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Messenger RNA Profiles in Liver Injury and Stress: A Comparison of Lethal and Nonlethal Rat Models

Niels Tygstrup,*[,1] Kristian Bangert,† Peter Ott,* and Hanne Cathrine Bisgaard‡
**Liver Unit and †Department of Clinical Biochemistry, Rigshospitalet, Copenhagen, Denmark; and ‡Department of Life Sciences and Chemistry, Roskilde University, Denmark*

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Liver damage activates processes aimed at repairing damage; simultaneously, liver functions required for survival must be maintained. The expression of genes responsible for both in rat models of lethal (lipopolysaccharide, 90% hepatectomy, and D-galactosamine) and nonlethal (turpentine, 70% hepatectomy, and acetaminophen) liver damage and stress was measured at 3, 6, 12, and 24 h after the intervention and quantitated as the area between the control curves and the test curves (AUC). The expression of genes for cell division and remodeling was upregulated most in the lethal models. The expression of most liver-specific function genes was reduced. Positive AUC was found for ARG, ASL, CPT1, Mdr1b, Mdr2, and PEPCK. It is concluded that a high expression of genes for repair of liver damage is associated with reduced expression of genes for several liver-specific functions, possibly reflecting a limited capacity for transcriptional activity. Maintained or increased expression of selected function genes indicates that the corresponding functions have high priority. The liver sustains metabolic homeostasis ensuring that other organs in the body function normally. Simultaneously, the processes required for the integrity of its own structure and function are maintained as a result of regulated expression of the genes that produce the proteins needed to perform both set of functions.

Key Words: **mRNA profiles; lipopolysaccharide; turpentine; D-galactosamine; acetaminophen; partial hepatectomy; toxic liver damage; liver function; liver regeneration.**

Abbreviations used: ARG, arginase; ASL, argininosuccinate lyase; ASS, argininosuccinate synthetase; AUC, area under curve; Bsep, bile salt exporting pump; C/EBPα, CAAT binding protein alpha; C/EBPβ, CAAT binding protein beta; CPS, carbamoylphosphate synthase-1; CPT1, carnitinepalmiotyl transferase-1; GCS h.c., glutamylcysteine synthetase heavy chain; GCS l.c., glutamylcysteine synthetase light chain; GSTπ, glutathione *S*-transferase Pi; IGFBP1, insulin growth factor binding protein 1; IGF-I, insulin growth factor-I; IL-6, interleukin-6; IL-6R, interleukin-6 receptor; Mdr1b, multidrug resistance protein 1b; Mdr2, multidrug resistance protein 2; mRNA, messenger ribonucleic acid; Mrp2, multidrug-resembling protein 2; Ntcp, Na-dependent cholate transporting protein; Oatp1, organic ion transport protein 1; Oatp2, organic ion transport protein 2; ODC, ornithine decarboxylase; OTC, ornithine transcarbamylase; PAI-1, plasminogen activator inhibitor 1; PEPCK, phospho*enol*pyruvate carbokinase; TNFα, tumor necrosis factor alpha; uPAR, urokinase plasminogen activator receptor; α-AGP, alpha1-acid glycoprotein; α2-M, alpha2-macroglobulin.

[1] To whom correspondence and reprint requests should be addressed at Laboratory of Hepatology, Rigshospitalet 2151, 9 Blegdamsvej, DK 2100 Copenhagen, Denmark. Fax: +45 3545 2354. E-mail: tygstrup@rh.dk.

Liver damage reduces the homeostatic metabolic functions more or less, if severe enough preventing survival. Severe clinical liver damage is characterized by multiorgan failure (1), demonstrating that the function of the affected organs depends on substrates produced by the liver. However, liver damage also triggers the activation of genes serving to repair the damage, e.g., by regeneration of liver cells. Survival is only possible if indispensable homeostatic metabolic functions and functions for repair of the liver are maintained before irreparable damage has occurred.

From a molecular point of view liver failure is insufficient capacity for transcription and translation of genes encoding vitally important proteins and peptides. It has been debated for about 70 years, ever since Higgins and Anderson (2) introduced partial hepatectomy in rats as an experimental model, whether regenerating hepatocytes have the same functional competence as normal, quiescent cells, or they "dedifferentiate." It is now generally agreed that hepatocytes retain differentiated functions during regeneration. However, while the overall pattern of RNA populations and their translation products during liver regeneration have been found to differ little from that of the normal adult rat liver (3), quantitative changes do exist (4).

The quantitative changes in the expression of genes coding for liver specific function proteins in liver failure are of interest. Reduced expression of function genes and concomitant reduction of the associated functions

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may contribute to the clinical features of fulminant hepatic failure (5). Therefore, changes in mRNA profiles may contribute to understanding of the molecular adaptation of the liver to damage. How are the resources shared between the requirements for function and repair?

Numerous studies have shown changes in the expression of liver specific function genes after liver damage from partial hepatectomy (5, 7, 8), in endotoxemia (6, 10), and following administration of hepatotoxins (9, 11). The purpose of the present study was to quantitate changes in the mRNA profiles of a set of liver function genes, using the "area under the curve" method as used in pharmacodynamic studies, and to relate changes to the profile of hepatic genes activated by injury or stress. Because the relation between function and repair could be more critical during potentially lethal liver damage, both lethal and nonlethal models were studied.

MATERIALS AND METHODS

Male Wistar 200 g rats were obtained from Møllegaard, Denmark and kept under a controlled 12-h light/dark cycle. Except for a fast over night before the interventions (performed between 7 and 10 AM) the animals had free access to Altromin tablets and water. Partial hepatectomy was performed in full anaesthesia (midazolam, 0.25 mg; and immobilonvet, 0.2 ml; followed by Revivon.vet, 0.2 ml). Rats were sacrificed after 3, 6, 12, and 24 h by cervical dislocation, blood was drawn from the aorta, and liver tissue was snap frozen and stored at −80°C for RNA extraction. The Danish Council for Supervision with Experimental Animals had approved the study.

Procedures. (i) Thirty-six rats were injected with lipopolysaccharide (L-2630 from Sigma, 15 mg/kg) intraperitoneally. Groups of 6 rats were killed at 3, 6, and 12 h after the injection. Four rats died between 12 and 24 h and the remaining 14 rats were killed at 24 h.

(ii) Twenty-four rats were injected with turpentine oil (BP93-255059, Unikem A/S, Denmark), 1 ml in both thighs. Groups of 6 rats were killed at each of the stated intervals. None died spontaneously.

(iii) Twenty-four rats were given an intraperitoneal injection of D-galactosmine (G 0264, Sigma, 375 mg/kg dissolved in 0.9% NaCl). Groups of 6 rats were sacrificed at the stated intervals. None died spontaneously.

(iv) Twenty-four rats were given an intraperitoneal injection of acetaminophen (1000 mg dissolved in 6 ml of 0.9% NaCl at 52°C). Groups of 6 rats were killed at the stated intervals. None died spontaneously.

(v) Twenty-four rats were subjected to a 90% surgical hepatectomy as described in Ref. (12), except that no glucose supplement was given. Groups of 5 rats were sacrificed at the stated intervals. Two rats died before 12 h.

(vi) Twenty-four rats were subjected to a 70% surgical hepatectomy as described in Ref. (2). Groups of 6 were sacrificed at the stated intervals. None died spontaneously.

(vii) Controls consisted of 3–6 rats given the corresponding vehicle or, for partial hepatectomy, sham operated rats. RNA was extracted from approximately 200 mg snap frozen liver tissue and steady-state mRNA levels determined by slot blot analysis according to the previously described protocol (9).

cDNA probes for genes encoding (i) proteins associated with repair represented by IGFBP1, c-fos, c-myc, histone 3, ODC, PAI-1, uPAR, α2-M, α1-AGP, β-fibrinogen, .. C/EBPβ TNFα. IL-6, IL-6R, β-actin, α-tubulin, C/EBPα, and (ii) proteins associated with function represented by albumin, IGF-I, plasminogen, Bsep, Ntcp, Oatp1, Oatp2, Mdr2, Mrp2, Mdr1b, GCS l.c., GCS h.c., GSTPi, Cyp1A2, Cyp2B1/2, Cyp2E1, CPS-1, OTC, ASS, ASL, ARG, glutamine synthetase, glutaminase, PEPCK, CPT-1 were donated from other workers, bought from ATCC, or cloned by RT-PCR. Where appropriate the identity of the cDNAs was confirmed by DNA sequencing. Specificity of all cDNA probes was assured by Northern blot analysis.

Specific hybridizations were visualized by autoradiography on an imaging plate BASIII and the hybridization signal analyzed in a FUJIX Bioimaging analyzer system BAS 2000 (Fuji Photo Film Co.). Afterward, filters were rehybridized with cDNA for 18S rRNA, to compensate for variations in RNA loading, the median variation being 9%.

Calculations. Levels of mRNA are given as mean ± SEM, normalized as ratio of the corresponding control (see above). As a measure of the change in expression during the experimental period the area of the mRNA/time curve (AUC; dimension: level × time) was calculated as the sum of the area of the intervals between the samplings, expressed as the mean of the mRNA level at the start and the end of the period times the duration of that period. No change (i.e., mRNA level equals to the control) was used as zero. Thus positive values indicate an increased expression and negative values a decreased expression during the period of observation relative to controls (see Fig. 1). Cluster analysis was performed using the Statistica v.6 software.

Statistics. After logarithmic transformation of AUC (from the baseline), analysis of variance or unpaired *t* test was used. *P* values <0.05 were taken as evidence of significant difference.

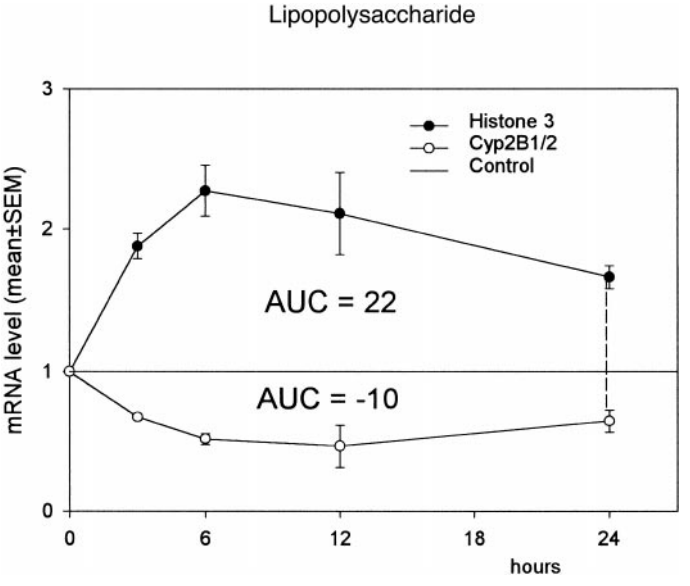

FIG. 1. Estimation of the area under the curve (AUC) for mRNA levels of histone 3 and Cyp2B1/2 in rats treated with LPS 15 mg intraperitoneally at zero time. mRNA levels are shown as ratio of control (means ± SEM).

RESULTS

Data were separated into mRNA encoding proteins involved in repair (replication, remodeling, cytokine response, acute phase proteins, and cell structure proteins) (Table 1), and in liver specific functions (Table 2).

TABLE 1

AUC of mRNA for Repair Proteins

	Lethal			Nonlethal		
	LPS	PHx90%	GalN	Turp	PHx70%	APAP
Cell division						
IGFBP1	20	1216	72	3	18	6
c-fos	9	398	2	−2	−3	2
c-myc	8	118	4	−5	1	4
Histone 3	22	31	44	1	25	−5
ODC	23	56	−10	13	−1	−12
Remodeling and structure						
PAI-1	530	491	72	9	14	12
uPA R	52	112	14	5	0	0
α-Tubulin	5	34	0	0	25	1
β-Actin	28	40	15	−2	29	2
Acute phase						
α2M	58	9	−7	30	49	7
α1AGP	49	10	−9	39	56	16
β-Fibrinogen	12	20	−3	16	12	8
C/EBPβ	20	36	−10	3	9	−2
Cytokines						
TNFα	66	0	24	4	−2	1
IL-6	20	−2	1	21	−1	2
IL-6 R	20	−1	−14	22	8	−2

Figure 2 shows the changes in mRNA levels over time in four examples of repair and function genes, respectively, with the area under the curve (AUC) of each set of observations. Both repair and function genes were significantly different between the models ($P < 0.001$, one-way analysis of variance), and within each model repair was significantly higher than function (unpaired t test) except for the APAP model ($P = 0.056$). The mRNA levels over time curves were significantly different between the models for repair as well as function, and between the lethal and the nonlethal models ($P < 0.001$; interaction by two-way analysis of variance).

The largest increase in repair gene expression was observed in the lethal models LPS, PHx90%, and GalN, those for cell replication most after PHx90%, remodeling most after LPS and PHx90%, and those for acute phase proteins most after LPS. TNFα expression was increased after LPS and GalN, and IL-6 and IL-6R after LPS. Among the nonlethal models the acute phase gene expression was increased after Turp and PHx70%. IL-6 and IL-6R expression after Turp was similar to that after LPS.

Three-fourths (109 of 150) measured function mRNA profiles were negative (mean −7). Nine function genes showed increased expression (>4) in one or more models. Seven were highest in the PHx90% model (CPT1, PEPCK, CPS, ASL, ARG, Mdr2, and Mdr1b), three in the APAP model (ASS, GCShc, and GSTπ), and one in the turpentine model (Cyp2B1/2). Positive values for ASL were also found in the LPS, the PHx70%, and the APAP models, for MDR1b in the LPS, the GalN, and the PHx70% model, and for CPT1 in the LPS, the PHx70%, and the APAP models. The relative mRNA level over time and the AUC of four function genes with substantial increases in one or more models is shown in Fig. 3.

The lethal and the nonlethal models were compared by cluster analysis. The lethal models showed the AUC of genes for cell division and for remodeling far separated from the rest; in the nonlethal models this was the case for the acute phase protein genes. Tree diagrams for the function genes are shown in Fig. 4. Variables may be regarded as members of the same cluster when the distance between them is below a given level. In this study the 6-percentile was chosen as point of discrimination, corresponding to 7 and 6.4 distance units in the lethal and the nonlethal models, respectively. This included the majority of function variables in one cluster, having from 1 to 10 links to other variables (means of 5 and 4, lethal and nonlethal, respectively). Excluded from this major cluster were Mdr1b, CPT1 and ASL in both models, additionally Mdr2, glutamine synthetase, PEPCK and ARG in the lethal models, and Cyp2B1 and GSTπ in the nonlethal

TABLE 2

AUC of mRNA for Function Proteins

	Lethal			Nonlethal		
	LPS	PHx90%	GalN	Turp	PHx70%	APAP
Exported proteins						
Albumin	−6	−6	1	−6	−2	0
IGF-I	−10	−1	−1	−7	0	−7
Plasminogen	−9	−3	−3	−3	−5	−4
Metabolism						
CPT	15	20	−15	1	9	9
PEPCK	−2	9	−10	−10	−4	6
C/EBPα	−1	−3	−8	−1	−2	−2
Urea metabolism						
CPS	−6	4	−6	−7	0	1
OTC	−7	−10	−3	−6	−6	−6
ASS	−4	2	−7	−9	0	8
ASL	39	99	−10	1	14	11
ARG	1	25	−6	−6	0	1
Glutamine synt	4	3	−7	1	3	−6
Detoxification						
Cyp1A2	−11	−10	−8	−3	−2	−10
Cyp2B1/2	−10	−14	−5	21	−3	−7
Cyp2E1	−12	−12	−4	−12	−5	−1
GCShc	−8	−6	−3	−11	0	7
GCSlc	−11	−7	−8	−14	−10	−8
GST Pi	−1	−4	1	0	−5	5
Bile transport						
Bsep	−9	−5	−7	−2	3	−8
Ntcp	−10	−10	−6	−8	−6	−7
Oatp1	−15	−12	−6	−6	−6	−7
Oatp2	−17	−15	−7	−16	−8	−11
Mrp2	−13	−6	−8	−5	−4	−11
Mdr2	−7	17	−6	−8	3	−5
Mdr1b	65	66	11	0	30	−1

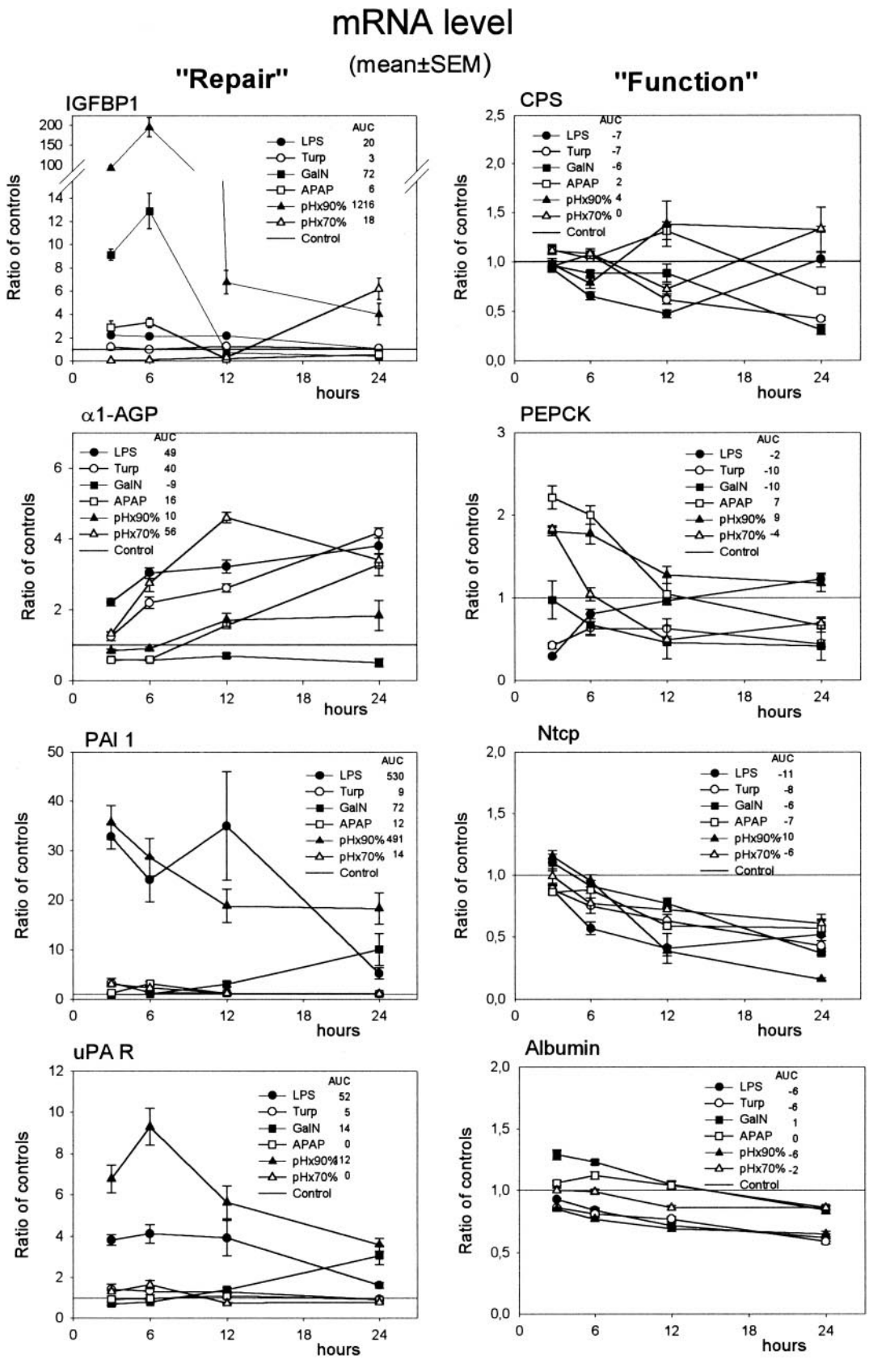

FIG. 2. mRNA levels over time during the 24-h experimental period in the six experimental models (see Materials and Methods) is presented for four genes related to repair and four genes related to liver specific function. The estimated area under the curve for each transcript is shown after the signature.

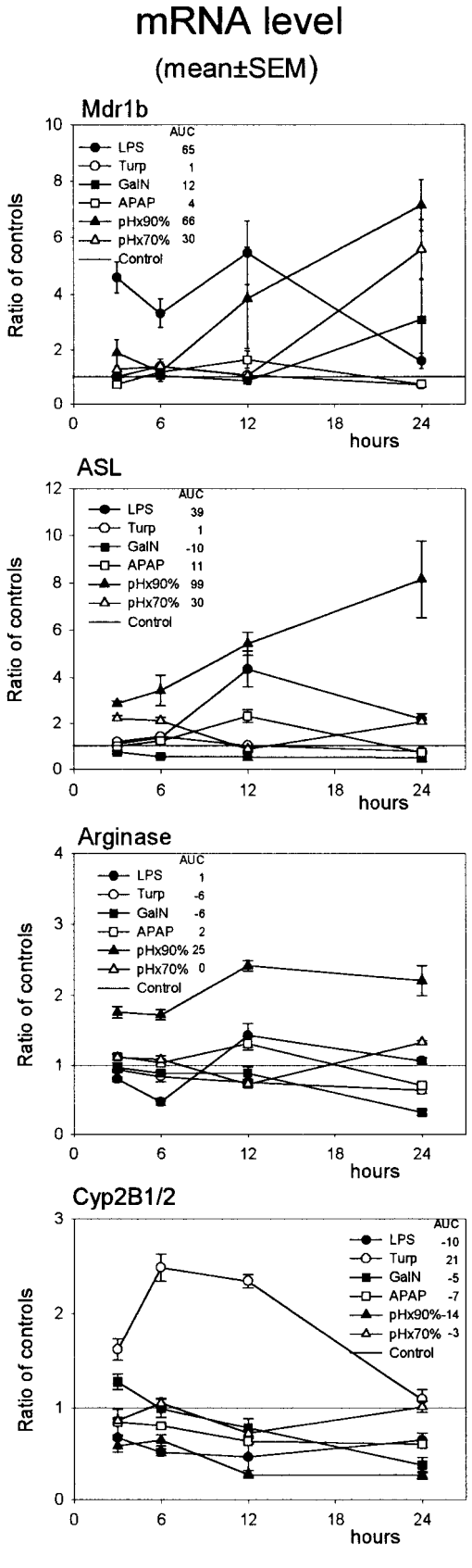

FIG. 3. mRNA levels over time during the 24-h experimental period in the six experimental models (see Materials and Methods) is presented for four genes of liver specific functions that were upregulated in one or more of the models. The estimated area under the curve for each transcript is shown after the signature.

models. In the nonlethal models CPT1 and ASL represented a cluster, in the lethal model they were further separated. Links between repair and function genes at the 6-percentile level were found In the nonlethal group, i.e., one cluster comprising α-tubulin, β-actin, histone 3 and Mdr1b, and one with c-fos, c-myc, albumin, ARG, CPS, and GSTPi. In the lethal group similar links were only found at the 25-percentile distance level, one including α1AGP and α2M, and one consisting of α-tubulin, β-fibrinogen, C/EBPβ, IL-6, IL-6R, ARG, PEPCK, Mdr2, CPT1, and glutamine synthetase.

DISCUSSION

The expression of several genes of liver specific functions were downregulated when genes required to protect and repair the liver were activated by the interventions applied. The six models differ as to severity, three being life threatening (lipopolysaccharide and D-galactosamine in the doses given, and 90% partial hepatectomy), and three have no immediate mortality (turpentine, acetaminophen in the relatively low dose, and 70% partial hepatectomy). The models also differ as to mechanism for injury and stress. LPS involves hepatotoxicity (13) as well as induction of an acute phase response, and turpentine induces an acute phase response (14) without apparent hepatotoxicity. GalN and the APAP are chemical hepatotoxins that damage hepatocytes, GalN mainly through blockage of RNA production by entrapment of uridine (15), and APAP by adduct formation and inactivation of proteins with the reactive metabolite NAPQI (16). The consequences of liver damage from hepatotoxins are twofold, loss of functional liver mass and release of cellular debris, like fragments of actin (17) through necrotic destruction of liver cells. In contrast, partial hepatectomy is a practically selective reduction of functional liver cell mass. The distinction between repair and function genes may be arbitrary, since the knowledge about the precise role of each of the corresponding proteins is limited. Genes related to cell replication, acute phase response, remodeling, cytokines, and transcription factors were regarded as repair genes and the others as function genes.

Gene expression was estimated at the level of their corresponding mRNA during the first 24 h after the intervention, i.e., the critical period after the lethal procedures. As a measure of gene expression during that period the area under the curve (AUC), i.e., the area between the lines connecting mRNA levels and zero (the control values) was calculated. Positive AUC

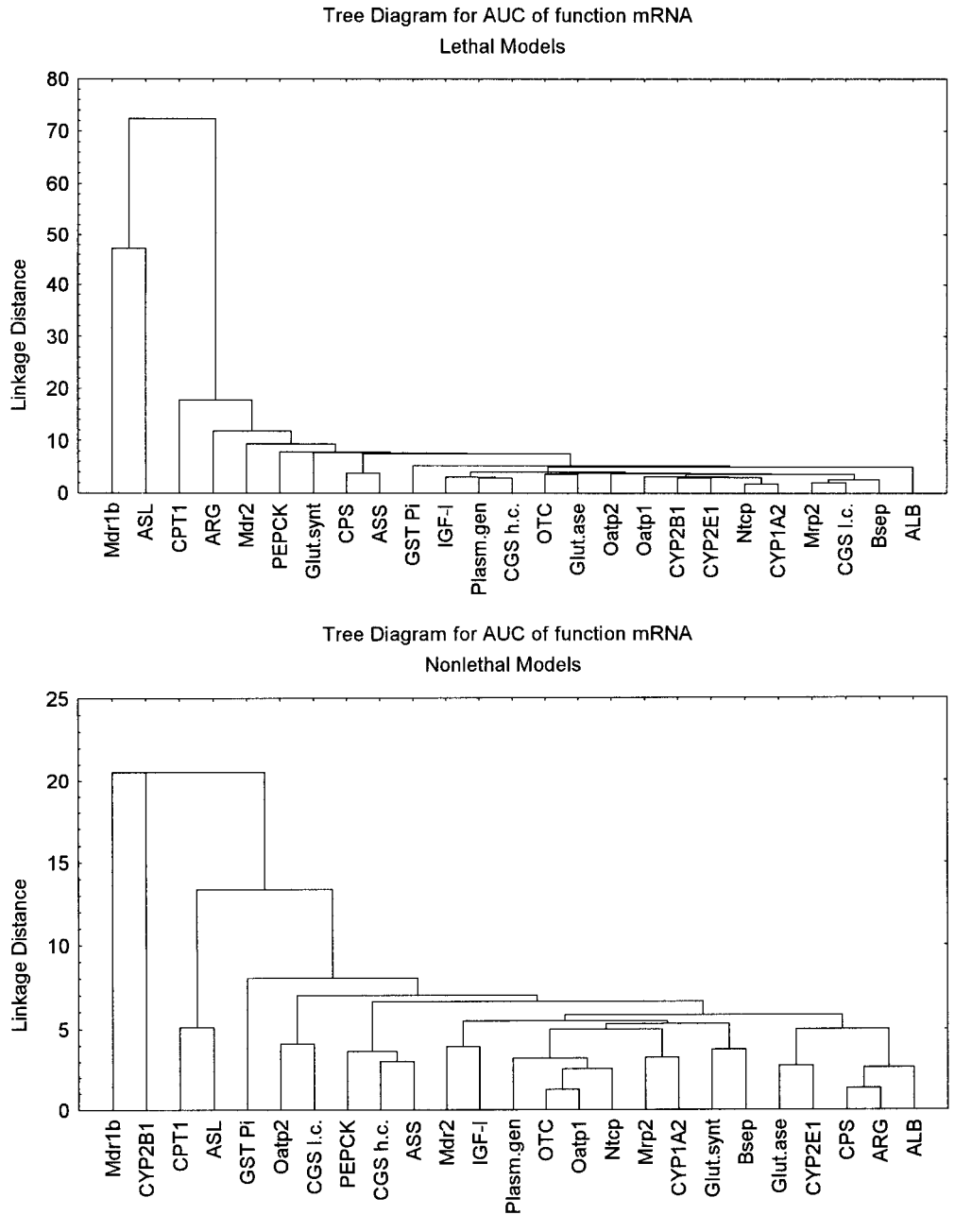

FIG. 4. Tree diagrams for AUC of function gene expression in the lethal (lipopolysaccharide, D-galactosamine, and 90% hepatectomy) and in the nonlethal (turpentine, acetaminophen, and 70% hepatectomy) models. The Euclidean distance (single-linkage method) is shown on the ordinate.

indicates increased mRNA level and negative AUC reduced level in relation to the controls. It should be noted that positive and negative AUC values couldn't be compared quantitatively. AUC was measured in relation to values in control animals, where the expression rate of repair genes is quite low, so that, e.g., a 10-fold increase, corresponding to an AUC of +200, may be a modest increase in number of transcripts produced in relation to the total number produced by the cell. In contrast, an AUC of −10 for the mRNA of a gene supporting essential liver specific functions means a reduction to about 60% of that of controls, which in terms of reduced number of transcripts produced may exceed the transcription rate of the repair gene. Furthermore, a negative AUC underestimates the actual reduction of transcription rate of the corresponding genes, in particular for those that have a relatively long half-life of the transcript. For some of them, e.g., albumin, it is possible that transcription was practically discontinued in several of the models.

The absolute value of the AUC of a transcript will depend on the period of observation and the frequency of sampling, and comparisons between profiles with different period and frequency may be misleading. This may also be the case if comparisons are based on observations made only at a single point in time due to a large variation in mRNA level over time for many transcripts (cf. Figs. 2 and 3). The AUC depends on the rate of transcription as well as degradation of mRNA which both may be modulated by the interventions (18). Nevertheless, the AUC is a measure of the mRNA available for translation to the corresponding proteins during the period of observation.

The mRNA profiles for repair genes differed markedly among the models. The AUC of mRNAs related to cell division was most increased in the PHx90% model, very high for IGFBP1 (19, 20) which together with histone 3 also was high after GalN, indicating a strong stimulus for cell division. Lower values were seen after LPS and PHx70%. The AUC of TNFα was high only in the LPS and the GalN models [in which very high ALT values found (not shown)], and close to zero in the PHx90% model. Our data suggest that in this setting the role of TNFα is to stimulate apoptosis rather than cell division (21). High AUC were found for PAI1 and uPAR in the LPS, the PHx90% and in the GalN model, i.e., in the lethal models. The uPA receptor and the inhibitor PAI-1 are important for remodeling of liver tissue, playing critical roles in regulation of hepatic repair via proteolysis of matrix elements, clearance of cellular debris from the field of injury, and proteolytic maturation of hepatocyte growth factor (HGF) (22, 23). Even though the lethal models differ widely as to mechanism of liver damage, the uniform upregulation of genes related to replication and remodeling suggests that the corresponding gene products are critical for survival. Since GalN limits the mRNA production, a high priority for expression of those genes in this model supports their critical role. In contrast, the expression of the acute phase genes was downregulated after GalN. The mRNA for acute phase proteins was elevated in the LPS, the turpentine, and the PHx70% models. While IL-6 is believed to be important for upregulation of transcription of these genes, mRNA of IL-6 and IL-6R only was increased in the first two models. In the PHx90% model the AUC of α2M, α1-AGP and β-fibrinogen were only moderately increased, but it is noteworthy that the transcription factor C/EBPβ. an important transcription factor for acute phase genes (24), was highest in this model. A possible interpretation is that in the PHx90% model signals for acute phase reaction were active but ineffective.

A majority of the function genes had a negative AUC, i.e., downregulated gene expression in relation to controls. It is notable, however, that some function genes showed a constant or increased expression. Thus substantial increases in expression were found for ASL, Mdr1b and CPT1 in the LPS and the PHx90% models. CPT1, the rate limiting enzyme for β-oxidation of fatty acids that provide energy for the hepatocytes (25), was downregulated only after GalN. The activity of this enzyme has been found to be temporarily reduced after partial hepatectomy (26). The role of ASL may be to stimulate the production of polyamines (27), assisted by high expression of ARG and ODC as found in the PHx90% model. Besides, high expression of ASL may increase the availability of arginine for NO synthesis (28). The data on the mRNA of enzymes for drug metabolism and detoxification were in accordance with reports of inhibition by inflammatory reactions, general as well as local, on the transcription and activity of the Cyp enzymes (29, 30). A notable exception was the selectively increased expression of Cyp2B1/2 after turpentine, which adds this compound to the known inducers of the enzyme. Upregulation of Mdr1b has been reported by others after high doses of LPS (10), PHx (31) as well as after bile duct ligation (32). Mdr1b mediates active transport of a of toxic hydrophobic substances from the liver cell to bile, and it has been suggested that its upregulation is a mechanism to limit cell injury by facilitating the elimination of toxic compounds (32) and perhaps compensates for the decreased expression of other ABC transporters. In contrast to those Mdr1b is upregulated by NFκB in response to stress-full stimuli such as endotoxin, radiation, heat chock and toxic anti-cancer drugs, under conditions where NFκB has antiapoptotic effects. Because pharmacological blockade of Mdr1b enhances apoptosis after experimental oxidative stress, Müller (33) speculated that Mdr1b upregulation could provide antiapoptotic protection for cells against oxidative induced cell damage. Since Mdr1b expression was increased in all three lethal and the PHx70% model this suggests that Mdr1b may be essential for replication. Finally, PEPCK, a key enzyme in gluconeogenesis, was moderately upregulated in the PHx models.

The cluster analysis confirms that the lethal and the nonlethal models differ most in respect to repair gene expression, while most of the function genes are equally affected, although preferentially expressed function genes, like ASL, ARG, CPT1, PEPCK, Mdr1b, Mrp2, and glutamine synthetase, showed highest mRNA levels among the lethal models.

The cause for the relative suppressed transcription of many function genes, and an increased transcription of a few, as a response to liver damage remains unknown. It may be speculated that the transcriptional capacity of the hepatocyte is limited and that an inherited "survival program" confers priority to expression of repair and function genes producing the vitally most important proteins.

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REFERENCES

1. Barie, P. S. (1998) Understanding the enigma of hepatic failure. *Crit. Care Med.* **26,** 995–996.
2. Higgins, G. M., and Anderson, R. M. (1931) Experimental pathology of the liver. I. Restoration of the liver of the white rat following partial surgical removal. *Arch. Pathol.* **12,** 186–202.
3. Petropoulos, C. J., Lemire, J. M., Goldman, D., and Fausto, N. (1985) Homology between rat liver RNA populations during development, regeneration, and neoplasia. *Cancer Res.* **45,** 5114–5121.
4. Huber, B. E., Heilman, C. A., Wirth, P. J., Miller, M. J., and Thorgeirsson, S. S. (1986) Studies of gene transcription and translation in regenerating rat liver. *Hepatology* **6,** 209–219.
5. Ito, Y., Hayashi, H., Taira, M., Tatibana, M., Tabata, Y., and Isono, K. (1991) Depression of liver-specific gene expression in regenerating rat liver: A putative cause for liver dysfunction after hepatectomy. *J. Surg. Res.* **51,** 143–147.
6. Milland, J., Tsykin, A., Thomas, T., Aldred, A. R., Cole, T., and Schreiber, G. (1990) Gene expression in regenerating and acute-phase rat liver. *Am. J. Physiol.* **259,** G340–G347.
7. Tygstrup, N., Bak, S., Krog, B., Pietrangelo, A., and Shafritz, D. A. (1995) Gene expression of urea cycle enzymes following two-thirds partial hepatectomy in the rat. *J. Hepatol.* **22,** 349–355.
8. Kurumiya, Y., Nozawa, K., Sakaguchi, K., Nagino, M., Nimura, Y., and Yoshida, S. (2000) Differential suppression of liver-specific genes in regenerating rat liver induced by extended hepatectomy. *J. Hepatol.* **32,** 636–644.
9. Tygstrup, N., Jensen, S. A., Krog, B., and Dalhoff, K. (1997) Expression of liver functions following sub-lethal and non-lethal doses of allyl alcohol and acetaminophen in the rat. *J. Hepatol.* **27,** 156–162.
10. Vos, T. A., Hooiveld, G. J., Koning, H. H., Childs, S., Meijer, D. K., Moshage, H., Jansen, P. L., and Müller, M. (1998) Up-regulation of the multidrug resistance genes, *mrp1* and *mdr1b*, and down-regulation of the organic anion transporter, Mrp2, and the bile salt transporter, spgp, in endotoxemic rat liver. *Hepatology* **28,** 1637–1644.
11. Zieve, L., Anderson, W. R., Lyftogt, C., and Draves, K. (1986) Hepatic regenerative enzyme activity after pericentral and periportal lobular toxic injury. *Toxicol. Appl. Pharmacol.* **86,** 147–158.
12. Gaub, J., and Iversen, J. (1984) Rat liver regeneration after 90% partial hepatectomy. *Hepatology* **4,** 902–904.
13. Portolés, M. T., Ainaga, M. J., and Pagani, R. (1993) The induction of lipid peroxidation by *E. coli* lipopolysaccharide on rat hepatocytes as an important factor in the etiology of endotoxic liver damage. *Biochim. Biophys. Acta Gen. Subj.* **1158,** 287–292.
14. Moshage, H. (1997) Cytokines and the hepatic acute phase response. *J. Pathol.* **181,** 257–266.
15. Decker, K., and Keppler, D. (1972) Galactosamine induced liver injury. *In* Progress in Liver Diseases (Popper, H., and Schaffner, F., Eds.), pp. 183–199, Grune & Stratton, New York.
16. Fujimura, H., Kawasaki, N., Tanimoto, T., Sasaki, H., and Suzuki, T. (1995) Effects of acetaminophen on the ultrastructure of isolated rat hepatocytes. *Exp. Toxicol. Pathol.* **47,** 345–351.
17. Lee, W. M., and Galbraith, R. M. (1992) The extracellular actin-scavenger system and actin toxicity. *N. Engl. J. Med.* **326,** 1335–1341.
18. Friedman, J. M., Chung, E. Y., and Darnell, J. E., Jr. (1984) Gene expression during liver regeneration. *J. Mol. Biol.* **179,** 37–53.
19. Mohn, K. L., Melby, A. E., Tewari, D. S., Laz, T. M., and Taub, R. (1991) The gene encoding rat insulin-like growth factor-binding protein 1 is rapidly and highly induced in regenerating liver. *Mol. Cell Biol.* **11,** 1393–1401.
20. Ghahary, A., Minuk, G. Y., Luo, J., Gauthier, T., and Murphy, L. J. (1992) Effects of partial hepatectomy on hepatic insulin-like growth factor binding protein-1 expression. *Hepatology* **15,** 1125–1131.
21. Bradham, C. A., Plümpe, J., Manns, M. P., Brenner, D. A., and Trautwein, C. (1998) Mechanisms of hepatic toxicity I. TNF-induced liver injury. *Am. J. Physiol.* **275,** G387–G392.
22. Schneiderman, J., Sawdey, M., Craig, H., Thinnes, T., Bordin, G., and Loskutoff, D. J. (1993) Type 1 plasminogen activator inhibitor gene expression following partial hepatectomy. *Am. J. Pathol.* **143,** 753–762.
23. Nieto-Rodríguez, A., Hérnandez-Pando, R., Kershenobich, D., and Rodríguez-Fragoso, L. (2001) Expression of urokinase-type plasminogen activator in an experimental model of hepatocarcinoma. *Toxicology* **161,** 13–23.
24. Takiguchi, M. (1998) The C/EBP family of transcription factors in the liver and other organs. *Int. J. Exp. Pathol.* **79,** 369–391.
25. Berk, P. D., and Stump, D. (1999) Acute hepatic failure and defective fatty acid transport: Clinical proof of a physiologic hypothesis. *Hepatology* **29,** 1607–1609.
26. Lai, H. S., and Chen, W. J. (1995) Alterations of remnant liver carnitine palmitoyltransferase I activity and serum carnitine concentration after partial hepatectomy in rats. *J. Surg. Res.* **59,** 754–758.
27. Minuk, G. Y., Gauthier, T., and Benarroch, A. (1990) Changes in serum and hepatic polyamine concentrations after 30%, 70% and 90% partial hepatectomy in rats. *Hepatology* **12,** 542–546.
28. Tabuchi, S., Gotoh, T., Miyanaka, K., Tomita, K., and Mori, M. (2000) Regulation of genes for inducible nitric oxide synthase and urea cycle enzymes in rat liver in endotoxin shock. *Biochem. Biophys. Res. Commun.* **268,** 221–224.
29. Morgan, E. T., Thomas, K. B., Swanson, R., Vales, T., Hwang, J., and Wright, K. (1994) Selective suppression of cytochrome *P*-450 gene expression by interleukins 1 and 6 in rat liver. *Biochim. Biophys. Acta Gene Struct. Expr.* **1219,** 475–483.
30. Siewert, E., Bort, R., Kluge, R., Heinrich, P. C., Castell, J., and Jover, R. (2000) Hepatic cytochrome P450 down-regulation during aseptic inflammation in the mouse is interleukin 6 dependent. *Hepatology* **32,** 49–55.
31. Vos, T. A., Ros, J. E., Havinga, R., Moshage, H., Kuipers, F., Jansen, P. L., and Müller, M. (1999) Regulation of hepatic transport systems involved in bile secretion during liver regeneration in rats. *Hepatology* **29,** 1833–1839.
32. Lee, J., Boyer, J. L. (2000) Molecular alterations in hepatocyte transport. Mechanisms acquired cholestatic liver disorders. *Semin. Liver Dis.* **20,** 373–384.
33. Müller, M. (2000) Transcriptional control of hepatocanalicular transporter gene expression. *Semin. Liver Dis.* **20,** 323–337.