INTRODUCTION

• Baxalta/Shire is marketing a human recombinant von Willebrand factor (rVWF) product to treat von Willebrand disease.

• As patients include women of reproductive potential, per ICH S6(R1) guidance, product development would require an embryo fetal developmental study (i.e. Segment II teratology) in rats and a combined embryo-fetal and peri-postnatal developmental study (ePPND) in rabbits. However, development of neutralizing antibodies is an expected immune response after repeated application of heterologous proteins and is not predictive of the situation in humans. The company therefore sought an alternative to animal testing in line with the principles of the 3Rs (Reduction, Refinement and Replacement).

OBJECTIVE

To assess the human fetal risk of rVWF in an ex vivo human placental transfer study

METHODS

This study was conducted at the Placenta Laboratory of the University Hospital Jena, Germany. Ex vivo perfusion experiments were performed using a closed system, each with one isolated cotyledon from one placenta for both maternal and fetal circuits according to Schneider et al.1

• All placenta (21 valid perfusions) derived from normal pregnancies at regular term. Ischemia time between delivery and installation in the perfusion equipment was max. 30 min, followed by 30 min re-oxygenation. Buffer (3 control cotyledons/group) or rVWF was added to the perfusion medium (maternal circuit) resulting in concentrations of approximately 1, 5, or 10 U VWF/mL (5 treated cotyledons/group). Perfusion was performed for 120 min.

• A schematic of the perfusion equipment is shown in Figure 1. The perfusion solutions were gassed by gas exchange oxygenators with 95% O2 / 5% CO2 in the maternal circuit and 95% N2 / 5% CO2 in the fetal circuit, with a flow rate of 12 mL/min and 3 (±0.3) mL/min, respectively.

• To control the membrane transfer, antipyrine (80 µg/mL), which shows complete time-dependent transfer through intact placenta, and creatinine (150 µg/mL), which shows slower transfer through intact placenta than antipyrine, were added to the maternal circuit.

• The following quality parameters were assessed throughout the experiment: perfusate volume, fetal circuit pressure, transfer of antipyrine and creatinine, ß-hCG release, and metabolic parameters. Assessment of a spectrum of quality parameters failed.

• Perfusates samples from the maternal and fetal circuit were collected throughout perfusion for detection of rVWF by VWF:Ag.

RESULTS

Antipyrine and creatinine transfer was regular, indicating that the placental barrier was intact and that a controlled physiological transfer from maternal to fetal circulation was possible.

Maternal Circuit

Results of the VWF-Ag measurement in control and treated cotyledons are shown in Table 1.

• As expected, for the control cotyledons the VWF-Ag concentration was low (< 0.034 U/mL) in all samples.

• In the group perfused with the lowest volume of r-VWF (mean of 0.46 U/mL), about 53% of the calculated concentration (0.86 U/mL) was recovered. At the end of perfusion (120 min), 50% (range: 37–77%) of the initially present rVWF was removed from the circuit.

• For the middle volume group, the measured VWF-Ag concentration was 14% lower than that applied to the circuit. At a mean concentration of 3.69 U/mL, 86% of the calculated concentration (4.29 U/mL) was recovered. At the end of perfusion (120 min), 15% (range: 1–25%) of the initially present rVWF was removed from the circuit.

• For the high volume group, the measured VWF-Ag concentration was 26% lower than that applied to the circuit. At a mean concentration of 6.31 U/mL, 74% of the calculated concentration (8.58 U/mL) was recovered. At the end of perfusion (120 min), 15% (range: 1–36%) of the initially present rVWF was removed from the circuit.

Fetal Circuit

• For all samples on the fetal side of the circuit, no VWF-Ag was detected at any time point.

In summary, the results of this study demonstrate that rVWF does not pass the human placenta.

Figure 1: Schematic assembling of the perfusion equipment

Table 1: Analysis of VWF-Ag in control and treated cotyledons

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Control cotyledons</th>
<th>Treated cotyledons</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.016</td>
<td>0.016</td>
</tr>
<tr>
<td>5</td>
<td>0.022</td>
<td>0.022</td>
</tr>
<tr>
<td>30</td>
<td>0.020</td>
<td>0.020</td>
</tr>
<tr>
<td>60</td>
<td>0.020</td>
<td>0.020</td>
</tr>
<tr>
<td>120</td>
<td>0.020</td>
<td>0.020</td>
</tr>
</tbody>
</table>

CONTROL

mean 0.016 0.022 0.020 0.020 0.020
range < 0.0016–0.018 < 0.0016–0.034 0.014–0.030 < 0.0016–0.034 0.014–0.026

TREATED

mean 0.46 0.27 0.28 0.25 0.23 50
range 0.38–0.58 0.18–0.32 0.19–0.35 0.15–0.37 0.10–0.37 23-63

mean 3.69 3.15 2.95 3.13 3.01 85
range 3.08–4.56 2.88–3.60 2.88–3.39 2.56–3.40 2.32–3.54 75-99

mean 0.46 0.27 0.28 0.25 0.23 50
range 0.38–0.58 0.18–0.32 0.19–0.35 0.15–0.37 0.10–0.37 23-63

mean 6.31 6.14 5.70 5.24 5.30 85
range 5.12–8.21 4.96–8.24 4.20–6.96 3.88–6.94 3.32–6.80 64-99

CONCLUSIONS

• In a human ex vivo placental transfer study, it was demonstrated that rVWF does not pass the human placenta

• The results indicate that the risk of direct harmful effects of rVWF on the fetus is low, obviating further in vivo studies

REFERENCE


DISCLOSURES

PL, UE, KE, TR and MT are employees of Shire Austria GmbH, Vienna. UE, JP, and KE were sponsored by Baxalta (now part of Shire) for conduct of the study.

1. Shire Austria GmbH, Vienna; 2. Placenta Laboratories, Jena University Hospital, Jena, Germany; 3. Medilys Laborgesellschaft, Hamburg, Germany