Hepatocyte decay in late irradiation liver failure

Nahed H. Koura, MD, PhD.

ABSTRACT

Objective: The purpose was to examine the morbid changes of the hepatocytes in late irradiation liver failure. This was in an attempt to understand the reasons behind this failure, the extent of ultrastructural destruction, and the disturbance in mitotic activity of the hepatocytes.

Methods: This study was carried out at King Khalid University Hospital, Riyadh, Kingdom of Saudi Arabia, from 2001-2002. Out of 80 adult albino rats of equally mixed sex, half of them were x-irradiated and the other half were employed as a control. At 12 and 24 weeks after irradiation the animals were sacrificed. The liver was examined under both the light and the electron microscopes. The hepatocytes and their mitotic figures in addition to their mitochondria were counted at both occasions of rat sacrifice.

Results: The counting of these structures, as mentioned in the above methods, exhibited a considerable decrease in their numbers particularly when liver failure had prevailed. At the last sacrifice of irradiated animals, the liver architecture was largely preserved despite the markedly damaged hepatocytes, the endoplasmic reticulum seemed to have undergone detriment before the mitochondria.

Conclusion: It seemed, in the light of the above results that impairment of the mitotic mechanism of the hepatocytes is the major factor in predisposition to liver failure rather than the influence imposed by the damage of other liver tissues apart from these cells. The study recommends re-estimation of irradiation dosage applied for therapeutic purposes to the liver. Further understanding of ultrastructural irradiation detriment may in addition to development in other respects of radiotherapy, help to minimize complications of this treatment.

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Many modern studies on irradiation of living tissues have well established, that irradiation energy initially causes "ionization", which is a detachment of an electron from its regular space in the structure of the atoms, molecules ions and in this event an electron can be carried into a more external orbit, normally empty, which constitutes an "atomic excitation". The ultimate result of this physical effect of radiation is a series of physicochemical reactions that end up by dissociation of molecules and the expression of radiobiological damage. The consequences of irradiating normal tissues have been widely investigated and there are 3 main factors that were found to govern the tissue response to radiation exposure. The first factor is the dose given, the number of cells in a tissue, the proportion of cells killed as a result of irradiation is never zero no matter how small the dose was, the defined" threshold dose" corresponds to the proportion of cells killed above which detriment becomes noticeable.[1-3] The 2nd factor is the species although the difference is little among mammalian species and interestingly the

From the Department of Anatomy, Faculty of Medicine & King Khalid University Hospital, King Saud University, Riyadh, *Kingdom of Saudi Arabia*.

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Address correspondence and reprint request to: Dr. Nahed H. Koura, Department of Anatomy, Faculty of Medicine, King Khalid University Hospital, King Saud University, PO Box 2925, Riyadh 11461, *Kingdom of Saudi Arabia*. Tel. +966 (1) 2050326 Fax. +966 (1) 2050324. E-mail: samir-elattar@hotmail.com

threshold dose is almost the same among individuals of a certain species.[4] The last factor is the individual tissues where the difference is great in relation to the special tissue organization and the kinetics of a certain tissue.[5] In the context of the present problem, further interpretation of organization of the liver tissues needed to be understood. The hepatocytes rarely divide under normal conditions and this may be related to their relatively long life span, which was estimated at almost one year.[6] Therefore, the presence of stem cells in the normal liver is doubtful and has never been claimed by authors.[7] However, the current understanding postulates that the well differentiated functioning hepatocytes are able to undergo replication only according to the natural organization of the organ or in response to stimuli in an attempt to maintain the physiological requirement. This pattern of liver regeneration is usually described as "flexible tissue model", in contrast to other tissues such as the bone marrow where several cell compartments are always present at the same time including stem cells "hierarchical model".[8] In the former model, after irradiation if mitosis does not give viable cells and as radiation killing of cells depends on the stage of cellular differentiation, some of the remaining mature functioning cells die by senescence. The homeostatic mechanisms will then provoke the already existing living and functioning hepatocytes for a limited number of divisions "subclonogenic proliferation". As a result to this pattern of response and entirely away from considering the acute liver reaction to irradiation, the clinical expression of the liver damage can be delayed for periods from months to years. This is however, explained on the ground of the majority of the hepatocytes ultimately losing their ability to further multiplication and also coming to an end of their life span. Death of the larger number of cells, thus occurring leads to a rapid organ failure described as a phenomenon of "avalanche death". A pronounced dose-response relationship was found to influence this late effect of liver irradiation.[9] The larger the dose was the much reduction of the latent period between exposure to radiation and the appearance of late organ failure. Considering the above literature, the late liver irradiation damage can be explained by the hepatocytes finally exhausting their mitotic ability and also becoming old to eventually pass away. However, this justification is not clear enough as it did not involve the ultrastructural changes of the hepatocytes following irradiation and also the pattern of mitosis of these cells in the same context.[10] This is although some studies showed ultrastructural liver irradiation damage but unfortunately these studies did not elaborate on the conditions of each of the cellular organelles under radiation exposure. Therefore, the present study gave a particular concern to looking at the changes of certain organelles in the hepatocytes and also to the manner of division of these cells at the onset of frank liver failure and amidst the time between this event and administering radiation. In pursuing the aim of the present work microscopical as well as numerical procedures were employed.

Methods. ***Experimental design.*** The animals were only once locally irradiated and at frequently regular short intervals, the onset of the liver failure was to be diagnosed through blood chemical analysis and also by watching the animal's activity. Microscopical examination was carried out only twice at 12 and 24 weeks following animals exposure to radiation and this was to assess the late condition of both of the hepatocytes mitosis and structural disturbances.

Experimental animals. Eighty D-strain albino rats (inbred at the King Khalid University animal house) of equal mixed sex were divided into 4 equal groups, 2 groups were used as controls and the other 2 were subjected to X-radiation energy. The animals were adults of almost 4-months of age and their weight ranged from 150-220 gm. Ordinary plastic cages were employed to keep the rats in the animal house under room temperature and were freely supplied with water and the standard food pellets.

Experimental procedures. Irradiation was carried out by a Marconi X-ray machine, routinely used in the Department of Radiology in King Khalid University Hospital, Riyadh, Kingdom of Saudi Arabia to give the required dose of irradiation in this experiment. The physical factors employed were 230 Kv, filter 0.5 mm, Copper ions 1mm Al, f.s.d. 30 cm and a single dose of 9 Gys was used at a dose rate 100 rads per minute every 10 minutes.[11] Ether was used as an anesthetic and the animals was immobilized under a specially adopted plastic holder lying on their back to ensure that the irradiation was applied accurately. The body of the animal was protected by a complete cover of lead 5 mm thick except the upper right quadrant of the abdomen.

Clinical and laboratory assessment of the liver function. The goal was to detect the possible deterioration of the performance of this organ as a result of irradiation. The clinical evaluation was approximately viewed daily by watching the animal's activity and also through weighing the animals each 2-weeks. Laboratory investigation involved measuring the serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes and the total serum bilirubin (TSB). A blood sample from all animals was taken as frequent as shown in **(Table 1)**.

Microscopical study. Using both the light and electron microscopes was employed to estimate the late liver damage in terms of feasible (seemingly clear outline of the cells or the mitochondria) counting of the total light microscopic number of the

hepatocytes and their mitotic figures and also the ultrastructural number of the hepatocyte mitochondria. The apparent changes of the endoplasmic reticulum and the mitochondria were to be observed. At the ends of both 12 and 24 weeks, an irradiated and a control group were sacrificed through intravenous injection with sodium pento-barbitone (10 mg/kg body weight) into the rat tail. The liver in all animals was immediately dissected and processed for Hemoxylin (H) & Eosin (E) paraffin sections and also for ultrastructural examination. The later procedure was conducted through immersion of small liver specimens into 4% glutaraldehyde in 0.1 M cacodylate buffer, PH 7.4 for 10 minutes then fixed in 2% osmium tetroxide in 0.1 M cacodylate buffer and processed for electron microscope grids.[12] The ultrasections were examined by the transmitting electron microscope.

Numerical study. It was conveniently conducted with the help of a slide micrometer. The liver lobule was divided into 4-quarters, in each of them the number of the apparently intact hepatocytes and their relatively clear mitotic figures were counted and the total numbers were then obtained. This undertaking involved 5 lobules in a section for a total of 10 from each animal and for all the groups. In a similar manner the number of the hepatocyte mitochondria was identified by reading 5 electromicrographs taken from 5 lobules for each animal and inclusive to all groups.

Statistical analysis. Statistical analysis of the data was based on a repeated measures-analysis of variance. The experimental unit was the animal and was statistically treated accordingly. Each measurement from the same rat was treated as a repeated measure rather than an independent sample. A significant level of 0.05 was used for all analysis.

Results.

The clinical observations. revealed declining activity of the treated animals during the initial 4 and last 6-8 weeks with the period in between showing a moderate improvement relative to the ordinary physical behavior of these animals. A pattern of change similar to that of the activity as mentioned above was seen in both of the appetite and the weight **(Table 1)** in both groups of the irradiated animals. All irradiated animals had a considerable decline in weight during the first 4 weeks and thereafter regained much weight 12 weeks but by almost the 20th week they began to lose weight. The above changes of the weight in the treated rates were of statistical significance ($p<0.01$). The laboratory tests of the aspartate transaminase (AST) and alanine transferase (ALT) enzymes and the total serum bilirubin(TSB) introduced a substantially high increase **(Table 2)** during the first 4 weeks after treatment and kept a moderate values thereafter except for the total bilirubin that its value had never come down. There was no sex difference.

Table 1 - Depicts the mean and the standard deviation of the animals weight taken regulary at 2 week intervals.

Time in weeks	Weight of animals (g)	
	Control	Irradiated
0	180 ± 40	178 ± 45
2	182 ± 45	145 ± 39
4	180 ± 46	139 ± 41
6	179 ± 42	150 ± 46
8	183 ± 39	161 ± 38
10	178 ± 43	166 ± 40
12	183 ± 42	165 ± 41
14	179 ± 44	166 ± 41
16	180 ± 41	164 ± 42
18	181 ± 40	160 ± 41
20	180 ± 41	150 ± 39
22	182 ± 41	143 ± 37
24	179 ± 43	135 ± 36

The microscopical examination. Using H & E staining, exhibited damage of the hepatocytes where the cytoplasm appeared severely degranulated and the nucleus shrank with darker staining. These changes **(Figure 2)** of the hepatocytes were seen in animals sacrificed by the end of the 12 week after irradiation. Similar changes **(Figure 3)** were observed by the end of the 24th week following irradiation but many hepatocytes nuclei became rather more faintly stained and disintegrated. The hepatocytes cytoplasm at this last stage of the experiment looked almost entirely degranulated in the majority of the liver cells. The same liver staining H & E did not reveal significant destruction of other liver structures, in particular at the last examination, the familiar hepatic architecture was essentially preserved. The electromicrographs presented gradually intensifying damage between the above two times of examination subsequent to irradiation. At the earlier time, **(Figure 5)** the hepatocytes had a markedly decreased density of the endoplasmic reticulum meshwork and the mitochondria became relatively darker and looked larger in size. The endoplasmic reticulum at this earlier time seemed to have suffered of more detriment than the mitochondria. The decreased density and the largely lost roughness of the former organelle and synchronous presence of almost the normal number of the mitochondria in the same hepatocytes **(Table 3)** supported this last observation. This is although the last organelle appeared relatively larger and darker. The late time examination **(Figure 6)** showed a greatly disappearing endoplasmic reticulum and the mitochondria became sparse and frequently coalesced together with much paler staining than normally encountered. There was no sex difference.

The numerical studies (Table 3). Disclosed that there are almost 3300-2300 hepatocytes and 300-120 mitotic figures in a liver lobule under normal

Table 2 - Presents the mean and standard deviation of serum levels for control and x-radiation treated rats with the enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT), in addition to the total serum bilirubin (TSB). All the values increased during the first 4 weeks but only the transeferases went down after that. The statistical significances of the above figure were high ($p<0.001$).

Time (week)	AST/IU		ALT/IU		TSB/μmol/L	
	Control	Treated	Control	Treated	Control	Treated
0	18 ± 10	20 ± 20	22 ± 10	21 ± 20	10 ± 5	10 ± 6
2	19 ± 9	600 ± 30	25 ± 9	850 ± 30	9 ± 4	30 ± 4
4	20 ± 10	550 ± 25	21 ± 11	600 ± 40	9 ± 5	35 ± 6
6	17 ± 8	500 ± 25	22 ± 9	650 ± 25	10 ± 6	32 ± 7
8	21 ± 10	400 ± 30	26 ± 8	500 ± 30	12 ± 7	35 ± 8
10	20 ± 10	300 ± 30	19 ± 10	500 ± 25	10 ± 5	39 ± 12
12	19 ± 8	350 ± 27	23 ± 8	200 ± 40	11 ± 7	35 ± 10
14	17 ± 8	200 ± 20	20 ± 10	150 ± 30	10 ± 6	35 ± 5
16	18 ± 10	200 ± 25	25 ± 9	200 ± 25	11 ± 5	37 ± 7
18	19 ± 7	180 ± 20	23 ± 10	180 ± 20	10 ± 6	40 ± 6
20	20 ± 9	200 ± 25	19 ± 9	150 ± 40	9 ± 4	60 ± 15
22	19 ± 7	220 ± 30	22 ± 11	120 ± 30	10 ± 6	70 ± 20
24	20 ± 10	200 ± 20	21 ± 10	100 ± 25	9 ± 5	75 ± 19

AST - aspartate aminotransferase, ALT - alanine aminotransferase, TSB - total serum bilirubin, IU - international unit

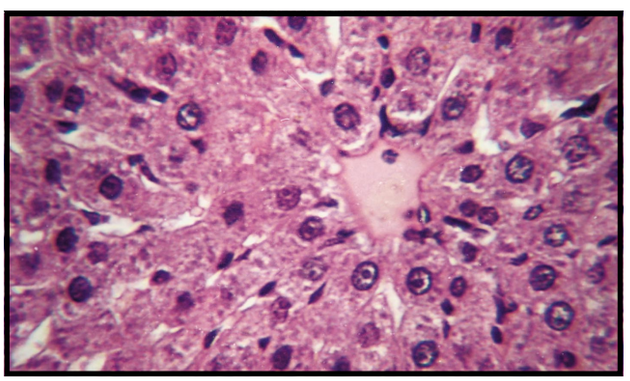

Figure 1 - A photomicrograph of the control rat liver showing the normal hepatocytes radiating from a central vein and some of the familiary seen mitotic figures (x 1000).

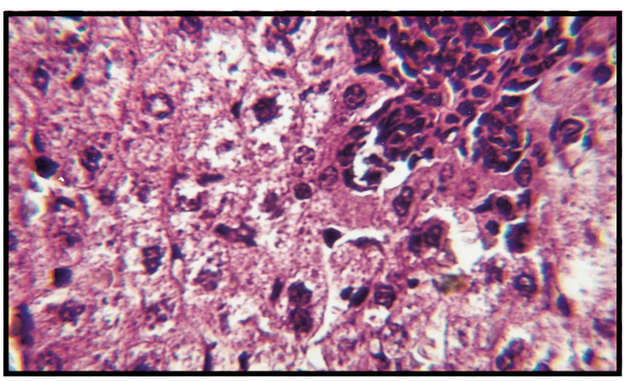

Figure 3 - A photomicrograph of rat liver 24 weeks after exposure to radiation. The hepatocyte cytoplasm became completely degranulated. The nuclei of these cells lost much of their density and looked disintegrated. The cellular outline of the hepatocytes was almost entirely absent. The central vein of hepatic lobule were markedly dilated and congested. The mitotic figures were scarce (x 1000).

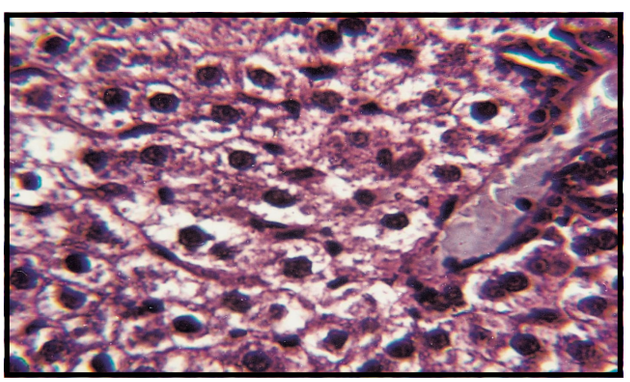

Figure 2 - A photomicrograph of rat liver 12 weeks after exposure to radiation. The hepatocyte cytoplasm became faint and the nuclei of these cells had a relatively darker color than their ordinary appearance and mitotic figures were greater in number than the normal encounter. The outlines of these cells were feebly observed. The central veins appeared dilated and congested (x 1000).

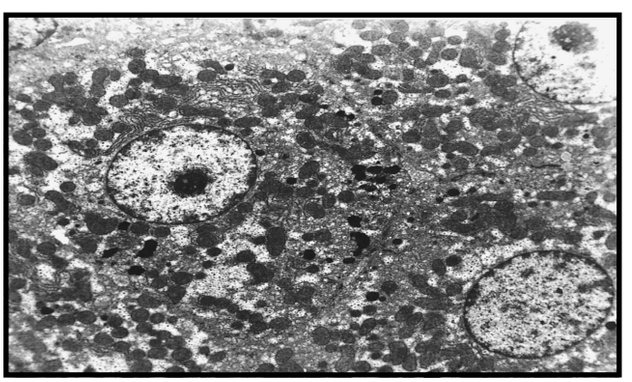

Figure 4 - An electromicrograph of hepatocytes of a control rat liver. The cytoplasm was abundant and rich in mitochondria (x 10000).

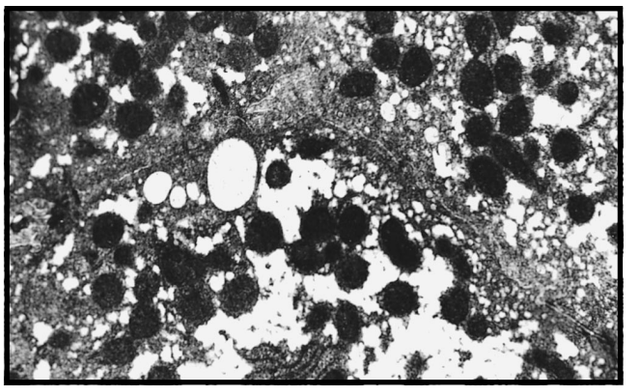

Figure 5 - An electromicrograph of rat liver 12 weeks after exposure to radiation showing areas of low cytoplasmic density in the hepatocytes and mitochondria were darker and larger in size. The rough endoplasmic reticulum was very much reduced and only occupied a small area in the section (x 10000).

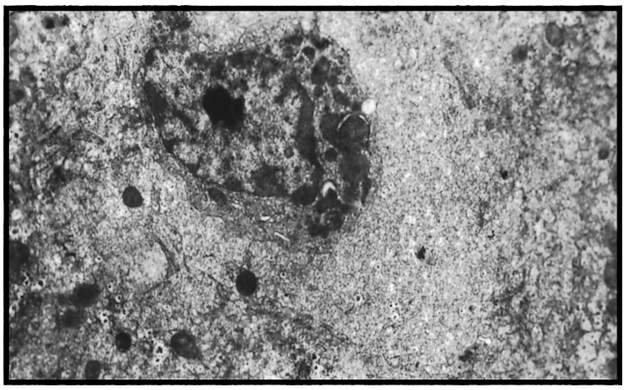

Figure 6 - An electromicrograph of rat liver 24 weeks after exposure to radiation. The hepatocyte outlines were greatly lost, the mitochondria were markedly diminshed in number and coalesced together to form relatively large and faintly stained bodies. The endoplasmic reticulum almost completely disappeared (x 10000).

Table 3 - Shows the mean and standard deviation of the numbers in a liver lobule of the control and x-radiation treated rates for total hepatocytes, total mitotic figures and the total mitochondria. The hepatocytes number and the mitotic figures fell to almost a quarter while the number of mitochondria was almost reduced to a half of that of the control by the end of the experiment. These results were statistically highly significant ($p<0.001$).

Time (weeks)	Hepatocytes		Mitotic figures		Mitochondria	
	Control	Treated	Control	Treated	Control	Treated
12	2800 ± 450	1350 ± 200	200 ± 75	330 ± 60	1200 ± 400	1100 ± 350
24	2900 ± 400	700 ± 180	220 ± 69	40 ± 27	1100 ± 430	500 ± 250

circumstances. At the first time of microscopical examination as mentioned before the number of the hepatocytes fell almost to only a half of the control while the number of mitotic figures was significantly higher than that of the control. The 2nd time of examination showed a further decline in the hepatocytes number as it became almost one quarter from the control and at this proportion the number of mitotic figures also came down relative to the control. The number of mitochondria at the earlier examination was almost the same as that normally found within the hepatocytes but was significantly decreased at the late examination (**Table 3**).

Discussion. The result of the present study reproduced a clear phenomenon of the incidence of liver failure at a relatively long time after this organ was exposed to ionizing radiation. However, many workers documented the same phenomenon.[13,14] These pioneer investigators explained this late irradiation liver failure through the temporal compensatory action of the unique normal mechanism which regulates hepatocytes division. In their work, for a short period of several days following irradiation, hepatocytes replication was scarce.[15,16] An enhanced mitosis was then observed for a period ranging from several months to even years until the whole mitotic mechanism finally was impaired with the eventual establishment of liver failure.

The above mentioned phenomenon is still largely obscure when viewed in terms of the changing mitotic activity and the role of the individual hepatocyte organelles in the final decay of this activity.[17] However, the present work focused the attention on some rather readily demonstrable organelles; the mitochondria and the rough endoplasmic reticulum and in reference to the known function of these 2 organelles, one may presume that they play an essential role in cell division. However, the present study detected degenerative signs in both of these 2 organelles by the end of the 12-week period past irradiation, and in the same time the endoplasmic reticulum seemed to have suffered of more damage than the mitochondria as it was shown before. In the same last event and despite the apparently severe injury inflicted on both the mitochondria and the endoplasmic reticulum, the number of mitotic figures greatly exceeded that of

the normal encounter. This incredible ability of the cell to replicate under the present experimental condition, is difficult to explain at the moment but it may be conceptualized that the individual cell organelles although each of them is known to have a certain function they are still anatomically as well as physiologically intimately interrelated. There, it seems that in each single cell activity the organelles are probably sequentially involved, and may be that under stress not only the same organelle is always the igniter of this activity. The theme of exchanging roles among the cell organelles may well be the foundation where by the seriously injured hepatocytes were able to recover and undergo significant reproduction. Comprehending further this functional flexibility concept, each cell organelle should have its own degree of vulnerability to injury where the quantity as well as the quality of the hurt imposed on the cell is the determinant of the whole cell response. However, as it is the case in other body organs it seems that this functional flexibility is predetermined to work only temporarily and fail to sustain long term normal functioning.[18] Other sequelae of irradiation effect on the liver tissues particularly on hepatic vasculature can not be ignored in the present context.[19] The liver architecture was largely preserved in this study as it was earlier shown, that indicated that the irradiation impact on liver pattern of the blood vessels especially on the fine termination of the portal vein presented by the lobular central veins was not significant.[20] These central veins escaping a substantial radiation damage can be interpreted in relation to radiation dose employed here and also to the length of the post-irradiation period after which the rats were examined.[21] Therefore, this study would favor explaining the late liver failure past irradiation as an ultimate blockage of the hepatocyte multiplication mechanism rather than other liver tissues destruction.

In conclusion, an immediate clinical significance of the present study is obviously difficult to determine but it may be recommended that using irradiation in the therapeutic field has to be rescrutinised with probably reconsideration of the appropriate dose. Better control of irradiation complications may be achieved through further ultrastructural study in addition to other currently used means.

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