The Adverse Cardiovascular Effects of Bruton’s Tyrosine Kinase Inhibitors on the Mouse Model

Dev Patel

Abstract. Introduction: Bruton’s tyrosine kinase (BTK) inhibitors have revolutionized the treatment of several hematologic malignancies. However, treatment of cancers such as chronic lymphocytic leukemia (CLL) with BTK inhibitors has produced many adverse cardiovascular side effects, such as atrial fibrillation (AF), myocardial fibrosis, and left atrial enlargement (Burger et al., 2015).

Methods: In this study, a mouse model was used to determine if the adverse cardiovascular effects caused by BTK inhibitors resolved 30 days after discontinuation of treatment. 10 mice were given the BTK inhibitor Ibrutinib and another 10 mice were given a control treatment of dimethyl sulfoxide (DMSO). Echocardiograms, electrophysiology studies, and histology analyses were utilized to determine if these adverse effects were resolved.

Results: A significant difference (p < 0.05) post-recovery between the Ibrutinib mice and the control mice was found in Atrial Fibrillation burden. It was also found that a significant difference remained (p < 0.05) in left atrium area between the Ibrutinib and control mice. Finally, myocardial scarring also did not resolve post-recovery.

Conclusion: There was a decrease in the magnitude of adverse cardiovascular effects after recovery. A significant difference in heart inducibility, myocardial fibrosis, and left atrial enlargement between the control and experimental groups still remained. This study will help physicians and pharmaceutical companies understand the irreversible cardiovascular side effects of BTK inhibitors. Future studies are needed to determine the metabolic pathway by which BTK inhibitors cause these cardiovascular sequelae. This information will better equip pharmaceutical companies for the development of newer generations of BTK inhibitors.

I. Introduction

Recently, targeted therapies have served to help revolutionize cancer treatment options and prognoses. Unfortunately, these therapies can also potentially have severe cardiovascular complications in both patients who are still undergoing cancer treatments and those who are cancer survivors.

CLL is a type of cancer that occurs in the blood-forming cells of the bone marrow. Kaplan-Meier survival curves indicate that CLL has a high mortality rate. Previous treatments, such as monoclonal antibodies, have been less effective in decreasing mortality than desired (Fleming et al., 2021). Due to this, effective treatments for CLL are needed to improve outcomes for those diagnosed with this disease. A promising development in oncology has been the use of biological drugs that are classified as small molecule kinase inhibitors, which have been used to treat CLL (Manouchehri et al., 2020).

An increasingly effective treatment strategy for CLL involves using drugs that inhibit BTKs. BTKs are involved in cell survival, angiogenesis, and growth of cancer cells in patients diagnosed with CLL. Angiogenesis is the process by which new blood vessels are formed through the differentiation of endothelial cells. Therefore, the on-target inhibition of these kinases helps prevent the proliferation of cancer cells in CLL patients (Woyach et al., 2018). On-target effects are the events that take place due to inhibition of the desired target.
Ibrutinib is known to be a BTK inhibitor. As previously mentioned, on-target effects of BTK inhibitors such as Ibrutinib include the prevention of the growth and survival of B-cells. Unfortunately, adverse cardiovascular effects are induced due to the off-target impacts of the small molecule inhibitors (Moslehi, 2016). Off-target effects are sequelae that take place that differ from normal on-target sequelae. Previous studies have determined that one of the off-target receptors for BTK inhibitors is the C-terminal Src kinase (CSK). BTK inhibitors bind to CSK and inhibit the kinase from promoting cell differentiation and growth. CSK functions as a protein tyrosine kinase that phosphorylates members of the C-terminal Src kinase family (Schmedt et al., 1998). This phosphorylation of the Src family kinases renders them inactive. Therefore, the inhibition of CSK leads to hyperactivity of Src family kinases.

The hyperactivity of Src family kinases has various cardiovascular sequelae. Some of the cardiovascular effects of drug-induced CSK inhibition include left atrial enlargement, AF, and myocardial fibrosis. An increase in left atrial area is known to reflect the burden of diastolic dysfunction (Patel et al., 2009). Diastolic dysfunction occurs when the muscles of the heart are not able to relax due to abnormal stiffness. AF occurs when there is an irregularity in heart rhythm. During AF, there is a chaotic irregularity in the beating of the upper atrium chambers of the heart, causing the atria to be out of sync with the ventricles. This may cause a clot in the atria; the atrial clot can dislodge and potentially cause a stroke if it travels to the brain. According to the American Heart Association (2016), 15–20% of people who have strokes also have heart arrhythmia. These effects have detrimental impacts on the overall health of patients. Due to the gravity of these conditions, more information is needed about the cardiovascular effects resulting from Ibrutinib treatment in order to prevent them from occurring.

In this study, we examined if the cardiovascular effects caused by Ibrutinib treatment in the C57BL/6J mouse model resolved following recovery from treatment. Given the clinical observations that AF is associated with Ibrutinib treatment, we sought to generate a pre-clinical model where the effects of Ibrutinib on the heart could be studied in more depth. In this study, groups of mice were treated with either Ibrutinib or a DMSO control for 30 days. Recovery from treatment was allowed for an additional 30 days after conclusion of the treatments and experiments were performed to determine if the cardiovascular effects of CSK inhibition were resolved. It is hypothesized that the cardiovascular effects will not resolve.

II. Materials and Methods

A. Mice

All animal studies were approved by the Vanderbilt University Medical Center Institutional Animal Care and Use Committee. The studies were performed on 3-month-old male C57BL/6J wild-type mice.

B. In Vivo Interventions

Mice were treated with either 25 mg·kg⁻¹·d⁻¹ of Ibrutinib or DMSO control via intraperitoneal injections. There were 20 male mice in total: 10 in the experimental group and 10 in the control group. Baseline echocardiograms were performed on all 20 mice. Mice were then injected with either Ibrutinib or DMSO vehicle control daily for 30 days. After 30 days, echocardiograms and electrophysiology studies were performed on the mice. 10 of the mice (5 from each treatment group) were sacrificed immediately after 30 days of treatment. The heart, lungs, liver, and kidneys of the sacrificed mice were harvested and histology was performed. The five sacrificed mice that were treated with Ibrutinib served as the positive controls and the five mice treated with DMSO served as negative controls.

The remaining 10 mice recovered in the Division of Animal Care facility for 30 days without further intervention. Following this recovery period, echocardiograms were performed. Electrophysiology studies were also performed on these mice. The remaining 10 mice were then sacrificed after 30 days of recovery and histology studies were performed on the harvested tissues.

C. In Vivo Electrophysiology Study

Mice were anesthetized using 5% isoflurane. Surface echocardiograms were recorded using a transesophageal electrode. Arrhythmias were imposed by standard burst pacing protocol (Yao et
al., 2018). An irregular atrial beating that lasted at least one second was defined as AF. The burden was measured 60 days after the first injections.

D. Echocardiography

Echocardiography was performed on anesthetized mice using a cardiac ultrasound system. Left atrial area was measured using the parasternal long axis view (Figure 1).

![Figure 1: Parasternal long axis view of the heart](image)

III. Histology

The heart, lungs, liver, and kidneys were collected for histology. Masson trichrome stain and hematoxylin and eosin stain were performed. All images were taken via NanoZoomer 2.0-RAS or confocal microscopy. Masson’s trichrome allows for differentiation between muscle and collagen in samples; muscles are stained red, cytoplasm is stained pink, and collagen is stained blue. If there is an increased presence of blue staining, this indicates that fibrosis has occurred because the excess connective tissue that is characteristic of fibrosis is composed mainly of collagen. The National Institutes of Health image processing program ImageJ was used to analyze the tissue images.

Statistical analyses were conducted with GraphPad Prism 8.4.3 software. The statistical significance was assessed using the two-tailed Student’s t-test for normally distributed data. AF inducibilities were assessed by Fisher’s exact test. A p-value of 0.05 or less was considered to be statistically significant.

IV. Results

A. Atrial Fibrillation

One of the previously proven side effects of CSK inhibition is that there is greater AF inducibility and AF burden when measured according to the electrophysiological procedure listed in the Methods section (Figure 2). This is as expected, as it has been previously proven through CSK knockouts that inhibition of CSK activity leads to greater AF inducibility (Xiao et al., 2020). Following 30 days of recovery from treatment, there was still a significant difference (p < 0.05) between post-treatment AF inducibility and burden between control and Ibrutinib-treated mice (Figure 3).
B. Left Atrium Enlargement

An additional side effect of CSK inhibition is an increase in the size of the left atrium in mouse models (Xiao et al., 2020). The echocardiograms were used to measure left atrium area. Prior to treatment with the control or Ibrutinib, there was no significant difference (p>0.05) in left atrium size between mice treated with Ibrutinib and mice treated with the control (Figure 4). Following 30 days of treatment, there was a significant difference in left atrium area between mice treated with the control and mice treated with Ibrutinib (Figure 5). After 30 days of recovery was allowed, there was still a significant difference (p < 0.01) in left atrium area between mice treated with Ibrutinib and mice treated with the control (Figure 6).

Figure 2: AF burden was compared between the C57BL/6J mice treated with Ibrutinib and the C57BL/6J mice treated with the DMSO control upon conclusion of the 30 days of treatment. The total number of mice was 20, with 10 in each treatment group. The p-value was less than 0.001.

Figure 3: AF burden was compared between the C57BL/6J mice treated with Ibrutinib and the C57BL/6J mice treated with the DMSO control after 30 days of recovery from the treatment. The total number of mice was 10, with 5 being from each initial treatment group. The p-value was less than 0.05.
Figure 4: Comparison of left atrium area between the two mice groups before they were treated with Ibrutinib or the DMSO control. The total number of mice treated was 20, with 10 in each treatment group. The p-value was greater than 0.05.

Figure 5: Comparison of left atrium area between the two mouse groups directly after they were treated with Ibrutinib and DMSO control daily for 30 consecutive days. The total number of mice treated was 20, with 10 in each treatment group. The p-value was less than 0.01.

Figure 6: Comparison of left atrium area between the two mouse groups post-recovery 28 days from DMSO control vs Ibrutinib. The total number of mice treated was 10 with 5 in each treatment group. The p-value was less than 0.05.
V. Discussion

In this study, we examined whether the cardiovascular effects in the mouse model caused by Ibrutinib treatment resolved following recovery from treatment. This study will help determine whether the cardiovascular complications that arise from CSK inhibition are permanent. The hypothesis that the cardiovascular effects would not resolve was supported by the study.

There was a significant difference in AF inducibility (determined by electrophysiological studies) between the mice treated with DMSO and the mice treated with Ibrutinib post-treatment. This did not deviate from previous findings (Xiao et al., 2020). However, we now show that AF inducibility does not completely resolve after 30 days of recovery from treatment. There is still a significant difference between post-recovery. This demonstrates that the cardiovascular complication of AF is persistent and does not resolve when a recovery period of 30 days is allowed. The average AF burden for the group treated with Ibrutinib did decrease from post-treatment to post-recovery. However, the reduction in AF inducibility post-recovery was not statistically significant between the two treatment groups.

There was no significant difference during pre-treatment between the two groups of mice when comparing the area of the left atrium. This is as expected since the mice were all C57BL/6J mice that had not been injected yet with Ibrutinib or control. After 30 days of treatment, there was a significant difference in left atrium area between the mice treated with Ibrutinib and the mice treated with DMSO control (p < 0.01). Previous studies achieved similar results (Xiao et al., 2020). However, our data shows that a significant difference remains in the left atrium area between the mice treated with Ibrutinib and the mice treated with DMSO control (p < 0.05) after 30 days of recovery are allowed following 30 days of injections with the specified treatment. This further demonstrates that adverse cardiovascular effects of CSK inhibition persist even post-recovery.

Nevertheless, additional data is needed to truly understand the accuracy of the results. To increase the statistical power of the study, a greater number of mice should be used in each treatment group. Also, a longer recovery period should be used to see if an increase in recovery time post-treatment affects the outcome at all.

A future study that investigates whether myocardial fibrosis resolves post-treatment or not could be used to further support this study by further establishing that adverse cardiovascular events do not resolve after recovery is allowed from treatment with Ibrutinib. Additional studies can also be conducted to determine the downstream pathway by which CSK inhibition leads to AF. As shown in this report, the adverse cardiovascular effects arising from treating CLL through CSK inhibition do not resolve. Therefore, it is important to determine the exact downstream mechanisms by which this CSK inhibition occurs so pharmaceuticals developed in the future can reduce the probability of these adverse effects occurring.

VI. Acknowledgements

I would like to thank Dr. Javid Moslehi for allowing me to perform these experiments in his lab. I would also like to acknowledge Dr. Matthew Fleming, a Cardiology fellow in the Moslehi lab at the Vanderbilt University Medical Center, for his support throughout the semester.

Funding: Dr. Moslehi and this research is supported by National Institutes of Health grants (R01HL141466, R01HL155990, and R01HL156021).
References


