SERUM TROPONINS AS BIOMARKERS OF DRUG-INDUCED CARDIAC TOXICITY

- OUTLINE -

STATEMENT OF PURPOSE

Define the current status of scientific evidence regarding the utility of serum troponins I and T as biomarkers of drug-induced cardiac injury in nonclinical and clinical drug evaluation studies. Identify scenarios in which this biomarker could be of benefit and identify barriers and knowledge gaps that limit such usage.

JUSTIFICATION

1) Experience/need for valid biomarkers of drug-induced cardiac toxicity
   a) Incidence of adverse cardiac events with pharmaceuticals
      - Attrition of pharmaceuticals from various clinical phases of development
      - Revocation of drug registrations
   b) Limitations of current biomarkers
      i) SPECIFICITY - Current biomarkers employed to assess drug-induced cardiac toxicity are not specific to the heart. These proteins are also localized in other tissues, especially skeletal muscle, and are released to the serum in response to non-cardiac tissue injury.
         (i) (Tsung and Tsung, 1988; Christenson et al., 1997; Apple, 1999; Fredericks et al., 2001).
      ii) SENSITIVITY – Current biomarkers of drug-induced cardiac toxicity are sometimes not detected in serum under conditions of confirmed cardiac histopathology.
      iii) There are large species-related differences in the utility of current biomarkers of drug-induced cardiac toxicity. Consequently, these do not lend themselves well to bridging between non-clinical and clinical studies.

INTRODUCTION TO BIOMARKERS

1) Categories of cardiac injury
   a) Structure – tissue integrity
   b) mechanical function – developed pressures, hemodynamics
   c) dysrrhythmias -
   d) homeostasis – ionic, metabolic

2) Characteristics of an ideal biomarker of tissue injury
   a) Specific
   b) Sensitive
   c) Favorable kinetics
   d) Robust detection assay
   e) Bridge non-clinical and clinical scenarios

BACKGROUND REGARDING THE TROPONINS

- Biology
  - Component of the thin myofilaments
  - Participation in the contractile process
- Isoforms
  - Expression and turnover

CHARACTERIZATION OF THE TROPONINS AS BIOMARKERS OF DRUG-INDUCED MYOCARDIAL INJURY:

a. Specificity
    i. High myocardium/serum ratio
        1. below detection limits in untreated "control" serum using commercially available assays
        2. Ventricular myocyte concentration (Dean, 1998):
            a. 10.8 mg/g cTnT
            b. 4 - 6 mg/g cTnI
            c. table of reported values (species: cardiac, skeletal, serum)
        3. cTnI is not expressed in non-cardiac tissues, even in pathological states (Bodor et al., 1995; ref., Apple, 1999)
        4. cTnT is reported to be expressed in non-cardiac (skeletal muscle) tissue under certain conditions:
            a. fetal (Saggin et al., 1990)
            b. skeletal muscle trauma/denervation (Saggin et al., 1990)
            c. End-stage kidney disease (Apple, 1999; Chapelle et al., 2002)
            d. forced expression of cTnT isoforms in cultured muscle and non-muscle cells (Warren and Lin, 1993)
        5. multiple isoforms of TnT mRNA in failing and compensated hearts (Mesnard-Rouiller, et al., 1997)
        6. Commercially available MAB specific for cardiac isoforms of cTnI and cTnT
            a. do not cross-react with skeletal muscle isoforms (<0.005%) (Muller-Bardoff et al., 1997)
            b. The detection of TnT in non-cardiac tissues may be an artifact of antibody specificity (Ricchiuti et al., 1998; ref., Apple, 1999; Fredericks et al., 2002)

b. Sensitivity

i. Low base line values in "control" sera

1. baseline near detection limit
2. 2nd generation cTnT MAB detection limit ~ 0.01-0.02 µg/ml (Mueller-Bardoff et al., 1997; Hallermayer et al., 1999)
3. The serum troponins are detected as early, if not earlier in the course of pathogenesis than are other biomarkers of myocardial injury
    a. examples of elevated serum troponins early and in the absence of cardiac histopathology. If allowed to proceed, eventually observe the cardiac histopathology.
    b. Increase serum cTnT precedes CK, CK-MB, LDH (Wu, 1999)
    c. In acute MI, sensitivity of cTnI > LD1/LD2 (Martins et al., 1996; Jaffe et al., 1996)
    d. Increased serum cTnT in situations of reversible injury (Clark et al., 1995; Wu and Ford, 1999)
    e. cTnI and cTnT released in parallel with each other and with myoglobin and CKMB with reperfusion during clinical CAB (Bleier et al.,1998)
    f. in acute MI model in swine, cTnI more sensitive and specific than myoglobin or CKMB (Feng et al., 1998).
    g. cTnI and cTnT and myoglobin increase earlier and are more sensitive than CK or CKMB in human acute MI (Mair et al., 1995)

c. Kinetics
    i. Sufficiently long half life allows detection in serum
        1. cTnI half-life ~ 2 hrs (Mair et al., 1991)
        2. longer T1/2 than myoglobin or CKMB
    ii. Small intracellular free fraction (<10%) of both cTnI and cTnT is released rapidly in response to cardiac injury.
        1. peak cTnI and cTnT at 4 hrs after acute isoprenanline in rats (Bertinchant et al., 2000). Neither CK nor LDH were elevated at this time.
    iii. Majority is bound to actin and released more slowly
        1. myofibrillar degradation (Katus et al., 1991; Mair, 1977; Voss et al., 1999).
    iv. Biphasic kinetics:
        1. initial appearance in serum within 24 hrs followed by a second phase over 4-14 days (Mair et al., 1991; 1992; Wu et al., 1992)
    v. Serum concentrations correlate with extent of myocardial injury
        1. cTnI and cTnT proportionate to extent of acute MI
            a. humans (Collinson, 1998)
            b. dog (Ricchiuti et al., 1998)
                i. loss of LV cTnT, but not cTnI, in ischemic myocardium ~ infarct size @ 3 weeks
                ii. serum cTnI and cTnT proportionate to infarct size
        2. serum cTnI and cTnT correlate with histopath following isoprenaline in rats (Bertinchant et al., 2000)
        3. maximum concentration and AUC for cTnI in coronary effluent of Langendorf rabbit heart correlates with contusion energy (Bertinchant et al., 1999)
            a. no such relationship for cTnT, CK or LD
        4. acute MI model in beagle dog (LAD ligation), peak cTnT and AUC cTnT correlate with infarct size at 96 hr (Remppis et al., 2000)
        5. acute MI model in mice (coronary artery ligation) H-FABP correlated with infarct size at 7 days
            a. no correlation for plasma cTnT (Aartsen et al., 2000)
        6. Both serum cTnT and histopath score correlate with cumulative dose of doxorubicin (Herman et al., 1999)
        7. lack of correlation between serum cTnT and LVEDP or LVDP in Langendorf rat heart model of ischemia reperfusion (Kawakami et al., 1999).
        8. cTnT correlates with various types of cardiac injury (>CK or LD isozymes) for various species such as dog, rat, mouse, and ferret (ref., O'Brien et al., 1997)

d. Robustness of the assay

    i. Simple
        1. Run time ~ 9-18 minutes (Mueller-Bardof et al., 1997; Gerhardt and Ljungdahl, 1998; Hallermayer et al., 1999)
    ii. Accurate
        1. Complex of cTnT stable for
            a. ~ 5 years at –70C
            b. 5 freeze-thaw cycles
        2. Complex of cTnI less stable to freeze-thaw cycles
    iii. Reproducible
    iv. Inexpensive
    v. Valid across species

e. Bridge preclinical and clinical
    i. Same technology can be employed for both preclinical and clinical monitoring
        1. Amino acid sequence highly conserved across species
        2. MAB to human cTnT cross-reacts with cTnT of rat, dog, cat, pig, goat, cow, horse, rabbit, sheep, chicken, turkey, and trout (O'Brien et al., 1998)
            a. Does not cross-react with skeletal TnT of these same species (less than 1%) (O'Brien et al., 1998)
        3. cTnI and cTnT less than 0.6% expressed in skeletal muscle of rats, dogs, pigs, monkeys (Fredericks et al., 2001)

CONCLUSIONS

1. Serum cTnI and cTnT are the most highly specific of the currently employed biomarkers of drug-induced myocardial injury.

2. Serum cTnI and cTnT, when measured in its critical diagnostic window of time, are highly sensitive indicators of myocardial injury

3. The serum troponins are detected as early, if not earlier in the course of pathogenesis than are other biomarkers of myocardial injury.

4. The troponins are released from cardiac tissue during the active phase of cell lysis and return to baseline following cessation acute, finite rounds of pathogenesis. In situations of progressive cell injury, such as occurs with doxorubicin, there is a propagation of cell injury as reflected by a long-sustained increase in serum troponins.

5. The increases in serum cTnI and cTnT, when measured in its diagnostic window, are proportionate to the extent of myocardial injury. Exceptions to this observation may be attributed to sampling times outside the critical diagnostic window.

6. The commercially available assays for cTnI and cTnT are simple, accurate, reproducible, and inexpensive.

7. The appearance of cTnI or cTnT in serum signifies a generalized disruption of the limiting cell membrane or the disruption of the myofilaments and leakage from the cell.
    a. Cardiac injury that does not result in altered cardiac cell membrane permeability may not be associated with increases in serum troponins.

8. It remains unresolved whether the troponins can leak from a single cell in the absence of irreversible cell degeneration. Perhaps the more important question, however, is whether any increase in troponin necessarily signifies a significant concern regarding the long-term cardiovascular health of the individual. It is the consensus of the Expert Working Group that, based on the current status of knowledge regarding the troponins, any significant elevation of in serum troponins warrants follow-up and further investigation of possible myocardial injury.

9. The troponins are the serum protein biomarker of choice for monitoring potential drug-induced myocardial injury

10. Monitoring serum troponins would be useful in investigating cases of concern for drug-induced myocardial injury

11. Monitoring serum troponin can be useful when included in nonclinical studies to assess the potential for drug-induced myocardial injury.

LIMITATIONS

- Must be measured within the critical diagnostic window
- cTnI assay kits are marketed by several vendors, but cTnT assay available from only a single vendor
- Baseline values and quantitative changes in serum troponins may be altered in various disease states, which may reflect secondary damage to the myocardium.
- Inter-assay validation

INFORMATION GAPS/DATA NEEDS

- Existing evidence does not distinguish the superiority of cTnI or cTnT
- Gather more definitive data in lab animals regarding quantitative correlation serum troponins to extent and type of drug-induced myocardial injury as assessed by other methods including histopathology
    - Gather more data regarding kinetics of serum troponins and correlation with active, reversible or persistent myocardial changes in structure and/or function
- Examples of clinical-to-nonclinical correlations of serum troponins and drug-induced myocardial injury
- Need for additional biomarkers of drug-induced cardiac toxicity.
    - Alternate types of cardiac toxicity, which may not be marked by the troponins
    - New technologies.

SUMMATION

a) cTnI and cTnT are specific, sensitive, and robust biomarkers that are released early in the course of pathogenesis and reflect the extent of drug-induced myocardial injury in nonclinical safety studies
   i) more specifc and sensitive than conventional serum proteins
   ii) robust detection assays
   iii) bridge across species, including humans
   iv) amenable to new technologies such as proteomic platforms

b) cTnI and cTnT mark active myocardial cell injury to yield elevations in serum concentration that resolve towards baseline once the ongoing cell injury process subsides.
   i) Troponins accurately report cardiac injury but are not, however, predictive of impending cardiac damage

c) cTnI and cTnT are not biomarkers of all forms of drug-induced cardiac toxicity
   i) need to identify biomarkers of other types (homeostasis, etc) of cardiac injury.

d) Plans to address information gaps and data needs regarding the troponins:
   i) Gather more definitive data in lab animals regarding quantitative correlation serum troponins to extent and type of drug-induced myocardial injury as assessed by other methods including histopathology
      (1) Gather more data regarding kinetics of serum troponins and correlation with active, reversible or persistent myocardial changes in structure and/or function
      (2) Prefer T or I or both
      (3) implementation- EWG to meet to discuss most effective experimental design – This will dictate most efficient means for conducting the needed experiments.

   ii) Examples of clinical-to-nonclinical correlations in serum troponins and drug-induced myocardial injury – data mining within the FDA, Pharma
      (1) is there value to adding troponins as an end-point?
      (2) develop partnership through ILSI, Soc Tox Path, kit developers, or other organizations to bring stake-holders together to agree on format for sharing existing data for evaluation

e) Need for additional biomarkers of drug-induced cardiac toxicity.
   i) Alternate types of cardiac toxicity, some of which may precede that which is marked by the troponins
   ii) New technologies.
   iii) Expert Working Group to discuss and coordinate a meeting amongst stakeholders to identify and evaluate most promising biomarkers

f) Peer reviewed publication of final troponin report.