ATP Metabolism as Biomarker Target for Cardiovascular Protection and Toxicity

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Biomarkers Symposia – My involvement

Drug development is a science, art, or both?

- 1970’s - Application of pharmacokinetics
- 1980’s - Controlled clinical studies for efficacy
- 1990’s - Pharmacodynamics and pharmacogenetics
- 2000’s - Focus on drug safety
- 2010’s – Biomarkers?
Potential Impacts of Biomarkers (Pharma 2010)

- Revitalize and sustain a vibrant pharmaceutical, biotechnology, and diagnostic industry.
- Develop more targeted drug therapies and reduce cost of drug development.
- Form the scientific basis of personalized medicines.
Definition of biomarkers

Biological marker (biomarker): “A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.”

e.g. Blood pressure, blood glucose, body weight, etc.

Clinical endpoint: “A characteristic or variable that reflects how a patient feels, functions, or survives”

e.g. Feeling depressed
Processes of New Drug Discovery & Development


Fig. 1. A schematic depiction of the new drug development process.
CONCEPTUAL MODEL FOR BIOMARKERS AND SURROGATE ENDPOINTS

Selection of Types of Biomarkers
ATP Metabolism (Yeung, et al. 1997)
Physiological Roles
Adenosine and ATP

- Regulate coronary blood flow
- Inhibit platelet aggregation
- Cardioprotection
- Neuro- and immuno- modulation
- Attenuate tissue necrosis (limit size of infarct)
- Mediator of ischemic or exercise preconditioning
- Others?
HPLC of Adenosine and its Oxypurine Metabolites in Plasma
Feng and Yeung, Ther Drug Monit 22:177 – 183, 2000

A Standard Solution
B Plasma Standard
C Plasma Standard

a. Uric acid
b. Hypoxanthine
c. Xanthine
d. Adenine
e. Inosine
f. Guanosine
g. Adenosine
h. Is
HPLC Assay of Purine Nucleotides in RBC

Inhibition of RBC uptake of adenosine

Yeung, Mosher, MacRae, Klassen. J. Pharm. Pharmacol. 43:685 - 689, 1991

- Rabbits (n = 6) pretreated with saline or DTZ (5 mg/kg sc bid for 5 doses).
- Adenosine administered by rapid iv infusion (over 10 min)
Effect of Anti-ischemia Drugs on ATP Metabolism in RBC *(Yeung et al., 2011)*

![Graphs showing the effect of anti-ischemia drugs on ATP metabolism in RBCs.](image)
Effect of Isoproterenol (Yeung et al., 2012)
# ATP Metabolism in Rats treated with isoproterenol

*(Yeung and Seeto, 2012)*

<table>
<thead>
<tr>
<th>Biomarker variables</th>
<th>Control (n=9)</th>
<th>Isoproterenol treatment (n=10)</th>
<th>Victims* (n=5)</th>
<th>Survivors** (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP vs. AMP r*</td>
<td>-0.051 ± 0.312*</td>
<td>-0.515 ± 0.421</td>
<td>-0.717 ± 0.362</td>
<td>-0.262 ± 0.380</td>
</tr>
<tr>
<td>ATP vs. AMPβ*</td>
<td>0.002 ± 0.032*</td>
<td>-0.202 ± 0.204</td>
<td>-0.318 ± 0.190</td>
<td>-0.058 ± 0.115**</td>
</tr>
<tr>
<td>ATP vs. ADP r</td>
<td>0.299 ± 0.308*</td>
<td>-0.277 ± 0.589</td>
<td>-0.429 ± 0.654</td>
<td>-0.088 ± 0.456</td>
</tr>
<tr>
<td>ATP vs. ADP β</td>
<td>0.103 ± 0.117*</td>
<td>-0.294 ± 0.542</td>
<td>-0.523 ± 0.642</td>
<td>-0.008 ± 0.194</td>
</tr>
<tr>
<td>ADP vs. AMP r</td>
<td>0.579 ± 0.260</td>
<td>0.787 ± 0.253</td>
<td>0.812 ± 0.248</td>
<td>0.767 ± 0.285</td>
</tr>
<tr>
<td>ADP vs. AMP β</td>
<td>0.132 ± 0.124*</td>
<td>0.392 ± 0.277</td>
<td>0.296 ± 0.282</td>
<td>0.469 ± 0.278</td>
</tr>
</tbody>
</table>

* Rats died within 5 hrs after isoproterenol
* Rats survived longer than 5 hrs after isoproterenol
* Correlation coefficient
* Regression coefficient
* Data represent mean ± SD
* p<0.05 vs. isoproterenol (t-test)
** p<0.04 vs. victims (t-test)

Table 3: Correlation between RBC concentrations of adenine nucleotides in rats after isoproterenol (30 mg/kg sc).
Effect of exercise on correlation between plasma DTZ concentrations and SBP


[Graphs showing relationship between plasma DTZ concentrations and SBP]
Effect of Exercise (Yeung, et al., 2010)
Effect of Exercise on Cardiovascular Protection

(Yeung et al., 2011)

Survival Rate increased from 50% to 80%
Effect of diltiazem on cardiovascular toxicity induced by isoproterenol (Yeung and Seeto, 2012)

After 10 mg/kg of DTZ bid for 4 doses, survival rate increased from 50% to greater than 80%
Opportunities for ATP metabolism as Biomarker target

- **Disease and health management:**
  - May be a measure of “Inner Energy”, “Reserves”, and “Cardiovascular homeostasis”
  - Cardiovascular and metabolic diseases, cancer, stroke, aging (inside age) and other neurodegenerated diseases.
  - Aging and other chronic illnesses.

- **Drug development:**
  - Cardiovascular protective agents (ARB, ACEI, CCB, Rennin and thrombin inhibitors, anti-platelet agent, B-blocker, ant-coagulant, NPH, and others)
  - Anti-cancer agents
  - Cardiovascular toxicities (cardio + vascular).

- **Complimentary medicine:**
  - Natural health products.
  - Traditional Chinese medicines
  - Energy supplements
Ideal biomarker of effect

(Sistare 2003)

- Should be drug or treatment related.
- Provide a diagnostic linkage to toxicity
- Represent a mechanistic intervention that may occur far upstream of actual toxicity for early detection of toxicity.
- Inter-species application.
Biomarker Checklist for ATP Metabolism

- Is ATP metabolism applied across species (or species independent)? √
- Is ATP metabolism quantitative and measurable in systemic circulation for pharmacodynamic study? √
- Could ATP metabolism help lead candidate selection and reduce the risk of attrition? √
- Is ATP metabolism a sensitive and specific biomarker for disease or therapeutic intervention? ?
- Could ATP metabolism be studied in a routine clinical setting? ?
Biomarkers and Critical Path (Karsdal 2009)
The imperative to produce high-value, innovative drugs will intensify, creating a higher performance hurdle for new therapeutics.

Basic biomedical science will churn out candidate biomarkers with tantalizing potential to improve value, whereas methods to use them effectively in drug development will evolve more slowly.

The balance between these forces may well determine the success or failure of the drug development enterprise over the next decade.
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Dalhousie University vs Lobster – which is better known for Nova Scotia?