# Aβ1-42 binding and uptake by primary human astrocytes *in vitro*:

# Effects of α1-Antichymotrypsin

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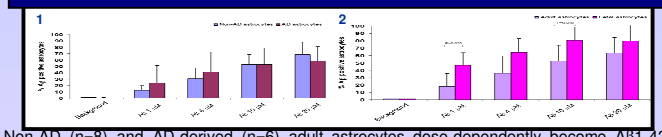
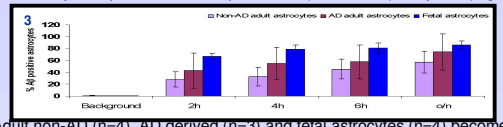
## BACKGROUND

Imbalance between the production and clearance of the amyloid β-peptide (Aβ) is a key event in the Alzheimer's disease (AD) pathogenesis[1]. Alpha1-antichymotrypsin (ACT) might influence the Aβ fibrillogenesis[2,3], biological effects[4,5] and clearance[6] leading to enhanced Aβ deposition in the brain[7]. Activated astrocytes are found surrounding amyloid plaques in AD brains[8] but their role in AD pathogenesis is still poorly understood. These reactive cells over-express ACT[9] and are able to release pro-inflammatory mediators[10]. Recent evidence also suggests that rodent astrocytes may internalize and degrade extracellular Aβ[11]. If also human primary astrocytes are capable of degrading Aβ1-42, is still to be determined.

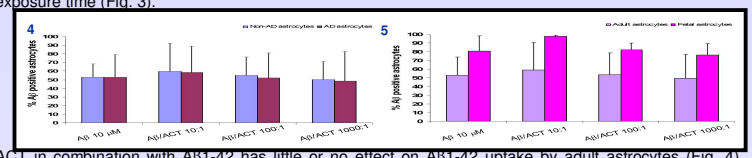
## RESULTS



Non-AD (n=8) and AD-derived (n=6) adult astrocytes dose-dependently become Aβ1-42 positive in a similar manner upon o/n incubation (Fig. 1). Significantly more fetal astrocytes than adult astrocytes become Aβ1-42 positive when exposed to 1 µM and 10 µM Aβ1-42(Fig. 2)



Adult non-AD (n=4), AD derived (n=3) and fetal astrocytes (n=4) become Aβ1-42 positive depending on exposure time (Fig. 3).

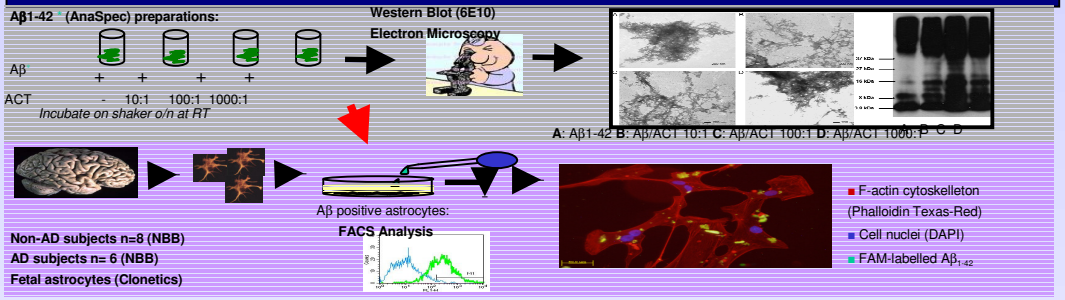


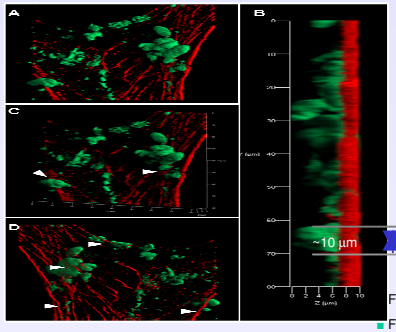
ACT in combination with Aβ1-42 has little or no effect on Aβ1-42 uptake by adult astrocytes (Fig. 4), whereas 17.34 % more fetal astrocytes become Aβ1-42 positive upon o/n treatment with 10 µM Aβ/ACT at a 10:1 molar ratio (Fig. 5)

## OBJECTIVE

- Can primary human fetal and adult astrocytes ingest Aβ1-42 *in vitro*?
- Do primary human fetal and adult astrocytes differ in *in vitro* Aβ1-42 uptake?
- Does α1-antichymotrypsin interfere with the Aβ uptake by primary human fetal and adult astrocytes?
- Is there a pro-inflammatory chemokine monocyte chemoattractant protein-1 (MCP-1) response to treatment with Aβ1–42 and Aβ/ACT combinations?

## METHODS



Confocal Laser Scanning Microscopy optical sectioning (Z-scanning (10 µm stack) was performed on astrocytes incubated with FluoAβ (green) and labelled with TR conjugated phalloidin (F-actin, red) (Fig. 6).

Images show one Z-stack processed for 3D reconstruction. (**A**) View from above, FluoAβ aggregates of various size (green) are seen located on the cell surface, on top of the F-actin cytoskeleton.

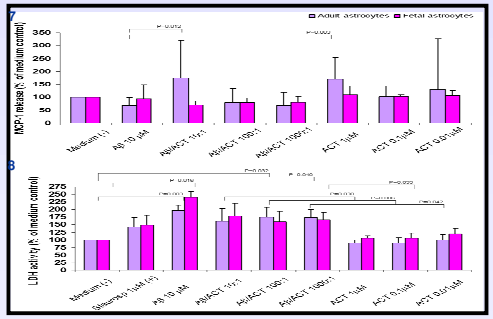
(**B**) View (rotated 90º) of the astrocyte cell layer (10 µm) (red) where different size FluoAβ aggregates (green) are confirmed to preferentially be located on the cell surface (arrows).

(**C**) View diagonally from the side with FluoAβ aggregates seen intermingled with F-actin (arrows), indicating intracellular uptake.

(**D**) View from inside the cell (from below) showing various size FluoAβ aggregates (green) being intermingled with the F-actin (arrows).

F-actin cytoskelleton (Phalloidin Texas-Red)

FAM-labelled $Aβ_{1-42}$



The MCP-1 release from treated versus untreated cells was largely unaffected in both adult and fetal astrocytes (Fig. 7). However, in adult astrocytes the MCP-1 release was enhanced upon 10 µM Aβ/1 µM ACT (10:1) treatment versus 10 µM Aβ alone. This difference might be mediated by the effect of ACT itself.

Treatment with 10 µM Aβ o/n had a significant cytotoxic effect on both fetal and adult astrocytes (Fig. 8). In addition, the combinations of Aβ/ACT had a significantly more toxic effect on the astrocytes compared to the different concentrations of ACT alone.

## CONCLUSIONS

- Primary human astrocytes are, during cytotoxic conditions, able to bind and take up Aβ1-42 *in vitro*, without a pro-inflammatory response
- Human adult astrocytes derived from non-AD and AD subjects become Aβ1-42 positive upon exposure, in a similar manner, whereas a greater percentage of human fetal astrocytes become Aβ1-42 positive upon 1 µM and 10 µM o/n treatment with Aβ1-42
- ACT has no or little effect on Aβ1-42 uptake by adult astrocytes whereas the uptake by fetal cells might be enhanced
- Enhanced MCP-1 release upon Aβ/ACT co-treatment versus Aβ alone in adult astrocytes, might be mediated by ACT itself

**Literature:** [1]Hardy and Higgins 1992. [2]Fraser et al 1993. [3]Eriksson et al 1995. [4]Sun et al 2002. [5]Baker et al 2007. [6]Abraham et al 2000. [7]Mucke et al 2003. [8]Veerhuis et al 2003. [9]Abraham 2001. [10]Dong and Benveniste 2001. [11]Wyss-Coray et al 2003.