REFERENCES

1. L. T. Williams, D. Mullikin and R. J. Lefkowitz, *J. biol Chem.* **253**, 2984 (1978).
2. K. H. Jakobs, K. Aktories, M. Minuth and G. Schultz, *Adv. Cyclic Nucleotide Res.* **19**, 137 (1985).
3. S. M. Periyasamy and P. Somani, *Fedn Proc.* **45**, 563 (1986).
4. S. M. Periyasamy and P. Somani, *Eur. J. Pharmac.* **116**, 17 (1985).
5. S. J. Korn, M. W. Martin and K. Harden, *J. Pharmac. exp. Ther.* **224**, 118 (1983).
6. J. W. Wei and P. V. Sulakhe, *Naunyn-Schmiedeberg's Archs Pharmac.* **314**, 51 (1980).
7. N. E. Larsen, D. Mullikin-Kilpatrick and A. Blume, *Molec. Pharmac.* **20**, 255 (1981).
8. N. Ferry, S. Adnot, A. Borsodi, M. L. Lacombe, G. Guellan and J. Hanoune, *Biochem. biophys. Res. Commun.* **108**, 708 (1982).
9. S. Y. Cech and M. E. Maguire, *Molec. Pharmac.* **22**, 267 (1982).
10. S. Y. Cech, W. C. Broaddus and M. E. Maguire, *Molec. cell. Biochem.* **33**, 67 (1980).
11. K. K. McMahon and M. M. Hosey, *Molec. Pharmac.* **28**, 400 (1985).
12. K. Aktories, G. Schultz and K. H. Jakobs, *Biochim. biophys. Acta* **676**, 59 (1981).
13. J. J. Mooney, W. C. Horne, R. I. Handin, J. J. Schildkraut and R. W. Alexander, *Molec. Pharmac.* **21**, 600 (1982).
14. L. E. Limbird and J. L. Speck, *J. Cyclic Nucleotide Res.* **9**, 191 (1983).

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Liver regeneration and hepatic microsomal changes in rats administered cyclosporin A

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Cyclosporin A (CsA), a cyclic immunosuppressant, is widely used in the transplantation of organs, including the liver. Generally, hepatotoxicity is less involved than nephrotoxicity (which can be ameliorated to some extent by dosage reduction and substitution therapy). Hepatic changes, notably hyperbilirubinemia, have been reported in human organ recipients on CsA [1–4], in dogs with orthotopic liver transplants [5] and in rats over periods of 21–49 days [6, 7] or with isolated rat liver perfusion [8]. It has been suggested that liver dysfunction in human organ recipients may stem from intercurrent infection [9]. The metabolites of CsA in humans, and in species such as the dog and the rat, retain the basic cyclic oligopeptide structure, the most prominent biotransformations being hydroxylation alone and in conjunction with N-demethylation [10]. Microsomal mixed-function oxidase system elements in rats administered 50 mg of CsA per kg, daily for 14 days, were followed by Cunningham *et al.* [11]; cytochrome P-450 decreased slightly, whereas aminopyrine *N*-demethylase (APdM) was little affected. These variables were elevated markedly in animals treated also with Aroclor 1254, an enzyme inducer. For 7 weeks, at an oral dose of CsA of 25 mg/kg daily, cytochrome P-450 was unchanged; APdM decreased during the first 4 weeks, rose during remission, and fell during the relapse period in relation to nephrotoxic effects. The levels of NADPH–cytochrome *c* reductase activity paralleled APdM activity from week 3 onward [12]. In another report by Cunningham *et al.* [13], enzyme induction in the presense of CsA ensued with continued i.p. injection of phenobarbital just as with Aroclor 1254, but not with 3-methylcholanthrene. Augustine and Zemaitis [14] corroborated in the rat, several of the above findings concerning cytochrome P-450 and demonstrated a type I binding spectrum for CsA. Depression of aniline hydroxylase was greater than that of APdM as observed by Cunningham *et al.* [11], and NADPH–cytochrome *c* reductase was most sensitive to the action of CsA at oral doses of 25 or 50 mg/kg daily for 9 days, whereas protein and cytochrome b_5 levels were not influenced appreciably at either level. CsA has also been shown to inhibit cytochrome P-450 and the enzymes, APdM and benzo[*a*]pyrene hydroxylase (BPH), in male Swiss mice [15]. With heightened hepatic microsomal drug metabolism or enzyme induction, concomitant nephrotoxicity of CsA appears to diminish as a result of a lower drug level in blood. Effects of enzyme induction are also apparent with phenytoin [4, 16] but not with inhibitors such as ketoconazole [17–19] and melphalan [18].

The current report describes findings on CsA in relation to the extent of liver regeneration in partially hepatectomized rats and hepatic microsomal variables as such and after enzyme induction by phenobarbital. The data are compared with those for intact animals. The intention of this study was to delineate liver regenerative changes that may be applicable to human post-transplantation grafts. According to a recent communication [20], CsA does not affect hepatic thymidine kinase or ornithine decarboxylase in Fischer male rats over a period of 48 hr after partial heptectomy and, in fact, pretreatment with CsA leads to potentiation of their levels.

Materials and methods

CsA, furnished by Sandoz, Inc., East Hanover, NJ, was dissolved in olive oil heated to 60° to yield a solution of 15.0 mg/ml. Phenobarbital was dissolved in low alkaline pH medium (80 mg/ml). Male rats of the Charles River (COBS) strain, averaging 300 g in weight, were partially hepatectomized in the mid-morning under ether anesthesia; two-thirds of the organ was removed [21] and dried to constant weight at 100°. All animals were housed in individual cages and given Purina rat meal and water *ad lib.* CsA (25 $mg \cdot kg^{-1} \cdot day^{-1}$) was administered by gavage in a volume of 0.5 ml for the first 7 days; controls received the vehicle. At least four animals of each group were injected with phenobarbital i.p. (80 $mg \cdot kg^{-1} \cdot day^{-1}$) on days 7–9, inclusive. All rats were killed by exsanguination on day 10, at which time the entire livers were removed, drained and weighed; tared amounts were deep-frozen for microsomal studies and the remaining organ was dried as stated above. Very small sections were reserved for microscopic examination. The amount of tissue regeneration (liver increment, g) was calculated from the dry weights at surgery (L_s) and necropsy (L_n) according to: $L_n - (0.46)L_s$ [22]. Other males from the same shipment were treated similarly except that they were not sham-operated; wet and dry liver weights as g/100 g body weight were measured for each rat. The precipitation method of Schenkman and Cinti [23] was used to isolate liver microsomes. Procedures for analyses of protein and nucleic acid contents of homogenates and subfractions as well as of cytochrome P-450

and the enzymes, APdM and BPH, were the same as those detailed previously [24].

Results and discussion

Figure 1 presents liver increment, microsomal protein, cytochrome P-450 and enzyme levels, for operated rats treated with CsA alone and in conjunction with phenobarbital and the phenobarbital controls, expressed as percentages of the oil-fed controls. The respective microsomal findings and wet liver weight percentages for the intact rats appear in Fig. 2. The non-enzyme-induced groups contained nine to thirteen rats, and the percentage body weight changes over the period of 10 days for oil controls, CsA, CsA + phenobarbital, and phenobarbital controls were 0.0, −8.4, −11.1 and −6.1, respectively, for the operated groups and 0.0, −5.5, 4.2 and 4.9, respectively, for the intact animals. The dosage and route of administration of CsA used promote pharmacological activity in the rat [25, 26] and, of a number of toxicological responses, weight loss, possibly due to anorexia, is quite prominent in this species [27].

CsA did not produce significant changes in liver increment nor in any of the microsomal variables, compared to the respective operated controls (Fig. 1). The phenobarbital-injected controls elicited a small but definite rise in cytochrome P-450 in contrast to phenobarbital + CsA. However, the latter appeared to be responsible for increases in APdM and BPH, the elevations with the phenobarbital controls just lacking statistical significance at the 5% level of probability. Both phenobarbital-injected groups were equally hepatotrophic, based on the liver increments; neither treatment caused any remarkable change in microsomal protein content.

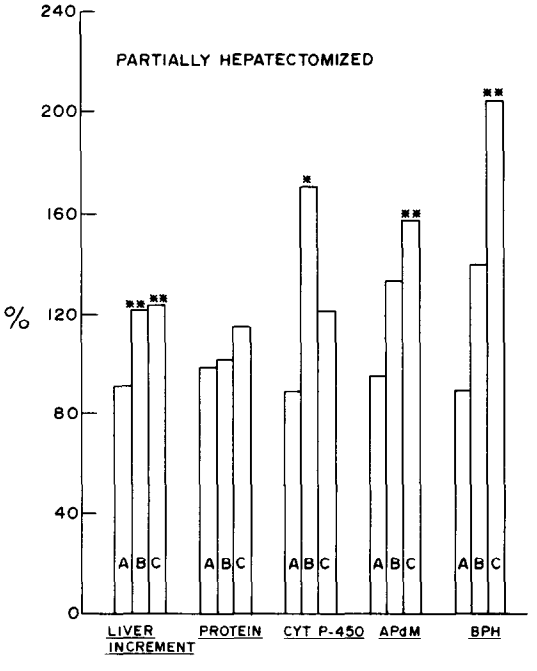

Fig. 1. Liver changes for operated rats as percentages of controls and based on the units (each per g wet liver): mg and nmol for microsomal protein and cytochrome P-450. respectively, and nmol product/min for the enzymes. The increment (g) was calculated from the dry weights of tissue removed at surgery and 10 days later, at necropsy. Treatments: A, CsA (N = 10); B, controls induced with phenobarbital (N = 4); and C, CsA + phenobarbital (N = 4). Variance per group was low to moderate on the basis of the SE of each mean. By analysis of variance, differences from the means of thirteen oil-fed controls are statistically significant where designated as: $^{*}P < 0.05$ and $^{**}P < 0.01$.

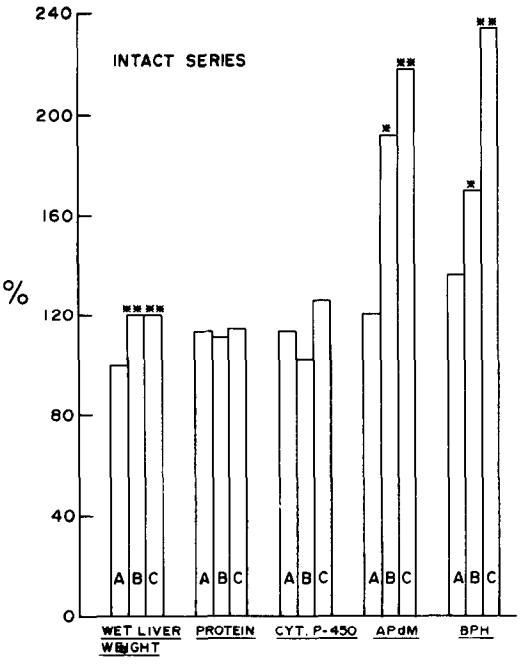

Fig. 2. Liver variables for unoperated males as in the legend of Fig. 1 except that for the oil-fed control group, N = 9. The dry liver weight percentages paralleled those shown for the wet organs. Key: $^{*}P < 0.05$ and $^{**}P < 0.01$.

As in the operated series, CsA did not affect the control liver weights or the microsomal components (Fig. 2). The two phenobarbital-treated groups displayed liver enlargement and heightened APdM and BPH activities, the respective values being greater for CsA + phenobarbital than for the phenobarbital controls in relation to the enzyme levels of the oil-fed controls, but the microsomal protein and cytochrome P-450 contents were not altered significantly.

To evaluate the contribution, if any, of CsA when given in conjunction with phenobarbital, comparisons were carried out between the phenobarbital and CsA + phenobarbital groups for each liver variable in both the operated and intact series. Only one difference proved to be significant; BPH of the partially hepatectomized rats on the combined treatment ranged higher than that of the induced controls (df, 8; Fisher *t*-value, 3.27; $P < 0.02$). With intact rats fed twice the dose of the undecapeptide for 14 days, Cunningham *et al.* [11] reported that CsA increases the inductive action of Aroclor 1254 on cytochrome P-450 but, in a later study employing similar conditions [13], that it reduces Aroclor and phenobarbital induction of cytochrome P-450 and APdM.

In summary, CsA had no significant action on the extent of liver regeneration in the partially hepatectomized male rat or on liver weight changes in the intact animal at the dosage studied. Except for a few components depressed by the drug, such as NADPH–cytochrome *c* reductase reported by others [13, 14], CsA had little influence on hepatic microsomal mixed-function oxidase elements such as cytochrome P-450 levels and APdM and BPH activities. These variables were induced in the presence of phenobarbital and, except for BPH in the operated rats, CsA

had no remarkable effect on the increases. As might be expected from the dosage and short experimental interval, the liver sections displayed no unusual light microscopic changes compared with the control groups. Further studies might explore wider CsA dosage ranges in several species of either sex, isolation of the relevant molecular form of cytochrome P-450, and the nature of the drug binding sites of hepatocytes from the organs of operated and intact rats, possibly, by such approaches as photoaffinity labeling [28]. Although the liver, following partial removal, is unique in that regenerative changes take precedence over those involved in drug detoxification [29–32], our findings with CsA in the operated animal were similar to those found with the intact rat.

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REFERENCES

1. G. G. C. Klintmalm, S. Iwatsuki and T. E. Starzl, *Transplantation* **32**, 488 (1981).
2. R. Loertscher, M. Wenk, F. Harder, F. Brunner, F. Follath and G. Thiel, *Lancet* **ii**, 635 (1981).
3. R. Loertscher, G. Thiel, F. Harder and F. P. Brunner, *Transplantation* **36**, 115 (1983).
4. P. A. Keown, C. R. Stiller, A. L. Laupacis, W. Howson, R. Coles, M. Stawecki, J. Koegler, G. Carruthers, N. McKenzie and N. R. Sinclair, *Transplantn Proc.* **14** (Suppl. 4), 659 (1982).
5. J. W. Williams, T. G. Peters, R. Haggitt and S. VanVoorst, *Surgery Gynec. Obstet.* **156**, 767 (1983).
6. P. H. Whiting, J. G. Simpson, R. J. L. Davidson and A. W. Thomson, *Br. J. exp. Path.* **64**, 437 (1983).
7. A. W. Thomson, P. H. Whiting, J. T. Blair, R. J. L. Davidson and J. G. Simpson, *Transplantation* **32**, 271 (1981).
8. F. S. Rotolo, G. D. Branum, B. A. Bowers and W. C. Meyers, *Am. J. Surg.* **151**, 35 (1986).
9. S. Rodger, J. H. Turney and I. Haynes, *Transplantation* **36**, 451 (1983).
10. G. Maurer, H. R. Loosli, E. Schreier and B. Keller, *Drug Metab. Dispos.* **12**, 120 (1984).
11. C. Cunningham, P. H. Whiting, M. D. Burke, D. N. Wheatley and J. G. Simpson, *Transplantn Proc.* **15** (Suppl. 1), 2712 (1983).
12. C. Cunningham, M. P. Gavin, P. H. Whiting, M. D. Burke, F. Macintyre, A. W. Thomson and J. G. Simpson, *Biochem. Pharmac.* **33**, 2857 (1984).
13. C. Cunningham, M. D. Burke, D. N. Wheatley, A. W. Thomson, J. G. Simpson and P. H. Whiting, *Biochem. Pharmac.* **34**, 573 (1985).
14. J. A. Augustine and M. A. Zemaitis, *Drug Metab. Dispos.* **14**, 73 (1986).
15. S. M. Moochhala and K. W. Renton, *Biochem. Pharmac.* **35**, 1499 (1986).
16. P. A. Keown, A. Laupacis, G. Carruthers, M. Stawecki, J. Koegler, F. N. McKenzie, W. Wall and C. R. Stiller, *Transplantation* **38**, 304 (1984).
17. R. M. Ferguson, D. E. R. Sutherland, R. L. Simmons and J. S. Naiarian, *Lancet* **ii**, 882 (1982).
18. G. R. Morgenstern, R. Powles, B. Robinson and T. J. McElwain, *Lancet* **ii**, 1342 (1982).
19. D. J. G. White, N. R. Blatchford and G. Cauwenbergh, *Transplantation* **37**, 214 (1984).
20. L. Makowka, G. Svanas, C. Esquivel, R. Venkataramanan, S. Todo, S. Iwatsuki and T. E. Starzl, *Surg. Forum* **37**, 352 (1986).
21. G. M. Higgins and R. M. Anderson, *Archs Path.* **12**, 186 (1931).
22. L. L. Gershbein and J. A. Labow, *Am. J. Physiol.* **173**, 55 (1953).
23. J. B. Schenkman and D. L. Cinti, in *Methods in Enzymology* (Eds. S. P. Colowick and N. O. Kaplan), Vol. 52, pp. 83–9. Academic Press, New York (1978).
24. A. C. White and L. L. Gershbein, *Res. Commun. Chem. Path. Pharmac.* **48**, 275 (1985).
25. K. Nooter, F. Schultz and P. Sonneveld, *Res. Commun. Chem. Path. Pharmac.* **43**, 407 (1984).
26. R. Wassef, Z. Cohen and B. Langer, *Transplantation* **40**, 489 (1985).
27. M. J. G. Farthing, M. L. Clark, A. Pendry, J. Sloane and P. Alexander, *Biochem. Pharmac.* **30**, 3311 (1981).
28. K. Ziegler and M. Frimmer, *Biochim. biophys. Acta* **855**, 147 (1986).
29. J. R. Fouts, R. L. Dixon and W. R. Schultice, *Biochem. Pharmac.* **7**, 265 (1961).
30. T. Hilton and A. C. Sartorelli, *J. biol. Chem.* **245**, 4187 (1970).
31. P. T. Henderson and K. J. Kersten, *Biochem. Pharmac.* **19**, 2343 (1970).
32. E. Roberts, M. B. Ahluwalia, G. Lee, C. Chan, D. S. R. Sarma and E. Farber, *Cancer Res.* **43**, 28 (1983).

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Influence of the sulphation inhibitor, 2,6-dichloro-4-nitrophenol, on the production, and conjugation, of 4-hydroxybiphenyl generated from 4-methoxybiphenyl by rat isolated hepatocytes

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A number of aromatic xenobiotics are metabolized by the cytochrome P450-dependent microsomal monooxygenase (MMO)* system leading to the production or unmasking of a phenolic functional group which is followed by conjugation with glucuronic acid or sulphate at the newly generated phenolic group. It is recognised that the balance of these two phases of metabolism may play an important role in determining the overall biological effect of the parent xenobiotic, and for this reason it is important to identify the factors that might influence the balance of these two phases of metabolism.

* Abbreviations used: DCNP, 2,6-dichloro-4-nitrophenol; 4-MBP, 4-methoxybiphenyl; MMO, microsomal monooxygenase; 4-OHBP, 4-hydroxybiphenyl.