International Journal of Research in Engineering, Science and Management
Volume-3, Issue-1, January-2020
www.ijresm.com | ISSN (Online): 2581-5792

A Rare Occurrence of Hepatoblastoma in a Study on Experimental Induction of Hepatocarcinogenesis

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***Abstract*: In the present study, experimental induction of hepatocarcinogenesis was done using Diethylnitrosamine in male Wistar albino rats. In most of the rats, development of various common hepatic tumors like hepatic adenoma, hepatic carcinoma, cholangioma and cholangiocarcinoma was observed. However, in one rat, there was induction of a very rare tumor of liver called hepatoblastoma. This was diagnosed based on the gross appearance of the tumor mass, histopathology and confirmed through negative staining of glutamine synthetase in contrast to the positive staining in other hepatic tumors.**

***Keywords*: Hepatoblastoma, Hepatocarcinogenesis, Glutamine Synthetase, Rat.**

1. Introduction

Hepatoblastoma is a poorly differentiated malignant embryonal liver tumor that occurs almost exclusively in infants and very young children. In rodents, hepatoblastoma develops spontaneously or can be induced by a number of chemicals. Various chemicals like oxazepam, nitrosamine-phenobarbital combination, primidone, pyridine, anthraquinone etc are known to induce hepatoblastoma occasionally after a treatment period of about one to two years (Turusov et al, 2002 [1]).

There was confusion in the literature between hepatoblastomas and poorly differentiated hepatocellular carcinoma, cholangiocarcinomas or cholangiomas as many scientists considered the hepatoblastomas as more embryonal and more malignant variants of these tumors (Diwan et al, 1989 [2]). However, Ishak and Glunz has delineated the morphologic and prognostic differences between hepatoblastoma and hepatocellular carcinoma (Abenoza 1987 [3]). In the present study, DEN alone has induced such a rare tumor and hence, it is being reported.

2. Materials and method

A. Source of chemicals

DEN - Merck KGaA, Darmstadt, Germany, Anti-Glutamine synthetase primary (Ab73593) and anti-rabbit secondary antibodies- Abcam, United Kingdom and all other chemicals were procured from local vendors

B. Experimental induction of hepatocarcinogenesis

Male Wistar albino rats of 8 weeks age weighing around 200 g were treated with Diethylnitrosamine at 100 mg/kg body weight intraperitoneally on first day followed by promotion using 0.01 % DEN in drinking water ad libitum for 90 days. The experiment was conducted after obtaining prior permission from IAEC and according to rules of CPCSEA, India.

C. Histopathology

The tissues were preserved in 10 % NBF, routinely processed by paraffin embedding technique and sectioned at 4-5 micron thickness using rotary type microtome and stained with haematoxylin and eosin (H&E) for microscopic examination (Luna, 1968 [4]).

D. Immunohistochemistry for glutamine synthetase

Routine immunohistochemistry protocol of Abcam was followed. Citric acid buffer for antigen retrieval, anti-glutamine synthetase antibody as primary antibody and anti-rabbit goat polyclonal antibody as secondary antibody were used for immune histochemical staining.

3. Result and discussion

Hepatoblastoma is a primary malignant hepatic tumor resembling the developing liver in the embryo and fetus which is also referred to as embryonal hepatoma. Hepatoblastoma being a rare tumor, it was neither induced after treatment with DEN alone nor occurred spontaneously. However, it was induced after sequential exposure to DEN followed by enhancement with phenobarbital for a very long period.

In our study, treatment of rats with DEN alone for just 90 days induced hepatoblastoma in one animal by the end of treatment period. Grossly, the tumor mass was observed in left lateral lobe of liver and it was solitary, brownish gray in color and firm in consistency. On incising the mass, central area of necrosis and many small vascular lakes within the tumor tissue were observed (Fig. 1). These findings were correlating the

findings of Turusov 1997 [5]. There was also hemorrhagic ascites in the rat having this tumor (Fig. 1).

The microscopic examination of the mass revealed high and dense cellularity, intense basophilia of cells (Fig. 2, a) as compared with the other hepatic tumors (Fig. 2, b) and there was no encapsulation of the tumor tissue. Invasion of the tumor cells into the surrounding was evident at certain areas indicating its malignant nature. Mitotic activity was high in the cancerous tissue. Small, round to elongated cells, with scant basophilic cytoplasm forming rosette like structures were seen. Occasionally, the cells were also arranged in palisade form surrounding the central vascular pockets, which were lined by the endothelium and contained either blood or eosinophilic material (Fig. 2, a). Similar findings were also observed by Turusov et al, 2002 [1]. These histological findings are characteristic of the embryonal cells capable of metaplasia. Similar to Wilms' tumor (nephroblastoma), hepatoblastoma manifests different maturational stages in its epithelial component and also has an ability to express divergent or heterologous differentiation into bone, cartilage, squamous epithelium, and neuroepithelium (Abenoza et. al, 1987 [3]).

On immunohistochemical staining of the tumor tissue for glutamine synthetase which is liver specific marker stained cytoplasm positive for the hepatoma and hepatocellular carcinoma (Fig 3, b). However, because of its embryonal nature, hepatoblastoma has lost the features of hepatic tissue and thus stained negative for glutamine synthetase (Fig. 3, a).

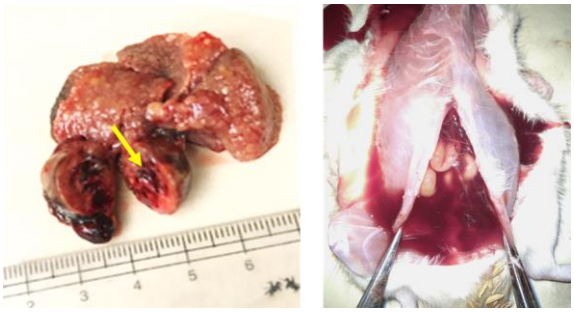

Fig. 1. Gross appearance of hepatoblastoma in liver. Solitary brownish gray mass in left lateral lobe with vascular pockets (arrow) and hemorrhagic ascites

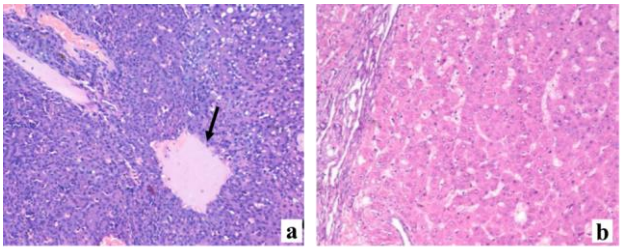

Fig. 2. Microscopic findings of liver tumors – comparison. a) Hepatoblastoma – highly basophilic dense cellularity, palisade arrangement of cells around vascular pocket (arrow) H&E 50x, b) hepatocellular carcinoma – eosinophilic and less dense cellularity H&E 100x

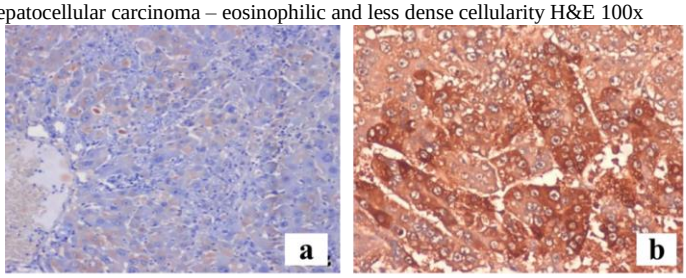

Fig. 3. Immunohistochemistry of liver tumors – Glutamine synthetase 200x. a) Hepatoblastoma – negative staining. b) Hepatocellular carcinoma – highly positive cytoplasmic staining

4. Conclusion

Diethylnitrosamine has also the capability to induced hepatoblastoma in addition to hepatic adenoma and hepatocellular carcinoma in rats within a short span of 90 days treatment without the requirement of any promoters. However, the percentage of incidence is very low as compared with the other hepatic tumors.

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