Original Article

Anatomy

A Morphological Study of the Effects of X-Rays on the Melanocytes of the Skin, Under Light Microscope

1. Furrakh Mustafa Memon 2. Ghulam Mujtaba Kolachi 3. Santosh Kumar

1. Assoc. Prof. of Anatomy, DIMC, DUHS, Karachi 2. Assoc. Prof. of Anatomy, DMC, DUHS, Karachi
3. Assoc. Prof. of Anatomy, SMC, JSMU, Karachi

ABSTRACT

Objective: To observe the effects of x-irradiation on the melanocytes of the skin under light microscope

Study Design: A prospective experimental study.

Place and Duration of Study: The study was carried out at Department of Anatomy, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre Karachi from 2008 to 2009.

Materials and Methods: Thirty animals were taken and were divided in to two groups. Each group was further 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–irradiation in a dose of 5 Gy was given. Animals were sacrificed under ether anesthesia after completion of their respective periods. Tissues were processed and 4-5 micron thick paraffin embedded sections were cut and stained with Masson's Fontana stain.

Results: Insignificant ($P>0.05$) decrease in number of melanocytes was obtained when compared to control in group B1 animals. In group B2, increase in size and number of melanocytes was noted with long dendritic processes and large amount of melanin seen scattered between the layers of epidermis was present and results were moderately significant ($P<0.01$) in case of face and abdomen and significant ($P<0.05$) in case of back. In group B3 Size and number of melanocytes were also increased with large amount of melanin scattered between the layers of epidermis. The results obtained were highly significant ($P<0.001$) in case of face and moderately significant ($P<0.01$) in case of abdomen and back.

Conclusion: X-irradiated skin of face, abdomen and back of the guinea pig depicted increased number and size of melanocytes with long dendritic processes and large amount of melanin scattered between the layers of epidermis.

Key Words: X-irradiation, Melanocytes, Masson's Fontana stain.

INTRODUCTION

The x-rays are composed of mass less particles of energy (photon) and were discovered by a German physicist Wilhelm Conrad Roentgen in 1895 and he was awarded Nobel Prize in 1901. Ionizing radiation act either by direct hit on the target organ atoms i.e. DNA, producing damages in the chromosomes or indirect by causing radiolysis of cellular water with formation of free hot radicles. These free radicles then interact with critical atoms and molecules particularly DNA to produce chemical modifications and deleterious effects (Robins, 1994)[1]. Morphologically x-irradiation produces epidermal loss, cristolysis, cytoplasmic vacuolization, hyperkeratinization and redistribution of biometals as well as basal cell carcinoma (Landthaler., et al 1995; Berry et al., 1976; Archambeau et al., 1984; Enokihara., et al 1993; De Chatterjee et al 1994)[2,3,4,5,6].

The ever increasing use of radioactive substances both in industry and medicine has made the study of radiation damages, of great practical importance. On human body its effects vary from local tissue necrosis to genetic damage, cancer and death (Walter and Talbot 1996)[7]. Since the discovery of x-rays and until now their effects attract much attention this interest is largely explained by the fact that in the humans first of all skin is exposed to the radiation during medical examination (Andronov et al., 1998, Bardychev et al., 1982)[8,9].

All mammalian cells are affected by ionizing radiation; moderate variability exists among different cell types and tissues with respect to their susceptibility to a specific effect such as cell death (Anderson 1990)[10]. X-rays can affect both normal and neoplastic cells especially rapidly growing one such as epidermal cells (Hussain et al 2005)[11]. The skin is the one of the largest structures in the body and is a complex organ system that forms a protective covering for the body (Moore and Persaud, 1998)[12]. Different component of skin react to radiation with different sensitivities (Song and Lambart, 1999)[13]. The color of human skin derives from and varies with the amount of blood and its degree of oxygenation in the cutaneous circulation and the thickness of stratum corneum and the activity of specialized cells (melanocytes) producing pigment melanin (William et al., 1995)[14]. Melanocytes are found scattered between keratinocytes of stratum germinativum and stratum spinosum, as well as with in hair follicle. The cells possess a small, spherical nucleus and numerous dendritic processes that extend between adjacent keratinocytes. The melanocytes

supposedly wear out and slough off with the epidermal scales but their number is maintained by proliferation of cells which are presumably melanocytes in an active phase of melanogenesis (Copenhaver et al., 1978)[15]. This study is designed to observe the effects of x-radiation on melanocytes of face, abdomen and back under light microscope.

MATERIALS AND METHODS

This study was conducted in the Department of Anatomy, Basic Medical Sciences Institute Jinnah Postgraduate Medical Center Karachi where 30 adult male guinea pigs weighing 400 to 450 G were taken. The animals were divided into two groups, A and B containing fifteen animals each and were further sub-divided into three sub-groups containing five animals each, according to the time of sacrifice, i.e. 48 hours, 15th day and 45th day. Group-A served as control. Group-B received whole body X-radiation in dose of 5 Gy at Karachi Institute of Radiotherapy and Nuclear Medicine Karachi. The animals were sacrificed at the end of their respective period of treatment under the ether anaesthesia. The skins of Guinea pigs were shaved and skin fragment (size one centimeter square) from face, abdomen and back were collected. Skin fragment from each side was fixed in 10% formalin for 12-18 hours. After that tissues were processed in ascending strength of alcohol, cleared in xylene and infiltrated and embedded with paraffin. Five micron thick vertical sections were cut at rotatory microtome and floated in hot water bath and placed on glass slide and stained with Masson's Fontana for the study the of any alteration in the melanin pigment. The morphology of the melanocytes was observed by visualizing the size and processes of melanocytes and the amount of melanin in 40x objective and 8x ocular under light microscope. Melanocytes were calculated with the help of ocular counting reticule in 40x objective and 8x ocular under light microscope.

RESULTS

In control group the melanocytes were dark black and brown black in colour and were present in the basal layer of epidermis and stratum spinosum and few were present at dermoepidermal junction. Melanocytes were rounded in shape, with pale staining cytoplasm and with varying dendritic processes scattered between keratinocytes of stratum basale. Melanin was also normally scattered between these layers of epidermis. Mean number of epidermal melanocytes in face, abdomen and back were; 2.99±0.08, 2.51±0.13 and 2.66±0.14 respectively.

In Group B1 melanocytes were dark black in colour with few dendritic processes, scattered between keratinocytes of stratum basale. There was little increase in size. Melanin was also seen scattered between the layers of epidermis. Mean number of epidermal melanocytes in face, abdomen and back were; 2.87±0.03, 2.51±0.07 and 2.68±0.13 respectively. Insignificant ($P>0.05$) decrease in number of melanocytes was obtained when compared to control.

In group B2 melanocytes appeared dark black and brown black, rounded cells with increase in size and number and long dendritic processes. The large amount of melanin was also seen scattered between the layers of epidermis (fig-1, 2 and 3). Mean number of epidermal melanocytes in face, abdomen and back were 3.36±0.03, 3.18±0.07 and 3.16±0.13 respectively. An increase in number and size of Melanocytes were moderately significant ($P<0.01$) in case of face and abdomen and significant ($P<0.01$) in case of back.

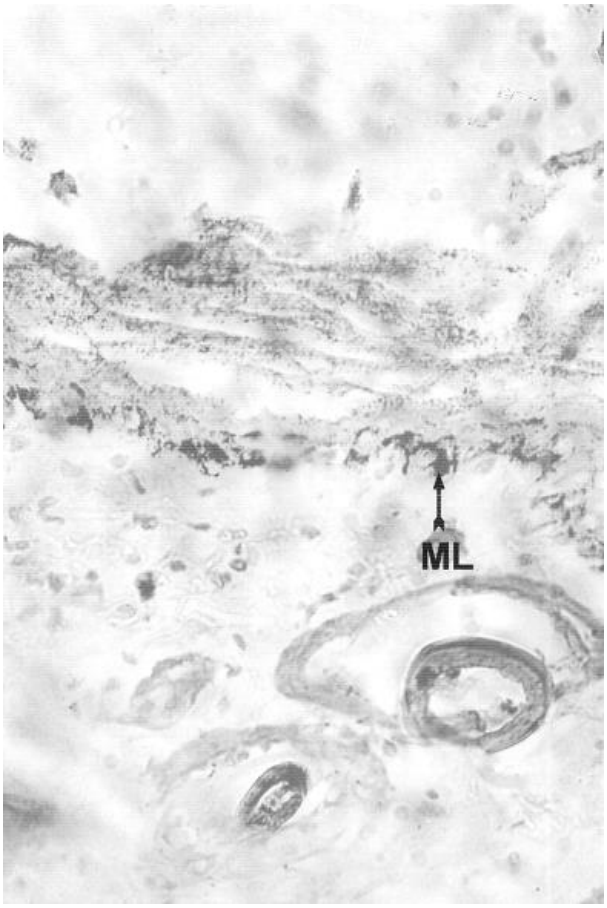

Figure No.1Masson's Fontana stained 5 µm thick longitudinal section of face skin showing increase in number, size, dendricity and pigment of melanocytes (ML) after 15 days treatment with x-radiation in guinea pig. Photomicrograph x400.

In group B3 melanocytes appeared dark black in colour with very long dendritic processes. Size and number of melanocytes were increased. Large amount of melanin was scattered between the layers of epidermis. Mean number of epidermal melanocytes in face, abdomen and back were 3.58±0.09, 3.23±0.09 and 3.36±0.08 respectively. The results obtained were highly

significant (P<0.001) in case of face and moderately significant (P<0.01) in case of abdomen and back.

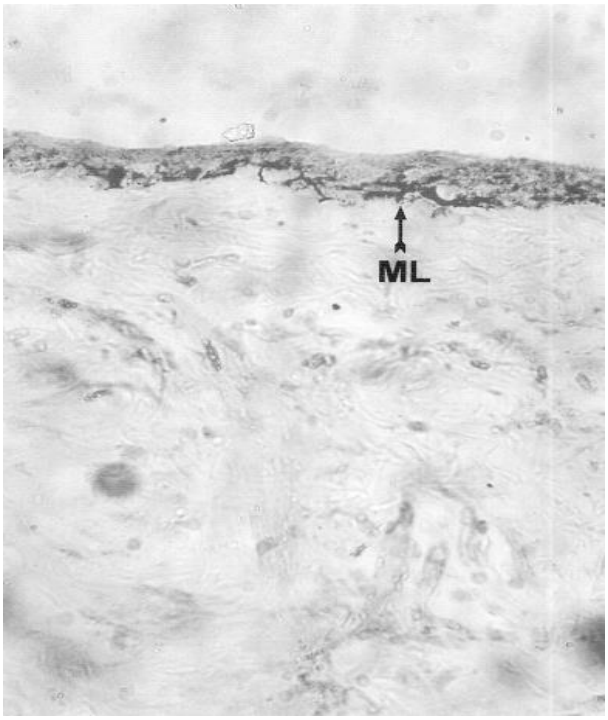

Figure No.2: Masson's Fontana stained 5 µm thick longitudinal section of abdominal skin showing increase in number, size, dendricity and pigment of melanocytes (ML) after 15 days treatment with x-radiation in guinea pig. Photomicrograph x400.

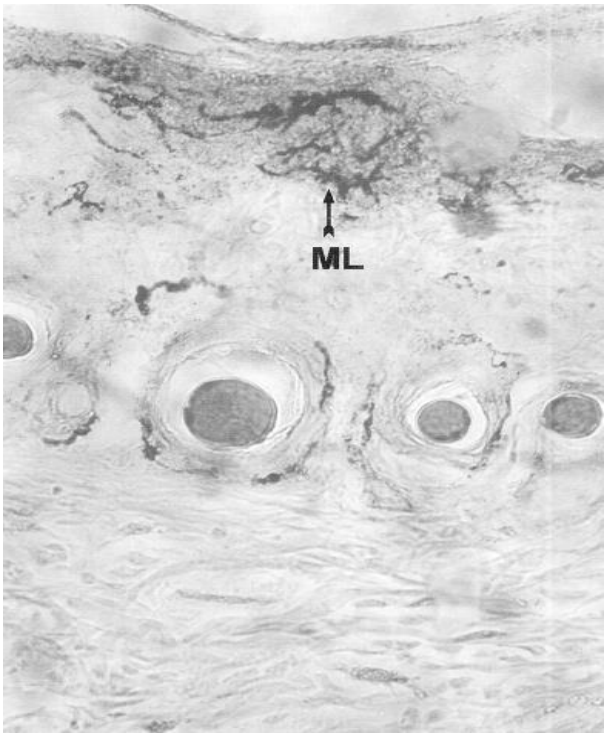

Figure No.3: Masson's Fontana stained 5 µm thick longitudinal section of back skin showing increase in number, size, dendricity and pigment of melanocytes (ML) after 15 days treatment with x-radiation in guinea pig. Photomicrograph x400.

DISCUSSION

Exposure to ionizing radiation and certain transition metals can increase cellular formation of free radicals under aerobic conditions this leads to formation of reactive oxygen species, reactivity of these oxyradicals lead to the deleterious changes in living cell including DNA strand breaks, protein oxidation and membrane damage (Halliwell and Gutteridge, 1989)[16]. All mammalian cells are affected by radiation, but moderate variability exists among different cell types and tissues with respect to their susceptibility to specific effects such as cell death. Skin injury has 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 somewhat among individuals (Koenig et al 2001)[17].

X-rays are widely used for both imaging and therapeutic processes our knowledge about their possible injurious effects on the skin is incomplete. Present study is designed to observe the effects of x-radiation on the melanocytes of face, abdomen and back under light microscope. In present study guinea pigs were taken as experimental animals because radiosensitivity of these laboratory animals is close to humans (Bardychev et al, 1982)[9] and these experimental animals were exposed to single whole body irradiation in dose of 5 GY (Melchilov et al; 2003, Song and Lambert, 1999)[13,18].

In Group B2 and B3 in all three locations the size, dendricity and pigmentation was increased. This cellular hypertrophy with well developed and elongated dendritic processes and proliferation of cells could be due to potent activation of melanocyte with the x-rays. This finding was in agreement with the observation of Hussein et al (2003)[11] in which x-ray of irradiated skin under electron microscope the melanocyte were large and highly branched. Their cytoplasm contains numerous cisternae of rough endoplasmic reticulum, Golgi saccules and melanosomes at the different stages of development. A similar finding was also observed by Middlekamp (2005)[19] who observed in his study that an increase in melanocyte size, dendricity and number as well as increased pigment was seen in irradiated epidermis.

Cell bodies of melanocyte are normally confined to the basal layer of epidermis but by ultraviolet radiation or x-radiation the cell bodies of melanocytes appear in suprabasal layer of epidermis and melanin formation is stimulated (Copenhaver et al., 1978)[15]. These findings were also in agreement with the observation of Bolognia et al., 1990 and Gilchrist et al., 1960[20] in which irradiated skin showed larger melanocytes with

an increase in dendricity compared to non irradiated skin.

CONCLUSION

X-irradiated skin of face, abdomen and back of the Guinea pig depicted increased number and size of melanocytes with large amount of melanin scattered between the layers of epidermis. Increase in size and number of dendritic processes were also observed.

REFERENCES

1. Robbins SL, Cotran RS, Kumar V. Environment and nutritional disease. In Robbins pathologic basis of disease. 5th ed. Philadelphia: WB Saunders; 1994.p.402-8.
2. Berry RJ. Skin response to X-irradiation in the guinea pig. Int J Radiat Biol Relat Study Phys Chem Med 1976; 30: 535-41.
3. Landthaler M, Hagspiel HJ, Late irradiation damage to skin caused by soft x-radiation therapy of cutaneous tumour Arch Dermatol 1995;131: 182-86.
4. Archambeau JO, Ines A. Response of swine skin microvasculature to acute single exposure of x-rays: quantification of endothelial changes. Radiat Res 1984; 98: 439-53.
5. Enokihara MM, Pacheco IP. Ultrastructure of convoluted proximal tubule of kidney mice before and after x-ray exposure. Rev Paul Med 1993;111: 403-6.
6. De Chatterjee. Low level x-ray exposure on rat skin, hyperkeratization and concomitant changes in biometal concentration. Biol Trace Elem Res 1984; 46:203-10.
7. Walter JB, Talbot IC. The effects of ionizing radiation. Walter and Israel general pathology. 7th ed. New York: Churchill Livingstone;1996.p. 557-68.
8. Andronov AV, Krymski VA, Labanov GV. Med Radiol Radiates Besopan 1998; 43 20-23.
9. Bardychev MS, Peteric VD, Krasnov AS. Med Radiol 1982; 6: 7-11.
10. Anderson RE, Berthrong M, Fajardo LF. Radiation injury in Anderson's pathology. 10th ed. St Louis: the CV Mosby Company; 1990.p.484-512.
11. Hussein MR, Eman E, Dief-Abu, Mohammad H, Raheem AE, Abdel–Rehman. Ultrastructural evaluation of the radioprotective effects of melatonin against x-ray induced skin damage in albino rats. Int J Exp Path 2005; 86: 45-55.
12. Moore KL, Persaud TVN. The integumentry system in the developing human, clinically oriented embryology. 6th ed. Philadelphia: WB Saunders; 1998.p.514-19.
13. Song S, Lambert PF. Different responses of epidermal and hair follicular cells to radiation correlate with distinct patterns of p53 and p21 induction. Am J Pathol 1999;155: 1121- 27.
14. William PL, Bannister LH, Berry MM, Collins P, Dyson M, Dussek JE, et al. Integumental system: skin and breast. In: Gray's anatomy. 38th ed. London: Churchill Livingstone; 1995.p.375-424.
15. Copenhaver WM, Kelly DE, Wook RL. The integument. In: Bailey's text of histology. 17th ed. USA: the Williams and Wilkins Company; 1978.p. 423-43.
16. Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. Oxford: Clarendron Press; 1989.
17. Koeing TR, Wolff D, Mettler FA, Wagner LK. Skin injuries from fluoroscopically guided procedure; characteristics of radiation injury. Am J Roentgenol 2001; 3-11.
18. Melchikov AS, Ryzhov AI, Medredev MA. Morphological changes in epidermal basal cells of different location induced by x-rays. Bull Experimental Biol Med 2003; 136: 224-27.
19. Middlekamp MA, Park HY, Lee J, Gilchrist BA, Gonzalev S. Detection of UV-induced pigmentary and epidermal changes overtime using in vivo reflectance conofocal microscopy. J Invest Dermatol 2006; 126:402-7.
20. Gilchrist BA, Park HY, EII MS, Yaar M. Mechanism of ultraviolet light induced pigmentation. Phochem Photobiol 1996; 63: 1-10.

Address for Corresponding Author:
Dr. Furrakh Mustafa Memon
Assistant Professor
Anatomy Department
Dow International Medical College
DUHS Karachi.
Cell # 0333-2185465
E-mail: drfarrukhmm@hotmail.com.