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ARTICLE

Elevated Hepatic Glucocorticoid Receptor Expression During Liver Regeneration in Rats

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In rats within the first week of partial hepatectomy reconstruction of the normal histological structure of the liver already starts. To approach the possible role of endogenous glucocorticoids in the process of regeneration we measured the changes in the expression of steroid glucocorticoid receptor gene after various regeneration intervals. After partial hepatectomy, between 0.5–168 hours from the surgery, the gene expression (mRNA) of glucocorticoid receptor was determined by reverse transcription followed by PCR and normalized to that of glycerolphoshate dehydrogenase. Two peaks of glucocorticoid receptor mRNA were detected first, between 3 and 6 hours (first peak) and a second between 24 and 36 hours. Immunoreactive glucocorticoid receptor was detected by immunohistochemistry using monoclonal anti-glucocorticoid receptor. Three days after the surgery immunohistochemical studies showed substantially more immunoreactive GcR protein in the regenerated liver than in the controls. These semiquantitative data provide evidence suggesting elevation of glucocorticoid receptor expression during regeneration of liver at mRNA and protein levels. (Pathology Oncology Research Vol 5, No 2, 107–109, 1999)

Keywords: glucocorticoid receptor, liver, regeneration, monoclonal antibody, mRNA

Introduction

Within the first week of partial surgical[4,5,7] or chemically induced[6] hepatectomy the rat liver starts to reorganize its normal histological structure; after the third week the regenerated and the original livers are practically indistinguishable. Different growth factors and cytokines are involved in the regulation of this regeneratory process. The role of interleukin-6 (IL-6) in hepatic regeneration is also obvious, since in IL-6 gene-targeted mice the rate of regeneration is markedly slowed down and can be re-accelerated again by exogenously added IL-6.[4] Moreover, NFκB inducing cytokines (such as IL-1 and tumor necrosis factor, TNF) by preventing apoptosis seem to enhance and maintain regeneration in liver.[8]

We the have previously shown that some of the inflammatory cytokines (IL-6, IL-1, TNF) increase the number of glucocorticoid binding sites in hepatic cells *in vitro*.[13] The regeneration process may change receptor expression as demonstrated earlier by increased availability of hepatic insulin receptor during liver-regeneration in rats.[5]

In our present experiment we monitored the rate of glucocorticoid receptor expression in regenerating rat liver by molecular biological and immunohistochemical assays.

Materials and Methods

Animals and hepatectomy

Home-bred 3 month old Wistar male and female rats were used according to criteria outlined in "Guide for the Care and Use of Laboratory Animals". A partial (two thirds) surgical hepatectomy[7] was performed between of 9 and 12 a.m. A small subxyphoid incision was made under ether anesthesia, the left and median (the two big) lobes of the liver were

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removed, then the incision was sutured. After various intervals (0, 0.5, 1, 3, 6, 12, 24, 36, 72, 120 hours after surgery) the animals were sacrifized and the liver remnant was perfused with sterile cold 0.9 % NaCl solution through the portal vein and placed in ice-cooled plates. For control purposes, sham operated rats were used.

RNA isolation and reverse transcriptase polymerase chain reaction (RT-PCR)

After homogenization total cellular RNA was isolated following the guanidin-isotiocyanate/phenol method.[3] From the isolated RNAs, RT-PCR was performed (the incubation mixture for RT was: $MgCl_2$ 2,5 mM, dNTP 10 mM, RNase inhibitor 20 U/µl, MULV reverse transcriptase 25 U (Promega), oligo dT 50 uM (Promega). For PCR the incubation mixture was the same as that for RT, except that Taq polymerase (Promega) was included, and specific GcR primers[9] (sense: 5' GGG TAA TTA AGC AAG AGA AAC TGG G 3', antisense: 5' TGG AGG AGA GCT TAC ATC TGG TCT C 3') were used instead of oligodT. The cycle conditions for RT were 42ºC 20 min, 100ºC 10 min for PCR 94ºC 4 min and 30 cycles; 95ºC 0.5 min, 65ºC 1 min, 72ºC 1 min, then 72ºC 5 min).

Using the same mRNA and cDNA samples RT-PCR mRNA of an internal control, glycerolphosphate dehydrogenase (GAPDH) (sense: 5' GGT ATC GTG GAA GGA CTC AT 3', antisense 5' ACC ACC TGG TGC TCA GTG TA 3') has been determined,[1] as well. After electrophoresis the RT-PCR bands were scanned and densitometric analysis was performed by Image Quant program. The densitometric values of GcR cDNA were normalized to those of GAPDH. This value provides a semiquantitative estimation of relative changes in GcR gene expression.

Immunohistochemical detection of glucocorticoid receptors

Immunohistochemical tests were carried out using intact remnants: perfusion made use of sucrose as the cryoprotective material, the liver-tissue was fresh-frozen, and cut in the cryostat. We used directly FITC labeled mouse monoclonal GcR antibody. The antibody was raised[2] against a peptide (27 amino acid motive) of human GcR; however, due to its phylogenic conservative feature it cross reacts with its rat counterpart.

Results

Macromorphological changes in liver regeneration are already visible within 24 hours of surgery: seven days after partial hepatectomy the liver remnants have almost regained the original tissue-properties (not shown)**.** Our semiquantitative RT-PCR studies *(Table 1)* demonstrated, that the expression of the GcR-gene (normalized to glycerolphoshate dehydrogenase mRNA, GAPDH) is time dependent and indicates two peaks. The first peak of gene expression of the GcR-gene appears between 3 and 6 hours, the second one is between 24–36 hours, then the relative value rapidly declines at 72 hours following surgery. Comparison to the house-keeping glycerolphosphate dehydrogenase gene indicates that these changes of GcR gene expression are relevant in regenerating hepatic tissue.

Table 1. **Relative amount of glucocorticoid receptor (GcR) and glycerolphosphate dehydrogenase (GAPDH) mRNA during liver regeneration of rats**

Time after partial hepatectomy (hr)	*Relative density of GcR cDNA**	*Relative density of GAPDH cDNA*	*GcR/GAPDH quotient*
0	1343±134	1914±132	0.6076
1	2874±199	1891±145	1.5198
3	4788±221	1821±149	2.6293**
6	3356±359	1885±201	1.7803
12	4434±339	2512±198	1.7651
24	4897±501	1679±156	2.916**
36	3456±401	1771±187	1.9514
72	2567±299	2976±231	0.8625
120	2287±198	1944±241	1.1764
168	2004±227	2431±178	0.8243

PCR bands corresponding to reverse transcribed GcR and GAPDH mRNA were scanned and analysed by densitometry (Image Quant) (results in *Table 1* correspond to mean ± sem of four experiments). ** $p < 0.05$ (Student t probe).
For details see Materials and Methods.

Immunohistochemical analysis *(Figure 1)* demonstrates the major quantity of the GcRs at 72 hours found in the cytoplasm of hepatocytes. Before three days (at 24 and 48 hours) we failed to detect similar strong reactions in regenerating liver tissues (not shown).

Discussion

Clarification of the molecular mechanism and hepatic regeneration may contribute towards the development of a therapy for liver lesions, carcinoma, viral- and of autoimmune liver-diseases as well as alcohol-related damage. One can speculate, that endogenous glucocorticoid might be one of the multiple factors regulating the complex procedure of liver regeneration. Therefore, molecular regulation and changes in the expression of hepatic GcR, an important element of the glucocorticoid sensitivity might influence the actual effectiveness of endogenously available glucocorticoids during regeneration. This effect could be both direct, through the regulation of hepatocyte proliferation and also indirect to modulate the local production of many cytokines

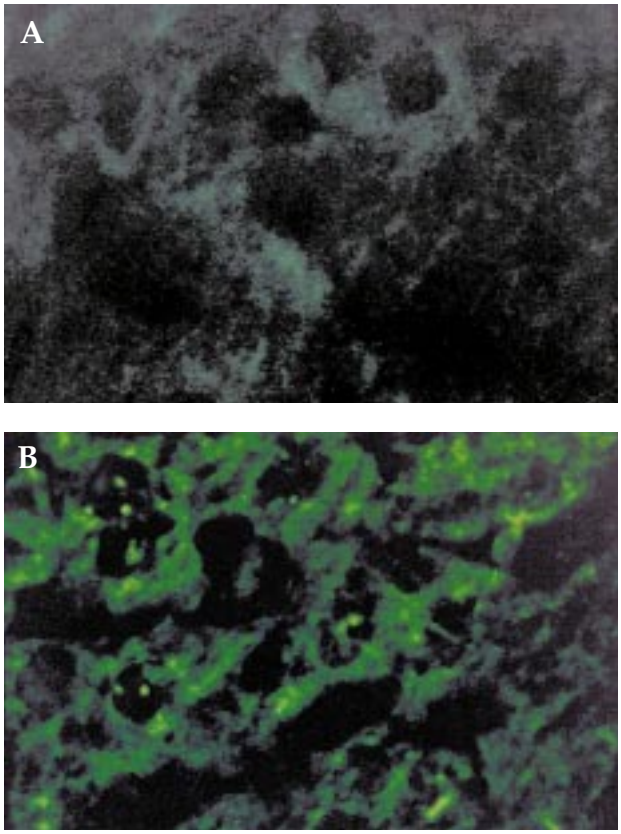

Figure 1. *Immunohistochemical localization of glucocorticoid receptors in the sham operated control (A) and in 72-hours regenerating (B) rat liver using FITC labeled monoclonal mouse antibodies raised against human glucocorticoid receptor (for details see Materials and Methods). X750.*

and expression of cytokine receptors. Since IL-6 elevates glucocorticoid binding in hepatocytes,[13] and *vice versa* glucocorticoids elevate the expression of IL-6 receptors on hepatocytes[11] a further link between cytokines and glucocorticoid metabolism in liver can be suggested. The recently recognized molecular association and "asymmetric" functional interaction between the Stat5 transcriptional factor and glucocorticoid receptor[12] also provides new hints for the role of various cytokines in down- and up-regulation of glucocorticoid receptor expression and function.

Our data showing upregulation of GcR mRNA and immunoreactive GcR in regenerating liver was confirmed by earlier experiments[10] demonstrating increase in the inducibility by dexamethasone of the activity of hepatic enzymes tyrosine aminotransferase and tryptophan oxygenase in regenerating rat liver after partial hepatectomy. Other receptor binding results using labelled dexamethasone in our laboratory (not shown here) provide preliminary evidences that during regeneration not only GcR mRNA and protein, but the number of functionally active (dexamethasone binding) GcR also increases.

The second peak of GcR mRNA (around 24 h) largely precedes the visible elevation (at 72 h) of immunoreactive GcR, this time we have no explanation why there is no clear concordance in these results, more data on the regulation of translation and catabolic processes of GcR in rat hepatocytes required. Our monoclonal antibody[2] however stains only cytoplasmic GcR. Confirmation of the functional relevance of endogenous glucocorticoids in hepatic regeneration process need further direct experimental evidence.

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