Reduced expression of *kan-1* (encoding putative bile acid-CoA–amino acid *N*-acyltransferase) mRNA in livers of rats after partial hepatectomy and during sepsis

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We isolated a cDNA clone, *kan-1*, from a rat liver cDNA library using a reverse transcriptase PCR cloning method. The *kan-1* cDNA encoded a polypeptide of 420 amino acids, and was 70 and 69% identical in nucleotide and amino acid sequences respectively with human liver bile acid-CoA–amino acid *N*-acyltransferase (BAT). Thus Kan-1 is probably a rat homologue of human BAT (rBAT). Kan-1/rBAT mRNA was mainly expressed in the livers of adult rats and rats immediately after, but not before, birth. It was expressed in the hepatocytes, the sinusoidal endothelial cells and the Kupffer cells of the liver. An anti-Kan-1/rBAT polyclonal antibody detected a protein of molecular mass 46 kDa in the liver. After partial hepatectomy, the levels of Kan-1/rBAT mRNA decreased at 6 and 12 h in the regenerating liver. In a sepsis model, hepatic expression of Kan-1/rBAT mRNA decreased at 6 and 12 h after caecal ligation and puncture. The kinetics of Kan-1/rBAT mRNA expression suggests that it may play a role in acute-phase reactions.

INTRODUCTION

The liver regenerates actively after partial hepatectomy and liver injury. In response to tissue loss and by partial hepatectomy, DNA synthesis in hepatocytes starts at about 14 h and peaks at 24 h after the operation [1]. The remaining intact lobes grow rapidly, and after 1 week 90% of the liver mass is restored. The hepatocytes then return to quiescence [2]. Liver cell DNA synthesis, cell division and return to quiescence are considered to be controlled by growth-stimulatory and/or -inhibitory factors [3]. Despite extensive analysis of the hepatic and humoral factors that may induce and subsequently terminate DNA replication in hepatocytes, the regulatory mechanisms of liver regeneration remain largely unknown.

We have studied changes in the mRNA levels of cytokines and protein tyrosine phosphatases in the liver in response to various stimuli such as partial hepatectomy and sepsis. We found that both interleukin-1α and β genes were induced in the regenerating liver after partial hepatectomy [4]. The expression of several protein tyrosine phosphatase genes was found to increase within 6 h, decrease to the normal level by 24 h and increase again by 48–72 h after partial hepatectomy [5]. By inducing sepsis in rats with caecal ligation and puncture (CLP), we demonstrated that interleukin 1 and intercellular adhesion molecule 1 mRNAs were induced in the liver [6]. During these experiments, we coincidentally obtained a novel cDNA fragment from a rat liver using a reverse transcriptase PCR (RT-PCR) with a pair of primers specific for rat tumour necrosis factor α (TNF-α) cDNA. We tentatively named the fragment *kan-1*. We obtained a full-length *kan-1* cDNA, the nucleotide sequence of which suggested *kan-1* encodes a rat homologue of human liver bile acid-CoA–amino acid *N*-acyltransferase (BAT; EC 2.3.1.65). We analysed the expression of *kan-1* in the rat liver during development, after partial hepatectomy and during sepsis.

MATERIALS AND METHODS

Animals and cell fractionation

Embryonic, neonatal and adult (weighing 250–300 g) Wistar rats were obtained from SLC (Shizuoka, Japan). The livers were perfused *in situ* with collagenase, and the harvested cells were fractionated using a centrifugal elutriation rotor (Beckman) as previously described [7]. The purities of the resultant hepatocyte, sinusoidal endothelial-cell and Kupffer-cell fractions were more than 90%. Liver plasma membranes were isolated by the method of Maeda et al. [8].

Operative procedures

Partial (about 70%) hepatectomy was performed by removing the median and left lateral lobes under ether anaesthesia [2]. The sham operation involved laparotomy and gentle movement of the liver. Sepsis was induced by CLP as previously described [6].

RNA extraction and cDNA cloning

Total RNA was extracted from a rat liver by the acid guanidium thiocyanate/phenol/chloroform method [9]. First-strand cDNA was synthesized from 5 μg of total RNA with random primers using a First-Strand cDNA Synthesis Kit (Pharmacia, Uppsala, Sweden) according to the manufacturer's recommendations. The cDNA was then subjected to 30 cycles of PCR using a DNA thermal cycler (Astec) *Taq* DNA polymerase (Toyobo) and

Abbreviations used: BAT, bile acid-CoA–amino acid *N*-acyltransferase; rBAT, rat homologue of human BAT; CLP, caecal ligation and puncture; RACE, rapid amplification of cDNA ends; RT-PCR, reverse transcriptase PCR; TNF-α, tumour necrosis factor α.

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The sequence will appear in the EMBL/GenBank/DDBJ Nucleotide Sequence Databases under the accession number D43964.

oligonucleotide primers. The PCR cycle consisted of 30 s at 94 °C, 1 min at 62 °C, 1 min at 72 °C, and a 5 min final extension at 72 °C. The sequences of the forward and reverse primers specific for rat TNF-α, which were designed to give an amplified fragment of 600 bp, were 5′-CCTCAGCCTCTTCTCATTCC-3′ and 5′-ACTCCAAAGTAGACCTGCCC-3′. The amplified fragments were electrophoresed in a 1.0% agarose gel, and, after ethidium bromide staining, bands were excised and the DNA was eluted from the gel. It was then cloned into the *Eco*RV site of plasmid vector pBluescript SK(−) (Stratagene) using the TA cloning technique as previously described [10,11]. The amplified plasmids were purified, and the inserts were sequenced from both ends by a dideoxynucleotide-chain-termination method using a BcaBEST DNA Sequencing Kit (Takara) according to the supplier's protocols.

Rapid amplification of cDNA ends (RACE) [12]

Amplification of 3′ end

Total RNA (5 μg), extracted from an adult rat liver, was reverse-transcribed to cDNA with a dT_{18}-adaptor primer (5′-AACTG-GAAGAATTCGCGGCCGCAGGAAT$_{18}$-3′). The 3′ end of *kan-1* cDNA was amplified by PCR using 1 μl of the resultant cDNA pool, the forward *kan-1*-specific 3′-amplification primer (5′-CGTGGTGGGAGAAGATGATA-3′) and the adaptor primer (5′-AATTCGCGGCCGCAGGAA-3′) as a reverse primer in 100 μl of PCR solution. They were subjected to 30 cycles of PCR by using a step programme (94 °C, 30 s; 60 °C, 1 min; 72 °C, 1 min), followed by a 5 min final extension at 72 °C. The identity of the 3′ end cDNA product of the amplification reaction was confirmed by Southern-blot analysis using a *kan-1* cDNA probe.

Amplification of 5′ end

For amplification of the 5′ end of cDNA, we used a 5′ RACE System (Gibco–BRL) according to the manufacturer's instructions. Briefly, 1 μg of total RNA extracted from an adult rat liver was reverse-transcribed to cDNA using the *kan-1*-specific 5′ reverse primer-1 with the nucleotide sequence 5′-ACTTTCTA-TGGCTATT-3′. For tailing of the cDNA first strands, 2 μl of 2 mM dCTP, 1 μl of 10× synthesis buffer, and 10 units of terminal deoxynucleotidyltransferase were added. The mixture was then incubated for 10 min at 37 °C and heated for 15 min at 65 °C. A 1 μl sample of the reaction mixture was used for amplification as described for the 3′-end procedure, except for the substitution of the forward anchor primer (5′-CUACUACUACUA-GGCCACGCGTCGACTAGTACGGGIIGGGIIGGGIIG-3′) and the *kan-1*-specific 5′-reverse primer-2 (5′-ATCTCTGCTCC-AATGCACAC-3′). The amplified products of 3′- and 5′-RACE were cloned into plasmid vectors and the sequences were determined.

Northern-blot hybridization

Northern-blot hybridization was performed as previously described [6]. Briefly, 10 μg of total RNA in each lane was separated by 1.0% agarose/formaldehyde-gel electrophoresis and transferred to a Hybond-N$^+$ nylon membrane filter (Amersham). Rat Kan-1 and albumin cDNA probes were labelled with [α-^{32}P]dCTP using a Megaprime random-primer DNA-labelling kit (Amersham). Hybridization was performed at 65 °C for 2 h in Rapid Hybridization Solution (Amersham) according to the manufacturer's recommendations. The filter was washed twice with 2×SSC (1×SSC is 150 mM NaCl and 15 mM sodium citrate)/0.1% SDS at room temperature for 10 min, and then once with 1×SSC/0.1% SDS at 65 °C for 15 min. The filter was autoradiographed overnight at −80 °C using an intensifying screen. To normalize the amount of RNA applied in each lane, the same RNA blots were rehybridized with an S26 ribosomal protein cDNA probe. The amounts of Kan-1 and S26 ribosomal protein mRNA were determined by densitometric scanning of autoradiograms with an ATTO Densitograph (Tokyo, Japan).

In situ hybridization histochemistry

In situ hybridization histochemistry was performed as previously described [11,13]. The liver was removed and immersed in 4% *p*-formaldehyde in PBS and embedded in paraffin. Sense and antisense RNA probes of Kan-1 were transcribed and labelled with digoxigenin-UTP using the digoxigenin RNA-labelling kit (Boehringer-Mannheim) and following the manufacturer's instructions. Some 50 μl of hybridization solution was placed on each section and the sections were then incubated at 50 °C for 16 h in a moist chamber. RNase A treatmented was carried out at 37 °C for 30 min. The immunodetection was performed with the Nucleic Acid Detection Kit of Boehringer-Mannhein according to the manufacturer's instructions. A control consisted of hybridization with a sense RNA probe under the same conditions as described above.

Antisera

For production of the anti-Kan-1 antibody, a peptide Gln-His-Leu-Asn-Pro-Gly-Phe-Asn-Ser-Gln-Leu, corresponding to the C-terminal region of Kan-1, was synthesized. The peptide was coupled to keyhole limpet haemocyanin, and then used to immunize rabbits.

Western-blot analysis

Fresh rat tissues and all liver cell fractions were homogenized in RIPA buffer (20 mM Tris/HCl, pH 7.4, 0.1% SDS, 1% Triton X-100) on ice, and aliquots containing 50 μg of protein in each lane were subjected to SDS/PAGE (8% gels). The proteins were electroblotted to Immobilon-P membrane (Millipore), and then subjected to a standard Western-blotting protocol using the anti-Kan-1 serum. Immunoblots were visualized by enhanced chemiluminescence (Amersham).

RESULTS

Isolation of rat *kan-1* cDNA

We tried to amplify rat TNF-α cDNA using TNF-α-specific primers and a first-strand cDNA library made from a normal rat liver. When we sequenced the cDNA inserts, which were each approx. 600 bp in length, four of the five cDNA clones were found to possess a novel sequence that was not found in the GenBank or EMBL nucleic acid databases. The remaining inserts corresponded to rat TNF-α cDNA. We named the novel cDNA fragment *kan-1*. We obtained the full-length cDNA by the RACE method. The amplified products of 3′ and 5′- RACE were 700 and 1000 bp respectively in length, and the sequences of three independent clones were compiled to correct for errors introduced by the PCR amplifications.

Primary structure of rat *kan-1*

The 1.88 kb rat *kan-1* cDNA sequence, shown in Figure 1(a), had a single open reading frame. The cDNA sequence of the 3′ untranslated region was rich in adenine and thymine, which suggested instability of the mRNA. The first ATG codon present

(a)

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                AAGACTTTTTCCCCCAGCCTTAACTGGATAGTCTGAAGTTTTCAAAACTCTTATCCCACAAAGT  -151
TGTCAGAACCTTGATTGGGAAGTCCTGTGCATCTGTGCTAACCTACAGGGCCTCCTTATCCAGAGCACTCTGCAT   -76
TTCAGAGGGTCGCTGTCGAACTACGGTTTTGGCGAAGACATTCCTGAAGAATTGTCTGAGGTTTCCTCTGCAAAA    -1
ATGGCCAAGCTGACAGCTGTTCCTCTCAGTGCACTTGTTGATGAGCCTGTGCATATCCGGGTCACAGGCCTGACC    75
M  A  K  L  T  A  V  P  L  S  A  L  V  D  E  P  V  H  I  R  V  T  G  L  T     25

CCCTTCCAGGTGGTGTGCCTTCAGGCATCACTGAAAGATGATAAGGGAAACCTGTTTAATTCTCAGGCCTTCTAC   150
P  F  Q  V  V  C  L  Q  A  S  L  K  D  D  K  G  N  L  F  N  S  Q  A  F  Y     50

AGGGCCAGTGAAGTGGGTGAGGTAGATCTGGAACGTGATTCCTCTCTTGGAGGAGACTACATGGGGGTCCACCCC   225
R  A  S  E  V  G  E  V  D  L  E  R  D  S  S  L  G  G  D  Y  M  G  V  H  P     75

ATGGGTCTTTTCTGGTCCATGAAACCTGAAAAGCTATTGACTAGATTGGTAAAAAGAGATGTGATGAATAGGCCC   300
M  G  L  F  W  S  M  K  P  E  K  L  L  T  R  L  V  K  R  D  V  M  N  R  P    100

CACAAAGTCCACATAAAACTTTGCCATCCATACTTTCCAGTAGAAGGCAAAGTTATCAGTTCCTCCTTGGATAGT   375
H  K  V  H  I  K  L  C  H  P  Y  F  P  V  E  G  K  V  I  S  S  S  L  D  S    125

CTGATTCTGGAAAGGTGGTATATGGCACCTGGTGTCACTAGGATCCATGTAAAGGAAGGCCGAATCCGGGGAGCC   450
L  I  L  E  R  W  Y  M  A  P  G  V  T  R  I  H  V  K  E  G  R  I  R  G  A    150

CTGTTTCTGCCTCCAGGAGAAGGTCCTTTCCCAGGGGTCATTGACTTGTTTGGAGGAGCTGGTGGACTGTTTGAG   525
L  F  L  P  P  G  E  G  P  F  P  G  V  I  D  L  F  G  G  A  G  G  L  F  E    175

TTCCGGGCCAGCCTCCTGGCCAGTCATGGCTTTGCCACTTTAGCTCTGGCTTACTGGGGCTATGATGACCTGCCC   600
F  R  A  S  L  L  A  S  H  G  F  A  T  L  A  L  A  Y  W  G  Y  D  D  L  P    200

TCTCGACTGGAGAAGGTAGATCTAGAGTATTTTGAAGAAGGTGTAGAGTTTCTCCTGAGACATCCTAAGGTCCTG   675
S  R  L  E  K  V  D  L  E  Y  F  E  E  G  V  E  F  L  L  R  H  P  K  V  L    225

GGCCCAGGGGTTGGCATCCTTTCTGTGTGCATTGGAGCAGAGATTGGACTTTCTATGGCTATTAACCTAAAACAG   750
G  P  G  V  G  I  L  S  V  C  I  G  A  E  I  G  L  S  M  A  I  N  L  K  Q    250

ATAACAGCCACTGTACTTATAAATGGGCCTAATTTTGTTTCTAGCAATCCACATGTATATCGTGGTAAGGTCTTC   825
I  T  A  T  V  L  I  N  G  P  N  F  V  S  S  N  P  H  V  Y  R  G  K  V  F    275

CAGCCTACACCCTGCAGTGAAGAATTTGTAACCACCAATGCTTTGGGACTTGTAGAGTTCTATCGAACCTTTGAG   900
Q  P  T  P  C  S  E  E  F  V  T  T  N  A  L  G  L  V  E  F  Y  R  T  F  E    300

GAAACTGCAGATAAGGATAGCAAATACTGTTTTCCCATTGAAAAAGCTCACGGACATTTTCTTTTCGTGGTGGGA   975
E  T  A  D  K  D  S  K  Y  C  F  P  I  E  K  A  H  G  H  F  L  F  V  V  G    325

GAAGATGATAAGAATCTCAACAGCAAAGTGCATGCTAAGCAAGCCATAGCCCAGCTGATGAAAAGTGGAAAGAAG  1050
E  D  D  K  N  L  N  S  K  V  H  A  K  Q  A  I  A  Q  L  M  K  S  G  K  K    350

AACTGGACTCTGCTGTCTTACCCTGGGGCAGGTCACCTGATTGAGCCTCCCTACTCCCCACTGTGCTCAGCCTCA  1125
N  W  T  L  L  S  Y  P  G  A  G  H  L  I  E  P  P  Y  S  P  L  C  S  A  S    375

AGGATGCCCTTTGTAATCCCAAGCATCAACTGGGGAGGAGAGGTTATCCCACACGCAGCTGCGCAGGAACATTCT  1200
R  M  P  F  V  I  P  S  I  N  W  G  G  E  V  I  P  H  A  A  A  Q  E  H  S    400

TGGAAGGAGATACAGAAATTTCTCAAGCAGCATCTTAATCCAGGTTTCAACAGTCAGCTGTGAGTGGACTTGATT  1275
W  K  E  I  Q  K  F  L  K  Q  H  L  N  P  G  F  N  S  Q  L  *               420

ATATTACTGGAAAGAGGAGCTGGGCATCTCCTGGCCAGCTCCACTCCTCACTTCCATAGAGGAATGTCTTTAATC  1350
TCTTATCACATGAGGAAGAAGAGTACCACCAGAAAATGCCGAAGGACAGAGAGTGATAACCTCATGACTTTGGAA  1425
GGGGAGACATGTTTTCCATGGAATAAAATGTCCCTCAGTGAGAGTCCTATATCTGTATAAATAAAATCTTAGGGT  1500
TTTCCTAAAATGTTCAACACCACAGCAACTTTCTGTGATGATAATTATCAAGGAAATTATCACTGATAATCCACA  1575
GGATACTTTAGTTTATAAAAGAGACATGAAAAGAATTATATATTGTTACTTATTAATTTCTTAAAACTCACATTA  1650
AATATGCTTAGATCATCAAAAA                                                        1677
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(b)

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Kan-1  MAKLTAVPLSALVDEPVHIRVTGLTPFQVVCLQASLKDDKGNLFNSQAFYRASEVGEVDL   60
hBAT   -IQ---T-V----------QA---I---M-SF----E-EN-DM-Y---H---N-F-----   60

Kan-1  ERDSSLGGDYMGVHPMGLFWSMKPEKLLTRLVKRDVMNRPHKVHIKLCHPYFPVEGKVIS  120
hBAT   NHA------------------L---------L--------FQ-QV--YDLELI-NN--A-  120

Kan-1  SSLDSLILERWYMAPGVTRIHVKEGRIRGALFLPPGEGPFPGVIDLFGGAGGLFEFRASL  180
hBAT   APKA--T-----V-------K-R---L-----------L----------L---L------  180

Kan-1  LASHGFATLALAYWGYDDLPSRLEKVDLEYFEEGVEFLLRHPKVLGPGVGILSVCIGAEI  240
hBAT   ---R---S-----HN-E---RKP-VT-------AAN--------F-S---VV---Q-VQ-  240

Kan-1  GLSMAINLKQITATVLINGPNFVSSNPHVYRGKVFQPTPCSEEFVTTNALGLVEFYRTFE  300
hBAT   ------Y---V--------T--PFGI-Q--H-QIH--L-H-AQLIS------L-L-----  300

Kan-1  ETADKDSKYCFPIEKAHGHFLFVVGEDDKNLNSKVHAKQAIAQLMKSGKKNWTLLSYPGA  360
hBAT   T-QVGA-Q-L----E-Q-Q---I---G--TI---A--E---G--KRH--N----------  360

Kan-1  GHLIEPPYSPLCSASRMPFVIPSINWGGEVIPHAAAQEHSWKEIQKFLKQHLNPGFNSQL  420
hBAT   ------------C--TTH  DLRLH--------------A-----R--RK--I-DVT---  418
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Figure 1 (a) Nucleotide and deduced amino acid sequences of rat *kan-1* cDNA and (b) alignment of amino acid sequences of rat Kan-1 and human BAT [14]

(**a**) Nucleotide and deduced amino acids are numbered to the right, starting with the proposed initiating methionine codon. The stop codon that ends the open reading frame is marked with an asterisk. (**b**) Amino acid numbers are given at the right. Identical amino acids are indicated by dashes in the sequence of hBAT.

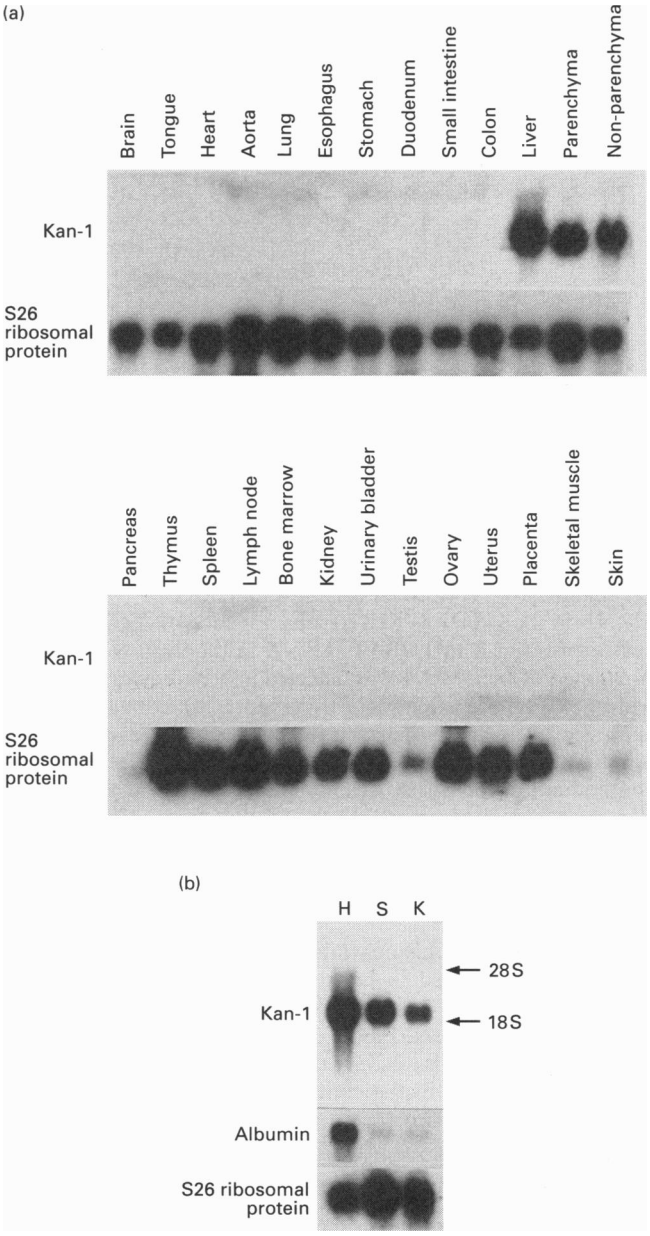

Figure 2 (a) Tissue distribution of Kan-1 mRNA expression in rats and (b) Kan-1 mRNA expression in the rat liver cell fractions enriched for hepatocytes (H), sinusoidal endothelial cells (S) and Kupffer cells (K)

(**a**) Total RNA (10 μg) from various tissues was hybridized with a Kan-1 cDNA probe. The same filter was rehybridized with an S26 ribosomal protein cDNA probe as an internal standard. (**b**) 10 μg of total RNA from each cell preparation was hybridized with a Kan-1 cDNA probe. The same filter was sequentially rehybridized with a rat albumin cDNA probe and an S26 ribosomal protein cDNA probe.

in this frame was assigned as the initiation codon, as it matched Kozak's consensus motif [15]. The *kan-1* cDNA encoded a polypeptide of 420 amino acids, and was 70 and 69% indentical in nucleotide and amino acid sequences respectively with human BAT [14] (Figure 1b). The predicted molecular mass was about 46 kDa. So *kan-1* cDNA probably encodes a rat homologue of human BAT (rBAT).

Expression of Kan-1/rBAT mRNA in the rat liver

As shown in Figure 2(a), Kan-1/rBAT mRNA was constitutively expressed mainly in the liver, in both parenchymal and non-

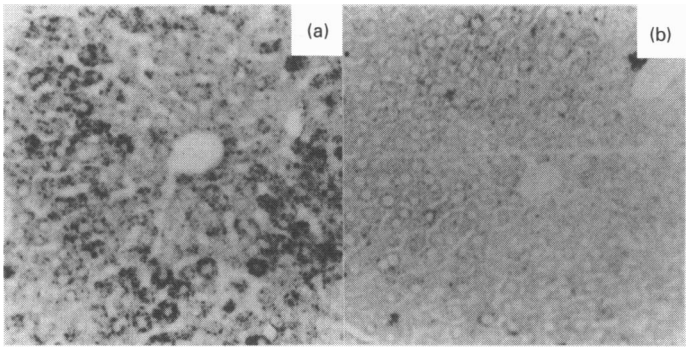

Figure 3 ***In situ*** **hybridization histochemistry of rat liver with a Kan-1 antisense RNA probe (a) and a kan-1 sense RNA probe (b)**

Positive signals are observed in the cytoplasm of the hepatocytes (**a**) but no signal is detected in (**b**).

parenchymal cells. As Kwakye et al. [16] identified BAT protein in rat kidney, we performed RT-PCR and detected Kan-1/rBAT mRNA in the kidney as well (results not shown). To identify the cells expressing Kan-1/rBAT mRNA in the liver, the liver was fractioned. Kan-1/rBAT was detected in hepatocytes, sinusoidal endothelial cells and Kupffer cells (Figure 2b). Albumin mRNA expression was barely detected in the sinusoidal endothelial or Kupffer cell fractions, which indicated that there was little contamination of these cell preparations with hepatocytes and that non-parenchymal cells of the liver also expressed Kan-1/rBAT mRNA.

In situ hybridization histochemistry

In situ hybridization histochemistry revealed that Kan-1/rBAT mRNA was expressed in hepatocytes. The hybridized signals were more intense in the periportal and intermediary areas than in the pericentral areas (Figure 3a). The cytoplasm of the non-parenchymal liver cells was very thin, and no signal was apparent. Staining was absent in the control experiment using the sense RNA probe (Figure 3b).

Identification of Kan-1/rBAT protein

To identify and characterize the Kan-1/rBAT protein, a polyclonal rabbit antibody was prepared against the C-terminal peptides of the predicted *kan-1* gene product. A normal rat liver, which expressed Kan-1/rBAT mRNA, was analysed by Western blotting. Figure 4 shows that a liver protein of molecular mass 46 kDa was detected by the antibody. No protein was detected in the spleen lysate. As this molecular mass was identical with that predicted from the *kan-1* cDNA sequence, the anti-Kan-1 polypeptide serum was considered to recognize Kan-1/rBAT protein.

To determine the subcellular localization of Kan-1/rBAT protein, rat liver cells were fractionated into plasma-membrane and cytoplasmic/nuclear fractions. Each fraction was subjected to Western blotting. Kan-1 was detected in the cytoplasmic/nuclear fraction but not in the plasma-membrane fraction (Figure 4).

Developmental expression of Kan-1/rBAT mRNA

To investigate the changes associated with development, Kan-1/rBAT mRNA expression in the liver during late fetal, neonatal and adult stages was analysed by Northern-blot hybridization.

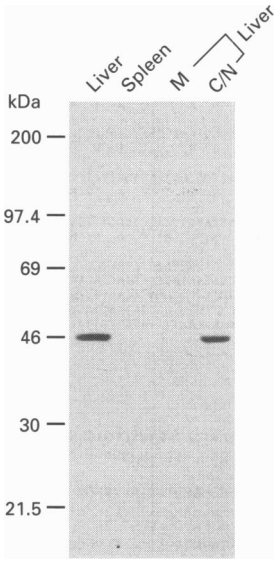

Figure 4 Western-blot analysis of rat Kan-1 protein

Lysate from whole liver, spleen, plasma-membrane fraction of liver (M) and cytoplasmic/nuclear fraction of liver (C/N) (50 μg in each case) was electrophoresed, transferred to a membrane and incubated with an anti-Kan-1 polyclonal antibody. A 46 kDa protein in the lysate of whole liver and cytoplasmic/nuclear fraction of the liver was detected.

As shown in Figure 5, the Kan-1/rBAT mRNA was not detectable until immediately after birth, and expression peaked on day 21.

Kan-1/rBAT mRNA expression in the regenerating liver

When we performed a 70% partial hepatectomy and analysed Kan-1/rBAT mRNA expression by Northern-blot hybridization, the level of the Kan-1/rBAT mRNA was found to decrease in the regenerating liver (Figure 6). At 12 h after hepatectomy, the message almost disappeared. However, 24 h after hepatectomy, the level of Kan-1/rBAT mRNA increased but was below that seen in the sham-operated rat liver. Only a slight decrease in Kan-1/rBAT mRNA was observed in the sham-operated rat liver.

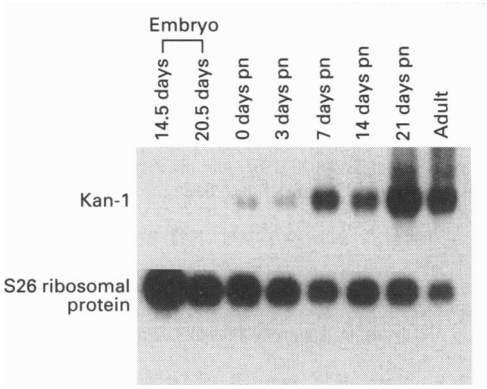

Figure 5 Northern-blot analysis of Kan-1/rBAT mRNA in embryonic and postnatal (pn) rat livers

Total RNA (10 μg/lane) from embryonic and postnatal (pn) rat livers was hybridized with a Kan-1 cDNA probe. The same filter was rehybridized with an S26 ribosomal protein cDNA probe as an internal standard.

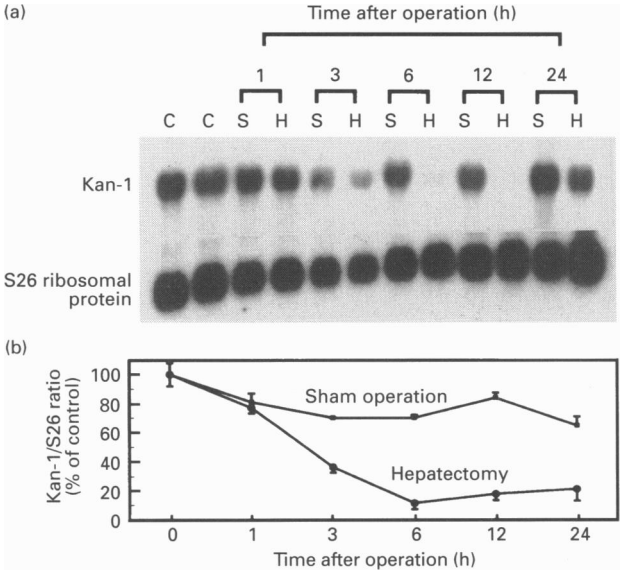

Figure 6 Nothern-blot analysis of Kan-1/rBAT mRNA during liver regeneration

(**a**) Total RNA (10 μg/lane) from the livers of untreated control rats (C), sham-operated rats (S) and partially hepatectomized rats (H) was hybridized with a Kan-1 cDNA probe. The same filter was rehybridized with an S26 ribosomal protein cDNA probe as an internal standard. (**b**) The Kan-1/rBAT mRNA levels of sham-operated rats (■) and partially hepatectomized rats (●) were quantified by densitometric scanning of autoradiograms and corrected for the amount of RNA loaded in each lane by comparison with the bands of the S26 ribosomal protein mRNA control (Kan-1/S26 ratio). Values are expressed relative to the value of untreated control liver (C), which was taken as 100%. Data are means ± S.D. from three rats.

Kan-1/rBAT mRNA expression in the livers of septic rats

We then examined Kan-1/rBAT mRNA expression by Northern-blot hybridization after CLP [6] (Figure 7). The level of Kan-1/rBAT mRNA decreased and was barely detectable at 6 and 12 h after CLP. At 24 h after the operation, it became detectable again but the levels were below those of untreated rat livers.

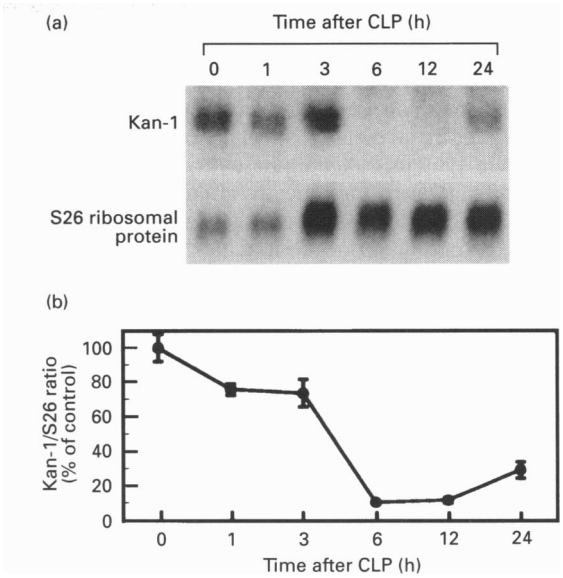

Figure 7 Northern-blot analysis of Kan-1/rBAT mRNA in the livers of septic rats

(**a**) Total RNA (10 μg/lane) from the livers of rats after CLP was hybridized with a Kan-1 cDNA probe. The same filter was rehybridized with an S26 ribosomal protein cDNA probe as an internal standard. (**b**) The Kan-1/rBAT mRNA levels in the livers of septic rats were quantified by densitometric scanning of autoradiograms and corrected for the amount of RNA loaded in each lane by comparison with the bands of the S26 ribosomal protein mRNA control (Kan-1/S36 ratio). Values are expressed relative to the value of untreated control liver (0 h), which was taken as 100%. Data are means ± S.D. from three rats.

DISCUSSION

In humans, more than 95% of the biliary bile acids are *N*-acyl amidates with glycine or taurine [17]. The conjugation of bile acids with glycine or taurine in the liver is catalysed by two sequential enzymes: the microsomal enzyme, cholyl-CoA synthetase, and the cytosolic enzyme, BAT. Recently, molecular cloning of human BAT cDNA was reported and the recombinant human BAT demonstrated significant enzyme activity for the conjugation of both taurine and glycine with cholic acid [14]. In other species, BAT cDNA has not yet been cloned. As described above, we obtained a novel rat cDNA, *kan-1*, which encodes a protein bearing 69% identity with human BAT protein. Kan-1 protein was detected by immunoblot analysis in the cytoplasmic/nuclear fraction of liver but not in the plasma-membrane fraction. On Northern-blot analysis of rat liver RNA, human BAT cDNA probe hybridized to a single band of the same size as Kan-1 mRNA (results not shown). As it is thought that significant structural differences exist between the forms of the enzyme in different species [14], the *kan-1* cDNA probably encodes a rat homologue of human BAT even though the two proteins exhibit relatively low similarity.

Two of three cysteine residues in the hydrophobic region of human BAT were conserved, and two additional cysteine residues were present in the hydrophobic region of Kan-1. Bile acid thioester is probably formed with these cysteine residues, as the hydrophobicity around the cysteines may favour an interaction with hydrophobic bile acids. It is of interest that Kan-1/rBAT mRNA was expressed not only in hepastocytes but also in hepatic sinusoidal endothelial cells and Kupffer cells (Figure 2b). These cells, as well as hepatocytes, may take part in bile acid conjugation.

The acute-phase response is a systemic reaction to inflammation or tissue injury such as partial hepatectomy and peritonitis [18]. It is characterized by complex changes that include fever, leucocytosis, increased muscle proteolysis, altered carbohydrate and trace metal metabolism and a pronounced change in hepatic protein synthesis. Acute-phase reactants are a group of secretory proteins with dramatically altered rates of synthesis during the acute-phase response of the liver. A marked decrease in mRNA levels of a major negative acute-phase reactant, α_2-HS glycoprotein, was observed after the administration of turpentine or TNF, or after partial hepatectomy [19]. Minimum levels were reached at 24 h. The kinetics of Kan-1/rBAT mRNA expression after partial hepatectomy and in sepsis was simliar to the kinetics of the negative acute-phase reactants. Kan-1/rBAT may play a role in acute-phase reactions.

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