An Assessment of Skeletal Muscle Troponin I as a Biomarker of Drug-Induced Skeletal Muscle (SM) Toxicity

Alan Knapton[1], David Peters[1], Elliot Rosen[1], Quynh-Anh Lu[2], Joel Estis[2], Sara J. Agee[2], John H. Todd[2], Eugene H. Herman[1]

[1]DAPR, CDER, FDA, Siver Spring, MD 20993. [2]Singulex Inc, Alameda, CA, 94502.

Program No. 1019.1

ABSTRACT

Many drugs can induce SM myotoxicity. Creatine kinase (CK) has been used as a biomarker of myotoxicity. The present study utilized ultrasensitive immunoassays (Errena) to determine whether slow and fast-twitch troponin I (ssTnI, fsTnI) might be useful biomarkers. Female Sprague-Dawley (SD) rats (12 wks) were dosed daily (po) with 80 mg/kg simvastatin (SVS) daily for up to 14 days. Tissues and blood samples were collected 24 hrs after the final dosing. Body weight declined significantly (21-27%) in 15 of 27 SD dosed for given 6-11days. SM alterations (inflammation, degeneration and necrosis) were detected primarily in the psoas muscle (fast twitch) in 11 of these animals (lesion severity=0.5-2.5). All 15 had elevated levels of fsTnI. Those with psoas lesions were elevated to higher mean levels (3667 u/ml) compared to 592 pg/ml (4 SD without lesions) and 129 u/ml (15 control SD). Soleus muscles (3 SD) were only minimally affected by SVS. ssTnI increased in 1 of 3 SDI. The levels of CK were not consistent. Body weight increased (0.5-2.7%) in 12 SD treated with SVS for 14 days. Psoas muscle lesions were found in 6 of the 12 rats (lesion severity 1-3). Serum levels of fsTnI were normal in 6 SD without lesions (147 u/ml) but were elevated in those with lesions (8,317 u/ml). Soleus lesions were detected in only 1 SD (minimal) and the ssTnI levels form all 12 SD was in the normal range (156 u/ml). CK levels increased from 199 IU (no lesions) to 526 IU (psoas lesions). These results indicate that ssTnI and fsTnI can be used as biomarkers to identify drug-affected SM fiber types and may offer advantages over CK.

INTRODUCTION

Drugs, including those under development or currently available, may sometimes cause unexpected toxicity to the muscles, resulting in a varying degree of symptomatology, from mild discomfort to permanent damage and even in some instances to rhabdomyolysis (death of a large number of skeletal muscle cells) and fatal renal failure (Bannwarth, 2002, Dalakas, 2009). Current clinical practice includes the monitoring of symptoms such as muscle pain, weakness or tenderness and serum creatine kinase (CK) levels (Victor and Sieb, 1994). Careful patient monitoring is vital to detect possible myotoxicity. Currently, CK is a non specific marker of injury that is used to monitor skeletal muscle injury. CK has limited sensitivity and specificity when used as a biomarker of skeletal muscle injury. For example, CK has been shown to be present in the heart, kidney and brain besides the skeletal muscles so its presence in the blood can not be totally attributed to skeletal muscle injury (Friedman and Perryman, 1991). It has been suggested that CK levels be allowed to increase 3 to 5 times over the acceptable baseline level before stopping any therapy (Bannwarth, 2002).

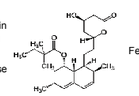
This laboratory has investigated the use of cardiac troponin I as a biomarker to detect injury specific to the cardiomyocytes in laboratory animals (Herman et al, 2011). This protein biomarker is normally present in very small amounts in the blood of healthy subjects, and is released only upon damage specifically to the cardiomyocytes (Herman et al, 1999). Skeletal muscle troponin (sTnI) is a protein that is part of the contractile apparatus in the skeletal muscle cells and is released into the blood following damage specific to these cells (Simpson et al., 2004). There are important advantages to using sTnI as a biomarker of skeletal muscle injury including increased sensitivity and specificity compared to CK. An ultrasensitive immunoassay to monitor the levels of sTnI in the blood has been developed by Singulex Inc. The present study utilized a rat model to evaluate the utility of monitoring circulating levels of sTnI following exposure to an agent (simivastatin) known to cause myotoxicity. It is hoped that sTnI could be used as an early biomarker which can determine those patients receiving treatment with current or new drugs that may be developing serious and potentially dose-limiting skeletal muscle toxicity.

MATERIALS/METHODS

Simvastatin

Gp I – 80 mg/kg Simvastatin Daily Oral Dosing (30 rats)

Gp II – 0.5% Methyl Cellulose Daily Oral Dosing (15 rats)



Female Sprague Dawley Rats (9-10 weeks old)

Dosing Protocol

1. Suspend Simvastatin (Sequoia Research Products) in 0.5% Methyl Cellulose (CMC).
2. Administer 80 mg/kg Simvastatin or 0.5% CMC by oral gavage daily for 7, 10 and 14 days.

Study Termination

1. Euthanize and necropsy each animal 24 hours after last dosing, or if animal's weight is 20% less than its initial body weight.
2. Collect blood for hematology, clinical chemistry and cardiac troponin analysis.
3. Collect and process tissues for light microscopic evaluation.

Evaluation of Tissues

1. A light microscopic evaluation of H/E stained sections of the psoas, soleus and liver tissue was made. The semi quantitative assessment of lesion severity was made on a scale from 0 to 4 (see figure 1).

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.

RESULTS

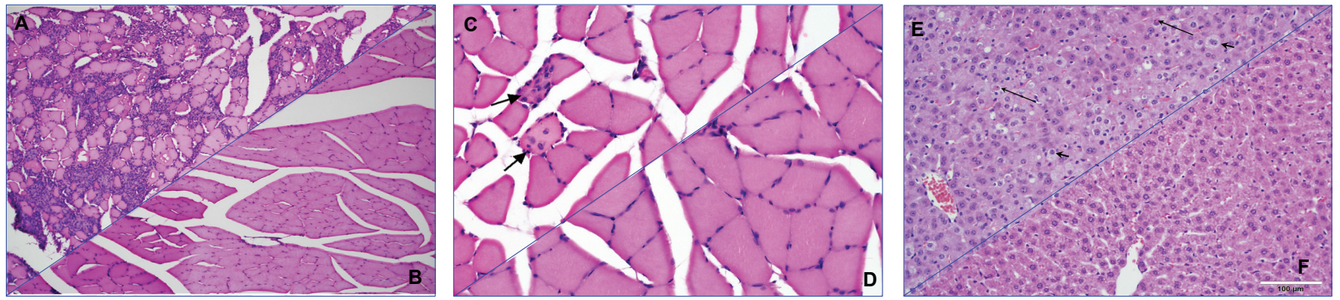


Figure 1. Light micrographs from H&E stained tissues from female SD rats showing 0.5% CMC controls and alterations in rats treated for 14 days with either 80 mg/kg Simvastatin. (A) Psoas muscle from a rat receiving 80 mg/kg of simvastatin for 14 days showing moderate myofiber degeneration, necrosis, inflammation and regeneration. (B) Normal control psoas muscle from a rat receiving 0.5% CMC for 14 days. (C) Soleus muscle from a rat receiving 80 mg/kg of simvastatin for 14 days showing minimal necrotic muscle fibers and inflammation (arrows). (D) Normal soleus muscle from a rat receiving 0.5% CMC for 14 days. Normal Liver (E) Liver from a rat receiving 80 mg/kg of simvastatin for 14 days , Marked hepatocellular swelling (short arrows), vacuolation and single cell necrosis (long arrows). (F) Normal control liver from a rat receiving 0.5% CMC for 14 days.

Table 1. Mean Severity* and Frequency of Lesions Found in the Psoas of Sprague Dawley Rats Treated with Either 80 mg/kg Simvastatin or 0.5% Methylcellulose

Treatment		No. of Animals	0.0 to 0.4	0.5 to 0.9	1.0 to 1.4	1.5 to 1.9	2.0 to 2.4	2.5 to 2.9	Lesion Score >1.0
Duration (Days)									
6 - 7	Simvastatin 80mg/kg	13	7	3	3	0	0	0	3/13[a]
	0.5% Methyl-Cellulose	5	4	1	0	0	0	0	0/5
9 - 12	Simvastatin 80mg/kg	4	0	1	1	2	0	0	3/4[b]
	0.5% Methyl-Cellulose	5	3	2	0	0	0	0	0/5
13-14	Simvastatin 80mg/kg	11	6	1	0	2	2	0	4/11
	0.5% Methyl-Cellulose	5	2	3	0	0	0	0	0/5

*Mean lesion severity score derived from the average score given to each animal for the following conditions: myofiber degeneration, necrosis and histiocytic inflammation.
Lesion scores were rated as 1, Minimal; 2, Mild; 3 Moderate and 4 Marked
[a]Where ratios are given, the numerator denotes the number of animals with a mean psoas lesion score of greater to or equal to 1.5 and the denominator denotes the number of animals examined.

Table 2. Mean Severity* and Frequency of Lesions Found in the Soleus of Sprague Dawley Rats Treated with Either 80 mg/kg Simvastatin or 0.5% Methylcellulose

Treatment		No. of Animals	0.0 to 0.4	0.5 to 0.9	1.0 to 1.4	Lesion Score = 0.5
Duration (Days)						
6 - 7	Simvastatin 80mg/kg	13	11	2	0	2/13[a]
	0.5% Methyl-Cellulose	1	1	0	0	0/1
9 - 12	Simvastatin 80mg/kg	4	3	1	0	1/4
	0.5% Methyl-Cellulose	5	3	2	0	2/5
13-14	Simvastatin 80mg/kg	10	9	1	0	1/9
	0.5% Methyl-Cellulose	5	5	0	0	0/5

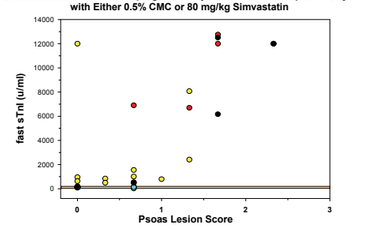
*Mean lesion severity score derived from the average score given to each animal for the following conditions: myofiber degeneration, necrosis and histiocytic inflammation.
Lesion scores were rated as 1, Minimal; 2, Mild; 3 Moderate and 4 Marked
[a]Where ratios are given, the numerator denotes the number of animals with a mean soleus lesion score>0.5 and the denominator denotes the number of animals examined.

Table 3. Mean Severity* and Frequency of Lesions Found in the Liver of Sprague Dawley Rats Treated with Either 80 mg/kg Simvastatin or 0.5% Methylcellulose

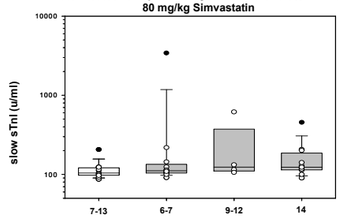
Treatment		No. of Animals	0.0 to 0.4	0.5 to 0.9	1.0 to 1.4	1.5 to 1.9	2.0 to 2.4	Lesion Score >0.5
Duration (Days)								
6 - 7	Simvastatin 80mg/kg	13	0	5	8	0	0	13/13[a]
	0.5% Methyl-Cellulose	5	5	0	0	0	0	0/5
9 - 12	Simvastatin 80mg/kg	4	0	1	3	0	0	4/4
	0.5% Methyl-Cellulose	5	5	0	0	0	0	0/5
13-14	Simvastatin 80mg/kg	10	8	0	2	0	0	2/10
	0.5% Methyl-Cellulose	5	5	0	0	0	0	0/5

*mean lesion frequency derived from the average score given to each animal for the following conditions: reduced hepatocellular clearing, single cell necrosis, lobular infarct, increased mitotic rate, hepatocellular degeneration, mononuclear cell infiltration, increased anisokaryosis, lipidosis and hematopoesis.
[a]Where ratios are given, the numerator denotes the number of animals with mean hepatic lesion scores of greater than or equal to 0.5 and the denominator denotes the number of animals examined.

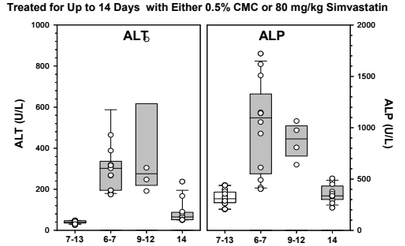
Lesion Score of the Psoas Type II Muscle and Serum Fast Skeletal Troponin I Concentrations in Female Sprague Dawley Rats Treated for Up to 14 Days with Either 0.5% CMC or 80 mg/kg Simvastatin



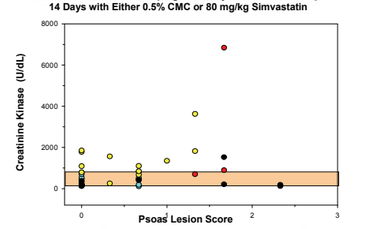
Serum Slow Skeletal Troponin I Concentrations in Female Sprague Dawley Rats Treated for Up to 14 Days with Either 0.5% CMC or 80 mg/kg Simvastatin



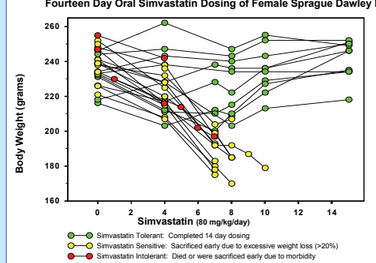
Serum (ALT) and (ALP) Concentrations in Female Sprague Dawley Rats Treated for Up to 14 Days with Either 0.5% CMC or 80 mg/kg Simvastatin



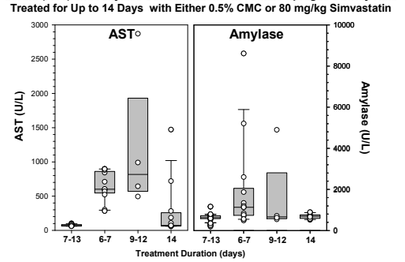
Lesion Score of the Psoas Type II Muscle and Serum Creatinine Kinase Concentrations in Female Sprague Dawley Rats Treated for up to 14 Days with Either 0.5% CMC or 80 mg/kg Simvastatin



Fourteen Day Oral Simvastatin Dosing of Female Sprague Dawley Rats



Serum (AST) and Amylase Concentrations in Female Sprague Dawley Rats Treated for Up to 14 Days with Either 0.5% CMC or 80 mg/kg Simvastatin



SUMMARY

Female Sprague-Dawley rats were treated with 80 mg/kg simvastatin for up to 14 days. The rats showed varying degrees of sensitivity to simvastatin. Approximately one half (13/28) of the animals were euthanized after 6-7 doses and others (4/28) after 9-12 doses because of excess weight loss. In contrast another group (11/28) survived the entire 14 day treatment period with little effect on body weight. Hepatic lesions were noted in all animals that were not able to tolerate the 14 dose regimen. Serum levels of ALT and ALP were elevated in these rats. Lesions (severity score >_ 1) were noted in a fast skeletal muscle (poas) in animals treated for 6-7 days (3/13), 9-12 days (3/4) or 14 days (4/11). Significant increases in fast skeletal muscle troponin I were detected in 9 of these 10 animals. In contrast, significant elevations of creatine kinase above baseline was found in only 4 of 10 rats with lesion scores of >_ 1. In contrast, the soleus muscle (a slow muscle) was resistant to simvastatin as no significant lesions were detected in 23/28 of the treated rats. Serum levels of slow skeletal muscle troponin I were also normal in 23/28 of treated animals. These results indicate that the tolerability of female SD rats to treatment with simvastatin is not uniform. The results also indicate that skeletal muscle troponin I (slow and fast) can be used as biomarkers to identify simvastatin-effected skeletal muscle fiber types and may offer advantages over creatine kinase

CONCLUSIONS

1. **Female SD rats are not uniformly tolerant to treatment with simvastatin.**
2. **Skeletal muscle alterations were noted after 6-14 days of simvastatin dosing.**
3. **The poas (a fast muscle) was more affected than the soleus (a slow muscle).**
4. **Increases in fast skeletal muscle troponin I levels were detected whereas levels of slow muscle skeletal muscle troponin I remained mostly in the normal range.**
5. **Increases in levels of creatine kinase were not uniform.**
6. **Monitoring skeletal muscle troponin I appears to be a sensitive means of detecting and identifying drug-induced skeletal muscle injury.**

REFERENCES

1. **Bannwarth B. Expert Opin. Drug Saf. 1(1): 65-67, 2002.**
2. **Dalakas MC. J Neurol. Neurosurg. Psychiatry 80:832-838, 2009.**
3. **Friedman DL and Perryman MB, J. Biol. Chem., 266(33):22404-22410, 1991.**
4. **Herman E, Agee S, Knapton A, Rosen E, Zhang J, Estis J, Lu Q, Todd J, Lipshultz S., Toxicol. Pathology (in Press).**
5. **Herman EH, Zhang J, Lipshultz SE, Rifai N, Chadwick D, Takeda K, Yu Z-X, Ferrans VJ. J. Clin. Oncol. 17:2237-2243, 1999.**
6. **Simpson JA, Van Eyk J, Iscoe S., J Physiol. 554: 891-903, 2004.**
7. **Victor M, Sieb JP. In:Engel AG, Frazini-Armstrong C (eds). Myology (2nd edn). McGraw-Hill, New York, 1697-1725, 1994**