EFFECT OF CYCLOSPORINE A ON CYTOCHROME P-450-MEDIATED DRUG METABOLISM IN THE PARTIALLY HEPATECTOMIZED RAT

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(Received June 12, 1998; accepted January 11, 1999)

This paper is available online at http://www.dmd.org

ABSTRACT:

Despite its hepatotoxic potential, cyclosporine A (CsA) has been reported to positively influence compensatory liver growth. To probe the physiological consequences of CsA on the recovery of liver function, studies were initiated in the 2/3 partially hepatectomized (PHx) rat, taking the recovery of cytochromes P-450-dependent drug metabolism as primary outcome. CsA was administered at a dose of 3.33 mg/kg/day for 10 days. Drug metabolism was evaluated by the recovery of 14CO₂ after administration of isotopically labeled model drugs and by studying the expression of the P-450 transcripts involved in their biotransformation before and 24 to 96 h after PHx. Before PHx, neither the steady-state mRNA nor the in vivo disposition of caffeine (CYP1A2), erythromycin (CYP3A2 and 3A1), or aminopyrine (CYP2B1 and 2C11) were influenced by CsA. Studies 24 h after PHx revealed a 29 to 39% reduction in the elimination of [14C]aminopyrine and [14C]erythromycin, which was unaffected by CsA. Their metabolism at 48 to 96 h after PHx also remained unaffected by CsA. By contrast, postPHx, [14C]caffeine elimination decreased to a level closely proportional to the loss in liver mass. In addition, CsA accelerated the recovery and/or prevented the decrease of caffeine elimination 24 h after PHx but not at later time points, indicating an early, but unsustained, beneficial effect of CsA on the recovery of CYP1A2-mediated activities. These data show that at the critical time of greatest loss in liver mass, CsA has only a selective influence on the biotransformation of cytochrome P-450 protein-dependent activities and that its effect on the regeneration process does not translate into an overall accelerated recovery of the hepatic drug-metabolizing function.

Several investigators have reported that cyclosporine A (CsA)¹ can positively influence the liver regeneration process after 2/3 partial hepatectomy (PHx) in laboratory animals (Garcia-Alonso et al., 1989; Francavilla et al., 1990; Mazzaferro et al., 1990; Kahn et al., 1990; Tanaka et al., 1993). However, despite numerous reports indicating an increase in the hepatic regeneration phenomenon, several studies have noticed that the liver weight restitution was not influenced by CsA in the postPHx period (Makowka et al., 1986; Kahn et al., 1990; Provencher et al., 1997). Moreover, CsA has also been shown to induce oxidative stress in rat hepatocytes (Wolf et al., 1997), to increase the growth of carcinoigen-induced liver foci (Yabu et al., 1991; Masuhara et al., 1993), to inhibit both the hepatic uptake and synthesis of bile acids (Kukonguiriyapan and Stacey, 1988; Levy et al., 1994), and to lead to cholestasis (Stone et al., 1987; Kassianides et al., 1990). These observations indicate that, in addition to its reported trophic effect on the hepatic regeneration process, CsA also harbors the potential for inducing serious hepatic side effects, which, despite a CsA-mediated acceleration in the regeneration process, could dampen the recovery of hepatic function after loss of liver mass.

The liver is the major site of drug metabolism and CsA administration has been reported to compete with drugs metabolized by CYP3A2 (rat; Zhang and Thomas, 1996) and CYP3A4 (human; Pichard et al., 1990), the two cytochromes P-450 primarily involved in the biotransformation of CsA (Combalbert et al., 1989; Prueksaritanont et al., 1993). By contrast, CsA has been reported not to influence per se the in vivo biotransformation of xenobiotics metabolized by other cytochromes P-450 as exemplified by studies on the N-demethylation of aminopyrine and the level of CYP2B2, 2C6, 2C11, and 2C13 in the rat (Gershbein, 1987; Isogai et al., 1993; Beckurts and Lauterburg, 1995). Although an important hepatic reserve exists for several biotransformation activities, a loss in liver mass secondary to either the progression of hepatic diseases, or toxic or surgical aggression is usually accompanied by a loss, albeit not always of the same magnitude (Rikkers and Moody, 1974; Lauterburg and Bircher, 1976; Sendama et al., 1985), in the liver-metabolizing capacity. Despite a steady stream of studies reporting a positive effect of CsA on hepatic compensatory hyperplasia, the physiological consequences of the CsA-mediated increase on the regeneration process have not been evaluated. The purpose of the present studies was, therefore, to investigate the influence of CsA on the functional recov-

¹ Abbreviations used are: CsA, cyclosporine A; 3-MC, 3-methylcholanthrene; ALT, alanine aminotransferase; βNF, β-naphthoflavone; CYP, cytochrome P-450 gene; CYP, cytochrome P-450 protein; DEX, dexamethasone; PB, phenobarbital; PHs, sham-operated animals; PHx, 2/3 partial hepatectomy/hepatectomized.
ery of the liver after PHx, taking the behavior of three cytochrome P-450 (CYP)-dependent activities as the primary outcome of the studies. We now report that in the postPHx period, CsA has no influence on the recovery of CYP2B1, 2C11, 3A1, and 3A2-dependent activities and their corresponding hepatic steady-state mRNA levels. By contrast, caffeine metabolism, a CYP1A2-dependent substrate, was shown to be increased by CsA 24 h after PHx but not at later time points.

Materials and Methods

Animal Treatment and Drug Regimen. The studies were designed to evaluate the effect of CsA administration on the recovery of the phase 1 drug-metabolizing function after PHx in the rat, a well characterized model of liver regeneration (Michalopoulos and DeFrances, 1997). Male Sprague-Dawley rats weighing 200 to 250 g were used in all studies. All animals used in this study were treated in accordance with the standards of ethics for animal experimentation of the Canadian Council on Animal Care. All protocols were approved by a local animal ethics committee.

A preliminary dose- and time-response study established that doses of 3.33 to 10 mg/kg/day CsA had similar effects on DNA synthesis, whereas a 20 mg/kg dose inhibited DNA synthesis after PHx. Further studies for periods of 3, 6, 9 (dose of 3.33 mg/kg/day), and 12 (doses of 3.33 and 10 mg/kg/day) days were found to have a similar effect of the regeneration process. The lowest effective dose (3.33 mg/kg/day) was chosen for all subsequent studies with a 10-day pretreatment period before partial liver resection. Drug administration was continued at the same dose during the postresection period. CsA was diluted in olive oil and administered i.p. Placebo-treated controls received the vehicle only and were subjected to the same experimental regimen as CsA-treated rats.

Partial hepatectomy was performed under light isoflurane anesthesia after pretreatment with CsA or placebo. A 3-cm abdominal midline incision was made and the median and left lobes of the liver were extruded and excised (PHx; Higgins and Anderson, 1931), or returned to the abdominal cavity (sham operation). The animals were then returned to their cages and had access to food and water ad libitum.

Parameters Indicative of Drug-Metabolizing Enzyme Activities. [14C]Aminopyrine, [14C]erythromycin, and [14C]caffeine metabolism in vivo. The influence of CsA administration on the in vivo disposition of [14C]aminopyrine, [14C]erythromycin, and [14C]caffeine (all obtained from NEN-DuPont Canada Inc., Markham, Ontario) was evaluated before PHx as well as at several time points (24–96 h) after liver resection. [Dimethylamine-14C]aminopyrine (0.25 μCi; sp. act. 99 mCi/mmol), [N-methyl-14C]erythromycin (0.25 μCi; sp. act. 55 mCi/mmol), or [1-methyl-14C]caffeine (0.50 μCi; sp. act. 53.3 mCi/mmol) elimination was measured essentially as described by Villeeneuve et al. (Villeneuve et al., 1978). Briefly, the compounds were injected i.v. under light isoflurane anesthesia. Rats were then immediately allowed to awaken and were housed in individual air-tight cages. Exhaled14 CO2 was first measured with a multianalyzer system (Paramax, Dade County, Brea, CA) in normal rat blood were linearly correlated with respect to expected CsA concentrations. The circulating concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (AP) were measured with a multianalyzer system (Paramax, Dade County, Brea, CA) in the Clinical Biochemistry department of our hospital. Statistical Analysis. Results are expressed as means ± S.E.M. Statistically significant differences between group means were evaluated by analysis of variance or by the Student’s t test as indicated in the table and figure legends.
3A2, 3-MC increased mRNA levels of CYP1A2, PB increased mRNA levels of CYP2B1, whereas βNF increased those of CYP1A2. Cyclophilin (the CsA-binding protein) mRNA levels were unaffected by CsA administration (data not shown).


**TABLE 1**

Influence of CsA on the circulating biochemical profile, body weight and liver weight

<table>
<thead>
<tr>
<th>Treatment</th>
<th>AST (U/l)</th>
<th>ALT (U/l)</th>
<th>AP (U/l)</th>
<th>CsA (ng/l)</th>
<th>B.Wt. (g)</th>
<th>Liver Weight (g/100 g b.wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>95 ± 4</td>
<td>30 ± 3</td>
<td>295 ± 24</td>
<td>N/D</td>
<td>6 ± 3</td>
<td>295 ± 6</td>
</tr>
<tr>
<td>CsA</td>
<td>87 ± 4</td>
<td>27 ± 4</td>
<td>282 ± 16</td>
<td>727 ± 77</td>
<td>253 ± 6</td>
<td>4.10 ± 0.14</td>
</tr>
<tr>
<td>p</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td></td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

Animals received CsA (3.33 mg/kg/day × 10 days) or olive oil (control). AST, ALT, and AP were determined in fresh serum while Ca²⁺ and CsA were analyzed in whole blood of sham-operated animals. n = 8/group except for CsA determination, where n = 3. N/D indicates not detectable. Statistically significant differences between group means were evaluated by the Student’s t test.

**FIG. 1.** Influence of CsA administration and PHx on the level of the hepatic CYP1A2, 2B1, 2C11, 3A1, and 3A2 mRNAs.

A representative Northern blot analyses of the hepatic CYPs. All measurements were done in individual animals after placebo (control rats receiving olive oil; lanes 1 and 3) administration, or CsA (lanes 2 and 4). At day 10 after the beginning of CsA administration, animals were subjected to sham operation (lanes 1 and 2) or to a 2/3 partial liver resection (lanes 3 and 4) and CYP mRNA levels were measured 48 h after PHx. Poly(A)⁺-enriched RNA (5 μg/lane) of individual rats were used for all samples. GAPDH mRNA levels were used to control for unequal loading of poly(A)⁺ RNA on the gels. Results are representative of at least three separate experiments per group in sham-operated rats and at least seven experiments per group in PHx rats. B, densitometric evaluation of mRNA levels obtained in the Northern analyses. Placebo □, CsA-treated rats □. N.D. indicates not detectable; PHs indicates sham-operated animals; PHx indicates partially hepatectomized animals. Statistically significant differences between group means were evaluated by analysis of variance. Effect of PHx, p < .001; intragroup test post hoc Bonferroni-Dunn: significantly different from PHs, *p < .03, **p < .009, ***p < .0001.
elimination before surgery. Partial hepatectomy similarly decreased the elimination of [14C]aminopyrine and [14C]erythromycin in placebo-control and CsA-treated animals but CsA administration did not significantly accelerate the biotransformation recovery of either substrate as illustrated in Figs. 3 and 4 and Tables 2 and 3.

In contrast, CsA significantly enhanced the biotransformation of [14C]caffeine 24 h after PHx (CsA versus control: p < .04; Fig. 5, Table 4) but its elimination progressively increased in placebo-treated rats between 48 and 96 h after surgery where no differences between the two groups were observed. A significant interaction between drug administration and time in relation to partial hepatectomy was observed (p, .02) due to the CsA-mediated early beneficial effects (early recovery and/or protection against a decrease) on [14C]caffeine elimination, which was followed by a gradual tapering off of its biotransformation activity at times 48, 72, and 96 h after partial hepatectomy. [14C]caffeine elimination was also shown to exhibit an overshoot in its recovery 72 and 96 h after surgery most particularly in placebo-controls. Sham-operated animals showed biotransformation activities similar to those observed before surgery (data not shown).

Discussion

The metabolizing capacity related to several cytochromes P-450-mediated biotransformation activities [CYP 2B1 and CYP2C11 (aminopyrine; Kotake et al., 1982; Ching et al., 1996) and CYP1A2 (caffeine; Butler et al., 1989)] revealed that neither their steady-state mRNA levels nor their in vivo disposition were influenced by the chronic administration of CsA in sham-operated animals. The N-demethylation of erythromycin (CYP3A2, 3A1; Craig et al., 1993) was shown to be slightly decreased, albeit in a nonsignificant manner (p < .055), by CsA administration, an observation concurring with that of others where the elimination of erythromycin was shown to be unaffected by CsA treatment in vivo (Watkins, 1996). In addition, we
reported here that CYP3A1 and 3A2 mRNAs were not influenced by CsA in both the pre- and posthepatectomy (48 h) periods. In vitro studies, however, have reported competition between CsA and many other substrates metabolized by CYP3As including erythromycin (Pi-3A1-mediated clinically relevant concentration, CsA has little effect on CYP3A2 less than 1 nM observed in the present studies, indicating that at operation (data not shown) or partial hepatectomy (n = 4/group). Data are presented as means ± S.E.M. for the cumulative 14CO2 recovered as a percentage of the injected dose. Statistically significant differences between group means at each time pre- and post-PHx were analyzed by a two-way analysis of variance for repeated measures.

Studies of the hepatic drug-metabolizing capacity after PHx revealed, however, a significant reduction in the elimination of both aminopyrine (29 to 32%) and erythromycin (29 to 39%), a reduction that was also shown not to be influenced by chronic CsA administration. The relatively small loss in the biotransformation capacity of the liver observed after 2/3 hepatic resection illustrates well the adaptive capacity of the organ as extrahepatic metabolism of aminopyrine or of erythromycin has been shown to be negligible in the rat and in humans (Sendama et al., 1985; Watkins et al., 1992). It also concurs with observations on the biotransformation of aminopyrine (Lauterburg and Bircher, 1976; Sendama et al., 1985) and on the circulating concentration of liver-specific proteins (Rikkers and Moody, 1974) where no concordance between hepatic function and liver mass were observed. Our data thus clearly show that CsA does not perturb nor accelerate the recovery of drug-metabolizing enzyme activity associated with three major cytochrome P-450 enzymes (CYP2B1, CYP2C11, and CYP3A2) after partial liver resection. This observation might be somewhat surprising in view of the apparent increase in the hepatic regeneration process reported by several investigators (Mazzaferro et al., 1990; Kahn et al., 1990; Francavilla et al., 1991) or of the acceleration in DNA synthesis reported by Garcia-Alonso et al. (Garcia-Alonso et al., 1990) as well as by our own laboratory (Provencher et al., 1997), observations certainly indicative of a CsA-mediated increased efficiency in the liver recovery process.

Data obtained during the study on caffeine elimination suggest that CsA might have mediated the early recovery of CYP1A2-linked drug-metabolizing activity although the breath test approach does not allow the exact metabolic pattern resulting from the biotransformation of the drug to be measured. However, as already shown by others, the caffeine breath test is a valuable method to measure CYP1A2-mediated N-demethylation activities as it has been shown to exhibit a high correlation with the in vivo metabolic clearance rate of caffeine in humans as well as in the rat (Willson and Hart, 1981; Renner et al., 1984). Moreover, using the breath test as a probe, data obtained in our laboratory (G. Raymond and J.P.V., unpublished data) have also shown that βNF (a known inducer of CYP1A2) administration increased the elimination of caffeine whereas treatment with PB, DEX, or 4-methylpyrazone had no influence on its biotransformation, indicating that the caffeine breath test is a good indicator of CYP1A2-dependent activity. Interestingly, the decrease in caffeine elimination (a 60% decrease) after PHx was also closely related to the loss of liver mass contrary to the smaller decrease observed in the elimination of aminopyrine and erythromycin. This observation indicates that the hepatic metabolic reserve related to CYP1A2 seems to be smaller (and closely related to liver mass) than that observed for the other cytochromes P-450 probed in this study, an observation also made by others in rats as well as humans (Renner et al., 1984; Schaad et al., 1995) and where extrahepatic metabolism has also been reported to be negligible (De Waziers et al., 1989). It is tempting to suggest a link between the accelerated first wave of DNA synthesis (which takes place in the PHx model 22 to 24 h after the 2/3 resection; Mazzaferro et al., 1990; Kahn et al., 1990; Francavilla et al., 1991) and in the regeneration process, mediated by CsA with the early recovery of the

![Fig. 4. Influence of CsA administration on the elimination of [14C]erythromycin before, and 24, 48, 72, and 96 h after PHx.](image-url)
caffeine biotransformation function. With time, however, the effect of CsA would be expected to diminish as liver regeneration will progress in placebo-treated rats to catch up with the CsA-treated animals. In fact, the early beneficial effect of CsA on the recovery of CYP1A2-mediated biotransformation was, indeed, short-lived, its effect gradually diminishing over the following 72 h. It is not excluded that the deleterious hepatic effect(s) of CsA might have played a role in counterbalancing the positive influence of the drug on the hyperplastic process dampening the expected accelerated recovery in liver mass and the hepatic drug-metabolizing function. The data obtained do not preclude, however, an accelerated CsA-mediated recovery (and/or protection from an initial decrease) on other substrates but they show that its positive effect is limited. Indeed, at the critical time of greatest loss in liver parenchyma, CsA was only successful in improving the in vivo biotransformation of CYP1A2-linked drug metabolizing activity, whereas it did not increase the biotransformation of CYP2B1-, 2C11-, and 3A2-dependent substrates. It illustrates, however, the lack of impact of the drug on the whole organ recovery function associated with these drug-metabolizing activities.

Our data, thus, lead us to conclude that the reported effect of CsA on the regeneration process does not translate into an overall accelerated recovery of the drug-metabolizing function of the liver despite an apparent early acceleration of the regeneration process. The effect of CsA may, then, mostly affect those drug-metabolizing activities exhibiting the lowest reserve that would be expected, therefore, to be the most sensitive to losses in liver mass or occur when hepatic mass

**TABLE 3**

Influence of CsA on [14C]erythromycin metabolism

<table>
<thead>
<tr>
<th>Group</th>
<th>Time in Relation to 2/3 Partial Hepatectomy</th>
<th>% dose exhaled [14CO2]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre 24h Post 48h Post 72h Post 96h Post</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>12.2 ± 0.5 (100%) 7.5 ± 0.6*** 6.6 ± 0.4*** 9.8 ± 0.9 9.1 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>CsA</td>
<td>11.3 ± 0.7 (100%) 8.0 ± 0.7* 7.3 ± 0.4** 9.5 ± 0.6 9.1 ± 0.7</td>
<td></td>
</tr>
</tbody>
</table>

Values represent the cumulative [14CO2] exhaled during the 2-h collection period as % of the dose injected (or in parenthesis, normalized to 100% for the value observed prior to surgery). n = 6 animals/group for each time period studied. Statistically significant differences between group means were analyzed by two-way analysis of variance. Effect of treatment: N.S. Interaction time/treatment: N.S. Effect of time: Control: p < .0001; CsA p < .003. Individual posthoc tests between pre and post PHx within the control or CsA group respectively were performed according to Bonferroni-Dunn: * p < .02; ** p < .003; *** p < .0002.

**FIG. 5.** Influence of CsA administration on the elimination of [14C]caffeine before, and 24, 48, 72, and 96 h after PHx.

CsA  △△△, placebo-control rats —— A single 0.50 μCi dose of [14C]caffeine was injected i.v. and exhaled [14CO2] was collected during eight consecutive 15-min periods starting immediately after drug administration. All animals were studied before and after PHx (n = 4/group). Data are presented as means ± S.E.M. for the cumulative [14CO2] recovered as a percentage of the injected dose. Differences between group means at each time pre- and postPHx were analyzed by a two-way analysis of variance for repeated measures.

**TABLE 4**

Influence of CsA on [14C]caffeine metabolism

<table>
<thead>
<tr>
<th>Group</th>
<th>Time in Relation to 2/3 Partial Hepatectomy</th>
<th>% dose exhaled [14CO2]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre 24h Post 48h Post 72h Post 96h Post</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.7 ± 0.5 (100%) 2.3 ± 0.3* 4.0 ± 0.4 (70%) 8.9 ± 0.5** 9.6 ± 0.9***</td>
<td></td>
</tr>
<tr>
<td>CsA</td>
<td>5.1 ± 0.5 (100%) 4.4 ± 0.8 (86%) 5.6 ± 0.7 (110%) 8.6 ± 0.5 (169%) 7.7 ± 0.9 (151%)</td>
<td></td>
</tr>
</tbody>
</table>

Values represent the cumulative [14CO2] exhaled during the 2-h collection period as % of the dose injected (or in parenthesis, normalized to 100% for the value observed prior to surgery). n = 4 animals/group for each time period studied. Statistically significant differences between group means were analyzed by two-way analysis of variance. Effect of treatment: N.S. Interaction time/treatment: p < .02. Effect of time: Control: p < .0001; CsA p < .006. Individual posthoc tests between pre and post PHx within the control or CsA group respectively were performed according to Bonferroni-Dunn: * p < .003; ** p < .006; *** p < .0008.
is inadequate for body size, such as after transplantation of livers that are small for recipients.

Acknowledgments. We thank Dr. G.N. Hendy from the Departments of Medicine and Physiology, McGill University for providing the rat cyclophilin probe, Dr. Pierre Banon from the Clinical Biochemistry Department, Centre Hospitalier de l’Université de Montréal, Campus St-Luc for measuring the CsA, and Manon Livernois for her excellent secretarial assistance.

References


