

## SINGLE-DOSE AND STEADY STATE PHARMACOKINETICS OF CSA AND TWO MAIN PRIMARY METABOLITES, AM1 AND AM4N IN PATIENTS WITH RHEUMATIC/AUTOIMMUNE DISEASES

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**Background.** Cyclosporine A (CsA) is an immunomodulatory agent used in standard immunosuppressive regimens in solid organ transplantations as well as in the treatment of autoimmune diseases such as rheumatoid arthritis (RA), systemic lupus and psoriasis. Its immunosuppressive activity is primarily due to parent drug. However, following oral administration, absorption is incomplete and varies between individuals. Further, there is a dearth of pharmacokinetic data for CsA in autoimmune patients compared to transplant recipients.

**Aim.** The goal of this study was to investigate the single-dose and steady state pharmacokinetics of CsA and two main primary metabolites, AM1 and AM4N, in patients with rheumatic/autoimmune diseases.

**Methods.** Thirty-eight subjects, average age (years $\pm$  SD) 46.8 ( $\pm$ 11.6) years with rheumatoid arthritis, systemic lupus erythematosus, psoriatic arthritis, ankylosing spondylitis and undifferentiated SpA were included in an observational open study. The single dose pharmacokinetics (area under the concentration-time curve of CsA and its metabolites (AUC) and other PK parameters) were determined over a 24 h period following oral administration of 1.3 mg/kg oral CsA. Two CsA formulations-Neoral and the Czech generic substitute Consupren®, were used.

Pharmacokinetic analysis was performed on all 38 patients after administration of a single dose of CsA (1.34 mg/kg/day). In 12 patients only, a second series of blood samples was taken to calculate monitored PK parameters under steady state conditions.

**Results.** Pharmacokinetic assessment showed  $AUC_{0-24}$  3009.66  $\pm$  1449.78 ng/ml.h and  $C_{max}$  827.84  $\pm$  425.84 after administration of a single dose of CSA,  $AUC_{0-24}$  3698.50  $\pm$  2147 ng/ml.h and  $C_{max}$  741  $\pm$  493 ng/ml after repeated dose. The proportion of the AM1 metabolite ( $AUC_{0-24}$ ) after a single dose of CsA corresponded to 40% of the parent compound and to approximately 35% of the parent compound in steady state conditions. The proportion of AM4N metabolite was low in both conditions and represented only 3 and 4.5% after a single dose and at steady state, respectively.

**Conclusion.** The pharmacokinetic data ( $AUC_{CsA}$ ,  $C_{max}$ ) for the whole 24 h interval were similar to the published findings, mainly under steady state conditions. The AM1 ( $AUC_{0-24}$ ) after a single dose of CsA and in steady state conditions represented about 40% of the parent drug. The ratio of AM4N metabolite was low in both conditions.

### INTRODUCTION

Cyclosporine A (CsA), a calcineurin inhibitor with potent immunosuppressive activity, is used in standard immunosuppressive regimens in solid organ transplantations as well as in the treatment of autoimmune diseases. Cyclosporine A, is effective in the treatment of rheumatoid arthritis (RA) as a single drug, as well as in combination with methotrexate or sulfasalazine. Several consensus conferences have provided guidelines for the use of CsA in rheumatology. Clinical researchers generally agree that starting low-doses of CsA should range from 2.5 to 3.5 per day<sup>1-3</sup>. However, data comparing its efficacy and safety over the long-term and in a large number of patients are limited.

CsA is also effective in the treatment of articular and cutaneous manifestations of psoriasis. A clearing effect on the dermatitis has been demonstrated in a number of studies; however, no data are available for the different forms of psoriatic arthritis<sup>4</sup>. In one controlled study from 1995, CsA was equally clinically effective as methotrexate. In another, from 2001, it was shown to be more effective than sulfasalazine or symptomatic treatment<sup>4,5</sup>. Apropos the use of CsA in other autoimmune/ rheumatic diseases, less data are available. In uncontrolled, small studies of systemic lupus erythematosus (SLE), a significant decrease in overall disease activity was described<sup>6-8</sup> with best results in controlling proteinuria, leukopenia and thrombocytopenia but with little effect on arthritis<sup>6,7</sup>.

In a subgroup of patients with lupus nephritis, CsA treatment was reported favourably in almost all studies and some data showed that it might have beneficial effects at a histological level<sup>9-12</sup>.

There are also case reports and retrospective case series supporting the efficacy of CsA in the treatment of inflammatory myopathies<sup>12-14</sup>. A randomised controlled clinical trial in patients with poly(dermato)myositis comparing a combination of glucocorticoids and methotrexate vs. glucocorticoids and CsA alone showed comparable efficacy for the two treatment arms<sup>15</sup>. Cyclosporine A acts more rapidly than azathioprine and may be a useful second-line drug. There are limited data on the use of CsA in patients with seronegative spondylarthropathies. No data are available for other spondyloarthropathies, including ankylosing spondylitis.

After administration of cyclosporine, there is an initial absorption phase, during which blood concentrations reach a peak level ( $C_{max}$ ). Typically,  $C_{max}$  occurs within the first 2-3 h CsA is strongly lipophilic, insoluble in water and has limited solubility in other solvents (olive oil, ethanol). Therefore, CsA is absorbed incompletely from the gastrointestinal tract, predominantly from the small intestine, and resulting bioavailability ranges widely between 5-80% (ref.<sup>15,16</sup>). The original oil-based preparation of CsA showed wide inter- and intra-patient variability. Absorption is significantly affected by solubilisation of CsA in bile and a number of other factors, e.g. diet, gastrointestinal transit time, concurrent medications and others<sup>16-21</sup>. The currently used CsA formulation, Neoral® has greater and more balanced gastrointestinal absorption and improved bioavailability than the older formulations. Neoral® is a microemulsion pre-concentrate of particle size <100 nm<sup>20,21</sup> compared with Sandimmun®, similar  $C_0$  levels, but higher  $C_{max}$  as well as AUC were achieved during the steady state in patients who received Neoral®. Pharmacokinetic data in patients with RA and psoriasis, as well as patients with solid organ transplants, showed better bioavailability for Neoral® than Sandimmun®. Average increase ranged from 22% to 54% (ref.<sup>19-21</sup>).

The main site of biotransformation for immunosuppressant drugs is the cytochrome P450-dependent monooxygenase system (P450) in the intestinal membrane and liver. Cyclosporine A is metabolized mainly by the P450 3A subfamily - P450 3A4, 3A5 and 3A43 (ref.<sup>23-25</sup>). Because multiple metabolites of CsA have been characterized, other enzymes may also be involved in CsA clearance (e.g. subfamilies P450 1A, 2A, 2B and 2C) (ref.<sup>23-25</sup>). The most active of the metabolites, AM1, followed by AM9, showed 10-20% of the immunosuppressive activity of CsA (ref.<sup>26-29</sup>).

The pharmacokinetics of CsA have been the subject of a large number of articles and reviews, mainly in the area of solid organ transplantation<sup>21</sup>. Data describing the pharmacokinetics of CsA in autoimmune diseases however are relatively rare. Few data are available for patients with rheumatoid arthritis<sup>30-32</sup>, but more consistent data were found for psoriasis treatment<sup>33-37</sup>. None of these stud-

ies however described the pharmacokinetics of the main primary CsA metabolites AM1 and AM4N.

The aim of our study was to evaluate basic pharmacokinetic parameters of parent drug and its primary metabolites AM1 and AM4N after single and repeated administration of cyclosporine A in patients with the autoimmune/rheumatic diseases listed.

## METHODS

### Subjects

The study was performed in the Rheumatology Department, Faculty Hospital Plzen, Czech Republic. After informed consent and with the approval of the local Ethics Committee, 38 subjects average age ( $\pm$  SD) 46.82 ( $\pm$ 11.67) years with rheumatoid arthritis, systemic lupus erythematosus, psoriatic arthritis, ankylosing spondylitis and undifferentiated spondylarthritis (off-label use in the last two indications, CsA was administered to patients with recurrent ocular complications of these diseases and failure of previous treatment). were included in an observational open study. Patient data are summarized in (Table 1). Inclusion criteria were if they had normal renal and hepatic function, were normotensive, and had stable concomitant medication one month prior to entry. Exclusion criteria were if they had severe hypertension, clinically relevant cardiovascular disease, active infection, malignancy or other unstable medical conditions, had received any drug with the potential to interact with cyclosporine metabolism or were pregnant or lactating.

### Study design

This was an open, observational, single centre study. In total 38 CsA naive patients were recruited. Pharmacokinetic data were obtained after administration of a single dose of CsA as well as during steady state conditions after the 3-month period of continual stable CsA therapy.

Single dose pharmacokinetics over a 24 h interval was determined in 38 patients by giving them 1.3 mg/kg oral CsA. In 12 patients who were willing to participate in follow-up, a second series of blood samples was taken in order to calculate area under the concentration -time curve of CsA and its metabolites (AUC) and other PK parameters in steady state conditions.

### Pharmacokinetic and Statistical Analysis

During each pharmacokinetics part of the study, 10 venous blood samples were collected (pre-dose and at times 0.5 h; 1 h; 1.5 h; 2 h; 4 h; 6 h; 8 h; 12 h; and 24 h after the morning dose of CsA). Venous blood samples (5 ml) were collected into tubes containing ethylenediaminetetraacetic acid (EDTA) for determination of CsA and its main metabolites (AM1 and AM4N) concentrations.

The following pharmacokinetic parameters were individually analysed by model-independent analysis:

- Absorption constant (Ka)
- Elimination constant (Ke)

- Half-life of drug elimination ( $t_{1/2el}$ )
- Maximum blood concentrations ( $C_{max}$ ) in the dosing interval and corresponding  $t_{max}$
- Area under the blood cyclosporine concentration/time curve over a 2 and 24 h interval ( $AUC_{0-2}$ ,  $AUC_{0-24}$ )
- Mean Residence Time (MRT)
- Volume of distribution (Vd)
- Clearance (Cl)
- Area under the blood AM1 and AM4N concentration/time curve during 24 h interval

Computations were performed by WinNonLin Pharsight software (Mountain View, CA, USA).

Area under the blood concentration-time curve over 2 h during absorption phase (for CsA) and 24 h interval (for CsA and metabolites AM1, AM4N) was determined using the trapezoidal rule.

The data were evaluated as means and standard deviation (SD). The significance of the intraindividual pharmacokinetic differences during the study period (single dose, steady state) was not tested statistically, due to the small number of patients who participated in the follow-up.

**Analyses:** Concentrations of CsA in whole blood were determined using high performance liquid chromatography (HPLC). Whole blood was precipitated with zinc sulphate, extracted with diethyl ether, evaporated, dissolved in aqueous methanol and partitioned twice with n-hexane. Chromatography was carried out using a microbore RP-column under isocratic elution with acetonitrile-methanol-water (200:80:140, v/v/v) at 70 °C and a detector set at 205 nm, as described previously (Brozmanova et al.<sup>38</sup>).

**Table 1.** Basic patient characteristics.

(n=38)

Mean age (years $\pm$ SD)	46.82 $\pm$ 11.67
Female	23 (60.3%)
Male	15 (39.5%)
CsA dose (mg/kg/day $\pm$ SD)	1.28 $\pm$ 0.34
Concomitant corticosteroids use	21 (55%)
Concomitant DMARDs use	21 (22%)
Concomitant NSAIDs use	22 (26%)

## RESULTS

The average dose of CsA was 1.28 $\pm$ 0.34 mg/kg/day. In individual patients, the same dose was maintained during the study, divided into equal doses, taken at 12 h intervals. Basic pharmacokinetic parameters for single and repeated dose administration expressed as means  $\pm$  SED are summarized in (Table 2).

**Table 2.** Pharmacokinetic parameters of CsA and its AM1 and AM4N metabolites after single dose and repeated administration.

CsA	Single dose (n=38) Mean $\pm$ SD	Steady state (n=12) Mean $\pm$ SD
Ka (1/h)	0.65 $\pm$ 0.31	0.90 $\pm$ 0.21
Ke (1/h)	0.10 $\pm$ 0.06	0.13 $\pm$ 0.09
$t_{1/2el}$ (h)	7.06 $\pm$ 2.10	13.90 $\pm$ 4.43
$C_{max}$ (ng/ml)	827.84 $\pm$ 425.84	741 $\pm$ 493
$t_{max}$ (h)	1.44 $\pm$ 0.14	1.70 $\pm$ 0.35
$AUC_{0-2}$ (ng/ml.h)	1154.5 $\pm$ 693.12	762.50 $\pm$ 485.58
$AUC_{0-24}$ (ng/ml.h)	3009.66 $\pm$ 1449.78	3698.50 $\pm$ 2147
MRT (h)	9.08 $\pm$ 2.20	21.70 $\pm$ 5.91
Vd (ml/kg)	11.20 $\pm$ 7.96	20.55 $\pm$ 15.65
Cl (ml/h/kg)	0.98 $\pm$ 0.80	1.18 $\pm$ 0.99
<b>AM1</b> $AUC_{0-24}$ (ng/ml.h)	1217.24 $\pm$ 850.34	1294 $\pm$ 768
<b>AM4N</b> $AUC_{0-24}$ (ng/ml.h)	111.6 $\pm$ 39.4	170.50 $\pm$ 202.99

## DISCUSSION

The aim of our study was to evaluate the basic pharmacokinetic parameters of low dose CsA and its metabolites, AM1 and AM4N.

Cyclosporine A is a drug with a narrow therapeutic window and large interindividual differences in pharmacokinetic parameters. The pharmacokinetic data showed that the absorption phase during the first four hours after oral administration of CsA is an area of the greatest intra- and interpatient variability. The interpatient variability in pharmacokinetic profiles is smaller for microemulsion forms of CsA than conventional oil-based formulations, indicating more consistent and more predictable absorption of CsA from microemulsion formulations. Due to the need to balance the efficacy and toxicity for this narrow therapeutic range drug, considerable effort has been made to define pharmacokinetic relationship for CsA therapies, mainly in transplant medicine. In autoimmune disease, clinical pharmacokinetic data are available for adult patients with psoriasis and those with rheumatoid arthritis and lupus nephritis (Table 3). When we compare these data with those of transplant patients, it is clear that the

doses used in autoimmune diseases are generally lower. In the case of our patients, the average CsA dose was very low (average dose 1,28 mg/kg/day), only half recommended dose. Consequently, the daily exposure (AUC) was lower and thus the risk of toxicity. In these patients, therapy is typically controlled through targeting the initial and maximal dose levels. Monitoring of blood CsA concentrations is usually not performed for the common adjustment of therapy. Routine measurements of serum creatinine and blood pressure can alert the clinician to the onset of toxic manifestations.

There are limited pharmacokinetic data for autoimmune diseases. These data were obtained mainly from pharmacokinetic conversion studies using two different CsA formulations - usually conventional oil in water emulsion (Sandimmun®) and microemulsion preconcentrate (Neoral®). None of these studies described the pharmacokinetics of the main cyclosporine primary metabolites (AM1 and AM4N).

The patients in our observational study were treated with two CsA formulations-Neoral and the Czech alternative Consupren®, which was developed as an emulsion albeit with a different type of emulsifier, resulting in dif-

ferent physical character of the emulsion (water/oil emulsion).

The pharmacokinetic data (CsA<sub>AUC</sub>) over the whole 24 h interval were very similar to the findings in other published sources, mainly during steady state conditions. According to the manufacturer's information, after oral administration, patients with RA achieved maximum concentrations (C<sub>max</sub>) between 1.5-2 h after administration C<sub>max</sub> (±SD) was 728 ± 263 ng/ml, through concentration 94 ± 37.7 ng/ml at a dose of 2.37 ± 0.36 mg/kg/day, average AUC (± SD) 2641 ± 877 ng/ml.h.

The available results from pharmacokinetic conversion studies are summarized in (Table 3).

The proportion of AM1 metabolite (AUC<sub>0-24</sub>) after a single dose of CsA corresponded to 40% of the parent compound and approximately 35% of the parent compound in steady state conditions. The proportion of metabolite AM4N was low in both conditions and represented only 3 or 4.5% after a single dose and steady state, respectively. We were unable to compare our data with findings from other studies due to the lack of relevant data on rheumatic diseases. During the 3 month follow- up, changes in some pharmacokinetic parameters were ob-

**Table 3.** Pharmacokinetics of Neoral® and Sandimmun® in Autoimmune Patients.

Population	Dose (mg/kg/day) <sup>1</sup>	AUC±SD (ng/ml*hod)	C <sub>max</sub> (ng/ml)	Reference
Psoriasis				
n=63 single dose (IV) <sup>+</sup> Steady state	2.5	10045±2133(S) 4245±2331	—	35
N=37 Steady state Switch S→N	300 mg*	3202±596 (S) 4940 ± 921(N)	623±173(S) 1166±201(N)	36
N=19 Steady state Switch N→S	229 mg**	2970±764(N) 2297±612 (S)	873±173(N) 511±140(S)	34
RA				
N=12 Steady state Switch N→S	2.5	2873±848(N) 2355±1128(S)	811±244(N) 495±291(S)	31
N=19 Steady state Switch S→N	3.0	2667±1155(S) 3335±1300(N)	811±244(N) 495±291 (S)	32
N=51 1) Switch S→N 2) Continual N	3.5 3.3	2288±428(S) 2708±647(N)	539±156 (S) 746±223 (N)	30

<sup>1</sup>Dose given in the form of two individual daily doses, unless otherwise noted

N-Neoral, S-Sandimmun

\*Average body weight approx. 80 kg

\*\* Average body weight approx. 72 kg

(Modified from 19).

served, such as reduction of AUC during the absorption phase, lower  $C_{max}$ , increase in Cl and Vd. Unfortunately, due the small number of patients with steady state pharmacokinetics, it was not possible to obtain relevant statistics evaluation.

In conclusion, this observation study provides basic pharmacokinetic data on the behaviour of cyclosporine and its metabolites in patients with different rheumatic diseases after single administration and under steady state conditions. In comparison with transplant medicine, this issue is not very well documented. Data on the behaviour of metabolites in this group of patients are still not well documented.

## ABBREVIATIONS

AUC, Area under the curve; C0, Concentration before drug administration; Cl, Clearance; Cmax, Peak concentration; CsA, Cyclosporine A; DMARDs, Disease-modifying anti-rheumatic drugs; EDTA, Ethylenediaminetetraacetic acid; HPLC, High performance liquid chromatography; Ka, Absorption constant; Ke, Elimination constant; MRT, Mean residence time; NSAIDs, Nonsteroidal anti-inflammatory drugs; RA, Rheumatoid arthritis; SD, standard deviation; tmax, Time to peak concentration;  $t_{1/2 el}$ , Half-life of drug elimination; Vd, Volume of distribution,

## REFERENCES

- Panayi GS, Tugwell P. The use of cyclosporin A in rheumatoid arthritis: conclusions of an international review. *Br J Rheumatol* 1994;33(10):967-9.
- Van den Borne BE, Landewe RB, Goei The HS, Breedveld FC, Dijkmans BA. Cyclosporin A therapy in rheumatoid arthritis: only strict application of the guidelines for safe use can prevent irreversible renal function loss. *Rheumatology (Oxford)* 1999;38(3):254-9.
- Tugwell P, Bombardier C, Gent M, Bennett K, Ludwin D, Grace E, Buchanan WW, Bensen WG, Bellamy N, Murphy GF. Low dose cyclosporine in rheumatoid arthritis: a pilot study. *J Rheumatol* 1987;14(6):1108-14.
- Spadaro A, Riccieri V, Silli-Scavali F, Sensi F, Taccari E, Zoppini A. Comparison of cyclosporin A and methotrexate in the treatment of psoriatic arthritis: a one year prospective study. *Clin Exp Rheumatol* 1995;13:589-93.
- Salvarani C, Macchioni P, Olivieri I, Marchesoni A, Cutolo M, Ferraccioli G, Cantini F, Salaffi F, Padula A, Lovino C, Dovigo L, Bordin G, Davoli C, Pasero G, Alberighi OD. A comparison of cyclosporine, sulfasalazine, and symptomatic therapy in the treatment of psoriatic arthritis. *J Rheumatol* 2001;28:2274-82.
- Tokuda M, Kurata N, Mizoguchi A, Inoh M, Seto K, Kinashi M, Takahara J. Effect of low dose cyclosporine A in systemic lupus erythematosus. *Arthritis Rheum* 1994;37:551-8.
- Manger K, Kalden JR, Manger B. Cyclosporin A in the treatment of systemic lupus erythematosus: results of an open clinical study. *Br J Rheumatol* 1996;35:669-75.
- Caccavo D, Lagana B, Mitterhofer AP, Ferri GM, Afeltra A, Amoroso A, Bonomo L. Long-term treatment of systemic lupus erythematosus with cyclosporine A. *Arthritis Rheum* 1997;40:27-35.
- Griffiths B, Emery P. The treatment of lupus with cyclosporin A. *Lupus* 2001;10:165-70.
- Dostál C, Tesař V, Rychlík I, Zabka J, Vencovský J, Bartůnková J, Stejskalová A, Tegzová D. Effect of 1 year cyclosporine A treatment on the activity and renal involvement of systemic lupus erythematosus: a pilot study. *Lupus* 1998;7:29-36.
- Dammacco F, Della Casa Alberighi O, Ferraccioli G, Racanelli V, Casatta L, Bartoli E. Cyclosporine A plus steroids versus steroids alone in the 12-month treatment of SLE. *Int J Clin Lab Res* 2000;30:67-73.
- Zabel P, Leimenstoll G, Gross WL. Cyclosporin for acute dermatomyositis (letter). *Lancet* 1984;343.
- Bendtzen K, Tvede N, Andersen V, Bendixen G. Cyclosporine for polymyositis (letter). *Lancet* 1984;792-3.
- Gruhn WB, Diaz-Buxo JA. Cyclosporine treatment of steroid resistant interstitial pneumonitis associated with dermatomyositis/polymyositis. *J Rheumatol* 1987;14:1045-7.
- Vencovský J, Jarošová K, Macháček S, Studýnková J, Kafková J, Bartůnková J, Nemcová D, Charvát F. Cyclosporine A versus methotrexate in the treatment of polymyositis and dermatomyositis. *Scand J Rheumatol* 2000;29:95-102.
- Fahr A. Cyclosporin clinical pharmacokinetics. *Clin Pharmacokinet* 1993;24(6):472-95.
- Faulds D, Goa KL, Benfield P. Cyclosporin: a review of its pharmacodynamic and pharmacokinetic properties and therapeutic use in immunoregulatory disorders. *Drugs* 1993;45:953-1040.
- Lindholm A. Factors influencing the pharmacokinetics of cyclosporine in man. *Ther Drug Monit* 1991;13(6):465-77.
- Yocom DE. Cyclosporine: Clinical application in autoimmune diseases. *Mosby - Wolfe*, Philadelphia 2000.
- Vrána A, Andrýsek T. The effect of particle size on bioavailability in cyclosporine preparations based on submicron dispersions. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub*. 2001;145(2):9-15.
- Anon. Neoral package insert. East Hanover, NJ: Novartis Pharmaceuticals; 2005.
- Gulliver WP, Murphy GF, Hannaford VA, Primett DRN. Increased bioavailability and improved efficacy in severe psoriasis of a new microemulsion formulation of cyclosporin. *Br J Dermatol* 1996;135(Suppl. 48):35-9.
- Aoyama T, Yamano S, Waxman DJ, Lapenson DP, Meyer UA, Fischer V, Tyndale R, Inaba T, Kalow W, Gelboin HV. Cytochrome P-450 hPCN3, a novel cytochrome P-450 IIIA gene product that is differentially expressed in adult human liver. cDNA and deduced amino acid sequence and distinct specificities of cDNA-expressed hPCN1 and hPCN3 for the metabolism of steroid hormones and cyclosporine. *J Biol Chem* 1989;264(18):10388-95.
- Westlind A, Malmbo S, Johansson I, Otter C, Andersson TB, Ingelman-Sundberg M, Oscarson M. Cloning and tissue distribution of a novel human cytochrome P450 of the CYP3A subfamily, CYP3A43. *Biochem Biophys Res Commun* 2001;281(5):1349-55.
- Lown KS, Kolars JC, Thummel KE, Lown KS, Kolars JC. Interpatient heterogeneity in expression of CYP3A4 and CYP3A5 in small bowel. Lack of prediction by the erythromycin breath test. *Drug Metab Dispos* 1994;22(6):947-55.
- Yatscoff RW, Copeland KR, Faraci CJ. Abbott TDx monoclonal antibody assay evaluated for measuring cyclosporine in whole blood. *Clin Chem* 1990;36(11):1969-73.
- Radeke HH, Christians U, Sewing KF, Resch K. The synergistic immunosuppressive potential of cyclosporin metabolite combinations. *Int J Immunopharmacol* 1992;14(4):595-604.
- Rosano TG, Brooks CA, Dybas MT, Cramer SM, Stevens C, Freed BM. Selection of an optimal assay method for monitoring cyclosporine therapy. *Transplant Proc* 1990;22(3):1125-8.
- Christians U, Schlitt HJ, Bleck JS, Schiebel HM, Kownatzki R, Maurer G, Strohmeyer SS, Schottmann R, Wonigeit K, Pichlmayr R. Measurement of cyclosporine and 18 metabolites in blood, bile, and urine by high-performance liquid chromatography. *Transplant Proc* 1988;20:609-13.
- Anderson IF, Helve T, Hannonen P, Leirisalo-Repo M, Gilboe IM, Nissilä M, Keystone EC, Kraag GR, Bjørneboe O, Chalmers A, Dovland H, Mueller E, Richard F, Whatmough I, Schmidt AG, Kovarik JM. Conversion of patients with rheumatoid arthritis from

- the conventional to a microemulsion formulation of cyclosporine: a double blind comparison to screen for differences in safety, efficacy and pharmacokinetics. *J Rheumatol* 1999;26:556-62.
- 31. van den Borne BE, Landewé RB, Goei The HS, Mattie H, Breedveld FC, Dijkmans BA. Relative bioavailability of a new oral form of cyclosporin A in patients with rheumatoid arthritis. *Br J Clin Pharmacol* 1995;39:172-5.
  - 32. Marchessoni A, Fantini F, Battafarano N, Zeni S, Ruiu G, Padula A, Taglione E, Pasero G, Leoni L. Pharmacokinetic effect of conversion to a new formulation of cyclosporin A in rheumatoid arthritis patients. *Rheumatol Int* 1998;17:197-202.
  - 33. Choc MG. Bioavailability and Pharmacokinetics of cyclosporine formulations: Neoral vs Sandimmun. *Int J Dermatol* 1997;36(suppl 1):1-6.
  - 34. Erkko P, Granlund H, Nuutinen M, Ritamo S. Comparison of cyclosporine A pharmacokinetics of a new microemulsion formulation and standard oral preparation in patients with psoriasis. *Br J Dermatol* 1997;136:82-8.
  - 35. Galla F, Marzochi V, Croattino L, Poz D, Baraldo M, Furlanut M. Oral and intravenous disposition of cyclosporine in psoriatic patients. *Ther Drug Monit* 1995;17:302-4.
  - 36. Elder CA, Moore M, Chang CT, Jin J, Charnick S, Nedelman J, Cohen A, Guzzo C, Lowe N, Simpson K. Efficacy and pharmacokinetics of two formulations of cyclosporine A in patients with psoriasis. *J Clin Pharmacol* 1995;35:865-75.
  - 37. Furlanut M, Baraldo M, Pea F, Marzocchini V, Croattino L, Galla F. Blood Concentrations and clinical effect of cyclosporine in psoriasis. *Ther Drug Monit* 1996;18:544-8.
  - 38. Brozmanová H, Grundmann M, Šafarčík K, Jegorov A. High-performance liquid chromatographic method for therapeutic drug monitoring of cyclosporine a and its two metabolites in renal transplant patients. *J Chromatography* 2000;749:93-100.