



**Valutazione della tossicità dell'aria attraverso test
in vitro su colture cellulari di
endotelio e fibroblasti esposte con strumento
Cultex**

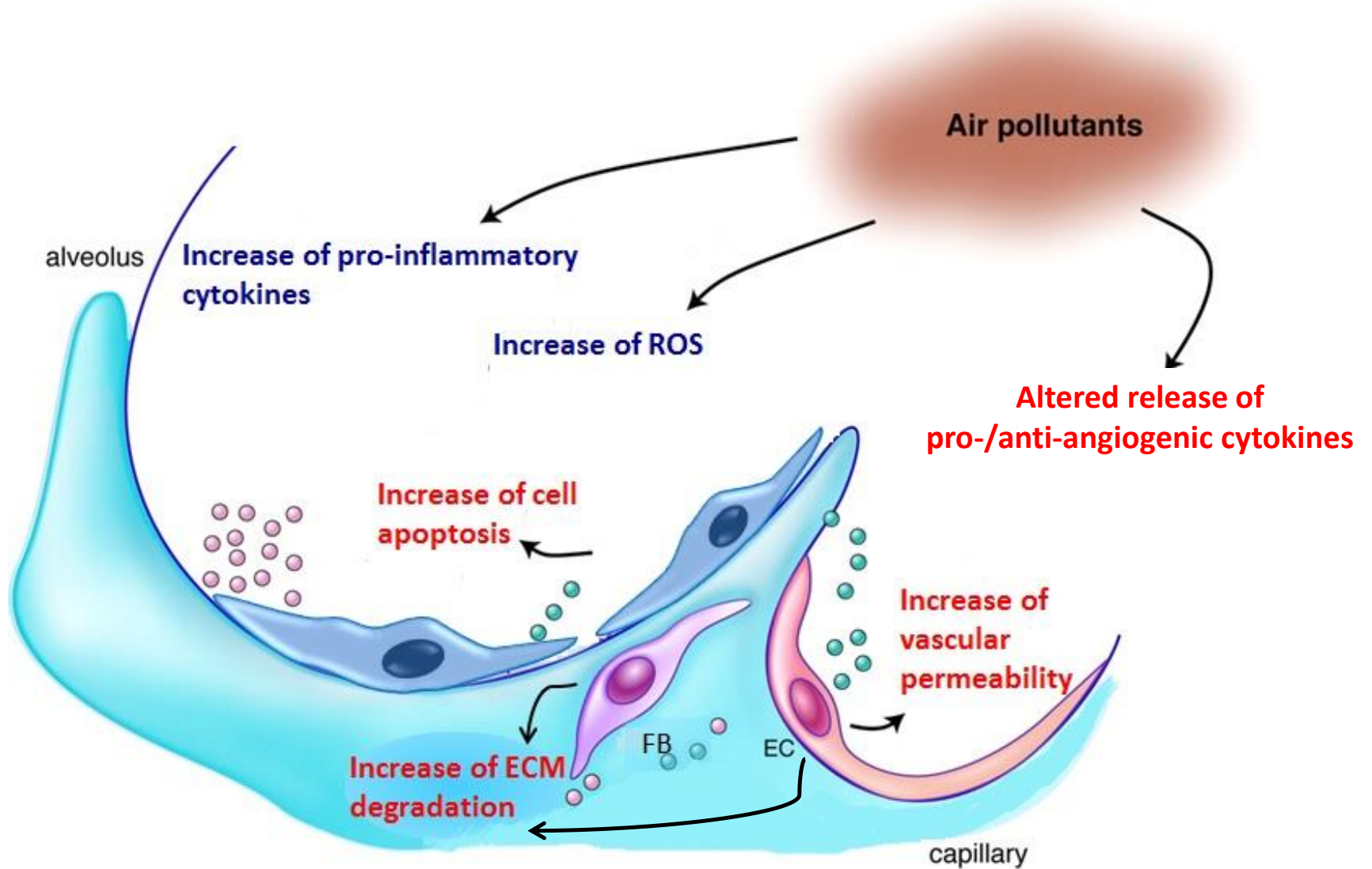
Progetto Jonico-Salentino

Kick off meeting

2 marzo 2016

Prof. Angelo Vacca

The role of air pollution in alveolus and pulmonary microvasculature



RESEARCH

Open Access

Particulate matter air pollution disrupts endothelial cell barrier via calpain-mediated tight junction protein degradation

Ting Wang¹, Lichun Wang¹, Liliana Moreno-Vinasco¹, Gabriel D Lang¹, Jessica H Siegler¹, Biji Mathew¹, Peter V Usatyuk¹, Jonathan M Samet², Alison S Geyh³, Patrick N Breyse³, Viswanathan Natarajan¹ and Joe G N Garcia^{1*}

PM sample (0.1-0.3 μm diameter) tested on human lung microvasculature ECs:

- Pro-inflammatory activity
- Increase of vascular permeability *in vitro* and *in vivo* via ROS generation
- Increase of NF-Kb signaling pathway
- Distruption of tight junctions

Aims of the study

Evaluate the cytotoxic effects of air pollution on Human Pulmonary Artery Endothelial Cells (HPAECs) and on human pulmonary fibroblasts (WI-38)

❖ HPAECs

- Proliferation
- Apoptosis
- Release of pro-angiogenic (VEGF, FGF-2, HGF-SF, IL-6), anti-angiogenic (IL-12, INF α , INF γ) and pro-inflammatory (IL-1 β , IL-6, IL-18, TNF α) cytokines
- MMPs release
- Angiogenic abilities (spontaneous migration, chemotaxis, spreading, adhesion, *in vitro* angiogenesis)
- *In vitro* vascular permeability
- Expression of cell adhesion molecules

❖ WI-38

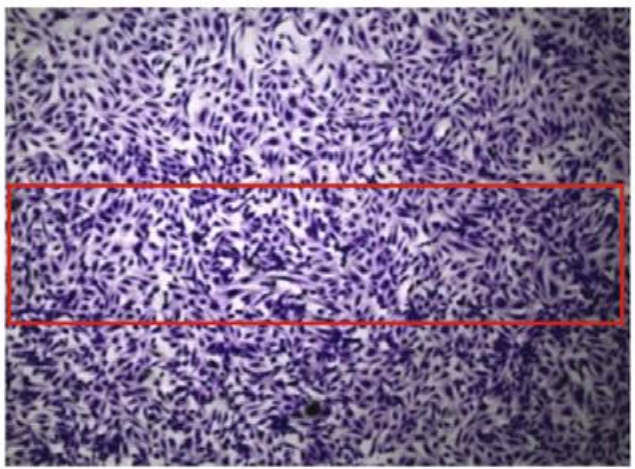
- Activation (FSP1, α SMA, FAP)
- Proliferation
- Apoptosis
- Release of pro-/anti- angiogenic and pro-inflammatory cytokines

Cytotoxic effects of Hemilipin, a PLA₂ from Hemiscorpius Leptorum venom, on ECs (I)

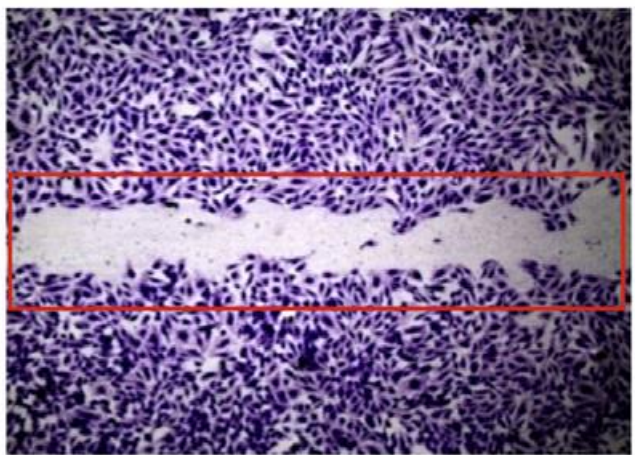
Jridi - Vacca , Toxicon 2015

Wound-healing assay

Ctrl

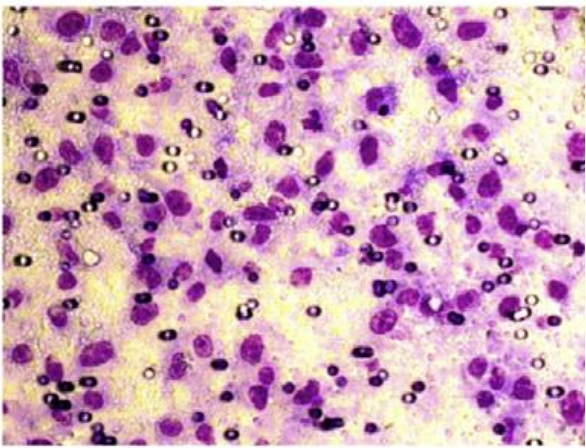


Hemilipin

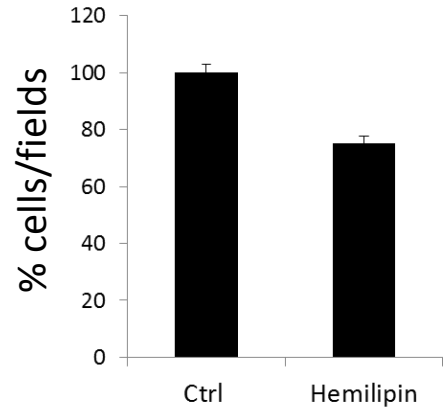
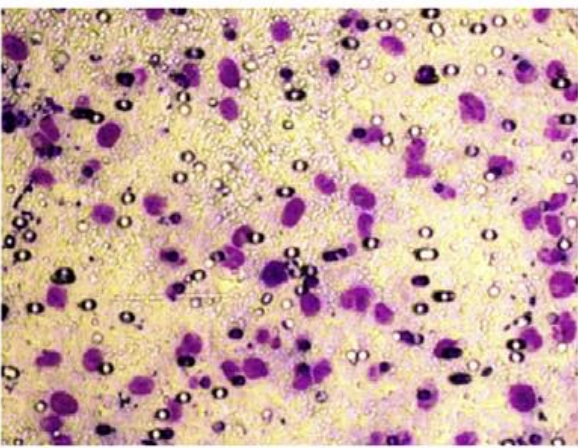


Chemotaxis assay

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Hemilipin

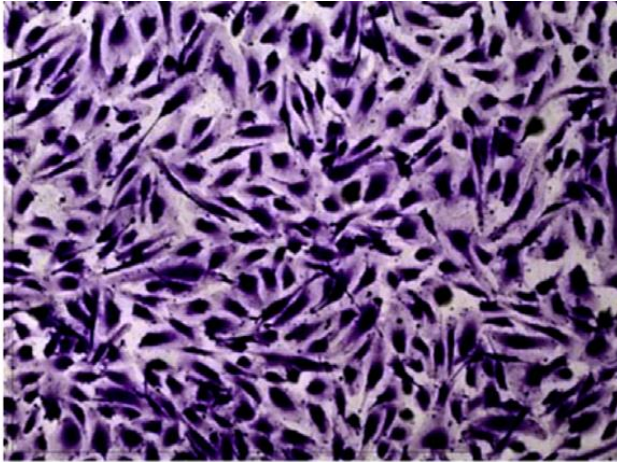


Cytotoxic effects of Hemilipin, a PLA₂ from Hemiscorpius Leptorum venom, on ECs (II)

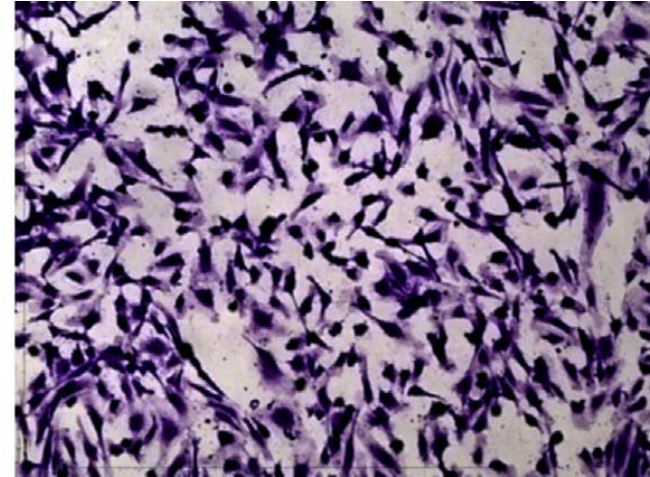
Jridi - Vacca , Toxicon 2015

Spreading assay

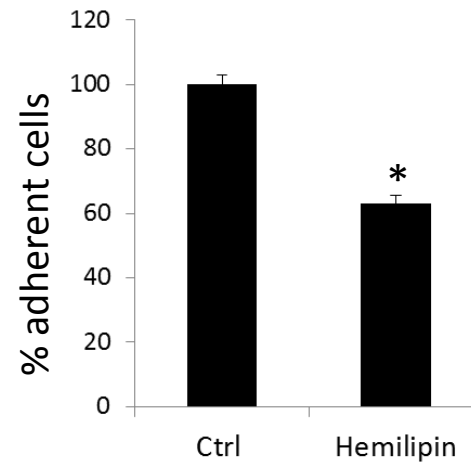
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Hemilipin



Adhesion assay

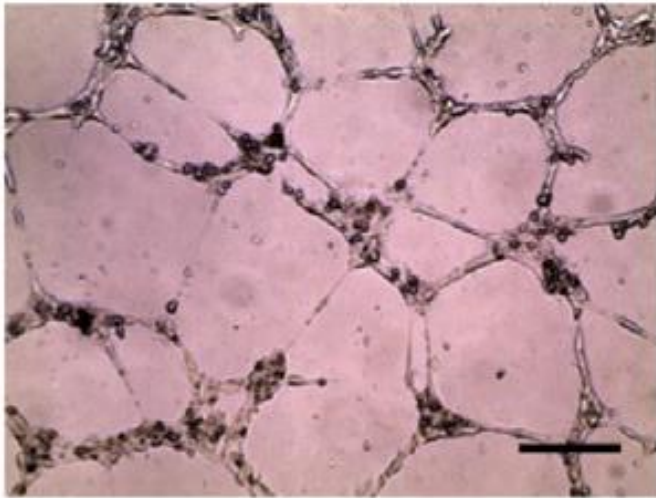


Cytotoxic effects of Hemilipin, a PLA₂ from Hemiscorpius Leptorum venom, on ECs (III)

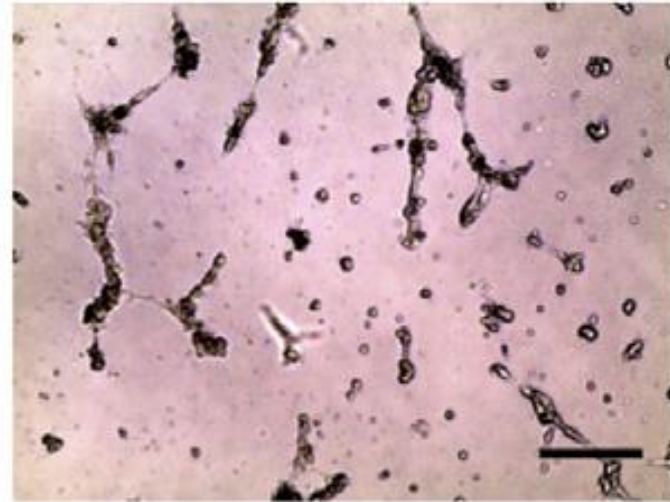
Jridi - Vacca , Toxicon 2015

In vitro angiogenesis assay (Matrigel®)

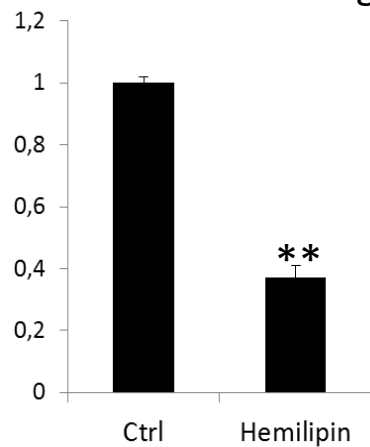
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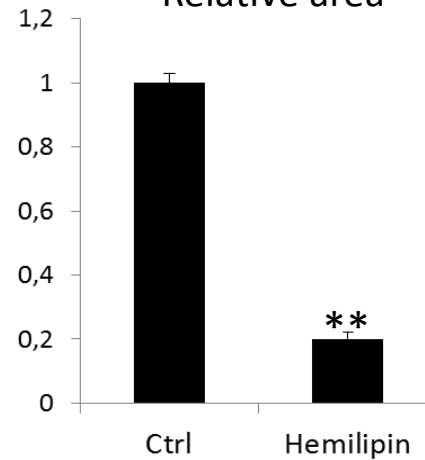
Hemilipin



Relative vessel length



Relative area



Aims of the study (II)

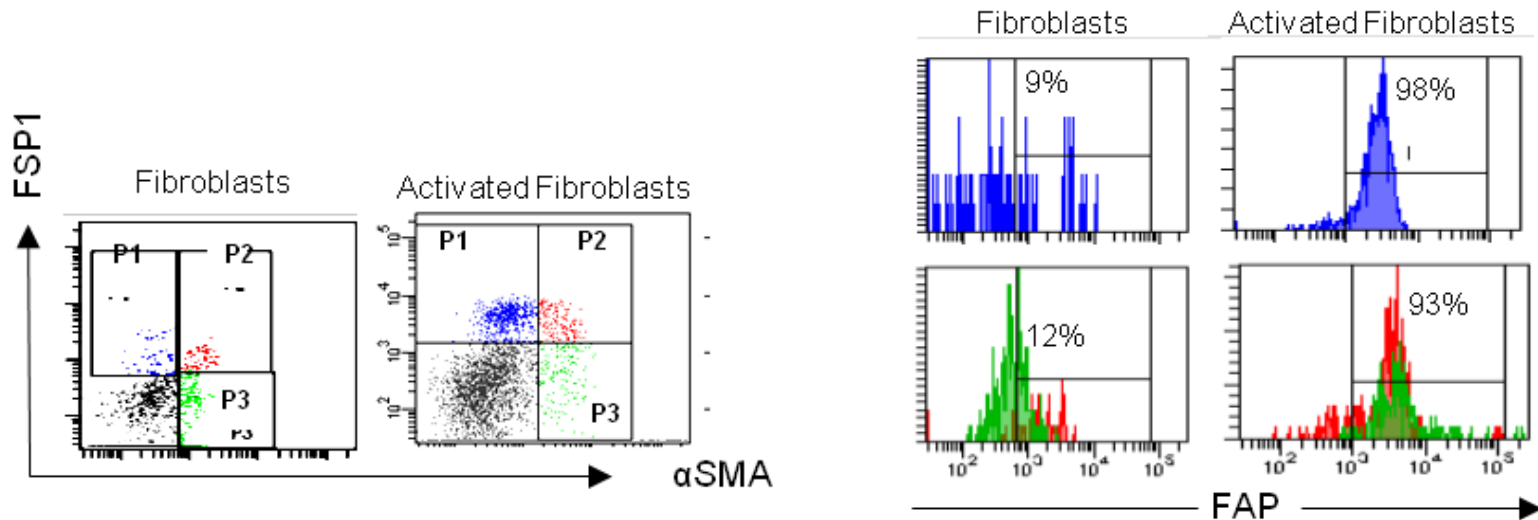
Evaluate the cytotoxic effects of air pollution on Human Pulmonary Artery Endothelial Cells (HPAECs) and on human pulmonary fibroblasts (WI-38)

❖ WI-38

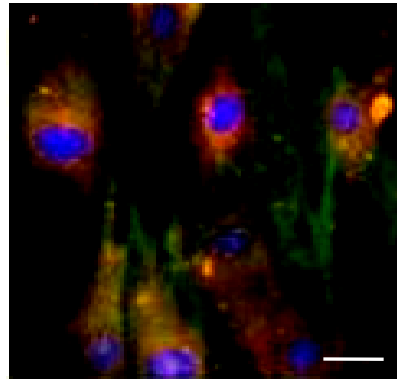
- Activation (FSP1, α SMA, FAP)
- Proliferation
- Apoptosis
- Release of pro-angiogenic (VEGF, FGF-2, HGF-SF, IL-6), anti-angiogenic (IL-12, $\text{INF}\alpha$, $\text{INF}\gamma$) and pro-inflammatory (IL-1 β , IL-6, IL-18, $\text{TNF}\alpha$) cytokines

Phenotypic analysis of Activated Fibroblasts

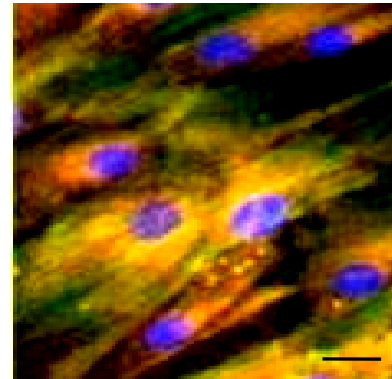
Frassanito - Vacca , Leukemia 2014



Fibroblasts



Activated Fibroblasts



FSP1/ α SMA/DAPI

Increased expression of FSP1, α SMA and FAP in Activated Fibroblasts