ARE STATINS TOXIC?
A "DENTISTRY" PERSPECTIVE

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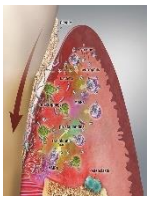
INTRODUCTION

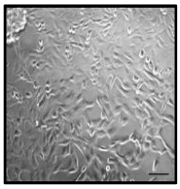
Periodontal diseases (PD) are a set of chronic disorders of infectious and inflammatory nature, highly prevalent world-wide, caused by specific microorganisms able to stimulate an excessive immune-inflammatory response, triggering the progressive destruction of periodontal tissue. (Carrillo *et al.* 2006).
Statins, drugs commonly used in the control of dyslipidemias, have additional effects ("pleiotropic effects"): modulation of immunologic/inflammatory response(s), inhibition of osteoclast activity and the dose-dependent stimulation of bone formation, anti-bacterial and anti-fungal effect, anti-oxidant effect, *etc.* (de Araujo *et al.* 2013; Dalcico *et al.* 2013).
Evidence to date suggests a potential role and benefit of statins (use) against periodontitis.

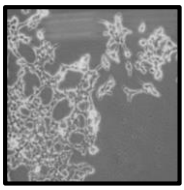



OBJECTIVE

Evaluate the Dose-dependant Viability and/or Cytotoxicity of Atorvastatin (ATV) and Simvastatin (SMV) on Epithelial and Fibroblast Cell lines.

METHODS

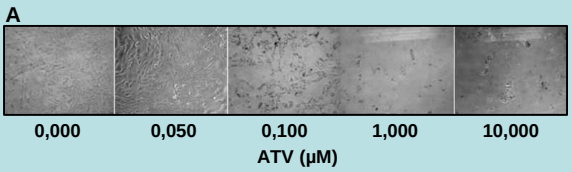

NIH/3T3 (murine fibroblast) cells

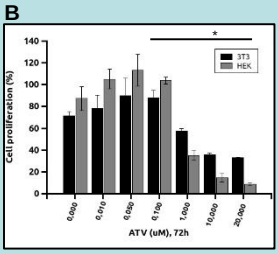

HEK293 (human epithelial) cells

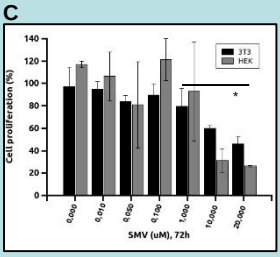
Cell Culture. NIH/3T3 (murine fibroblast) and HEK293 (human epithelial) cells were cultured for 24 hours in DMEM medium supplemented with 10% FBS. Cells were then treated for another 72 hours with the different concentrations of ATV and SMV.

Viability/Cytotoxicity Assays. Cell viability was evaluated using a commercially-available mitochondrial-activity based kit (PrestoBlue/LifeTechnologies). Cytotoxicity was determined via staining the cells with AnexinV and DAPI (early-apoptosis and necrosis markers) for detection and quantification using flow cytometry.

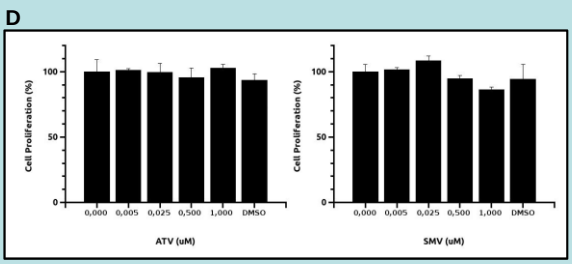
RESULTS

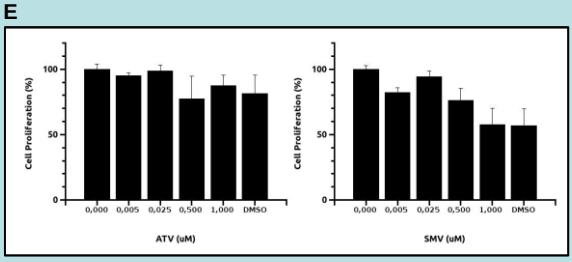




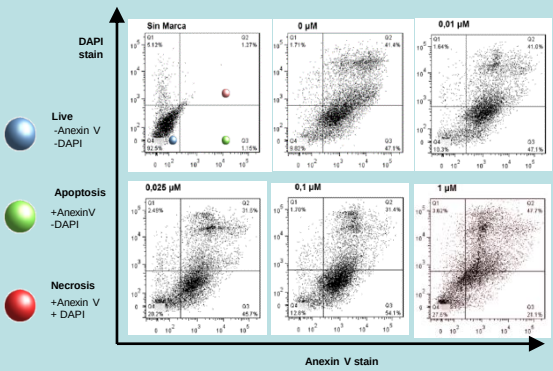


Prospective, full range of statin doses viability. A. Representative image sequence of NIH/3T3 cell line stimulated with a prospective range of ATV doses (0.00 to 10.00 µM). **B.** Column chart showing NIH/3T3 and HEK-293 cell viability at different doses of ATV. **C.** Column chart showing NIH/3T3 and HEK-293 cell viability at different doses of SMV. * **ANOVA $\rho < 0.05$**.

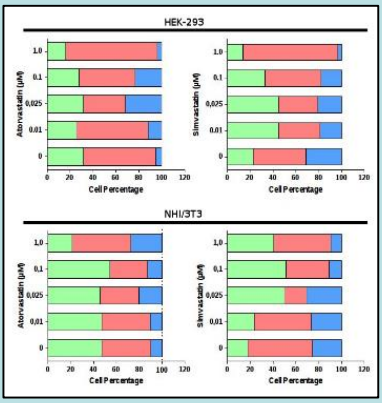




Cell viability at low doses of statins. Cell viability was evaluated by the PrestoBlue kit, at doses of statins between 0.00 and 1.00 µM. Column charts show the effect of both statins (ATV and SMV) in **D.** NIH/3T3 cells and **E.** HEK-293 cells.



Cytotoxicity assay by Flow Cytometry. Cells were cultured and stimulated for 72 h with different doses of ATV, then were detached by 1% Tripsin and suspended in PBS buffer. Cell suspension was stained with Anexin V (apoptosis marker) and DAPI (necrosis marker) for 15 minutes at RT. After the staining process, cells were measured by Flow Cytometry. Point dispersion charts shown the distribution of 10.000 cells on four squares **(Q1 and Q2: Necrotic/death cells ; Q3: Apoptotic live cells; Q4: Live cells)**.



Statins cytotoxic effect measure by Flow Cytometry. Hek-293 (upper panel) and NIH/3T3 (down panel) cells were stimulated with 0 to 1 µM doses of statins. Live, apoptotic and necrotic cells (death) are color-coded as in the figure above.

CONCLUSIONS

Understanding dose-responsiveness of statins is vital. While concentrations below 0,1 µM of ATV or SMV seem cyto-safe and compatible, therapeutic (clinical) potential; especially against periodontal disease remains to be tackled: a subject of ongoing research at our BioMAT'X laboratories in Chile. zhaidar@uandes.cl