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- ADDENDUM -

THE IMPACT OF PROMOTER POLYMORPHISM ON HEME OXYGENASE-1 EXPRESSION IN HUMAN LIVER TRANSPLANTS

Submitted
INTRODUCTION

In a clinical study, recently published in the American Journal of Transplantation (Am. J. Transplant. 2005; 5: 1875-1885), we have shown that the level of heme oxygenase-1 (HO-1) expression in human livers before orthotopic liver transplantation (OLT) is associated with outcome after transplantation \(^{(1)}\). Liver grafts with an initial low HO-1 mRNA expression (defined as <3.4 times controls) suffered significantly less ischemia/reperfusion injury and had better hepatobiliary function after transplantation than grafts with an initial high HO-1 expression (>3.4 times controls). In this previous study, we have attempted to identify risk factors that could explain the observed variation in HO-1 expression among different grafts. A large number of donor characteristics and procurement-related variables were compared between the two groups. Unfortunately, we were unable to identify any variable that could discriminate the low HO-1 expression group from the high expression group. Moreover, there were no differences in the expression of the stress protein heat shock protein 70 (HSP 70), indicating that the observed differences were also not likely explained by the presence of more compromised or pre-injured livers in the group with high HO-1 expression. In our previous study we have also explored the possibility that the variation in initial HO-1 expression could be explained by a well described repeat polymorphism in the promoter region of the HO-1 gene. This repeat polymorphism is characterized by different lengths of \((GT)_n\) dinucleotide repeat and was previously shown to be associated with outcome after kidney transplantation \(^{(2,3)}\). Although a short \((GT)_n\) repeat (<25) has been suggested to result in enhanced inducibility of the HO-1 gene, we did not find a higher number of short alleles in the initial high HO-1 mRNA group, leaving the question what discriminates liver grafts with low HO-1 expression from those with high HO-1 expression unanswered.

Recently, Ono et al. \(^{(4,5)}\) have described another polymorphism of the HO-1 promoter, which was shown to have an effect on gene transcription rate. This variation is characterized by a single nucleotide polymorphism (SNP), \(A(-413)T\). By using a luciferase reporter assay in in vitro studies, these investigators have shown that \(A(-413)\)-alleles result in a 6-fold higher HO-1 promoter activity, compared to \(T(-413)\)-alleles. Although this SNP was suggested to be dominant over the \((GT)_n\) polymorphism, it has been much less studied in patient populations. We, therefore, analyzed the \(A(-413)T\) SNP in the promoter region of the HO-1 gene in our donor liver samples. \(A/A\)-alleles were present in 36% of the donors, 50% of the donors were heterozygous \((A/T)\) and 14% of the donors were non-carriers of the \(A(-413)\)-allele \((T/T)\) (Table 1). In contrast to the \((GT)_n\) repeat poly-
morphism, we found a significant difference in the distribution of livers with at least one A(-413)-allele (A/A and A/T) and livers without an A(-413)-allele (T/T) in the groups with low or high HO-1 expression. None of the liver grafts that did not carry an A(-413)-allele (T/T group) exhibited a high HO-1 mRNA expression. Apparently, the A(-413)T SNP is the only variable that discriminates livers with initial high HO-1 mRNA expression from the ones with low HO-1 expression. Other investigators have shown HO-1 to be upregulated in kidneys from brain-dead donors\(^\text{6,7}\). Although the exact mechanisms underlying this increased HO-1 expression are yet unidentified, our data suggest that the degree of upregulation in donor livers may be affected by the A(-413)T SNP, but not by the (GT)\(_n\) polymorphism. The different impact of the two types of HO-1 polymorphism could possibly be explained by different binding sites of transcription factors responsible for the upregulation of HO-1 under the condition of brain-death.

Based on these interesting new findings, we conclude that the large variation in HO-1 expression in livers from an otherwise indiscernible group of brain-dead donors is, at least partly, explained by the A(-413)T SNP in the HO-1 gene. The T/T variant of the A(-413)T SNP in the HO-1 promoter is significantly associated with a low induction of HO-1. These results are the first to show an association between the HO-1 A(-413)T SNP and levels of HO-1 mRNA in human tissue. More large scale, clinical studies, with adequate statistical power, will be needed to define the impact of this HO-1 polymorphism on clinical outcome after OLT.

### Table 1. Distribution of HO-1 genotype in the livers with initial low or high HO-1 mRNA levels.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Initial HO-1 expression</th>
<th>(n=19)</th>
<th>High (n=18)*</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(GT)(_n) repeat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Short allele (SS or SL)</td>
<td></td>
<td>8 (50%)</td>
<td>8 (50%)</td>
<td></td>
</tr>
<tr>
<td>Long allele (LL)</td>
<td></td>
<td>11 (52%)</td>
<td>10 (48%)</td>
<td>1,000</td>
</tr>
<tr>
<td>A(-413)T SNP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A-carriers (A/A or A/T)</td>
<td></td>
<td>14 (44%)</td>
<td>18 (56%)</td>
<td></td>
</tr>
<tr>
<td>Non-A carriers (T/T)</td>
<td></td>
<td>5 (100%)</td>
<td>0 (0%)</td>
<td>0,046</td>
</tr>
</tbody>
</table>

*) Genomic DNA for SNP analysis was not available in one donor.

FIGURE 5. Distribution of HO-1 genotype in the livers with initial low or high HO-1 mRNA levels.
REFERENCES


