Pharmacology: Profiles, Parameters, Interpretations, and Drug Interactions

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DURING the last few years, many clinical pharmacology studies of cyclosporine (CsA) have been completed and, as shown in the Proceedings of this Congress, there continues to be much interest in this area. For example, over 50 papers in this volume discuss various aspects of CsA pharmacology in humans compared with 13 papers in the First International Congress on CsA in 1983. Of particular interest are papers that discuss CsA metabolites or new immunoassays for the measurement of CsA in biologic fluids. In addition, an entire meeting was devoted to CsA pharmacology in July 1986, which was summarized in a separate 272 page issue of *Transplantation Proceedings* (December 1986). Despite the large number of clinical pharmacology studies of CsA published during the last few years, many important questions remain and many divergent opinions were shared during the Congress.

PROFILES AND PARAMETERS

Nearly every study has reported wide interpatient variability in CsA pharmacokinetics. Although some of this variability is related to erratic and incomplete bioavailability after oral administration, considerable variability in CsA pharmacokinetics has also been observed after intravenous (IV) administration. Many studies have attempted to identify factors that account for the observed variability. Although some factors have been identified, the reasons for most of the observed variability are not known.

The oral absorption of CsA has not been well characterized. Acute or chronic diarrhea, postoperative ileus, and other factors have been reported to influence oral absorption of CsA but few of these have been confirmed in carefully controlled studies. The effect of food on oral CsA absorption is not clear; studies have reported both increased and decreased CsA absorption after oral administration. One recent study has reported that metoclopramide, a drug that increases gastric emptying, increases the maximum CsA concentration, time to achieve maximum concentration, and area-under-the-curve after oral CsA administration. Alterations in bile production or flow, such as those observed in patients after orthotopic liver transplantation, patients with severe liver disease, and patients with external bile drainage, would be expected to have impaired absorption of CsA. Further, concurrent administration of chenodeoxycholic acid or removal or clamping of a patient's T tube can improve oral CsA absorption in some patients. There may also be diurnal variations in oral CsA absorption after transplantation. Since the oral CsA dose required to achieve a given CsA concentration decreases with increasing time posttransplant, some individuals have stated that the oral absorption of CsA increases with chronic administration. However, as emphasized by several participants at the Congress, it is important to include studies of IV CsA when studying oral CsA pharmacokinetics to distinguish between changes in oral absorption v changes in clearance.

It is known that CsA is widely distributed into many body tissues, as evidenced by volume of distribution values that exceed body weight and by relatively high CsA concentrations in most body tissues. The specific value is

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influenced by both assay method and biologic fluid. Several recently published papers have suggested that differences in lipoprotein or erythrocyte concentrations—the major proteins that bind to CsA—can influence CsA concentrations or CsA pharmacokinetics. In one study, age-dependent changes in serum CsA clearance and volume of distribution were apparently related to differences in triglyceride concentrations between the various age groups. Higher triglyceride concentrations were associated with lower clearance and volume of distribution values. Although free or unbound CsA concentrations were not measured, these observations support the hypothesis that increases in triglyceride concentration are associated with decreases in the fraction of CsA unbound in plasma (ie, free fraction). According to theoretical pharmacokinetic concepts, changes in the fraction unbound can influence both the clearance and volume of distribution of drugs that are extensively metabolized and have a low extraction ratio and a large (>100 L) volume of distribution, all of which are characteristics of CsA. Since other lipoproteins were not measured, it is not known if changes in other lipoproteins would also correlate with CsA clearance and volume of distribution. Hypertriglyceridemia has been associated with high serum CsA concentrations. In a paper by Lindholm et al at this Congress, however, the free fraction of CsA in plasma correlated with serum cholesterol, HDL-cholesterol, and apolipoprotein A1 but not with triglycerides. Furthermore, de Groen et al reported at the Congress that the occurrence of central nervous system and renal toxicities and hypertension during the first few weeks after liver transplantation were found to be related to low cholesterol levels. None of the patients studied with a cholesterol level >120 mg/dL had central nervous system toxicity and none of the patients with a value >230 mg/dL had nephrotoxicity or hypertension. The development of toxicity in that study did not correlate with whole blood CsA concentrations measured by HPLC. Changes in hematocrit can also influence blood CsA pharmacokinetics, although the observed differences are less than those observed with triglycerides.

Since CsA is lipophilic and since obesity can influence the distribution of lipophilic drugs, several studies by Yee et al have described the effect of obesity on CsA pharmacokinetics. Obesity was defined as actual body weight >125% of ideal body weight. Serum or blood CsA clearance and volume of distribution, expressed in mL/min or L, respectively, were similar in age-matched obese and nonobese patients. However, significant differences were observed when clearance and volume of distribution were expressed as mL/min/kg actual body weight or L/kg actual body weight, respectively, which suggests that CsA dosage in obese patients should not be based on actual body weight but on ideal body weight. The lack of a difference in volume of distribution between obese and nonobese patients was somewhat surprising because of the lipophilicity of CsA.

CsA is biotransformed in man to many metabolites. More than 25 CsA metabolites have been identified in human blood, urine, or bile. A proposed scheme for the metabolism of CsA is shown in Fig 1; CsA metabolites are numbered according to studies by Maurer et al. It should be emphasized that our understanding of CsA metabolism is incomplete, and that other metabolites are probably present in biologic fluids in humans. It is also important to note that the metabolite patterns in one biologic fluid, such as in bile or urine, may differ from that in blood or tissue.

Several HPLC assays are now available to measure CsA metabolites in biologic fluids, and the availability of these assays are primarily responsible for the rapid increase in knowledge concerning CsA metabolites. Many participants in the Congress cautioned that before these assays are routinely used, their specificity, accuracy, and precision should be carefully studied. Papers at this Congress have shown that symmetric peaks

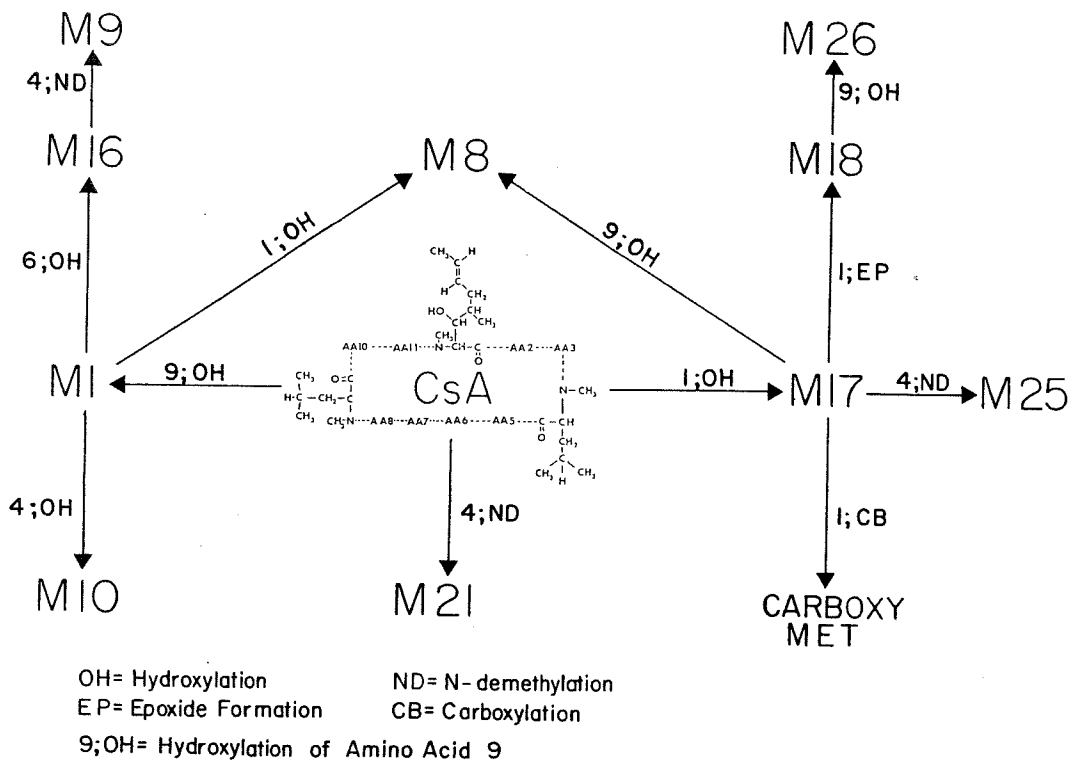

Fig 1. Proposed metabolism of CsA in humans. Metabolites are numbered according to the paper by Maurer et al.

that were reported to consist of a specific CsA metabolite actually contain one or more additional compounds. It is therefore important to not rely on HPLC retention time alone but to confirm the purity and identity of peaks of interest with other analytical methods.

Several transplant groups have shown that relatively high concentrations of CsA metabolites no. 17 (M17) and no. 1 (M1) are present in human blood and that trough M17 concentrations can exceed those of the parent drug. The trough blood M17 or M1 to CsA concentration ratio may differ according to patient population. The highest ratio of M17 to CsA in blood has been observed in liver transplant recipients. Additional studies have also shown that blood concentrations of these metabolites can be measured for the duration of the dosing interval. Several studies indicate that CsA metabolites vary in their affinity for cellular binding sites. CsA-M17 and M1 have greater affinity for the cellular fraction of blood compared with parent drug while M21 has less affinity for cells. Very little information is available on the amount or pattern of CsA metabolites in plasma or serum.

The contribution of CsA metabolites to the biologic activity of CsA is controversial. In some studies, the immunosuppressive activity of M17 is similar to that of parent drug while other studies have shown minor activity compared with CsA. In the present Congress, Drs Zeevi and colleagues reported that M17 and M1—at concentrations that are similar to CsA—inhibit lymphocytes grown from human liver allografts. These investigators also observed that CsA and M17 exhibited synergistic activity when tested in combination. Furthermore, Dr Kahan's group has reported that metabolite E, a monohydroxylated deriv-

ative of CsA found in plasma, inhibited interleukin 2 production as effectively as the parent drug. Most investigators agree that the degree of immunosuppressive activity depends on the in vitro assay used for immunosuppression. However, it is not known which in vitro immunosuppressive assay correlates most accurately with in vivo immunosuppression.

The contribution of CsA metabolites to nephrotoxicity is not clear. Ryffel et al have reported that animals given purified quantities of M17 do not develop renal dysfunction. However, one study has reported that serum CsA concentrations measured by RIA correlates more accurately with renal dysfunction than those measured by HPLC, which suggests that immunoreactive CsA metabolites play a role in CsA-induced renal dysfunction. Several other investigators have also observed that CsA concentrations measured by radioimmunoassay can increase before the development of renal dysfunction while those measured by HPLC show no significant change.

INTERPRETATION

The interpretation of CsA concentrations varied, depending on the institution and sometimes on the individual within each institution. Some transplant centers have found that CsA concentration does not correlate with graft rejection or nephrotoxicity and are therefore not clinically useful. However, other individuals at other centers have observed that CsA concentrations do correlate with clinical effects and routinely adjust CsA dosage to maintain CsA concentration within a specific therapeutic concentration range. Both groups agree that many patients with CsA concentrations within the therapeutic concentration range experience graft rejection, acute graft-*v*-host disease, or drug-related toxicity, which indicates that CsA concentration, like other laboratory tests, is not 100% accurate in predicting the occurrence of therapeutic efficacy or drug toxicity. Rather, the use of CsA concentration should be viewed as a method to increase the likelihood of therapeutic success, defined as a lack of graft rejection, acute graft-*v*-host disease, or drug-related toxicity.

Pharmacodynamic studies have been conducted in several different transplant populations. The therapeutic range for CsA depends on the type of transplant procedure, length of time posttransplant, degree of immunologic compatibility between donor and recipient, and immunosuppressive drug regimen. In solid organ transplant recipients, higher CsA concentrations are usually required to maintain graft function in the early postoperative period as compared with those required several months posttransplant. Several studies in renal transplant recipients, initially reported by Kahan et al and confirmed by other investigators, have shown that serum CsA concentrations measured by radioimmunoassay (RIA) correlated with the risk of graft rejection and nephrotoxicity. Henny et al have also reported a significant correlation between blood CsA concentrations measured by RIA and the incidence of nephrotoxicity in renal transplant recipients. Savoldi et al have reported that graft rejection after renal transplantation was strongly associated with a CsA bioavailability of $\leq$30%, serum half-life of $\leq$6 hours, or peak serum concentration of $\leq$1,500 ng/mL after oral CsA administration. In addition, patients who received CsA in divided doses had a higher incidence of CsA-induced nephrotoxicity, especially if their CsA clearance was $<$12 mL/min/kg. Most (12 of 13) patients had an improvement in their nephrotoxicity when their daily CsA dose was switched from twice daily to once-a-day administration. It should be noted, however, that not all studies have found CsA concentrations to be a useful predictor of either graft rejection or nephrotoxicity. For example, Henry et al presented at this Congress data that suggest that CsA concentrations "are not helpful in decision-making." One of the difficulties in interpretation of these studies is the use of different clinical endpoints. Graft and patient survival, incidence of rejection, biopsy

results, steroid requirements, general laboratory evaluation of organ function, mixed lymphocyte response inhibition, T4:T8 ratios, and plasma interleukin 2 receptor concentrations have all been used as clinical endpoints. Few studies have examined the relationship between CsA concentration and clinical effects in liver and cardiac transplant recipients and patients with autoimmune diseases.

In marrow transplant recipients, serum CsA concentration measured by radioimmunoassay have been shown to correlate with both the risk of renal dysfunction and acute graft-*v*-host disease. Renal dysfunction, defined as a doubling of baseline serum creatinine, occurred in 85% to 90% of CsA-treated patients. The risk of developing renal dysfunction was related to serum CsA concentration. The median day of onset of renal dysfunction was 46, 29, and 20 in patients with a mean trough CsA concentration of <150, 150 to 250, and >250 ng/mL. Eight of nine patients who did not develop renal dysfunction had a mean trough CsA concentration of <150 ng/mL.

Since the RIA measures both parent drug and crossreactive metabolites, several investigators have stated that CsA concentrations measured by this assay method can be misleading and not clinically useful. However, if some of these crossreactive CsA metabolites are nephrotoxic, then CsA concentration measured by RIA may correlate more accurately with acute renal dysfunction than those measured by HPLC. To test this hypothesis, CsA concentration was remeasured in the same serum samples by HPLC. After adjustment for baseline serum creatinine, CsA concentration measured by RIA was the only factor that significantly influenced the risk of renal dysfunction. CsA concentrations measured by HPLC did not correlate with the risk of developing renal dysfunction, which suggests that CsA metabolites play a role in the development of renal dysfunction.

The risk of developing acute graft-*v*-host disease in marrow transplant recipients is also related to trough serum CsA concentration measured by RIA. The relationship between patient characteristics including CsA concentration and the risk of acute graft-*v*-host disease was analyzed with a relative risk regression model. Patients with low trough CsA concentration, older patients, patients treated with CsA alone (*v* the combination of CsA and methotrexate), and patients transplanted after 1983 had a significantly higher risk of developing acute graft-*v*-host disease. The risk of acute graft-*v*-host disease was estimated to be about 30% less for every 100 ng/mL increase in CsA concentration.

The most appropriate assay method or biologic fluid for monitoring CsA concentration is not clear. Most of the participants at the Congress monitored CsA therapy with one of three methods: (1) serum CsA concentrations measured by RIA, (2) whole blood CsA concentrations measured by HPLC, or (3) whole blood CsA concentrations measured by RIA. Proponents of plasma argue that plasma transports the CsA and CsA metabolites to peripheral tissue and therefore provides the most accurate index of drug and drug metabolites concentration in peripheral tissues. Proponents of whole blood argue that blood measurements do not depend on sample processing conditions such as temperature and that CsA and CsA metabolites have a greater affinity for the cellular fraction of blood. Changes in blood CsA and CsA metabolite concentrations should therefore reflect changes in target tissue concentrations. Studies presented at this Congress in both humans and rats appear to support that the pattern of CsA metabolites relative to CsA in blood correlates with the pattern observed in tissues. However, it has not been shown that a change in the absolute tissue concentration of CsA and CsA metabolites is reflected by a parallel change in blood concentration. Availability of tissue biopsy specimens and specific methods of analysis will be needed to address this question.

The definitive study to determine the most appropriate assay method or biologic fluid for

monitoring CsA concentration in transplant patients must compare the ability of each different CsA concentration to predict for the occurrence of inadequate immunosuppression (ie, the occurrence of graft rejection or graft-*v*-host disease) or toxicity. Until such a study is conducted, recommendations concerning assay method or biologic fluid are premature. The selection of an assay method and biologic fluid for routine monitoring of CsA concentration should therefore be based on specific needs of each institution or transplant group. It should be noted that a task force recently concluded that "whole blood is the preferred matrix for CsA measurement. . . ." and that "the method for measurement of CsA should be specific (ie, HPLC or monoclonal antibody)." However, no study has demonstrated that whole blood CsA concentrations measured by HPLC correlates more accurately with clinical events than other assay methods or biologic fluids. Further, as reviewed above, no study has reported a correlation between whole blood CsA concentrations measured by HPLC and clinical effect (ie, graft rejection or nephrotoxicity) while several studies have observed that either whole blood or serum CsA concentrations measured by RIA correlates with clinical effect. In addition, as summarized above, several investigators have observed that CsA concentrations measured by RIA correlate more accurately with renal dysfunction than those measured by HPLC.

Several new CsA immunoassays are being developed or tested in various laboratories. The two immunoassays that have generated the most interest are the fluorescence polarization immunoassay developed by Abbott Laboratories and monoclonal radioimmunoassays developed by Quesniaux and coworkers. The major advantages of the nonspecific fluorescence polarization immunoassay are rapid turn around time and excellent accuracy and precision, particularly at low (<100 ng/mL) CsA concentrations. The assay is currently available for measurement of CsA concentration in serum or whole blood. At least two different RIAs have been developed from monoclonal antibodies. The most interesting one distinguishes between parent drug and CsA metabolites. Several papers at the Congress have confirmed that CsA concentrations measured with this assay are nearly identical to those measured by HPLC. The other monoclonal RIA does not distinguish between CsA and CsA metabolites and results in higher CsA concentrations than those measured by the present polyclonal RIA. Rosano et al have reported at this Congress that the total blood concentrations of CsA, M17, and M1, as measured by HPLC, closely approximate those measured by the nonspecific monoclonal RIA. However, the close correlation between the two measurements does not mean necessarily that the nonspecific monoclonal RIA only measures CsA, M17, and M1. Both new assays have the same disadvantages of the present polyvalent RIA: long turnaround time and probably poor precision at low CsA concentrations. It is clear that the specificity of each immunoassay for CsA metabolites differs but it is not clear whether measurement of crossreactive CsA metabolites is an advantage or disadvantage when monitoring CsA concentrations in transplant patients.

DRUG INTERACTIONS

Although the number of drugs reported to interact with CsA continues to increase, only a few drugs have been shown to change CsA pharmacokinetics or CsA concentrations in controlled studies.

The absorption of CsA may be altered by concurrent therapy with other drugs. Drugs that change gastrointestinal motility such as metoclopramide or loperamide have been reported to alter the oral absorption of CsA. Concurrent administration of CsA and chenodeoxycholic acid can also increase the oral absorption of CsA.

The metabolism of CsA can be altered by concurrent therapy with agents that inhibit or induce drug metabolizing enzyme activity.

One drug interaction presented at this Congress that may be related to CsA metabolism is the effect of the calcium blocker diltiazem on CsA pharmacokinetics. The pharmacokinetic interaction between steroids and CsA is still unclear but is an important research question because of the widespread usage of this combination in transplant patients.

CONCLUSION

Although knowledge concerning CsA pharmacology has expanded since the last CsA Congress in 1983, many of the same questions remain and many new questions are being asked. Examples of the questions that have not been answered include "what is the most appropriate biologic fluid (whole blood *v* serum/plasma) or assay method (HPLC *v* RIA) for monitoring CsA concentration?" With the advent of HPLC assays that can measure CsA metabolites in biologic fluids, new questions include "do CsA metabolites contribute significantly to either immunosuppression or renal toxicity?" Or, "what is the role of the newly introduced immunoassays?" It is encouraging to note the continued interest in CsA pharmacology. It is hoped that present and future studies will provide answers to some of these questions.