HUMAN ADIPOSE-DERIVED STEM CELLS OBTAINED FROM LIPOASPIRATES ARE HIGHLY CYTOGENOTOXIC SUSCEPTIBLE TO HYDROGEN PEROXIDE

Alencar Kolinski Machado, Francine Carla Cadoná, Verônica Farina Azzolin, Gustavo Scola, Marta Maria Medeiros Frescura Duarte, José Raul Pinto Saldanha, Luana Lenz, Thais Doeler Algarve, Ana Cristina Andreazza, Ivana Beatrice Mânica da Cruz.

Chicago, march 24th, 2015.
INTRODUCTION

STEM CELLS

EMBRYONIC STEM CELLS

Proliferation  Differentiation
INTRODUCTION

STEM CELLS

Cellular renovation and tissue repair

Apoptosis → Cellular Renovation

Proliferation | Differentiation

Maintaining the homeostasis
INTRODUCTION

STEM CELLS

Chronic Degenerative Diseases

Therapeutic Strategies!!!

Stem Cells (SCs) on Medicine Alternative

Advance in the SCs researches
INTRODUCTION

STEM CELLS

Detection of specific receptors

Recognition of SCs in the adult body

Clinic studies and experimental researches

Facility to obtain

Positive therapeutic response

Mesenchymal Stem Cells
INTRODUCTION

STEM CELLS

In vivo

Adult SCs

Cell renovation

Differentiation

There isn’t senescence

Cell aging

In vitro

Adult SCs

Premature senescence

Proliferation and differentiation

Inability to use for regenerative therapy
- Stem cells use: high number of cells
  - Long time in cell culture conditions
  - Cellular senescence

PROBLEM???
METHODS

ASCs ISOLATION AND CULTURE

Lipoaspirates → PBS Washes → Enzymatic Digestion

CO₂ Incubator (5%), 37°C
H₂O₂ EXPOSITION

ASCs at 4th passagem

1-1000 μM H₂O₂

2 h

ACUTE EXPOSITION

CYTOGENOTOXIC EFFECT

EXPERIMENTAL EVALUATIONS

(ZHANG et al., 2012)
**METHODS**

**Free dsDNA quantification**

**Cytotoxicity Evaluation**

Cell death and membrane rupture → Free dsDNA on medium → PicoGreen → Fluorescence

480nm – excitation

520nm - emission

**Caspases 1, 3 e 8 determination**

Quantikine Imunoassay Caspase Human® → 450 nm
**METHODS**

**Dichlorofluorescein assay (DCF)**

**ROS quantification**

DCFH-DA → DCFH → DCF

Esterase enzymes

**Fluorescence**

488nm – excitation
525nm – emission

(AHN, 1996)

**Oxidative Biomarkers**

**TBARS**
Lipid peroxidation

(OHKAWA et al., 1979)

**SOD activity**
Epinephrine

(BOVERIS e CADENAS, 1997)

**CAT activity**

H$_2$O$_2$

(AEBI, 1984)
DNA comet alkaline assay

Genotoxicity evaluation

- Cell lysis
- Electrophoresis
- Staining with silver nitrate

Cell morphology evaluation

- Fixation
- Giemsa staining

(SINGH et al., 1979; NADIN et al., 2001)

Fronza e colaboradores (2010)
RESULTS

ASCs isolation

Cytotoxic effect and cell morphology alterations
RESULTS

Caspases 1, 3 e 8 induction
Table 1: Oxidative metabolism parameters of ASCs exposed to different concentrations of H$_2$O$_2$.

<table>
<thead>
<tr>
<th>H2O2 Concentration (µM)</th>
<th>(0)</th>
<th>(1)</th>
<th>(3)</th>
<th>(10)</th>
<th>(30)</th>
<th>(100)</th>
<th>(200)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROS</td>
<td>100.3 ± 7.5$^a$</td>
<td>106.0 ± 9.7$^b$</td>
<td>108.8 ± 11.9$^b$</td>
<td>166.8 ± 23.5$^c$</td>
<td>165.1 ± 22.0$^c$</td>
<td>185.8 ± 31.3$^d$</td>
<td>368.3 ± 11.1$^e$</td>
</tr>
<tr>
<td>TBARS</td>
<td>100.4 ± 5.3$^a$</td>
<td>305.4 ± 18.0$^b$</td>
<td>656.3 ± 40.7$^c$</td>
<td>776.3 ± 40.7$^d$</td>
<td>838.2 ± 28.6$^e$</td>
<td>915.0 ± 90.6$^f$</td>
<td>1823.0 ± 35.2$^g$</td>
</tr>
<tr>
<td>CAT</td>
<td>100.1 ± 3.3$^a$</td>
<td>152.3 ± 11.5$^b$</td>
<td>151.5 ± 10.5$^b$</td>
<td>154.1 ± 8.8$^b$</td>
<td>159.3 ± 10.8$^b$</td>
<td>156.3 ± 7.6$^b$</td>
<td>156.3 ± 7.6$^b$</td>
</tr>
<tr>
<td>SOD</td>
<td>100.0 ± 1.5$^a$</td>
<td>211.7 ± 25.8$^b$</td>
<td>89.9 ± 0.8$^c$</td>
<td>22.1 ± 3.5$^d$</td>
<td>24.9 ± 2.8$^d$</td>
<td>30.5 ± 6.3$^d$</td>
<td>34.12±4.6$^d$</td>
</tr>
</tbody>
</table>
RESULTS

Genotoxic effect

Damages

0 1 2 3 4

DNA damage index

Concentrations H$_2$O$_2$ (µM)

0 1 3 10 30 100 200

a b
DISCUSSION

Oxidative Metabolism: proliferation, differentiation and senescence of SCs

Murry et al (1986): were the first to study the effect of H$_2$O$_2$ in adult stem cells;

Zhang et al (2012): *in vitro* effect of H$_2$O$_2$ in the cell expansion adult stem cells;

Choe et al (2012): differentiation to osteoblasts from SCs using H$_2$O$_2$.
Our results...

- ASCs high sensibility to the increase of ROS;
- 100% of death from 200 µM treatment;
  - Apoptotic process activation: caspase 1, 3 and 8;
DISCUSSION

Our results...

- Significant increase in the CAT activity;

- SOD modulation not dose dependent manner, agreeing with Gottfredsen et al (2013);

- Embryonic stem cells + H$_2$O$_2$ = SOD inhibition

- Significant DNA damages, with a decrease of damages 0;

- Cytomorphologics alterations.

(Enzymatic function: GAETANI et al., 1996)
Despite the H$_2$O$_2$ is able to increase the adult SCs proliferation, but the acute exposition, specially from 3µM, of this kind of treatment is also able to conduct to citotoxic effects, oxidative stress and mainly genomic damages.
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