Influence of Dexamethasone on Pharmacokinetic Parameters of Cyclosporine in Rabbits

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ABSTRACT

The present study was designed to investigate the presence of significant pharmacokinetic (PK) interaction between Dexamethasone (DEX) at different concentrations on Cyclosporine (CsA) PK parameters in healthy male rabbits. The rabbits were selected and divided into three groups: Control group (n=6) received orally CsA solution (7.5 mg/kg/day) prepared from soft gelatin capsule for five days and on the fifth day, serial blood samples were withdrawn from marginal ear vein of rabbits at different time intervals post-dosing. In the first and second groups, rabbits were given orally (7.5 mg/kg/day) CsA solution concomitantly with DEX at two doses (0.33 and 0.66 mg/kg/day), respectively. On the fifth day of administration, each test group's serial blood samples were collected for over 24 hours as in the control group. Different PK parameters of CsA for the three groups were determined using non-compartmental analysis. It was observed that, there were statistically insignificant differences between control and test groups when co-administered with DEX at both concentrations. The present study results demonstrated that concurrent administration of DEX at both concentrations had not influenced the PK parameters of CsA.

Keywords: cyclosporine, dexamethasone, drug-drug interaction, pharmacokinetic parameters.

INTRODUCTION

CsA is a calcineurin inhibitor and potent immunosuppressive agent, which significantly impacts organ transplantation ⁵. It has considerably improved the first and second-years graft survival rates and decreased morbidity in kidney, liver, heart, lung, and pancreas transplantation. In addition to that, several studies have supported the efficacy of CsA in preventing graft-versus-host disease in bone marrow transplantation ⁶. CsA is extensively metabolized in the liver by CYP3A4 system ⁷. Sustained and clinically significant drug-drug interactions (DDIs) can occur during long-term therapy. Thus, the co-administration of multiple drugs with CsA could increase the risk of treatment failure, nephrotoxicity, and other adverse effects ⁸.

DDIs are one of the commonest causes of medication errors in developed countries, mainly in the elderly due to poly-therapy, with a prevalence of 20-40% ⁹; thus, Poly-therapy increases the complexity of therapeutic management and the risk of clinically significant DDIs which can both induce the development of adverse drug

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reactions or reduce the clinical efficacy 6. CsA soft gelatin capsule (Neoral®) is a modified micro-emulsion formulation with uniform and slightly increased bioavailability compared to (Sandimmune®) 7, 8. After oral administration of CsA (Neoral®), the time to peak blood concentration (t_{max}) is 1 to 2 hours (h), the elimination of CsA is primarily biliary with only 6% of the dose (parent drug and metabolites) excreted in urine, the disposition of CsA from blood is generally biphasic with a terminal half-life (t_{1/2}) approximately 8.4 h (range 5 to 18 h) 9. Dexamethasone (DEX) is a synthetic adrenocortical steroid agent used in treating chronic inflammatory and autoimmune diseases and is also effective as an antiemetic agent in cancer chemotherapy 10. Cytochromes P450 (CYP) is a major source of variability in drug PKs, and response 11 and CYP3A4 is the most common and versatile one 12. Many clinically significant drug interactions result from induction or inhibition of CYP3A4 enzymes, the major drug-metabolizing enzymes mainly in the liver 13, 14. A remarkable feature of CYP3A4 is its extreme promiscuity in substrate specificity and cooperative substrate binding, which often leads to undesirable DDIs and toxic side effects. Owing to its importance in drug development and therapy, CYP3A4 has been the most extensively studied 15. This study aims to investigate the presence of clinically significant PK interaction between DEX at different concentrations on CsA (A narrow therapeutic index drug) PK parameters by using healthy male rabbits as an animal model.

1. MATERIALS AND METHODS

2.1 Animals

Several experimental trials were performed on 18 New Zeland strains of adult male rabbits weighted (3.1-3.4 kg) and aged 8-10 months were enrolled in this study. The Research and Ethics Committee approved animals of the Experimental Animal Care Facility, College of Pharmacy, Al-Azhar University of Gaza (AUG), Palestine. The rabbits were selected randomly and divided into three groups (six for each group). All rabbits were kept under standard laboratory conditions in a 12-hour light/dark cycle at 25°C ± 2°C provided with pellet diet with water ad libitum and were fasted overnight before the experiments.

2.2 Study design and blood sampling

In an in-vivo drug-drug interaction, a randomized designed study was conducted in eighteen healthy male rabbits. The rabbits were selected and divided into three groups: Control group (n=6) received orally CsA solution (7.5 mg/kg/day) prepared from soft gelatin capsule (Neoral®) for five days, and on the fifth-day blood samples (1.5-2 mL) were withdrawn from marginal ear vein of rabbits at 0.00, 0.50, 1.00, 1.50, 2.00, 3.00, 6.00, 12.00 and 24 hr post-dosing 16. The rabbits in the first and second groups (test groups) receive d orally CsA solution (7.5 mg/kg/day) and DEX concomitantly at two different doses (0.33 and 0.66 mg/kg/day), respectively. On the fifth day of the administration, serial blood samples from each group were collected for over 24 hours as in the control group. Whole blood samples in EDTA tubes were kept at (2-8)° C until analyzed (Whole blood sample is stable for up to 3 days).

2.3 Analysis of blood samples

Analysis of whole blood samples to determine the concentrations of CsA was performed at the laboratory of Medical Relief Society-Gaza using Maglumi 800 System and Maglumi 800 CsA detection kit (Shenzhen New Industries Biomedical Engineering Co., Ltd.). The Kit is based on chemiluminescent immunoassay (CLIA). It is used in hospitals for rapid CsA assaying in whole blood to monitor the CsA dose.

2.4 CsA PK and statistical analysis

PK parameters for control and test groups including C\text{max}, t_{max}, K_e, t_{1/2}, AUC_{0-24}, AUC_{0-\infty}, and MRT were determined. Both parameters (C\text{max}) and (t_{max}) were directly determined from the plasma concentration versus time curves. The linear trapezoidal rule calculated the AUC0-24. The AUC_{0-\infty} was determined by the following equitation: AUC_{0-\infty} = AUC_{0-24} + Ct / K_e, where Ct is

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defined as the final measured serum concentration at time \( t \) and \( K_e \) is the elimination rate constant. The \( K_e \) was determined by the least-squares regression of plasma concentration-time data points lying in the terminal region using semilogarithmic dependence that corresponds to first-order kinetics. The \( t_{1/2} \) was calculated as \( 0.693/K_e \). PK analysis was determined using model-independent method (Non-Compartmental Approach) WinNonlin Professional Software (Version 6.3, Pharsight Corporation, Cary, NC) and (GraphPad Prism versión 4.00; San Diego, CA, USA). Statistical methods, including descriptive analysis and Mann-Whitney test, were applied to compare the PK parameters of CsA alone (control group) or co-administered with DEX in first and second groups (test groups). (SPSS) program (version 16.0) was applied to analyze data. A statistically significant difference was considered when \( P \leq 0.05 \).

2. RESULTS

The DEX-CsA interaction study was carried out to determine the influence of DEX at different concentrations on the PK parameters of CsA. Whole blood concentration-time profiles of CsA in the control group (Received only CsA 7.5 mg/kg/day) and test groups (First and second groups), which received CsA concomitantly with DEX at doses (0.33, and 0.66 mg/kg/day) respectively are shown in Figure 1. The PK parameters of CsA were determined for the control and test groups, including \( C_{\text{max}}, t_{\text{max}}, t_{1/2}, K_e, \text{AUC}_{0-24}, \text{AUC}_{0-\infty}, \text{and MRT} \). The control group's obtained PK parameters were compared with those of the first test group (Table 1) and those with the second test group (Table 2).

![Figure 1: Plot of blood CsA concentration-time profile. Control group: Rabbits received CsA alone (7.5mg/kg/day) orally for five days, first and second groups (test groups): Rabbits received CsA (7.5mg/kg/day) concurrently with DEX (0.33 and 0.66mg/kg/day) orally for five days, respectively (n=6).](image-url)
Table 1. PK parameters of CsA in control and first test group (n=6).

<table>
<thead>
<tr>
<th>PK Parameters</th>
<th>Groups</th>
<th>N</th>
<th>Mean ± SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/ml)</td>
<td>Control group</td>
<td>6</td>
<td>254.9±106.4</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>First group</td>
<td>6</td>
<td>290.0±144.9</td>
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<tr>
<td>t&lt;sub&gt;max&lt;/sub&gt; (h)</td>
<td>Control group</td>
<td>6</td>
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<td>0.58</td>
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<tr>
<td></td>
<td>First group</td>
<td>6</td>
<td>1.83±0.75</td>
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<tr>
<td>Ke (h⁻¹)</td>
<td>Control group</td>
<td>6</td>
<td>0.052±0.001</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>First group</td>
<td>6</td>
<td>0.054±0.02</td>
<td></td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>Control group</td>
<td>6</td>
<td>13.38±1.82</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>First group</td>
<td>6</td>
<td>13.29±2009</td>
<td></td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-24&lt;/sub&gt; (ng/ml.h)</td>
<td>Control group</td>
<td>6</td>
<td>2221±730</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td>First group</td>
<td>6</td>
<td>2009±946</td>
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</tr>
<tr>
<td>AUC&lt;sub&gt;0-∞&lt;/sub&gt; (ng/ml.h)</td>
<td>Control group</td>
<td>6</td>
<td>3069±808</td>
<td>0.71</td>
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<tr>
<td></td>
<td>First group</td>
<td>6</td>
<td>2863±985</td>
<td></td>
</tr>
<tr>
<td>MRT (h)</td>
<td>Control group</td>
<td>6</td>
<td>9.22±0.62</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>First group</td>
<td>6</td>
<td>9.16±1.12</td>
<td></td>
</tr>
</tbody>
</table>

Control group: Rabbits received CsA (7.5mg/kg/day) orally for five days; First group: Rabbits received CsA (7.5mg/kg/day) concurrently with DEX (0.33mg/kg/day) orally for five days; Statistical significance: P≤0.05.

Table 2. PK parameters of CsA in control and second test groups (n=6).

<table>
<thead>
<tr>
<th>PK Parameters</th>
<th>Group</th>
<th>N</th>
<th>Mean ± SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/ml)</td>
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<td>6</td>
<td>254.9±106.4</td>
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<td>Second group</td>
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<td>213.5±72.6</td>
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<td>1.50±0.87</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td>Second group</td>
<td>6</td>
<td>1.92±0970</td>
<td></td>
</tr>
<tr>
<td>Ke (h⁻¹)</td>
<td>Control group</td>
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<td>0.06</td>
</tr>
<tr>
<td></td>
<td>Second group</td>
<td>6</td>
<td>0.049±0.012</td>
<td></td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>Control group</td>
<td>6</td>
<td>13.38±1.82</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>Second group</td>
<td>6</td>
<td>18.97±5.61</td>
<td></td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-24&lt;/sub&gt; (ng/ml.h)</td>
<td>Control group</td>
<td>6</td>
<td>2221±730</td>
<td>0.59</td>
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<tr>
<td></td>
<td>Second group</td>
<td>6</td>
<td>1975±333</td>
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<tr>
<td>AUC&lt;sub&gt;0-∞&lt;/sub&gt; (ng/ml.h)</td>
<td>Control group</td>
<td>6</td>
<td>3069±808</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>Second group</td>
<td>6</td>
<td>2747±497</td>
<td></td>
</tr>
<tr>
<td>MRT (h)</td>
<td>Control group</td>
<td>6</td>
<td>9.22±0.62</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>Second group</td>
<td>6</td>
<td>8.79±0.83</td>
<td></td>
</tr>
</tbody>
</table>
Control group: Rabbits received CsA (7.5mg/kg/day) orally for five days; Second group: Rabbits received CsA (7.5mg/kg/day) concurrently with DEX (0.66mg/kg/day) orally for five days; Statistical significance: \( P \leq 0.05 \).

In this study, \( C_{\text{max}} \) of CsA (control group) was \( 254.9\pm 106.4 \) ng/mL, and \( t_{\text{max}} \) was \( 1.50\pm 0.87 \) h. The rabbits treated with DEX 0.33 mg/kg/day (first group) produced \( C_{\text{max}} \) and \( t_{\text{max}} \) of \( 290.0\pm 144.9 \) ng/mL and \( 1.83\pm 0.75 \) h, respectively. A slight increase of \( C_{\text{max}} \) and \( t_{\text{max}} \) of CsA were found when DEX (first group) was co-administered with CsA compared to the control group. The differences were statistically insignificant. A slight decrease in \( AUC_{0-24} \) and \( AUC_{0-\infty} \) was manifested in the first test group in comparison to control, but it was statistically insignificant (\( P \geq 0.05 \)). Other PK parameters, including \( t_{1/2} \) and \( K_e \) were also insignificant (Table 1). Blood concentration-time profiles of CsA were comparable for control, and second test groups (Figure 1).

PK parameters of CsA (\( C_{\text{max}}, t_{\text{max}}, K_e, t_{1/2}, AUC_{0-24}, AUC_{0-\infty} \) and MRT) were unaffected (\( P \geq 0.05 \)) by co-administration of DEX at a concentration of 0.66 mg/kg/day (second group) as shown in (Table 2).

3. DISCUSSION

Most immunosuppressive agents have narrow therapeutic indexes, so dosing most of the immunosuppressive agents is applied under careful monitoring of their blood concentrations. Knowing the potential factors that can modify immunosuppressive therapy and pharmacokinetics and metabolic drug interactions can decrease the fluctuation of immunosuppressant blood concentrations. CsA is extensively metabolized in the liver by the CYP3A4 system, a member of the \( \text{CYP450} \) family of oxidizing enzymes. Sustained and clinically significant DDIs can occur during long-term therapy with CsA. The co-administration of multiple drugs with CsA could result in graft rejection, renal dysfunction, or other undesirable effects. Potential CsA drug interaction is of great clinical importance. Drugs affecting CYP3A4 metabolic activity are a possible candidate for such interactions. Synthetic glucocorticoids as dexamethasone or prednisolone are known substrates and inducers of CYP3A enzymes. The regulation of these enzymes, particularly CYP3A4, has been extensively studied. In vitro studies of DEX in cultures of human hepatocytes showed potent CYP3A4 inducing effects. In vivo studies had shown that co-administration of DEX with some drugs as phenytoin resulted in reduced plasma level of the drug. When DEX was discontinued, plasma concentration increased by 300% and increased CYP3A4 activity in healthy volunteers and human hepatocyte cultures. The conducted study of DEX-CsA interaction showed statistically insignificant differences in PK parameters when CsA was administered alone or in combination with DEX given at two different doses. Similar results were obtained in another research work realized by Villikka and collaborators who studied the PK of triazolam (CYP3A4 substrate) when co-administered with DEX (4-day course of 1.5 mg dexamethasone daily). The impact of an enzyme inducer like DEX increases gradually over time. The effect of DEX doses on PK of tacrolimus was manifested after three months of treatment after one month. This could explain the obtained results since DEX was co-administered with CsA for five days. The possibility that higher doses of dexamethasone could induce CYP3A4 and thereby cause clinically significant drug interactions cannot be excluded. This is particularly relevant in cancer chemotherapy as many anticancer agents are CYP3A4 substrates, and DEX as an antiemetic agent is used at high doses.

In conclusion, the present study results demonstrated that concurrent use of DEX at the examined regimen with CsA had not influenced PK parameters of CsA. Furthermore, PK studies of CsA using DEX at higher doses and for a longer duration and other clinically relevant CYP inducers or inhibitors are advised.

Conflict of interest

The authors proclaim no conflict of interest.
REFERENCES

Challenges. 2018; 409-440.


تأثير دواء الديكساميثازون بجرعات مختلفة على حركة دواء السيكلوسبيرون

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ملخص

تم تصميم هذه الدراسة للتحقيق في وجود تفاعل حركي بين دواء الديكساميثازون (DEX) والسيكلوسبيرون (CsA) في الأ.ARRائف. تم اختبار الأربعة وتقييمها إلى ثلاث مجموعات: المجموعة الضئيلة (ع = 6) تقلص محلول السيكلوسبيرون عن طريق الفم (7.5 ملم، كجم / يوم) بعد تحضيره من كبسولة الجيلاتين الطبرية لمدة خمسة أيام. وفي اليوم الخامس تم سحب عينات الدم الشاملة من الوريد الذقنى للأزالاب في فترات زمنية متشابهة بعد الجرعات. أعطيت الأربعة في المجموعتين الأولى والسادسة على طريق الفم محلي السيكلوسبيرون (7.5 مجم / كجم / يوم) و دواء الديكساميثازون بالتزامن مع جرعة (33 مجم، 0.66 مجم / كجم / يوم) على التوالي. في اليوم الخامس من اعطاء الدوانيين ، تم جمع عينات دم متسلسلة لكل مجموعة اختبار على مدى 24 ساعة كما في المجموعة الضئيلة ومن ثم تم تحديد الحركيات الدوائية للسيكلوسبيرون في مجموعة الضياع والاختبار. لاحظ أنه كان هناك اختلافات غير ملموسة في مستويات السيكلوسبيرون بين مجموعات الضياع والاختبار عند اعطاء محلول السيكلوسبيرون مع دواء الديكساميثازون بالتركيزين المذكورين اعلاه مما يشير إلى أن الديكساميثازون لا يؤثر على حركة دواء السيكلوسبيرون.

الكلمات المفتاحية: السيكلوسبيرون، الديكساميثازون، التفاعلات الدوائية، الملاحظات الحركية الدوائية.