Original Article

The Effects of X-Rays on the Hair Follicles, Blood Vessels, Collagen and Elastic Fibers, of the Skin of Guinea Pigs and the Role of Vitamin C, A Morphological Study under Light Microscope

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ABSTRACT

Objective: To observe the effects of x-rays on hair follicles, blood vessels and collagen and elastic fibers of the skin and role of vitamin C.

Study Design: A prospective experimental study.

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

Materials and Methods: Twenty seven animals were taken and were divided in to three groups. Each group was further subdivided into three subgroups containing three 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. Group C animals were also given injection of vitamin-C intraperitoneally in the dose of 1 mg/G/day. Animals were sacrificed under ether anaesthesia after completion of their respective periods. Tissues were processed and 4-5 micron thick paraffin embedded sections were cut and stained with Hematoxylin and eosin for morphology of hair follicles and blood vessels, Masson's trichrome for study of collagen and Van Geison for elastic fibers.

Results: Group A served as control. In Group 'B' hair follicles were reduced in size and number of cells per bulb was also reduced, blood vessels were dilated their endothelial cells were swollen lumen was narrow and vessel wall was sclerosed, collagen and elastic fibers were reduced in amount. In group 'C' early effects of x-rays subsided to great extent in less than 15 days but there was no sign of improvement in elastic fibers.

Conclusion: X-rays have hazardous effects on hair follicles, blood vessels and collagen fiber elastic fiber. Vitamin C minimizes these hazardous effects.

Key Words: Hair follicle, Masson's trichrome, Van Gieson.

INTRODUCTION

The ever increasing use of radioactive substances both in industry and medicine has made the study of radiation damages, of great practical importance (Walter and Talbot, 1996). Irradiation has been reported to produce multiple negative effects on wound healing process inhibits inflammatory reaction, connective tissue proliferation, maturation of granulation tissue, transcription of mRNA, secretion of collagen and neovascularization (Gu et al., 1998; Bernstein et al., 1993). Different components of skin react to radiation with different sensitivities, loss of hair occur after treatment with relatively low doses of radiation indicating that hair follicles are highly sensitive to ionizing radiation (Malkinson 1981, Prasad 1995). Hair loss or alopecia is one of the earliest responses following exposure to ionizing radiation (Hopewell 1990). This observation suggests that hair follicles are sensitive to radiation, induced damage (Song and Lambert 1999).

Vascular damage is extremely important consequence of irradiation (Anderson 1999). In acute radiodermititis capillary endothelium may be hypertrophic and congested; hemorrhages and thrombosis are often observed (Spittle 1998).

Irradiation of animals caused a significant reduction in collagen synthesis with heavy dose of radiation there is decrease in proliferation of fibroblasts which is responsible for collagen synthesis, a direct negative impact of ionizing radiation on fibroblast proliferation (Grant et al., 1973, javanovic, 1993). In irradiated skin fibroblasts were relatively small in size and had few branches; their nuclei were hyperchromatic (Hussein 2005). Cell culture studies of fibroblasts exposed to ionizing radiation have demonstrated that irradiated fibroblasts has a significant prolonged generation time when compared to normal fibroblast (Rudolph 1988).

Ascorbic acid treatment has been reported to confer protection against radiation in vitro and in vivo (Nevas et al 1993; Barerstock, 1979) and it has beneficial effects on the course of radiation-induced skin injuries (Decosse, 1988). Ascorbic acid plays an important role in the maintenance of collagen (Naidu, 2003). Treatment with ascorbic acid before irradiation enhanced the synthesis of collagen hexosomamine

(Jagetia et al., 2003). The use of natural radio protector/antioxidants to overcome direct negative effect of ionizing radiation and for targeting reactive oxygen species could be an important therapeutic strategy to improve healing of irradiated wounds (Cabbabe and Kroack 1986). Therefore keeping in mind the radioprotective role of ascorbic acid we designed this study to observe these effects on hair follicles, blood vessels and collagen and elastic fibers.

MATERIALS AND METHODS

This study was conducted in The Department of Anatomy, Basic Medical Sciences Institute Jinnah Postgraduate Medical Center Karachi where 27 adult male Guinea pigs weighing 400 to 450 G were taken and were divided into three groups, A, B and C. each group was further sub-divided into three sub-groups containing three animals each according to the time of sacrifice, i.e. 48 hours, 15th and 45th day. Group-A served as control. Group-B received whole body X-irradiation in dose of 5 Gy at Karachi Institute of Radiotherapy and Nuclear Medicine. Group-C received whole body X-radiation in dose of 5 Gy and injection of vitamin-C intraperitoneally in the dose of 1 mg/G/day (Injection vitamin-C manufactured by Shanghai Medicines and Health Products Shanghai, China).

The animals (Guinea pigs) were sacrificed at their respective time of treatment under the ether anaesthesia. The skins of Guinea pigs were shaved and skin fragment (size one centimeter square in shape) from face, back and abdomen were collected at 48 hours, 15th and 45th day. Skin fragment from each side was fixed in 10% formalin and 10% buffered neutral 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 were placed on glass slide and stained with Hematoxylin and eosin for morphology of skin and Masson's trichrome for study of collagen fibers, and with Van Geison stain for study of elastic fibers. The condition of hair follicle was observed in 40x objective and 8x ocular under light microscope (by observing the size of hair follicle and number of cells per bulb and pyknosis to assess the degree of apoptosis). The changes in blood vessels were assessed by visualizing the size of lumen, size of endothelial cells and sclerosis of the vessel wall. The arrangement of collagen fibers were observed in 10x objective and 8x ocular under light microscope. Elastic fibers were observed to assess the degeneration of these fibers in 10x objective and 8x ocular under light microscope.

RESULTS

In control group the hair follicles were scattered through the dermis and consisted of three to four concentric layers of epithelial cells with rounded nucleus at its base hair bulb was also seen with hair matrix. Blood vessels were seen at the junction of papillary and reticular layer of dermis with adequate lumen and endothelial cells. Collagen fibers were blue in colour and arranged irregularly in the form of extensive network just beneath the epidermis. Elastic fibers appeared black against red stained collagen, in papillary layer they were thin and scanty, and in reticular layer they were long, thick and followed the course of collagen bundle.

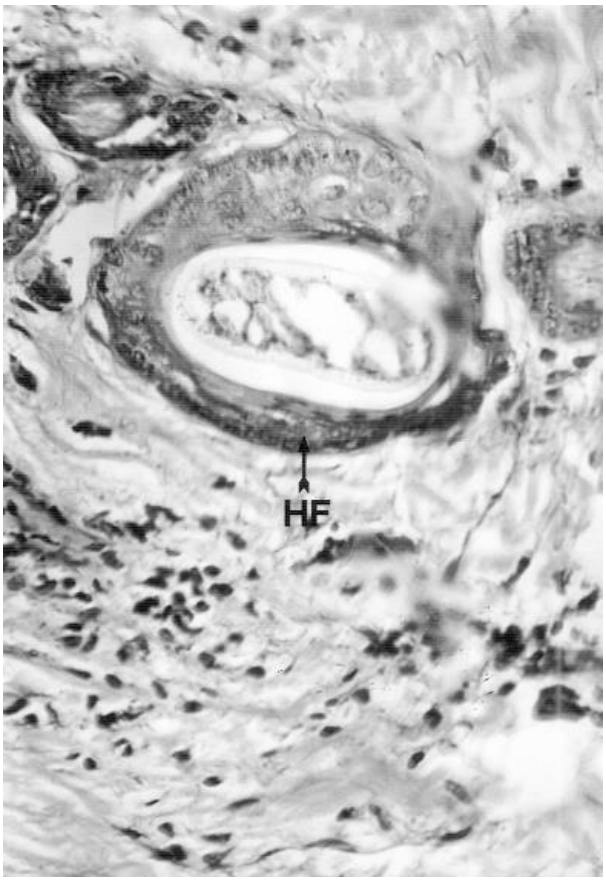

Figure No.1: H&E stained, 5µm thick longitudinal section of back skin showing blood Vessel (BV) swelling of endothelial cells and narrowing of lumen (L) 48 Hours after treatment with X-radiation in Guinea pig Photomicrograph x 400.

In group 'B1' the hair follicles were reduced in size. Follicular matrix was smaller and total numbers of cells per bulb were 20-30% less than the control. The nucleus of the cell was pyknotic fig-1.

Blood vessels were seen dilated, their endothelial cells were swollen and lumen was narrower than control and there was also sclerosis of vessel wall fig-2. There was no change in collagen and elastic fibers compared with control. Group B2: The hair follicles were reduced in size and follicular matrix was smaller and total numbers of cells per bulb were less than the control and the nucleus of the cell was pyknotic fig-3.

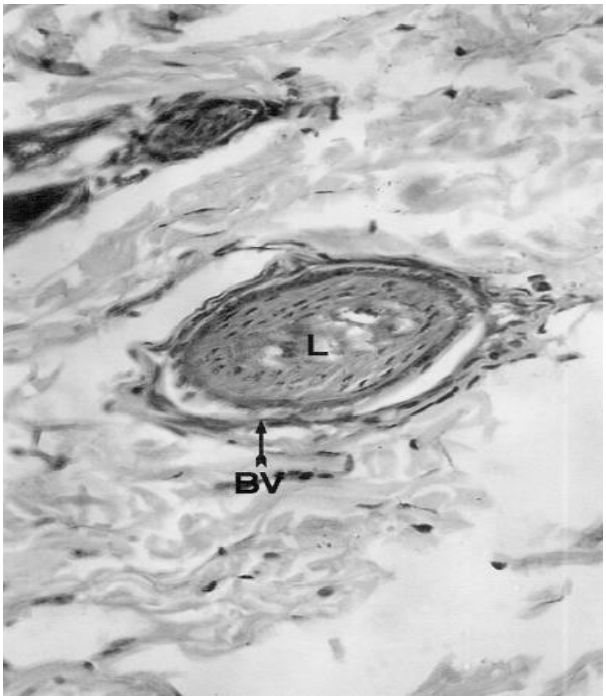

Figure No.2: H&E stained 5 µm thick longitudinal section of back skin showing Blood vessels (BV) swelling of endothelial cells and narrowing of lumen (L) After 48 hours of treatment with X-radiation in Guinea pigs. Photomicrograph x 400.

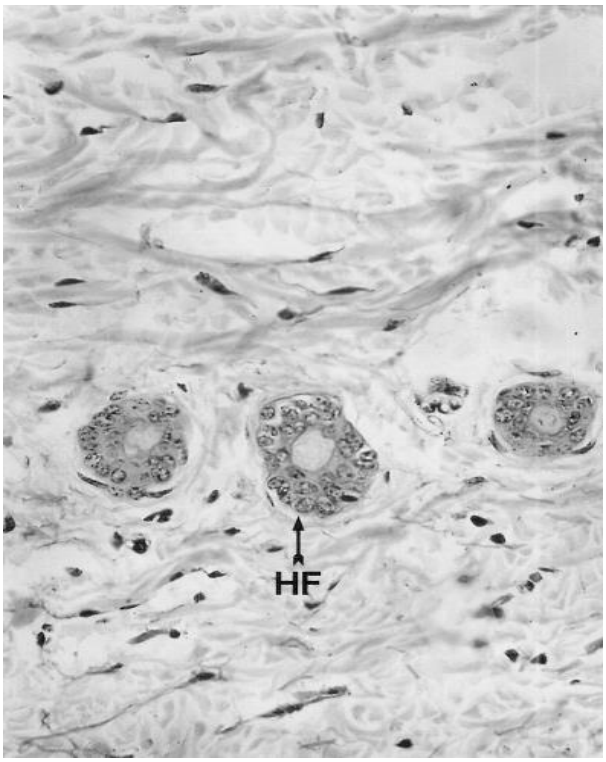

Figure No.3: H&E stained 5 µm thick longitudinal section of back skin showing hair follicle (HF) decrease size and total number of cells per bulb with pyknotic nucleus after 15 days treatment with x-radiation in guinea pig. Photomicrograph x400.

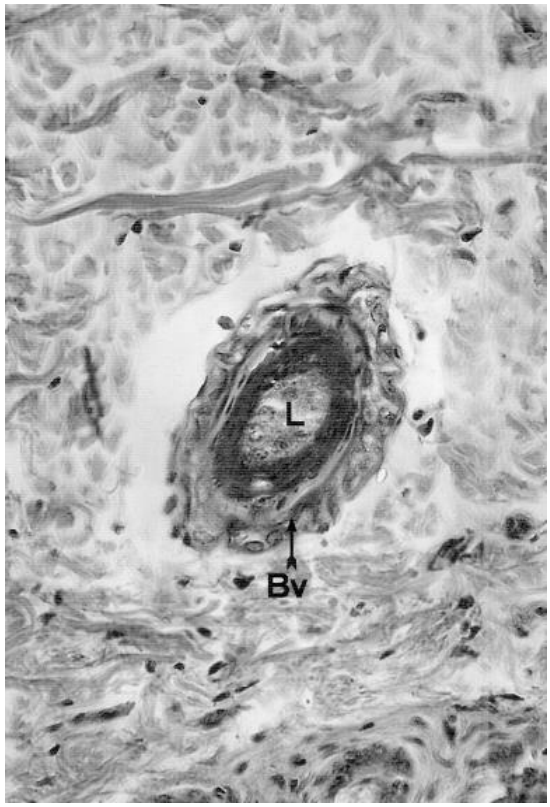

Figure No.4: H&E stained, 5 µm thick longitudinal section of back skin showing blood vessel (BV) decreased swelling of endothelial cells and increase lumen (L) size after 45 days treatment with x-radiation in guinea pig Photomicrograph x400

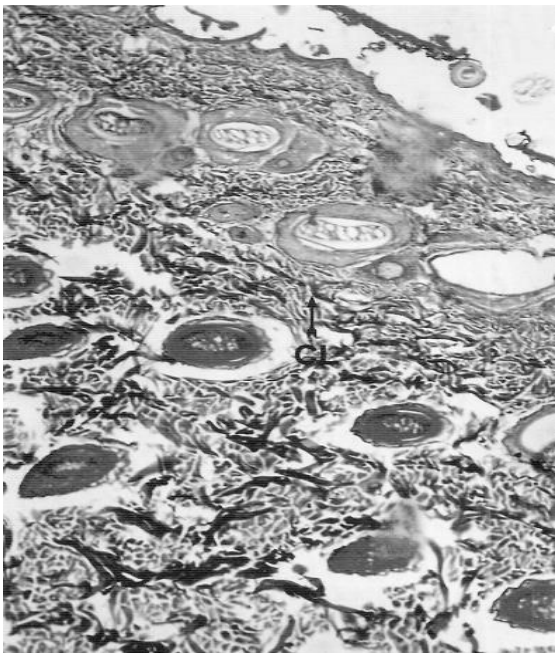

Figure No.5: Masson's trichrome stained,5µm thick longitudinal section of back skin showing thinning and reduced amount of collagen fibers (CL) after 15 days treatment with x-radiation in guinea pig. Photomicrograph X100.

Figure No.6: Elastic Von Geison stained 5 µm thick longitudinal section of back skin showing thinning of elastic fiber (EL) after 15 days treatment with x-radiation in guinea pig. Photomicrograph x 100.

Blood vessels were seen dilated and their endothelial cells were swollen and lumen was narrower than control with sclerosis of vessel wall fig-4. Collagen fibers were thin and reduced in amount fig-5. Elastic fibers became thinner than control showing degeneration fig-6. Group B3: The hair follicles were reduced in size, follicular matrix was smaller and total numbers of cells per bulb were less than the control. Dilatation of Blood vessels was reduced and swelling of endothelial cells was subsided lumen was like control. Collagen fibers were thin and reduced in amount. Elastic fibers were thinner than control showing degeneration.

Group C1: Hair follicles were reduced in size. Follicular matrix was smaller and total numbers of cells per bulb were less than control, and nucleus of the cell became pyknotic. The blood vessels were seen dilated and their endothelial cells were swollen and lumen was narrower than control. There was also sclerosis of vessel wall. Collagen fibers were blue in colour (in Masson's trichrome stain) there was no change in collagen than control. Elastic fibers appeared black against red stained collagen there was no change in elastic fibers than control. Group C2: Hair follicles were reduced in size. Follicular matrix was smaller and total numbers of cells per bulb were less than control, nucleus of the cell became pyknotic. The dilatation of the blood vessels was reduced and swelling of endothelial cells was subsided. Lumen was wide like control the sclerosis of vessel wall was very much reduced. The collagen fibers were thicker and elastic fibers became thin than control. Group C3: Hair follicles were reduced in size. Follicular matrix was smaller and total numbers of cells per bulb were less than control. The dilatation of the blood vessels was reduced and swelling of endothelial cells was subsided and lumen was wide like control. The collagen fibers were increased and thicker and elastic fibers became thin than control.

DISCUSSION

In present study as mentioned was designed to observe the radioprotective role vitamin C on hair follicles, blood vessels, collagen and elastic fibers. Vitamin C was chosen in injectable form to attain high serum concentration level rapidly and adult male Guinea pigs were selected because radiosensitivity is close to humans (Bardychev et al 1982) these both were in also agreement with the selection criteria of Raziq and Jafarey (1987) and Melchikov et al (2003). Hair loss (reduced hair follicles) was seen in all three sites, i.e. face, abdomen and back. This finding was supported by similar observation made by song and Lambart (1999) who found that after exposure of mice to 5 Gy of ionizing radiation, cell in the matrix of hair follicle underwent apoptosis but not growth arrest. The above findings were also in agreement with Hopewell (1990) and Malkinson and Prasad (1995). They observe loss of hair occurs after treatment with relatively low doses of radiation, indicating that hair follicles are highly sensitive to ionizing radiation.

In the x-irradiated groups the blood vessel were seen dilated, endothelium cells were swollen then control, and the lumen was narrower and sclerosis of vessel wall was present, this is due to post irradiation inflammatory response called radio-dermatitis these findings were in accordance with Anderson,(1990) in which early after irradiation the vascular dilatation may cause skin erythema and regressive changes, including swelling and vacuolization of endothelium and necroses of vessel wall and some time haemorrhage. Hussein et al (2005) reported that in irradiated skin under electron microscope the endothelial cell had marked irregularly of their luminal surface, hetrochromic nuclei, numerous pinocytotic vesicles and widening of intercellular spaces.

In X- irradiated groups B2, B3 and the collagen fibers became thin in all sites and irregularly arranged this finding was in agreement with the Walter and Talbot (1996) according to it irradiation of animals caused a significant reduction of collagen synthesis, both newly formed and original collagen became hyalinized. In groups-B1, B2, B3 the elastic fibers became thinner

indicating the sign of degeneration which is in agreement with the observations of Robbins, 1994.

In group C2 and C3 the amount of collagen was increase near to control this is because vitamin 'C' improves the collagen deposition and reduce the hyalinization and increases the vascularity and fibroblast density. These finding were in agreement with the observation of Frie et al (1989) and Navas et al (1994) in which indicated the beneficial effects of ascorbic acid on wound healing through changes in cell regeneration and collagen synthesis. These observations were further supported by the observations of Frie., et al (1989), Navas .,et al (1994) who mentioned in there studies that beneficial effect of ascorbic acid on wound healing through changes in cell regeneration and collagen synthesis. Jagetia et al (2004) also observed that ascorbic acid inhibited the radiation induced decrease in collagen syntheses.

CONCLUSION

The study concludes that the damaging effects of x-rays on hair follicle, blood vessels and collagen and elastic fibers (of the skin) could be minimized by the treatment of vitamin C.

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