ORIGINAL ARTICLE

Pharmacogenetic analysis of the absorption kinetics of cyclosporine in a population of Spanish cardiac transplant patients

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Pharmacogenetics; Cardiac transplant; Cyclosporine; Pharmacokinetics

Abstract
Objective: To determine how single nucleotide polymorphisms located on genes MDR1, CYP3A4, and CYP3A5 affect the absorption kinetics of cyclosporine in cardiac transplant patients.
Method: We selected a sample of 30 adult patients having previously undergone a primary cardiac transplant and who had received cyclosporine as an immunosuppressant. During the first month after the transplant, we performed a pharmacokinetic study of each patient to determine values in the cyclosporine concentration area under the 12-hour curve, steady-state cyclosporine concentration, maximum cyclosporine concentration, and time to reach that concentration. Single nucleotide polymorphisms were genotyped in all patients: MDR1 3435C > T, CYP3A4-390A > G, and CYP3A5 6986A > G.
Results: Being a carrier of the T-allele for polymorphism MDR1 3435C > T is associated with higher values in the cyclosporine concentration area under the 12-hour curve (P=.01) and in steady-state cyclosporine concentration (P=.05), compared with those from patients who do not carry that allele.
Discussion: Our results show that genotype differences in MDR1 3435C > T can explain part of the variability in cyclosporine absorption among individuals in the population of Spanish cardiac transplant recipients.

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Introduction

Cardiac transplantation continues to be the definitive treatment in patients suffering from terminal cardiac failure. Cyclosporine A (CsA) is one of the most frequently used drugs to avoid rejection in this type of patient. As is the case with other calcineurin inhibitors, its pharmacokinetics is characterised by the presence of a generally poor, highly variable, and unpredictable oral bioavailability and great interindividual variability during first-pass metabolism and systemic clearance. 1 For some years now it has been known that a great deal of such variability is due to differences of activity in the glycoprotein P-gp, CYP3A4 and CYP3A5. 2

The product of the gen MDR1 (ABCB1) is the glycoprotein P-gp which belongs to the family of ABC membrane transporters (subfamily B). Its ATP-dependent activity consists of acting as a pump for a wide range of both endogenous and exogenous substances including immunosuppressant drugs used in cardiac transplant patients such as calcineurin inhibitors, sirolimus and corticosteroids, in such a way that it eliminates these drugs from the cytoplasm and moves them to extracellular space. 3

The CYP enzymatic family is made up of more than 50 casual isoenzymes of the oxidative metabolism of many endogenous and exogenous compounds. 4 The CYP3A subfamily, which represents the majority of CYP proteins in the human liver, metabolises more than 50% of all drugs used today. 5 It is made up by the isoenzymes CYP3A4, CYP3A5, CYP3A7, and CYP3A43. CYP3A4 and CYP3A5 have a very similar substrate specificity profile and are, quantitatively, the most important forms of this subfamily.

In recent years various studies have been published on the effect that the genotype of single nucleotide polymorphisms (SNP) of the genes MDR1, CYP3A4, and CYP3A5 may have in the function of the proteins which they codify and therefore in the pharmacokinetics of the CsA. In spite of the evidence accumulated in this time, there are still discrepancies in the clinical interpretation of the results of such studies. 6 One of the factors which may help to explain such discrepancies is the highly diverse ethnical origin of the populations in which the different studies have taken place. The genetic context of each population is an element of confusion which introduces important differences into the population frequencies of the allele of these SNP, which makes it difficult to extrapolate to the Spanish population results obtained in other populations genetically distant.

As no such study has been carried out to date amongst patients from our area, in this study we proposed determining the role of MDR1 3435C > T, CYP3A4-390A > G and CYP3A5 6986A > G in the absorption kinetics of CsA in a Spanish population of adult cardiac transplant patients.

Methods

Population

The study involved a total of 30 patients of Caucasian origin (23 males and 7 females) who had an average (standard deviation) in age of 43 (14) (18-67) years when they underwent their first orthopaedic cardiac transplant in the Hospital Universitario Reina Sofía (Córdoba). All patients received treatment with CsA together with corticosteroids, mycophenolate mofetil or azathioprine, or sirolimus, associated or not to induction treatment using basiliximab or ATG. The treatment with CsA began within the first 24 hours following the transplant, the dosage was 4 mg/kg/day divided into 2 doses. Then the dosage was modified to...
Primer sequences

The sequences of (Isogen Life Bioscience BV, Cordoba, Spain) and 5 U/µL, used 300 ng of genomic DNA, 0.2 µL of Taq polymerase for each SNP was carried out by means of polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). For each PCR reaction we used 300 ng of genomic DNA, 0.2 µL of Taq polymerase 5 U/µL, 2 µL of 10x PCR Buffer without Mg²⁺, 1.6 µL of MgCl₂ 25 mmol, 1.6 µL of dNTPs 2 µmol (Dominion MBL, Cordoba, Spain) and 1 µL (15 pmol/µL) of each primer (Isogen Life Bioscience BV, Maarsen, the Netherlands), all in a final volume of 20 µL of H₂O. The sequences of the primers used for each SNP can be seen in Table 1.

The genotyping of each SNP was separated by means of non-denaturalising agarose electrophoresis in an agarose gel (2%) and finally, visualised in the Gel Printer Plus ultraviolet transilluminator (Tecnología para Diagnóstico e Investigación S.A., Madrid, Spain).

Table 1 Sequence of the primers used for genotyping the polymorphisms analysed

<table>
<thead>
<tr>
<th>SNP (dbSNP)</th>
<th>Primer sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDR1 3435C &gt; T</td>
<td>Fw: 5'-TGT TTT CAG CTG CTT GAT GGC AAA-3'</td>
</tr>
<tr>
<td>(rs1045642)</td>
<td>Rev: 5'-GGT AAC AAC TAA CCC AAA CAG GA-3'</td>
</tr>
<tr>
<td>CYP34A-290A &gt; G</td>
<td>Fw: 5'-GGA ATG AGG ACA GCC ATA GAG ACA AGG GGA-3'</td>
</tr>
<tr>
<td>(rs11539969)</td>
<td>Rev: 5'-CCT TTC AGC TCT GTG TTG CTC TTT GCT G-3'</td>
</tr>
<tr>
<td>CYP3A5 6986A &gt; G</td>
<td>Fw: 5'-CAA TTT TTC ACT GAC CTC ATA TTC T-3'</td>
</tr>
<tr>
<td>(rs776746)</td>
<td>Rev: 5'-TGC GTT CCG AAG TAT ACT ACG CAT TAC ACC ACC AGG TGT CCT TCT TTA T-3'</td>
</tr>
</tbody>
</table>

dbSNP indicates single nucleotide polymorphism database reference number; SNP: single nucleotide polymorphism.
Pharmacogenetic analysis of the absorption kinetics of cyclosporine in a population of Spanish cardiac transplant patients

ng/h/mL), calculated by the trapezoidal method and the plasmatic concentration in the stationary state (Css, ng/mL), calculated as Css = AUC0-12/ dosage interval. The AUC0-12 values were also adjusted according to the dose (D, mg/kg) and weight (kg) of each patient, thereby obtaining the variables AUC0-12/D, and AUC0-12/D/weight.

Ethical aspects

The analysis was carried out adhering to the fundamental principles established in the Declaration of Helsinki (1964), the Council of Europe Convention on Human Rights and Biomedicine (1997) the UNESCO Universal Declaration on the Human Genome and Human Rights (1997) and also in line with the requirements established by Spanish legislation in the areas of biomedical investigation, the protection of personal data and bioethics. The project underwent evaluation by the Ethics and Clinical Investigation Committee of the Reina Sofia University Hospital. All patients received information about the analysis and written informed consent was requested before taking part therein.

Data analysis

To examine population homogeneity, the genotypic frequencies of the different SNPs were analysed according to the expected frequencies according to the Hardy-Weinberg equilibrium, using the $\chi^2$ test with Yates’ correction for continuity. The Shapiro-Wilk test was used to check the level of conformance to a normal distribution model of said allele, without there being statistically significant differences in the other pharmacokinetic parameters ($P>.05$) in comparison to patients who were not carriers of said allele, without there being statistically significant differences in the other pharmacokinetic parameters analysed.

<table>
<thead>
<tr>
<th>SNP (dbSNP)</th>
<th>Genotypes</th>
<th>Genotypes</th>
<th>Genotypes</th>
<th>Genotypes</th>
<th>T max, h</th>
<th>C max,N, ng/mL</th>
<th>Ccss, ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDR1 3435C &gt; T</td>
<td>TT/TC (n=22)</td>
<td>6686 (5583-7050)</td>
<td>40.1 (27.7-47.2)</td>
<td>2508 (2111-2927)</td>
<td>2 (1-2)</td>
<td>1137 (1030-1380)</td>
<td>554 (481-587)</td>
</tr>
<tr>
<td>rs1045642</td>
<td>CC (n=8)</td>
<td>4569 (4165-4811)</td>
<td>31.6 (23.7-38.7)</td>
<td>2479 (1960-3059)</td>
<td>2 (1-2)</td>
<td>1326 (865-1511)</td>
<td>340 (301-435)</td>
</tr>
<tr>
<td>P</td>
<td>0.010</td>
<td>0.241</td>
<td>0.815</td>
<td>0.794</td>
<td>0.959</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>CYP3A4-390A &gt; G</td>
<td>AA (n=26)</td>
<td>6188 (4701-6977)</td>
<td>34.5 (27.0-45.2)</td>
<td>2333 (1931-2917)</td>
<td>2 (1-2)</td>
<td>1107 (975-1380)</td>
<td>554 (460-593)</td>
</tr>
<tr>
<td>rs2470574</td>
<td>GA/GG (n=4)</td>
<td>5420 (5084-6522)</td>
<td>50.8 (41.2-52.5)</td>
<td>3104 (3015-3432)</td>
<td>2 (1-2)</td>
<td>1340 (1306-1364)</td>
<td>562 (507-598)</td>
</tr>
<tr>
<td>P</td>
<td>0.833</td>
<td>0.202</td>
<td>0.239</td>
<td>0.771</td>
<td>0.372</td>
<td>0.762</td>
<td></td>
</tr>
<tr>
<td>CYP3A5 6986A &gt; G</td>
<td>GG (n=27)</td>
<td>5970 (4694-6913)</td>
<td>32.4 (27.0-46.1)</td>
<td>2333 (1931-2929)</td>
<td>2 (1-2)</td>
<td>1137 (1000-1380)</td>
<td>554 (459-593)</td>
</tr>
<tr>
<td>rs776746</td>
<td>GA/AA (n=3)</td>
<td>7052 (6236-7364)</td>
<td>43.8 (42.1-49.0)</td>
<td>2927 (2874-3021)</td>
<td>2 (1-2)</td>
<td>1188 (1078-1288)</td>
<td>587 (519-613)</td>
</tr>
<tr>
<td>P</td>
<td>0.285</td>
<td>0.175</td>
<td>0.516</td>
<td>0.770</td>
<td>0.893</td>
<td>0.656</td>
<td></td>
</tr>
</tbody>
</table>

AUC0-12 indicates area of concentration beneath the curve between 0 and 12 hours after the dose; AUC0-12/D, area of concentration beneath the curve between 0 and 12 hours after the dose, dose and weight-normalised; C max, maximum concentration observed; Ccss, average concentration in a state of equilibrium; T max, time taken to achieve the maximum observed concentration. The values are expressed as a median (first quartile - third quartile). For hypothesis contrasts, the Kruskal-Wallis test was used and differences were considered statistically significant when the P value was <.05.
Discussion

To date, studies aimed at analyzing pharmacogenetic aspects of CsA have not found a clear relation between the different MDR1, CYP3A4 and CYP3A5 polymorphisms and their pharmacokinetic parameters.7,9 Min et al carried out an analysis (14 healthy volunteers of which 11 were of African-American origin and 3 Caucasian) of the effect of MDR1 3435C > T and CYP3A4-290A > G in the kinetic of absorption of the CsA (AUC0-24 h, T1/2, Cmax, t1/2g, and CL/F).10 They found that carriers of the A allele of CYP3A-290A > G presented greater values of AUC0-24 h/D and lesser values of CL/F. With regard to MDR1 3435C > T, although the Cmax and the el AUC0-24 h/D the CT and TT Group was 15% and 22 % greater than in the CC, neither of these differences were statistically significant. Anglicheau et al11 studied in 100 renal transplant patients of Caucasian origin, in a stable condition, the influence of the polymorphisms MDR1 [—129T > C], 1236C > T, 2677G > (T/A), 3435C > T], and CYP3A5 6986A > G in concentration values of the dose of CsA (C0), Cmax, AUC0-24 h, AUC0-12 h, Cmax/D and AUC0-24 h/D in absolute values and normalised by the dose. They only found a weak association between MDR1 1236C > T and the values of Cmax/D and AUC0-24 h/D which they considered insufficient for dose optimisation in clinical practice. None of the pharmacokinetic parameters were associated with CYP3A5 6986A > G. Mai et al12 carried out a retrospective analysis of the effect of different MDR1 haplotypes in the pharmacokinetics of CsA on 98 renal transplant patients of Caucasian origin in a stable condition. They observed no differences in their analysis of haplotypes in the C0 values and of CsA concentration 2 hours after the dose, (C0) nor AUC0-12 h. Kuzuya et al13 carried out a study of 97 renal transplant patients of Asian origin amongst whom they analysed the effect of MDR1 [—129T > C], 1236C > T, 2677G > (T/A), 3435C > T]. The interval since the transplant was of 2-17 (average, 7) years. The pharmacokinetic parameters analysed were AUC0-24 h, Cmax and Cmin. These authors did not observe significant differences between the required doses of CsA and the MDR1 polymorphisms studied.

On the other hand, other studies do exist which do find, in a manner similar to ours, a relation between some MDR1, CYP3A4, and CYP3A5 polymorphisms and the pharmacokinetic parameters of the CsA. As a result, the study carried out by Balram et al14 on a subgroup of Asian cardiac transplant patients demonstrated that patients with the CC genotype presented an AUC0-24 h, 11% less than carriers of the TT genotype. In our study we observed an even greater difference as TT genotype carriers presented an AUC0-24 h, 5.3% and 38% greater in comparison to CT and CC genotype carriers, respectively. Chowbay et al15 analysed, in 275 healthy volunteers and 14 Asian cardiac transplant patients, the influence of MDR1 [1236C > T, 2677G > (T/A), 3435C > T] and CYP3A-290A > G in the values of AUC0-12 h, AUC0-24 h, Cmax and Cmin in stable condition after the transplant. These authors found that the values of AUC0-12 h, AUC0-24 h, and Cmax were greater in those patients presenting the MDR1 haplotype T-T-T. In line with the above, Barnard et al16 recently observed that greater doses of CsA were necessary for the MDR1 genotype CC carriers, in comparison to CT and TT carriers, for 3 and 12 months after the transplant.

Unlike other authors, we could not observe significant differences in the kinetic parameters analysed for the SNP of the CYP3A4 and CYP3A5 genes, probably due to insufficient statistical power due to the low allele frequency of the SNP analysed and our study’s limited sample size.

The determining factors for the different results observed were the following: genotypic variability associated to the race/ethnic group of the chosen populations, the type of transplant, the variables chosen for the pharmacokinetic analysis as well as the point in time following surgery in which the study was carried out. These would justify, in part, some of the discrepancies in the results of the studies published to date. For example, with regard to the distribution of the frequencies of the MDR1 3435C > T alleles, great differences according to the race of the population studied have been observed.17 Thus, in African-American population the C allele is much more predominant than in Caucasian population, which implies that the majority of people of African-American origin present a CC genotype, associated to a greater expression of P-gp. However, there is a low frequency of the C allele in Asians. Therefore, these variations have an influence on the studies results on the bioavailability of many drugs, including the CsA. We consider that it is important to carry out a study on the pharmacogenetic factors which may influence the kinetic of CsA absorption based on the genotype frequencies of said SNP in the Spanish population.18

To conclude, our results are in accordance with the data published by certain authors in respect of the MDR1 polymorphism 3435C > T because the genotype differences of said SNP may explain part of the interindividual variability in the absorption of CsA observed in the population in our context.

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References

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