

The Complete Pharmacokinetic Profile of Serum Cardiac Troponin I in the Rat and the Dog

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Recent improvements in assays have allowed serum cardiac troponin I (cTnI) to be measured at previously undetectable concentrations, which may have implications for cardiotoxicity studies. We characterized the pharmacokinetics of cTnI after a single iv administration of purified cTnI in rats at doses of 0.005, 0.05, and 0.5 $\mu\text{g}/\text{kg}$ and in beagle dogs at doses of 0.05, 0.1, and 0.2 $\mu\text{g}/\text{kg}$. Serum cTnI concentration-time profiles were well described by a two-compartment pharmacokinetic model with first-order elimination in both species. The estimated mean (SD) values of total serum clearance, volume of distribution of the central compartment, and terminal half-life were 318 ml/h/kg, 52.9 ml/kg, and 0.8 h in rats and 481 (135) ml/h/kg, 230 (70) ml/kg, and 1.85 (0.5) h in dogs, respectively. In both species, a fast distribution phase was followed by a relatively slow elimination phase. These data indicate that the current practice in cardiotoxicity studies of unguided blood sampling should be revised. A targeted case-by-case approach is required whereby samples are collected not only relative to the kinetics of the test article but also in relation to the kinetics of the biomarker in the test species and the type and severity of anticipated cardiovascular perturbation. This approach is essential for the identification of subtle increases of serum cTnI concentrations in the low dynamic range.

Key Words: cardiac troponin I; kinetics; rat; dog; ultrasensitive assay; biomarker.

Cardiac troponin, comprised of the subunits T, C, and I, is the calcium-modulated complex involved in forming the actin-myosin cross-bridges responsible for myocardial contraction. Cardiac troponin I (cTnI) is exclusively expressed by the myocardium and is bound primarily to the myofibril, although small amounts of cTnI also are present in the cytosol (Adams *et al.*, 1994; Katus *et al.*, 1991). It is highly conserved across species; therefore, increased serum cTnI concentrations are used ubiquitously as a highly sensitive and specific biomarker of myocardial tissue injury in humans (Thygesen *et al.*, 2007) and in several species of laboratory animals (Apple *et al.*, 2008; Christiansen *et al.*, 2002; Clements *et al.*, 2010; Mikaelian

et al., 2009; O'Brien, 2008; O'Brien *et al.*, 1997; Ricchiuti *et al.*, 1998).

After myocardial insult, the time to peak serum cTnI concentrations across species and the size of this elevation depends largely on the mechanism of cardiovascular injury (Mikaelian *et al.*, 2010), test article dose, and the duration of exposure (Clements *et al.*, 2010). However, the effect of minor serum cTnI elevations independent of extensive cardiomyocyte damage has not been evaluated thoroughly because current assays, in which the lower limit of quantification (LLOQ) is 30 pg/ml, are not sensitive enough for this purpose. In addition, transient or slight cardiomyocyte damage may not generate a large and persistent release of cTnI (Hickman *et al.*, 2010; Mikaelian *et al.*, 2011), making it difficult to identify a treatment-related transient increase using limited sampling times.

Coincident with the LLOQ limitations of previous assays is that the pharmacokinetic clearance of serum cTnI also could not be measured because baseline serum cTnI values are well below 30 pg/ml (Mikaelian *et al.*, 2009, 2011; Schultze *et al.*, 2009). A new ultrasensitive cTnI assay with an LLOQ of 0.8 pg/ml (Mikaelian *et al.*, 2009; Schultze *et al.*, 2009; Todd *et al.*, 2007) can now establish baseline ranges of serum cTnI and allow its pharmacokinetics to be profiled completely.

We characterized the pharmacokinetics of serum cTnI in the Wistar Han rat and beagle dog because they are the species of choice for the toxicity studies used in drug development. Exogenous species-specific purified cTnI was injected iv as a bolus, and serum cTnI concentrations were measured using the new ultrasensitive immunoassay. These data serve as the foundation for sound scientific protocol design and interpretation of serum cTnI data in safety studies.

MATERIALS AND METHODS

All experiments were conducted in accordance with the guidance of the Roche Animal Care and Use Committee. The Nutley site of Hoffmann-La

Roche, Inc. is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

Wistar Han rats. Male Wistar Han rats, aged 8–9 weeks, 240–290 g (Charles River Laboratories, Raleigh, NC), were acclimated for 3 days before catheter implantation. Rats were housed individually in polycarbonate, solid-bottom cages in a controlled environment (temperature, $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$; humidity, $50 \pm 20\%$; light/dark cycle, approximately 12 h) with free access to Purina Certified Rodent Diet #5002-9 (pellets) and reverse osmosis filtered water.

To accommodate serial phlebotomy, all rats received a femoral catheter and harness with a stainless steel spring guard that allowed the sampling port to be placed outside the cage (Supplementary data). After catheter surgery and a 48-h recovery period, 18 rats were randomly divided evenly into three cTnI dose groups (0.5, 0.05, 0.005 $\mu\text{g}/\text{kg}$). A single bolus dose of purified rat cTnI (Life Diagnostics Inc., Westchester, PA) was delivered iv through a warmed tail vein. Dose selections were based on a targeted C_{max} at the low level of what would be expected following a mild to moderate myocardial necrotic event. The calculated iv dose ($\mu\text{g}/\text{kg}$) per rat was based on a 250 g body weight and a total blood volume of 14 ml. Blood samples (0.3 ml) were collected immediately before dosing and at 5 and 30 min and at 1, 2, 4, 6, and 24 h after dosing.

Beagle dogs. Male beagle dogs, aged 24–36 months, 9–13 kg (Marshall BioResources, North Rose, NY), were acclimated for at least 4 weeks before the study. Dogs were housed individually in stainless steel cages in a controlled environment (temperature, $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$; humidity, $50 \pm 19\%$; light/dark cycle, approximately 12 h), provided 2021C-Teklad Global Certified 21% Protein Dog Diet, and were allowed free access to reverse osmosis filtered water provided by an automatic watering system.

Nine dogs were randomly divided evenly into three cTnI dose groups in which a single bolus iv dose (0.2, 0.1, or 0.05 $\mu\text{g}/\text{kg}$) of purified dog cTnI (Life Diagnostics Inc.) was delivered through the saphenous vein. Dose selections were based on a targeted C_{max} at the low level of what would be expected following a mild to moderate myocardial necrotic event. The calculated iv dose ($\mu\text{g}/\text{kg}$) per dog was based on a 10 kg body weight and a total blood volume of 900 ml. Blood samples (1–1.5 ml) were obtained from the jugular vein of each animal before and at 5 and 30 min and at 1, 2, 4, 6, and 24 h after dosing.

Clinical chemistry. All blood samples were collected into labeled serum separator tubes and allowed to clot for at least 30 min at room temperature. After cold centrifugation (15 min at 3000 RPM) serum samples were transferred to individually labeled amber screw-cap vials and stored at -70°C until analysis.

All serum samples (50 μl each) were run in duplicate, and serum cTnI concentrations were measured according to the manufacturer's recommendations using the Singulex Erenna Ultrasensitive Immunoassay system (Singulex, Alameda, CA). For individual animals, final serum cTnI concentrations reported here are the means of the two samples. Group means were determined from all serum cTnI concentrations.

Pharmacokinetic analysis. Pharmacokinetic data were analyzed using Phoenix WinNonlin version 6.0 software (Pharsight, St Louis, MO). Before analysis, each animal's baseline serum cTnI value obtained prior to dosing was subtracted from all subsequent serum cTnI values for the purpose of excluding endogenous cTnI from the compartment model. The analysis reflects only the pharmacokinetic properties of the exogenous, administered, cTnI. Terminal half-life ($t_{1/2}$) was estimated with noncompartmental analysis.

In developing the compartment model, we weighted the residuals using the 1/Y option. Models were selected using the Akaike information criterion and the Bayesian information criterion. A two-compartment model was determined to be the most appropriate choice. Goodness-of-fit diagnostic plots (i.e., predicted vs. observed concentrations and residuals vs. predicted concentrations) defined in the Phoenix software were examined to verify the adequacy of the model and the best-fit characteristics parameters.

Mean pharmacokinetic characteristics for the rat were fitted from a composite concentration-time profile of each dose; therefore, descriptive statistics were not applied. Serum concentration-time data were fitted for each individual dog, and pharmacokinetic characteristics were reported as means and SDs.

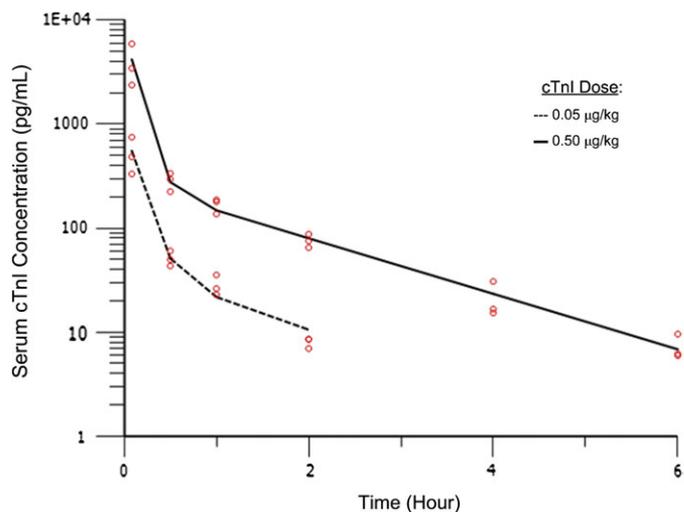


FIG. 1. The pharmacokinetics of cTnI in the Wistar Han rat analyzed with a two-compartment model. Open circles are serum cTnI concentrations from individual animals.

RESULTS

In both the rat and the dog, a biphasic disposition of serum cTnI after iv injection was observed. Inspection of a semilog plot of the concentration-time profile of serum cTnI in both rats (Fig. 1) and dogs (Fig. 2) indicated that a two-compartment model with first-order elimination was appropriate for characterizing the pharmacokinetics of cTnI after dosing.

The goodness-of-fit diagnostic plots indicated that a two-compartment model could adequately describe the biexponential concentration-time profile of cTnI in both test species (data not shown). Mean (SD) baseline serum cTnI concentrations (Fig. 3) were 4.6 (2.3) pg/ml with a range of 1.9–9.3 pg/ml in

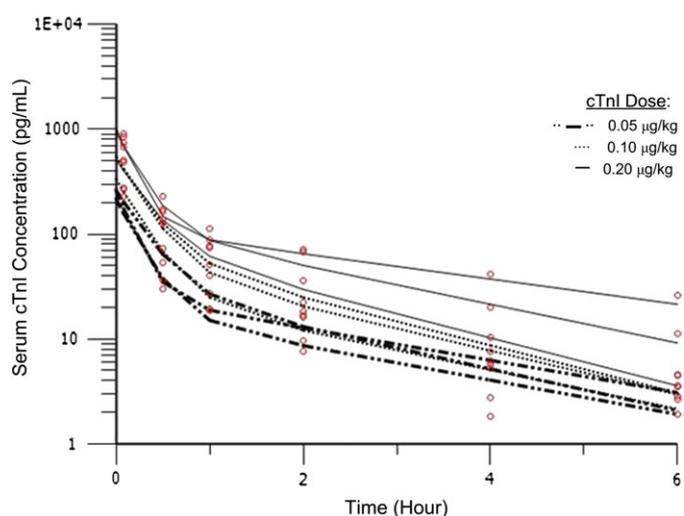


FIG. 2. The pharmacokinetics of cTnI in the beagle dog analyzed with a two-compartment model. Open circles are serum cTnI concentrations from individual animals. Each data line tracks the serum cTnI concentration from the same dog post dosing. Values are reported as means and SDs.

TABLE 1
Two-Compartment Model Characteristics after a Single IV Administration of cTnI in Wistar Han rats

Dose ($\mu\text{g}/\text{kg}$)	CL (ml/h/kg)	Vc (ml/kg)	Ke (h^{-1})	K12 (h^{-1})	K21 (h^{-1})	$t_{1/2}$ (h)	$C_{5\text{min}}$ (pg/ml)	$\text{AUC}_{0-\infty}$ ($\text{h} \times \text{pg}/\text{ml}$)
0.05	267	49.4	5.40	1.99	1.00	0.58	521	220
0.5	370	56.5	6.55	2.63	0.89	1.00	3900	1680
Mean	318	52.9	5.97	2.31	0.943	0.8	2211	950

Note. $\text{AUC}_{0-\infty}$, area under the curve from 0 to infinity; $C_{5\text{min}}$, maximum serum cTnI concentration as assayed 5 min post dose; CL, total serum clearance; Ke, central compartment elimination rate constant; K12, distribution rate constant from central to peripheral compartment; K21, distribution rate constant from peripheral to central compartment; $t_{1/2}$, terminal half-life; Vc, volume distribution of the central compartment.

the rat and 3.0 (1.0) pg/ml with a range of 2.0–5.4 pg/ml in the dog. To exclude endogenous serum cTnI from the compartment model, these baseline serum cTnI concentrations were subtracted from the cTnI concentrations observed at each sampling time point after dosing.

In the rat, serum cTnI concentrations after a dose of 0.005 $\mu\text{g}/\text{kg}$ rapidly returned to baseline. As a result, concentrations after normalization to baseline values were reliable only at 5 and 30 min after the 0.005 $\mu\text{g}/\text{kg}$ dose, so this dose group was excluded from pharmacokinetic analysis. Pharmacokinetic characteristics were estimated from the concentration-time profiles of serum cTnI at the 0.05 and 0.5 $\mu\text{g}/\text{kg}$ doses (Fig. 1). The observed mean serum cTnI concentrations were 521 and 3900 pg/ml at 5 min after the 0.05 and 0.5 $\mu\text{g}/\text{kg}$ doses, respectively. Within 30 min after dosing, serum cTnI concentrations had declined sharply, but they declined more gradually thereafter (Table 1). The results showed a serum clearance of 318 ml/h/kg with a $t_{1/2}$ of 0.80 h and a Vc of 52.9 ml/kg, which was similar to the blood volume of the rat (Lee and Blaurock, 1985).

In the dog, biexponential concentration-time profiles were observed after a single iv injection of cTnI at doses of 0.05, 0.1, and 0.2 $\mu\text{g}/\text{kg}$. A quick distribution phase completed within the first hour after dosing was followed by a relatively slow elimination phase (Fig. 2). The observed mean (SD) serum cTnI concentrations were 258 (26), 635 (133), and 752 (214) pg/ml 5 min after iv doses of 0.05, 0.1, and 0.2 $\mu\text{g}/\text{kg}$, respectively. Pharmacokinetics were analyzed using a two-compartment model for each individual animal. In general, linear pharmacokinetics were observed within the dose range tested (Table 2). The overall mean (SD) total serum clearance was 481 (135) ml/h/kg with a $t_{1/2}$ of 1.85 (0.51) h. Mean (SD) Vc was 230 (70) ml/kg, which was approximately 3 times higher than the blood volume in the dog (Johnson *et al.*, 1985).

DISCUSSION

We characterized the full pharmacokinetic profile of serum cTnI after an iv dose of exogenous species-specific purified cTnI in the rat and dog. The selected doses were chosen to generate the kinetic profile seen with low-level or transient serum cTnI release after slight cardiomyocyte damage. After iv delivery of

exogenous cTnI, a biexponential concentration-time profile was observed in both species (Figs. 1 and 2). At the low levels of serum cTnI tested, a rapid species-specific distribution phase was followed by a longer and more gradual elimination phase.

These data reveal the need for an ultrasensitive assay with an LLOQ in the single-digit picogram per milliliter range because baseline values (Fig. 3) for both the rat and the dog were far below the 30 pg/ml or greater LLOQ of traditional immunoassays (Apple *et al.*, 2008). In addition, these data show that selecting appropriate sampling times is critical to measuring the release of cTnI into the serum during minor cardiac perturbations.

The time over which substantial concentrations of serum cTnI are released after administration of known cardiotoxics has been well characterized (Clements *et al.*, 2010; Mikaelian *et al.*, 2009; York *et al.*, 2007). In the rat, acute myocardial toxicity induced by the β -agonist isoproterenol results in a robust increase of serum cTnI as early as 30 min after dosing, with values peaking at 2–4 h and returning to near baseline after 24 h (Clements *et al.*, 2010; Mikaelian *et al.*, 2009; O'Brien *et al.*, 2006; Schultz *et al.*, 2011). The relatively quick clearance of serum cTnI after acute cardiotoxicity in the rat is apparent; however, to the authors knowledge, all

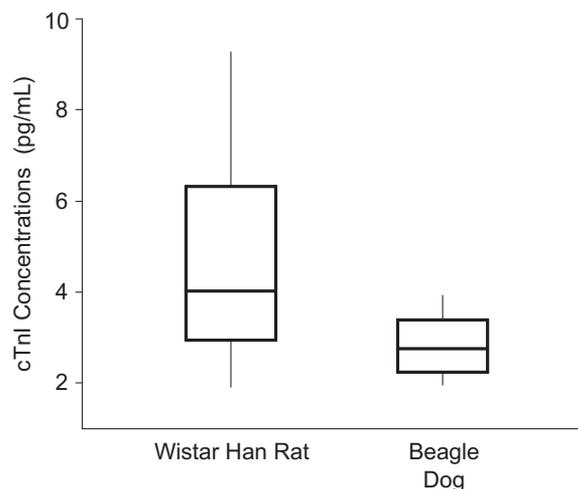


FIG. 3. Baseline serum cTnI concentrations in the Wistar Han rat and beagle dog. Shown are maximum, minimum, interquartile range, and median values.

TABLE 2
Two-Compartment Model Characteristics after a Single iv Administration of cTnI in Beagle Dogs

Dose ($\mu\text{g}/\text{kg}$)	CL ($\text{ml}/\text{h}/\text{kg}$)	Vc (ml/kg)	Ke (h^{-1})	K12 (h^{-1})	K21 (h^{-1})	$t_{1/2}$ (h)	C _{5min} (pg/ml)	AUC _{0-∞} ($\text{h} \times \text{pg}/\text{ml}$)
0.05	447 (80.5)	204 (23)	2.18 (0.21)	1.96 (0.86)	0.813 (0.089)	2.02 (0.129)	258 (26)	145 (25)
0.1	496 (165)	220 (67)	2.24 (0.115)	1.25 (0.01)	0.821 (0.113)	1.42 (0.361)	635 (133)	299 (85)
0.2	500 (191)	265 (109)	1.90 (0.112)	2.18 (1.12)	0.945 (0.073)	2.1 (0.698)	752 (214)	536 (178)
Mean	481 (135)	230 (70)	2.11 (0.207)	1.80 (0.82)	0.860 (0.103)	1.85 (0.511)	548 (257)	327 (197)

Note. AUC_{0-∞}, area under the curve from 0 to infinity; C_{5min}, maximum serum cTnI concentration as assayed 5 min post dose; CL, total serum clearance; Ke, central compartment elimination rate constant; K12, distribution rate constant from central to peripheral compartment; K21, distribution rate constant from peripheral to central compartment; $t_{1/2}$, terminal half-life; Vc, volume distribution of the central compartment.

published reports on the release of endogenous cTnI during instances of myocardial damage in any laboratory animal species have employed assays that were not sensitive enough to establish baseline values (Clements *et al.*, 2010; O'Brien *et al.*, 2006; Schultze *et al.*, 2011).

Given recent evidence suggesting that small elevations of serum cTnI above baseline are correlated with an increased risk of myocardial-related mortality in humans (Mills *et al.*, 2011), the ability to establish and monitor baseline concentrations of serum cTnI in the rat or dog is essential to assessing the cardiovascular safety of therapeutic compounds in development.

There are reports of cTnI being released independently of apparent cardiac injury in which circulating cTnI was detected for a considerably shorter time than that associated with the onset of necrosis (Hickman *et al.*, 2010; Wu and Ford, 1999). Transient elevations of serum cTnI independently of histological findings of cardiac injury have also been reported (Mikaelian *et al.*, 2011). Although cTnI is bound predominantly to the myofibril and its release is correlated with necrosis, a small percentage (3–4%) is unbound and resides in the cytoplasmic compartment (Adams *et al.*, 1994; Katus *et al.*, 1991).

Release into the circulation of the cytoplasmic pool of cTnI has been speculated to occur independently of cardiomyocyte injury, possibly as a result of blebosome formation after cell membrane disruption (Hickman *et al.*, 2010). Accordingly, the reported low-level elevations of serum cTnI in the apparent absence of myocardial necrosis (Hickman *et al.*, 2010; Mikaelian *et al.*, 2011; Wu and Ford, 1999) are most likely the result of a release of the cytoplasmic pool into circulation caused by disruption of the cardiomyocyte membrane or a release from minute areas of necrosis that are not identified on routine histologic examination because of the inability to examine the entire heart histologically.

Regardless of how cTnI is released, the pharmacokinetic data presented here become extremely important in study design and in accurately interpreting data from minor cardiac perturbations accompanied by elevations of serum cTnI in the picogram per milliliter range. The rapid clearance and short $t_{1/2}$ of serum cTnI identified in this study indicate that serum cTnI concentrations after minor cardiac perturbations must be

measured soon after the expected maximal cardiac effect of the test article. Without appropriate sampling times, the likelihood of false-negative interpretations is increased, especially during instances of minimal or undetectable necrosis. Furthermore, these data indicate that the pharmacokinetics of other biomarkers used in toxicity studies must be characterized to guide the selection of sampling times.

Pharmacokinetic characteristics in the *in vivo* system are inherently interdependent (Rowland and Tozer, 1989). Thus, $t_{1/2}$ depends on the Vc—the volume that cTnI appears to occupy within the central compartment—and the CL—the volume of plasma cleared of cTnI per unit of time, along with the peripheral compartment kinetics (Rowland and Tozer, 1989). The perceived disconnect between the rate of clearance and the reported $t_{1/2}$ in the rat and dog (Tables 1 and 2) is likely not the result of plasma protein binding and general metabolic activity but rather of differences in physiology, specifically of distribution volumes and clearance rates. Compared with dogs, rats have a faster heart rate, greater hepatic blood flow, a faster glomerular filtration rate, and more robust urine and biliary excretion (Lin, 1995). Although their CL is lower than that of dogs (Table 2), their Vc (Table 1) is also markedly lower. Thus, rats have a smaller central compartment volume, which contributes to a more rapid clearance and a shorter $t_{1/2}$ of serum cTnI. These data indicate that sampling times to detect low-level changes in serum cTnI need to be selected after considering species-specific variations in clearance rates.

Following an acute myocardial infarction (AMI) in humans, cardiac troponins I, T, and C appear in the blood as a mixture of free subunits, covalent complexes, and posttranslationally modified forms (Madsen *et al.*, 2006; McDonough *et al.*, 1999; Peronnet *et al.*, 2006). The peak and area under the curve concentration of cardiac troponins in the blood correlate with the size of the infarct area (Giannitsis *et al.*, 2008; Kragten *et al.*, 1996), and serum troponin concentrations can remain elevated for days (Thygesen *et al.*, 2007). This persistent elevation is not due to slow clearance but rather prolonged release kinetics as a result of dissociation of the myofibril-bound troponin subunits and their eventual release into the blood following a necrotic event (Kragten *et al.*, 1996). The data presented here are not intended to characterize

the kinetics of cTnI following AMI, after which a heterogeneous mixture of cTn subunits and complexes are present in the serum. Rather, the pharmacokinetic data reported here serve to identify the kinetic profile of cTnI following minor cardiac damage, when a suspected release of the cytoplasmic cTnI pool occurs (Hickman *et al.*, 2010). These kinetic data could prove valuable for the clinician when using the ultrasensitive cTnI assay for the cardiovascular risk stratification of patient populations.

LIMITATIONS OF THE STUDY

An inherent limitation of a two-compartment model is the uncertainty of the constituents of the peripheral compartment. The exact location of this compartment cannot be clearly defined physiologically. We can only speculate as to the source of the redistributed serum cTnI. Investigations using labeling and advanced imaging modalities, such as positron emission, are required to identify this source. Two-compartment modeling as performed in this study, however, is appropriate for selecting adequate sampling times when incorporating the ultrasensitive cTnI assay in toxicity studies.

CONCLUSIONS

Our data identified a rapid clearance rate of serum cTnI in the rat and dog. These data show the difference in pharmacokinetic clearance of serum cTnI between species and thus the need to select blood sampling times not only in relation to the kinetics of the test article but also to the pharmacokinetics of the biomarker in the test species and the type and severity of anticipated cardiovascular perturbations. Furthermore, characterizing the pharmacokinetics of serum cTnI in these species, which are widely used in toxicology studies, is necessary for further investigation into the biological importance of low-level cTnI changes. An ultrasensitive assay for measuring values in the low picogram per milliliter range could make serum cTnI a more valuable biomarker of cardiac damage with important implications in both clinical and preclinical toxicology studies of compounds in drug development and in the pursuit of personalized medicine.

SUPPLEMENTARY DATA

Supplementary data are available online at <http://toxsci.oxfordjournals.org/>.

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