Relationship between CES2 genetic variations and rifampicin metabolism

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Objectives: Rifampicin is known to be deacetylated in vivo, resulting in its metabolite 25-desacetyl rifampicin, but the enzyme metabolizing rifampicin and the association of this process with any genetic variation have not yet been elucidated. In this study, genetic variations of a surrogate enzyme, carboxylesterase 2 (CES2), and their association with the metabolism of this drug, were investigated.

Methods: Plasma concentrations of rifampicin and 25-desacetyl rifampicin were measured in 35 patients with tuberculosis receiving a first-line antituberculosis treatment. Direct PCR-based sequencing of the CES2 gene, covering all 12 exons, the 5′-untranslated region (UTR), the 3′-UTR and intronic and promoter regions, was performed. A dual luciferase reporter assay was carried out to assess whether variations in the promoter region affected the transcription of this gene.

Results: Ten variations were detected, of which two were in the candidate promoter region, five in introns and three in the 3′-UTR. One of the variations in the 3′-UTR was a novel variation. Genotypes at three closely linked variations (c.-2263A>G, c.269-965A>G and c.1612+136G>A) and c.1872*302_304delGAA were associated with significantly different plasma rifampicin concentrations. The mean plasma rifampicin concentration significantly increased with the number of risk alleles at the three closely linked variations, while the plasma concentration decreased along with an increase in the number of risk alleles at c.1872*302_304delGAA. When HepG2 cells were transfected with a luciferase reporter construct bearing the c.-2263G allele, luciferase activities were consistently decreased (by 5%–10%) compared with those harbouring the c.-2263A sequence.

Conclusions: Variations in CES2, especially c.-2263A>G in the promoter region, may alter rifampicin metabolism by affecting expression of the gene.

Keywords: tuberculosis, promoters, carboxylesterase 2, pharmacogenetics

Introduction

The dosage and the duration of tuberculosis treatment with the antituberculosis drugs are well established and widely followed. However, modification of the treatment protocol may be inevitable in cases of delayed or minimal response, resistance to the drugs, risk of drug interaction, changes in drug metabolism or changes in drug level in patients with conditions such as diabetes, HIV infection, renal failure or liver dysfunction.1,2 In such cases, a pharmacogenetic approach is promising to enhance treatment efficacy and to lower the incidence of tuberculosis and the emergence of drug-resistant organisms.3 There are some antituberculosis drugs whose metabolism is correlated with genetic variations, e.g. isoniazid with the NAT2 gene and rifampicin with the SLCO1B1 gene.4,5

Rifampicin is known to metabolize mainly to 25-desacetyl rifampicin via deacetylation by B-esterase or another kind of esterase in liver microsomes.6 Human carboxylesterase (CES), acetylcholinesterase and butyrylcholinesterase belong to the B-esterase family. Among these enzymes, CES2 is the most important enzyme and has the broadest substrate specificity. Furthermore, the structure of rifampicin is similar to that of
CPT-11, which is the representative prodrug that is metabolized by CES. For these reasons, we hypothesized that CES2 could be the enzyme responsible for the metabolism of rifampicin. CES2 is a member of the serine esterase family and metabolizes esters and some other functional groups. Several variations in the CES2 gene have been reported, including non-synonymous variations and variations in non-coding regions. A number of studies have demonstrated contradicting associations between variations in CES2 and the metabolism of drugs such as irinotecan, capecitabine, mycophenolic acids and isoniazid. However, there have been no reports on the relationship between variations in CES2 and the metabolism of rifampicin.

In this study, we investigated genetic variations in CES2 in the Korean population and their relationships with plasma rifampicin levels and the metabolism of this drug.

Methods

This study was approved by the Institutional Review Board of the Seoul National University Bundang Hospital and written informed consent was obtained from all subjects. EDTA samples were collected from 35 patients with tuberculosis at 2 h after the oral administration of rifampicin after overnight fasting to estimate the peak concentration. For analysis of the frequency of genetic variations in CES2 in the general population, 100 healthy persons were enrolled.

Rifampicin and 25-desacetyl rifampicin were measured using ultra-performance liquid chromatography-tandem mass spectrometry, as described in our previous study. For analysis of the frequency of genetic variations in CES2 in the general population, 100 healthy persons were enrolled.

For amplification and sequencing of CES2, GenBank accession number AY851164 was used as a reference sequence. Primers for PCR were designed to include the entire 12 exons, the 5'-untranslated region (UTR), the 3'-UTR and intronic and promoter regions. The primer sequences and their predicted product sizes are listed in Table S1 (available as Supplementary data at JAC Online). For sequencing of the PCR product, additional primers were designed to cover sections of the product that were as long as possible. The primer sequences for the sequencing reaction are listed in Table S2 (available as Supplementary data at JAC Online).

SNaPshot (Applied Biosystems, Foster City, CA, USA) analysis was performed for 10 variations from five regions with genomic DNA from 100 normal controls. The nine single nucleotide polymorphisms (SNPs) were analysed using two sets of SNaPshot reactions, while a small deletion (c.1872+302_304delGAA) was analysed by sequencing. The target SNPs, PCR primers and SNaPshot primers, and the sizes of SNaPshot primers, are listed in Table S3 (available as Supplementary data at JAC Online).

A dual luciferase reporter assay was performed following a previously described method with minor modifications. Briefly, the promoter region of wild-type CES2 (c.3000 to c.-984 of the CES2 gene) was synthesized by RT-PCR amplification of RNA isolated from human blood, followed by cloning into the luciferase reporter vector pcDNA3-Based (Promega, Madison, WI, USA). CES2 promoter constructs encoding sequence variations revealed in this study were generated by site-directed mutagenesis. The variations tested were as follows: CA, CG, TA and TG (the first nucleotide at c.2548a and the second nucleotide at c.2263 of CES2). These firefly luciferase constructs were transfected into HepG2 cells. A luciferase assay was performed with a dual-luciferase reporter assay system (Promega). The experiments were performed three times in duplicate. The mean luciferase activities, normalized for cell transfection efficiencies, were calculated relative to the activity of the common allele type construct (common allele, set as 100).

The mean differences in drug concentration between different nucleotide variants were analysed by analysis of variance after log transformation. The normality of the log-transformed data was evaluated using the Shapiro–Wilk test. Paired t-tests were performed to compare the luciferase reporter activities between the transfected cell lines of different alleles.

Results

Variations in CES2

We found 10 different variations in the CES2 regions sequenced (Table 1). Two were in the promoter region, five in introns and three in the 3'-UTR. None was found in the exonic regions. Of these variants, three SNPs (c.-2263A>G, c.269-965A>G and c.1612+136G>A) were found to be closely linked, to the extent that these three SNPs showed identical allelic distributions. The frequencies of the variations were not significantly different from those reported from other studies, except for the frequency of the alleles of c.1872+302_304delGAA, which was 0.67 in our study, but 0.06 in dbSNP hosted by the National Center for Biotechnology Information (NCBI). The variation c.1872+445C>T has not been reported previously and had a low frequency of variation. The frequencies of the 10 variations in CES2 in 100 healthy persons were very close to those in the tuberculosis patients, except in the case of c.1872+302_304delGAA, where the frequency was 0.35 in healthy persons, but 0.67 in tuberculosis patients. The variation at position c.1872+445 was not found in the healthy persons.

Association of CES2 variations with plasma rifampicin concentrations and the ratio of 25-desacetyl rifampicin

When the plasma concentrations of rifampicin in the 35 patients with tuberculosis were analysed, some associations with CES2 variations were found. Variations at c.2263 (including c.269-965 and c.1612+136), c.615+120, c.1872+69 and c.1872+302_304 showed trends of a single direction of change in the mean plasma rifampicin concentration as the number of risk alleles increased (Table 1). The 25-desacetyl rifampicin to rifampicin ratio was significantly higher in individuals heterozygous at c.1872+445 than in individuals homozygous for the major allele (0.379±0.439 versus 0.037±0.215, P<0.001). No significant differences were found in this ratio for the other nine loci. There were eight patients with diabetes or liver dysfunction. The plasma concentrations of rifampicin were not significantly different in the patients with diabetes or liver dysfunction. The dose per weight was also not significantly correlated with the plasma rifampicin concentrations.

Promoter assay of CES2

As c.-2263A>G in the promoter region resulted in a significant increase in plasma rifampicin concentration in tuberculosis patients, luciferase reporter analysis was performed to test whether c.-2263A>G affected transcription of the CES2 gene. Interestingly, the luciferase activities of CG and TG constructs were consistently decreased compared with those of CA and TA constructs, respectively (by 5%–10%), even though these decreases were not statistically significant, probably due to the
Intron 10 c.1613-87G
Intron 10 c.1612
Intron 3 c.615
Intron 2 c.474-152T
Intron 1 c.269-965A
Location

3

3

3

3

3

Intron 10 c.1613-87G
Intron 10 c.1612
Intron 3 c.615
Intron 2 c.474-152T
Intron 1 c.269-965A

Intron 2 c.474-152T
C
G
T
A

Promoter

Intron 10 c.1613-87G

Promoter
c.2263A>T

Intron 1 c.269-965A

Intron 2 c.474-152T

Intron 3 c.615+120G

Intron 10 c.1612+136G

Intron 10 c.1613-87G

3’UTR
c.187269A>G

3’UTR
c.1872302,304delGAA

3’UTR
c.1872445C>T

Promoter
c.2263A>T

Intron 1 c.269-965A

Intron 2 c.474-152T

Intron 3 c.615+120G

Intron 10 c.1612+136G

Intron 10 c.1613-87G

3’UTR
c.187269A>G

3’UTR
c.1872302,304delGAA

3’UTR
c.1872445C>T

Table 1. Frequencies of CES2 variations and the corresponding plasma rifampicin concentrations

<table>
<thead>
<tr>
<th>Location</th>
<th>Polymorphismsa</th>
<th>Allele frequencies</th>
<th>Rifampicin concentrations (mg/L)b</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>nomenclature 1</td>
<td>nomenclature 2</td>
<td>patients</td>
</tr>
<tr>
<td>Promoter</td>
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<td>g.453C&gt;T</td>
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<tr>
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<td>g.738A&gt;G</td>
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<tr>
<td>Intron 1</td>
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<td>g.4629A&gt;G</td>
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<tr>
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<td>g.6622T&gt;C</td>
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<tr>
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<tr>
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<tr>
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</tr>
<tr>
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<td>g.11884,11886delGAA</td>
<td>0.67</td>
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<tr>
<td>3’UTR</td>
<td>c.1872445C&gt;T</td>
<td>g.12027C&gt;T</td>
<td>0.03</td>
</tr>
</tbody>
</table>

aSequence variations in nomenclature 1 are based on the coding DNA reference sequence and those in nomenclature 2 are based on the genomic reference sequence (GenBank accession number AY851164).
bPlasma rifampicin concentration was measured in samples taken 2 h after oral administration of rifampicin in tuberculosis patients. Data are presented as mean±SD values and the number of patients in each group is given in parentheses. P values were calculated by analysis of variance test after natural log transformation of the rifampicin concentration.
cMajor, homozygotes carrying major allele; hetero, heterozygotes; minor, homozygotes carrying minor allele.

These variations, three closely linked variations, c.2263A>G, c.269-965A>G and c.1612+136G>A, may alter the metabolism of rifampicin by affecting the efficiency of transcription of the gene. To the best of our knowledge, this is the first report to reveal a relationship between CES2 polymorphisms and the metabolism of rifampicin.

Unlike previous studies on CES2 variations,7,8,17 we did not detect any non-synonymous or splice site variations. This was quite unexpected, as the previous studies focused on a Japanese population, who are ethnically similar to Koreans. The allele frequencies of all variations identified in this study were similar to those reported in NCBI and previous reports, except for that of the 3 bp deletion.

There was a clear trend for increased or decreased plasma rifampicin concentration, depending on the number of risk alleles of the six variations present. This suggests that the variants may be associated with each other and may affect the metabolism of this drug in any way. The result of promoter analysis suggests that, of the two variant loci within the promoter, c.2263A>G affects the transcription of the enzyme. The change from A to G was associated with a consistent decrease in luciferase activity, which may result in decreased metabolism of the drug and increased plasma concentration. The inter-individual variability of rifampicin concentration related to the genetic variations in the SLCO1B1 gene5 or the CES2 gene suggests that higher dosages of rifampicin may overcome such problems.

The limitations of this study include the small number of patients sampled and the low statistical power of the results of the promoter analysis. Additionally, the functional consequences of the variations other than c.2263A>G have not been studied. Further study is required to apply the results to patient care.

In conclusion, variations in CES2 were associated with changes in plasma rifampicin concentration, and variation in the promoter region, at c.2263A>G, may decrease the metabolism of rifampicin.

Discussion

In this study, we identified 10 variations in CES2 in 35 tuberculosis patients who had been treated with a first-line antituberculosis regimen. Plasma rifampicin concentration increased or decreased as the number of risk alleles of six variations increased. Among small number of experiments (P=0.092 for CA versus CG and P=0.059 for TA versus TG; Figure 1).

Figure 1. Dual luciferase reporter analysis of two variants in the promoter region of CES2. The heights of the filled bars represent the mean luciferase activities and lines indicate standard deviations. P values were obtained by paired t-tests.
rifampicin, resulting in increased plasma rifampicin concentration, by affecting expression of the gene.

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Transparency declarations
None to declare.

Supplementary data
Tables S1, S2 and S3 are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References
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