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CYP3A4 and CYP2A6 activities marked by the metabolism of lignocaine and coumarin in patients with liver and kidney diseases and epileptic patients

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1 The *in vitro* hepatic metabolism of lignocaine to monoethylglycinexylide (MEGX) is mediated by CYP3A4 and that of coumarin to 7-hydroxycoumarin (7OHC) by CYP2A6. We investigated the usefulness of monitoring serum MEGX concentrations (after 1 mg kg^{-1} lignocaine i.v.) and urinary 7OHC excretion (after 5 mg coumarin p.o.) to reflect liver function in patients with liver (n = 36), kidney (n = 12) and epileptic (n = 12) disease and in control subjects (n = 20). The extent of liver disease was assessed using measurements of serum aminoterminal propeptide (PIIINP) and Child-Pugh grades.

2 Serum concentrations of MEGX were decreased in severe (4.6 ± 3.0 s.d. ng ml^{-1}), moderate (19.1 ± 11.6 s.d ng ml^{-1}) and mild (32.8 ± 14.2 s.d ng ml^{-1}) liver disease as compared with controls (53.4 ± 15.8 s.d ng ml^{-1}). The excretion of 7OHC over 2 h was decreased in severe (18.0 ± 10.3 s.d % of dose) and moderate (34.2 ± 15.6 s.d %), but not in mild (49.7 ± 19.0 s.d %) liver disease as compared with that in controls (56.2 ± 11.6 %).

3 In epileptic patients the urinary recovery of 7OHC was increased (2 h value 69.5 ± 13.2 s.d %) suggesting enzyme induction. In contrast, serum MEGX concentration were low (40.0 ± 14.1 s.d ng ml^{-1}), possibly due to competition for CYP3A4 between lignocaine and antiepileptic drugs.

4 In patients with kidney disease serum MEGX concentration (56.5 ± 26.1 s.d ng ml^{-1}) was similar to that in controls. The excretion rate of 7OHC was reflected in the creatinine clearance (r = 0.664).

5 Serum PIIINP values correlated better than Child-Pugh grades with serum MEGX concentration and excretion of 7OHC.

6 The case history, extent of liver disease, kidney function and drug therapy must be considered when evaluating liver function with probe drugs known to be metabolized by specific hepatic isoforms of cytochrome P450.

Keywords cytochrome P450 isoforms MEGX coumarin hepatic function kidney function

Introduction

Patients with hepatobiliary diseases need functional assessment for prognostic evaluation and drug dosage adjustments, and knowledge of hepatic drug metabolizing ability is important when choosing liver donors before transplantation. Recent developments allow a degree of quantification of hepatic drug metabolism using probe drugs which mark the activity of isoforms of cytochrome P450.

Lignocaine is *N*-deethylated to monoethylglycinexylide (MEGX) mainly by CYP3A4 [2]. Its hepatic extraction is 0.6–0.8 [2] and after i.v. administration its clearance should depend upon both

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oxidative metabolism and liver blood flow. Thus, plasma concentrations of MEGX after i.v. injection of lignocaine (MEGX test) reflect CYP3A4 activity [1, 2]. In patients with hepatobiliary diseases lignocaine elimination is relatively slow [2].

Coumarin is hydroxylated to 7-hydroxycoumarin (7OHC) by CYP2A6 and urinary excretion of 7OHC after oral administration of coumarin (coumarin test) reflects the activity of CYP2A6 [3–5]. Use of both drugs has been suggested for measurement of liver function in clinical practice [1, 4]. However, the influence of kidney function and simultaneous drug therapy on these tests needs further investigation and was the subject of the present study.

Methods

Patients with liver disease

Thirty-six patients with alcoholic liver disease (29 men and 7 women) were studied (Table 1). The diagnosis had been confirmed histologically by methods described previously [7]. Twenty-six patients had hepatic cirrhosis and 10 had severe fibrosis with or without fatty liver. They were classified into three groups (severe, moderate, mild disease) on the basis of fibrotic activity indicated by serum aminoterminal-propeptide level (PIIINP) (Tables 1 and 2, Figure 1), and this classification was compared with histological diagnosis and with a modified Child-Turcotte score [8] (Table 3). None of the patients had significant cardiac decompensation and kidney function, estimated by creatinine clearance, was normal. Twenty-six patients were taking drugs (digoxin, frusemide, thiazides, β-adrenoceptor blockers (atenolol/sotalol) and nitroglycerin), but none was receiving compounds known to alter hepatic CYP3A4 or CYP2A6 activities. Eight subjects were regular smokers, the others were non-smokers.

Patients with kidney disease

Twelve patients (11 male and 1 female) with stable chronic renal impairment due to hypertension [5], glomerulonephritis [3], chronic pyelonephritis [2], polycystic kidneys and renal artery stenosis (one each) were studied. They had low creatinine clearances (0.7 ± 0.4 s.d ml min^{-1}) and low serum albumin (31.5 ± 2.8 s.d g l^{-1}). Liver function tests were normal. They were receiving drugs for the treatment of heart failure (digoxin), angina pectoris (nitroglycerin) or hypertension (frusemide, atenolol). Four patients had radiological bone changes associated with chronic renal disease (renal osteodystrophy), and they had elevated PIIINP (Figure 1) and alkaline phosphatase levels. The elevation of PIIINP in these patients reflected bone marrow fibrosis [9].

Epileptic patients

Twelve patients (six of each sex) with chronic epilepsy, undergoing therapy with anticonvulsant agents (six with carbamazepine, three with carbamazepine and clonazepam, and three with phenobarbitone and phenytoin) for 5 to 22, years, were studied. The diagnosis had been confirmed by EEG findings and clinical data. The patients had normal liver and kidney function. Three were receiving drugs for cardiovascular diseases (digoxin, nitroglycerin, atenolol, frusemide).

Table 1 Clinical and laboratory data for patients with liver and kidney disease, epileptic patients and healthy control subjects

Subjects	*Age (years)*	*Sex*	*BMI ($kg\ m^{-2}$)*	*Drug use*	*Bil ($mmol\ l^{-1}$)*	*ASAT ($u\ l^{-1}$)*	*ALAT ($u\ l^{-1}$)*	*AP (%)*	*ALB ($g\ l^{-1}$)*	*T-T (%)*	*Creatinine clearance ($ml\ min^{-1}$)*
1 *Alcoholic liver disease*											
severe	52 ± 9	8 M	27.5	8/12	43.1[a]	62.7[a]	41.2	371.9[a]	32.7[b]	65.7[a]	1.33
(*n* = 12)		4 F	3.9		21.6	31.9	27.9	156.8	5.8	30.2	0.35
moderate	56 ± 9	11 M	27.4	9/12	17.5	45.9[b]	61.0	262.8[c]	37.4	96.2	1.40
(*n* = 12)		1 F	2.9		11.2	28.8	19.8	189.5	4.1	15.9	0.51
mild	56 ± 8	10 M	27.2	9/12	12.4	37.7	47.3	173.7	39.0	98.2	1.60
(*n* = 12)		2 F	4.0		5.9	26.1	31.5	84.9	5.6	6.7	0.50
2 *Kidney disease*	62 ± 11	11 M	25.7	12/12	11.6	32.9	37.2	213.5[a]	31.5[a]	95.2	0.7[a]
(*n* = 12)		1 F	1.9		7.8	21.8	36.7	43.7	2.8	6.9	0.4
3 *Epilepsy*	53 ± 11	6 M	23.8	12/12	9.6	23.5	29.3	144.5	37.5	100.0	1.1
(*n* = 12)		6 F	1.8		1.5	6.1	12.6	43.7	2.8	0	0.3
4 *Controls*	54 ± 9	10 M	25.3	None	10.9	27.7	25.5	135.6	37.7	100.0	1.6
(*n* = 20)		10 F	4.1		3.5	6.2	9.3	39.0	1.8	0	0.6

Data are mean ± s.d.
BMI = Body mass index, liver and kidney test values, Bil = total bilirubin, ASAT = aspartate transferase, ALAT = alanine transferase, AP = alkaline phosphatase, ALB = albumin, T-T = thrombotest; N = normal *P* values compared with controls
a < 0.001, b < 0.01, c < 0.05

Table 2 Serum type III procollagen (PIIINP), serum monoethylglycinexylidide (MEGX) concentration and urinary 7-hydroxycoumarin (7-OHC) excretion in patients with liver (n = 36), kidney (n = 12) and epileptic (n = 12) disease and in control subjects (n = 20)

Subjects	*Fibrotic activity PIIINP ($\mu g\ l^{-1}$)*	*Serum MEGX ($ng\ ml^{-1}$)*	*Urinary excretion of 7-OHC (% of dose) at* 2	4	24 h
1 *Alcoholic liver disease*					
severe	14.8[a] ± 3.4	4.6[a] ± 3.0	18.0[a] ± 10.3	13.0 ± 5.6	21.5[a] ± 12.8
moderate	6.6[a] ± 1.7	19.1[a] ± 11.6	34.2[a] ± 15.6	14.5 ± 7.1	10.4[a] ± 15.5
mild	3.4 ± 0.5	32.8[a] ± 14.2	49.7 ± 19.0	10.7[c] ± 6.6	12.2[b] ± 13.6
2 *Kidney disease*	5.7 ± 4.2	56.5 ± 26.1	27.8[a] ± 15.7	26.3 ± 9.2	23.7[a] ± 12.5
3 *Epilepsy*	3.5 ± 0.4	40.0[b] ± 14.1	69.5[b] ± 13.2	11.3 ± 6.7	0.0 ± 0.0
4 *Controls*	3.4 ± 0.4	53.4 ± 15.8	56.2 ± 11.6	16.7 ± 8.2	2.5 ± 3.4

P values compared with controls a < 0.001, b < 0.01, c < 0.05.

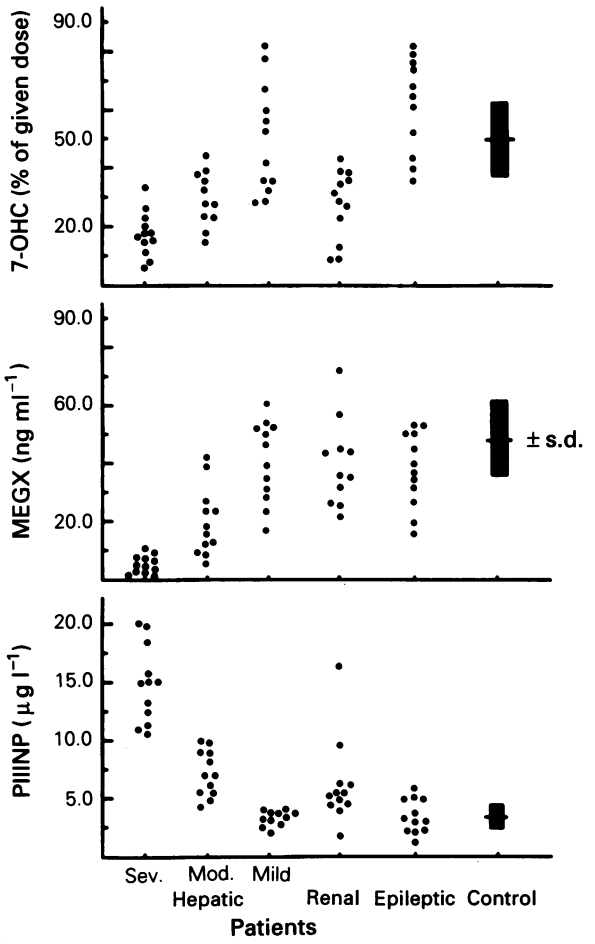

Figure 1 Serum type III procollagen (PIIINP) levels, serum concentrations of monoethylglycinexylidide (MEGX) at 15 min after i.v. injection of lignocaine (1 mg kg^{-1}) and urinary excretion of 7-hydroxycoumarin (7OHC) at 2 h after an oral 5 mg dose of coumarin in patients with liver and kidney disease, epileptic patients and healthy control subjects. (High PIIINP values in two kidney patients reflected bone marrow fibrosis).

Control subjects

Twenty age and sex-matched subjects who had no evidence of liver disease, and who were not receiving any medication known or suspected to induce or inhibit CYP3A4 or CYP2A6, were studied. Liver and kidney function tests were performed as part of their clinical evaluation.

Table 3 Liver histology and Child – Pugh scores of the liver disease patients (n = 36) classified by fibrotic activity (PIIINP)

Classification	*PIIINP ($\mu g\ l^{-1}$)*		
	≤ 4.2	*4.3 – 9.9*	*≥ 10.0*
Histology	6 Cir 6 FL + Fib	9 Cir 3 FL + Fib	12 Cir
Child – Pugh			
– class A	6	5	0
– class B	6	5	3
– class C	0	2	9

Cir = cirrhosis, FL + Fib = Fatty liver and fibrosis.

Protocol

The study protocol was approved by the local Ethics Committee and the subjects gave their written informed consent. The subjects were investigated as inpatients after clinical and laboratory examination. Blood and urine samples for liver and kidney function tests were taken after an overnight fast. Lignocaine hydrochloride (1 mg kg^{-1}, Astra, Södertelje, Sweden) was given as a 1 min i.v injection. Blood samples for the measurement of MEGX were taken before and 15 min after the injection [1]. A Venalot® capsule (containing 5 mg coumarin and 25 mg rutosides, Schaper & Brummer, Ringelheim, Germany) was given orally with 200 ml water after the 15 min blood sample for MEGX assay had been taken [4]. The patients had breakfast 2 h after administration of the coumarin capsule. Urine was avoided before and at 2, 4 and 24 h after capsule administration. Blood and urine samples were centrifuged immediately and kept frozen (–20° C) until assay.

Assay of MEGX and 7OHC

MEGX was determined by a fluorescence polarization immunoassay (FPIA) [1] using a commercial kit (Abbott Diagnostics, Illinois, USA), as described previously [10]. The intra- and inter-assay coefficients of

variation of the assay were 2.4% and 1.2% at a concentration of 310 nM (n = 10).

7OHC was assayed by an h.p.l.c. method described previously [4, 11]. Urine (0.25 ml) was incubated with β-glucuronidase (500 units ml^{-1}) for 30 min at 37° C. The mixture was then filtered and 20 µl injected into the h.p.l.c. system, which consisted of an Interchom Nucleosil C18 (5 µm 25 cm) column, an Altex model 110 pump, a Cecil Ce 2112 UV monitor and a Hitach D-2000 integrator. The elution buffer used as 1.5% v/v acetic acid: acetonitrile (75:25), and pH was adjusted to 4.80 with ammonium hydroxide. The flow rate was 1 ml min^{-1} and u.v. detection was at 313 nm. 4-Hydroxycoumarin was used as internal standard. The intra-assay and interassay coefficients of variation for duplicate analyses were 1.5% (n = 10) and 1.7% (n = 10), respectively, at a 7OHC concentration of 20 nM.

Assay of serum collagen antigen content

PIIINP concentration was measured by an equilibrium-type radioimmunoassay as described previously [12, 13]. The method overcomes the problem of non-parallelism between the standard antigen and human samples encountered with commercial PIIINP radioimmunoassays. Intra- and interassay coefficients of variation were about 5% at all antigen concentrations.

Liver function tests

Serum albumin concentration, total bilirubin content, and the activities of aspartate aminotransferase and alkaline phosphatase were measured using standard automatic analyzer techniques. Prothrombin time was determined using an automatic instrument.

Results

Patients with liver disease

The fibrotic process was related to serum MEGX concentrations and 7OHC excretion and the results of liver function tests (Figure 1, Tables 1–3). Serum MEGX concentrations were lower in patients with severe (−58.27 to −39.33, 95% confidence interval of difference), moderate (−45.04 to −23.56) and mild liver (−31.96 to −9.24) disease relative to control subjects. The patients with severe (−46.51 to −29.89) and moderate (−31.85 to −12.15) liver disease had lower 7OHC excretion at 2 h and increased excretion at 24 h relative to controls. In patients with mild liver disease 7OHC excretion was normal at 2 h (−17.50 to 4.50), but differed at 24 h (Table 2). Comparison of serum PIIINP values with MEGX and 7OHC values (Figure 2) revealed inverse correlations (r^2 = 0.485, $P < 0.01$; and r^2 = 0.373, $P < 0.05$, respectively).

Epileptic patients

In patients treated with antiepileptic drugs (Tables 1 and 2, Figure 1) 7OHC excretion was relatively rapid.

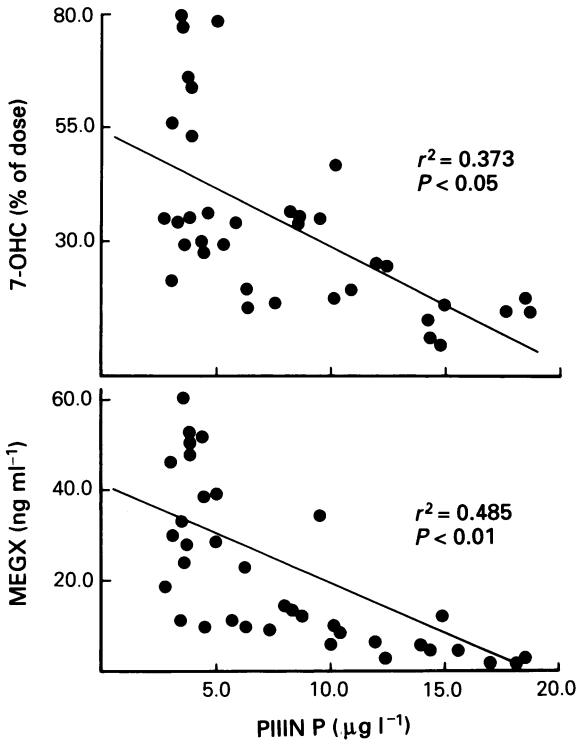

Figure 2 Relationship between serum PIIINP and serum MEGX concentrations (lower) and 2 h 7OHC excretion (upper) in patients with liver disease (n = 36).

At 2 h the patients excreted more metabolite than the controls (95% confidence interval of difference 4.19 to 22.41), and recoveries were lower than those in the controls (−24.73 to −2.07).

Patients with kidney disease

Patients with impaired kidney function had delayed 7OHC excretion compared with controls (Table 2). Recovery at 2 h was related to serum creatinine level (r = −0.854, $P < 0.001$) and creatinine clearance (r = 0.664, $P < 0.05$). The total recovery of 7OHC in the renal disease patients (78%) was similar to that in epileptic patients (81%) and controls (75%), suggesting that excretion rather than formation of 7OHC was compromised in renal impairment (Table 2). Serum MEGX concentrations were similar in patients with renal disease and controls (Table 2).

Correlations

Serum MEGX concentrations and 2 h urinary 7OHC recovery were correlated, r^2 = 0.451 ($P < 0.01$). A positive correlation between the Child-Pugh score and serum PIIINP (r^2 = 0.421, $P < 0.01$) indicated that both measures reflected liver function. The PIIINP values were more closely related to CYP isoform activities (MEGX, r^2 = 0.286, NS and 7OHC, r^2 = 0.335, $P < 0.05$; Figure 2) than were the Child-Pugh scores. Child-Pugh scores incorporating PIIINP values as sixth parameter correlated with serum MEGX (r^2 = 0.395, $P < 0.05$) and 2 h 7OHC recovery (r^2 = 0.458, $P < 0.01$).

Discussion

Major problems in the investigation of drug metabolism in liver disease include the assessment of hepatic integrity and functional capacity, the need for simple yet informative markers of functional disturbances, and the determination of the extent to which abnormal function affects drug metabolism. Clinical, biochemical and histological methods and their combinations have been utilized in various scoring systems [8, 14, 15], but no single index provides information about the nature and severity of liver disease.

The assay of collagen antigens allows evaluation of the activity of the fibrotic process, thought to be a central phenomenon in different manifestations of liver disease and injury. PIIINP, liberated during the stoichiometric conversion of type III procollagen to collagen by a specific peptidase [16–18], reflects *de novo* synthesis of type III procollagen in the liver, although the possibility that serum PIIINP is derived from breakdown of extracellular type III procollagen cannot be excluded in patients with subfulminant hepatic failure [13]. Our patients with kidney disease, who had normal liver function associated with bone marrow fibrosis, had elevated serum PIIINP values. Thus, chronic renal disease must be considered when using serum PIIINP as an index of fibrosis.

In patients with liver disease the synthesis of procollagen is stimulated by the inflammatory process, necroinflammation of hepatocytes, activation of cytokines and lymphokines and by compounds released from macrophages [19–22], phenomena associated with a decrease in drug metabolizing enzyme activity [23]. Collagen production then exceeds its regeneration by the liver and fibres together with other extracellular matrix components, non-collagenous glycoproteins and proteoglycans, accumulate in the extracellular spaces [24]. This is associated with disturbances in hepatic microcirculation even in the early phase of the process [25, 26]. In advanced liver cirrhosis, thick trabeculae distort lobular architecture and the vascular bed, leading to arteriovenous shunting [25, 26] and decreased substrate availability to already impaired enzymes.

Our data demonstrate that high PIIINP and low serum MEGX concentrations both indicate impaired liver function. Decreased enzyme activity together with vascular changes associated with the fibrotic process decrease lignocaine metabolism in patients with liver disease. However, like lignocaine, many other drugs (e.g. erythromycin, steroids, cyclosporine A, nifedipine, midazolam, phenobarbitone and carbamazepine) are metabolized by CYPA3 enzymes [27], and interactions may occur [28, 29]. Consequently, concurrent use of these drugs may lead to erroneous conclusions when using MEGX as a measure of liver function.

Coumarin is at present the only drug known to be oxidized by CYP2A6 [3]. The 7-hydroxymetabolite is conjugated with glucuronic acid and excreted rapidly into urine [5]. Our findings indicate that in patients with severe or moderate hepatic disease 7OHC excretion is delayed, indicating an impairment of coumarin metabolism. However, the 2 h coumarin test could not distinguish patients with mild liver disease from controls. Furthermore, the coumarin test is not applicable in patients with impaired kidney function.

CYP3A4 is the major cytochrome P450 enzyme in human liver [30]. It can be induced by many drugs including phenytoin [31], phenobarbitone [32] and carbamazepine [33, 34], the drugs used by our epileptic patients. Rapid 7OHC excretion in these patients relative to controls may indicate such induction of the hepatic microsomal enzyme system including also CYP2A6 isoform. However, in contrast, lignocaine *N*-deethylation was delayed. Since, we presume that these patients had normal hepatic blood flow, it is likely that this observation can be explained by competition between the anticonvulsant agents and lignocaine for CYP3A4, as seen in patients treated with erythromycin and amiodarone [8, 28, 29]. Nine out of 12 epileptic patients were treated with carbamazepine and epoxidation of this compound is mediated by CYP3A4 [35]. The use of drugs competing for CYP3A4 may delay MEGX production invalidating the MEGX test as an index of decreased liver function.

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References

1 Oellerich M, Raude E, Brudelski M, *et al.* Monoethylglycinexylidide formation kinetics: A novel approach to assessment of liver function. *J clin Chem clin Biochem* 1987; **25**: 845–853.

2 Bargetzi MJ, Aoyama T, Gonzalez FJ, Meyer UA. Lidocaine metabolism in human liver microsomes by cytochrome P450 IIIA4. *Clin Pharmac Ther* 1989; **46**: 521–527.

3 Raunio H, Syngelmä T, Pasanen M, *et al.* Immunochemical and catalytical studies on hepatic coumarin 7-hydroxylase in man, rat and mouse. *Biochem Pharmac* 1988; **37**: 3889–3895.

4 Rautio A, Kraul H, Kojo A, Salmela E, Pelkonen O. Interindividual variability of coumarin 7-hydroxylation in healthy volunteers. *Pharmacogenetics* 1992; **2**: 227–233.

5 Pelkonen O, Raunio H, Rautio A, Mäenpää J, Lang MA. Coumarin 7-hydroxylase: Characteristics and regulation in mouse and man. *J Ir Coll Phys Surg* 1993; **22** (Suppl 1): 24–28.

6 Sotaniemi EA, Niemelä O, Risteli L, *et al.* Fibrotic process and drug metabolism in alcoholic liver disease. *Clin Pharmac Ther* 1986; **40**: 46–55.

7 Sotaniemi EA, Ahlquist J, Pelkonen RO, Pirttiaho H,

Luoma P. Histological changes in the liver and indices of drug metabolism in alcoholics. *Eur J clin Pharmac* 1977; **11**: 295–303.

8 Pugh RNH, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding eosophagial varices. *Br J Surg* 1973; **60**: 646–649.

9 Hasselbach H, Junker P, Horslev-Petersen K, Lisse J, Bentsen KD: Procollagen type II aminoterminalpeptide in serum in idiopathic myelofibrosis and allied conditions. Relation to disease activity and effect of chemotherapy. *Am J Hematol* 1990; **33**: 18–26.

10 Arvela P, Leinonen A, Rautio A, Salonpää P, Sotaniemi EA, Pelkonen O. Comparison of two methods to measure lidocaine metabolite as a liver function test. (Abstract) *Sixth Southeast Asian/Western Pacific Regional Meeting of Pharmacologists*. Hong Kong 1991, p 167.

11 Fasco MJ, Cashin MJ, Kaminsky LS. A novel method for the quantitation of warfarin and its metabolites in plasma. *J liquid Chromatogr* 1979; **2**: 565–575.

12 Risteli J, Niemi S, Trivedi P, Mäentausta O, Mowat AP, Risteli L. Rapid equilibrium radioimmunossay for the amino-terminal propeptide for human type III procollagen. *Clin Chem* 1988; **34**: 715–718.

13 Sotaniemi EA, Mungan Z, Risteli L, *et al.* Serum type III procollagen and basement membrane antigens in hepatitis B virus liver disease. *Eur J int Med* 1992; **3**: 131–139.

14 Chopra S, Griffin PH. Laboratory tests and diagnostic procedures in evaluation of liver disease. *Am J Med* 1985; **79**: 221–230.

15 Popper H, Udenfriend S. Hepatic fibrosis. Correlation of biochemical and morphological investigation. *Am J Med* 1970; **49**: 707–721.

16 Rohde H, Vargas L, Hahn EG, Kalbfleisch H, Brugera M, Timpl R. Radioimmunoassay for type III procollagen peptide and its application to human liver disease. *Eur J clin Invest* 1979; **8**: 451–459.

17 Prockop DJ, Kivirikko KI, Tuderman L, Gunzman NA. The biosynthesis of collagen and its disorders. *New Engl J Med* 1979; **301**: 13–23, 77–85.

18 Risteli L and Risteli J. Non-invasive methods for detection of organ fibrosis. In *Connective tissue in health and disease*, ed Rojkind M. CRC press, Boca Raton, FL, 1990, pp. 61–98.

19 Leibovich SJ, Ross RA. Macrophage-dependent factor that stimulates the proliferation of fibroblasts in vitro. *Am J Path* 1976; **84**: 501–514.

20 Wahl SM, Wahl LM, McCarthy JB. Lymphocyte mediated activation of fibroblast proliferation and collagen production. *J Immunol* 1978; **121**: 942–949.

21 Casini A, Ricci OE, Paoletti F, Surrenti G. Immune mechanisms for hepatic fibrogenesis. T-lymphocyte-mediated stimulation of fibroblast collagen production in chronic active hepatitis. *Liver* 1985; **5**: 134–141.

22 Clement B, Loreal O, Levavasseur F, Guillongo A. New challenger in hepatic fibrosis. *J Hepatol* 1993; **18**: 1–4.

23 Saarni HU, Savolainen E-R, Sotaniemi EA. Effect of medroxyprogesterone acetate on liver collagen and drug metabolism in rats after chemical liver injury. *Res Comm Chem Path Pharmac* 1983; **42**: 61–69.

24 Lieber CS. Precursor lesions of cirrhosis. *Alcohol Alcoholism* 1983; **18**: 5–20.

25 Schaffner F, Popper H. Capillarization of hepatic sinusoids in man. *Gastroenterology* 1963; **44**: 239–242.

26 Orrego H, Medline A, Blendis LM, Rankin JG, Kresden DA. Collagenization of the disse space in alcoholic liver disease. *Gut* 1979; **20**: 673–679.

27 Wrighton SA, Stevens JC. The human hepatic cytochromes P450 involved in drug metabolism. *Crit Rev Toxicol* 1992; **22**: 1–21.

28 Siegmund JB, Wilson JH, Imhoff TE. Amiodarone interaction with lidocaine. *J cardiovasc Pharmac* 1993; **21**: 513–515.

29 Olkkola KT, Aranko K, Luurila H, *et al.* A potentially hazardous interaction between erythromycin and midazolam. *Clin Pharmac Ther* 1993; **53**: 298–305.

30 Wrighton SA, Ring BJ, Watkins PB, VandenBranden M. Identification of a polymorphically expressed member of human cytochrome p-450 III family. *Mol Pharmac* 1989; **36**: 97–105.

31 Shaw PM, Barnes TS, Cameron D, *et al.* Purification and characterization of an anticonculsant-induced human cytochrome P-450 catalysing cyclosporin metabolism. *Biochem J* 1989; **263**: 653–663.

32 Waxman DJ, Azarnoff L. Phenobarbital induction of cytochrome P-450 gene expression. *Biochem J* 1992; **281**: 577– 592.

33 Ohnaus EE, Breckenridge AM, Park BK. Urinary excretion of 6β-hydrocortisol and the time course measurement of enzyme induction in man. *Eur J clin Pharmac* 1989; **36**: 39–46.

34 Ged C, Rouilloin JM, Pichard L, *et al.* The increase in urinary excretion of 6β-hydrocortisol as a marker of human hepatic cytochrome P450IIIA induction. *Br J clin Pharmac* 1989; **28**: 373–387.

35 Pirmohamed M, Allott R, Green VJ, Kitteringham NR, Chadwick D, Park BK. Lymphocyte microsomal epoxide hydrolase in patients on carbamazepine therapy. *Br J clin Pharmac* 1994; **37**: 577–581.

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