



Imatinib Cardiotoxicity in Hypertensive and Normotensive Rats.



Eugene Herman¹, Alan Knapton¹, Elliot Rosen¹, Quynh Anh Lu², Joel Estis², John Todd², Steven Lipshultz³, Jun Zhang¹

¹DAPR, CDER, FDA, Silver Spring MD 20993, ²Singulex Inc, Alameda, CA 94502, ³University of Miami School of Medicine, Miami FL 33136.

ABSTRACT

Imatinib (IMB) is a new generation chemotherapeutic agent that specifically targets tyrosine kinase pathway-dependent tumors. However, cardiotoxicity has been reported to occur with clinical use of IMB. The characteristics of IMB-induced cardiac alterations have not been completely determined. The present study sought to evaluate the influence of a coexisting disease such as hypertension on the cardiotoxic effects of IMB. Groups of adult male SD or SHR were dosed with 50 (5/group) or 100 mg/kg (10/group) IMB or water (10/group) (p.o.) daily for 14 days (dose of 100 mg/kg is approximately 2x recommended human dose of 600 mg/m²). Tissues and blood samples were collected 24 hours after the last dose. The 100 mg/kg dose caused a slight reduction in the rate of body weight gain. This dose also decreased serum glucose and increased serum alanine transaminase (ALT) concentrations in both SD and SHR. Changes from control were most pronounced in SD given 100 mg/kg (ALT=175 vs. 118% and glucose=81 vs. 91% compared to SHR). White blood cell counts were depressed in SHR but not in SD rats. Dose-dependent cardiac lesions were noted in the groups of SD and SHR given either the 50 or 100 mg/kg dose of IMB (blinded evaluation). Myocardial alterations included cytoplasmic vacuolization, myofibrillar loss, interstitial infiltration with chronic inflammatory cells and fibrosis (proliferation of myofibroblasts). Mean lesion scores (based on a scale of 0 to 3) were higher in SHR than in SD (100mg/kg-1.9 vs 1.25 and 50 mg/kg-1.5 vs 1.1, p<0.05) (Tukey-Kramer Test). Increased serum levels of cardiac troponin I (ultrasensitive Erenna immunoassay) were detected in animals from all IMB-treated groups. The overall mean concentrations were higher in SHR (31.5±29.0, 41.3±29.0 and 53.8±12.3 pg/ml) compared to SD (8.0±5.7, 25.20 and 30±25 pg/ml) at the control, 50 and 100 mg/kg doses, respectively. These results indicate that hypertension as expressed in SHR appears to be a factor that can intensify the cardiotoxic effects of IMB and that monitoring for cardiac troponin I may be a potential means of detecting IMB toxicity.

INTRODUCTION

Tyrosine kinase inhibitors (TKI) represent a relatively new class of drugs which target specific cellular pathways that are over expressed in certain types of tumors (Sawyers, 2004). The targeting of tumor specific pathways was intended to represent an improvement over more conventional chemotherapeutic drugs which tend to effect all rapidly dividing cells. These same targeted pathways may also be important for the viability of normal cells in tissues such as the heart. Despite the potential for an improved safety profile with use of TKI's, cardiac toxicity has been reported following clinical use of imatinib (IMB), one of the first specific targeting-type agents (Force et al, 2007; Kerkela et al, 2006). At present, experimental studies examining IMB cardiotoxicity have been carried out on a limited basis. IMB-treated mice develop left ventricular contractile dysfunction (Kerkela et al, 2006). In other studies a modest though consistent decrease in LV function and a loss of myocardial mass also was noted in IMB-treated mice (Atallah et al, 2007). Saad et al (2008) gave 30 mg/kg IMB orally for 10 days and detected mild cardiac alterations. Will et al (2008) detected alterations in isolated H9C2 cells exposed to IMB. Hasinoff (2010) examined the effects of several TKI in isolated neonatal rat ventricular myocytes and has related the alterations to target kinase specificity. A recent *in vitro* and *in vivo* study suggests that IMB causes myocardial alterations only at high concentrations (Wolf et al, 2010). The pathogenic mechanism responsible for TKI-induced cardiac alterations appears to be multi factorial. Studies have shown that hypertensive rats are more sensitive than normotensive rats to the cardiotoxic effects of doxorubicin (Herman et al, 2008). The present study was initiated to determine whether the hypertensive or normotensive rat could also serve as an animal model with which to explore questions related to the pathogenesis and characteristics of IMB-induced cardiotoxicity.

MATERIALS/METHODS

Study No. 1

Male SHR dosed orally with 50 (5), 100 (5), 50 (5) mg/kg imatinib or water(5) daily for 10 days

Study No. 2

Male SHR or SD dosed orally with 50 (10) mg/kg imatinib or water(10) daily for 14 days.

Animals

Male spontaneously hypertensive rats (SHR) or Sprague-Dawley (12-14 wks)

Dosing Protocol

1. Dissolve imatinib mesylate in distilled water (15.5 mg/ml base)
2. Administer imatinib orally by gavage daily for 10 or 14 days

Study Termination

1. Euthanize and necropsy each animal 24 hours after last dosing
2. Collect blood for hematology, clinical chemistry and cardiac troponin analysis
3. Collect and process tissues for light microscopic evaluation

Evaluation of Cardiac and Non cardiac Tissues

1. Light microscopic evaluation of toluidine blue cardiac slides
 - A) lesion severity based on semi quantitative assessment of extent of myocyte vacuolization, myofibrillar loss, interstitial lymphocytic infiltration, fibrosis (myofibroblasts and chronic inflammatory cells).
 - B) overall lesion severity score based on a scale of 0-3
2. Light microscopic evaluation of H/E non cardiac tissue slides

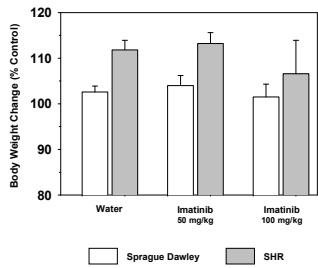
Cardiac Troponin Analysis

1. Singulex ultrasensitive cTnI assay (Singulex, Alameda, CA)

Statistical Analysis

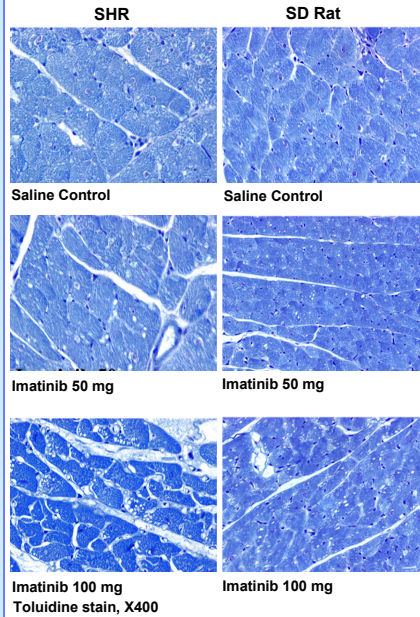
1. Tukey-Kramer multiple comparisons test used to assess differences in Hematological, clinical chemistry and cardiac troponin values.
2. Significance of differences in myocardial lesion scores among the groups was determined by the Mann-Whitney test.
3. For all tests, P<0.05 was taken as the level of significance.

Mean Body Weight Changes (%) in SHR and Sprague Dawley Rats Treated Daily with Imatinib for 14 Days



RESULTS

Light Micrographs Showing Myocardial Alterations in SHR Treated Daily with Either Saline or Imatinib for 14 Days

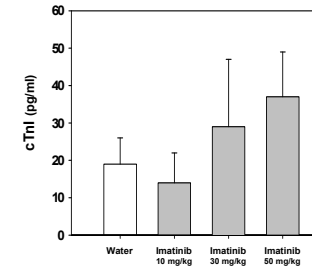


Cardiomyopathy Scores in SHR and SD Rats After Treatment with Imatinib for 10 and 14 Days

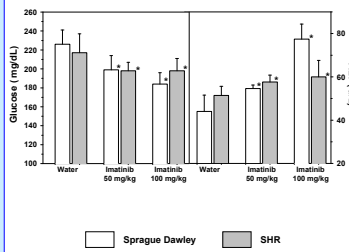
| Imatinib (mg/kg) | Days | Type of Rat | Cardiomyopathy Score | | | | | | |
|------------------|------|-------------|----------------------|-----|-----|-----|-----|-----|--------------------|
| | | | 0 | 1.0 | 1.5 | 2.0 | 2.5 | 3.0 | |
| 10 | 10 | SHR | 2 | 2 | 1 | 0 | 0 | 0 | 3/6 |
| 30 | 10 | SHR | 0 | 1 | 4 | 0 | 0 | 0 | 5/6 ^a |
| 50 | 10 | SHR | 0 | 0 | 5 | 0 | 0 | 0 | 5/6 ^a |
| Control | 10 | SHR | 5 | 0 | 0 | 0 | 0 | 0 | 0/5 |
| 50 | 14 | SHR | 0 | 0 | 5 | 0 | 0 | 0 | 5/5 ^{a,c} |
| 100 | 14 | SHR | 0 | 0 | 3 | 3 | 3 | 0 | 9/9 ^{a,c} |
| Control | 14 | SHR | 8 | 0 | 0 | 0 | 0 | 0 | 0/8 |
| 50 | 14 | SD | 0 | 4 | 1 | 0 | 0 | 0 | 5/5 ^{a,c} |
| 100 | 14 | SD | 0 | 6 | 3 | 0 | 0 | 0 | 9/9 ^{a,c} |
| Control | 14 | SD | 10 | 0 | 0 | 0 | 0 | 0 | 0/10 |

^a Where ratios are given, the numerator denotes the number of animals with a cardiomyopathy score greater than or equal to 1.0 or 1.5, respectively and the denominator denotes the number of animals examined.
^b Lesion scores significantly greater than those of the control group (p<0.05)
^c Lesion scores significantly greater than those of SD given 50 mg/kg Imb (p<0.05)
^d Lesion scores significantly greater than those of SD given 100 mg/kg Imb (p<0.05) (Mann-Whitney Test)

Serum cTnI Levels of SHR Treated Daily with Imatinib or Water for 10 Days

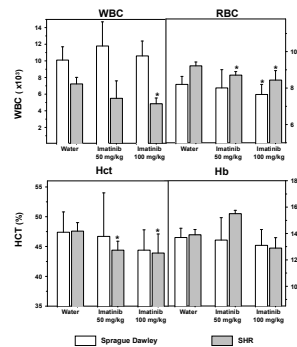


Mean Serum Glucose and ALT Concentrations in SHR and Sprague Dawley Rats Treated Daily with Imatinib for 14 Days



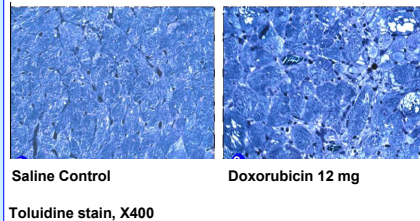
*Significantly different from control group, P<0.05, Tukey-Kramer multiple comparisons test

Hematological Changes in SHR and Sprague Dawley Rats Treated Daily with Imatinib for 14 Days

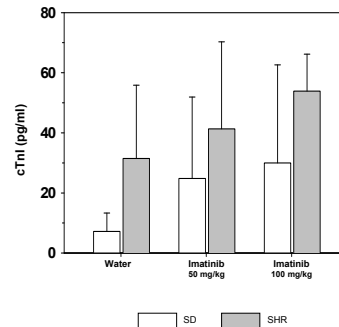


*Significantly different from control group, P<0.05, Tukey-Kramer multiple comparisons test

Light Micrographs Showing Cardiac Lesions (myocardial vacuolization and myofibrillar loss) in Female SHR Treated with 1 mg/kg Doxorubicin Weekly for 12 Weeks.



Serum cTnI Levels of SHR and Sprague Dawley Rats Treated Daily with Imatinib or Water for 14 Days



SUMMARY

1. Both SHR and SD rats were able to tolerate daily doses of up to 100 mg/kg imatinib given daily for up to 14 days.
2. Gains in body weight were comparable between treated and control groups.
3. Imatinib (both doses), caused slight but significant decreases in serum glucose and increases in ALT serum levels.
4. Decreases in RBC, WBC and hematocrit occurred primarily in SHR.
5. Myocardial lesions were observed in SHR after 10 days (10-50 mg/kg) and in both SHR and SD after 14 days (50-100 mg/kg) of treatment.
6. Cardiac alterations consisted of myocyte vacuolization, myofibrillar loss and fibrosis and were significantly more severe in SHR.
7. Small increases in cardiac troponin I were noted in both SHR and SD rats.
8. Imatinib at doses up to 100 mg/kg had relatively little effect on non cardiac tissue.

CONCLUSIONS

1. Imatinib causes myocardial alterations after 10 to 14 days of treatment.
2. Myocardial lesions are more severe in SHR than SD indicating that hypertension could be a cardiotoxicity risk factor.
3. Either SHR or SD could be used as a model to explore imatinib cardiotoxicity.

REFERENCES

1. Atallah E, Durand JB, Kantarjian H, Cortes J. Blood 110:1223-1237, 2007.
2. Force T, Krause DS, Van Etten RA. Nat Rev Cancer 7:332-344, 2007.
3. Hasinoff BB. Toxicol Appl Pharmacol 244:190-195, 2010.
4. Herman EH, Knapton A, Zhang J, et al. FASEB J 22, 2008.
5. Kerkela R, Grazette G, Pacobi R, et al. Nat Med 12: 908-916, 2006.
6. Saad SY, Alkhatry KM, Arafah MM. J Pharm Pharmacol 58:567-573, 2006.
7. Sawyers CL. Genes Dev 17:2998-3010, 2003.
8. Will Y, Dykens JA, Nadanaciva S, et al. Toxicol Sci 106:153-161, 2008.
9. Wolf A, Couttet P, Dong M, et al. Leuk Res, 2010.