Demonstration of Glucose 6-Phosphatase Activity in the Oval Cells of Rat Liver and the Significance of the Oval Cells in Azo Dye Carcinogenesis

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SUMMARY

Glucose 6-phosphatase activity was studied histochemically in the oval cells of rat livers during the early stage of hepatocarcinogenesis induced by 3'-methyl-4-dimethylaminoozobenzene. Short-period perfusion fixation of the liver preserved both the ultrastructure of the liver cells and glucose 6-phosphatase activity excellently throughout the whole hepatic tissue. Under light microscopic examination of the liver of rats fed the azo dye diet, glucose 6-phosphatase activity was found in the oval cell proliferating area in varying degrees. The electron microscopic study demonstrated that the oval cells formed ductular structures with elaborated basal laminae, and were occasionally found within the space of Disse between the hepatocytes. Ultrastructurally, some oval cells were identical to bile ductular cells, while others showed various transitional cytostructural characteristics between ductular cells and hepatocytes. An electron microscopic histochemical study revealed various intensities of glucose 6-phosphatase activity in the endoplasmic reticulum and nuclear envelope of the oval cells. The intensity of glucose 6-phosphatase activity in the oval cells was nearly proportional to their grade of morphological resemblance to the hepatocytes. The findings presented in this report indicate that the oval cells transform into hepatocytes through various transitional phases during azo dye carcinogenesis.

INTRODUCTION

Various hepatocarcinogens as well as other noxious stimuli have been known to induce proliferation of the so-called "oval" cells in the liver preceding or accompanying the development of hepatoma (5—7, 11, 12, 21, 24). The oval cells have been described as cells with elongated or vesicular nuclei, small nucleoli, and scant cytoplasm, developing in the portal space (5—9, 11, 12, 16, 19, 21, 22, 24–26, 31) and, electron microscopically, showing characteristics similar to bile ductular cells (8, 9, 11, 12, 18, 25).

The fate of the proliferated oval cells and the significance of their proliferation are matters of dispute. Some investigators considered the possibility of the transformation of the oval cells into hepatocytes (5, 16, 21, 31), but many others were not in agreement as to the existence of such transformation and rather considered the cells to disappear by cell death and removal (9, 22, 23, 25, 26). Inaoka (11), in our laboratory, examining the early stage of azo dye hepatocarcinogenesis in rats, concluded that the oval cells were able to transform into hepatocytes through various transitional stages. This is one of the series of studies (2, 12) to reevaluate this conclusion, investigating the oval cells during azo dye carcinogenesis with application of the electron microscopic histochemical method for examining glucose 6-phosphatase activity which was known to be demonstrable in hepatocytes in normal livers (1).

MATERIALS AND METHODS

Male Wistar rats with an average initial weight of 200 g were fed a synthetic diet containing 0.06% 3'-Me-DAB2 for 1 to 6 weeks. The control rats were fed the same diet, but without carcinogen, throughout the experiment. After cessation of the azo dye feeding, the rats were fasted for 12 hr overnight and the liver was fixed by the perfusion-fixation method for light and electron microscopic examinations, as follows. Every rat was laparotomized under light ether anesthesia, and the liver was perfused through the portal vein with 1.25% glutaraldehyde buffered by 0.1 M cacodylate (pH 7.4) at a pressure of 90 cm H2O. The perfusate was drained through the hepatic vein. Perfusion with the fixative was always suspended within 1 min after the start of perfusion, and immediately thereafter ice-cold 0.1 M cacodylate buffer was used to wash out the fixative remaining within the liver.

For light microscopy, paraffin sections and frozed sections were made for hematoxylin and eosin staining and glucose 6-phosphatase histochemistry (28), respectively. Electron microscopic examination was performed chiefly on the livers of rats fed the azo dye diet for 4 weeks, because they contained every stage of oval cell proliferation. For electron microscopic histochemistry of glucose 6-phosphatase (4, 17), the liver specimen was cut by an Oxford Vibratome into 40-μm slices, which were rinsed in 0.1 M cacodylate buffer containing 7% sucrose for several hr. After examination by light microscopy of a 20 μm-thick section next to 40-μm

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2 The abbreviations used are: 3'-Me-DAB, 3' methyl-4-dimethylaminoazobenzene; ER, endoplasmic reticulum; AFP, α-fetoprotein.
sections, the portions of oval cell proliferation in the 40-μm liver slices were cut out and were incubated in Wachstein and Meisel’s medium (28). Following the incubation, they were washed with distilled water and fixed in 2% osmium tetroxide, dehydrated through the graded ethanol series, and embedded in Epon 812. Ultrathin sections were made by an LKB ultratome and examined by a JEM 100C or a Hitachi HS7D electron microscope.

RESULTS

Light Microscopic Findings. In the normal liver fixed by the perfusion method described above, hepatocytes showed glucose 6-phosphatase activity almost equally throughout the hepatic tissue, but within the liver lobule it was slightly weaker in the centrolobular area (Fig. 1). Light microscopy showed that glucose 6-phosphatase-positive cells were exclusively hepatocytic in character (Fig. 2).

In the liver of rats fed the azo dye diet, proliferation of the oval cells began to occur 1 week after initiation of the azo dye feeding, and became more pronounced with time. In the early stages, the oval cells were seen in the perportal spaces and then gradually appeared in rows or clusters between parenchymal cells in the midzonal and perportal areas of the liver lobule. In a more advanced stage, most of the preexisting hepatocytes in the periportal and midzonal areas disappeared, being replaced by the oval cells (Fig. 3). Some of the oval cells in this stage showed round nuclei and relatively ample cytoplasm, appearing to be small hepatocytes. After glucose 6-phosphatase incubation, some of the cells in the oval cell proliferating area showed various intensities of glucose 6-phosphatase activity, but they could not be identified as oval cells light microscopically (Fig. 4).

Electron Microscopic Findings. After glucose 6-phosphatase incubation, the hepatocytes in the normal livers showed electron-opaque fine granular reaction products in the rough and smooth ER and in the nuclear envelope (Fig. 5), while other hepatic cells including bile ductular cells (Fig. 6), littoral cells of the sinusoid, fat storage cells, and capillary cells showed little or no reaction product.

In the area of oval cell proliferation of the livers of rats fed the azo dye diet for 4 weeks, a few oval cells usually formed a small ductular structure which was surrounded by the basal lamina (Figs. 7 to 9). The ductules occasionally appeared in the space of Disse adjacent to the hepatocytes. On the luminal surface of the ductule, microvilli developed (Figs. 7 to 10), and between the ductules, sinusoids were occasionally seen (Figs. 7 and 10).

The oval cells were varied in both intensity of glucose 6-phosphatase activity and cytounstructural characteristics. Some of them, with negative or a very small degree of glucose 6-phosphatase activity, showed definite characteristics of ductular cells, with irregularly shaped nuclei and scant cytoplasm containing a few small mitochondria and poorly developed ER (Fig. 8), whereas some others appeared to be of hepatocytic nature, showing intense glucose 6-phosphatase activity and containing round nuclei, numerous large mitochondria, well-developed ER, lysosomes, microbodies, lipid droplets, and no basal lamina (Fig. 10). Between these 2 types of cells, various transitional types could be observed. Fig. 9 illustrates the cell that shows evident glucose 6-phosphatase activity, in spite of its resemblance to the ductular cell, and those that are more hepatocytic in their ultrastructure. Intensity of the glucose 6-phosphatase reaction of the oval cells was nearly proportional to the grade of resemblance to hepatocytes.

DISCUSSION

The short-period perfusion fixation of the liver with a low concentration of glutaraldehyde proved to preserve excellently the ultrastructure of the liver cells and their glucose 6-phosphatase activity. In normal liver, hepatocytes showed an intense glucose 6-phosphatase reaction, while ductular cells showed a very weak or no reaction. With this histochemical method, the oval cells proliferating during the early stage of 3'-Me-DAB carcinogenesis clearly showed various intensities of glucose 6-phosphatase activity as well as transitional morphological features between ductular cells and hepatocytes. This finding seemed to demonstrate the intermediate character of the oval cell between hepatocytes and ductular cells, and most of them should be considered the “transitional cells” of Inaoka (11) and Iwasaki et al. (12).

With regard to the role of oval cell proliferation, Inaoka (11) stated that they might serve for restoration of severe and prolonged liver damage. That is, regeneration through the division of preexisting hepatocytes, damaged by the azo dye, may be insufficient for restoration and, consequently, it may be necessary to promote new hepatocyte production from the oval cells, which might be resistant enough to the toxic action of 3'-Me-DAB to proliferate under azo dye feeding. On the other hand, oval cell proliferation does not necessarily precede development of hepatoma by every hepatocarcinogen. For example, 3'-Me-DAB or ethionine induces prominent oval cell proliferation, whereas proliferation does not occur in diethyl-nitrosamine carcinogenesis in rats (10). This difference is considered to be due mainly to different degrees of cytotoxicity of these agents on the liver cells. On the other hand, Stein et al. (24) reported that cortisone almost completely prevented oval cell proliferation caused by feeding with ethionine, but did not influence parenchymal cell damage by ethionine. This indicated that some other factors might concern induction of oval cell proliferation.

Recently, some interesting findings concerning the function of oval cells were reported. Watabe (30) described the transient appearance of serum AFP in the early stage of azo dye carcinogenesis in rats, which nearly coincided with the time of rapid oval cell proliferation (18, 30). Dempo et al. (2) and Uriel et al. (27) demonstrated localization of AFP in the oval cells by immunofluorescence techniques or radioautography utilizing the affinity of estrogen to AFP. These findings strongly suggest that oval cells might have some functional similarity to fetal liver cells. Furthermore, the isozyme patterns of some liver enzymes demonstratedly deviate, becoming similar to the patterns of fetal liver in the early stage of 3'-Me-DAB carcinogenesis (3, 13, 14, 15, 20, 29). These changes of the liver during the preneoplastic stage of azo dye
carcinogenesis might also be caused in part by the biological properties of the proliferated oval cells.

In the present study, the following questions, among others, have not been answered, i.e., how do oval cells make up the normal liver architecture and are those induced by agents other than 3'-Me-DAB also able to transform into hepatocytes? Studies on these problems are now in progress.

REFERENCES

Fig. 1. Glucose 6-phosphatase reaction in normal rat liver fixed by perfusion fixation. X 190.

Fig. 2. High-power view of liver cells incubated for glucose 6-phosphatase activity. All of the cells positive in glucose 6-phosphatase reaction show features of hepatocytes. X 790.

Figs. 3 and 4. Rat liver at the 4th week after initiation of feeding with 0.06% 3'-Me-DAB.

Fig. 3. Oval cell proliferating area occupies the large part of the liver lobule, replacing original hepatocytes. H & E. X 260.

Fig. 4. Glucose 6-phosphatase reaction of the liver of rat fed the azo dye diet. Dark area (left) is composed mainly of original hepatocytes, and light area (right) is composed mainly of oval cells. Cells with various intensities of glucose 6-phosphatase activity are present in oval cell-proliferating area. X 190.

Figs. 5 to 10. Electron micrographs of glucose 6-phosphatase histochemistry. Figs. 5 and 6 show normal rat livers and Figs. 7 to 10 show the livers of rats fed with 0.06% 3'-Me-DAB for 4 weeks.

Fig. 5. Glucose 6-phosphatase reaction in a normal rat hepatocyte. An electron-opaque reaction product is distributed in the rough and smooth ER, and the nuclear envelope. X 12,900.

Fig. 6. Bile ductular cells (Bd) in normal rat liver, which show scant cytoplasm containing small number of mitochondria, poorly developed ER, and microvilli on the luminal surfaces. These cells show no or only a very small amount of reaction product in the nuclear envelope. X 12,900.

Fig. 7. A low-power survey view of the oval cell-proliferating area. Oval cells (Oc) form ductular structures, surrounded by basal laminae (arrows). On the luminal surfaces, microvilli (Mv) are developed. Sinusoids (S) are seen between the ductules. Some oval cells are negative in glucose 6-phosphatase activity, while others show electron-opaque reaction products in the ER and perinuclear space. X 5,200.

Fig. 8. Oval cells (Oc) with features of ductular cells showing a slight degree of glucose 6-phosphatase activity in the ER and perinuclear space (arrows). A basal lamina (Bl) can be seen. X 17,300.

Fig. 9. Oval cells with glucose 6-phosphatase activity. An oval cell (Oc) on the right shows scant cytoplasm containing a few small mitochondria and poorly developed ER, resembling ductular cells. The oval cells (Oc*) on the left show greater resemblance to the hepatocytes, which have many large mitochondria and relatively developed ER. X 12,200.

Fig. 10. These cells appear as small hepatocytes (Hp), but they form a ductular structure in association with an oval cell resembling ductular cells (Dc). A basal lamina (arrow) can be seen around the Dc, but obscure around the Hp; S, the sinusoid. X 5,200.
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