

Fine Structure Alteration of Rat Liver induced by Nitrosohexamethylenamine

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(Introduced by P. Shubik)

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(Received for publication; 20, Aug. 1970)

ABSTRACT

The ultrastructural alterations in rat liver by feeding NHM(nitrosohexamethylenamine).

These are described at intervals of 10 days, 5 weeks, 11 weeks, 14 weeks, 19 weeks, and 22 weeks.

The group at 5 and 11 weeks showed hyperplastic lesions but, no nuclear change.

There were dilated rough endoplasmic reticulum with detached ribosomes, and alteration of mitochondria.

The mitochondria showed a dense matrix which often included membranous materials.

In the 14, 19, and 22 week groups, it showed nodular lesion which had atypical cells, and it was observed that the nucleus were enlarged and nucleoli were segregated.

The bile canaliculi were dilated and contained dense materials.

INTRODUCTION

In 1968 Goodall, Lijinsky, and Tomatis observed a high incidence of liver tumors in rats fed nitrosohexamethylenamine (NHM).

The liver tumors appeared to be multicentric and histologically were of two distinct types, liver cell carcinomas and endothelial sarcomas.

Much of the interest in the N-nitrosamines centers around the possibility that they may be potential human carcinogens produced by nitrosation of secondary amines(Lizinsky and Epstein, 1970) and because of the possibility (Druckrey, 1962) and that alkylation of guanylic acid may be responsible for their carcinogenic action.(Magee, 1968).

In these studies, we observed the ultrastructural alterations in rat liver parenchyma and endothelial cells sequentially in animals fed NHM for designated intervals.

MATERIALS AND METHODS

Female MRC Wistar derived rats weighing about 250gm were used, housed in plastic cages in group of 5 female rats and given Rockland

* The investigation was supported by a grant CA-5070-06, National Institute of Health, United States Public Health Service. I wish to thank Dr. W. Lizinsky for his help in supplying this material.

Table 1. Experimental Schedule

No. of weeks treated	No. of animals	Total dose of NHM (each animal)
10 days	5	40 mg
5 weeks	5	100 mg
11 weeks	5	220 mg
14 weeks	5	280 mg
19 weeks	5	380 mg
22 weeks	5	440 mg

rat and mouse diet and tap water ad libitum.

NHM (nitrosohexamethylenamine, Eastman Organic Chemicals, Rochester, N.Y.) was given in the drinking water as a 20ml solution containing 4mg NHM for 5 days each week. 5 animals were sacrificed at time intervals of 10 days, 5, 11, 14, 19 and 22 weeks after the start of treatment (Table 1).

Samples of all animals were fixed in neutral buffered formalin, sections were made by standard procedures and stained with Ehlich's Hematoxylin and Eosin for light microscopy.

For electron microscopy, animals were killed by a blow on the head and bled by severing the jugular vein, liver, tissue was taken from the median lobe and fixed for 4 hours at 4°C in phosphate buffer, pH 7.4 in 6% Glutaraldehyde using the method of sabatini (1968), and additional samples were fixed for 2 hours in one percent OsO_4 in phosphate buffer (palade, 1962). All tissues were dehydrated with graded alcohol and embedded in Epon 812 according to standard procedures (Luft, 1961).

Thin sections were cut with an LKB ultratome, then stained with uranyl acetate (Millonig, 1961) and lead citrate (Reynolds, 1963) and observed in an HITACHI 11-E electron microscope.

RESULT

For light microscopic finding:

It was present as periportal hyperplasia, occasional mitosis, and double nucleated cells which were observed on 5 weeks and 11 weeks. In a few nodular lesions occurred on 14 weeks, 19 weeks, and 22 weeks, the cells were so atypical and varied in size and shape.

For Electron microscopic findings:

Since there was no apparent difference between the effective treatment for animals fed NHM for 10 days and 5 weeks these will be described together.

In animals exposed for 10 days and in the group treated for 5 weeks, the hepatic cells showed no nuclear change. There was dilated endoplasmic reticulum, dilated golgi membranous and increased number of lysosomes (Fig. 1). After 11 weeks of feeding, the nucleoli were slight enlarged due to an increase in both granular and fibrillar constituents, but segregation was not apparent (Fig. 2) and pronounced cytoplasmic changes with increased vesiculation in smooth endoplasmic reticulum. Mitochondria appeared relatively normal but some showed a dense matrix and relatively long cristae which often included membranous materials (Fig. 3).

After 14 weeks of feeding, there was marked enlargement of the nucleus and nucleoli. The nucleoli of occasional cells showed nucleolar aggregates fibrillar condensations (Fig. 4).

The mitochondria contained dense droplets often covered with membranous materials. The smooth endoplasmic reticulum was distinct and tubules and vesicles were formed (Fig. 5).

There were some electron dense granules contained within in cisternal space (Fig. 6).

After 19 weeks of feeding. The endoplasmic reticulum cisternae were dilated.

Some mitochondria interlocking protrusions between adjacent profiles (Fig. 7). There were thickening on the endothelial lining cells and spaces of Disse.

After 22 weeks of feeding, the findings previously described persisted, there was swelling of endothelial cells and increased number of kupffer cells. There were prominent hyperplasia of smooth endoplasmic reticulum and increased glycogen particles (Fig. 8).

Bile canaliculi were severely dilated and microvilli often contained vesicles and dens droplets (Fig. 9).

There was swelling of endothelial cells and increased number of Kupffer cells.

DISCUSSION

The morphological effects of nitrosohexamethylenamine(NHM) on liver cells have been evaluated in the present study by electron microscopy. Fine structure alterations occurred in the nucleus, nucleoli, and in cytoplasmic organelles especially in the smooth endoplasmic reticulum and the mitochondria.

Hruban, Swift(1963), Enmelot et al. (1960) and Gonato and Rosenthal(1968) described the alterations of mitochondrial structure may be the result of leakage of reactive metabolites from the detoxification systems of the endoplasmic reticulum.

The appearance of intracisternal granules in groups treated longer than 11 weeks is of considerable interest. The morphologic appearance of these granules is similar to those described in the pancreas of rats induced by puromycin as described by Longnecker, et al. (1968). They have also been described by Ekholm, Edlund, and Zelandar(1967) in rat pancreas after treatment with ethionine. Swift, et al. (1965) after treatment with β -3-Thienylalanine. They suggested that the intracisternal granules may be the result of a defect in the transport of enzymatic proteins from the cisternal space to the golgi vacuoles, based on biochemical analysis of

intracisternal granules in the exocrine cells of the pancreas.

Palade (1956) has also suggested a similar origin.

The disorganization of rough endoplasmic reticulum and the nucleolar and nuclear changes observed in animals fed longer than 19 weeks are also observed in hepatoma.

In 1965 Hruban, et al. described the structural changes in transplantable hepatomas of the rat and pointed out abnormalities in nucleoli, dilated golgi membranes and free ribosomal particles.

In 1968 Svovoda and Higginson (1968) suggested that nuclear and nucleolar abnormalities in carcinogenesis do not fall into any single specific pattern. Each hepatoma was characterized by a particular abnormality of its cytoplasmic organelles, by the quantity of particular organelles, and by the interrelationships between them.

The proximity of mitochondria described in animals fed longer than 14 weeks with protrusion and interdigitation of mitochondria are of considerable interest as was the observation of myeloid figures. Similar bundles of smooth membranes were observed by Greenwalt (1962) and Dalton (1964).

The alterations in endothelial and Kupffer cells observed, which included osmophilic droplets and other dense bodies, are interpreted as phagocytic ingestion of material from the degenerating hepatic cells.

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Legends of Figures

- Fig. 1.** HNM feeding after 10 days' Rat Hepatocyte showing nucleus (Nu) and cytoplasm with dilated endoplasmic reticulum, decreased numbers of ribosomes, dilated golgi complex(Go), increased number of Lysosome (Ly) and microbodies (Mb). $\times 22,000$
- Fig. 2.** HNM feeding after 11 weeks rat hepatocytes showing part of nucleus(Nu), it has increased dense chromatin and increased size of the nucleus. $\times 30,000$
- Fig. 3.** NHM feeding after 11 weeks hepatocytes showing irregular shape of mitochondria(mi) and contained membranes. Tubular and vesicular of smooth endoplasmic reticulum (ser) and increased number of microbodies (mb). $\times 29,000$
- Fig. 4.** NHM feeding after 11 weeks showing part of Sinusoid(Sin) and Parenchymal cells, showing RBC, endothelial cell (E), space of Diss. (D) and microvilli (vi). There is significant proliferation of the smooth endoplasmic reticulum (ser). Some of the mitochondria (mi) contained opaque materials(\uparrow). $\times 8,900$
- Fig. 5.** NHM feeding after 11 weeks showing part of hepatocyte cytoplasm. Mitochondria (mi) contained dense droplets covered by membrane (\uparrow) and contained granules. There are dense granules in the intracisternal space also. $\times 32,000$
- Fig. 6.** NHM feeding after 19 weeks showing part of Nucleus(Nu) and cytoplasm. There is nuclear sergegation of fibrillar and granular components(\uparrow). $\times 9,600$.
- Fig. 7.** NHM feeding after 19 weeks showing part of cytoplasm, mitochondria showed protrusions between adjacent profiles (\uparrow). There was prominent dilated rough endoplasmic reticulum (RER) and hyperplasia of smooth endoplasmic reticulum. $\times 22,500$
- Fig. 8.** NHM feeding after 22 weeks showing part of Nucleus (Nu) and cytoplasm, it shows prominent hyperplasia of smooth endoplasmic reticulum (\uparrow) and glycogen particles (gl). $\times 13,500$
- Fig. 9.** NHM feeding after 22 weeks showing part of bile canaliculi(Bc). It shows marked dilation and contained rounded dense bodies and vesicles (\uparrow). $\times 34,000$









