Original paper

SIGNIFICANCE OF THERAPY MONITORING OF IMMUNOSUPPRESSIVE MEDICINES IN RENAL TRANSPLANTATION PATIENTS

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Immunosuppressive medicines are characterized by specific pharmacokinetic profile which requires therapy monitoring by means of measuring their blood concentrations. Therapy monitoring, by means of determining blood concentration of the medicine, enables application of an optimal individual immunosuppressive therapy. Due to its variable pharmacokinetics, and small therapeutic index and potential interaction with numerous other medicines, the post-operative monitoring of immunosuppressive medicines is an essential element of therapy protocol for renal transplantation patients. Therapy monitoring represents an efficient way to reduce adverse effects of immunosuppressive medicines and to prevent transplantation rejection by means of adapting the doses in renal transplantation patients. Determining the concentration of immunosuppressive medicines is of special importance in the modified dosing for patients with renal insufficiency. Pharmacokinetic analysis is important for proper interpretation of immunosuppressive medicines' blood concentrations. The interpretation of the received results must be multidisciplinary, considering that there are numerous factors of variability of patients and immunosuppressive medicines. *Acta Medica Medianae 2009;48(2):22-27.*

***Key words**: therapy monitoring, immunosuppressive medicines, interactions, renal transplantation*

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Introduction

Immunosuppressive medicines prevent organ rejection by developing immunotolerance to specific antigens with preservation of immunological ability of the organism as a whole (1).

Out of immunosuppressive medicines we most commonly use: cyclosporine (CsA), sirolimus, tacrolimus (Tc), azathioprine, mycophenolate mofetil (MFM) and corticosteroids (KS). As of recently, immunosuppressive therapy also includes monoclonal antibodies, biomolecules (for example basiliximab,daclizumab).

Immunosuppressive medicines are characterized by a specific pharmacokinetic profile which requires therapeutic monitoring (Therapeutic Drug Monitoring- TDM), by means of recording blood level concentrations. Reasons for application of TDM immunosuppressive medicines are as follows:

- Small therapeutic index (TI).
- Correlation of concentrations and therapeutic or toxic effects.
- Metabolism through cytochrome P450.
- Great individual differences.
- Risk for developing interactions with other medicines.
- Monitoring patient adherence (2).

Tc and CsA plasma concentrations above upper limits of therapeutic index are associated with the increased incidence of adverse reactions (nephrotoxicity, hepatotoxicity), while their lower concentrations increase the risk for organ rejection. Therapeutic monitoring of immunosuppressive medicines is done by determining their levels in patient's blood. When TDM is applied in order to adjust the dosage, medication concentration must be in a state of dynamic balance, that is, there must elapse 5 half-life times for medicine elimination. Deviation of this rule is only in the case of suspicion that there is toxicity. When the state of dynamic balance is achieved, it is important to timely take blood samples, have a valid analytical procedure and interpret the results accurately (2-4).

Characteristics of therapeutic monitoring for immunosuppressive medicines

In general, TDM by determining plasma concentration of the drug, enables the application of optimal individual immunosuppressive therapy. When monitoring immunosuppressive medicines and their metabolites, plasma concentrations must be precisely planned and accompanied by

appropriate sampling of biological material and valid analytical procedure. The interpretation of the received results is of special importance and must be multidisciplinary considered that there are numerous factors of variability of patients and immunosuppressive medicines (Table 1) (4-6).

Table 1. Variability factors influencing the concentration of immunosuppressive medicines

Patient factor	**Medicine factor**
Adherence	Medicine quality
Individual capacity, absorption and distribution characteristics	Manner and time of application
Individual capacity, metabolism characteristics and medicine excretion	Treatment type
Body mass index (BMI), body surface	Treatment mistakes
Significant co morbid states	Time of sampling
Other medicines	Interferences in analytical procedure
Physiological/pathophysiological factors	Selectivity of the chosen method
Factors of environment	Efficiency of the chosen method

Pharmacokinetic analysis is important for correct interpretation of the received immunosuppressive medicines plasma concentrations. Concentrations of immunosuppressive medicines lower or higher than expected can be the result of inadequate cooperation between the patient and his physician (adherence), wrong choice, medicine dosage of the time of biological material sampling (5,7).

Concentrations of the medicine lower than expected are most often the result of a poor bioavailability of the medicine, its elimination faster than expected, increased distribution volume and inability to achieve balance (1,2,5).

Alternative immunosuppressive medicine monitoring is a much simpler method that includes following the function and reaction of organ rejection in patients on immunosuppressive therapy based on appropriate biochemical parameters (urea, creatinine, creatinine clearance) and radiological examinations (echo, Doppler).

Immunosuppressive protocols are dominated by calcineurine inhibitors (cyclosporine, tacrolimus) which are used in combination with other immunosuppressive medicines (mycophenolate mofetil, sirolimus, corticosteroids, basiliximab).

Due to its variable pharmacokinetics and a small therapeutic index, post-transplant monitoring of immunosuppressive medicines is an essential part of patient therapeutic monitoring, which enables optimal treatment with significant reduction of risk for nephrotoxicity, heaptotoxicity and transplant rejection (8,9). New formulations of immunosuppressive medicines with specific pharmacokinetics require constant monitoring of medication blood levels. Therapy monitoring enables titration of the dosage depending on patients individual characteristics and considerations for their inter and intra- individual differences (8-10).

Specificities of therapy monitoring of immunosuppressive medicines

Calcineurine inhibitors are the most commonly used immunosuppressive medicines in the medical practice. Their similar pharmacokinetic features spring from the same mechanism of action and similar metabolism, dependant on the activity of CYP 3A4 enzyme and P-glycoprotein (Figure 1).

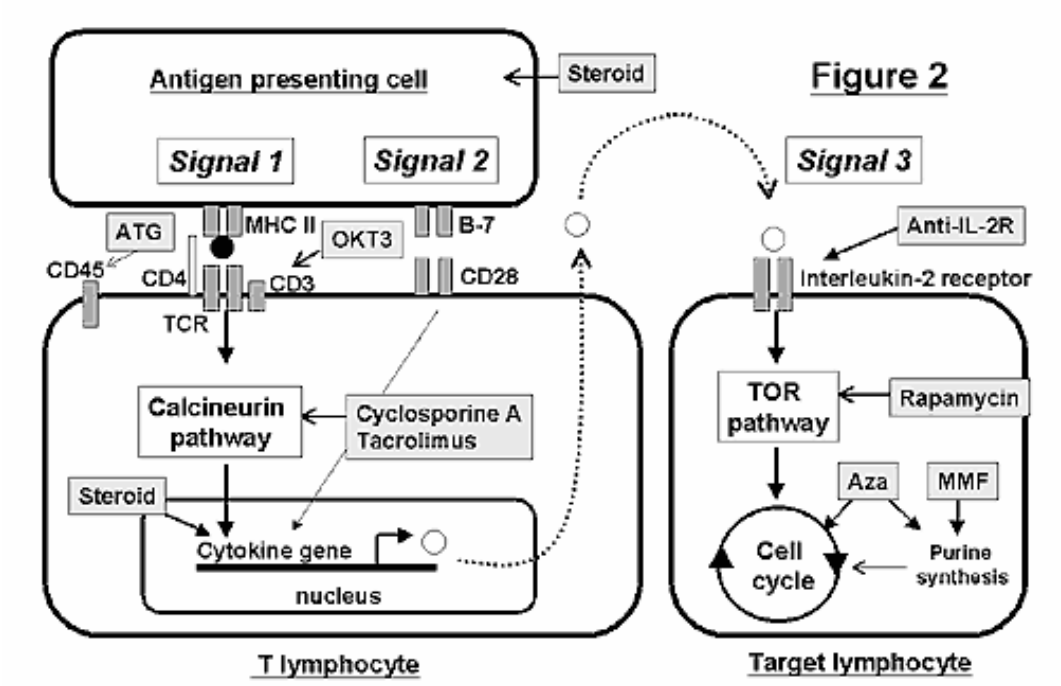

Figure1. Mechanism of action for calcineurin immunosuppressive medicines

Cyclosporine (CsA)

Cyclosporine is one of the first immunosuppressive medicines to be applied. Its molecule represents neutral lipophile undecapeptide which within cells easily makes complex with cyclophilin, cell protein, inhibiting the activity of calcineurin phosphatase. As a consequence there occurs dephosphorization and nuclear translocation in T-cells, which thereby become inhibited. The efficiency of cyclosporine depends on specific reversible lymphocyte inhibition in G0 and G1-phase of cell cycle. T-lymphocytes are inhibited with greater specificity. T-helper cells are the main target, although T-suppressor cells can also be affected. Cyclosporine also inhibits creation and release of lymphokines including interleukin-2. CsA does not affect phagocyte function and does not cause bone marrow suppression (5,11).

CsA formulation determines the degree of its absorption from oral medicines and consequently its bioavailability. Cyclosporine is distributed mostly outside blood volume, penetrating deeply into tissues and bodily fluids, including liver, pancreas, and lungs. Distribution volume during the state of balance, during intravenous dosing is 3-5 L/kg (4-6) in transplant recipients (children younger that 10 have higher clearance values). Distribution of medicine in blood depends on its concentration. Approximately, 33-47% of the medicine is in plasma, 4-9% in lymphocytes, 5-12% in granulocytes and 41-58% in erythrocytes. In higher concentrations of CsA there is a saturation of its binding to leukocytes and erythrocytes. In plasma, approximately 90% of the medicine is bound to proteins, primarily lipoproteins. Cyclosporine goes through placenta and is excreted with human milk. Plasma concentrations in the state of balance are 75-150 μg/l (4,6).

CsA metabolism is performed through system 3A oxigense CYP 450 in the liver, and in a smaller degree in the gastrointestinal tract and the kidneys. After oral application, it is characterized by the phenomenon of the first pass. All medicines whose metabolism is conducted by the same enzyme system can affect CsA metabolism. Around 25 metabolites have been identified from human bile, feces, blood and urine. Main metabolites (M1, M9, and M4N) are created as products of oxidation at position 1-beta, 9-gama, and 4-N-demethilation. Secondary metabolite M19 and cyclic forms of main metabolites M1c and M1c9 are responsible for reduction in renal function and nephrotoxicity. Elimination of cyclosporine is performed mainly through bile, while only 6% of the dose (basic medicine and metabolites) is excreted by urine.

Table 2. Clinically significant interactions of calcineurine inhibitors (CNI)

Medicine interacting with CNI	**Action mechanism**	**Interaction effect**
trimetoprim+sulfametoxazole	Unclear mechanism	↓ conc..CNI
ditiazem	Competition in metabolism	↑ conc.CNI
phenytoin	Liver enzyme induction	↓ conc.CNI
phenobarbitone	Liver enzyme induction	↓ conc.CNI
rifampicin	Liver enzyme induction	↓ conc.CNI
isoniazid	Liver enzyme induction	↓ conc.CNI
sirolimus	addition	↑ immunosupppresion
ketoconazole	Liver enzyme induction	↑ conc. CNI
erythromycin	Competition in metabolism	↑ conc. CNI
corticosteroids	Liver enzyme induction	↑ conc. CNI
Hormones of synthetic origins	Competition in metabolism	↑ conc CNI
amphotericin B	addition	↑nephrotoxicity
Aminoglycoside antibiotics	addition	↑ nephrotoxicity
alopurinol	Metabolism inhibition	↑ conc. CNI
diclofenac	Increased absorption of dikl.	↑ conc diklofenak
methotrexate	Increased absorption metot.	↑ conc.metotreksat
Hyperici herba	Liver enzyme induction	↓ conc. CNI
Echinaceaae herba	immunostimulation	Contraindicated application
naringenin	Increased absorption of the medicine	↑ conc.of medicine
Oral contaceptives	Metabolism modification	Extended half-time of elimination
nifedipine	Metabolism inhibition	↑ conc. CNI
bromocriptine	Metabolism inhibition	↑ conc. CNI
Potent inhibitors CYP 3A4	Metabolism inhibition	↑ conc. CNI
Potent inductors CYP 3A4	Metabolism induction	↓ conc. CNI

Adverse effects for CsA include renal insuficiency, hyperkalemia, hypertension, gingive hypertension, gastrointestinal and neurological toxicity (1,12). Cyclosporine is substrate for CYP3A4 and moderate inhibitor of its activity. It also occurs as a weak inhibitor of CYP2C8/9. These characteristics of CsA indicate a high potential for interactions with other medicines. Many interactions with antibiotics have been registered (Table 2). Nepphrotoxicity is enhanced in simultaneous application of aminoglycosides, vancomycin and trimetoprim- sulphametoxazol (13). Macrolide antibiotics (clarithromycin, erythromycin) as well as norfloxacin increase CsA by inhibiting its metabolism. Rifampicin and ciprofloxacin can reduce immunosuppressive action of CsA by inducing its metabolism (12,13). Imipenem should be avoided due to its neurotoxicity. Insignificant pharmacokinetic interactions with aspirin, piroxicam, ketoprofen and indomethacin were observed. CsA increase diclofenac concentration (3,5,10).

Grapefruit juice increases absorption and bioavailability of CsA, and should be avoided during the therapy.

In recent times, more importance is given to monitoring CsA plasma concentrations two hours after oral administratio (C2), because of better correlation with therapeutic effect of the medicine (11,12).

Tacrolimus (Tcr)

Tacrolimus (Tcr) is macrocyclic lactone. Its inhibitory activity is more selective compared to CsA. Tcr binds to the proteins in the cell which are included into calcineurine and in this way inhibits its activity.

Tacrolimus is characterized by poor absorption from oral medicines and its bioavailability is only 20%. Elimination half-life is variable and is 3-40h. Tcr metabolism is performed in the liver through actions of oxidases, most of all CYP 3A4, when a large number of metabolites is produced, out of which 5 have immunological activity. Excretion is mostly done through the bile. Tcr potential for interactions is high. In vitro studies have shown that medicines which inhibit CYP 3A4 have an influence on Tcr plasma concentrations inhibiting its metabolism (10,11). Such medicines are: bromocriptine, cortisone, dapsone, ergotamine, etinilestradiol, gestodene, itraconazole, josamycine, lidocaine, mephenytoin, miconazole, midazolam, nicardipine, nifedipine, nilvadipine, norethisterone, hinidin, tamoxifen, verapamil. It is also important to mention kaptopril, chloramphenicol, cimetidine, clarithromycin, cyclosporine, danazol, fluconazole, ketoconazole, metoclopramide, omeprazole, prednisolone and sulindac. Naringenin, ingredient of grapefruit juice, known as inhibitor of most of all intestinal CYP 3A4, influence concentration of Tcr by changing bioavailability of the medicine.

Enzyme inductors cytochrome 3A generally reduce Tcr concentration. In the same way act barbiturates, rifampicin, phenytoin, carbamazepine, metamizole and isoniazid. Tcr inhibits CYP 3A4 activity, which can influence the efficiency of oral contraceptives or prolong half-time of CsA elimination, if they are applied at the same time (4,10,11,15).

Sirolimus (Syr)

Sirolimus is macrocyclic lactone structurally similar to tacrolimus. Similarly as other immunosuppressive medicines Syr requires TDM due to great variability in pharmacokinetics, most of all, distribution volume, clearance and maximal plasma concentration (16). Monotherapy by sirolimus shows therapeutic effects at concentrations 16,9-29,6 μg/l. When the protocol also includes CsA, therapeutic concentration range is somewhat lower and is at 5-15 μg/l. Syr metabolism is performed through enzyme CYP 3A4, with pronounced presystemic metabolism of the medicine because of action of intestinal CYP 3A4. Syr metabolism also includes P-glycoproteine which also causes the variability of pharmacokinetics and high potential for interaction with other medicines which are metabolized through the same enzymes. Great number of papers deals with interaction between CsA and Syr. It is established that sirolimus does not have significant influence on the level of reabsorbed CsA, expressed through AUC (area under the curve), while the influence of CsA on AUC of sirolimus is much greater (4,7,15). These data favor monitoring as a way for rational therapy with sirolimus (Table 2).

Mycophenolate Mofetil (MMF)

Mycophenolate is a precursor and chemically represents estar of mycophenolic acid. MMF is applied perorally, its bioavailability is around 94% and is susceptible to variability. Active form of the medicine is quickly released in the organism (MPA), which by means of glucuronidation, most of all in liver, is transformed into non-active β-glucuronide and eliminated by means of urine. Apart from this inactive form, it is considered that there is one active metabolite MPA but this is not yet proven. In the active form, the medicine inhibits the production of guanine nucleotides which hinder the immunological reaction of the organism. In practice, MMF is used in combination with other immunosuppressives (15). Therapeutic concentrations in combination with CsA are 14,1-34,9 μg/l, and in combination with Tcr 11,9-25,4 μg/l. Interactions with other medicines are possible (Table 3). Antacids reduce its absorption. Simultaneous application of acyclovir and MMF leads to increased concentrations of both medicines probably due to competition on the level of renal tubules in glucuronidation reaction. Because of possible change in plasma concentrations and the consequent activity of the medicine it is necessary to take care in all medicines susceptible to enterohepatic circulation. Simultaneous application of MMF and azathioprim can lead to bone marrow depression. Corticosteroids and CsA reduce MPA concentrations.

Table 3. Clinically significant interactions of MMF with other medicines

Medicine interacting with MMF	**mechanism**	**effect**
azathioprine	sinergism	Bone marrow depression
aciklovir	competition	↑ conc. of both medicines
cyclosporine	-	↓ conc.MPA za 30-40%
antacides	Reduced absorption	↓conc. MPA
corticosteroids	Enzyme expression	↓conc. MPA

Basiliximab (Bas)

Basiliximab is a representative of a new generation of immunosuppressive medicines from the group of biomolecules. Basiliximab represents himar monoclonal antibody Ig1k, produced by recombinant DNA technology. As antagonist of IL2 receptor with high affinity for binding, it blocks function of IL2R that is cell immune response and the rejection of the transplanted organ. In basiliximaba pharmacokinetics there is a dosage depandance of the parameters (C_{max}, AUC), but the area in which the medicine is distributed has not been defined yet. Distribution volume and clearance point to interindividual variability. Sex, age and race do not have influence on half-time of elimination which is 7,2±3,2 days. Simultaneous use of mycophenolate mofetil and azathioprim can significantly reduce the clearance of the medicine (do 22%) (17,18).

Conclusion

Therapy monitoring represents an efficient way to reduce adverse effects of immunosuppressive medicines and to prevent transplantation rejection, by means of adapting the doses in renal transplantation patients. Determining concentrations of immunosuppressive medicines is of especial importance in modified dosing in patients with renal insufficiency. Therapeutic moni-toring of plasma concentrations of immunosu-ppressive medicines and metabolites in biological fluids requires correct sampling of the biological material, accurate measurements of concentra-tions and detailed knowledge of pharmacokinetic principles, parameters as well as adequate interpretation of the obtained results. The choice and correct application of analytical procedure are most of all dependent on technical and material capacities of clinics and laboratories.

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ZNAČAJ TERAPIJSKOG PRAĆENJA IMUNOSUPRESIVNIH LEKOVA KOD BOLESNIKA SA PRESAĐENIM BUBREGOM

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Imunosupresivne lekove karakteriše specifičan farmakokinetski profil koji nameće potrebu za terapijskim praćenjem njihove koncentracije u plazmi. Terapijsko praćenje, određivanjem koncentracije leka u plazmi omogućava sprovođenje optimalne individualne imunosupresivne terapije. Zbog svoje varijabilne farmakokinetike, malog terapijskog indeksa i potencijalnih interakcija sa brojnim lekovima, praćenje imunosupresivnih lekova je esencijalni segment terapijskog protokola kod bolesnika sa presađenim bubregom. Terapijsko praćenje predstavlja efikasan način za smanjenje neželjenih efekata imunosupresivnih lekova i sprečavanje odbacivanja organa, prilagođavanjem režima doziranja kod bolesnika sa presađenim bubregom. Određivanje koncentracije imunosupresivnih lekova je od naročitog interesa u modifikovanom doziranju kod bolesnika sa bubrežnom insuficijencijom. Farmakokinetička analiza je značajna u pravilnoj interpretaciji dobijenih koncentracija imunosupresivnih lekova u plazmi. Tumačenje dobijenih rezultata mora biti multidisciplinarno, s obzirom na postojanje brojnih faktora varijabilnosti bolesnika i imunosupresivnih lekova. *Acta Medica Medianae 2009;48(2):22-27.*

***Ključne reči**: terapija, praćenje, imunosupresivni lekovi, interakcije, presađivanje, bubreg*