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Clinical Pharmacokinetics of Everolimus

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Contents

Abstract

Everolimus is an immunosuppressive macrolide bearing a stable 2-hydroxyethyl chain substitution at position 40 on the sirolimus (rapamycin) structure. Everolimus, which has greater polarity than sirolimus, was developed in an attempt to improve the pharmacokinetic characteristics of sirolimus, particularly to increase its oral bioavailability. Everolimus has a mechanism of action similar to that of sirolimus. It blocks growth-driven transduction signals in the T-cell response to alloantigen and thus acts at a later stage than the calcineurin inhibitors ciclosporin and tacrolimus. Everolimus and ciclosporin show synergism in immunosuppression both *in vitro* and *in vivo* and therefore the drugs are intended to be given in combination after solid organ transplantation. The synergistic effect allows a dosage reduction that decreases adverse effects.

For the quantification of the pharmacokinetics of everolimus, nine different assays using high performance liquid chromatography coupled to an electrospray mass spectrometer, and one enzyme-linked immunosorbent assay, have been developed.

Oral everolimus is absorbed rapidly, and reaches peak concentration after 1.3–1.8 hours. Steady state is reached within 7 days, and steady-state peak and trough concentrations, and area under the concentration-time curve (AUC), are proportional to dosage. In adults, everolimus pharmacokinetic characteristics do not differ according to age, weight or sex, but bodyweight-adjusted dosages are necessary in children.

The interindividual pharmacokinetic variability of everolimus can be explained by different activities of the drug efflux pump P-glycoprotein and of

metabolism by cytochrome P450 (CYP) 3A4, 3A5 and 2C8. The critical role of the CYP3A4 system for everolimus biotransformation leads to drug-drug interactions with other drugs metabolised by this cytochrome system. In patients with hepatic impairment, the apparent clearance of everolimus is significantly lower than in healthy volunteers, and therefore the dosage of everolimus should be reduced by half in these patients.

The advantage of everolimus seems to be its lower nephrotoxicity in comparison with the standard immunosuppressants ciclosporin and tacrolimus. Observed adverse effects with everolimus include hypertriglyceridaemia, hypercholesterolaemia, opportunistic infections, thrombocytopenia and leucocytopenia.

Because of the variable oral bioavailability and narrow therapeutic index of everolimus, blood concentration monitoring seems to be important. The excellent correlation between steady-state trough concentration and AUC makes the former a simple and reliable index for monitoring everolimus exposure. The target trough concentration of everolimus should range between 3 and 15 μg/L in combination therapy with ciclosporin (trough concentration 100–300 μg/L) and prednisone.

Everolimus is a derivative of sirolimus (rapamycin) bearing a 2-hydroxyethyl chain at position 40 (figure 1). Sirolimus is a macrolide antibiotic produced by *Streptomyces hygroscopicus*, an actinomycete that was isolated in 1975 from a soil sample obtained from Rapa-Nui (Easter Island).[1,2] Like sirolimus, everolimus has potent antiproliferative and immunosuppressive effects,[3-5] but with greater stability and solubility as well as more favourable pharmacokinetics.[6] In 2003, everolimus was authorised for marketing in some European countries, and approval in the US is expected in 2004.

Everolimus has a mode of action different from that of ciclosporin or tacrolimus, which are calcineurin inhibitors.[7-10] Instead, everolimus (and sirolimus) inhibit cell proliferation by blocking cell cycle progression from the G_1-phase to the S-phase. This inhibition is mediated via the complex formed by the association of everolimus with the immunophilin FK506-binding protein 12 (FKBP12), which also binds tacrolimus. The everolimus-FKBP12 complex inhibits the protein kinase mammalian TOR ('target of rapamycin'), which causes an arrest in the G_1 cell cycle.[11-14] This also inactivates the p70 S6 kinase in mammalian cells *in vitro*, resulting in selective inhibition of the synthesis of ribosomal proteins and induction of mRNA for new ribosomal proteins.[15,16] As a result, cell cycle progression is prolonged at the G_1-S interface.[5,17] Everolimus binds to FKBP12 with only 2-fold less affinity than sirolimus,[5,18] and X-ray crystallographic studies of the FKBP12-everolimus complex revealed a three-dimensional structure for bound drug resembling very closely that of sirolimus.[18,19]

Since everolimus and ciclosporin inhibit adjacent steps in the T-cell-mediated immune response, combination of ciclosporin and everolimus results in synergistic immunosuppressive activity.[20-26] Tacrolimus can be also combined with sirolimus or everolimus. Tacrolimus and sirolimus or everolimus have the same intracellular binding protein FKBP12; therefore, competitive antagonism was of concern.[27] Dumont et al.[28] showed that competitive antagonism is possible in cultured T-lymphocytes, but only if the concentration of one drug exceeds that of the other by 3-fold. When using immunosuppressive doses of both drugs in humans, approximately 5% of FKBP12 is blocked by sirolimus and tacrolimus. Recent studies have shown that sirolimus can be successfully combined with tacrolimus.[27,28] Therefore, combination therapy of everolimus with tacrolimus may also be useful, but at the moment no studies are available. Everolimus can also be combined with anti-interleukin-2 antibodies.[29,30] Studies with a combination of mycophenolate mofetil and everolimus have not so far been performed.

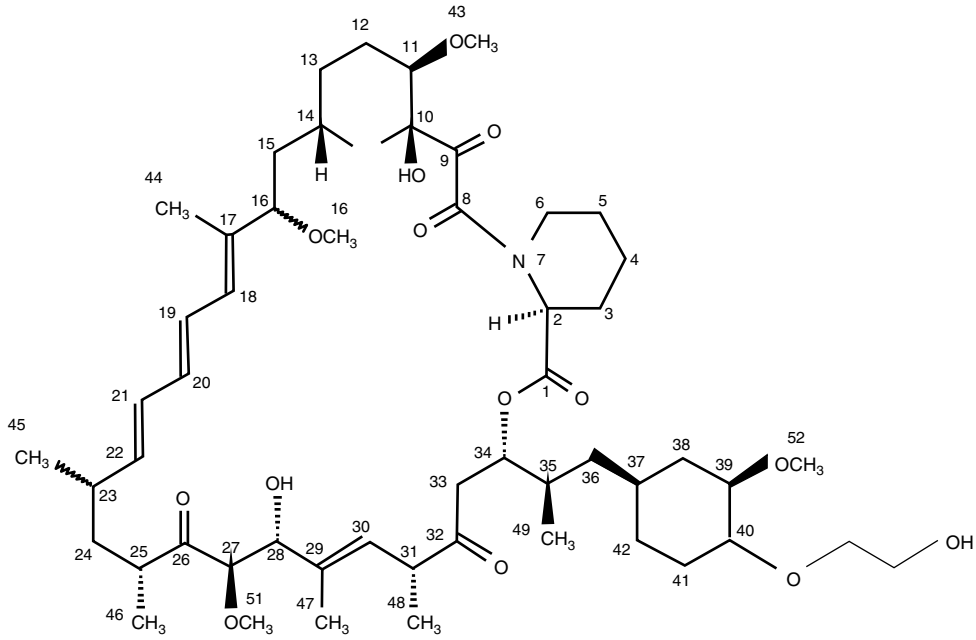

Fig. 1. Chemical structure of everolimus [40-*O*-(2-hydroxy)ethyl-rapamycin].

This review will summarise the methods for therapeutic drug monitoring and the data on clinical pharmacokinetics of everolimus.

1. Chemical Characteristics

Everolimus is a macrolide immunosuppressant with a molecular mass of 957.6Da ($C_{53}H_{83}NO_{14}$). Everolimus has greater polarity than sirolimus. Everolimus is soluble in alcohols, acetonitrile, ethers and halogenated hydrocarbons, and practically insoluble in water and aliphatic hydrocarbons. Its triene group is responsible for an ultraviolet absorption maximum at 276 nm.

Everolimus is sensitive to light and temperature. Everolimus stock solutions are stable for at least 6 months when stored at –80°C.[31] In a refrigerator (+4°C), everolimus stock solutions lose about 14% of their concentrations over 14 days.[31]

Everolimus in blood samples resists at least three freeze-thaw cycles.[32] Blood samples stored at –80°C are stable for at least 8 months.[32] After storage of extracted samples at –20°C for 1 week, the mean deviations from immediately analysed controls were between –1.3% and +10.5% at different concentrations.[31] Extracted blood samples in an autosampler (at room temperature) are stable for at least 48 hours.[33]

2. Analytical Assays

Like sirolimus, everolimus has a narrow therapeutic index and a variable oral bioavailability.[6] For the performance of pharmacokinetic and drug interaction studies, several analytical assays for the quantification of everolimus have been developed.

Since high-performance liquid chromatography (HPLC) with UV detection is not sensitive enough to assay concentrations lower than 2 μg/L, several different HPLC/electrospray-mass spectrometric methods and one enzyme-linked immunosorbent assay (ELISA) have been described for the quantification of everolimus in whole blood and other biological fluids.[31-40] Table I shows the linear ranges, the lower limit of quantification (LOQ) and the interday precisions of all these methods. Until now no data are available on the correlation between mass spectrometric and ELISA methods. It is also unknown if the ELISA can detect metabolites.

In human blood, more than 75% of everolimus is bound to erythrocytes.[34] Therefore, whole blood samples (EDTA tubes) are recommended for phar-

Table I. Comparison of the quantification methods for everolimus

Assay	Linear range (μg/L)	LOQ (μg/L)	Interday CV (%)	Reference
LC-ESI-MS	0.15–30	0.5	<7	33
LC-ESI-MS	0.1–100	0.1	<15	31
LC-ESI-MS	0.5–100	0.5	<10	35
LC-ESI-MS	0.25–100	0.25	<10	36
LC/APCI-MS	0.38–250	0.38	<12	37
LC/ESI-MS	0.3–200	0.3	<9	40
LC/ESI-MS/MS	0.37–400	0.37	<15	38
LC/ESI-MS/MS	0.5–100	0.5	<7.6	32
LC/ESI-MS/MS	0.25–100	0,25	<15	39
ELISA	2–80	2.0	<16	34

APCI = atmospheric pressure chemical ionisation; **CV** = coefficient of variation; **ELISA** = enzyme-linked immunosorbent assay; **ESI** = electrospray-interface; **LC** = liquid chromatography; **LOQ** = lower limit of quantification; **MS** = mass spectrometry; **MS/MS** = tandem mass spectrometry.

macokinetic studies and therapeutic drug monitoring.

2.1 High Performance Liquid Chromatography with Detection by Electrospray Mass Spectrometry

In the last 5 years, nine HPLC/electrospray-mass spectrometry methods have been developed for the quantification of everolimus in whole blood (EDTA) and other biological fluids.[31-40] Several off-line and on-line sample extraction methods based on liquid-solid, liquid-liquid and solid phase extraction have been established. As recommended, all detection methods were designed for whole blood samples collected in EDTA tubes.

Everolimus is often given in combination with ciclosporin. Therefore, seven of the nine methods allow the simultaneous quantification of ciclosporin.[33,35-40] The described HPLC/electrospray-mass spectrometry techniques can be very simply modified for simultaneous quantification of everolimus and tacrolimus. Measurement of mycophenolic acid (MPA) with HPLC/electrospray-mass spectrometry is also possible, but due to the high concentration differences between everolimus and MPA in blood, no simultaneous but sequential measurement will be possible.

Table I presents the characteristics of the quantification methods, including the linear range and the LOQ. Only one published LC/electrospray-mass spectrometric technique describes the simultaneous quantification of everolimus, ciclosporin and their main metabolites.[35] The disadvantages of the mass spectrometric techniques are the high cost of purchase and the demanding technical knowledge.

2.2 Enzyme-Linked Immunosorbent Assay

Kovarik et al.[34] quantified everolimus whole-blood concentrations with a validated ELISA. Performance was assessed on the basis of a five-point quality control concentration range from 2 to 80 μg/L of everolimus. Coefficients of variation ranged from 13.3% to 16.1% and bias ranged from –7.0% to –1.8%. The assay quantification limit was 2 μg/L.[34] Until now, no commercial kit for the quantification of everolimus is available.

3. Pharmacokinetics

Up to now only a few pharmacokinetic studies (phases I–III) have been published. In these studies, healthy volunteers and kidney, liver, heart and heart/lung transplant recipients were treated with everolimus. A summary of the pharmacokinetic parameters of everolimus is shown in table II. Nothing is known about pharmacokinetics of everolimus in patients with pancreas, small bowel or bone marrow transplantation.

3.1 Absorption and Distribution

The oral bioavailability of everolimus is low (16%), but higher than that of sirolimus (10%) in rats.[6] After a single oral dose of everolimus 4mg in 12 healthy volunteers, everolimus was absorbed rapidly (within 30 minutes after drug intake). The maximum concentration (C_{max}) of everolimus amounted to 44.2 ± 13.3 μg/L and was reached (t_{max}) after 30 minutes (range 0.5–1 hour).[41] The area under the concentration-time curve (AUC) was 219 ± 69 μg • h/L.[41]

In the entry-into-human study,[42] 54 stable renal transplant patients received a single oral dose of everolimus 0.25–25mg in addition to ciclosporin Neoral®[1] and low-dosage corticosteroids. C_{max} ranged from 2.3 to 179 μg/L, and t_{max} ranged from

1 Use of tradenames is for product identification only and does not imply endorsement.

Table II. Pharmacokinetic parameters of everolimus. The values were obtained in various phase I to III studies of everolimus in healthy volunteers or in kidney, liver, lung or lung/heart transplant recipients. In addition, all patients received ciclosporin and corticosteroids

Study details				Pharmacokinetic parameters					Reference
Assay	Organ	n	Dosage range (mg)	Dosage (mg)[a]	C_{max} (μg/L)	t_{max} (h)	AUC (μg • h/L)	$t_{½β}$ (h)	
LC/APCI-MS	Healthy	12	4	4 single dose	44.2 ± 13.3	0.5 (0.5–1)	219 ± 69	32.2 ± 6.1	41
LC/APCI-MS	Kidney	54	0.25–25[b]	2.5 single dose	45 ± 21	1.3 ± 0.4	344 ± 141	25 ± 6	42
LC/APCI-MS	Kidney	24	0.75–7.5 once daily	2.5 once daily	33 ± 12	1.8 ± 0.8	211 ± 83	18.1 ± 7.6	43
ELISA	Kidney	101	0.5–2 twice daily[c]	1 twice daily	11.6 ± 4.4	2 (1–5)	81 ± 34	ND	34
LC/APCI-MS	Liver	26	7.5[b]	7.5 single dose	53 ± 16	2.5 (1–4.1)	735 ± 227	32 ± 8	44
LC/ESI-MS	Lung, lung + heart	20	0.035/kg (≤2.5/day) + 0.10/kg (≤7.5/day)[b]	≤2.5 single dose	13.8 ± 3.1[d]	1.5	135 ± 34[e]	25.7 ± 3.6	45

a Listed pharmacokinetic parameters are related to this dosage.

b Capsules.

c Tablets

d Dose-normalised C_{max} in patients without cystic fibrosis.

e Dose-normalised AUC.

APCI = atmospheric pressure chemical ionisation; **AUC** = area under the concentration-time curve; **C_{max}** = maximum blood concentration; **ELISA** = enzyme-linked immunosorbent assay; **ESI** = electrospray-interface; **LC** = liquid chromatography; **MS** = mass spectrometry; **ND** = not determined; **t_{max}** = time to C_{max}; **$t_{½β}$** = elimination half-life.

1.0 to 2.2 hours. The AUC of everolimus (doses of 2.5–25mg) ranged from 344 to 2400 μg • h/L and was dose-proportional.[42] Increasing doses of everolimus had no significant influence on the absorption or distribution of ciclosporin.[42]

The overall absorption of everolimus, like that of sirolimus, is probably affected by the activity of P-glycoprotein.[46-48] It is recommended that patients should take the drug consistently with or without food to reduce fluctuations in drug exposure. In the two long-term studies with 24 stable and 101 *de novo* renal transplant recipients, the patients were treated with several doses of everolimus in combination with a basal immunosuppression of ciclosporin Neoral® and prednisone.[34,43] Everolimus was absorbed rapidly, with mean t_{max} values ranging across dose levels from 1.3 to 1.8 hours for the first dose, and from 1.5 to 2 hours at steady state (table III). Steady state was reached within 7 days,[34,43] with a median 3-fold accumulation of everolimus exposure compared with that after the first post-operative dose.[34] Steady state C_{max}, trough concentration (C_{min}) and AUC showed a dose-proportional increase (table III and figure 2), and steady-state C_{min} correlated well with the AUC of everolimus during the year-long study ($r^2 = 0.88$).[34] The inter-individual pharmacokinetic variability for AUC was 85.4% and intraindividual interoccasion variability was 40.8%,[34] suggesting that therapeutic drug monitoring is needed. As C_{min} of everolimus correlates well with dose and AUC, this variable is therefore recommended for therapeutic drug monitoring.

Everolimus pharmacokinetic characteristics did not differ with age, sex and weight in adults.[34,49] The apparent clearance (CL/F, i.e. dose/AUC) for a representative patient, 44 years old and weighing 71kg, was 8.82 L/h. A 1kg increase in bodyweight resulted in a 0.44% increase in clearance.[49]

Table III. Steady-state pharmacokinetic parameters of everolimus in renal *de novo* transplant recipients receiving long-term triple immunosuppression with ciclosporin, corticosteroids and the indicated twice-daily doses of everolimus (reproduced from Kovarik et al,[34] with permission). The concentrations of everolimus were measured by an enzyme-linked immunosorbent assay. Values are means ± SD, or median (range) for t_{max}

Parameter and unit	0.5mg	1mg	2mg
C_{min} (μg/L)	1.5 ± 1.8	4.7 ± 2.6	9.5 ± 5.2
t_{max} (h)	2 (1–5)	2 (1–5)	2 (1–8)
C_{max} (μg/L)	5.0 ± 2.9	11.6 ± 4.4	21.9 ± 10.5
Dose-normalised C_{max} (μg/L/mg)	10.0 ± 5.8	11.6 ± 4.4	11.0 ± 5.3
AUC (μg • h/L)	34 ± 23	81 ± 34	164 ± 78
Dose-normalised AUC (μg • h/L • mg)	68 ± 46	81 ± 34	82 ± 39

AUC = area under the concentration-time curve; **C_{max}** = maximum blood concentration; **C_{min}** = trough blood concentration; **t_{max}** = time to C_{max}.

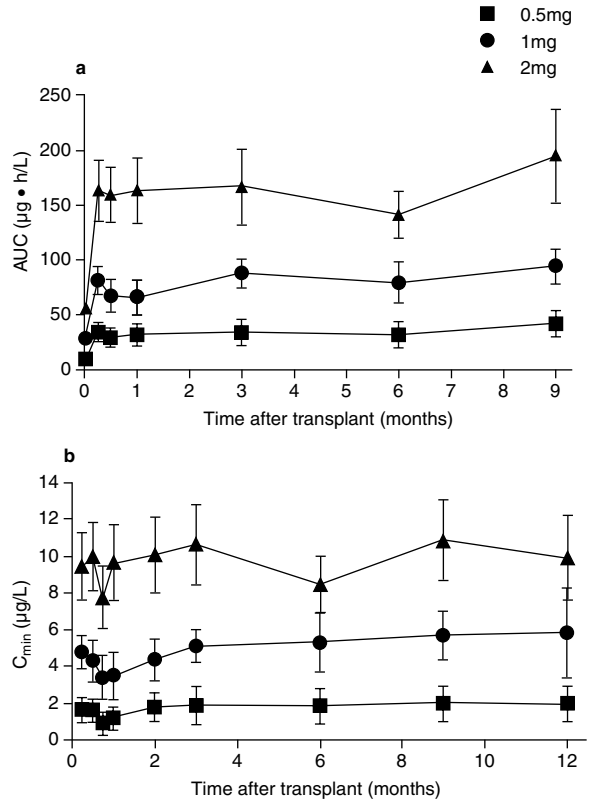

Fig. 2. Pharmacokinetics of everolimus after *de novo* renal transplantation: (**a**) area under the concentration-time curve (AUC) and (**b**) trough concentration (C_{min}). Groups of patients received three different dosages of everolimus (0.5, 1 and 2mg twice daily) in combination with ciclosporin and corticosteroids. The concentrations of everolimus were measured by an enzyme-linked immunosorbent assay. Bars designate 95% CIs (reproduced from Kovarik et al.,[34] with permission).

The different everolimus dosages had no significant influence on the C_{min} of ciclosporin.[34,43]

In *de novo* renal transplant recipients receiving immunosuppressive therapy with ciclosporin, corticosteroids and everolimus, Asian ethnicity did not significantly affect everolimus clearance.[49] In contrast, clearance was 20% higher in Black patients than in non-Black patients.[49]

Twenty-six *de novo* liver allograft recipients received everolimus in addition to ciclosporin Neoral® and corticosteroids.[44] It was shown that the route of administration (nasogastric or nasoduodenal) of everolimus had no significant influence on its pharmacokinetic parameters. Patients with a T-tube had a significantly lower C_{max} value than those without a T-tube. Over the first transplant month, C_{max} increased significantly by 41% in recipients with a T-tube and by 26% in recipients without a T-tube. The presence or absence of bile in the gastrointestinal tract did not significantly affect C_{max} (35 ± 10 µg/L with T-tube open versus 46 ± 19 µg/L with T-tube closed) or AUC (608 ± 234 µg • h/L with T-tube open versus 608 ± 226 µg • h/L with T-tube closed) of everolimus.[44] t_{max} was reached significantly earlier when the T-tube was closed (1.5 hours) in comparison with open (2.5 hours). A single dose of everolimus did not affect the steady-state pharmacokinetics of ciclosporin, regardless of whether the patients had external bile diversion or not.

In a phase I crossover study, 20 stable lung and heart/lung transplant recipients were treated with 0.035 mg/kg (2.5mg maximum) or 0.10 mg/kg (7.5mg maximum) of everolimus.[45] All patients

were stable transplant recipients and had a basal immunosuppression with ciclosporin and prednisone. Eight patients had pancreatic insufficient cystic fibrosis (group I) and 12 patients (group II) did not have cystic fibrosis. The ciclosporin dosage in the eight patients with pancreatic insufficient cystic fibrosis was double that in the 12 patients without cystic fibrosis. It has already been described that patients with cystic fibrosis have a poor absorption of ciclosporin.[50] The reason for this seems to be structural and functional abnormalities of the gastrointestinal system in these patients. At both everolimus dosages (0.035 mg/kg and 0.10 mg/kg), cystic fibrosis patients had significantly lower C_{max} of everolimus compared with the non-cystic fibrosis patients. However, the extent of everolimus absorption (AUC/dose) did not differ significantly between patients with and without cystic fibrosis when pooled across dose levels ($p = 0.63$).[45] t_{max} was not significantly influenced by different everolimus dosage levels or the different patient groups (cystic fibrosis versus non-cystic fibrosis). The steady-state pharmacokinetics of ciclosporin were not affected by coadministration of everolimus, nor did the presence of cystic fibrosis have an effect on ciclosporin pharmacokinetics.

To investigate the influence of liver impairment on the pharmacokinetics of everolimus, eight patients with moderate hepatic impairment (liver cirrhosis Child-Pugh B) and eight healthy subjects received a single oral 2mg dose of everolimus (tablets).[51] Absorption of everolimus was not altered, as evidenced by comparable C_{max} (liver cirrhosis vs healthy, 11.7 ± 4.3 vs 15.4 ± 8.6 μg/L) and t_{max} (liver cirrhosis vs healthy, 0.7 ± 0.3 vs 0.8 ± 0.5 hours).[51] The protein binding of everolimus was not influenced by moderate hepatic impairment (liver cirrhosis vs healthy, 73.8 ± 3.6% vs 73.5 ± 2.4%).[51]

At therapeutic concentrations, more than 75% of everolimus is partitioned into red blood cells and approximately 75% of the plasma fraction is protein bound.[34] In monkey lung transplant recipients, the highest everolimus concentrations were measured in gall bladder, pancreas, the transplant lung, cerebellum, kidneys and spleen.[52] The tissue distribution of everolimus in humans is unknown.

3.2 Metabolism and Elimination

Everolimus is metabolised mainly in the gut and liver by cytochrome P450 (CYP) 3A4, 3A5 and 2C8.[53] About 98% of everolimus is excreted in the bile in the form of metabolites and only 2% of everolimus is eliminated in the urine. The elimination half-life ranged from 18 to 35 hours across the different treatment groups.[42-44] Because of its rapid clearance, everolimus requires twice-daily administration, whereas the long half-life of sirolimus allows once-daily administration.[54,55]

In 12 healthy volunteers who received a single oral 4mg dose of everolimus, the CL/F of everolimus was 19.7 ± 5.4 L/h and the elimination half-life was 32.2 ± 6.1 hours.[41]

Ciclosporin acts synergistically with everolimus and is therefore given as combination therapy. All studies in humans showed that the steady-state pharmacokinetics of ciclosporin were not affected by everolimus coadministration.[34,42,43,56] One study has investigated the pharmacokinetics of everolimus metabolites during concomitant therapy with ciclosporin in seven stable renal transplant patients. Everolimus and four main metabolites, hydroxy-everolimus, dihydroxy-everolimus, demethyl-everolimus and the ring-opened form of everolimus, were found in blood.[35,56,57] Hydroxy-everolimus was the most important metabolite, with a dose-normalised AUC_{24} nearly half that of the parent compound (16.0 ± 6.5 vs 35.4 ± 13.1 μg • h/L), followed by demethyl-everolimus (AUC_{24} 10.7 ± 15.8 μg • h/L), dihydroxy-everolimus (AUC_{24} 8.5 ± 5.7 μg • h/L) and ring-opened everolimus (AUC_{24} 2.3 ± 2.1 μg • h/L). All metabolites appeared relatively soon after administration (t_{max} 1.2–2.0 hours vs 1.5 hours for everolimus).[56] The immunosuppressive or toxic activity of everolimus metabolites is unknown. This single oral dose of everolimus did not influence the pharmacokinetics of ciclosporin or its metabolite pattern, since the AUC and C_{max} of hydroxy-ciclosporin and dihydroxy-ciclosporin did not change significantly in the presence of everolimus. Ciclosporin clearance was not significantly influenced by increasing everolimus doses.[56]

In *de novo* liver transplant recipients who received ciclosporin and a single oral dose of everolimus, the interpatient coefficients of variability for

C_{max} and AUC were 35% and 34%, respectively.[44] This large interindividual variability in everolimus biotransfomation is caused by different activities of the efflux pump P-glycoprotein and of CYP3A4, 3A5 and 2C8.[6,53]

The effect of two different ciclosporin formulations (Sandimmun® and Neoral®) on the pharmacokinetics of everolimus has been investigated in healthy volunteers.[58] Coadministration of Sandimmun® (gelatin capsule filled with a corn oil suspension) with everolimus increased the AUC of everolimus by an average of 74% ($p = 0.0001$), whereas C_{max} and the elimination half-life of everolimus were not influenced by Sandimmun®.[58] Simultaneous administration of Neoral® (gelatin capsule filled with a microemulsion preconcentrate) with everolimus increased everolimus C_{max} by 82% and AUC by 168%. The elimination half-life of everolimus was not influenced by Neoral®.[58] If Sandimmun® or Neoral® is removed from an everolimus/ciclosporin immunosuppressive regimen, a 2- to 3-fold decrease in everolimus exposure is expected. In this situation, therapeutic drug monitoring of everolimus concentrations is recommended.[58]

Compared with healthy subjects, patients with moderate hepatic impairment (liver cirrhosis Child-Pugh B) had significantly lower CL/F of everolimus, by 53% on average (hepatic impairment vs healthy, 9.1 ± 3.1 vs 19.4 ± 5.8 L/h).[51] This was manifested as 115% higher AUC (hepatic impairment vs healthy, 245 ± 91 vs 114 ± 45 μg • h/L) and 84% prolongation in half-life (hepatic impairment vs healthy, 79 ± 42 vs 43 ± 18 hours).[51] The AUC of everolimus showed a significant positive correlation with the bilirubin concentration ($r^2 = 0.86$), and a significant negative correlation with albumin concentration ($r^2 = 0.72$).[51] The dosage of everolimus should be reduced by half in patients with hepatic impairment.[51] No data are available on the metabolism or elimination of everolimus in non-cirrhotic patients with hypoalbuminaemia.

At least eleven everolimus metabolites have been elucidated *in vitro*.[53,59-62] Hydroxylation and demethylation of everolimus appear to be the major metabolic pathways involved. The structure of the following metabolites have been identified: 46-, 24-, 25-, 12-, 11-, 14- and 49-hydroxy-everolimus, 39-*O*-, 27-*O*- and 16-*O*-demethyl-everolimus, and 40-*O*-dehydroxyethyl-everolimus.[53,59,60] Because of the steric configuration of everolimus, the different CYP enzymes (3A4, 3A5 and 2C8) showed different preferences for these metabolism sites.[53] CYP3A4 is the most important enzyme involved in the metabolism of everolimus.

3.3 Pharmacokinetics in Children

A phase I trial in stable paediatric renal transplant patients (median 4 years post-transplant) investigated the single-dose pharmacokinetics, safety and tolerability of everolimus in combination with ciclosporin Neoral® and corticosteroids, with or without azathioprine.[63] Nineteen patients (mean age 9.1 ± 3.8 years) were included in the study and received a single 1.2 mg/m^2 dose of everolimus. The patients were divided into two age-groups: 13 children (3–11 years) and six adolescents (12–16 years). There was a wide distribution of weight (range 16.4–68.0kg) and body surface area (range 0.67–1.75m^2). The average dose administered was 1.3 ± 0.4mg as tablets. The pharmacokinetics of everolimus and ciclosporin were determined in whole blood by a liquid chromatography-mass spectrometric method. Everolimus was well tolerated. The C_{max} of everolimus was 20.7 μg/L and was reached after 1 hour. The mean AUC was 220 ± 63 μg • h/L. CL/F of everolimus (mean 5.9 ± 1.7 L/h/m^2) showed a significant positive linear correlation with age ($r^2 = 0.71$, $p < 0.001$), bodyweight ($r^2 = 0.82$, $p < 0.001$) and body surface area ($r^2 = 0.80$, $p < 0.001$).[63] The apparent distribution volume (mean 250 ± 103 L/m^2) increased linearly with age, weight and body surface area ($p < 0.001$ for all), whereas the elimination half-life was similar regardless of age ($p = 0.15$). Compared with adults from a previous study, CL/F and distribution volume were lower in paediatric patients, whereas the elimination half-life was similar.[63,64] The steady-state pharmacokinetics of ciclosporin were not influenced by a single dose of everolimus. Based on these results, paediatric patients should receive bodyweight-adapted dosages of everolimus.[63]

In paediatric *de novo* kidney allograft recipients the steady-state pharmacokinetics of everolimus were longitudinally assessed during a 6-month period.[65] In addition to ciclosporin and corticosteroids, 19 paediatric patients received everolimus 0.8

mg/m^2 (maximum 1.5mg) everolimus twice daily as a dispersible tablet in water. Everolimus was administered at least 1 hour before or after meals and within 10 minutes of the corresponding morning and evening dose of ciclosporin. Nine boys and ten girls participated in this study. The median age was 9.9 years (range 1–16). The median bodyweight amounted to 29.0kg (range 11–77). In the first month the ciclosporin C_{min} was between 200 μg/L and 350 μg/L and between 100 μg/L and 300 μg/L thereafter. Everolimus and ciclosporin were measured simultaneously in whole blood by a liquid chromatography-mass spectrometric method. Seventeen out of 19 patients completed 6 months of treatment. Pharmacokinetics of everolimus were measured on day 7 and month 3. Everolimus and ciclosporin C_{min} concentrations were quantified on days 3, 5, 6 and 7 and at months 1, 2, 3 and 6. Following steady-state pharmacokinetics parameters (median) were calculated: C_{min} 4.7 μg/L; peak concentration 13.5 μg/L; AUC 77 μg • h/L; and apparent oral clearance 10.2 L/h/m^2. Positive correlation was found for the clearance with weight (r = 0.67), bodysurface area (r = 0.68), and age (r = 0.66). During the treatment time at months 1, 3 and 6 the C_{min} of everolimus was stable. The AUC of everolimus showed an intra- and interpatient variability of 29% and 35%, respectively. Everolimus was well tolerated. The median C_{min} value of ciclosporin was 156 μg/L, 83 μg/L, and 69 μg/L at months 1, 3 and 6 respectively. In conclusion, paediatric patients should receive bodysurface-adjusted dosages of everolimus.[65]

In this study of Hoyer et al.,[65] the children received everolimus formulated as a dispersible tablet in water. In contrast to this, in other studies in adults, patients received everolimus as a conventional tablet. In their study, Kovarik et al. compared the bioavailability of both types of tablets.[66] It was shown that the bioavailability of everolimus from the dispersible tablet was 10% lower relative to the conventional tablet. But the authors concluded that if a child is switched from the dispersible to the conventional tablet, it should be done by 1 : 1mg and tight therapeutic drug monitoring. As already reported in other studies, the tablets (dispersible or conventional) should be taken consistently either with or without food to minimise the fluctuations of C_{min} and AUC.[66]

3.4 Drug Interactions

In phase III trials of everolimus, about 51% of patients receiving an everolimus/ciclosporin/prednisone regimen presented with elevations in serum cholesterol and triglycerides. These patients were treated with the HMG-CoA reductase inhibitors pravastatin or atorvastatin (both 20 mg/day). Atorvastatin is a known substrate of CYP3A4 (like everolimus), but pravastatin does not interact with CYP3A4. In healthy men it could be shown that everolimus C_{max} was reduced by 9% and 10% with atorvastatin and pravastatin coadministration; the corresponding decreases in everolimus AUC were 5% and 6%, respectively.[67] Everolimus coadministration increased the C_{max} of atorvastatin by 11%, but the AUC of atorvastatin remained unchanged.[67] Coadministration of everolimus with pravastatin was associated with a 10% decrease in pravastatin C_{max} and a 5% decrease in the AUC. Everolimus had no influence on the elimination half-lives of the two statins.[67] In conclusion, the pharmacokinetics of everolimus, atorvastatin or pravastatin were unaffected by single-dose administration of everolimus with either atorvastatin or pravastatin.[67]

The influence of the CYP3A4 inducer rifampicin (rifampin) on the pharmacokinetics of everolimus in healthy volunteers was assessed by Kovarik et al.[41] When everolimus was administered during rifampicin treatment, the CL/F of everolimus was significantly increased, on average by 172%. Although t_{max} was not affected, C_{max} decreased in all volunteers, on average by 58% (p = 0.0001). The AUC decreased in 11 of 12 volunteers, and in one subject the AUC remained unaffected.[41] The average decrease in AUC in the full study population was 63% (p = 0.0001). Everolimus half-life was shortened significantly, from an average of 32 hours to 24 hours (26%, p = 0.0001). During combination therapy with everolimus and rifampicin, therapeutic drug monitoring is recommended to adjust the dosage of everolimus individually.[41]

The effect of comedications was investigated in *de novo* renal transplant recipients receiving immunosuppression with ciclosporin, corticosteroids and everolimus (0.75 or 1.5mg). Coadministration of

erythromycin or azithromycin resulted in a significant decrease in everolimus clearance by 22% and 18%, respectively.[49] Fluconazole had no significant influence on everolimus clearance.[49] By contrast, one patient receiving itraconazole had a 74% reduction of everolimus clearance.[49]

Everolimus should be administered consistently either with food or without food, because a high-fat meal influences the pharmacokinetics of everolimus.[68] The effect of a high-fat meal on the pharmacokinetics of everolimus was investigated in 24 healthy volunteers who received everolimus 2mg orally under fasting conditions and after a high-fat meal. Under the same food conditions, six stable renal transplant patients received oral everolimus 2.5 mg/day in addition to ciclosporin and prednisone. In the healthy volunteers, a high-fat meal delayed the t_{max} of everolimus by a median 1.25 hours, reduced C_{max} by 60% and reduced AUC by 16%.[68] In the renal transplant patients, a high-fat meal delayed t_{max} by a median of 1.75 hours, reduced C_{max} by 53% and reduced AUC by 21%.[68] Everolimus C_{min} values showed no food effect, whereas the fluctuation of C_{max} was reduced by 52%.[68]

4. Therapeutic Drug Monitoring

Studies in animals and humans showed that the immunosuppressive efficacy and the occurrence and severity of adverse effects of everolimus correlated with blood concentrations.[43] In the entry-into-human study, stable renal transplant recipients on steady-state immunosuppression with ciclosporin and corticosteroids received a single oral dose of between 0.25 and 25mg of everolimus. All everolimus doses were well tolerated, with no discontinuations due to adverse events, serious adverse events or deaths.[42] In the phase Ib study, stable renal transplant recipients (n = 6 for each dose) on basal immunosuppression with ciclosporin and corticosteroids received three different everolimus dosages (0.25, 2.5 and 7.5 mg/day) for 4 weeks. Of the patients treated with the highest everolimus dosage (7.5 mg/day), 43% had serious adverse events. Among all everolimus groups, there was an increased incidence of infectious episodes, including herpes simplex (n = 3), upper respiratory infection (n = 3), pharyngitis (n = 3) and one case each of pneumonia and sinusitis. There was an increased incidence of gastrointestinal disorders such as diarrhoea (n = 3), nausea (n = 3) and vomiting (n = 2), probably related to drug administration.[43] Patients treated with everolimus 0.75 or 2.5 mg/day had no significant changes in leucocytes or thrombocytes compared with placebo, but in patients treated with everolimus 7.5 mg/day leucocytes and thrombocytes were significantly decreased.[43]

In patients receiving combination therapy with ciclosporin, corticosteroids and everolimus, the incidence of moderate and severe rejection episodes was found to be significantly lower among patients in the 1 and 2mg everolimus twice daily group than in the 0.5mg everolimus twice daily group.[26]

Several studies showed that there was an increasing incidence of transient thrombocytopenia ($<100 \times 10^9$/L) with increasing everolimus AUC ($p = 0.03$).[34,43,45] To define the therapeutic dosage of everolimus and to correlate the dosage with the adverse effects of everolimus, two randomised, double-blind phase III trials in *de novo* kidney transplant patients have been performed.[69] A total of 695 patients received everolimus 0.75 or 1.5mg twice a day in combination with corticosteroids and ciclosporin (C_{min} 150–400 μg/L in month 1 and 100–300 μg/L thereafter). Everolimus tablets and ciclosporin capsules were given simultaneously. At weeks 1 and 2 and months 1, 2, 3 and 6 after kidney transplantation, blood samples into EDTA were taken and everolimus and ciclosporin C_{min} values were quantified by a liquid chromatography-mass spectrometric method. There was a significant ($p = 0.03$) relationship between incidence of freedom from acute rejection and everolimus C_{min}, being 68% at 1.0–3.4 μg/L, 81–86% at 3.5–7.7 μg/L and 91% at 7.8–15.0 μg/L.[69] Thus, a significantly increased risk of acute rejection was observed at everolimus C_{min} lower than 3 μg/L. The upper limit of the therapeutic range of everolimus appears to be defined by a 15% incidence of leucopenia ($<4 \times 10^9$/L) and a 17% incidence of thrombocytopenia ($<100 \times 10^9$/L) in the C_{min} range of 7.8–15 μg/L.[69-71] No difference in pharmacokinetics between male and female patients was observed.[34]

In phase III trials of everolimus, hypercholesterolaemia and hypertriglyceridaemia were observed. In these studies, HMG-CoA reductase inhibitors

were prescribed as part of post-transplant management in 51% of patients receiving an everolimus/ciclosporin/prednisone regimen and in 35% of patients in the control arm of the study, who received mycophenolate mofetil, ciclosporin and prednisone.[67] No correlation was found between dosage or concentration of everolimus and incidence of abnormal cholesterol or triglyceride serum levels.[34,43,45] The maximum cholesterol and triglyceride levels occurred on average by days 35 and 29, respectively.[34]

Surprisingly, significant elevations of serum creatinine were identified among patients receiving everolimus in combination with full therapeutic dosages of ciclosporin Neoral® in phase III studies of everolimus.[72] It has been suggested that this nephrotoxicity was associated with the calcineurin inhibitor ciclosporin. Available evidence indicates that everolimus is not associated with clinically demonstrable renal toxicity.[72] Encouraging results have been observed with everolimus in a reduced-dosage ciclosporin Neoral® regimen. Thus, in an ongoing, randomised, open-label, parallel-group study in 111 *de novo* renal transplant patients receiving quadruple immunosuppressive therapy with everolimus 3 mg/day and either full-dose Neoral® or reduced-dose Neoral® (plus an anti-interleukin-2 antibody and corticosteroids), rates of acute rejection were lower in patients receiving the reduced versus full-dosage ciclosporin Neoral® regimen (7.0% vs 16.7%, respectively).[29,70] Furthermore, renal function was significantly improved by the reduced-dosage Neoral® regimen, as measured by the indices of glomerular filtration rate and creatinine clearance.[29] Lipid levels were consistently lower and the incidence of notably high systolic and diastolic blood pressures was lower in patients receiving the reduced-dosage Neoral® regimen.[29,70]

Everolimus has a narrow therapeutic index and a variable oral bioavailability.[6] Therefore, therapeutic drug monitoring will be needed. It has been shown that in *de novo* renal transplant recipients who received everolimus 0.5, 1 or 2mg twice a day, C_{max}, C_{min} and AUC increased proportionally to dosage.[34] In phase III studies, the grade of thrombocytopenia correlated well with everolimus C_{min} values. Therefore, C_{min} of everolimus should be used for therapeutic drug monitoring.

5. Conclusion

The macrolide immunosuppressant everolimus has a different mode of action to that of ciclosporin, which leads to synergy of both drugs. Therefore, everolimus is currently under clinical investigation in combination with ciclosporin. The advantage of everolimus is its much lower nephrotoxicity in comparison with the calcineurin inhibitors ciclosporin and tacrolimus. Everolimus has a shorter half-life than sirolimus and is given twice daily. Steady-state pharmacokinetics are reached within 7 days. Steady-state C_{max}, C_{min} and AUC show a dosage-proportional increase. The main adverse events of everolimus are thrombocytopenia, hypercholesterolaemia, hypertriglyceridaemia and gastrointestinal disorders (diarrhoea). The interindividual pharmacokinetic variability in AUC is caused by the different activities of the efflux pump P-glycoprotein and of CYP3A4, 3A5 and 2C8. Due to its narrow therapeutic index and the variable oral bioavailability of everolimus, therapeutic drug monitoring is recommended. Efficacy for the prevention of acute rejection episodes, and the rate of common adverse effects (thrombocytopenia), correlate well with C_{min}. Therefore, C_{min} of everolimus should be used for therapeutic drug monitoring. In combination therapy with everolimus, ciclosporin and prednisone, everolimus C_{min} should be between 3 and 15 μg/L in whole blood (EDTA tube) to avoid acute rejection episodes and to reduce toxicity.

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