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COMPARATIVE HISTOPATHOLOGICAL STUDIES ON THE EFFECTS OF IONIZING X-RAY IRRADIATION ON NORMAL LIVER AND HEPATOMA

By

MAHMOUD A. EL-BANHAWY
Department of Zoology
Faculty of Science
Ain Shams University
Cairo - Egypt

IBRAHIM M. ANWAR
Department of Zoology
Faculty of Science
Assiut University
Assiut - Egypt

ABSTRACT

Normal mice exposed to X-rays showed distinct external symptoms and died mostly on the 13th or 14th day after irradiation. Pathological symptoms were more exaggerated in mice diseased with hepatoma and subjected to the effects of irradiation. These animals did not survive beyond the 4th day following treatment.

Histologically, the normal liver displays the characteristic pattern of lobulation and disposition of hepatic cells. The whole tissue is enveloped by a layer of squamous epithelium.

The liver of X-ray irradiated mice shows cloudy swelling in its cells, which became lightly stained and the nuclei display distinct signs of karyolysis. Hepatic blood vessels are markedly enlarged, engorged with blood and their limiting membranes and deteriorated.

Hepatoma has lost completely the characteristic pattern of the healthy liver. Cells are much smaller in size, being oval or spindle shaped and irregularly disposed with their nuclei obviously injured.

Hepatoma treated with X-rays show prominent signs of deterioration. Most of the tissue has been occupied by prominent vacuoles of variable sizes. The inter-vacuolar tissue is formed of necrotic cells.

INTRODUCTION

In spite of the great importance of the biological effects of irradiation on normal and malignant liver tissues, a few reports only are available in this respect (Semenoa, 1956; Trotter, 1962; Banhawy, 1963, 1964, 1969, Daoust, 1963 and Reid *et al.*, 1963).

Banhawy (1963, 1964 and 1969) noticed in his studies on enzyme equipment of liver and malignant tissues that these cells were apt to considerable deformities under the effect of X-ray irradiation. In general, cells were markedly deteriorated and nuclear membranes acquired a wavy irregular appearance.

According to Trotter (1962), hepatoma tissues lose completely the lobular pattern characteristic of normal liver tissue and its component cells are markedly smaller in size as compared to healthy hepatic cells. Reid *et al.* (1963) observed that hepatoma tissue displayed cloudy swelling, vacuolation, patchy necrosis and distinct signs of haemorrhage in some cases.

The present investigation includes a comparative histopathological investigation on the effects of X-ray irradiation on normal liver and hepatoma of mice of the C_3HA strain.

MATERIAL AND METHODS

Fully-grown mice of the C_3HA strain were used in the present experiments. All animals were of nearly the same age, varying from 6-8 months old. These were divided into two categories; the first category was subjected to a dose of total-body X-ray irradiation of 800 rads, delivered at the rate of approximately 110 r/minute. The source of X-rays was obtained from a 160 KV machine, operating at 4 ma., at a distance of 25-30cm, using filters of 0.5mm Cu and 1 mm. Al. Animals of the second category were subcutaneously injected with a suspension of transplantable hepatoma under sterilized conditions. When hepatoma was developed, the diseased mice were divided into two groups; one was left as control and the other was exposed to a dose of total-body X-ray irradiation of 800 r, also delivered at the rate of 110 r/minute.

Some healthy mice of the same strain and age were always kept as control. Healthy mice, X-ray irradiated mice, hepatoma-bearing ones and hepatoma-diseased animals treated with X-ray irradiation, were maintained in separate cages exposed to food and water *ad libitum* until they were sacrificed.

Animals were killed by decapitation. Anaesthetics or poisonous agents were not used, so as to avoid any confusing results. The liver, or the tumor, was rapidly dissected and cut into small pieces (approx. 2-3mm thick). In every case, a similar piece of normal liver, obtained from a healthy individual, was treated simultaneously for the sake of comparison and also to be sure that the procedure was progressing in its proper way and that any obtained changes would not be attributed to any defect in the technique.

The specimens obtained were fixed in Bouin's and Zenker-formol fluids. Paraffin sections (4-5 u thick) were stained by Harris haematoxylin and eosin stains, which have proved to be very satisfactory for both normal and diseased liver.

RESULTS AND OBSERVATIONS

External symptoms:

Pathological symptoms were apparent externally on the X-ray irradiated mice (devoid of hepatoma) on the tenth day post-treatment. Animals were withdrawn, lost their appetite and their hair became markedly rough. Most of these animals died on the thirteenth to the fourteenth day after X-ray irradiation. These animals represent the category, which was examined on the twelfth day following exposure to X-ray irradiation. X-ray irradiated hepatoma-bearing hosts died mostly on the fourth day post-irradiation. Such animals were sacrificed on the second day after X-ray treatment.

Histology of liver and hepatoma before and after X-ray treatment

Normal liver: Examination of cross sections of normal liver of mice of the C_3HA strain, after

staining with haematoxylin and eosin, reveals that the liver is enveloped by an outer sheath derived from the peritoneum, within which is a delicate connective tissue capsule. These two structures are usually referred to as the tunica serosa and capsule of Gilsson respectively (Copenhaver *et al.*, 1979).

The liver tissue is disposed in rather separate lobules, joined together by a scant amount of connective tissue. Each lobule is formed of a large number of hepatic cords, extending from the periphery of the lobule to a rather wide controlobular vein lined by flattend cells. These cords have an irregular appearance and each is formed of one or more cells thick, depending on the plane of the section (Fig. 1).

Between the hepatic cords, exist narrow blood sinusoids which converge from the outer surface of the lobule to the central vein. These are lined by two types of cells; the first, namely the endothelial cells, form a discontinuous layer and are resting on a complete basal lamina. The other type, Kupffer cells, are much larger in size and have branching processes (Fig. 1).

Bile canaliculi are found between the hepatic cords, being limited by low cuboidal cells.

The hepatic cells are distinctly eosinophilic with numerous fine granules distributed at random in their ground cytoplasm. Each cell is mostly mononucleated, except for a few ones which appear bi- or multinucleated. Copenhaver *et al* (1979) explained the presence of more than one nucleus as being due to a mitotic division of the mononucleate cell without accompanying division. In the mononucleated cells, the nucleus is centrally located. Nuclei, in general, have a spherical shape, being limited with definite nuclear membranes. The nucleus contains numerous dispersed fine chromatin granules, beside a few particles which are mostly adherent to the internal boundary of the nuclear membrane. In the majority of cases, one nucleolus exists in each nucleus; two nucleoli are observed sometimes (Fig. 1). A few mitotic figures are apparent in some hepatic cells.

X-ray irradiated liver: Treatment with X-ray irradiation has resulted in conspicuous alterations in the normal configuration and structure of hepatic tissue (Fig. 2). Generally, the hepatic cells display clear signs of cloudy swelling and are prominently larger in size as compared to the corresponding untreated ones. These cells are stained faintly with eosin and numerous fine faintly coloured granules are observed in their cytoplasm. The nuclei show distinct signs of karyolysis. Two nuclei are prevailing in each of these cells. It is apparent that this binucleated condition is a result of amitotic division. Chromatin granules are more abundant in these treated cells, being located in a homogeneously stained nucleoplasm. Most of these particles tend to adhere to the inner surfaces of the nuclear membranes. The nucleoli are markedly reduced in size and in some cells they are not clearly demonstrated.

One of the most striking changes, which had resulted in the liver tissue under the effect of X-ray irradiation, is the marked dilation of hepatic blood vessels. Figure 2 shows an enlarged centro-lobular vessel engorged with blood. Its lining epithelium is disrupted at certain places and the component cells of the remaining parts are obviously pyknotic.. Similarly, the blood sinusoids appear wider than the untreated case and some of them appear confluent with the central vein. The lining cells of these sinusoids display distinct signs of pyknosis.

Hepatoma

Hepatoma cells are far smaller in size than normal hepatic cells, being arranged mainly in the form of interlacing bundles (Fig. 3). These cells acquire an oval or fusiform appearance, each having a prominently basophilic ground cytoplasm displaying a few darkly stained granular inclusions. Each cell possesses a darkly coloured nucleus with one or two prominent nucleoli and a few chromatin particles. However, a considerable number of these cells are distinctly necrotic.

The characteristic hepatic blood vessels are no longer apparent in the hepatoma tissue. Instead, there are several irregular spaces which sometimes contain remnants of degenerated cells.

Mitotic figures, which were demonstrated in the healthy liver tissue, are not observed in hepatoma, but there is clear indication of amitosis (Figs. 3 and 4).

The limiting capsule of the hepatoma tissue is not exhibiting the regular thickness observed in normal liver, but it appears much thicker in some regions than in others. This thickening is attributed to the fact that the usual squamous epithelium of healthy tissue has been converted into swollen stratified squamous layer (Squamous cell carcinoma). These smaller cells contain a homogeneous material, which is presumably a fluid substance that is usually referred to as the serous fluid. Pyknotic nuclei are frequently observed in their abnormal squamous cells, having a central location. Large extensions of these growing squamous cells are observed within the hepatic tissue (Fig. 4).

It is also worth mentioning, that hepatoma tissue is invaded by bundles of connective tissue of considerable size (Fig. 4).

X-ray indicated hepatoma

Following treatment with X-rays, the hepatoma cells become markedly swollen and their boundaries could not be easily distinguished. Similarly, the nuclei are enlarged, being rather elongated with clear dentitions in some of them and few faintly stained chromatin particles. However, a considerable number of these nuclei are obviously pyknotic. No nucleoli could be clearly identified in these nuclei (Fig. 5).

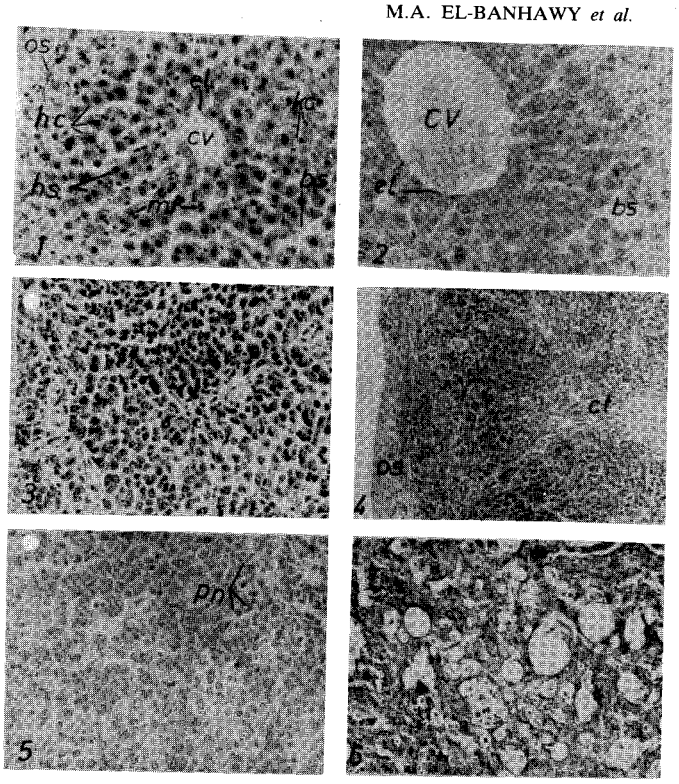
The ground cytoplasm of these X-ray treated hepatoma cells is faintly stained and contains a few fine basophilic granules.

The influence of X-ray irradiation is much more exaggerated in advanced (old) hepatoma (Fig. 6). The malignant tissue is highly vacuolated in an irregular manner. These vacuoles show marked variation in size and some of them contain debris of unknown nature. The little remaining tissue is completely different from the untreated one and if any cells could be identified, they appear highly necrotic.

DISCUSSION

A relatively few histological notes could be obtained from the extensive histochemical and biochemical studies dealing with the effects of irradiation and other chemical agents on several types of malignant tissues (Semenoa, 1956; Larinov, 1962; Trotter, 1962; Banhawy, 1963, 1964 a,b,c,d, 1969; Daoust, 1963; De Man and Blok, 1966 and Moussa *et al.*, 1979 a,b).

However, the findings of Trotter (1962), as well as those of Banhawy (1963, 1964 a,b,c,d, 1969) have shown that hepatoma tissue exhibited marked histological alterations, as compared to the healthy liver. These phenomena were examined in detail in the present communication, which shows that hepatoma tissue has lost completely the normal configuration and characteristic histological features of the corresponding liver tissue. Hepatoma cells were also found to be much smaller in size than the normal liver cells and the binding connective tissue of the hepatic cords has been highly deteriorated in the hepatoma tissue. Besides, it was found that the simple squamous epithelium bonding the liver tissue, has been converted into malignant squamous cell carcinoma, which was markedly swollen and contained serous fluid. Moreover, connective tissue trabeculae were noticed to extend from the growing squamous cell carcinoma, invading



EXPLANATION OF FIGURES

Harris haematoxylin and eosin staining.

Figure 1: Section of normal liver of mice (X 320)

Figure 2: Section of X-ray irradiated liver of mice (X 320)

Figure 3: Section of hepatoma (X 320)

Figure 4: Same as above (X 160)

Figure 5: Section of X-ray irradiated hepatoma (X 320)

Figure 6: Section of X-ray irradiated advanced (old) hepatoma (X 320).

ABBREVIATIONS

bs: blood sinusoids

ct: connective tissue

cv: central vein

el: endothelial lining

hc: hepatic cells

hs: hepatic strands

kc: Kupffer cells

mf: mitotic figures

os: outer sheath

pn: pyknotic nuclei

the hepatoma tissue. Cloudy swelling and necrosis are also common features in hepatoma, especially in the advanced or old types.

It was also found in the present material, that X-ray treatment of normal liver brings about considerable alterations in its structure. Cells suffer from cloudy swelling and their nuclei are enlarged and most of them exhibit signs of pyknosis. The hepatic vessels are also found in this case to be highly distended and engorged with blood which may lead to severe haemorrhage in the liver. Dilatation of blood vessels in the X-ray irradiated liver has also been previously reported by Semenoa (1956).

However, the effects of X-ray irradiation on normal liver and hepatoma have been extensively studied by Banhawy (1964b, c,d, 1969), but these studies were mainly carried out from the histochemical point of view. Nevertheless, a few histological remarks have been sporadically mentioned by this author. These indicate that X-ray irradiation produced generally considerable damage in hepatoma tissue, as well as complete deterioration of the binding connective tissue. These findings receive strong support from the present observations, which demonstrated cloudy swelling, haemorrhage and vacuolation of hepatoma tissue after treatment with X-rays.

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دراسة مقارنة لتأثير الأشعة السينية على الخلايات الكبدية السامة والسرطانية

محمود أحمد البنهاوى وابراهيم محمد أنور

اتضح من البحث الحالي ان الخلايات الكبدية السرطانية تختلف اختلافا بينيا في شكلها وتركيبها عن خلايا الكبد السليمة . فهى في الحالة الأولى مرتبة بطريقة مميزة على هيئة فصيصات صغيرة يتوسط كل منها وعاء دموى تبطنه خلايا طلائية حرشفية ، ولا يشاهد شيء في هذا الوعاء .

فاذا تحول هذا الكبد الى الحالة السرطانية اختل هذا النظام وفقدت الخلايا نظامها وشكلها المميز واصبحت صغيرة الحجم جدا وتأخذ شكلا مغزليا غير مألوف في الحالات العادية .

وعند التعرض للأشعة السينية تتأثر كل من الكبد والخلايا السرطانية تأثرا بالغا ينعكس في شكل الخلايا وحجمها والأوعية الدموية الموجودة بها .