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CONFERENCE REPORT

Liver Regeneration after Partial Hepatectomy in Rats with Defective Bilirubin Conjugation or Biliary Excretion

R.A.F.M. CHAMULEAU, D.C. ARONSON, W.M. FREDERIKS, D.K. BOSMAN, J.J.H. SMIT, M.A.W. MAAS, and P.L.M. JANSEN

The role of conjugated bilirubin in liver regeneration after partial hepatectomy (PH) was studied in Gunn rats, in transport-mutant (TR^-) rats, and in rats with extrahepatic biliary obstruction. Ornithine decarboxylase (ODC) and thymidine kinase (TK) activities in liver homogenates and immunohistochemistry of in vivo *bromodeoxyuridine (BrdU) incorporation in hepatic DNA were followed as regeneration parameters at 24 and 48 hr after PH. The results relative to TK activity and BrdU incorporation were consistent with significantly delayed hepatic DNA synthesis in Gunn rats in comparison to control Wistar and TR^- rats. This delay in DNA synthesis was not reflected in the hepatic ODC activity. After one week of complete common bile duct obstruction (CBO), an increased TK activity and BrdU incorporation was seen. PH following CBO resulted in a further increase in ODC activity and BrdU incorporation. TK activity did not change, however. These data relative to the regulation of hepatic DNA synthesis after PH in Gunn rats and in rats with extrahepatic biliary obstruction suggest a possible stimulatory role for conjugated bilirubin in hepatic regeneration; however, the normal hepatic DNA synthesis in TR^- rats studied before PH and the subnormal DNA synthesis seen 24 hr after PH in TR^- rats and in rats with CBO indicate that conjugated bilirubin does not stimulate hepatic DNA synthesis.*

KEY WORDS: liver regeneration; Gunn rat; transport-mutant rat; conjugated bilirubin; biliprotein; cholestasis.

Among the many postulated hepatotrophic factors capable of stimulating DNA synthesis during liver regeneration is the albumin–bilirubin complex, also called "biliprotein" (1). Because conjugated bilirubin is a prerequisite for the formation of the covalent bilirubin–albumin complex (2), one would expect a diminished regenerative capacity in the Gunn rat following a partial hepatectomy (PH) as Gunn rats have a defect in hepatic bilirubin glucuronyltransferase activity that results in a hyperbilirubinemia of the unconjugated type. In contrast, one might expect an increased regeneration response in the presence of increased plasma levels of conjugated bilirubin. Transport-mutant (TR^-) rats with hereditary conjugated hyperbilirubinemia are defective in the excretion of conjugated bilirubin and other anions into bile (3). As a consequence, TR^- rats have increased levels of biliprotein, and therefore one might expect at least a normal or possibly a supranormal regeneration response following PH in these animals.

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From the Laboratory of Experimental Medicine, Academic Medical Centre, University of Amsterdam, Amsterdam; Department of Surgery, Academic Hospital University of Leiden, Leiden; Department of Cellbiology and Histology, and Department of Gastroenterology and Hepatology, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands.

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Address for reprint requests: Dr. R.A.F.M. Chamuleau, Laboratory of Experimental Medicine, G-2, room 131 Academic Medical Centre, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands.

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A second experimental model of increased levels of conjugated bilirubin is complete extrahepatic obstruction of the common bile duct in the otherwise normal rat. In order to evaluate the effect of different concentrations of conjugated bilirubin on regenerative capacity, we studied several parameters of hepatic DNA synthesis after partial hepatectomy in three different models: the Gunn rat, TR^- rats, and Wistar rats with complete extrahepatic biliary obstruction.

MATERIALS AND METHODS

Male Wistar rats and male Gunn rats, body weight 300 ± 40 g, were obtained from TNO, Zeist, The Netherlands. TR^- rats (300 ± 40 g) were obtained from our own inbred colony (3).

Surgical Procedure. A partial hepatectomy (PH) of about 70% was performed in Wistar, Gunn, and TR^- rats according to the procedures of Higgins and Anderson (4) between 9 and 10 AM. Common bile duct obstruction (CBO) was performed in Wistar rats by double ligation of the common bile duct with duct transsection between the two ligatures (5). All surgical procedures were performed under ether anesthesia.

Regeneration Parameters. Ornithine decarboxylase activity (ODC) was measured in liver homogenate by the production of $^{14}CO_2$ from ^{14}C-labeled ornithine (6). Thymidine kinase (TK) activity was assessed by measuring the amount of [^{3}H]thymidine phosphate formed in liver homogenate after *in vitro* incubation with [^{3}H]thymidine under standard conditions (7). For the immunocytochemical detection of incorporated BrdU, an indirect two-step labeling technique was used with peroxidase-conjugated IgG using the method described by Schutte et al (9). The labeling index was determined as the number of BrdU-positive nuclei related to the total amount of nuclei. Corrections were made for BrdU incorporation at prior sampling times. These parameters were determined in liver tissue obtained at 0, 24, and 48 hr after PH.

Experimental Protocol. The following four groups of rats were studied: group 1, Gunn rats (N = 5); group 2, TR^- rats (N = 5); group 3, normal Wistar rats (N = 5); and group 4, normal Wistar rats with one week CBO (N = 11).

On the day of PH, BrdU (50 mg BrdU/kg body wt, dissolved in 0.9% NaCl) was injected intraperitoneally between 8 and 9 AM; T = −1 hr. (This procedure was repeated at T = 23 and T = 47 hr). One hour later, the PH was performed. Zero time (T = 0) values for TK, ODC, and BrdU were obtained. Twenty-four hours after PH, a small biopsy (about 1.0 g) was taken under ether anesthesia. At T = 48 hr the rats were sacrificed by an overdose of ether. Animal welfare was in accordance with the institutional guidelines of the University of Amsterdam.

Statistical Analysis. Statistical analysis was performed using Student's t test.

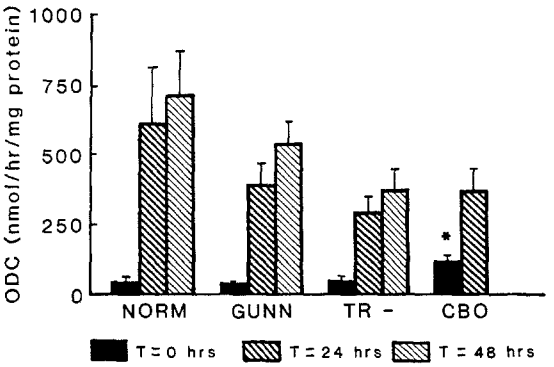

Fig 1. Ornithinedecarboxylase activity in liver tissue after partial hepatectomy. Measurements have been done before (T = 0) and 24 and 48 hr after partial hepatectomy. Values are means ± SEM. *P < 0.05 compared to normal rats at the same time.

RESULTS

Changes in the enzyme activities of ODC and TK as well as the BrdU incorporation of the three groups of rats and their controls are shown in the Figures 1–3. A significant stimulation of ODC activity was found at 24 and 48 hr after PH in all four groups. No significant differences between the groups were evident. After one week of CBO, hepatic ODC activity was increased significantly prior to PH.

In normal rats a significant increase in hepatic TK activity and BrdU incorporation at 24 and 48 hr after PH was found (Figures 2 and 3). In Gunn rats TK activity and BrdU incorporation 24 hr after PH was reduced significantly in comparison to both normal and TR^- rats (P < 0.05). Both parameters increased at 48 hr after PH. The pattern for the TR^- rats was similar to that seen in normal Wistar rats.

Figures 2 and 3 show that CBO for one week induced a significant increase in DNA synthesis as

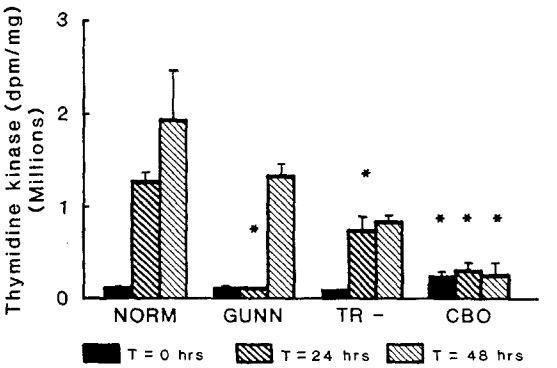

Fig 2. Thymidine kinase activity in liver tissue after partial hepatectomy. Measurements have been done before (T = 0) and 24 and 48 hr after partial hepatectomy. Values are means ± SEM. *P < 0.05 compared to normal rats at the same time.

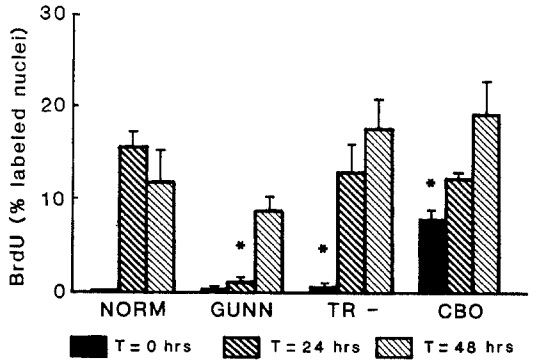

Fig 3. *In vivo* bromodeoxyuridine incorporation in DNA after partial hepatectomy. Measurements have been done before ($T = 0$) and 24 and 48 hr after partial hepatectomy. Values are means ± SEM. $^*P < 0.05$ compared to normal rats at the same time.

both the TK activity and BrdU incorporation parameters were increased prior to the PH. In this group, a significant further increase in BrdU incorporation occurred 24 hr ($P < 0.01$) and 48 hr ($P < 0.05$) after PH.

DISCUSSION

The present data shed interesting light on the role of conjugated bilirubin in the regenerative capacity of the liver following a partial hepatectomy (PH). In addition to such putative hepatotrophic factors as epidermal growth factor (EGF), transforming growth factor alpha, and hepatic stimulator substance [for a recent review see Chamuleau and Bosman (10)], a hepatotrophic role has been attributed also to biliprotein (1). Because biliprotein is a complex between albumin and conjugated bilirubin (2), one would expect a diminished regeneration response in the Gunn rat, and a failure of TK activity and BrdU incorporation to increase 24 hr after PH in Gunn rats. The observed increase in ODC activity is not considered to be an entirely specific parameter for DNA synthesis. The data of Diaz Gil et al (11), in which a noncovalent complex of unconjugated bilirubin and albumin was thought to be a liver growth factor was not confirmed by the present experimental data obtained in Gunn rats. The delayed DNA synthesis response seen in Gunn rats suggests that unconjugated bilirubin may actually inhibit DNA synthesis. Thus, the molecular nature of the hepatotrophic factor that Diaz Gil et al have isolated from serum remains unclear. Increased DNA synthesis in the presence of high levels of conjugated bilirubin as observed in liver one week after CBO and before PH is in agreement with a putative hepatotrophic activity for conjugated bilirubin. However, this observation could be explained also by an accumulation of any of many other cholephilic factors that could serve as a hepatotrophic agent, such as EGF. The latter possibility is likely as hepatic DNA synthesis was not increased in TR^- rats at $T = 0$. Furthermore both intrahepatic cholestasis (TR^- rats) and extrahepatic cholestasis (CBO rats) are associated with subnormal DNA synthesis following PH. Thus the current data suggest that some factor other than conjugated bilirubin stimulates DNA synthesis during experimental cholestasis.

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