Cardiotoxicity of Tyrosine Kinase Inhibitors in Renal Cell Carcinoma.

Introduction

Renal cell carcinoma (RCC) is the 6th most common cancer in the U.S. and nearly half of these patients develop cardiovascular (CV) side-effects from the gold-standard therapy—tyrosine kinase inhibitors (TKIs)1. Most patients with cancer now die from CV disease rather than from cancer itself. Hence it is imperative to explore the mechanisms by which these life-saving drugs induce heart failure (HF) in order to develop strategies to mitigate these effects and sustain cancer treatment for as long as possible.

Pazopanib is a TKI that inhibits vascular endothelial growth factor receptors (VEGFRs) and it is approved by the FDA for the treatment of various cancers including RCC. The clinical use of pazopanib and other TKIs is strongly limited by their association with serious CV side effects. Although little is known about the mechanism of TKI-induced cardiotoxicity, this drug causes serious adverse effects in 50% of treated patients, including HTN, HF and myocardial ischemia1,2. We are currently using the murine model to study the effects of pazopanib in vivo.

We have created a structural heart disease model in mice which display early signs of HF similar to humans. These mice (Biss-KO mice) lack cardiac β-1-spectrin, a cytoskeletal protein that is important for the structural integrity of the cell. Our previous work has shown that these mice have arrhythmias, spontaneous Ca2+ release and abnormal expression/localization of cardiac membrane proteins1. Wild type (WT) mice with induced HF show downregulation of β-1-spectrin accompanied by breakdown peptides which may serve as early blood and urine markers for HF.

Methods

Mice

8 Week old black male WT mice were orally dosed with 30 mg/kg of pazopanib twice daily for 43 days. Transgenic mice (Biss-KO) mice which lack cardiac β-1-spectrin were observed at baseline. Mouse Measurements

Organ weights and libra length from mice were measured immediately after termination of the experiment. Echocardiography (Echo)

The Small Animal Imaging Core’s Vevo2100 echo machine was used to obtain images once per week. Surface Electrocardiograms (ECG)

Surface ECG’s were obtained at each week. Immunoblots

Mouse whole heart tissue was electrophoresed and tested for several antibodies of interest. Blood Pressure

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

Ventricular cardiomyocytes were isolated from mice and used for electrophysiology testing in vitro.

Results

Mean arterial blood pressure over course of treatment

<table>
<thead>
<tr>
<th></th>
<th>APD(50) +/- SE</th>
<th>APD(75) +/- SE</th>
<th>APD(90) +/- SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 WT</td>
<td>2.08 +/- 0.13</td>
<td>5.29 +/- 0.63</td>
<td>12.20 +/- 2.22</td>
</tr>
<tr>
<td>0.5 DRUG</td>
<td>3.33 +/- 0.60</td>
<td>8.26 +/- 1.32</td>
<td>16.14 +/- 2.12</td>
</tr>
<tr>
<td>1.0 WT</td>
<td>1.92 +/- 0.15</td>
<td>4.77 +/- 0.58</td>
<td>10.11 +/- 1.31</td>
</tr>
<tr>
<td>1.0 DRUG</td>
<td>3.13 +/- 0.58</td>
<td>7.74 +/- 1.27</td>
<td>15.20 +/- 3.96</td>
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Protein expression at conclusion of treatment

Figure 1. Mean arterial pressure over time of control group (black) compared to mice treated with pazopanib (red). A two-way ANOVA showed a significant increase (p<0.05) in mean arterial pressure due to drug treatment but not due to time (n=7). * indicates statistical significance. Error bars represent ± standard error of mean.

Electrophysiology results at conclusion of treatment

Figure 2. Pazopanib treatment causes precursors to arrhythmias. Average action potential duration (APD) 50, 75 and 90 of myocytes from treated mice (red) was significantly elevated compared to control myocytes (black). 25 myocytes from treated mice developed delayed afterdepolarizations (DADs) while these were not observed in 5 myocytes from control mice (n=2).

Discussion

These results in mice resemble the hypertensive CV effects of TKIs seen in humans and warrant further investigation. We plan to repeat this experiment using various susceptible mouse populations in order to observe the advanced HF phenotype. Transverse aortic constriction (TAC) will be used to induce HF. TAC mice and β-1-spectrin knockout mice will be treated with pazopanib under the same dosing conditions used in this experiment. A portion of these mice will be treated with a renin-angiotensin-aldoestosterone antagonist (RAAA). Mass spectrometry will be used to identify β-1-spectrin breakdown peptides in blood and urine from mice treated with pazopanib. This experimental design will allow us to:

- Unravel the cardiotoxic molecular mechanism of TkIs within the cell
- Assess the role of β-1-spectrin in sustained HTN
- Observe the downstream effects of TKI treatment on β-1-spectrin
- Test a potential new biomarker for early signs of TKI-induced cardiotoxicity

References