The possibility that dexfenfluramine (especially at high doses) might be toxic to 5-HT neurons in the human brain needs to be investigated.

We thank Lynda Roggio for her technical assistance. This work was supported by NIDA grant DA05707 to G. A. R., NIDA grant DA04341 to M. E. M., and by a grant from the Retirement Research Foundation.

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First-pass metabolism of cyclosporin by the gut

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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.


Administration of cyclosporin, a potent immuno-suppressant used to prevent transplanted organ rejection, is complicated by its poor and unpredictable oral

Serotonin-immunoreactive axons in somatosensory cortex.

Left panel: control monkey. Right panel: monkey given subcutaneous 5 mg/kg dexfenfluramine twice daily for four days two weeks previously. Abnormalities were not seen in controls. Two weeks after treatment, there was a striking reduction in density of 5-HT axons in the cerebral cortex of dexfenfluramine-treated monkeys (figure); 5-HT axons with abnormal features were uncommon. Our results indicate that at the doses studied dexfenfluramine is toxic to 5-HT neurons in the brain of non-human primates. Furthermore, dexfenfluramine neurotoxicity is dose-related and more severe in primates than in rodents. Since the 1-25 mg/kg dose is only about five times that typically prescribed to patients (0-2 to 0-3 mg/kg), we believe that caution is necessary in the clinical use of dexfenfluramine because, in man, it might have a narrow margin of safety. However, in our study, dexfenfluramine was given subcutaneously (in man the drug is taken orally) and there may be differences in the distribution, metabolism, or elimination of dexfenfluramine in primates compared with man.

Despite these uncertainties, there are several reasons why our findings might be relevant to the clinical use of dexfenfluramine. Firstly, since the oral to parenteral ratio for fenfluramine and related neurotoxic amphetamines approaches unity, an oral route of administration is unlikely to give substantial protection against neurotoxicity. Secondly, although doses of dexfenfluramine used in our study are several-fold higher than doses typically prescribed to patients, man could be more sensitive than animals to the effects of this drug. Thirdly, there is great individual variability in the pharmacokinetics of dexfenfluramine in man: a safe dose in one patient could be neurotoxic in another. Fourthly, in our study, animals received dexfenfluramine for only four days, whereas patients usually receive the drug for twelve weeks, and sometimes for a year. Finally, since the half-life of dexfenfluramine is much longer in man (17-8 h) than in rodents (2-5 h), there is greater risk to patients, man could be more sensitive than animals to the effects of this drug.

If dexfenfluramine produces 5-HT neurotoxicity in man, what might the clinical consequences be? Although the function of 5-HT in human brain has yet to be fully defined, clinical evidence suggests that 5-HT loss may lead to disturbances of mood, sleep, impulse control, sexual activity, and neuroendocrine function. These changes in 5-HT function may be subtle and difficult to detect. Systematic studies of 5-HT function in man are needed, and the possibility that dexfenfluramine (especially at high doses) might be toxic to 5-HT neurons in the human brain needs to be investigated.
Concentrations of and above 5 ng/ml. The low cyclosporin concentrations in blood from patient B probably reflect an absence of bile, which facilitates cyclosporin absorption from the gut lumen. 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 P450III(A) (which metabolises cyclosporin to M1, M17, and M21) in tissues other than the liver or gut, and studies in rats have shown that P450III(A) enzymes in enterocytes do metabolise cyclosporin.

Our data probably underestimate total intestinal cyclosporin metabolism in both patients studied: P450III(A) also produces the M17 metabolite of cyclosporin, 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 P450III(A) in enterocytes. Co-treatment with oral erythromycin, a known inhibitor of P450III(A), 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, 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 [14C]-erythromycin breath test, treatment with rifampicin to induce his donor liver P450IIIA led to a normal [14C]-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 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.

REFERENCES

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 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 polymorphism.9 The aim of our study was to see how common these mutations might be in such families.

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 MgCl₂, 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-polynucleotide kinase and ATP, filled in by means of Klenow enzyme (DNA polymerase 1, large fragment) and dNTP, ligated to SmaI-cut dephosphorylated M13mp18 (Amersham, UK), and cloned into Escherichia coli (strain XL1 Blue, Stratagene, La Jolla, USA) by standard methods. 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 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 CCCGGAGGATTTGGAATCT and CCGGAAACCGTAGCTG before digestion of the amplified product with BstUI (Boehringer, Mannheim, Germany).

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.


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