Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2023 Feb 11; 11(A):105-110.
https://doi.org/10.3889/oamjms.2023.8826
eISSN: 1857-9655
Category: *A - Basic Sciences*
Section: *Histology*

A Histologic Study of Imatinib Cardiotoxicity in Adult Male Rats

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Edited by: Branislav Filipović
Citation: Al-Allaf L, Alnuaeimy W. A Histologic Study of Imatinib Cardiotoxicity in Adult male Rats. Open Access Maced J Med Sci. 2023 Feb 11; 10(A):105-110. https://doi.org/10.3889/oamjms.2023.8826
Keywords: Cardiotoxicity; Imatinib; Rat; Histopathology
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Received: 01-Jan-2022
Revised: 28-Jan-2023
Accepted: 01-Feb-2022

Funding: This research did not receive any financial support
Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: Cardiotoxicity is an unanticipated adverse effect associated with some chemotherapeutic agents. There are conflicting results about imatinib-induced cardiac toxicity.

AIM: This study aims at investigating the possible cardiotoxic effects of imatinib in rat model through assessing the possible histopathological alterations that might develop.

MATERIALS AND METHODS: This is a case–control and experimental study conducted over a period of 3 months at laboratory of postgraduate studies, Department of Anatomy, College of Medicine, University of Mosul, Mosul, Northern Iraq. Sixteen adult male Albino rats were randomly assigned to either "control group" or "imatinib-treated group." The control group was gavaged with distilled water daily for 4 weeks while the second group was given oral imatinib (200 mg/kg/day) for the same duration. Animals were sacrificed by euthanization after 24 h of the last dose. Hearts were obtained and cardiac specimens were immersed in paraffin. Sections' staining by hematoxylin (Harris)-eosin (H&E) and Massons' Thrichrom.

RESULTS: Rats treated with imatinib showed decreased physical activity and food intake. Regular arrangements of myofilaments were noticed during light microscopic examination of cardiac sections of control rats. However, sections from imatinib group showed several histological alterations (mainly myofibrillar loss in myocardium with vacuolated cytoplasm). Necrosis of cardiac muscle fibers was also noticed in some sections. Appearance of deeply staining cells with pyknotic nucleus, in addition to shrinkage of cardiac muscle fibers, was also noticed in some sections. The mean score of cardiac injury in the treated group was 2.1 (vs. 0.6 in controls). Some sections of treated group showed an increment (mild) in collagen fibers in-between cardiac myocytes.

CONCLUSIONS: The observations concluded that imatinib has targeted action on cardiomyocytes. Oncologists should be cautious regarding imatinib dose and duration besides the close cardiac monitoring throughout and beyond therapy duration.

Introduction

The cytotoxic chemotherapy has reduced patients suffering and increased the survival rates in many malignant conditions. However, this kind of treatment is related with many organs dysfunction [1]. Throughout the evaluation of cancer chemotherapy, one of the important issue of consideration is cardiotoxicity, due to the suggestions that chemotherapy-induced myocardial damage may be permanent and even fatal [2]. Heart failure due to cytotoxic chemotherapy is a major challenge in clinical practice.

Imatinib mesylate is an effective cytotoxic drug of high selection that belongs to the tyrosine kinase inhibitors' class (TKIs) and may produce congestive heart failure [3]. The tyrosine kinase endeavor of the (BCR-ABL) fusion protein (which is the target of imatinib) results from the philadelphia chromosome translocation (known translocation) which is evident in chronic myelogenous leukemia (CML) [4]. The imatinib has an inhibitory action on the stem cell factor (SCF), the SCF-mediated cellular events, the platelet derived growth factor (PDGF), and the receptors for PDGF [5].

Cardiac toxicity – once developed – is usually an indication for discontinuation of the given chemotherapy while tumors are still sensitive to that treatment [2]. Treatment with imatinib therapy is, in fact, has a life-long use; however, after around three decades since introduction into clinical practice, side effects are being identified including congestive heart failure [6].

The possible imatinib-induced cardiotoxicity was investigated by researchers. It was unexpected according to the International Randomized Study of STI571versus Interferon [7]. Other investigators did not report such toxicity [8]. There is a noticeable discrepancy about such relation in different studies. The present work aims to use the rat as an imitation to scrutinize the imatinib's cardiotoxicity spotlight on the cardiac histopathological alterations that might develop due to this treatment.

Materials and Methods

After obtaining the ethical approval from the Committee of Medical Researches at college of Medicine, university of Mosul, adult male Albino rats were obtained from Animal House of Veterinary College, university of Mosul, Mosul, Northern Iraq to perform this experimental study. This study was conducted over a period of 3 months. Animals were housed in standard plastic cages (18 × 47 × 34 cm) lined with wood chips [9] under well-controlled environmental conditions and subjected to 12 h light/dark cycle. They were local breaded and let to accommodate about 1 week before any interventions [9], [10]. A standardized food and water *ad libitum* were used to feed them [11]. They received the humane care following the standard ethical rules of dealing with laboratory animals. The experiments were conducted during the light time [12].

Experimental protocol

Animals were divided into groups. The first group included eight young adult rats which served as the "control group." They were administered distilled water daily for 30 days. The second group included eight rats which were treated with oral imatinib (100 mg/capsule) at dose of 200 mg/kg/once/day for 4 weeks and they served as the "treated group." Using formula in rats, this dose is lying within the range of that given clinically in treatment protocols (400–800 mg/d) [10], [13]. Animals were randomly assigned to the experimental groups and were gavaged using the 24 gauge needle.

Firmly restraining of animals was done as they were grasped at the area of loose skin (back and neck), to prevent the head's movement, then animal was positioned the animal vertically. Careful passing of the gavage needle through the lateral part of mouth, followed the mouth's roof and advanced into the esophageal region and then the stomach. Once the needle was passed into the proper length and position, either imatinib or water was injected as assigned [14].

Signs of toxicity were carefully observed throughout the study period. Animals in each experiment group were euthanized with ether [13], [15] beyond the 24 h after giving the last dose and the heart of each animal was obtained for further investigations.

Histological analysis

Each heart obtained was dissected out, plotted dry on a filter paper, and kept in 10% neutral formaldehyde while preparing for processing before the histopathological assessments [2], [16]. The left ventricular cardiac specimens (wash-out) [17] were embedded in paraffin, stained with hematoxylin (Harris)-eosin (H&E), and Massons' Trichrom (MT) [17] and examined microscopically. The evaluation was performed in a blind way in respect to treatment model.

The histopathological observations were judged constructed on the existence and the harshness of the following points; edema, muscle necrosis, leukocytic infiltration, inflammation (chronic), and fibrosis [16]. Semi-quantitatively, marking for each constituent was made using scale as Grade 0 was normal and 1–4 represented a score of mild to marked alterations. For each heart, calculation of the integral cardiac injury outcome was done with taking the norm of all the constituents injury marks [2], [10], [16].

Results

The present work aims to investigate the possible imatinib-induced cardiotoxicity. Assessment was based mainly on the cardiac histopathological changes that might develop due to this treatment. However, the main general findings while observing animals receiving the drug throughout the experiment included decreased physical activity and poor food intake.

Organ (heart) assessment at necropsy

Macroscopic observations

No gross tissue abnormalities, at necropsy, were noted in the hearts of all rats enrolled in the study.

Microscopic observations

1. Using H&E Stain

This work revealed that cardiac sections of control rats by light microscopic evaluation showed regular fine arrangements of myofilaments of cardiac myocytes (Figure 1). On the contrary, cardiac tissues from rats gavaged with imatinib showed different histological changes including myofibrillar loss and

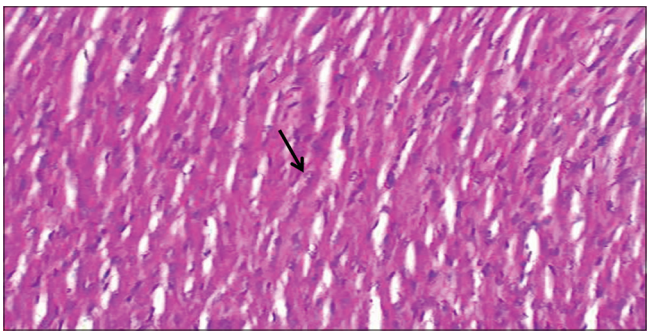

Figure 1: A section of cardiac wall from the control group in a microphotograph with myocardium. Oval centrally localized nuclei of cardiac myocytes is seen (arrow) (H&E ×400)

cytoplasmic vacuolization in the myocardial tissue (Figure 2).

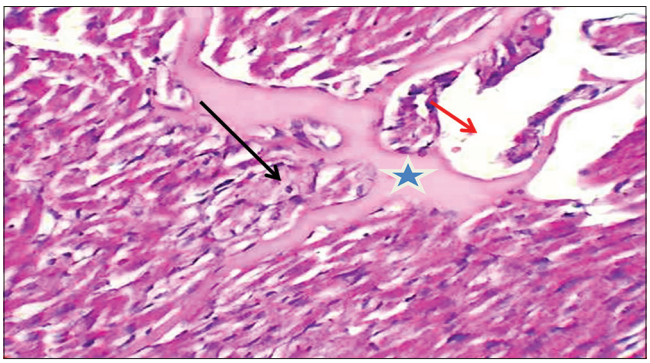

Figure 2: A microphotograph of a cardiac section from the imatinib-gavaged group. Cytoplasmic vacuolation (black arrow), widening in the intercellular space (red arrow), and intracellular edema (Star) are noticed (H&E, ×400)

Necrosis of cardiac muscle fibers (esinophilic cytoplasm with dark staining and karyolysis of nucleus) was also seen in some sections of imatinib-treated animals (Figure 3). Appearance of deeply staining cells with pyknotic nucleus and shrinkage of cardiac muscle fibers were also noticed in some sections of the treatment group (Figure 4) with sometimes hyalinization of cardiac muscle fibers (Figure 5).

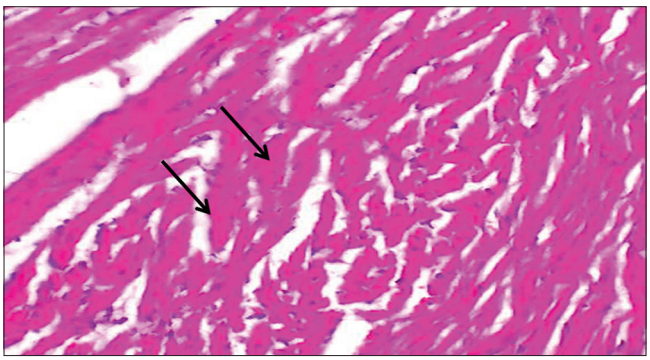

Figure 3: A microphotograph of a section from the cardiac wall of a rat-treated animal with the evidence of necrosis (karyolysis of nucleus and deeply eosinophilic cytoplasm) (arrow) (H&E ×400)

Comparison of total cardiac injury score between control animals and imatinib-treated ones is shown in Table 1.

2. Using (MT)

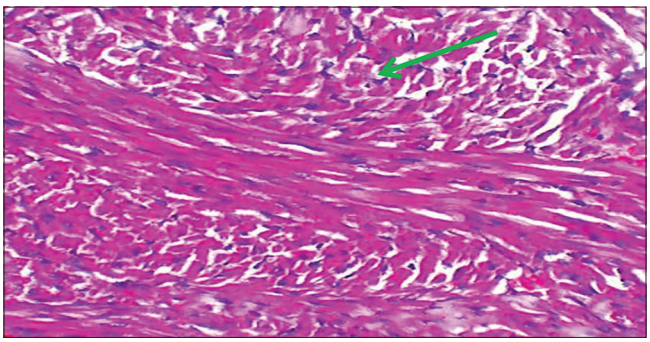

Figure 4: A microphotograph of a section in the cardiac wall of a rat treated with imatinib showing deeply stained cells with pyknotic nucleus (arrow) in addition to widening in the intercellular space and shrinkage of cardiac muscle fibers (H&E ×400)

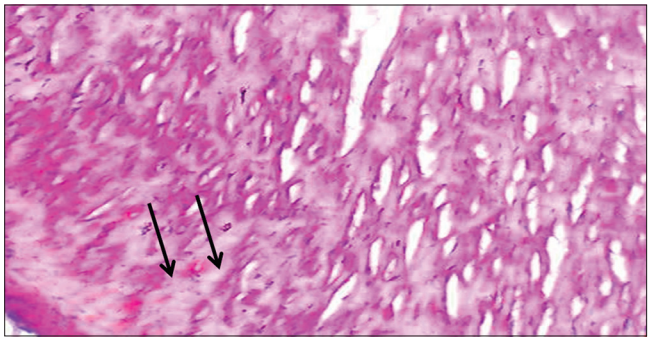

Figure 5: A microphotograph of a cardiac section of rat treated with imatinib showing hyalinization of cardiac muscle fibers (arrow) (H&E ×400)

Table 1: Comparison of total cardiac injury score between control animals and imatinib-treated ones

Group (n)	Histopathological score, mean ± SD
Control (8)	0.6 ± 0.2
Imatinib-treated (8)	2.1 ± 0.6

Data are expressed as mean ± SD. SD: Standard deviation.

This work revealed that sections from the treatment group showed mild increase in collagen fibers in-between the cardiac myocytes (Figure 6).

Discussion

The concept of using the molecularly targeted chemotherapeutic agents is based on the specific inhibition of those cellular molecules that has an association with tumor development and growth. These therapies shall be highly effective and theoretically devoid of harmful effects in regard of normal tissues [17]. Targeted anticancer drugs (as TKIs) are designed to be selectively working on molecules which are overexpressed in malignant cells but, however, a lot of these molecules are also biologically active in non-malignant and have crucial functions in the normal physiological phenomenon of many human body systems and enrolling the cardiovascular [18].

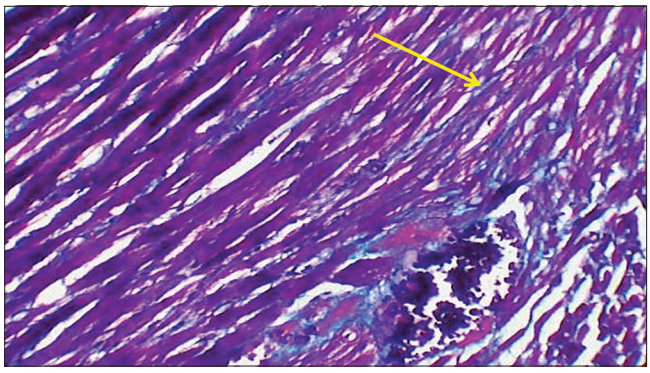

Figure 6: A microphotograph of a cardiac section of rat treated with imatinib. Collagen fibers (blue color) in-between cardiac myocytes (arrow) are few (MT ×400)

Imatinib, a first generation tyrosine kinase inhibitor was obtained the approval for the treatment

of CML cases during the chronic phase and beyond the failure of interferon therapy to control the blast crisis as well as the accelerated phase [19]. Several studies have revealed conflicting results about the imatinib-related cardiac effects although features of imatinib-induced cardiac toxicity were shown in many studies [2], [10], [20]. However, more investigations are needed to highlight its potential adverse effects on the heart.

Spectrum of histological changes in the hearts of rats exposed to imatinib including focal hemorrhage, interstitial edema, cytoplasmic vacuolation of myocytes, pyknosis, and necrosis of cardiac muscles fibers and even hylinization were discerned in this work. These findings are in accordance with those of other investigators [8], [20], [21].

Wolf *et al*. have reported more comprehensive features than those already reported such as myocyte necrosis, loss of myofibrils, and cytoplasmic vacuoles when animals (rats) were exposed to higher doses of imatinib (120 or 200 mg/kg) for 28 d [8]. Maharsy *et al*. have reported that 200 mg/kg of imatinib-induced loss of myocytes and fibrosis [3].

Researchers tried to explain the mechanisms of imatinib-induced cardiotoxicity. The observations of Kerkela *et al*. indicated that this drug inhibits the C-Abl of the cardiomyocytes and meanwhile activates the Junk pathway leading to cardiotoxicity [20].

The tyrosine kinase Abelson C-Ab1 is enmeshed in the response of cells to the DNA damage and the oxidative stress according to the reports of Mauro and Deininger. Imatinib inhibited C-Ab1 and PDGF depleting the endogenous antioxidant capacity and contributed to attenuating the activity of cardiac glutathion peroxidase [2], [22].

The formation of harmful and powerful reactive oxygen species (ROS) could be a pathogenic element in the development of imatinib-induced necrosis of heart muscles. However, Herman *et al*. showed no evidence of generation of ROS on exposing isolated cardiomyocytes to imatinib [10].

In addition, the imatinib-induced C-Abl inhibition in cardiac muscles promotes activation of the response to endoplasmic reticulum stress in addition to mitochondrial membranes collapse, reduction in total ATP contents, release of cytochrome C into cellular cytosol, and ultimately cell death [23]. Furthermore, the hypothesis of oxidative stress is further supported by the presence of vacuoles in the heart cells [3]. On the other hand, imatinib has potential alterations and theoretical impact on cell cycle progression by Cycline dc2 inhibition as suggested by workers [24].

Using the transmission electron microscope to examine the cardiac tissue obtained from mice treated with imatinib revealed some morphologic alterations in the sarcoplasmic reticulum beside abnormalities in mitochondria [20]. A study revealed that treatment with imatinib induced three kinds of changes in cardiac muscles: Necrosis, apoptosis, and autophagy. Imatinib treatment in animals has been also found to increase cardiac nitrotyrosine reactivity [10].

Moreover, there have been evidences of myocyte apoptosis, necrosis, and autophagy in failing human hearts on imatinib treatment [25]. Any or all of these processes could be incorporated in the heart failure's development among patients who were administered with imatinib or sunitinib [26], [27]. These findings may indicate that cardiac toxicity due to imatinib involves multiple pathways.

Morphologically, this study revealed that imatinib-induced alterations in myocyte which appeared to be somewhat similar to those noticed among hearts of animals treated with doxorubicin for long times [10] as the adverse effects of later drug have been studied more extensively [3].

Through humoral factors, there is a cross talking between non-myocytes and cardiac myocytes and, that play important roles in the development of cardiac growth. Consequently, impediment of PDGF receptors by imatinib may contribute to the neovascularization's inhibition and generating structural hypoxia and oxidative disfigurement [28], [29]. On the other hand, cardiac toxicity due to imatinib may be further promoted by other cofactors such as previous cardiovascular diseases or renal failure [30].

The present study revealed mild degree of fibrosis in sections of imatinib-treated rats using MT. This finding is consistent with those revealed by other workers who showed that although imatinib induces cardiotoxicity. There is only collagen fibers' increase (mild) among cardiac myocytes in differentiation with control group through Masson's trichrome staining [3], [31], [32]. They explained that on the basis that imatinib inhibits TGF-B (which promotes fibrogenesis) and thus reducing fibroblasts proliferation [30], [31], [32].

Finally, and in the contrary of other anticancer drugs, the reports on the consequence of imatinib on the cardiac tissue in experimental works are still limited and need further clarifications [3], [10].

Conclusions

Our study indicates that imatinib may affect cardiomyocytes adversely as indicated histologically. This is an alarm for oncologists to avoid any improper use of this drug mainly in terms of dose and duration of use in addition to cardiac monitoring throughout and beyond treatment period. Analysis of cardiac biomarker (as myoglobin, troponin, and creatinin kinase) in next works that related to the same topic is advocated.

Acknowledgments

Dr. Amjad Hazim Al-Naemi, Department of Biochemistry, College of Medicine, University of Mosul is sincerely thanked for his comments.

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