METHYLENE BLUE DECREASES MITOCHONDRIAL OXIDATIVE STRESS AND ALLEVIATES CARDIAC DYSFUNCTION IN DIABETES

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Introduction

- **DIABETES MELLITUS** is associated with increased prevalence of cardiovascular morbidity.
- Mitochondrial alteration appears to contribute substantially to the development of cardiac dysfunction in diabetes → **DIABETIC CARDIOMYOPATHY** → HF

**Proposed mechanisms:**
- Fatty acid-induced mitochondrial uncoupling
- Mitochondrial ROS production
- Mitochondrial proteomic remodelling
- Impaired mitochondrial Ca\(^{2+}\)-handling
- Altered mitochondrial biogenesis

*Bugger & Abel, Cardiovasc Res, 2010, 1;88(2):229-40*
Introduction

- Modulation of mitochondrial function - an important therapeutic target
- Of particular interest: pharmacological agents able to prevent ROS production via a redox effect and/or to improve mitochondrial function

E.g.: Methylene blue (MB): a tricyclic phenothiazine compound

A redox active agent with mild redox potential
**Introduction**

MB: artificial electron donor to mitochondria ETC complexes (→ increases energy) and to oxygen (→ prevents superoxide formation):

- in low doses
- without intramitochondrial excessive accumulation

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Objectives:

I. Assessment of the effects of ACUTE administration of MB on mitochondrial function in diabetic rat hearts

Department of Pathophysiology, Center for Translational Research and Systems Medicine, “Victor Babeș” University of Medicine and Pharmacy Timișoara

II. Assessment of the effects of in vivo CHRONIC treatment with MB on cardiac function in diabetic rats

Department of Foundational Sciences, Central Michigan University College of Medicine, USA
II. Material and Methods

2-month old male Sprague Dawley rats

- NORMAL (NO DIABETES) (N=5/group) :
  - NORM_CTRL
  - NORM_MB

- 5 week-diabetic rats – DM (N=5/group) :
  - DM_CTRL
  - DM_MB

- A single intraperitoneal injection of streptozotocin: 50 mg/kg body weight
- NO INSULIN TM!

- Ventricles were sampled for analysis

- Anesthesia: ketamine (30 mg/kg) & xylazine (10 mg/kg) administered i.p.

- Rat heart mitochondria (RHM) were isolated by differential centrifugations at 4°C:

NFBG = non-fasting blood glucose
I. Material and Methods

- mitochondrial respiratory rates → HRR

The SUIT protocol:
- chamber A: \( \text{GMSt2} + \text{DP} + \text{c} + \text{OmySt4} + \text{FETS} + \text{AmaROX} \)
- chamber B: \( \text{S(Rot)St2} + \text{DP} + \text{c} + \text{OmySt4} + \text{FETS} + \text{AmaROX} \)

- ROS generation → Amplex Red
- Sensitivity to \( \text{Ca}_2^+ \)-induced mPTP opening → CRC

→ In the presence/absence of 0.1 µM MB
II. Results

Clinical & biochemical characteristics

<table>
<thead>
<tr>
<th></th>
<th>NORM</th>
<th>DM</th>
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</thead>
<tbody>
<tr>
<td>Weight (grams):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Beginning</td>
<td>513.4 ± 13.8</td>
<td>521.7 ± 24.8</td>
</tr>
<tr>
<td>• End</td>
<td>589.0 ± 25.9</td>
<td>382.5 ± 49.6*</td>
</tr>
<tr>
<td>NFBG (mg/dL)</td>
<td>104.5 ± 4.4</td>
<td>382.5 ± 49.6*</td>
</tr>
</tbody>
</table>

NFBG = non-fasting blood glucose,
* P <0.05 CTL (N=10) vs DM (N=10). Mean ± SEM.
Mitochondrial respiration is increased by MB in normal and diabetic groups.

Mitochondrial respiration is decreased in diabetic vs. normal group regardless the presence of MB.

*P <0.05 DM_CTRL (N=5) vs DM_MB (N=5), and NORM_CTRL (N=5) vs NORM_MB (N=5).

Mean ± SEM.
I. Results

Mitochondrial respiration is increased by MB in normal and diabetic groups.

Mitochondrial respiration is decreased in diabetic vs. normal group regardless the presence of MB.

*P < 0.05 DM_CTRL (N=5) vs DM_MB (N=5), and NORM_CTRL (N=5) vs NORM_MB (N=5). Mean ± SEM.
I. Results

ROS production is increased by MB in mitochondria energized with GM, and decreased in mitochondria energized with (Rot)S in normal and diabetic groups.

Mitochondrial ROS production is increased in diabetic vs. normal group regardless the presence of MB.

*P <0.05 DM_CTRL (N=5) vs DM_MB (N=5), and NORM_CTRL (N=5) vs NORM_MB (N=5).
Mean ± SEM.
I. Results

Sensitivity to Ca\textsuperscript{2+}-induced mPTP opening is NOT modified by MB in normal and diabetic groups

Sensitivity to Ca\textsuperscript{2+}-induced mPTP opening is increased in diabetic group vs. normal group regardless the presence of MB

*P <0.05 DM_CsA (N=4) vs DM_CTRL (N=4), and NORM_CsA (N=4) vs NORM_CTRL (N=4). Mean ± SEM.
Conclusions (I)

- **ACUTE administration** of 0.1 µM MB elicited:
  
  1. A substrate-independent improvement of mitochondrial respiratory function in diabetic and normal rat hearts
  2. A substrate-dependent modulation of ROS production in diabetic and normal rat hearts
  3. No effect on calcium retention capacity in normal and diabetic hearts
Objectives:

I. Assessment of the effects of ACUTE administration of MB on mitochondrial function in diabetic rat hearts

Department of Pathophysiology, Center for Translational Research and Systems Medicine, “Victor Babes” University of Medicine and Pharmacy Timişoara

II. Assessment of the effects of \textit{in vivo} CHRONIC treatment with MB on cardiac function in diabetic rats

Department of Foundational Sciences, Central Michigan University College of Medicine, USA
II. Material and Methods

- 2-month old male Lewis rats

- A single intraperitoneal injection of streptozotocin: 55 mg/kg body weight
- Low doses (~ 2 units twice per week) of NPH insulin – subcutaneously
- MB in drinking water: 10 mg/kg/day

- control: CTL, CTL+MB groups
- 11 week-diabetic rats: DM, DM+MB groups

Day 0

2 weeks: DM

Euthanasia: pentobarbital (100 mg/kg)
LV was sampled for analysis

11 weeks

NFBG = non-fasting blood glucose, FBG = fasting blood glucose
II. Material and Methods

- **Cardiac function** was assessed by two-dimensional guided M-mode, two-dimensional and Doppler flow echocardiography.
- **Systolic and diastolic hemodynamic parameters** were evaluated using a Millar pressure transducer catheter introduced into the LV via the right carotid artery.
- **ROS generation in mitochondria isolated from LV** → Amplex Red

Pressure conductance catheter

Catheter insertion:
- right carotid artery
II. Results

Clinical & biochemical characteristics

<table>
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<tr>
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<th>CTL + MB</th>
<th>DM</th>
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<tbody>
<tr>
<td>Weight (grams):</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>• Beginning</td>
<td>285.0 ± 6.9</td>
<td>282.0 ± 9.9</td>
<td>285.8 ± 4.4</td>
<td>285.8 ± 3.4</td>
</tr>
<tr>
<td>• End</td>
<td>359.0 ± 15.9</td>
<td>369.0 ± 16.5</td>
<td>273.0 ± 3.8*</td>
<td>265.0 ± 11.5*</td>
</tr>
<tr>
<td>NFBG (mg/dL)</td>
<td>98.6 ± 1.3</td>
<td>97.3 ± 4.5</td>
<td>400.2 ± 14.9*</td>
<td>456.8 ± 23.9*</td>
</tr>
<tr>
<td>FBG (mg/dL)</td>
<td>99.0 ± 0.5</td>
<td>95.3 ± 2.3</td>
<td>310.6 ± 10.8*</td>
<td>320.1 ± 8.9*</td>
</tr>
<tr>
<td>Hb A1C (%)</td>
<td>2.5 ± 0.1</td>
<td>2.5 ± 0.1</td>
<td>5.6 ± 0.1*</td>
<td>5.6 ± 0.2*</td>
</tr>
</tbody>
</table>

NFBG = non-fasting blood glucose,
FBG = fasting blood glucose
* P <0.05 CTL (N=7) vs DM (N=7). Mean ± SEM.
## III. Results

### Cardiac structure and function: EXTRATHORACIC ECHOCARDIOGRAPHY

<table>
<thead>
<tr>
<th></th>
<th>CTL</th>
<th>CTL + MB</th>
<th>DM</th>
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</thead>
<tbody>
<tr>
<td><strong>Left ventricular mass (grams)</strong></td>
<td>0.81 ± 0.04</td>
<td>0.79 ± 0.05</td>
<td>0.90 ± 0.03</td>
<td>0.84 ± 0.04</td>
</tr>
<tr>
<td><strong>Heart rate (/min)</strong></td>
<td>363 ± 10</td>
<td>354 ± 7</td>
<td>284 ± 11*</td>
<td>273 ± 14*</td>
</tr>
<tr>
<td><strong>End-diastolic diameter (cm)</strong></td>
<td>0.79 ± 0.02</td>
<td>0.77 ± 0.02</td>
<td>0.84 ± 0.02*</td>
<td>0.81 ± 0.01</td>
</tr>
<tr>
<td><strong>End-systolic diameter (cm)</strong></td>
<td>0.45 ± 0.03</td>
<td>0.45 ± 0.01</td>
<td>0.50 ± 0.03*</td>
<td>0.44 ± 0.01</td>
</tr>
<tr>
<td><strong>Ejection time (msec)</strong></td>
<td>73 ± 2</td>
<td>74 ± 2</td>
<td>105 ± 7*</td>
<td>108 ± 5*</td>
</tr>
<tr>
<td><strong>Isovolumetric (IV) contraction time + IV relaxation time (msec)</strong></td>
<td>31 ± 2</td>
<td>31 ± 1</td>
<td>49 ± 3*</td>
<td>49 ± 3*</td>
</tr>
<tr>
<td><strong>MPI</strong></td>
<td>0.43 ± 0.04</td>
<td>0.42 ± 0.02</td>
<td>0.48 ± 0.05</td>
<td>0.46 ± 0.03</td>
</tr>
<tr>
<td><strong>Fraction shortening</strong></td>
<td>0.44 ± 0.03</td>
<td>0.43 ± 0.01</td>
<td>0.41 ± 0.01</td>
<td>0.45 ± 0.01</td>
</tr>
<tr>
<td><strong>End diastolic volume (ml)</strong></td>
<td>0.52 ± 0.03</td>
<td>0.50 ± 0.03</td>
<td>0.64 ± 0.05*</td>
<td>0.57 ± 0.03</td>
</tr>
<tr>
<td><strong>End systolic volume (ml)</strong></td>
<td>0.09 ± 0.01</td>
<td>0.08 ± 0.01</td>
<td>0.13 ± 0.02*</td>
<td>0.10 ± 0.01</td>
</tr>
</tbody>
</table>

MPI = Myocardial performance index  
* P <0.05 CTL (N=7) vs DM (N=7). Mean ± SEM.
### III. Results

*In vivo LV hemodynamic analyses*

<table>
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<th>DM</th>
<th>DM + MB</th>
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</thead>
<tbody>
<tr>
<td>Maximal volume (µL)</td>
<td>504 ± 29</td>
<td>473 ± 26</td>
<td>624 ± 50*</td>
<td>550 ± 10*</td>
</tr>
<tr>
<td>Minimal volume (µL)</td>
<td>123.6 ± 11.0</td>
<td>105.5 ± 8.4</td>
<td>169.9 ± 21.7*</td>
<td>131.2 ± 10.8**#</td>
</tr>
<tr>
<td>End systolic volume (µL)</td>
<td>132.8 ± 11.4</td>
<td>133.4 ± 14.7</td>
<td>199.5 ± 28.5*</td>
<td>149.6 ± 11.4#</td>
</tr>
<tr>
<td>End diastolic volume (µL)</td>
<td>477.7 ± 6.3</td>
<td>450.8 ± 27.2</td>
<td>579.7 ± 43.3*</td>
<td>486.8 ± 26.1#</td>
</tr>
<tr>
<td>End systolic p. (mmHg)</td>
<td>118.9 ± 4.8</td>
<td>124.1 ± 6.4</td>
<td>98.6 ± 4.5*</td>
<td>109.0 ± 3.3*</td>
</tr>
<tr>
<td>End diastolic p. (mmHg)</td>
<td>6.3 ± 0.5</td>
<td>7.6 ± 0.9</td>
<td>9.3 ± 0.9*</td>
<td>8.8 ± 0.2*</td>
</tr>
<tr>
<td><strong>EJECTION FRACTION (%)</strong></td>
<td>75.6 ± 1.6</td>
<td>78.0 ± 0.9</td>
<td>72.9 ± 2.7*</td>
<td>76.3 ± 1.3#</td>
</tr>
<tr>
<td>+dP/dt (mmHg/sec)</td>
<td>6829 ± 638</td>
<td>7580 ± 559</td>
<td>4464 ± 168*</td>
<td>4870 ± 401*</td>
</tr>
<tr>
<td>-dP/dt (mmHg/sec)</td>
<td>10292 ± 1027</td>
<td>10551 ± 1346</td>
<td>4734 ± 420*</td>
<td>4773 ± 407*</td>
</tr>
</tbody>
</table>

* P <0.05 CTL (N=7) vs DM (N=7), and # p<0.05 DM (N=7) vs DM+MB (N=7). Mean ± SEM.  

*In press*
III. Results

Chronic treatment with MB decreased ROS production in mitochondria energized with palmitoylcarnitine + M / (Rot)S

Mitochondrial ROS production is increased in diabetic vs. normal group

*P <0.05 CTL (N=3) vs DM (N=3), and # p<0.05 DM (N=3) vs DM+MB (N=3).
Mean ± SEM.

In press
Conclusions (II)

- CHRONIC treatment with MB (10 mg/kg/day/ 11 weeks):
  1. Improved cardiac function in the experimental model of diabetic cardiomyopathy
  2. Decreased oxidative stress in the diabetic hearts
Acknowledgments

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Mariana Rosca

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THANK YOU!

KEEP CALM?
NO WAY!
FIGHT LIKE HECK
AND CURE
MITO NOW!!