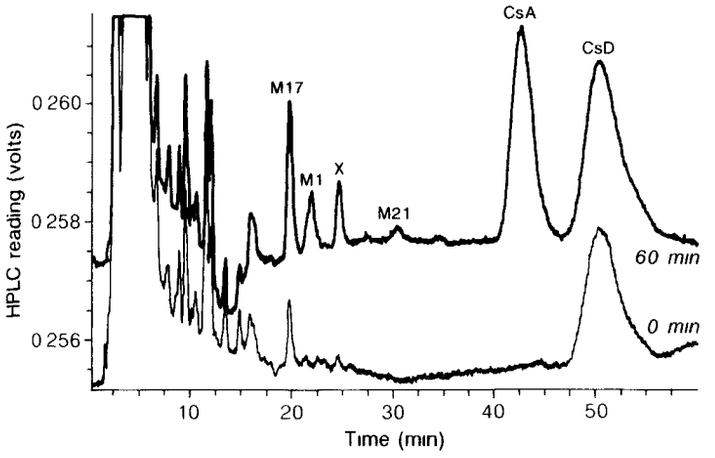
First-pass metabolism of cyclosporin by the gut

JOSEPH C. KOLARS WALID M. AWNI
ROBERT M. MERION PAUL B. WATKINS

Cyclosporin is thought to be exclusively metabolised in the liver. We instilled cyclosporin into the small bowel of 2 patients during the anhepatic phase of liver transplantation; cyclosporin metabolites were readily detected in portal venous blood. Our findings indicate that the small intestine is a major site of cyclosporin breakdown: such intestinal metabolism might help to explain the poor oral bioavailability and drug interactions of cyclosporin.



HPLC trace of portal vein blood (patient A) before and 60 min after duodenal instillation of cyclosporin during anhepatic phase of liver transplantation.

bioavailability.[1,2] Cyclosporin is widely assumed to be metabolised exclusively in the liver, but the principal enzyme (cytochrome P450IIIA) that produces the three major cyclosporin metabolites (M1, M17, and M21) in liver[3] is also found in enterocytes.[4] Could first-pass metabolism of cyclosporin in the gut account in part for its poor oral bioavailability? To test this hypothesis, we instilled cyclosporin into the small bowel of 2 patients during the anhepatic phase of liver transplantation and measured cyclosporin metabolites in portal blood.

2 patients with chronic cryptogenic cirrhosis who had not previously received cyclosporin agreed to the study, which was approved by the committee for the conduct of human research at the University of Michigan Medical School. Routine venovenous bypass was established during the anhepatic phase of orthotopic liver transplantation, and portal blood, with blood from the femoral vein, was diverted to the axillary vein with the assistance of a centrifugal pump (average flow rate 3 l/min). An enteric feeding tube was passed transnasally to the distal duodenum, through which 2 mg/kg cyclosporin ('Sandimmune' oral solution, Sandoz, East Hanover, New Jersey, USA) was given, flushed with 30 ml normal saline, and gently massaged into the proximal jejunum by the surgeon. In the first patient (A), 12 ml bile was aspirated from the gallbladder and given with cyclosporin to facilitate its absorption. 5 ml blood samples were obtained simultaneously from the portal vein cannula and from an indwelling catheter in the femoral artery immediately before cyclosporin administration. Samples were then drawn at intervals during the anhepatic phase, and stored with edetic acid at 4°C; the final samples were obtained just before the transplanted liver was reperfused. After solid-phase extraction, samples were analysed by high-performance liquid chromatography (HPLC), as previously described,[5] with purified M1, M17, M18, and M21 metabolites used as standards; this technique accurately measures cyclosporin and metabolite concentrations of and above 5 ng/ml.[5]

CYCLOSPORIN AND METABOLITE (M1 AND M21) CONCENTRATIONS IN PORTAL VEIN (PV) AND FEMORAL ARTERY (FA) AFTER DUODENAL INSTILLATION DURING ANHEPATIC PHASE

Time after instillation (min)	Cyclosporin (ng/ml)		M1 + M21 (ng/ml)	
	PV	FA	PV	FA
Patient A				
0	0	0	0	0
30	56	37	12	16
60	124	92	42	32
Patient B				
0	0	0	0	0
40	14	29	0	0
120	22	18	23	10

Cyclosporin was absorbed from the intestinal lumen during the anhepatic phase of liver transplantation and detected by 30 min in patient A (see figure) and by 40 min in patient B (see table). The low cyclosporin concentrations in blood from patient B probably reflect an absence of bile, which facilitates cyclosporin absorption from the gut lumen.[1] M1 and M21 were also readily identified in both patients; towards the end of the anhepatic phase these metabolites represented 25% and 51%, respectively, of total identifiable cyclosporin in portal blood (table). M17 could not be measured accurately by HPLC because a confounding peak in this region (see figure) was found in baseline samples from both patients.

Our findings show unequivocal and striking extrahepatic metabolism of cyclosporin. This metabolism almost certainly took place in the small intestine. At the end of the anhepatic phase, concentrations of cyclosporin metabolites were higher in portal vein samples than in blood from the femoral artery. Moreover there is little evidence of cytochrome P450IIIA (which metabolises cyclosporin to M1, M17, and M21[3,6]) in tissues other than the liver or gut, and studies in rats have shown that P450IIIA enzymes in enterocytes do metabolise cyclosporin.[7]

Our data probably underestimate total intestinal cyclosporin metabolism in both patients studied: P450IIIA also produces the M17 metabolite of cyclosporin,[3,6] which could not be measured accurately because of a baseline HPLC peak in this region. It is also likely that other HPLC peaks, such as that at 24·5 min (X on figure), also represent a cyclosporin metabolite. The gut's contribution to cyclosporin metabolism might therefore rival or even exceed that of the liver.

Several drug interactions of cyclosporin are consistent with substantial cyclosporin metabolism by P450IIIA in enterocytes. Cotreatment with oral erythromycin, a known inhibitor of P450IIIA,[8] leads to a striking increase in maximum and area-under-the-curve (AUC) blood concentrations of cyclosporin—changes which are not observed when erythromycin is given intravenously;[9] inhibition of enterocyte P450IIIA by erythromycin might allow more non-metabolised cyclosporin to be absorbed. We have also described a liver transplant recipient who had low hepatic P450IIIA activity as measured by the [^{14}C]-erythromycin breath test:[10] treatment with rifampicin to induce his donor liver P450IIIA led to a normal [^{14}C]-erythromycin breath test result, but effectively abolished oral bioavailability of cyclosporin. The suggestion that induction of enterocyte P450IIIA by rifampicin accounted for the fall in the oral bioavailability of cyclosporin[10] is supported by our finding of substantial cyclosporin metabolism in the small intestine. Enterocyte metabolism may partly account for the poor oral availability of cyclosporin and the effects of drugs and diet on cyclosporin absorption. The small intestine is rarely thought of as an important site of drug metabolism, but our observations for cyclosporin might also be relevant to the poor oral bioavailability of other substrates of cytochrome P450IIIA, including erythromycin, lignocaine, and oestrogens.

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ADDRESSES. **Departments of Internal Medicine** (J C Kolars, MD, P. B Watkins, MD) **and Surgery** (R M. Merion, MD), **University of Michigan Medical Center, Ann Arbor, Michigan, and Drug Evaluation Unit** (W M Awni, PhD), **Hennepin County Medical Center, Minneapolis, Minnesota, USA.** Correspondence to Dr Paul B Watkins, A7119 University Hospital, Kughn Clinical Research Center, University of Michigan Hospital, Ann Arbor, Michigan 48109-0108, USA

p53 germline mutations in Li-Fraumeni syndrome

MAURO F. SANTIBÁÑEZ-KOREF
JILLIAN M. BIRCH ANN L. HARTLEY
PATRICIA H. MORRIS JONES
ALAN W. CRAFT TIM EDEN
DEREK CROWTHER ANNA M. KELSEY
MARTIN HARRIS

Germline mutations within a defined region of the p53 gene have recently been found in families with the Li-Fraumeni syndrome (LFS). In the present study this region of p53 was sequenced in affected individuals from 8 families with LFS. In only 2 of them were such mutations detected. Our findings suggest that the p53 mutation could be the primary lesion in some but not all families with LFS, and confirm that there is a "hot spot" for these mutations at the CpG dinucleotide moiety of codon 248. Assigning risks and counselling families on the basis of presence of p53 mutations should be approached with caution.

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The principal features of the Li-Fraumeni syndrome (LFS) include sarcomas in children and young adults and premenopausal breast cancer in their close relatives.[1] Germline mutations within a defined region of the p53 gene have recently been found in affected members and obligate carriers in families with LFS.[2,3] These mutations were located in a stretch of 25 codons evolutionarily conserved.[2] In sporadic tumours, this region of the gene often contains a mutation.[4] The aim of our study was to see how common these mutations might be in such families.

INDIVIDUALS TESTED FROM EACH FAMILY

Patient	Cancer	Age at diagnosis (yr)	DNA source	p53 status
Family 1				
II 2	Liposarcoma	39	Peripheral blood	Wt
III 2	ALL	15	Peripheral blood	Wt
Family 2				
III 2	Bilateral breast	25	Peripheral blood, lympho-blastoid cell line	Mut.248/Wt CGG→CAG (Arg→Gln)*
	Leiomyosarcoma	44		
IV 1	Medulloblastoma	3	Fixed tissue	
	Osteosarcoma	8		
Family 3				
III 3	Bilateral breast	44	Peripheral blood	Wt
Family 4				
IV 5	ALL	14	Fibroblasts	Wt
Family 5				
II 4	Rhabdomyo-sarcoma	3	Peripheral blood	Mut.248/Wt CGG→TGG (Arg→Trp)*
	Chondrosarcoma	16		
I 2	Breast	33	Fixed tissue	
Family 6				
III 2	Adrenocortical	1	Peripheral blood	Wt
	Rhabdomyo-sarcoma	15		
Family 7				
III 3	Synovial sarcoma	25	Peripheral blood	Wt
Family 8				
IV 1	Liposarcoma	30	Peripheral blood	Wt
	Breast carcinoma	35		
III 6	Rhabdomyosarcoma	52	Peripheral blood	Wt

ALL = acute lymphoblastic leukaemia, Mut 248 = mutation at codon 248 (full pedigrees of families tested are available from J M B); Wt = wild type
*Both patients

Families were eligible for the study if they fulfilled the criteria defined by Li et al.[5] Blood samples were taken from at least 1 affected member of 8 such families. DNA was extracted from blood or lymphoblastoid cell lines and from paraffin-embedded tissue.[6,7] The conserved region in the 7th exon of p53 was amplified with the oligonucleotides GTTGTCTCCTAGGTGGCTC and TGGCAAGTGGCTCCTGACCT. Amplification was done in the presence of 1·5 μmol/l $MgCl_2$, 50 μmol/l of each dNTP, and 1 μmol/l of each oligonucleotide for 30 to 35 cycles (94°C, 1 min; 58°C, 1 min; and 74°C, 1 min): the first cycle was preceded by a step at 95°C for 5 min after which 2 units of *Taq* polymerase were added. The final elongation step was extended by 5 min.

The amplified product was phosphorylated with T4-polynucleotidekinase and ATP, filled in by means of Klenow enzyme (DNA polymerase 1, large fragment) and dNTP, ligated to *Sma*I-cut dephosphorylated M13mp11 (Amersham, UK), and cloned into *Escherichia coli* (strain XL1 Blue, Stratagene, La Jolla, USA) by standard methods.[6] For at least 1 individual of each family a minimum of eight clones from at least two independent amplifications were sequenced with the 'Multiwell Sequencing System' (Amersham). Direct sequencing[8] was done to confirm the presence of mutations in other affected members of the families in which mutations were found. For family 8, material from 16 members (affected and unaffected) was available and a polymorphism in exon 4 of p53 was analysed.[9] This polymorphism was detected by amplification with the pair of oligonucleotides CCCGGACGATATTGAACAATGGT and CCAGA-CGGAAACCGTAGCTGC before digestion of the amplified product with *Bst*UI (Boehringer, Mannheim, Germany).