Ultrastructural Effects of Irradiation on Squamous Cell Carcinoma of the Uterine Cervix

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Nineteen patients with previously untreated invasive squamous cell carcinoma of the uterine cervix were treated by irradiation alone at the Keimyung University Hospital from January, 1990 to July, 1991. The serial samplings of the tissue taken before and during radiation of the uterine cervix were studied by light and electron microscopic examination.

Radiation-induced cellular changes, particularly nuclear degeneration was pronounced. The tumor invasion pattern remained unchanged but the number of mitosis and tumor cells decreased. The number of infiltrating inflammatory cells, multinucleated giant cells and karyolytic cells were increased with radiation. Fibrosis was also increased. Electron microscopically, the amount of tonofilament in the tissue samplings was increased in the postirradiated state, but the desmosomes were decreased in numbers. Fibroblasts began to appear after an irradiation dose of 2700 cGy. After an irradiation dose of 3600 cGy or more, tumor cells were nearly completely degenerated and displaced with mature fibrotic tissue. There was an increase of activated fibroblasts and collagen fibers but a decrease of inflammatory cells in the interstitial tissue. Swelling of the mitochondria and endoplasmic reticulum, loss of intercellular bridges and an increased number of secondary lysosomes were also found with radiation.

Key Words: Ultrastructural effect, Radiation, Squamous cell carcinoma, Uterine cervix

INTRODUCTION

Radiaiton therapy plays an important role in the treatment of carcinoma of the cervix with surgery. Now there is a place for surgery in the management of early disease, and radiotherapy in advanced disease. Recently, there have been reports of concomittant chemotherapy and irradiation in patients with advanced disease. Although this is an intriguing concept, its usefulness is uncertain as yet[1~14].

Most of our knowledge concerning the morphologic effects of irradiation are based on experimental studies on animals. The lack of information concerning the cellular effects of radiation in human tumors is due to the fact that biopsy specimens are rarely taken from acutely irradiated regions. Only a few studies concerning the effects of irradiation mainly in the alimentary tract, salivary glands, pancreas and skin have been published[15,16]. In carcinoma of the cervix, many studies have been made of cellular changes of postirradiation cervicovaginal smears only[17,18].

They have shown that the cellular effects of irradiation such as cellular hypertrophy, cytoplasmic vacuoles, fibrils, pseudopode like projections, well defined perinuclear halos, multinucleation, nuclear vacuoles, large numbers of inflammatory cells and atypical fibroblasts are unspecific effects that may occur in reactions due to injury other than irradiation[19~23]. Malignant cells often show marked irradiation changes similar to benign cells[24].

The ultrastructural effects of well defined doses of irradiation on squamous cell carcinomas of the human head and neck are currently studied by Kellokumpu-Lethinen et al[25]. There are no earlier systematic studies of human carcinoma of the cervix at the light and electron microscopical level during irradiation. We obtained serial samples of the tissue taken before and during radiation of the squamous cell carcinoma and these were studied by light and electron microscopic examination.

계명의대 동산의료원 조사 및 을종연구비에 의하여 이루어 졌음 (1991).

MATERIALS AND METHODS

Nineteen patients with previously untreated invasive squamous cell carcinoma of the uterine cervix were treated by irradiation alone at the Keimyung University Hospital from January, 1990 to July, 1991. Fifteen of them had large cell nonkeratinizing types and four had large cell keratinizing types. The mean age of the patients was 49.3 years. Following FIGO classification, there was one of stage I, fourteen of stage IIa, three of stage IIb and one of stage IIIb. One biopsy specimen was taken before radiation and durging radiation five specimens were taken at 900 cGy, 1800 cGy, 2700 cGy, 3600 cGy and 4500 cGy by punch biopy, the total being one hundred and fourteen. Radiation was delivered using megavoltage equipment (6 MV, 23 MV linear accelerator) with a conventional fraction schedule (180 cGy per day, five fractions per week) with a total tumor dose of 4500 cGy. Radiation was used in an AP and bilateral box technique at 100 cm SAD with the patient prone.

The samples were prepared for light and electron microscopic studies as follows:

1. Light Microscopic Examination

The biopsy specimens were fixed in 10% neutral-buffered formalin, dehydrated, embedded in paraffin, cut into 2~4 μm thick sections and stained with hematoxilin and eosin stain.

2. Electron Microscopic Examination

For electron microscopic study, tissue pieces were cut into 1 mm^3 size, and prefixed with 2.5% glutaraldehyde solution (0.1M phosphate buffer, pH7.4) at 1~4°C for 2 hours. The specimens were washed with 0.1M phosphate buffer solution, then postfixed with 1% OsO4 for 2 hours, washed with the same buffer, dehydrated in a series of ethanols, replaced with propylene oxide, embedded in an epoxy compound as described by Luft's method[26] and conjugated at 37°C for 12 hours, at 45°C for 12 hours, and at 60°C for 48 hours.

Thick (1 μm) sections were cut and stained with toluidine blue for light microscopic study and thin sections (40~60 nm) were cut from the desired area with a Dupont diamond knife on a Sorvall MT-500 Ultramicrotome and attached to a gride and stained with uranyl acetate and lead citrate as described by the Watson and Reynolds's method[27] for electron microscopic study. Electron microscopic specimens were studied with a Hitachi H-600 transmission electron microscope.

RESULTS

1. Light Microscopic Findings

Preirradiated state tumor cells had large irregular nuclei, prominant nucleoli in the nucleus with mitosis and tumor cells invading the stroma. After an irradiation dose of 900 cGy, we found that the nuclear membrane was not prominent, cytoplasm was darkly stained and there was lymphocytic infiltration among tumor cells. After 1800 cGy-2700 cGy, we found that inflammatory cells and fibrosis were markedly increased and surrounded the tumor nests which decreased in size.

After 3600 cGy, tumor cells had more degenerative changes, fibrosis and the infiltration of inflammatory cells were greatly increased. After 4500 cGy, an increase was found in the number of multinucleated giant cells in the tumor nests and mitosis was rarely visible. In summary, an increase

Table 1. Ultrastructural Fundings of Irradiation in Uterine Cervical Carcinoma

	Before therapy	900 cGy	1800 cGy	2700 cGy	3600 cGy	4500 cGy
Desmosome	+++	+++	++	++	+	+
Tonofilament	–	+	++	++	+++	++++
ER dilatation	–	±	+	++	+++	++++
Mitochondrial swelling	–	+	+	++	+++	++++
Loss of intercellular bridge	–	±	+	++	+++	++++
Secondary lysosome	–	±	+	++	++	+++
Fibroblast	+	++	++	+++	+++	++++
Nuclear degeneration	–	±	+	++	+++	++++

of the nuclear size, an increased number of karyolytic tumor cells, and a decrease of tumor cells and mitosis were found with the radiation dose. Fibrosis and the number of multinucleated giant cells and infiltration of the inflammatory cells were increased, but the tumor invasion pattern remained unchanged.

2. Electron Microscopic Findings

Before radiation, we found a round nuclear margin, prominant nucleoli in the nucleus, an intact intercellular bridge and desmosomes (Fig. 1, 2). After 900 cGy, when compared to normal cells, nuclear morphology was more irregular and folding occurred, and we found an intranuclear cytoplasmic inclusion due to this folding (Fig. 3, 4). After 1800 cGy, swelling of the mitochondria, shortening of the intercellular bridge, an increased number of secondary lysosomes, and an increased amount of cytoplasmic tonofilament in the tumor cells and lipid droplets were found (Fig. 5, 6). After 2700 cGy, we found a marked increase of fibroblastic proliferation and infiltration of collagen fiber (Fig. 7). After 3600 cGy, swelling of the mitochondria and an

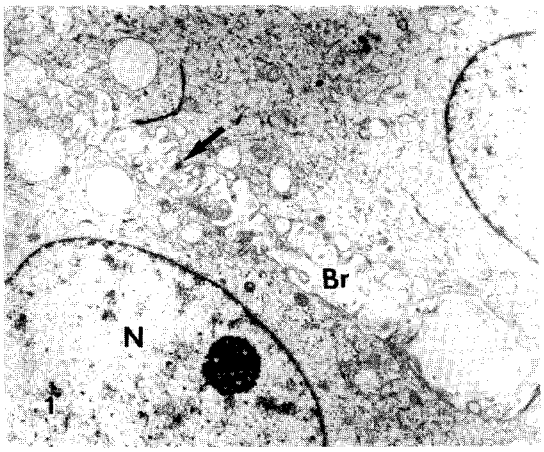

Fig. 1. Electron micrograph before radiation. The tumor cell shows well developed intercellular bridge and desmosome (arrow) (×8,500).

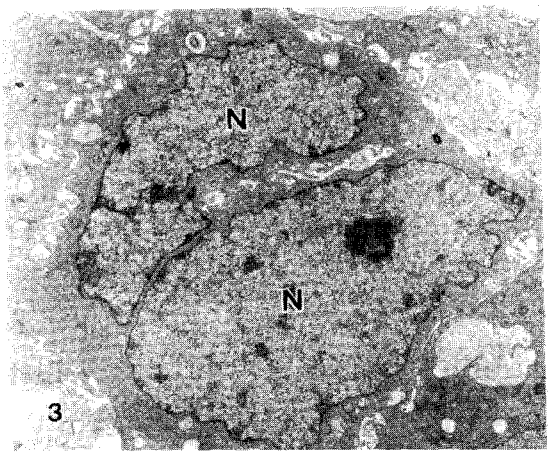

Fig. 3. Electron micrograph after 900 cGy radiation. The nuclei of the tumor cells are irregular (×6, 800).

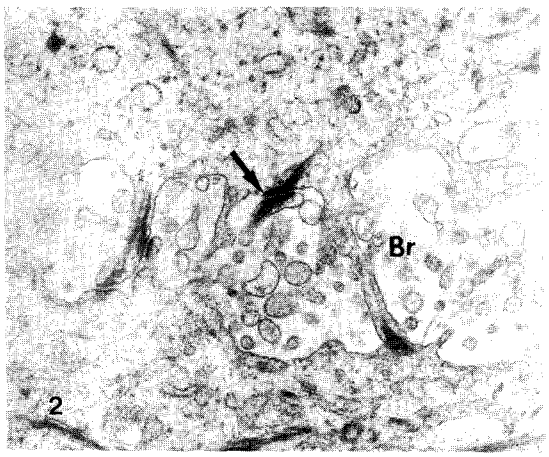

Fig. 2. Electron micrograph before radiation. Many intercellular bridges and desmosomes (arrow) are seen (×289,000).

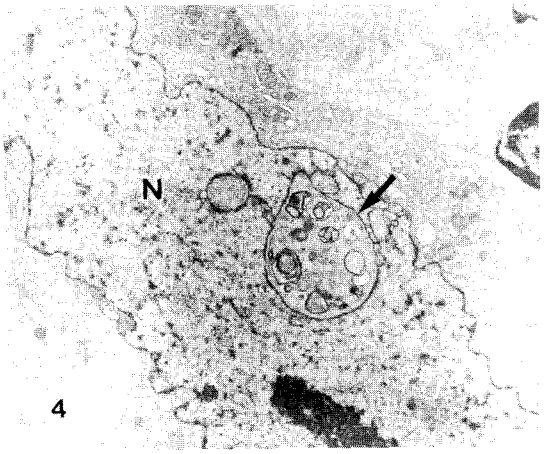

Fig. 4. Electron micrograph after 900 cGy. The nuclear membrane is folded and the pseudoinclusion (arrow) is seen (×8,500).

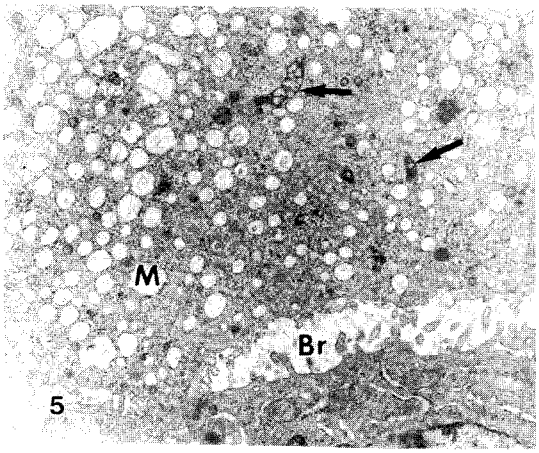

Fig. 5. Electron micrograph after 1800 cGy. The intercellular bridges are blunt. The cytoplasm of the tumor cell shows mitochondrial swelling and increased numbers of secondary lysosomes (arrow) (×13,600).

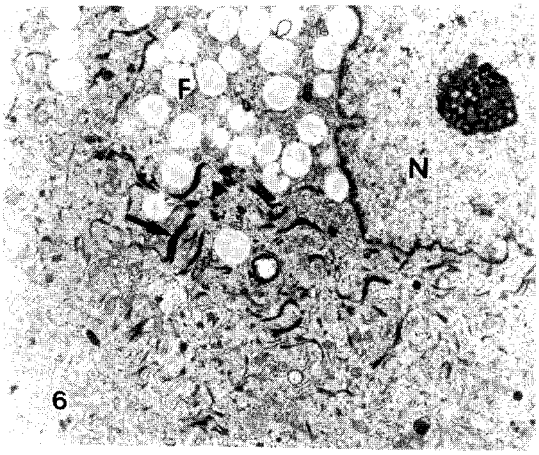

Fig. 6. Electron micrograph after 1800 cGy. Many fat droplets and increased numbers of tonofilaments (arrow) are seen in the cytoplasm of the tumor cell (×10,200).

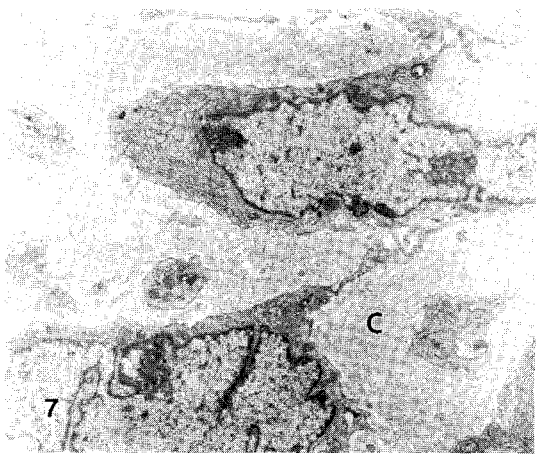

Fig. 7. Electron micrograph after 2700 cGy. Fibroblasts and collagen deposition are increased in the intercellular space (×10,200).

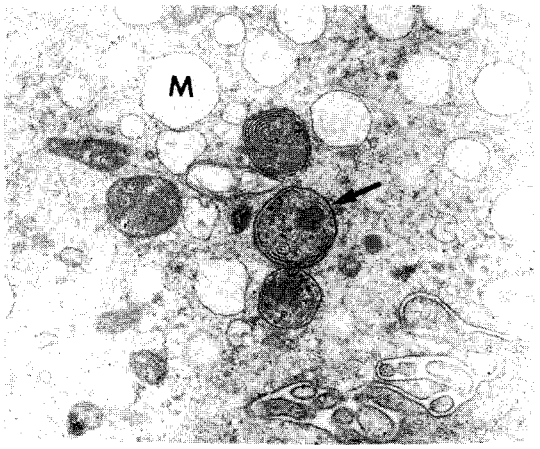

Fig. 8. Electron micrograph after 3600 cGy. Swollen mitochondria and abnormal mitochondria (arrow) are increased in the tumor cells (×34,000).

increase of abnormal mitochondria were also found (Fig. 8).

After 4500 cGy, local destruction of the nuclear membrane, dispersed intranuclear cytoplasm, visible chromatin outside the nuclear membrane, an increased amount of swelling of the endoplasmic reticulum and mitochondria, and loss of the intercellular bridge were found (Fig. 9, 10).

In summary, the swelling of cytoplasm, a decreased number of desmosomes due to a loss of the intercellular junction and blunting of the intercellular bridge, an increased amount of tonofilament, an increase of swelling of the mitochondria and endoplasmic reticulum, and an increased number of secondary lysosomes were found with radiation. Fibrosis and nuclear degenerative changes increased with the radiation dose and were the same as light microscopic findings.

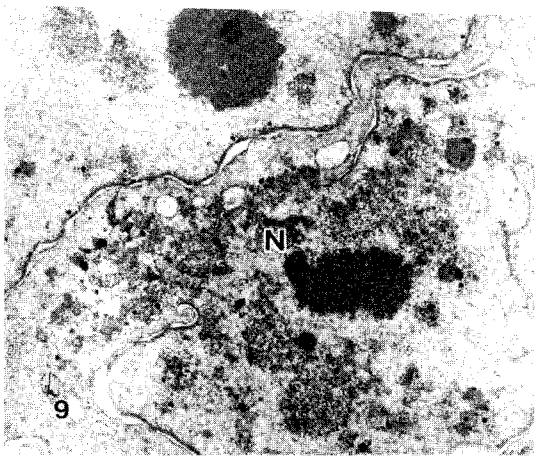

Fig. 9. Electron micrograph after 4500 cGy. Nuclear membrane of the tumor cell is partially destructed and the chromatin is seen outside the nucleus (×20,400).

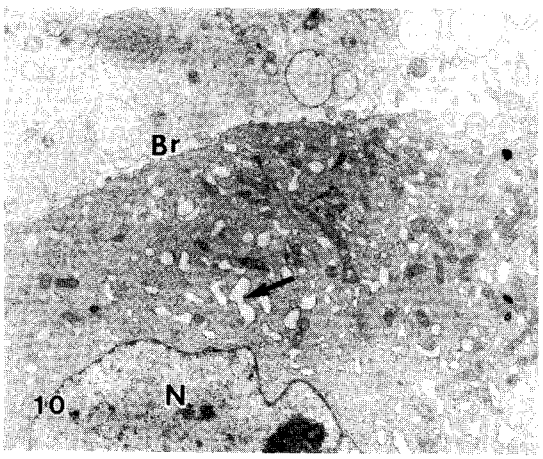

Fig. 10. Electron micrograph after 4500 cGy. The cytoplasm of the tumor cell shows loss of intercellular bridge and dilated endoplasmic reticulum (arrow) (×10,200).

DISCUSSION

Irradiation causes various changes from subtle to complete cell destruction depending upon the given dose and histologic type. Important factors of cell to radiation are the age of the cell, the amount of cytoplasm, the thickness of the cytoplasmic membrane and the size of the nucleus[28]. There are also physical factors, such as cell temperature and cellular pH, that may influence sensitivity to radiation.

Cellular responses to the same radiation dose, are different even though they are similar cell types. The basal squamous cells begin to show some changes during the first or second day following radiation, while it takes 5 to 6 days for the superficial squamous cells to show some changes. By preventing synthesis of DNA protein, irradiation may cause cellular death, inhibition of mitosis, and/or changes in the chromosomal or genetic informaiton that can be transmitted to its descendants.

Most cellular changes resulting from irradiation appear first in the nuclei, then in the cytoplasm[21,22]. But in our study, swelling of cytoplasmic organelles was found earlier than nuclear changes in electron microscopic examination.

According to experimental studies, changes in the nuclei to irradiation occurred as follows; hypertropy, well defined perinuclear halos, vacuolization, finely granular and uniformly distributed chromatin, an occasionally decreased amount of DNA, and pale and empty appearing nuclei with a loss of chromatin detail. Also noted was multinucleation (2 ~6 nuclei) caused by the impairment of normal mitotic activity, nuclear molding or overlap, and condensation of chromatin at the periphery; nuclear pyknosis, vacuolization, fragmentation, or karyorrhexis were also seen suggesting cell death in the benign or malignant, degnerate cells.

Cytoplasmic changes to radiation were cytoplasmic edema and hypertrophy, amorphous cytoplasm, cytoplasmic fibrils, and rupture and fragmentation of the cytoplasmic membrane. These bizarre cytoplasmic changes are not always specific for irradiation injury, because these changes can be seen at least partially in other degenerative cellular processes such as after chemotherpy, ultraviolet irradiation, or cautery. Similar changes can also be seen in the cells of patients with folic acid deficiency[29]. Other changes in the cytoplasm after irradiation are an increased number of inflammatory cells, epithelial repair, histiocytes, and foreign body giant cells.

Nakano et al reported a better five year survial rate in patients with Langerhans' cell infiltration in tumor tissues than in those without infiltration[31].

The efficacy of radiotherapy depends on the size and extension of the tumor, sensitivity of the tumor to irradiation, the amount of irradiation given, and tumor cell differentiation[29].

In addition to the above findings, in our study an increased size of the nucleus, a decreased number

of cancer cells and a decrease of mitosis were also found with radiation. The infiltration of inflammatory cells, the number of multinucleated giant cells and karyolytic cells were continuously increased with an increasing radiation dose, but inflammatory cells were decreased in the last part of irradiation which may be due to an increase of fibrosis in the ongoing process of healing.

Glucksmann et al reported that keratinization has been shown to occur during irradiation in cervical carcinoma which responds more favorably to irradiation[32], but no visible changes in the keratinization pattern are found in our light microscopic findings.

Electron microscopically, the swelling of the mitochondria and endoplasmic reticulum, amount of cytoplasmic tonofilament, the loss of the intercellular bridge and a number of secondary lysosomes increased with the radiation dose; and also found an increase of fibrosis and nuclear degenerative changes but a decreased number of chromosomes.

In the current study of Kellokumpu-Lethinen et al[25] intracellular filaments and desmosomes which are part of the kertinization pattern also increased during irradiation in some tumors in the electron microscopic examination. An explanation for this also might be that in the tumors there is variation among the differentiation level of the individual tumor cells. However, in our study the amount of intracellular filament increased but the number of desmosomes decreased with radiation.

Desmosomes are associated with intercellular junctions, since one of their important functions is to ensure cell to cell adhesion[24,33]. Numbers of desmosomes have been estimated for many types of tumors[34,40]. McNutt and Weinstein were the first to observe that desmosomes are less frequent in invasive cervical squamous cell carcinoma than in the normal cervix[35]. In a more detailed study, Wiernik and his associates found a small and yet statistically significant decrease in the total number of desmosomes per cell with malignant transformation[36~38]. Johnson and Shieridan found that desmosomes are absent or are very rare in Novikoff hepatoma cells (N1S1-67)[39].

Weinstein and his associates reported that the number of desmosomes was decreased in frequency in low grade invasive tumors and increased in frequency in noninvasive higher grade tumors. It has also been shown in a study of bladder carcinoma that when no invasion occurred the desmosome level remained similar to that of the controls, but when invasion was present, their level decreased[40~42].

Kellokumpu-Lethinen et al explained that the increase of the level of desmosomes during irradiation shown in his study might be responsible for the adhesion of tumor cells to the irradiated region, but a decreased number of desmosomes in our study may be due to the loss of cohesiveness with adjacent tumor cells by cellular swelling and degeneration.

In some studies the number of desmosomes was increased more than the initial tumor tissue after a sufficient time of radiotherapy. Therefore, it is important to carefully observe the level of desmosomes following irradiation. Continuing a follow up of these patients, we might find a clue to correlate the prognosis of carcinoma of the cervix with these electron microscopic findings in our study.

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═ 국문초록 ═

자궁경부의 편평상피암의 방사선치료에 수반되는 초미형태학적 변화

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1990년 1월 22일부터 1991년 7월 31일까지 계명대학교 동산의료원 치료방사선과에 내원한 자궁경부의 편평상피암 환자 19명을 대상으로 방사선치료에 수반되는 조직병리학적 및 초미형태학적 변화를 보기 위해 체외방사선 치료전 후(방사선조사량 900 cGy, 1800 cGy, 2700 cGy, 3600 cGy, 4500 cGy)에 조직생검을 시행하여 방사선 치료가 암조직에 미치는 효과, 즉 암세포의 퇴행성 변화에 따른 추이를 관찰하였다.

본 실험을 통하여 방사선 조사가 종양세포에 심한 상해를 미침을 알 수 있었다. 이는 종양세포에 퇴행성 변화를 초래할 뿐 아니라 각질의 형태에도 변화를 가져왔다. 방사선에 의한 핵의 퇴행성 변화는 현저하였고 종양의 침윤형태는 변화 없었으나 핵분열수와 종양세포수는 감소하였다. 염증세포의 침윤과 다핵거대세포의 수와 핵용해된 세포의 수는 방사선량이 증가될수록 증가하였다.

전자현미경적으로는 조직에 장세사의 양이 방사선 조사 후 증가하였으나 교소체 수는 감소하였다. 섬유아세포가 2700 cGy 이상에서 나타나기 시작하였고 3600 cGy 이상에서는 종양세포는 급격히 그 수가 감소되고 진행된 섬유화 조직에 의해 대치되었다. 방사선량이 증가함에 따라 활성화된 섬유아세포의 수가 늘어나는 반면 염증세포는 줄어 들었고 간질조직내 교원질이 증가되었다. 미토콘드리아와 내형질 세망의 종창, 세포간교의 소실 및 2차 리소좀의 수는 방사선량이 증가함에 따라 증가되었다