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Thymidine Kinase and Mitotic Index: Methods of Determination of Liver Cell Regeneration after Two Third Partially Hepatectomised Albino Rats

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Abstract: Liver regeneration after two third partial hepatectomised of albino rat was studied by ^{3}H Thymidine kinase assay (TK: EC 2.7. 1.21), mitosis count/1000 hepatocytes and DNA content. TK, rate determining enzyme of DNA biosynthesis, was observed maximum after at 24 h where as mitotic index and DNA content was observed maximum after 48 h after partial hepatectomised albino rats. These results suggest that thymidine kinase and mitotic cell division are an essential event in liver regeneration and can be used as a marker of cell regeneration.

Key words: Thymidine kinase, cell proliferation, salvage pathway, mitosis, partial hepatectomy, DNA synthesis

INTRODUCTION

The mechanism regulating liver regeneration following two third partial hepatectomy has received much attention as an ideal model to investigate the network of biological information. Liver regeneration can be considered as a particular example of controlled tissue increase. Liver regeneration after partial hepatectomy (PH) has been employed as an ideal model to investigate the regulatory mechanism of cellular proliferation *in vivo* (Tsukamoto and Kojo, 1989). Thymidine kinase (TK) catalyses the transformation of thymidine to thymidine monophosphate (TMP) in the presence of adenosine triphosphate (ATP). TMP is transformed to triphosphate in several steps and then is incorporated into DNA, since thymidine is incorporated in the phosphorylated form only.

Thymidine kinase uses as a substrate either exogenous thymidine from food or the endogenous thymidine released by degradation. It has thus designated as a *Salvage enzyme*. TK activity is very low in quiescent cells and increases dramatically during rapidly proliferative phases of eukaryotic cell (Margeli *et al*., 1996). In the rat liver regeneration following partial hepatectomy, the transition of the hepatic cell form Go to G_1 phase may take place within 4 h and the second signaling from G_1 to S phase is assumed to occur at 10-12 h (Tsukamoto and Kojo, 1989). TK is a key enzyme in the salvage pathway of nucleotide metabolism and catalyzes the first rate-limiting step in the synthesis of dTTP, transfer of a gamma-phosphate group from a nucleoside triphosphate to the 5'-hydroxyl group of thymidine, thus forming dTMP. TK is cytosolic and its activity fluctuates during cell cycle coinciding with the DNA synthesis rate and disappears during mitosis. This fluctuation is important for providing a balanced supply of dTTP for DNA replication (Zhu *et al*., 2006). We conducted experiments designed to provide an understanding of the regulatory role of TK in the activity of mitosis cell division and DNA synthesis in regenerating liver.

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MATERIALS AND METHODS

The [Methyl-3H] Thymidine (2 mci) was purchased from Board of Radioisotope Technology, Mumbai, India and Durapore membrane filter, from Milipore, USA. Diphenyl amine and Tris purchased from SRL, Mumbai, India. ATP, Marcaptoethanol and Phenyl Ethyl Sulphonyl Fluoride were purchased from Sigma (St. Louis, MO, USA). Optiphase Hi safe scintillation cocktail LKB scintillation was purchased from FSA Laboratory (Lough Boorough, England LE 110 RG). Radio isotope study was carried out at Nuclear Research Laboratory, Indian Veterinary Research Institute, Izzatnagar, India. Male Wister rats weighing 200 to 250 g obtained from Laboratory Animal Resources, Indian Veterinary Research Institute, Izzatnagar, UP, India and were maintained under temperature-controlled rooms with 12 h alternating light and dark cycles were given adequate nutrition and water *ad libitum* at Division of Physiology, IVRI, Izzatnagar. All experimental protocols using animals were performed according to the Principles of Laboratory Animal care (NIH, 1985) adopted with institutional animal ethical committee permission. Two-third partial hepatectomy (PH) was performed under diethyl ether anesthesia following the procedure of Higgins and Anderson, 1931.The rats were divided into three groups with 6 animals each and studies were made at 24 h, 48 h and on 7th day post operation intervals. Group I served non operated group, Group II served as only partial hepatectomy (PH) At 24 h, 48 h and on 7th day liver samples were collected from each group and used for the determination of enzyme activity of TK.

Analytic Procedure

The study carried out at Nuclear Research Laboratory, Indian veterinary Research Institute, Izatnagar, India. Excised liver was homogenized with 5 volumes of 50 mM Tris-HCl buffer (pH 7.3) containing 0.25 mM sucrose, 10 mM β-mercaptoethanol, 1 mM phenylmethylsufonyl fluoride and 1 mM EDTA. The supernatant fractions of liver homogenate centrifuged at 36000 g for 30 min at 4°C, was used for determining the enzymatic activity. Activity of TK was measured (Nakata *et al*., 1985) and expressed as pmol of product formed/min/mg of protein at 37°C. DNA content was measured by Diphenylamine reaction (Burton, 1956).The mitosis count were assessed histologically after hematoxylin and eosin staining (H and E).The number of cells in metaphase was determined 1000 hepatocytes.

Statistics

The experimental data were expressed as mean±SD. The significance of difference among the normal group and PH group were analyzed by means of one way ANOVA followed by Tukey's post-hoc test. $p<0.05$ was considered statistically significant.

RESULTS

Effect of PH on TK and DNA Level

The results of TK and DNA are shown in Table 1. The TK actions increased seven folds when compared with normal activity (resting in Go state) at 24 h. At 24 h, the TK activity was found to be maximum level when compared with 48 h and 7th day. DNA activity was found maximum at 48 h in PH group when compare with other PH group at 24 h and 7th day.

Effect of PH on the Histopathology

After 24 h PH (Fig. 1) observed kuffer cell hyperplasia, karyomegali and anosocytois. Also some mitosis was observed at periphery region.

Table 1: TK, DNA and Mitotic index level after Partial hepatectomisd albino rat liver after 24 h, 48 h and 7th day

Parameters	TK level (pmol/min/mg of protein)	DNA (mg/g of liver)	Mitotic index (Mitosis division/1000 hepatocytes)
Normal rats			
0	30.21±2.06[a]	3.12±0.96[a]	0
24 h	31.23±7.06[a]	3.65±1.02[b]	0
48 h	40.07±6.24[b]	3.21±0.72[b]	0
7th day	37.40±3.69[a]	3.50±0.93[b]	0
PH rats			
0	42.10±6.20[b]	3.43±1.05[a]	0
24 h	283.77±6.96[b]	4.64±0.49[b]	1 to 4
48 h	160.28±3.97[a]	5.83±0.84[a]	2 to 9
7th day	91.34±4.54[a]	3.17±0.96[b]	0 to 1

*Mean±SD, n = 6. [a]Significantly different from normal rat ($p<0.01$). [b]Significant different from PH rat ($p<0.01$)

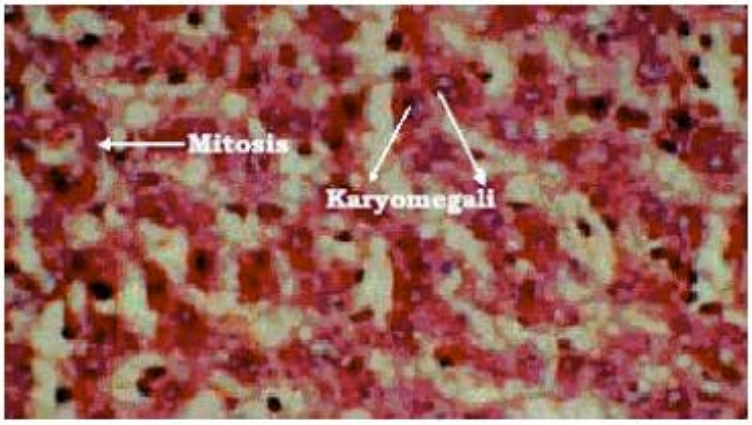

Fig. 1: Karyomegali, anosocytois and mitosis after 24 h of PH. (H/E x 320)

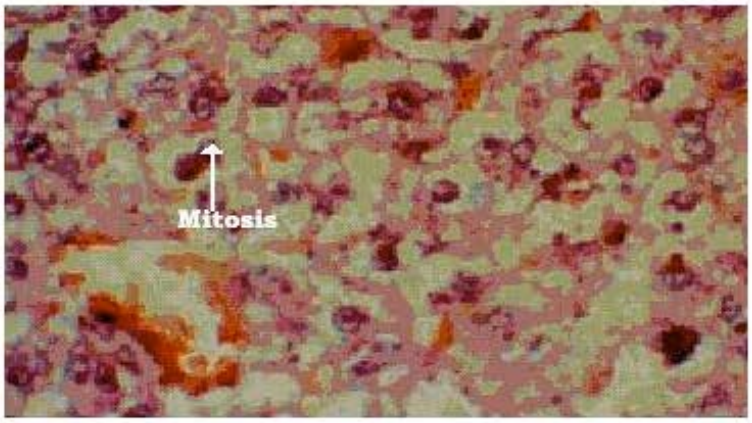

Fig. 2: Anosocytois, binuclear regenerative, swollen nucleus and mitosis observed at central region after 48 h of PH. (H/E x 320)

After 48 h hepatocytes show some regenerative appearance. It was observed that hepatocytes were higher karyomegali and anisocytosis when compared to PH group at 24 h. Mitosis also observed higher number in periphery and central region (Fig. 2).

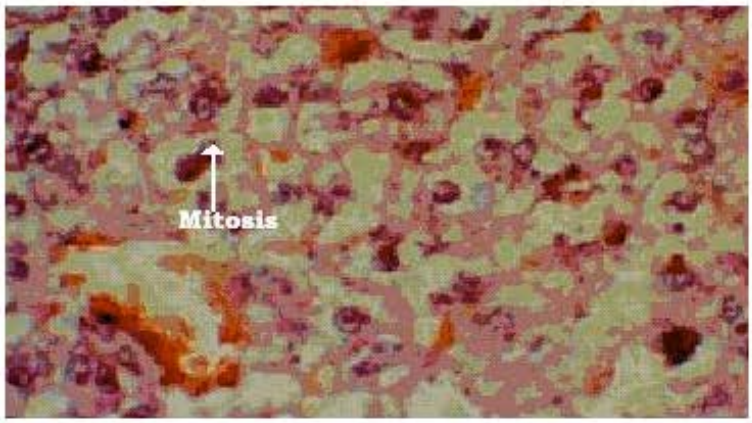

Fig. 3: Necrosis, occasionally showing fatty vacuoles, accumulated of erythocytes and very small mitosis in the dilated sinusoids on 7th day of PH. (H/E x 320)

On 7 th day after PH hepatocytes were bi-nuclear, swollen and some fatty vacuoles observed. Hepatocytes observed pykonitic condensed nuclei, there was accumulation of erythrocytes in the dilated sinusoids. Some of the hepatocytes showed cytoplasmic vacuolation. No mitosis was observed in this stage (Fig. 3).

DISCUSSION

TK has been shown to exhibit enhanced activity at 24 h in 24 h PH rats. Similar observation was also found in our previous study (Chattopadhyay *et al*., 2006a). TK is almost undetectable in non proliferating tissues (Beltz, 1967). TK enzyme is present in the cytosol of dividing cells during the G1 to S phases and is not present in resting cells (Borovansky and Netikova, 1994). In present observation it is found that the mitosis cell divisions were increased along with increased TK content. Interestingly on 7th day, TK levels were minimum and no mitosis was observed. Our previous our studies suggested that tri-iodo thyroxine (T_3) (Chattopadhyay *et al*., 2006b) controlled proliferation of hepatic cell. The kinetics of the regenerative response in liver cell after partial hepatectomy has been well established. It is apparent that in first 12 h after PH, hepatocytes more from a state of quiescence enter the cell cycle and progress toward DNA synthesis. DNA synthesis starts 12-16 h after PH and reaches a peak within 22-24 h, followed by a mitosis wave 6-8 h later. Second peak of DNA synthesis emerges at 48 h. (Bonkovski *et al*., 1985). The proliferative activation process quite synchronized. The regenerative liver is a system in which the cell cycle-dependent gene expression can be examined during a physiologic growth *in vitro*. Using this system, the regulatory mechanisms for the periodic changes in TK activity during cell cycle were investigated *in vivo*. The present results are consistent with the view that TK and Mitotic cell division are marker during liver cell regeneration. Thus, measurement of TK and mitosis count may be of use as an accurate and inexpensive marker of cell proliferation. Further studies are needed to evaluate the relative contribution and relationship of gene which are thought to involve in TK activity in liver regeneration.

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