both ionizing radiation and the certain transition metals can increase cellular formation of free radicals, under aerobic conditions. This can lead to formation of reactive oxygen species, reactivity of these oxyradicals lead to deleterious changes in living cell including DNA strands and breaks, protein oxidation and membrane damage⁹.

X-rays are widely used for both imaging and therapeutic purposes; our knowledge about their possible injuries effects on the skin is incomplete. Previous studies are based on the morphological X-rays induced skin changes. The present study was designed to observe the morphometric changes in skin of different locations induced by X-rays. Guinea pigs were taken as experimental animals because radio sensitivity of these laboratory animals is close to humans and these experimental animal were exposed to single whole body irradiation in dose of 5 GY^{10,11}.

The gross examination of treated animals of Group B show hair loss in face, abdomen and back. This finding was supported by similar observation made by Song and Lambert who found that after exposure of mice to 5 GY of ionization radiation, cell in the matrix of hair follicle under went apoptosis but not growth arrest. These findings are in agreement with Hopewell and Malkinson, they observed that loss of hair occurred after treatment with relatively low doses of radiation

indicating that hair follicles are highly sensitive to ionizing radiation^{11, 12, 13}.

In the present study the animals of group B1 with the dose of 5 GY radiation the thickness of epidermis in all three sites i.e. face, abdomen and back was reduced to half as compare with control, due to decrease in number of cell layers in stratum spinosum, stratum granulosum and stratum corneum this was probably due to growth arrest of epidermal cells. This finding was in agreement with the observation of Hussein et al who observed in their study that in X rays irradiated skin the stratification of epidermis was reduced to one layer each of basal, spinous and granular as well as few layer of corneocytes.

These findings are further supported by study of Song and Lambert et al, who observed the epidermal cells, responded to radiation by under going growth arrest. Growth arrest was more severe in 24 hours after (5 GY) irradiation¹¹.

Decrease in thickness of epidermis was in agreement with the observations by John et al in which degenerative phase of radiation effects consists of lost in prickle cells and basal cell layer. This loss is maximum or complete by day 21. The mitotic index falls to zero¹⁴.

In the group B2 the thickness of epidermis was near to control due to increase in the thickness in stratum corneum. The increase in thickness in stratum corneum was due to increase in ratio of cell loss in pathological states. This observation was in agreement with the observation of Berry, et al, Dechatterjee et al, Landthaler et al. They observed the morphologically X-radiation can produce epidermal loss and hyper keratinization^{15,16}.

In the group B3 thickness of epidermis in all three location i.e. face abdomen and back was near to control, this is because the cells of stratum basale shows the mitotic activity. This finding was in agreement with the observation of Hopewell, in which there is recovery of epidermis occurs as a result of proliferation of surviving clonogenic basal cells form with in the irradiated area. Furthermore, these finding were in agree with in the observation of Song and Lambert, in which epidermal cells respond to radiation undergoing growth arrest, not the apoptosis.

CONCLUSION

The study concludes that X-radiation produce deterministic damaging effects on epidermis in guinea pigs in 3 sites i.e. face, abdomen and back.

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