Pharmacokinetics of cyclosporine A, tacrolimus and sirolimus given subcutaneously in normal rats

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Introduction: **Immunosuppressive drugs, e.g. cyclosporine A, tacrolimus or sirolimus, are routinely given orally. However these drugs are characterized by a very individual profile of absorption from gastrointestinal tract, hepatic metabolism and elimination. Therefore the blood concentrations of these drugs vary significantly from patient to patient and must be frequently monitored. Alternative way of immunosuppressive drugs delivery is the intravenous one, but unfortunately this route is connected with increased toxicity of these drugs. Till now these drugs were not used by subcutaneous administration. Therefore the aim of the study was to examine the pharmacokinetics of cyclosporine A, tacrolimus or sirolimus given subcutaneously in normal rats.** ***Materials and Methods:*** **18 Sprague-Dawley rats were randomly allocated to 3 groups: treated with cyclosporine A (dose 3.3mg/kg body mass/24hour), tacrolimus (dose 0.33mg/kg body mass/24hour), sirolimus (dose 0.5mg/kg body mass/24hour), respectively. The drugs were administered subcutaneously once daily for 4 days. On day 4 blood concentrations of the drugs were measured: before administration and 1, 3, 7, 11, 15 and 23 hours after administration.** ***Results:*** **The blood concentrations of the drugs were reproducible with low variability.** ***Discussion:*** **The subcutaneous route is notable for administration of cyclosporine A, tacrolimus or sirolimus in rats. It is an alternative to oral drug administration in experimental studies. The potential clinical application of these results needs further investigation.**
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Key words:
• immunosuppressive drugs
• pharmacokinetics
• subcutaneous route
• cyclosporine A
• tacrolimus
• sirolimus

Farmakokinetyka cyklosporyny A, takrolimusu i syrolimusu podawanych drogą podskórną u szczurów

Wstęp: **Leki immunosupresyjne, takie jak cyklosporyna A, takrolimus, syrolimus, są stosowane w praktyce klinicznej po przeszczepieniu narządów głównie drogą doustną. Leki te charakteryzują się indywidualnym profilem wchłaniania z przewodu pokarmowego oraz metabolizmem, co powoduje dużą zmienność osiąganych stężeń leków we krwi i konieczność częstego monitorowania stężeń leków w trakcie leczenia. Dożylna droga podawania w/w leków immunosupresyjnych jest związana z większą toksycznością tych leków i stąd wykorzystywana jest jedynie w nagłych sytuacjach klinicznych. Jak dotąd cyklosporyna A, takrolimus i syrolimus nie były podawane w postaci iniekcji podskórnych. Celem badania było określenie farmakokinetyki cyklosporyny A, takrolimusu i syrolimusu podawanych podskórnie u szczurów.** ***Materiał i Metody:*** **Osiemnaście szczurów rasy Sprague-Dawley zostało przydzielonych w sposób losowy do 3 grup, którym podawano: cyklosporynę A (w dawce 3,3mg/kg masy ciała/dobę), takrolimus (w dawce 0,33mg/kg masy ciała/dobę) lub syrolimus (w dawce 0,5mg/kg masy ciała/dobę). Poszczególne leki podawane były podskórnie jeden raz na dobę przez 4 dni. W 4 dniu oznaczono stężenia poszczególnych leków: przed kolejną podażą leku oraz po 1, 3, 7, 11, 15 i 23 godzinach po podaniu leku.** ***Wyniki:*** **Stężenia cyklosporyny A, takrolimusu i syrolimusu oznaczone we krwi po podaniu tych leków drogą podskórną były powtarzalne i stabilne.** ***Dyskusja:*** **Podskórna droga podawania leków może być polecana dla takrolimusu, cyklosporyny A oraz sirolimusu u szczurów i może stanowić dobrą alternatywę dla podaży tych leków doustnie w badaniach doświadczalnych. Zastosowanie podskórnej drogi podawania w/w leków immunosupresyjnych w praktyce klinicznej wymaga jednak dalszych badań.** **(NEPHROL. DIAL. POL. 2013, 17, 53-55)**

Słowa kluczowe:
• leki immunosupresyjne
• farmakokinetyka
• droga podskórna
• cyklosporyna A
• takrolimus
• syrolimus

Introduction

Calcineurin inhibitors such as cyclosporine A, tacrolimus, and the mTOR (mammalian target of rapamycin) inhibitor sirolimus are used to prevent allograft rejection after solid organ transplantation [1, 2]. In patients they are mainly administered orally. However they are characterized by a narrow therapeutic index and variable absorption from the gastrointestinal tract causing intra- and interpatient variability of drug concentration, necessitating therapeutic drug monitoring [3,4,5,6]. The oral administration of these drugs is also not easily applicable in animal

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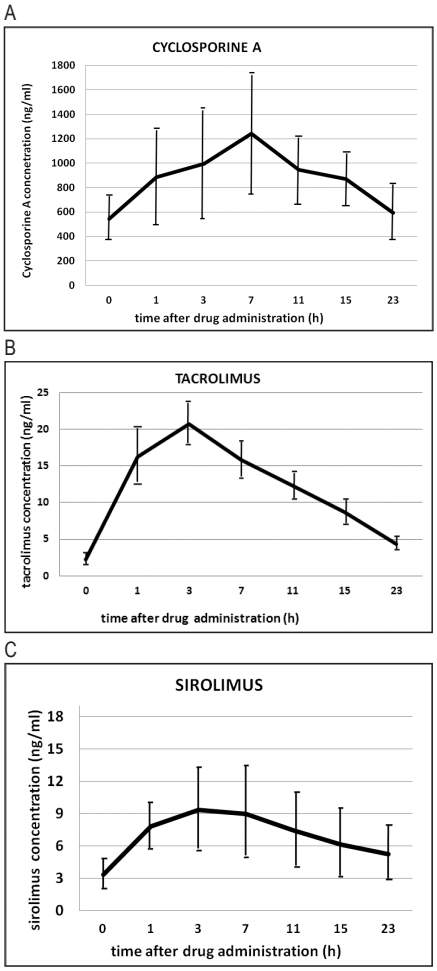

Figure1

Mean (±SD) concentration-time profile of cyclosporine A (A), tacrolimus (B) and sirolimus (C) after subcutaneous administration in Sprague-Dawley rats.

Stężenia cyklosporyny A (A), takrolimusu (B) oraz syrolimusu (C) we krwi w ciągu 24-godzin po podskórnym podaniu.

experiments. Until now, with few exceptions [7], no detailed studies in the pharmacokinetics of the drugs administered s.c.to rats have been reported. In 1985 Wassef et al. [7] studied cyclosporine A plasma concentration after oral and subcutaneous administration and found little variation of steady state cyclosporine A plasma levels following subcutaneous administration. The data concerning drugs pharmacokinetics are important for the appropriate planning of experimental studies with these drugs. The aim of the study was the precise systematic pharmacokinetics analysis of cyclosporine A, tacrolimus and sirolimus given subcutaneously in normal rats.

Materials and methods

Experimental animals

Eighteen Sprague-Dawley rats (weighted 300-400g) were provided by the Center for Experimental Medicine of the Medical University of Silesia in Katowice, Poland. The animals were maintained under standard laboratory conditions. Food and water were provided ad libitum.

Drugs

In this study: cyclosporine A (Novartis, Sandimmun^{R}Injection, containing 50 mg cyclosporine in 1ml, dissolved in Cremophor® EL (polyoxyethylated castor oil)) at a dose 3.3 mg/kg body mass for 24 hours, tacrolimus (Astellas, Prograf^{R}Injection, containing 5 mg tacrolimus in 1ml, dissolved in polyoxyl-60-hydrogenated castor oil and dehydrated alcohol) at a dose 0.33 mg/kg body mass for 24 hours and sirolimus (Wyeth, Rapamune^{R} Oral Solution, containing 1 mg sirolimus in 1 ml, dissolved in Phosal 50 PG® (phosphatidylcholine, propylene glycol, mono- and di-glycerides, ethanol, soy fatty acids, and ascorbylpalmitate), polysorbate 80 and ethanol) at a dose 0.5 mg/kg body weight every 24 hours were analyzed. All these drugs were diluted in 0.9% sodium chloride injection prior to use and only fresh solutions were used (to avoid decreased drug stability and other drug-specific reactions).

Experimental protocol

Eighteen Sprague–Dawley rats were randomly allocated to cyclosporine A, tacrolimus or sirolimus, respectively, given subcutaneously once daily for 4 days. On day 4 rats were anesthetized with isoflurane and blood were obtained by ocular puncture into the periorbital venous sinuses using heparinized microcapillary tubes. Serial blood samples (0,5ml) were collected into ethylene diaminetetraacetic acid (EDTA)-containing tubes: just before next drug administration, as well as at 1, 3, 7, 11, 15, 23 hours after drug administration. Throughout the study, aliquots of blood were frozen and stored at -20°C until analysis. The protocol was approved by the Ethic Committee of the Medical University of Silesia in Katowice, nr 36/2008.

Analysis of drugs' blood concentrations.

The blood concentrations of immunosuppressive drugs were measured in whole blood samples using immunoassay: for tacrolimus and sirolimus concentrations a microparticle enzyme immunoassay (MEIA) by Abbott and for cyclosporine A concentration a radioimmunological competition assay (RIA) by Biker.

The area under the serum concentration versus time curve (AUC) was calculated by the linear trapezoidal rule.

Results

A mean (±SD) blood concentration-time profile of cyclosporine A, tacrolimus and sirolimus during 24 hours after subcutaneous administration are presented in Fig. 1. The mean (±SD) pharmacokinetics parameters for the above drugs are summarized in Table 1. The maximum blood concentration (Cmax.), time to attain Cmax. (t max.), trough level (Cmin.) and AUC (area under the curve) were calculated from individual blood-concentration profiles. In rats treated with cyclosporine A and sirolimus a stable drug blood concentrations was observed during 24 hours with the ratio of peak to through level about two (i.e. 2.1 and 1.8, respectively). In the tacrolimus group a greater 24 h variation of drug concentration was observed (ratio of peak to through level 4.81) after a single subcutaneous drug administration. T max. was: 3±2 hours after tacrolimus and sirolimus administrations and 7 hours after cyclosporine administration. The area under the curve, which is the best predictor of drug exposure, also suggests that subcutaneous route provides reproducible and appropriate drugs level (Table I).

Table I

Mean (±SD) pharmacokinetic parameters of cyclosporine A, tacrolimus and sirolimus after subcutaneous administration in Sprague-Dawley rats.

Właściwości farmakokinetyczne cyklosporyny A, takrolimusu oraz syrolimusu po podaniu podskórnym u szczurów rasy Sparague-Dawley (średnia ±SD).

	Cyclosporine A	Tacrolimus	Sirolimus
C max (ng/ml)	1241 ± 550	20.7 ± 4.1	9.4 ± 5.4
C min (ng/ml)	592 ± 229	4.3 ± 1.1	5.2 ± 3.1
t max (h)	7.0 ± 0.0	3.3 ± 1.96	3.4 ± 2.1
AUC (ng/ml*h)	20918 ± 7108	266.5 ± 29.2	165 ± 98.3

C max - maximum blood concentration of drug, t max. – time to attain C max., C min.- through drug level, AUC- area under the curve

Discussion

The present study shows that in rats the subcutaneous route is feasible for the administration of cyclosporine A, tacrolimus or sirolimus, however no studies were made using oral route in parallel to compare the pharmacokinetic profiles. The subcutaneous administration of these drugs provides reproducible and steady drug levels, with little variation over 24-hours. Subcutaneous administration of tacrolimus is characterized by approximately fivefold ratio of peak to through level; the decline of the initial peak concentration is followed by a slower decline over the next 24 hours. Therefore twice daily tacrolimus administration by the subcutaneous route is presumably necessary to achieve more stable drug concentration over 24 hours. The differences in t max. between the studied drugs indicate different rates of absorption from subcutaneous tissue. The subcutaneous route eliminates many parameters which influence the bioavailability after oral administration, e.g. intake and composition of food, need for solubilization of cyclosporine in bile, gastrointestinal transit time and other [8,9].

Conclusions: The subcutaneous route is notable for administration of cyclosporine A, tacrolimus or sirolimusin in rats. It is an alternative to oral drug administration in experimental studies. The potential clinical application of these results needs further investigation.

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