Determination of Everolimus Concentrations for Monitoring Patients on Immunosuppressive Therapy after Heart and Kidney Transplantation

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Abstract

Background: Heart transplantation has become the treatment of choice for patients with intractable advanced heart failure after conventional medications and surgical procedures are being exhausted. With adequate immunosuppression we want to prevent or to treat allograft rejection while minimizing risk for infection or cancer. Everolimus (EVL) inhibits the manifestation of chronic allograft rejection.

Methods: We determined the concentration of EVL in patients after heart and kidney transplantation using immune turbidimetric assay and liquid chromatography-tandem mass spectrometry (LC-MS/MS). We analysed the samples of 36 patients after kidney transplantation and followed 6 patients after heart transplantation.

Results: The measurements of parallel samples using both techniques were in statistically significant correlation (r = 0.825, P < 0.0001).

With therapeutic drug monitoring (TDM) of EVL concentrations in heart recipients, we established that intra- and inter-subject variability in pharmacokinetics and pharmacodynamics contribute to changeable responses on EVL dosing. EVL concentrations were varying and depended on concomitant therapy, and the expression of cytochrome CYP3A4 and P-glycoprotein. Very potent inhibitor of CYP3A4 Sporanox® markedly increases the EVL whole blood concentrations. On the other hand, moderate and weak inhibitors have smaller impact on EVL concentrations.

Conclusions: Therapeutic drug monitoring of EVL concentrations is due to a narrow therapeutic index and optimal efficacy with minimal toxicity essential for individual dosing of EVL. TDM of EVL concentrations with immune turbidimetric assay is reliable, simple, specific, sensitive and without any interferences.

Keywords: Everolimus; Transplantation; Immunosuppressive Therapy; Determination in Blood

Introduction

The first successful transplantation of the kidney was performed by dr. Josepf E. Murray in Boston, 1954. This success was followed by a successful liver transplant in Denver (Dr. Thomas E. Starzl) and the heart in Cape Town (Dr. Christiaan N. Barnard) in 1967 [1].

An important milestone in the history of organ transplantation is also the finding by British biologist Peter B. Medawar that the rejection of the graft is responsible for the immune reaction. In 1953, he find out that the acquired tolerance was immune-specific and that it occurred due to the specific deviation of the host immune system [2,3].

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This was followed by Jean Dausset's important discovery of human leukocyte antigens ("Human Leukocyte Antigens-HLA") and Jon J. van Rood's discovery of anti-leukocyte antibodies. The event triggered further research on the classification of HLA antigens [4].

In Slovenia in 2017, 47 kidneys, 24 hearts and 23 liver were transplanted. At the end of 2017, a total of 115 individuals were listed on the waiting list for organ transplantation, of which 53 for kidney, 42 for the heart, 18 for liver and 2 for combined kidney transplant and pancreas [5].

Heart transplantation

Heart transplantation is an optional method of treating patients with a final heart failure after all other methods of pharmacological and/surgical treatment. They are used in patients with advanced heart failure caused by the virus, severe coronary artery disease and myocardial infarction, cardiac valve defects or congenital heart failure. Heart failure occurs when the heart muscle is no longer capable of pumping sufficient blood volume to meet the needs of the whole organism. Because of this, patients experience tiredness, difficulty in breathing, decreased physical capacity, and swelling of the legs or abdomen [6].

Transplantation of the kidneys

Among all organ transplants, kidney transplantation has the most successful history since the invention of the dialysis by William Kolff in 1944. In practice, the search for a tissue-related donor came into play only after dialysis techniques developed to the extent that it was possible to find the appropriate donor. After 1960, matching in HLA antigens has become a priority in finding a suitable donor for transplantation of the kidneys [7].

Transplantation of the kidneys is a safe and, compared to dialysis, a more favourable selection method of treatment that improves the survival and quality of life of patients with final renal failure [8]. Due to the advances and improvements in transplantation medicine, the 1-year survival, despite a higher age and severely ill patients, reached a level of 95% compared to 60% in the 1970s [9]. Indications for renal transplantation include chronic kidney disease (the consequence of diabetes, hypertension, glomerulonephritis, renal cysts,) and kidney tumours (Wilms's tumour in children and renal cell carcinoma in adults) [10].

Pharmacological treatment

The goal of immunosuppressive therapy is to prevent the rejection of the transplanted organs and at the same time reduce the side effects of medicines such as infections, cancer, diabetes, hypertension and renal impairment [11]. An immunosuppressive organ transplant protocol is a combination of drugs that act on different pathways of T cell activation [12]. The discovery of effective immunosuppressants represents a great progress and success of transplantation. In the early 1980s, the introduction of cyclosporine A (CYA) into immunosuppressive treatment protocols contributed significantly to the survival of organ recipients. Later, other immunosuppressants have been discovered, which in many studies have been studied still today, in particular, the need for early induction therapy, the most effective and safest combination of immunosuppressants, the safety of early steroid discontinuation and the lowest possible maintenance dose of immunosuppressants [13]. Immunosuppressive treatment protocols are generally divided into induction, maintenance and anti-refusal therapy.

Everolimus (EVL)

EVL is a macrolide immunosuppressant with a 2-hydroxyethyl chain in position 40, with a molecular weight of 958.224 g/mol ($C_{53}H_{83}NO_{14}$). It is a derivative of sirolimus (rapamycin) (SRL), a macrolide antibiotic produced by Streptomyces hygroscopicus. Just like SRL, EVL exhibits antiproliferative and immunosuppressive activity with even better stability, solubility and pharmacokinetic properties [14,15]. European Medicines Agency in 2003 approved EVL (Certican®) in Europe for immunosuppressive treatments after kidney and heart transplantation (first in Sweden) and Food and Drug Administration in the United States (Zortress®) in the case of kidney transplantation in 2010 [16].

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Pharmacological properties

EVL inhibits the cellular proliferation of hematopoietic and non-hematopoietic cells with the formation of a complex with the FK506-binding protein 12 (FKBP12) by growth factors. The EVL-FKBP12 complex inhibits mTOR protein (protein rapamycin) that stops the cell cycle in the G1 phase [14,15].

The pharmacokinetic properties of EVL are dose-dependent. Absorption is rapid and the highest concentration of the product is measured in a full blood sample for 1 - 2 hours after taking the medicine. Balance is established after 4 days of taking the medicine. More than 75% of the product is transferred to erythrocytes. 75% of the plasma fraction of EVL is bound to plasma proteins.

The half-life of EVL is between 18 and 35 hours, which requires a twice daily dosing of the drug (SRL has a half-life of 60 hours which dictates only a single daily dose).

Proliferation signalling pathway inhibitors allow dose reduction, abortion or avoidance of calcineurin inhibitors due to nephrotoxicity, which in addition to effective immunosuppression is the consequence of co-administration of EVL and CYA, in carefully selected patients. By exploiting the antiproliferative and antineoplastic effect of the proliferation pathway signal inhibitors, the acute rejection reaction with a reduced long-term renal impairment, coronary vasculopathy of the graft and cancerous diseases is achieved.

Side effects of EVL

Despite good tolerance, the occurrence of adverse events with the use of EVL is quite common [16]. The most common side effects are hyperlipidaemia (↑ total cholesterol, triglycerides, LDL cholesterol, ↓ HDL cholesterol), leukopenia, thrombocytopenia, peripheral oedema (changed permeability of endothelium with oxidative stress and release of prostacyclin), poor healing of the surgical wound (due to antiproliferative effect and wound infections), nephrotoxicity (↑ creatinine: severe nephrotoxicity with concomitant CYA, proteinuria due to reduced tubular reabsorption of proteins), acne, infections (bacterial infections: pneumonitis, inflammation of the throat; viral infections: sinusitis, herpes simplex, CMV) and GIT disorders (nausea, vomiting, diarrhoea) [15-20].

Monitoring of EVL concentrations

To maintain EVL concentrations in a narrow therapeutic range between 3 and 8 μg/L, as with other immunosuppressants, the concentration of the drug in blood must be followed. In addition to the narrow therapeutic area, EVL also has a variable bioavailability among individuals [14,15]. By maintaining the concentration of the drug in whole blood in the therapeutic area, the potential for adverse effects (especially in parallel therapy with CYA) is reduced while maintaining the beneficial effects of the drug [16]. Initially, the concentration of the drug in whole blood was determined only by liquid chromatography coupled with mass spectrometry (LC-MS). Lately, more and more laboratories are trying to introduce a cheaper and friendlier method for measuring EVL concentrations with the principle of immunochemical reaction [21].

In order to monitor EVL concentrations in patients with renal and cardiac transplantation in University Medical Centre Ljubljana, we first compared the EVL concentration with both methods in the group of recipients of the kidney. The results of the comparison are given in tables 1-3 and in graphs 1 and 2. The second aim of our study was to follow heart transplanted patients. The results are given in graph 3.

Materials and Methods

Patient group

In order to calculate the correlation between the immunochemical method and the LC-MS/MS, we used the determination of the concentrations of EVL in 9 recipients of the kidneys (5 males, 4 females, age of women 50 ± 14 years, age of males 46 ± 20 years) obtained by measuring concentrations EVL using an immunochemical method and liquid chromatography with tandem mass spectrometry from a series of two parallel samples of blood recipients. To monitor suitability the efficacy of EVL therapy included 6 patients aged 57-75 years

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(mean age 65 years), of which 1 female (58 years) and 5 men (66 ± 9 years old) who underwent cardiac transplantation and later with catheterization and UZ heart confirmed coronary vasculopathy of the graft. These patients receive EVL as part of immunosuppressive therapy. The concentration of EVL in whole blood was measured in patients to monitor the concentration of the drug, to determine the suitability of the concentration of EVL in whole blood in relation to the therapeutic range of this medicine. Blood samples were taken from patients in the nephrologic and cardiac department and in the transplantation clinic and cardiologic clinic in the steady state of the medicine (after 4 days of taking the medicine, before the next dose) according to the need to monitor the concentration of the active substance.

In the second part, we describe some interesting cases where the adequacy of EVL concentrations in the recipients of the heart with coronary vasculopathy confirmed with respect to its therapeutic area and accompanying therapy was established.

Analytical methods used

QMS method

To determine the EVL concentrations we used the turbidimetric method (QMS Everolimus Immunoassay) and the reagents of the manufacturer Thermo Scientific. The method allows quantitative determination of the immunosuppressive EVL in whole blood samples. In addition, the method makes it possible to monitor the Therapeutic Drug Monitoring of EVL concentrations, on the basis of which the clinician decides to adjust the dose of the medicinal product, insofar as it is not located in the therapeutic area.

Principle of the method

The immuno turbidimetric method for the quantitative determination of EVL is based on the interaction of the drug in the whole blood sample and the drug on the micro particles for binding sites in the anti-EVL Pt reagent. The EVL coated micro particle reagent is rapidly agglutinated in the presence of an anti-EVL Pt reagent in the absence of other competing drugs in the sample. The change of the absorbance is measured photometrically. When a sample containing EVL-containing sample is added, a partial inhibition of the agglutination reaction with a drop in absorbance occurs. The EVL-dependent classical agglutination inhibition curve is obtained with the highest level of agglutination at low EVL concentrations and with the lowest level of agglutination at high EVL concentrations.

The coefficient of variation for repeatability in the series ranges from 4.2 to 12.8%, and for repeatability outside the batch, it ranges from 4.9 to 18.4%.

LC-MS/MS method

Liquid chromatography with tandem mass spectroscopy is a wide-dynamic method that allows the simultaneous measurement of the concentrations of EVL, SRL, tacrolimus (TAC) and CYA in whole blood. The approach with tandem mass spectroscopy excludes the influence of interference of hydroxylated and/or demethylated metabolites of immunosuppressants and other commonly used drugs at the same time. The measurement of concentrations of EVL (and other immunosuppressants) is rapid, repeatable, and specific with the LC-MS / MS method [22].

This method yields the following coefficients of variation: for repeatability in the series, 10.1% for repeatability outside the series, 13.0% [23].

Statistical data processing was performed by the statistical software IBM SPSS Statistics Version 19 and Microsoft Office Excel 2007. We performed the Kolmogorov-Smirnov normality test and calculated the average value, the standard deviation of the measured concentrations, and the Pearson coefficient between the two methods.

Results

The basic calculations show that the results are distributed normally. Calculations of the two sets of results are shown in table 1.

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	Immunoturbidimetric	LC-MS/MS
Sample size	36	36
Mean	4.836	5.044
95% Cl	4.288 - 5.384	4.472 - 5.617
SD	1.6201	1.6912
Variance	2.6247	2.8603
Standard error of the mean	0.2700	0.2819
Median	4.900	5.050
95% Cl	4.266 - 5.700	4.500 - 5.934
Minimum	1.7	2.0
Maximum	8.7	8.3
2.5 - 97.5 P	1.82 - 8.14	2.08 - 8.02
Normal Distribution	0.7800	0.4741

Table 1: *Basic calculations of the comparison of both methods using the Kolmogorov-Smirnov test.*

The next step in evaluating the comparability of the methods was the calculation of the correlation and the use of paired t-test to evaluate the method differences. The calculations are given in tables 2 and 3.

Variable Y	Immunoturbidimetric
Variable X	LC-MS/MS
Sample size	36
Correlation coefficient r	0.8250
Significance level	P < 0.0001
95% CI for r	0.6810 to 0.9075

Table 2*: Correlation coefficient between the two methods.*

	1 - 2
Sample size	36
Mean difference	0.2083
Standard deviation	0.9819
95% CI	-0.1239 to 0.5406
Test statistic t	1.273
Degrees of Freedom (DF)	36
Two-tailed probability	P = 0.2114

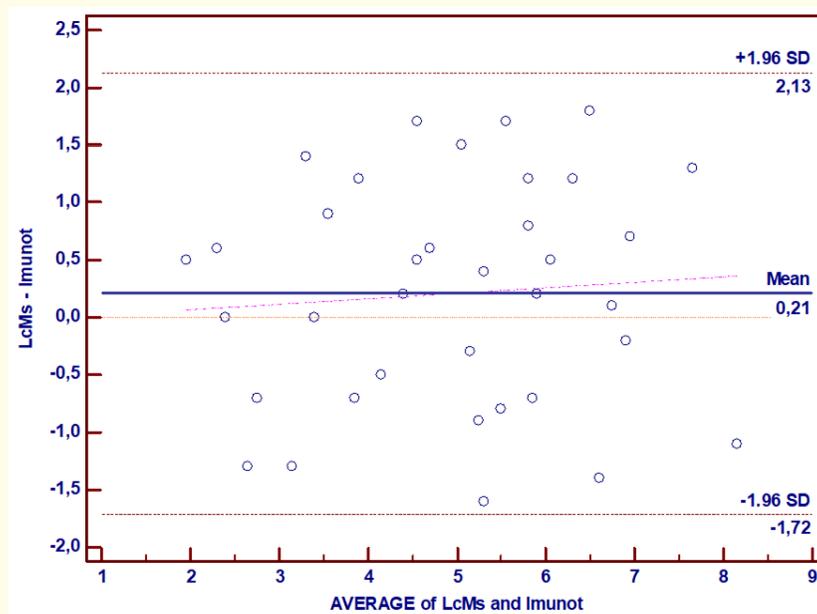***Table 3:*** *Paired t-test.*

Immunoturbidimetric - 1; LC-MS/MS – 2.

Using further statistical calculations we show what is the cause of the differences and somewhat poorer correlation. Regression shows that this is a slightly lower sensitivity of the immunochemical method and a systematic displacement of results, which is not significant (Graph 1).

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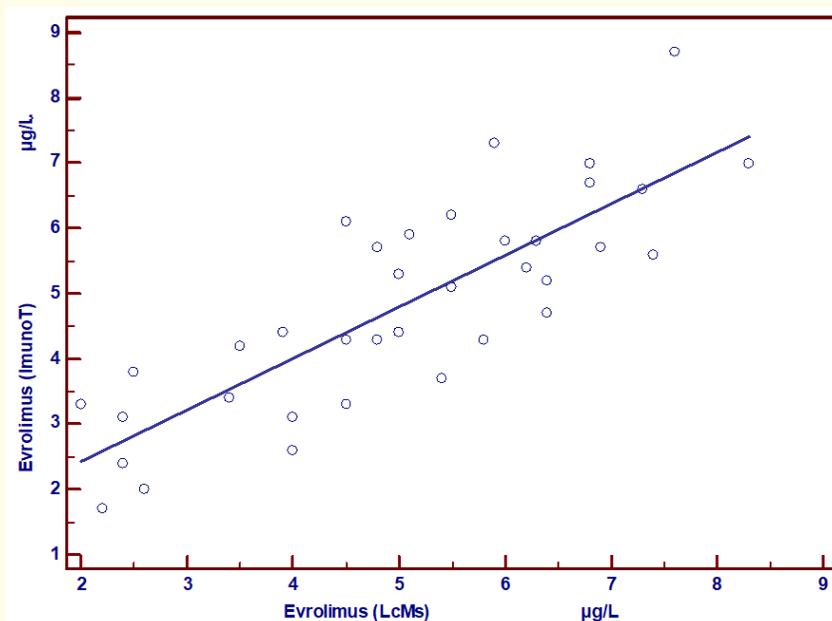
Graph 1: The clinical manifestations of menopause and andropause. Extent of the severity is guides the initiation, maintenance and monitoring (Sex)-Hormone Replacement Therapy.

The Bland-Altman graph shows (Graph 2) that this is a uniform distribution of results across the entire concentration range. The methods are comparable, but it should be taken into account that the immunochemical method is less sensitive. If we are looking for low concentration patients, then this method is not the most appropriate, but if the method is intended to monitor treatment and in most cases above the detection limit, then there are no barriers to it in routine work.

Graph 2: Bland-Altman graph of comparability of both methods used.

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In graph 3, data of patients receiving cardiac therapy who have been on everolimus therapy are collected.
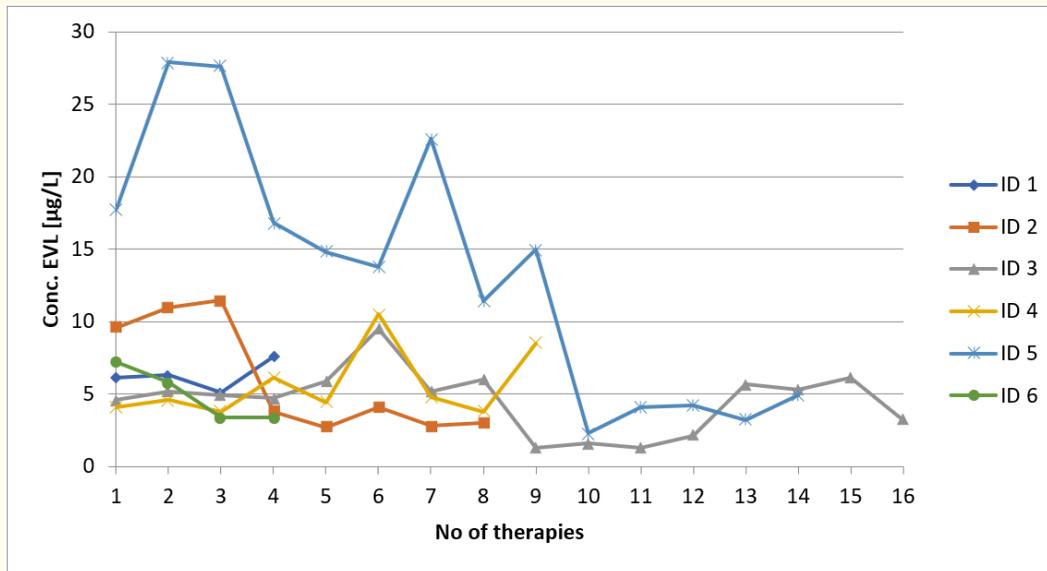
Graph 3: *EVL concentrations in the blood in the recipients of the heart with confirmed coronary vasculopathy.*

Discussion

Monitoring (TDM) concentrations of immunosuppressants is crucial in the diagnosis of the transplant recipient. Ever since the first days after the transplant, until the later outpatient monitoring of stable transplant recipients, TDM plays a crucial role in the long-term survival of recipients and transplants, despite the fact that problems with the onset of chronic rejection and the occurrence of a greater number of long-lasting adverse reactions still represent unresolved riddle [24]. EVL is a medicinal product with a narrow therapeutic area and variable bioavailability. By inhibiting cellular proliferation of smooth muscle, the reduction of neointima and transplantation arteriosclerosis inhibits the occurrence of both acute and chronic rejection, which contributes to the loss of graft function. Due to its mode of action it is used in conjunction with calcineurin inhibitors (synergy), which allows lower doses of these and thus a reduction in dose-related toxic effects of calcineurin inhibitors, in particular nephrotoxicity. Low therapeutic doses and the narrow therapeutic range of EVL, to express adverse reactions in the absence of a therapeutic area (risk of rejection or toxic effects), extensive interactions with other medicines, as well as large intra- and interindividual variability in drug metabolism are causes that, due to difficult maintenance of concentrations medicines in the therapeutic area require precise monitoring of concentrations in blood. In the first weeks following the introduction of the drug, the right dose relative to the therapeutic area is difficult to detect. Contemporary therapeutic protocols, such as a combination of drugs and dose reduction in cases of partial immunological tolerance, require precise measurements at even lower drug concentrations. With modern immune methods, a reliable measurement of immunosuppressive concentrations lower than the lower limit of the therapeutic index is possible [25].

The optimal, and at the same time the universal dosage regimen of EVL to maximize the benefit of immunosuppression and at the same time minimize the incidence of adverse events is most often difficult to achieve. Due to the variability within the individual patient and the accompanying therapy (inhibitors and inducers of CYP3A4), maintenance of blood concentrations within the boundaries of the therapeutic area of EVL is difficult. The lower limit of the therapeutic area of EVL is a concentration that provides a minimal but clinically relevant level of immunosuppression. The upper limit of the EVL of the therapeutic area of EVL indicates a concentration that is usually associated with side effects that occur when taking a particular (higher) dose [26]. Clinical studies have found that graft rejection does not occur with the maintenance of EVL concentrations in the blood below 3.5 µg/L in 65% of patients, and at a concentration between 3.6 and 7.3 µg/L, 69 - 80% the likelihood that a rejection reaction will not occur. The probability is increased to 85% at concentrations between 7.4 and 21.8 µg/L, but with the simultaneous increase in serious adverse drug reactions [27].

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The most important factors affecting the pharmacokinetics of EVL are absorption, black race, gene polymorphisms of P-glycoprotein, CYP3A4, 3A5, and 2C8 and interactions with other drugs [21]. About 60% of all medicines used are metabolized by cytochrome CYP3A isoenzymes. Due to the genetic polymorphism of CYP3A isoenzymes, the expression or activity of these isoenzymes can be variable among individuals. For individuals who have greater CYP3A expression, it is necessary to inhibit more enzyme than in individuals with less expression in order to achieve the same metabolic effect. Consequently, drug interactions in the presence of CYP3A inhibitors in subjects with lower CYP3A expression may be more potent. Medication with drugs that share the same pathway of elimination can lead to pharmacokinetic drug interactions. Concomitant administration of CYP3A inhibitors significantly reduces clearance and increases blood concentrations of EVL. Strong CYP3A inhibitors are ketoconazoles (antifungicides), moderate erythromycin, verapamil, and cyclosporine, and the weak inhibitor is atorvastatin [27].

Our studied patients are receiving cyclosporine in the form of gelatine capsules (Neoral®) which, in contrast to cyclosporine in the form of micro emulsion (Sandimmune®), is absorbed faster and causes 2-3 times higher EVL exposure (Certican®). The reason for this is competition of EVL and CYA for binding sites on P-glycoprotein and CYP3A4 and about 100-fold higher doses and cyclosporine concentrations in blood compared to EVL [28]. Weak CYP3A inhibitors minimize EVL growth. When monitoring EVL concentrations in whole blood and individual dose adjustments, both weak and moderate inhibitors can be taken in parallel with EVL [27].

For the first (ID 1) and sixth patient (ID 6), all the measured EVL concentrations are located within the boundaries of the therapeutic area. The dose of the medicine in the first patient (0.75 + 0.50 mg) is the same throughout, except CYA (Neoral®), which is a moderate inhibitor of CYP3A4, other inhibitors, inducers were not prescribed, what did not significantly change the concentration in the blood. The dose of EVL is slightly higher in the sixth patient (0.75 + 0.75 mg), lowering it to 0.75 + 0.50 mg in the meantime and eventually increasing to the starting dose again. The patient receives CYA and a mild inhibitor of CYP3A4 atorvastatin (Atoris). In the second patient (ID 2), EVL concentrations at a dose of 1.00 + 0.75 mg were found slightly above the therapeutic range, and at a 0.75 + 0.75 mg dose alternately fluctuated in and out the lower limit of the therapeutic index. In addition to the EVL, the patient is prescribed CYA. The third patient (ID 3) at the dose of EVL is 0.50 + 0.25 mg or 0.50 + 0.50 mg has concentration in whole blood within the therapeutic range, and the increase in dose increases the concentration that exceeds the upper limit of the therapeutic area. The EVL dose was then lowered to 0.50 + 0.25 mg, and blood concentrations dropped below the lower level of the therapeutic range. With the gradual increase in the dose of the active substance, concentrations were able to maintain within the recommended limits. The patient receives CYA and atorvastatin in parallel, which, due to interactions with CYP3A4, may contribute to slightly higher levels of EVL in blood. In the fourth patient (ID 4), EVL concentrations are initially found within the boundaries of the therapeutic range (0.75 + 0.75 mg and 1.00 + 0.75 mg). After the first measured excessive concentration, the dose is even increased (1.00 + 1.00 mg), thus reaching the prescribed concentrations of the therapeutic area of the drug. For some reason, the concentration of EVL slightly rises above the therapeutic range, causing a slight decrease in the dose (1.00 + 0.75 mg). CYP3A4 inhibitors of CYA and atorvastatin, which were co-administered with EVL, may contribute to the increase in concentrations. Itraconazole (Sporanox®) in the fifth patient (ID 5) caused significantly high levels of EVL in the blood at a relatively small dose (0.50 + 0.50 mg, 0.50 mg/2 days and 0.50 mg/day). By abolishing Sporanox® and adjusting the dose of EVL to 0.25 + 0.50 mg and 0.50 + 0.50 mg, the blood concentration level are maintained within the boundaries of the therapeutic range. Because in this case it is difficult to adjust the dose to concentration within the therapeutic range, ketoconazole is not recommended [29].

Conclusion

Due to the narrow therapeutic index of EVL and the optimum efficiency of its efficacy with minimal toxicity, the monitoring of EVL concentrations in whole blood is essential for individualizing the dose adjustment. Evaluation of EVL concentrations is reliable and easy with the immunochemical turbidimetric method. Monitoring blood concentrations of EVL is essential for improving the balance of efficacy and toxicity and for better long-term tolerance.

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