Association between Benzene Exposure, Circulating Angiogenic Cell Levels, and Cardiovascular Disease Risk in the Louisville Healthy Heart Study

Background
- Cardiovascular (CVD) burden
- CVD and pollution
- Endothelial progenitor cells (EPCs)/Circulating angiogenic cells (CACs)
- Benzene exposure effects

Methods of the Healthy Heart Study

Results

Summary

Burden of Cardiovascular Disease

Effect of risk factors on number of EPCs

Endothelial Progenitor Cells (EPCs)

Biological Plausibility

EPCs and CVD Risk

Diseases of the Heart

Association between the FRS and EPC Colony Counts

Mensah and Brown, 2007

JACC

Hill et al. 2003

Vasa et al. 2001

Shantsila et al. 2007

Jujo et al. 2008

Fadini et al. 2007, 2012

Nawrot et al. 2011

Heidenreich et al. 2011

Go et al. 2013

Mensah and Brown, 2007
Methods

Patient recruitment from localized urban collection during

Discussion

• Benzene-CVD Risk
  - Bone marrow toxicant
  - Associated with increased cardiovascular mortality
  - Chronic exposure linked with hypertension
  - Found in high amounts in traffic, industrial emissions, and cigarette smoke
  - Benzene metabolites (trans, trans-muconic acid (t,t-MA) linked with environmental exposure

Chronic exposure linked with increased cardiovascular mortality

Textbook

Results

Table 1. Antigenic identity of CACs.

<table>
<thead>
<tr>
<th>CAC Identification</th>
<th>Statistical Analysis</th>
<th>Cell Differentiation State</th>
<th>Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD34+ 45—AC133+</td>
<td>Description statistics</td>
<td>Cell type 8: CD34+ 45—AC133+ 0.1%</td>
<td></td>
</tr>
<tr>
<td>CD31− 34+ 45−AC133− 0.3%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD31+ 34+ 45+AC133+ 0.2%</td>
<td></td>
<td></td>
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<tr>
<td>CD31− 34− 45−AC133− 0.4%</td>
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<tr>
<td>CD31− 34+ 45−AC133− 0.5%</td>
<td></td>
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</tbody>
</table>

Figure 1. Id-i

Stains of Cell types 2, 8, 11, and 14. Presence of Id-1 green stain and 11 blue stain indicates validated angiogenic cells.

Experimental Procedures

EPCs/CACs and Pollution

Association between Human PECs and 45−

Association between Mouse EPCs and 45−

GAP exposure (days)

Traffic Proximity

Traffic Proximity Increases Odds for Atherosclerosis

Benzene

Benzene Metabolism

Benzene-CVD Risk

- Bone marrow toxicant
- Associated with increased cardiovascular mortality
- Chronic exposure linked with hypertension
- Found in high amounts in traffic, industrial emissions, and cigarette smoke
- Benzene metabolites with trans, trans-muconic acid (t,t-MA) linked with environmental exposure

Objective

Experimental Procedures

CAC Identification

Table 1. Antigenic identity of CACs.
**Summary/Discussion**

1. **t,t-MA levels were positively associated with cigarette smoke exposure.**
2. Levels of cell types 2, 8, and 14 were inversely associated with t,t-MA levels. Benzene may increase CVD risk in part through the suppression of these cells.
3. **t,t-MA levels were higher in people with high CVD risk regardless of smoking status.**
4. Future research should determine which adverse CVD outcomes are linked with ambient air benzene exposures.

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**Table 3. Significant associations (adjusted) between t,t-MA and circulating angiogenic cell levels.**

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Total Population</th>
<th>Non-smokers</th>
<th>African American</th>
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<tr>
<td>CD34+</td>
<td>β</td>
<td>0.003</td>
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<td>β</td>
<td>0.014</td>
<td>0.017</td>
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<td>0.014</td>
<td>0.017</td>
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<tr>
<td>AC133-8</td>
<td>β</td>
<td>0.016</td>
<td>0.017</td>
<td>0.017</td>
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* p < 0.05
† Nonsmokers have cotinine < 200µg/g creatinine.

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**Table 2. Significant associations (adjusted) between t,t-MA and circulating angiogenic cell levels.**

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**Results**

- **Figure 3A.** Healthy Heart Study geographic distribution. Participant residences are geographically masked.
- **Figure 3B.** No association between t,t-MA and distance from a major roadway. Residential proximity to a major road (annual mean of 10,000 vehicles/day).
- **Figure 4A.** Association between CVD risk and t,t-MA levels for low (Framingham Risk Score (FRS) < 20) and high (FRS ≥ 20) experienced a cardiovascular event; CVD risk categories: Low = 0.14 ± 0.03 (n=44) and 0.19 ± 0.02 (n=156) mg/g creatinine, respectively.
- **Figure 4B.** Association between CVD risk and t,t-MA levels for non-smokers. Mean t,t-MA levels for low and high CVD risk categories in non-smokers (cotinine < 200 µg/g creatinine) were 0.10 ± 0.03 (n=28) and 0.21 ± 0.04 (n=98) mg/g creatinine, respectively.
- **Figure 5A.** Positive association between cotinine and t,t-MA. Cotinine levels were regressed against t,t-MA, verifying the link between smoking and benzene exposure.
- **Figure 5B.** Positive association between t,t-MA and 3-HPMA. t,t-MA levels were regressed against 3-HPMA, further verifying the link between smoking and benzene exposure.
Discussion

Strengths

• Novel investigation of t,t-MA and CVD risk factors
• Large population study
• Individual measure of benzene exposure
• Empirical cigarette smoke exposure measurement

Limitations

• Biomarkers only collected at 1 time point
• Many other benzene metabolites
• Cotinine misclassification
• Possible selection bias
• No ambient benzene measurements

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