International Journal of Biological, Life and Agricultural Sciences
ISSN: 2415-6612
Vol:4, No:8, 2010

Effect of Dose Rate of Irradiation on Ultrastructure of Duodenal Mucosa

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***Abstract*—U**ltrastructure of duodenum mucosa of irradiated rat was studied versus dose rate of irradiation following exposure to gamma rays from 60-Cobalt source. The animals were whole body irradiated at two dose rates (1 $Gy.mn^{-1}$ and 1 $Gy.h^{-1}$) and three total doses (1, 2 or 4 Gy) for each dose rate. 24 or 48 h after irradiation, their small intestine was removed and samples of duodenum were processed for observations under a transmission electron microscopy. Samples of duodenum mucosa of control rats were processed in the same way. For the lower dose rate of 1 $Gy.h^{-1}$, main lesions characteristic of apoptosis were detected within irradiated enterocytes at a total dose of 2 Gy and 24 h after exposure. Necrosis was noted in the samples, 48 h after exposition. For the higher dose rate of 1 $Gy.mn^{-1}$, fewer changes were detected at all total doses 24 or 48 h irradiation. Thus, it was shown that the appearance of radiation-induced alterations varies not only with increasing total dose and post-irradiation time but especially with decreasing dose rate.

***Keywords*—**Dose rate**,** Radiation Inury, Apoptosis, Small Bowel

I. Introduction

EXPOSURE of the abdomen to ionizing radiations for treatment of a wide variety of abdominal and pelvic tumors may lead to chronic gastrointestinal disturb that affects quality of life for patients [1-4]. Radioinduced side effects are due to lesions occurring in normal cells irradiated in the vicinity of tumors. They can be avoided by reducing the dose or durtation of exposure, but these procedures may compromise the treatment. Light microscopy studies have showed loss of tissue in the intestinal mucosa after exposure to ionizing radiation may be responsible for these side effects of radiotherapy [5-7]. It is assumed that apoptosis may be one of the processes involved in loss of tissue that is dependent on the dose of radiation to which it is administrated [8-14]. No study has been conducted to demonstrate the effect of dose rate of radiation on radioinduced lesions of duodenum mucosa. The objective of this study was to examine whether there is influence of dose rate on the ultrastructure of the mucosa of the small intestine to investigate at subcellular level the type of injuries induced by gamma radiation in relation to the dose rate, total dose and post-irradiation time.

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Financial support by CNPq (Centro Nacional de Pesquisa e Desenvolvimento) and UESC (Universidade Estadual de Santa Cruz).

II. Material and Methods

A. Animals and Irradiation Procedure

This study were conducted with twenty-eight adult male Wistar rats weighting approximately 250 grams, held in laboratory conditions with water and food *ad libitum* and an alternating light/dark of 12 hours. For radiation, pairs of animals were placed in plastic boxes of two narrow compartments to prevent their movement and to allow a homogeneous distribution of total body irradiation. Then they were divided into three groups and two animals at once were irradiated by a 60-Cobalt source of an irradiador of CDTN/CNEN (Centro de Desenvolvimento de Tecnologia Nuclear-Comissão Nacional de Energia Nuclear, UFMG) as follows (Table I): - twelve animals of the first group were irradiated at a dose rate of 1 gray per minute (1 $Gy.mn^{-1}$), four rats during 1 minute for a total dose of 1 gray (1 Gy), four rats during 2 minutes for a total dose of 2 Gy, four rats during 4 minutes for a total dose of 4 Gy; - twelve rats of the second group were irradiated at a slower dose rate of 1 gray per hour (1 $Gy.h^{-1}$), four rats during 1 hour for a total dose of 1 Gy, four rats during 2 hours for a total dose of 2 Gy, four rats during 4 hours for a total dose of 4 Gy; - the third group of four animals controls was not irradiated. Back in their cages, half of the animals of each group were anesthetized by intraperitoneal injection of thiopental sodium, 24 hours and the other half, 48 hours after irradiation for the dissection of the small intestine. All procedures were performed in accordance with international laws of the protection and welfare of laboratory animals and were approved by the Ethics Commitee of the Federal University of Minas Gerais.

B. Sampling for electron microscopy

The upper part of the small intestine of all animals was dissected and the first two centimeters of duodenum were removed, cut longitudinally and then into samples of 5 mm side. They were immediately fixed by immersion with a solution of 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4), post-fixed with 1% osmium tetroxyde and then epon-embedded in order to achieve six blocks of oriented tissue, per specimen and per animal, for transversal sectioning of wall duodenum. From each samples block, 2 μm-thick semithin sections were cut using an ultramicrotome (Ultacut

E, Reichter-Junger/Optische-Germany/UESC), placed on glass slides, and stained with a 1% Toluidine Blue solution. Blocks of interesting areas and junctions such as desmosomes are observed.

TABLE I
IRRADIATION PROTOCOL

Dose Rate	Irradiation Duration	Total Dose	Post-Irradiation Time of Sacrifice	
			24 h	48 h
1 Gy.mn^{-1}	1 mn	1 Gy	n	n
	2 mn	2 Gy	n	n
	4 mn	4 Gy	n	n
1 Gy. h^{-1}	1 h	1 Gy	n	n
	2 h	2 Gy	n	n
	4 h	4 Gy	n	n

(n= 2 animals)

III. RESULTS

A. Ultrastructural appearance of control mucosa

The non-irradiated rats were handled and maintained under the same conditions as the irradiated. Mucosas presented the characteristics commonly found in healthy duodenum with columnar and goblet cells. Fig. 1 shows the ultrastructural morphology of classical enterocytes containing in their clear cytoplasm, round ou oblong mitochondria with visible crests, rough and smooth reticulum, and free ribossomes. The nucleus has a visible nucleolus, a smooth nuclear membrane, and a regular distribution of pores. The chromatin is heterogeneous and clear with very little heterochromatin against the inner nuclear membrane (Fig. 1a and b). The brush-border of the apical membrane of the enterocytes is regular with straight microvilli (Fig. 1b). The interdigitations of the lateral plasmatic membranes are touching without intercellular spaces highlighted on stained semithin sections observed by light microscope were cut again into 60 nm-thick ultrathin sections and placed under copper grids for observation by transmission electron microscope (80 kV, Morgani 268 D FEI Compagny-UESC and Zeiss-Fiocruz).

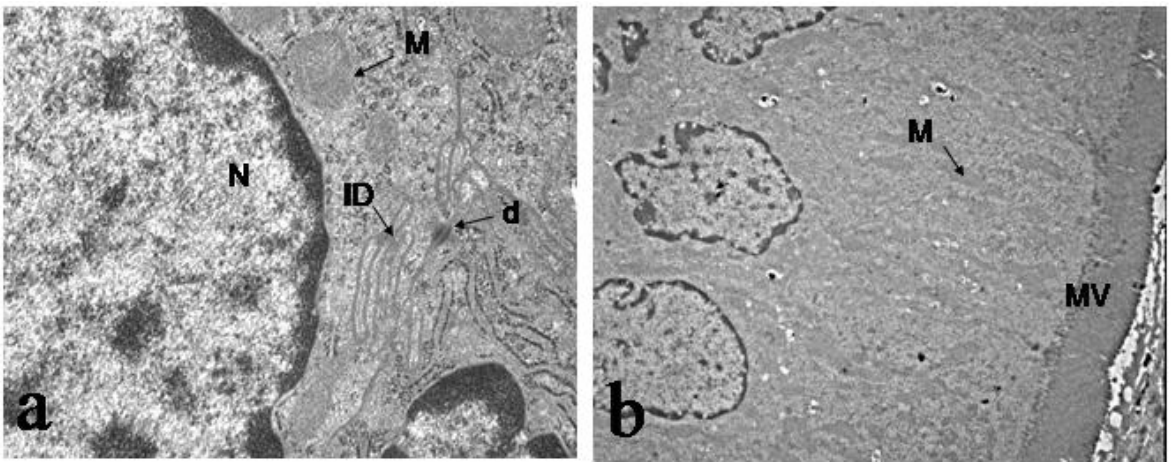

Fig. 1 Transmission electron micrographs of non irradiated duodenum mucosa showing transversal (a) and longitudinal (b) sections of columnar cells. Nucleus (N), mitochondria (M), brush border microvilli (MV), interdigitations (ID) of lateral membrane and desmosome. Magnifications: 20,000 X (a); 4,400 X (b).

B. Ultrastructural lesions at lower dose rate

The observations of duodenum mucosa of rats irradiated at the lower dose rate of 1 Gy.h^{-1} and a total dose of 1 Gy showed alterations that were not detected in controls. Apart from a slight widening of intercellular spaces between columnar and goblet cells, all sections showed similar characteristics to those of controls at any post-irradiation time, 24 ou 48 hours (Fig. 2). The nucleus and cytoplasm of these cells presented a classical ultrastructure identical to that of controls. It has been observed no change in shape and structure of the nuclei and distribution of chromatin. Cytoplasm showed no alterations of the organelles, mitochondria had a normal appearance as those of controls.

For a total dose of 2 Gy, others subcellular lesions were detected in columnar cells such as condensation of nuclei, cytoplasm and mitochondria, 24 h after irradiation (Fig. 3a). In

some cells which began the process of cell death by apoptosis, we noted the presence of numerous large mitochondria occupiyng the whole cytoplasm (Fig. 3b). Intercellular spaces are enlarged at this dose and pos-irradiation time (Fig. 3a and 3b) when compared with 1 Gy-samples. It was noted that the interdigitations of apoptotic cells are separated from those of neighboring cells still with their original form in glove-fingers (Fig 3a and 3d). Obvious injuries were noted, such as changes in the morphology of brush border microvilli that appeared bent or broken (Fig. 3c). These subcellular changes of mucosa cells, specific of cell death by apoptosis, were also present in all sections, 48 h following exposure to radiation but still near large areas of necrosis (Fig. 3d, e and f).

For a total dose of 4 Gy, large areas of necrosis were observed near areas containing cells that have some features of programmed cell death. Fig. 4 shows a tissue completely desorganized, scattered mitochondria of normal appearance seem to come from cells whose plasma membrane has been ruptured during the process of necrosis. At the center of the Fig. 4a, an altered cell seems to be a plasmocyte that presente a dense nucleus with irregular outlines and an endoplasmatic reticulum with dilated cisternae. It is near a blood vessel that appears to be damaged, containing some erytrocytes. As with other total doses, it was observed enlargement of intercellular spaces but the cells are still bound to each other by cytoplasmatic expansions may be due to the presence of junctions such as desmosomes (Fig. 4b). Some samples showed changes in cytoplasm such as swollen mitochondria and numerous clear vesicles not surrounded by a membrane in the apical part of columnar cells (Fig. 4c).

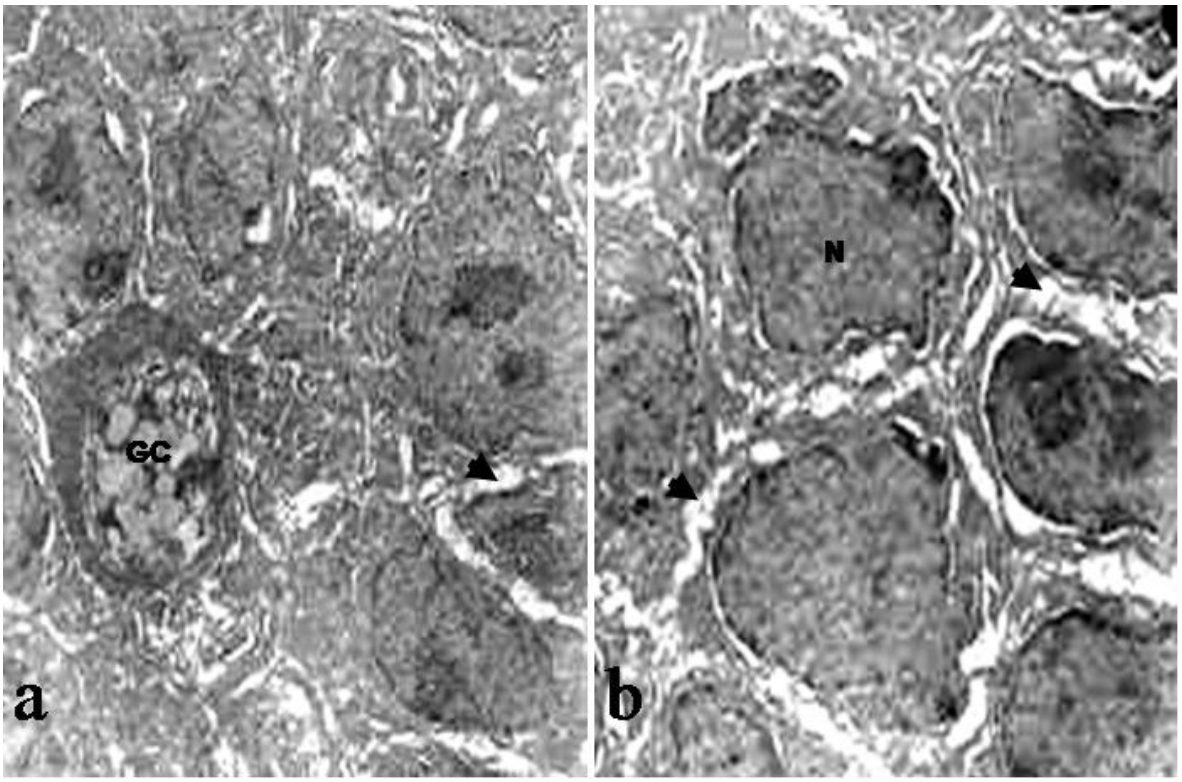

Fig. 2 Transmission electron micrographs of irradiated duodenum mucosa at a low dose rate of 1 $Gy.h^{-1}$, a dose of 1 Gy, 24 h (a) ou 48 h (b) after irradiation. On these transversal sections, note the presence of enlarged intercellular spaces within interdigitations (ID), usual shape nuclei (N) , and mucinogen granules of a goblet cell (GC). Magnifications: 3,950 X (a, c); 4,850 X (b).

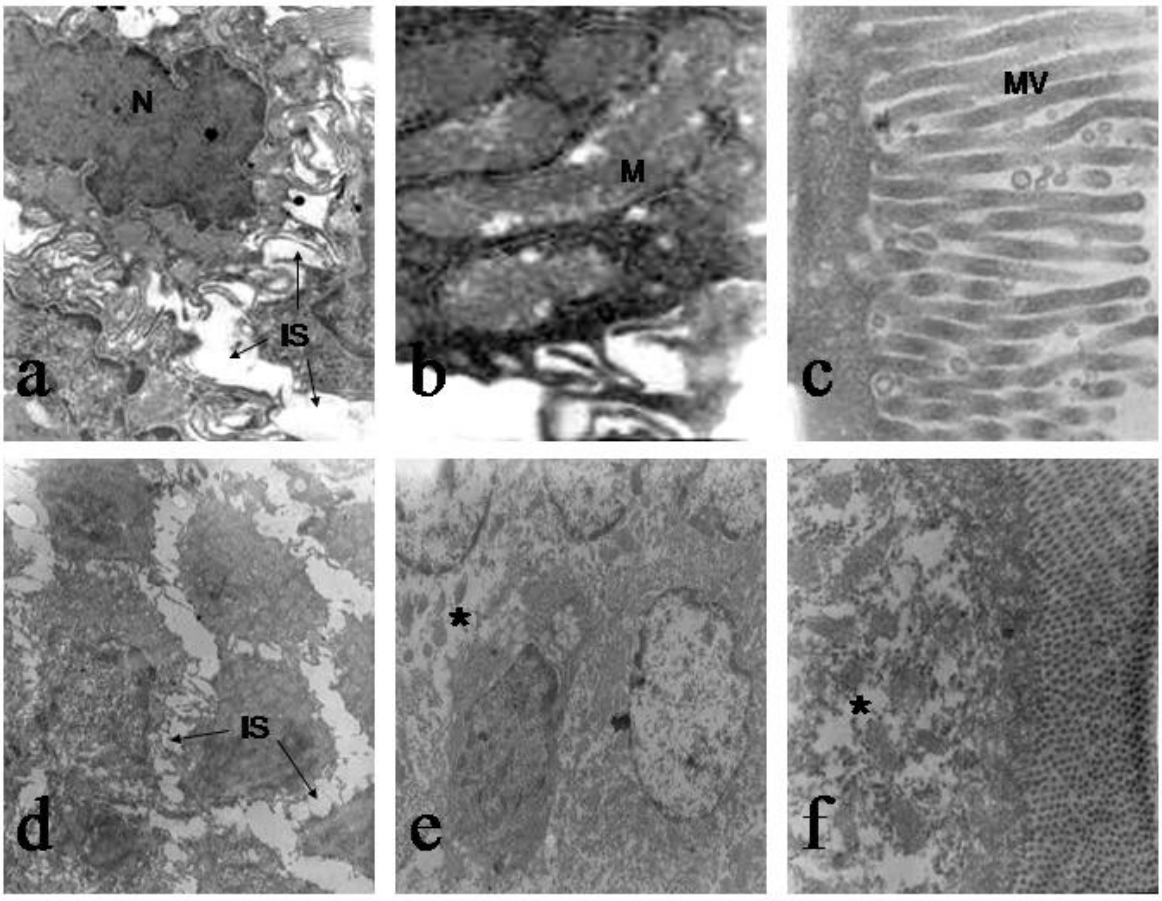

Fig. 3 Transmission electron micrographs of irradiated duodenum mucosa at a dose rate of 1 Gy.h^{-1}, a dose of 2 Gy, 24 h (a, b, c) and 48 h (d, e, f) after irradiation showing dense nucleus with irregular outlines (N), enlarged mitochondria (M), altered brush border microvilli (MV), intercellularspaces (IS) and necrotic areas (asterisk). Magnification: 12,500 X (a, c); 16,200 X (b); 40,000 X (c); 6,400 X (d, e); 16,200 X (f).

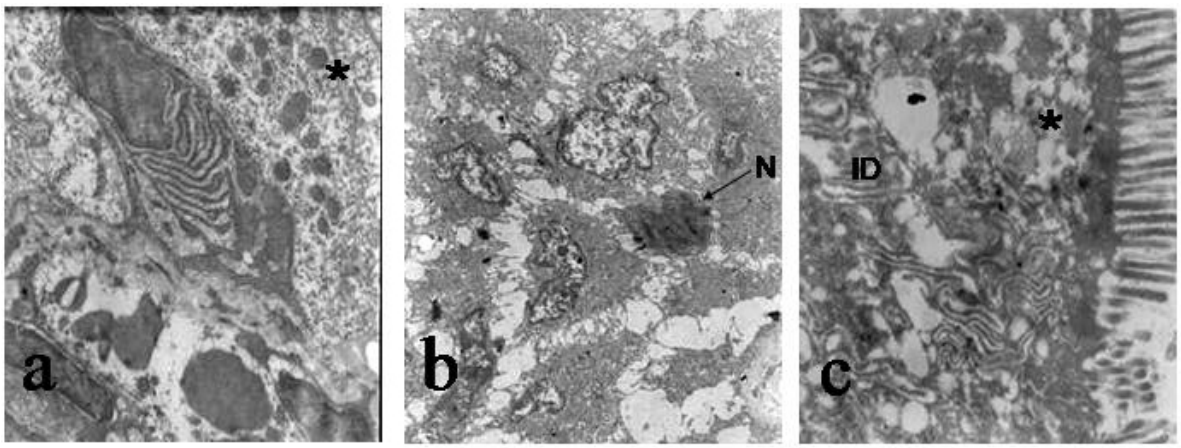

Fig. 4 Transmission electron micrographs of irradiated duodenum mucosa at a dose rate of 1 Gy.h^{-1}, a dose of 4 Gy, 24 h (a, b, c) and 48 h (d, e, f) after irradiation showing large necrotic aeras (asteris9k) within enterocyte cytoplasm, intercellular spaces and enlarged interdigitations (ID). Magnification: 12,500 X (a, c); 3,950 X (b); 20,000 X (c).

C. Ultrastructural lesions at higher dose rate

At the higher dose rate of 1 Gy.mn^{-1} and for doses of 1 and 2 Gy, few lesions were observed 24 or 48 h following irradiation (Fig. 5 an 6). Only swollen mitochondria were noted within some condensed cytoplasm. The main morphological change observed for the dose of 1 Gy was the presence of rounded structures which seem to affect only the membranes. They were detected in all cells and all endomembranes, on the cisternae of the endoplasmic reticulum, inside the mitochondria at the inner membrane that forms the cristae, and between the mucinogen granules of goblet cells. Many enterocytes have presente no morphological alterations of their organelles or the specializations of the plasma membrane, microvilli ou interdigitations. Some lesions were also observed for the dose of 2 Gy. Only a slight enlargement of the intercellular spaces have been detected but with points of contact between the plasma membranes of neighboring cells as shown in Fig. 6b. Cells that have begun the process of cell death by apoptosis were also observed for this dose. Their cytoplasm was dark and their nucleus with highly condensed chromatin. Fig 6c shows an apoptotic cell between healthy ones and its cytoplasmic expansions of the interdigitations highlighted by the strong condensation of its cytoplasm. For a dose of 4 Gy much more apoptotic alterations (Fig. 7) were detected at any post-irradiation time, as swollen mitochondria, irregular outlines of nuclear membrane, condensed chromatin and dark cytoplasm still near to necrosis areas. Fig. 7 shows two juxtaposed goblet cells without columnar cells between them. This is an unusual pattern which may be explained by the fact that the columnar cells, normally sited between them, had been detached from epithelium layer during the process of apoptosis.

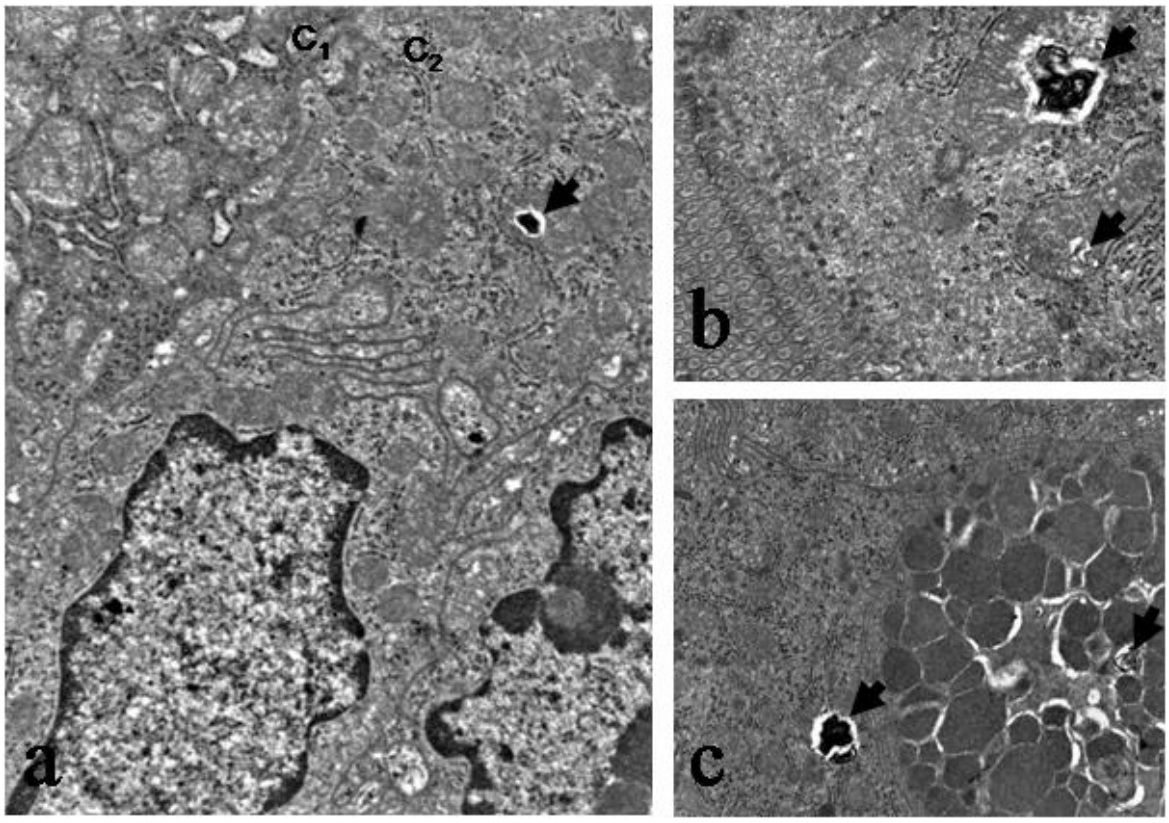

Fig. 5 Transmission electron micrographs of irradiated duodenum mucosa at a dose rate of 1 Gy.mn^{-1}, a dose of 1 Gy, 24 h (a) and 48 h (b, c) after irradiation showing an apoptotic cell (C_1) next to a non altered cell (C_2), nuclei with irregular outlines, clear mitochondrias, and lesions of membranes (arrows). Magnification: 20,000 X (a, b); 12,000 X (c).

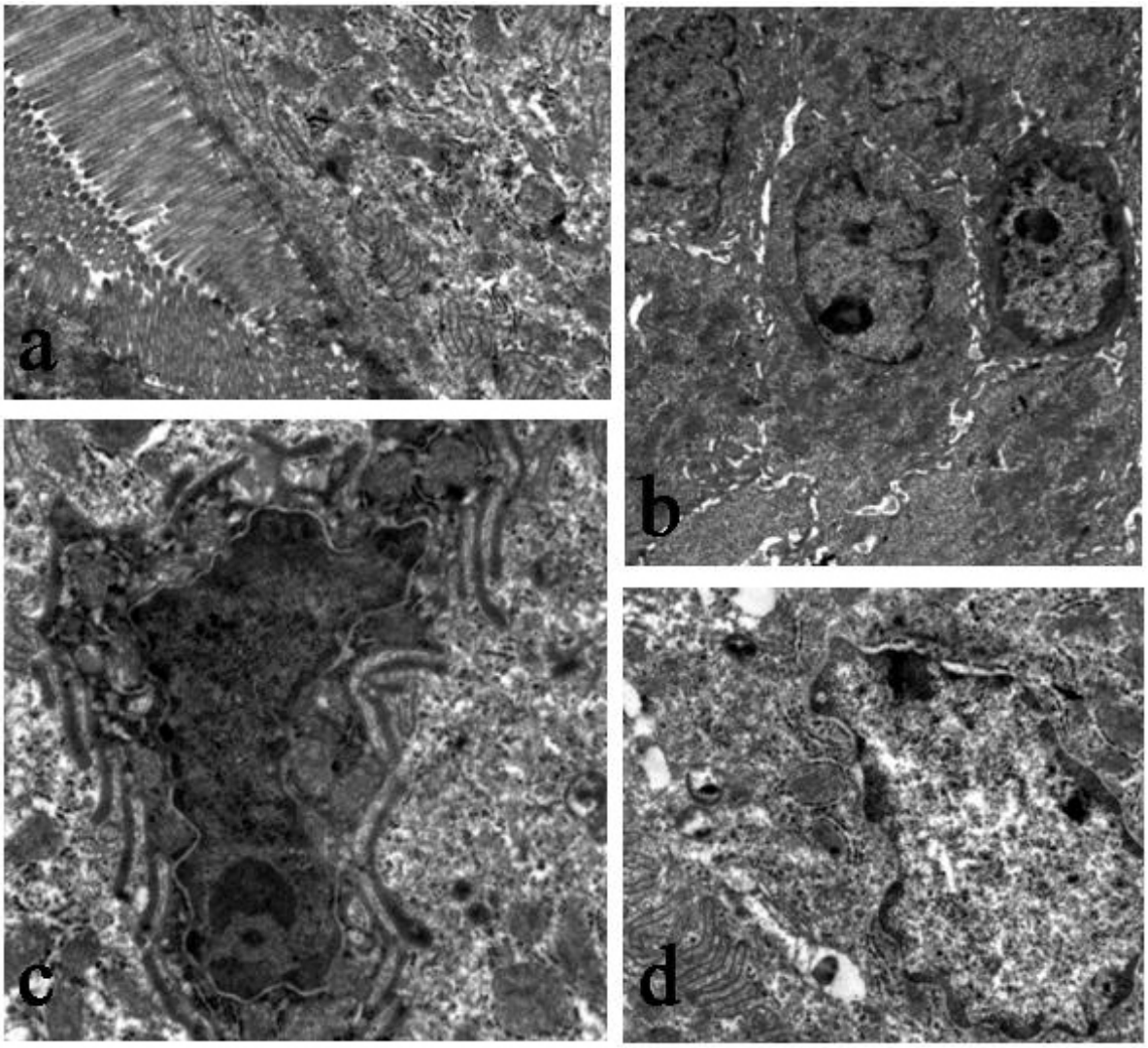

Fig. 6 Transmission electron micrographs of irradiated duodenum mucosa at a dose rate of 1 $Gy.mn^{-1}$, a dose of 2 Gy, 24 h after irradiation showing a longitidinal section of a crypt of Liberkühn (a) with non altered mitochondria, microvilli and interdigitations; a transversal section of columnar cells separated by thin intercellular spaces (b); an apoptotic cell next to healthy cells (c); and indentations od the nuclear membrane. Magnification: 4,400 X (a, b); 7,000 X (c, d).

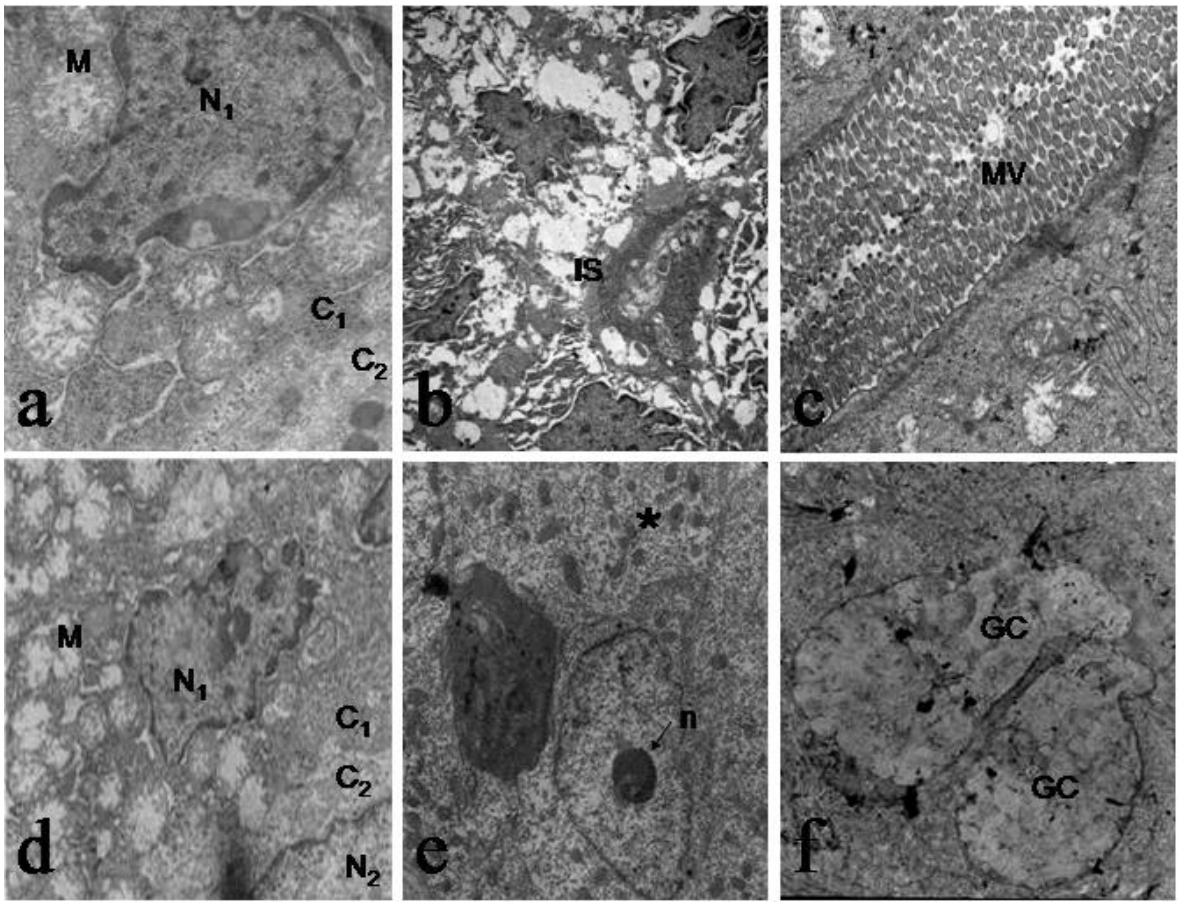

Fig. 7 Transmission electron micrographs of irradiated duodenum mucosa at a dose rate of 1 Gy.mn^{-1}, a dose of 2 Gy, 24 h (a, b,c) and 48 h (d, e, f) after irradiation showing the difference in condensation of the nuclei and cytoplasm of an apoptotic cell (N_1, C_1) and a neiboring cell (N_2, C_2) with a normal appearence (a, d), clear mitochondria (M), large intercellular spaces (b), non alterated microvilli, necrosis area (asterisk), nucleolus (n) and two juxtaposed goblet cells (GC) without beeing separated by columnar cells. Magnification: 4,400 X (a, b); 7,000 X (c, d); 6,420 X (e); 3,200 X (f).

IV. Discussion and conclusions

In contrast with the assumption that damages to cell are higher for higher doses and higher dose rates, the presente study has shown that damage to enterocytes are more importante at a low dose rate. At a dose rate of 1 Gy.h^{-1} apoptiosis were observed for the dose of 2 Gy and necrosis for the dose of 4 Gy, 24 or 48 h after exposure to radiation. The mucosas irradiated with the higher dose rate, 1 Gy.mn^{-1}, did not show these types of damage but few injuries as the presence of narrow intercellular spaces. The lack of injuries at this dose rate does not mean that the cells were not damaged but may mean that the exposure was too high to allow survival of cells that die by apoptosis. Thus during the irradiation and up to 24 h after exposure, they were eliminated by the physiological process of mucosa renewal. For the lower dose rate (1 Gy.h^{-1}), we found several lesions which may be due to a prolonged exposure to ionizing radiation inducing continuous process of lesions that may be observable 24 h and 48 h after exposition to radiation.

This work demonstrated that the appearance of these alterations varies not only with total dose increase but especially with the dose rate decrease and little with the post-irradiation time.

ACKNOWLEDGMENT

The author wishes to thank Dr Adriana for allowing us to use the transmission electron microscope (Unidade de Microscopia Eletrônica of Fiocruz Salvador/Bahia, Brazil).

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