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Pharmacokinetic drug–drug interaction study between clindamycin and cyclosporin in rabbits

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ABSTRACT

Cyclosporine A (CSA) is an immunosuppressant drug, metabolized mainly by CYP3A4 that is one of the Cytochrome P450 enzymes. Clindamycin (CLN) is a lincosamide antibiotic, inducing CYP3A4 activity *in vitro*, and thereby may alter CSA pharmacokinetics (PK). The current research was performed to investigate the PK parameters changes of CSA up on co-administrating with CLN in healthy male rabbits. Twelve healthy male rabbits randomly were selected and divided into two groups: Control set ($n = 6$) in which the rabbits were received oral normal saline CSA solution (10 mg/kg/day), meanwhile rabbits in the test group ($n = 6$) were treated with oral normal saline CSA solution (10 mg/kg/day) concomitantly with normal saline solution of CLN (8 mg/kg/day) at the same time for 7 days. Blood samples (2 ml) were collected and CSA concentrations were measured in whole blood at the predetermined time points by using Chemiluminescent Immunoassay (CLIA) detection kit. PK profiles of CSA for both groups in the control and test groups including C_{max}, t_{max}, $AUC_{0\text{-}24}$, the area under the blood concentration–time curve from 0 hour to infinity ($AUC_{0\text{-}\infty}$), $t_{½}$, and K_e were compared. The results showed a statistically insignificant differences in the PK parameters of CSA alone or combined with CLN with $p > 0.05$. In conclusion, it has been found that CLN does not affect the CSA PK. Further confirmation of our findings is requiered in humans before these results can be applied in patient care.

INTRODUCTION

Cyclosporine (CSA) is an immunosuppressant drug with narrow therapeutic index, extracted from fungus *Tolypocladium inflatum* (Borel 2002; Yang *et al*., 2018). It works by reducing the function of T-lymphocytes through blocking the phosphatase activity of calcineurin that leads to decrease the T-cell inflammatory cytokines (Matsuda and Koyasu, 2000). CSA is widely used as the first line therapy in prophylaxis and treatment of organ transplant rejection (Azzi *et al*., 2013), rheumatoid arthritis, psoriasis, and other autoimmune diseases (Faulds *et al*., 1993). Moreover, CSA is a substrate of CYP3A4. Modulation of CYP3A4 activity can alter PK of CSA as asequence (Larry 2008; Kronbach *et al*., 1988). The hepatic microsomal cytochrome P450 (CYP450) superfamily includes enzymes that are responsible for the metabolism of many chemical substances (Estabrook, 2003; Kelly and Kahan, 2002). Consequently, the modification of CYP450 enzyme activity can alter the biological activity of xenobiotic substrates via drug–drug interaction (DDI) (Rendic and Di Carlo, 1997). DDI is a specific term used when the efficacy or toxicity of a drug is modified by another administered drug, which may be beneficial or harmful. In last decades, safety and efficacy of DDI have been widely evaluated. Harmful effects; as they are remarkable through (10%–20%) of adverse to be hospitalized (Şimşek *et al*., 2019). Accordingly, sustained and clinically significant DDIs can occur during long-term therapy with CsA when co-administered with other drugs having CYP3A4 inducing or inhibitory effect, resulting in graft rejection or renal dysfunction (Campana *et al*., 1996). Clindamycin (CLN) is a lincosamide antibiotic that can be used against Gram-positive aerobic bacteria as well as some antiprotozoal actions (Dhawan

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et al., 1982). CLN induces CYP3A4 activity, suggesting a possible DDI between CSA and CLN when those drugs are concomitantly administered. There are limited data about the influence of CLN on CYP3A4 activity. The effect of CLN on CYP3A4 transcription in human hepatocytes and intestinal cells was moderately activated in HepG2 cells but in DPX-2 cells, CYP3A4, mRNA, and protein expression were weakly enhanced through the activation of Pregnane X receptor (PXR) (Yasuda *et al*., 2008). Suspected interaction were reported when CLN was added to CSA therapy, where serum CSA concentrations reduced continuously as the daily dose of CLN was increased. Interestingly, after CLN treatment was stopped, the dose of CSA was reduced to the initial one before starting antibiotic treatment (Thurnheer *et al*., 1999). In another study, a novel interaction between tacrolimus and CLN was reported in a renal transplant recipient with *Pneumocystis jirovecii* pneumonia, where the co-administration of CLN and tacrolimus led to a significant decrease in tacrolimus plasma level (Jouret *et al*., 2010). Moreover, Wynalda *et al*. (2003) found that, the incubation of CLN (100 µM) with human liver and gut microsomes inhibited 26% of CYP3A4 activity (Wynalda, 2003). This study aims to figure out the PK changes of CSA upon coadminestering of both CLN and CSA in male rabbits.

MATERIALS AND METHODS

Chemicals

Cyclosporine soft capsule (100 mg, Sandimmun Neoral®, Novartis, Basel, Switzerland) was gifted from the Ministry of Health, Gaza-Palestine. CSA content from three soft gelatin capsules was diluted with 30 ml normal saline. 3.5 ml were given daily for each rabbit. CLN capsule (300 mg, Dalacin C®, Pfizer, New York, USA**)** was bought from a private pharmacy (Gaza, Palestine). Capsule contents were transferred and suspended in 16 ml of normal saline and then 1.5 ml was administered for each rabbit daily.

Study design

Twelve healthy male rabbits (Weighted between 3.2 and –3.4 kg) were enrolled in this research and randomly selected and divided to two groups: Control and test groups (six for each). The study was approved by the Experimental Animal Care Center, Faculty of Pharmacy, Al-Azhar University, Gaza, Palestine. The rabbits were maintained under the regulations of the "Guide for the Care and Use of Laboratory Animals." The rabbits were maintained under standard laboratory conditions of a 12-hour light/dark cycle at 25°C ± 2°C and given pellet diet with free access to water (ad libitum) and fasted overnight prior to the experiments. Each rabbit of the control group received a single oral dose (10 mg/kg/day) of CSA solution prepared in normal saline daily, whereas the rabbits of the test group were treated with similar dose of CSA along with an oral dose of CLN (8 mg/kg/day) in normal saline. Doses were given using of a syringe linked to a mouth gavage that was placed in rabbit mouth's corner and the dose was step-wised to prevent choking.

Blood sampling

Fifteen minutes before starting the practical part and in a restraining box the rabbits were put. Hair around ear was removed and lidocaine 4% as local anesthetic was applied to avoid rabbits jerking due to venepuncture. On the seventh day after receiving the last dose, blood samples (2 ml) were collected using a small needle (23 gauge) linked to the marginal ear vein (Parasuraman *et al*., 2010), into heparinized vacutainer tubes at 0.0, 0.5, 1.0, 1.5, 2.0, 3.0, 6.0, 12, and 24 hours. The samples were kept and adjusted at (2°C–8°C)(whole blood sample is stable for up to 3 days; Shenzhen New Industries Biomedical Engineering Co., Ltd., China).

Analysis of blood samples

Adjusted amount of reagent as above was added to each sample, mixed together, and put at the vortex mixer for 2 minutes (2,000 rpm). All samples were tested when placed on board of the Maglumi system. The analysis was performed by Maglumi CSA (Cyclosporine detection kit, 130207001M) Maglumi 800 analyzer based on chemiluminescent immunoassay (CLIA) (Shenzhen New Industries Biomedical Engineering Co., Ltd and Snibe. The results for CSA concentrations were given automatically for each sample in ng/ml by applying of a calibration curve that is calculated using two-point calibration curve method. The MAGLUMI CSA (CLIA) Kit is an *in vitro* competitive, and non-enzyme involved flash chemiluminescence immunoassay for the rapid quantitative determination of CsA concentrations in whole blood samples with the Maglumi 800, fully-auto chemiluminescence immunoassay analyzer. Chemiluminescent Immunoassay (CLIA) system is the combination of chemiluminescence determination technology and immunoreaction technology, which possess high sensitivity and specificity.

Determination of pharmacokinetic parameters

The PK parameters (C_{max}), (t_{max}), the area under the blood concentration–time curve from 0 hour to last measurable concentration (AUC_{0-t}), and the area under the blood concentration–time curve from 0 hour to infinity ($AUC_{0-\infty}$) were calculated in both groups at the mentioned intervals. C_{max} and t_{max} were calculated from blood concentration versus time curves, while AUC_{0-t} was calculated by the linear trapezoidal rule meanwhile, $AUC_{0-\infty}$ was calculated by summing the area of AUC_{0-t} and $AUC_{0-\infty}$, using the equation $^{ref}AUC_{0-\infty} = AUC_{0-t} + C_t/K_e$, where "$C_t$" defined as the last measured serum/blood concentration at time t, and K_e is the elimination constant. K_e was calculated using least square regression of plasma/blood concentration–time data points lying in the terminal region using semi-logarithmic dependence that corresponds to first-order kinetic. The $t_{1/2}$ was calculated from the equation ($K_e = 0.693/t_{1/2}$). PK analyses were run 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).

Statistical analysis

The data is shown in the term of mean with standard deviation (SD) for each time point in each group. Differences in PK parameters of CSA between the control and test groups were assessed by independent (unpaired) *t*-test, using general linear model procedures. Statistical analyses were achieved using SPSS (Version 16) consistent with significance criteria ($p < 0.05$).

RESULTS AND DISCUSSION

Mean blood concentrations-time profile of CSA plot for both groups is given in (Fig. 1). The mean of C_{max} in control group was 244.7 ± 115.87 ng/ml, while in test group was 242.90 ± 104.45 ng/ml, *p*-value = 0.968. Time to reach the maximum blood concentration (t_{max}) of CSA in control group was 1.75 ± 0.76 hours. In test group, t_{max} was slightly decreased to 1.25 ± 0.42 hours which was not significant (*p*-value = 0.314). The results of PK parameters are shown in Table 1. The differences in the mean of PK parameters of CSA estimated for the two groups were statistically insignificant, $p > 0.05$. Regarding AUC_{0-24} and $AUC_{0-\infty}$, the means were 2,057.8 ± 778.6 and 3,492.9 ± 1,449 ng.hour/ml in the control group and were 2,948.0 ± 1,321.44 and 4,605.4 ± 2205.20 ng.hour/ml in the test group, respectively. Regarding $t_{1/2}$, we found that, the mean was 19.73 ± 14.01 versus 17.40 ± 5.54 hour, ($p = 0.574$) in control and test groups, respectively. Moreover, the means of K_e were 0.0519 ± 0.03 versus 0.0439 ± 0.01 $hour^{-1}$, ($p = 0.483$) in control and test group, respectively.

CYP450 enzymes, plays a vital role in DDI–s due to their critical function in drug metabolism. In addition, the importance of the CYP450 system for drug interactions depends on the fact that the activity of CYP enzymes can be inhibited or induced by certain drugs or exogenous substances leading to an alteration of the predicted plasma level of the affected drug. CYP3A and CYP2D6 are responsible for more than 50% of known drug metabolism, therefore, drug interactions are frequently seen when drugs metabolized by these enzymes are co-administered (Gonzalez *et al*., 2018; Snyder *et al*., 2012). The entire study was run with neither death nor replacing rabbits.

The introduction of therapeutic drug monitoring for CSA is extremely useful because of the wide inter- and intra-individual variability of CSA PK parameters, narrow therapeutic window, and the severity of CSA adverse effects beside therapeutic failure consequences (Liu *et al*., 2016).

CSA is metabolized by CYP3A4 (Dai *et al*., 2004). Combined of CSA with CYP3A4 induction or inhibition can affect its plasma concentration. CSA blood concentration was decreased by 63% and the dose of CSA was elevated, when it was concomitantly administered with rifampicin (Perona *et al*., 2013). In another study, concomitantly using of St. John's wort, a CYP3A4 induction led to a significant decrease in CSA levels, resulting in organ transplant rejection and graft loss (Ruschitzka *et al*., 2000). *In vivo* PK interaction between CLN with CSA is poorly described in the literature. This investigation was performed in our study. According to our results regarding the PK parameters AUC_{0-24} and $AUC_{0-\infty}$; there was statistical insignificance through *p* values as illustrated in Table 1. There was a clear similarity of our findings and López-Gil; who found insignificant alterations in CSA concentration/dose ratio in the patients undergoing bone marrow transplantation receiving fluconazole 100 mg/dl (López-Gil, 1993). In addition, ticagrelor and its active metabolite had statistically insignificant effect on CSA PK parameters (Teng *et al*., 2014).

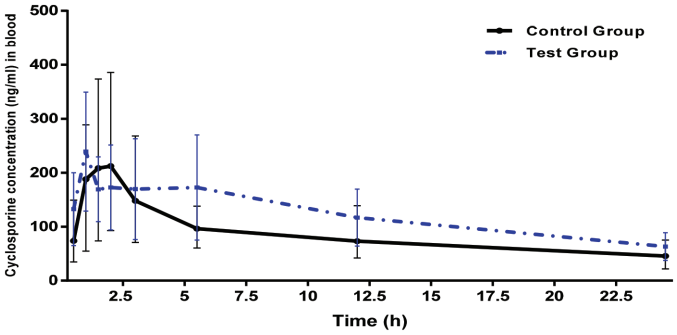

Figure 1. Mean blood concentrations-time profile of CSA in control and test groups of rabbits ($n = 6$ per group).

Table 1. Mean PK parameters of CSA in cotrol and test groups of rabbits ($n = 6$ per group).

PK Parameters	Control group* (Mean ± SD)	Test group** (Mean ± SD)	*p*-value
C_{max} (ng/ml)	244.67 ± 115.87	242.90 ± 104.45	0.968
t_{max} (hour)	1.75 ± 0.76	1.25 ± 0.42	0.314
$t_{1/2}$ (hour)	19.73 ± 14.01	17.40 ± 5.54	0.574
K_e ($hour^{-1}$)	0.0519 ± 0.03	0.0439 ± 0.01	0.483
AUC_{0-24} (ng.hour/ml)	2,057.8 ± 778.6	2,948.0 ± 1,321.44	0.127
$AUC_{0-\infty}$ (ng.hr/mL)	3,492.9 ± 1,449.70	4,605.4 ± 2,205.20	0.258

CONCLUSION

In conclusion, it has been found that CLN does not affect the CSA PK parameters. Further confirmation of our findings is requiered before to be applied in humans by using larger number of animals to decrease interindividual variability of CSA PK parameters between both groups before these results can be applied in patient care.

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CONFLICTS OF INTEREST

The authors declare that there are no conflict of interest.

LIST OF ABBREVIATIONS

Maximum blood concentration (C_{max}); The time to reach C_{max} (t_{max}); ouThe elimination rate constant (K_e); The half-life ($t_{1/2}$); The plasma concentration at time *t* (C_t); Nanograms (ng); Miligrams (Mg); Kilogram (Kg); Mililiters (ml); Hour (h).

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