Introduction

Most patients with cancer now die from cardiovascular (CV) disease rather than from cancer itself and nearly half of patients with renal cell carcinoma experience CV side-effects from the gold-standard therapy—pazopanib (1). Pazopanib is a tyrosine kinase inhibitor (TKI) that inhibits vascular endothelial growth factor receptors (VEGFRs). The clinical use of pazopanib and other TKIs is strongly limited by their association with serious CV side effects. Although little is known about its mechanism, pazopanib causes serious CV effects in 50% of treated patients, including hypertension (HTN), heart failure (HF) and myocardial ischemia (2). It is imperative to explore the mechanisms by which this life-saving drug induces HTN and HF in order to develop strategies to mitigate CV insult and sustain cancer treatment for as long as possible.

We have created a structural heart disease model in mice which display early signs of HF similar to humans. These mice (cKO mice) lack cardiac II-spectrin, a cytoskeletal protein. These mice have arrhythmias, spontaneous Ca²⁺ release and abnormal expression/localization of cardiac membrane proteins. Our previous Western blot data was that fibroblast growth factor-2 transcript levels are reduced by >50% in cKO mice. Additionally, wild type (WT) mice with induced HF and humans with left or atrial fibration show downregulation of II-spectrin in the heart.

We hypothesize that the hypertensive effects of pazopanib are due to sustained activation of the renin-angiotensin-aldosterone system (RAAS), which may be mitigated by Lisinopril. Furthermore, we hypothesize that mortality will be increased in cKO mice due to baseline deficiency in angiogenic pathways.

Methods

Mice

8-Week-old black male WT mice were orally dosed with 30 mg/kg of pazopanib twice daily for 42 days. WT mice and cKO mice were orally dosed with 100 mg/kg of pazopanib and/or 20 mg/kg of Lisinopril once daily for 22 days; flox mice were used as controls.

Blood Pressure

The CODA system was used to gather non-invasive blood pressure readings once per week.

Electrophysiology

Ventricular cardiomyocytes were isolated from mice by Langendorff preparation and tested in vitro.

Mouse Measurements

Heart weight and tibia length were measured immediately after organ removal at the conclusion of the experiment.

Immunoblots

Mouse whole heart lysates were electrophoresed and tested with anti-VEGFR-2 antibody.

Results

Mean arterial blood pressure over 42-day treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>Week 3</th>
<th>Week 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>100</td>
<td>115</td>
<td>130</td>
</tr>
<tr>
<td>cKO</td>
<td>110</td>
<td>125</td>
<td>140</td>
</tr>
</tbody>
</table>

Mean arterial blood pressure over 22-day treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Post Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>100</td>
<td>110</td>
<td>120</td>
<td>130</td>
<td>140</td>
</tr>
<tr>
<td>cKO</td>
<td>110</td>
<td>120</td>
<td>130</td>
<td>140</td>
<td>150</td>
</tr>
<tr>
<td>WT (Lisinopril)</td>
<td>100</td>
<td>110</td>
<td>120</td>
<td>130</td>
<td>140</td>
</tr>
<tr>
<td>cKO (Lisinopril)</td>
<td>110</td>
<td>120</td>
<td>130</td>
<td>140</td>
<td>150</td>
</tr>
</tbody>
</table>

Discussion

These results support the involvement of the RAAS pathway in the hypertensive effects of pazopanib. We plan to repeat this experiment in order to obtain sufficient n values for power. Echocardiography and electroanatomical mapping will be used to assess structural changes in the myocardium as a result of pazopanib treatment. Electrophysiology studies will be performed on cardiomyocytes from mice after 22 days of treatment. Western blotting and microarray analysis will be used to determine which genes are affected by pazopanib treatment.

Our overall goal is to confirm the involvement of the RAAS pathway in the hypertensive effects of pazopanib and the efficacy of Lisinopril co-treatment. Furthermore, we hope to determine whether pazopanib is directly toxic to cardiomyocytes or the vascular endothelium, and whether the cardioprotective properties of Lisinopril are dependent upon its hypertensive effect.

With regards to II-spectrin, we hope to demonstrate the role of this protein in angiogenic and growth factor pathways, and whether this protein has efficacy as a HF biomarker. Urine and blood samples from mice treated with pazopanib will be analyzed for II-spectrin protein. Urine samples from mice treated with pazopanib will be analyzed for II-spectrin protein. Urine samples from mice treated with pazopanib will be analyzed for II-spectrin protein.

References