Endothelial Colony Forming Cells Dysfunction Relates to Cardiovascular Alterations in Preterm Born Adults

Mariane Bertagnolli, PhD
Postdoctoral fellow
Sainte-Justine University Hospital Research Center
Université de Montréal
Canada
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NO CONFLICT OF INTEREST TO DISCLOSE
Preterm birth and hypertension: is there a link?

Luu et al. CMAJ, 2015
Endothelial colony-forming cells

Origins

EPC subtypes

ECFC in vitro

CFU-EC
CD45+
CD14+
KDR-

CAC
KDR+
CD34
CD133

ECFC
CD34+,CD133d
KDR+,CD31+
CD45-,CD14-

Angiogenesis
Hindlimb ischemia

Tissue repair
Neonatal hyperoxia-induced lung injury (BPD)

Asahara et al. AJP Cell Physiol, 2004
Prater et al. Leukemia, 2007
Schwarz et al. ATVB, 2012
EPC and ECFC in premature birth

- Preterm birth and EPC: 18 eligible studies were systematically reviewed.

EPC counts at birth are either similar or increased compared to full term.

Lower EPC counting in cord blood following pregnancy complications.

Cord blood ECFC are dysfunctional in preterm newborns and more susceptible to hyperoxic stress.
Objective

We aim to assess if ECFC function relates to cardiovascular risks in preterm born adults
Subjects enrolment and data collection

• ECFCs isolated from peripheral blood of 30 young adults (21-28 years old) born extremely preterm (<29 gestational weeks) and 30 full term (≥37 gestational weeks)
• Participants were paired by gender, age and socioeconomic status
• Birth and neonatal data were obtained by reviewing birth records
• Cardiovascular assessments: cardiac ultrasound, brachial blood pressure measurements and 24h blood pressure monitoring
## Baseline characteristics of subjects

<table>
<thead>
<tr>
<th></th>
<th>Preterm</th>
<th>Term</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=30</td>
<td>N=30</td>
</tr>
<tr>
<td>Mean age ± SD, years</td>
<td>24.3±2.2</td>
<td>23.4±2.3</td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>11 (37)</td>
<td>11 (37)</td>
</tr>
<tr>
<td>Median height (range), cm</td>
<td>169 (151-189)</td>
<td>170 (155-186)</td>
</tr>
<tr>
<td>Median weight (range), kg</td>
<td>64 (43-100)</td>
<td>68 (53-117)</td>
</tr>
<tr>
<td>Median BMI (range), kg/m²</td>
<td>22 (17-35)</td>
<td>22 (18-35)</td>
</tr>
<tr>
<td>Smoking, n</td>
<td>7 (23)</td>
<td>6 (20)</td>
</tr>
<tr>
<td>Systolic blood pressure (mean ± SD), (mmHg)</td>
<td><strong>125 ± 10</strong>*</td>
<td>119 ± 7</td>
</tr>
<tr>
<td>Diastolic blood pressure (mean ± SD), (mmHg)</td>
<td><strong>74 ± 6</strong>*</td>
<td>71 ± 5</td>
</tr>
<tr>
<td>Mean VO2max ± SD, mL/min</td>
<td>89 ± 27</td>
<td>92 ± 24</td>
</tr>
</tbody>
</table>

SD, standard deviation; BMI, body mass index; VO2, maximal oxygen consumption
# Clinical neonatal characteristics

<table>
<thead>
<tr>
<th>Maternal</th>
<th>Preterm n=30</th>
<th>Term n=30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational hypertension</td>
<td>4 (13)</td>
<td>0</td>
</tr>
<tr>
<td>Gestational diabetes</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PROM (%)</td>
<td>8 (27)</td>
<td>0</td>
</tr>
<tr>
<td>Antenatal steroids (%)</td>
<td>6 (21)</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Neonatal</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean GA ± SD, weeks</td>
<td>27±1</td>
<td>39±1</td>
</tr>
<tr>
<td>Mean Birth weight ± SD, g</td>
<td>1020 ± 218</td>
<td>3309 ± 336</td>
</tr>
<tr>
<td>Median days of ventilation (range)</td>
<td>14 (0-53)</td>
<td>-</td>
</tr>
<tr>
<td>Median days of supplemental O2 (range)</td>
<td>22 (0-130)</td>
<td>-</td>
</tr>
<tr>
<td>Median days of hospitalization (range)</td>
<td>70 (40-139)</td>
<td></td>
</tr>
<tr>
<td>BPD (%)</td>
<td>6 (21)</td>
<td>-</td>
</tr>
<tr>
<td>PDA (%)</td>
<td>9 (30)</td>
<td></td>
</tr>
<tr>
<td>ROP (%)</td>
<td>3 (10)</td>
<td>-</td>
</tr>
<tr>
<td>IVH (%)</td>
<td>5 (18)</td>
<td>-</td>
</tr>
<tr>
<td>Postnatal steroids (%)</td>
<td>5 (18)</td>
<td>-</td>
</tr>
<tr>
<td>Infections (%)</td>
<td>2 (7)</td>
<td>-</td>
</tr>
</tbody>
</table>

PROM, premature rupture of membranes; GA, gestational age; SD, standard deviation; O2, oxygen; BPD, bronchopulmonary dysplasia; PDA, patent ductus arteriosus; ROP, retinopathy of prematurity; IVH, intraventricular hemorrhage.
ECFC isolation from peripheral blood

- 25 mL of peripheral blood
- Participants were fasting for 12 hours
- Ficoll-Plaque PLUS (GE Healthcare)
- Mononuclear cells (PBMC) were separated by density gradient

Density = $2 \times 10^5$ cells/cm²
Rat tail collagen (Corning)
3-4 days
Endothelial cell growth media (Lonza)

Colonies are formed in 7-30 days

Frequency distribution of ECFC colony formation and growth (62% of total participants)
ECFC function in preterm and term born adults

ECFC proliferative (A) and tube formation (B) properties negatively correlate with ECFC colony growth in preterm-born subjects (preterm=18 vs term=19).

Cell proliferation (Click-it EdU)

Tube formation Matrigel assay
ECFC dysfunction relates with higher brachial (A) and day-time (B) systolic arterial pressure, as well as with increased left ventricular mass (C) in individuals born prematurely with late ECFC colony growth. Two-way ANOVA, mean±SEM.
Preterm born adults exposed to severe neonatal complications have dysfunctional ECFCs

**Figure A** – Time to ECFC colony growth in preterm-born subjects according to time of exposure to supplemental oxygen (O₂) as newborns. T-test, mean±SEM.

**Figure B** – Frequency distribution of preterm born subjects that were exposed to more severe neonatal complications as newborns.

*BPD, bronchopulmonary dysplasia* – O₂ at 36 weeks postmenstrual age; *PDA, patent ductus arteriosus* – treated with indomethacin or ligation; *IHV, intraventricular hemorrhage*; *ROP, retinopathy of prematurity*. 
Summary and Clinical Implications

Our findings demonstrate, for the first time, that ECFC dysfunction in preterm-born adults significantly relates with important cardiovascular risk factors, such as higher blood pressure and increased left ventricular mass.

Exposure to a proxy of severe neonatal complications relates with later in life ECFC dysfunction in preterm born adults.

Perspectives

Use of circulating ECFCs for the investigation of:

• Molecular mechanisms related with prematurity.

• The effects of clinical interventions, such as exercise and anti-hypertensive drugs on ECFC function, as well as on its relationship with clinical cardiovascular characteristics.
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  Cardiology Unit, CHUSJ
- Dr Bernard Thebaud, M.D.
  Ottawa Hospital Research Institute

Mariane Bertagnolli, Ph.D.
Postdoctoral fellow, University of Oxford
mariane.bertagnolli@cardiov.ox.ac.uk

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