Are endogenous cardiotonic steroids responsible of the trophoblastic impairment associated with preeclampsia?

Unusual investigations for unusual Stem Cells.

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Introduction

Preeclampsia is a **sever widespread pathology** occurring only during pregnancy and affecting both the mother and the conceptus (5-8% of all pregnancies; 20% of fetal and pregnancy associated mortality, up to 80% in emerging countries). Principal causes of death are **pulmonary oedemas**, **heart attacks** and **cerebral ischemia** (Bellamy, 2007; Hladunewich, 2007).

The understanding of this rapidly progressive condition is dramatically complicated by the extremely **late detection of macroscopic symptoms** (hypertension and proteinury) **compared to the defect in the invasion of trophoblast** (Meads, 2008).

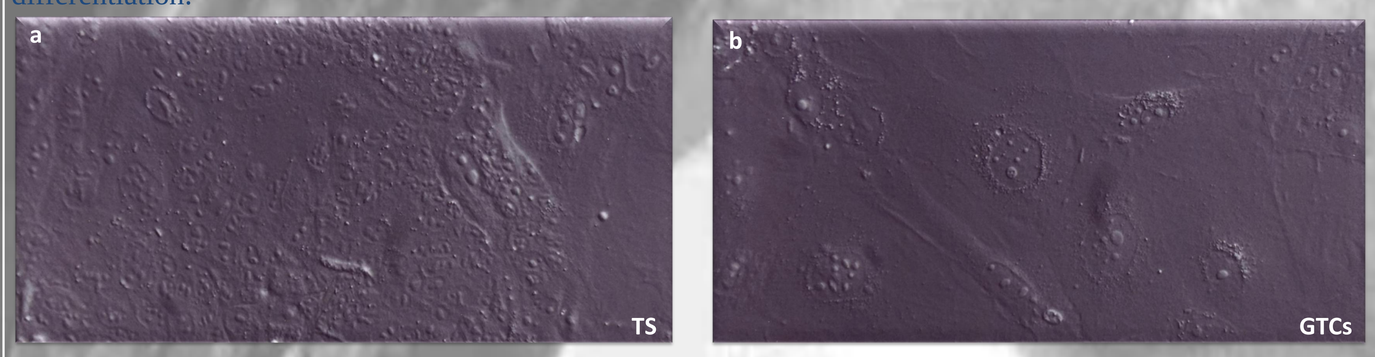
Despite some observations of an increase probability to develop preeclampsia in case of diabetes and obesity, the key feature of this pathology is an **abnormally elevated concentration** of endogenous inhibitors of Na/K-ATPase (e.g. marinobufagenin and ouabain like molecule, also called **cardiotonic steroids** or CTS; Bagrov, 2008). As recent studies point out the sodium pump as a versatile signal transducer involved in cell adhesion, polarity, migration and proliferation (Rajasekaran, 2001, 2003; Barwe, 2005; Soshani, 2005; Vagin, 2006), CTS should not be considered anymore as simple ionotropic modulator.

The study of the CTS's effects on the trophoblast invasion / proliferation is a critical step to discover early biomarkers of preeclampsia and represents the aim of this work in progress.

Project and first results

In vitro models

Permanent trophoblast stem cell lines (TS, a) were isolated from mouse blastocysts and cultured in a medium complemented with FGF4 and TGFβ1 (Tanaka *et al.*, 1998; Erelbacker *et al.*, 2004). Removing these factors initiates the differentiation of TS into Giant Trophoblast Cells (GTC, b). Epithelial to mesenchymal transition (EMT), invasiveness acquisition together with DNA endoreplication are the main features of this differentiation.



The effects of CTS on GTC invasion / differentiation will be evaluated by confocal microscopy (fig. 1), computer assisted videomicroscopy (fig. 3) and by metabonomics (fig. 4).

Prior to studying CTS, we describe for the first time the localisation and expression of Na/K-ATPase isoforms and several markers associated with EMT (e.g. Na/K-ATPase α1, α3, β1 and e-cadherin) during the differentiation of TS cells in GTCs (from d0 to d6, fig. 1). A strong correlation with their roles in cell polarity and motility acquisition was observed (fig. 2; videomicroscopy – *data not shown*). Eventual relocation of the Na/K-ATPase induced by CTS will be shortly evaluated.

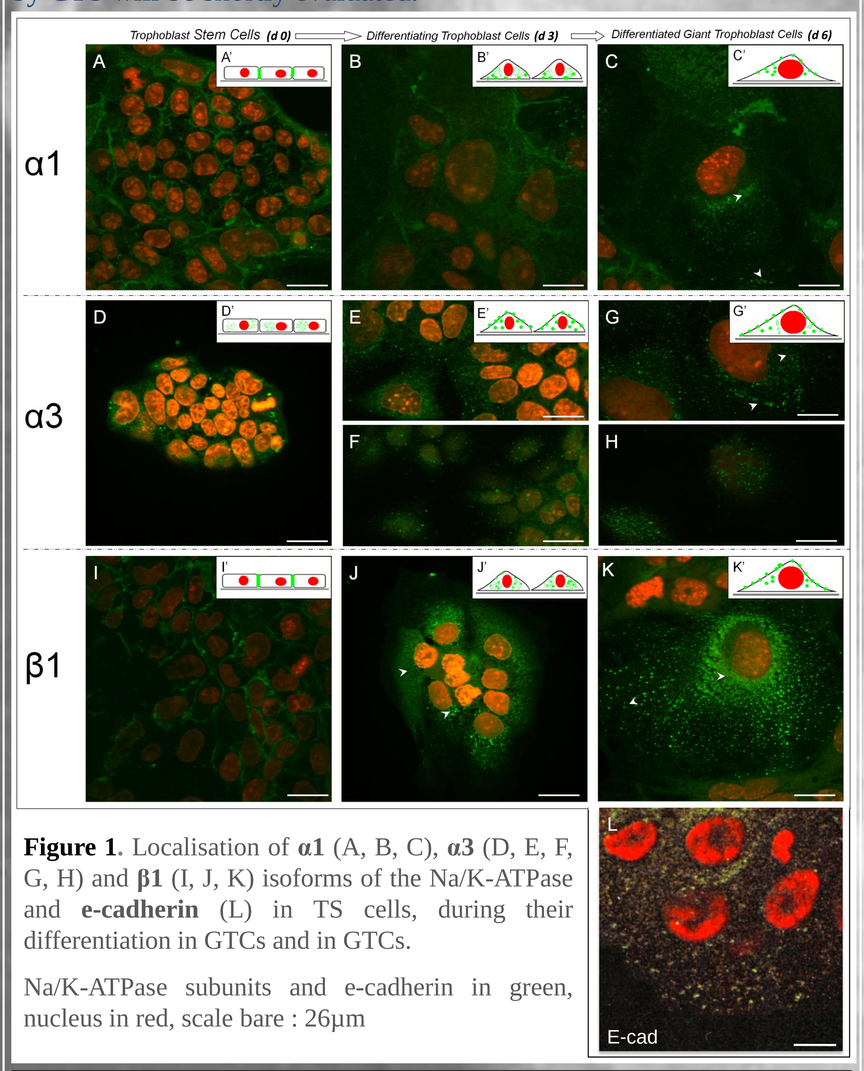


Figure 1. Localisation of **α1** (A, B, C), **α3** (D, E, F, G, H) and **β1** (I, J, K) isoforms of the Na/K-ATPase and **e-cadherin** (L) in TS cells, during their differentiation in GTCs and in GTCs.

Na/K-ATPase subunits and e-cadherin in green, nucleus in red, scale bare : 26µm

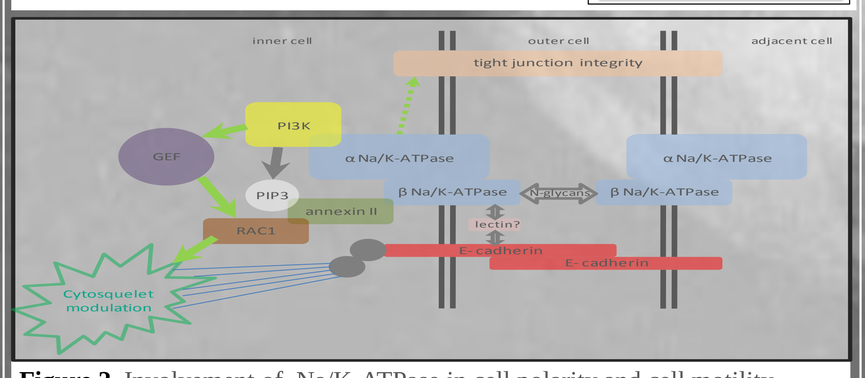


Figure 2. Involvement of Na/K-ATPase in cell polarity and cell motility.

Quantitative evaluation of growth, proliferation and motility of TS cells and GTCs will be performed by computer assisted videomicroscopy after MTT LD50 determination for ouabain and marinobufagenin.

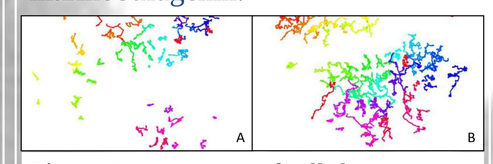
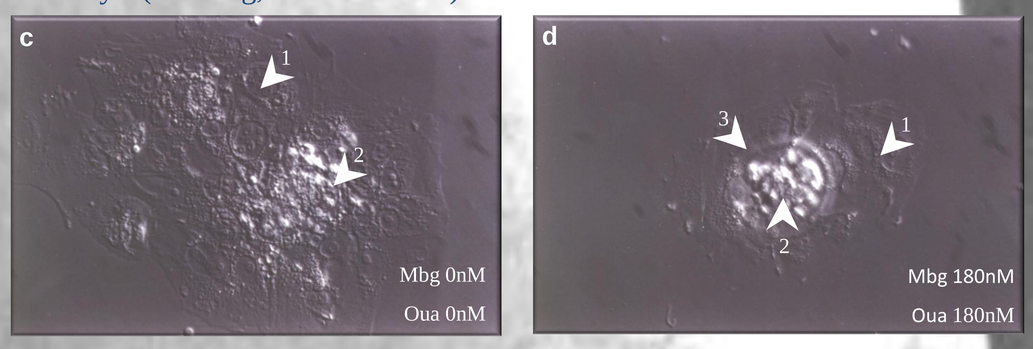


Figure 3. Monitoring of cells by computer assisted videomicroscopy, in presence or absence of an anti-migratory substance (respectively A and B; Hayot, 2006).

We firstly used mice blastocyst's outgrowths as cheap and convenient *in vitro* model to assess marinobufagenin and ouabain toxicities (c and d). It appears that both affect the differentiation of GTCs (arrows 1) and the emergence of the blastocyst (hatching, arrows 2 and 3).



This model will still be relevant in the future because marinobufagenin has been shown to induce the same responses as TGFβ in several models (Elkareh, 2007; Fedorova, 2009) and still remains dubious as a TGFβ mediator. As blastocyst's outgrowth is producing itself TGFβ in the epiblast, the comparison with TS cells which are / are not complemented in growth factors is very challenging.

In addition to classical investigations (Simmons, 2007; tab. 1), GTC's subpopulations will be characterized by a metabonomic approach, before and after the inhibition of Na/K-ATPase. Mainly based on the analysis of biofluids by spectrometry (High Resolution NMR / MS; fig. 4), this new-born "omic" approach studies the unique chemical fingerprint that cellular processes leave behind.
So, we could expect to point out specific biomarkers associated with preeclampsia and invasiveness.

	Ctsq	Plf	Pl1	Pl2
sinusoidal GTCs				
canal GTCs				
spiral arteries associated GTCs				
parietal GTCs				

Table 1. Specific markers expressed or not (green or red) in different subpopulations of GTCs. (from Simmons 2007)
Ctsq: cathepsin Q, Plf: proliferin, Pl1: placental lactogen 1, Pl2: placental lactogen 2.

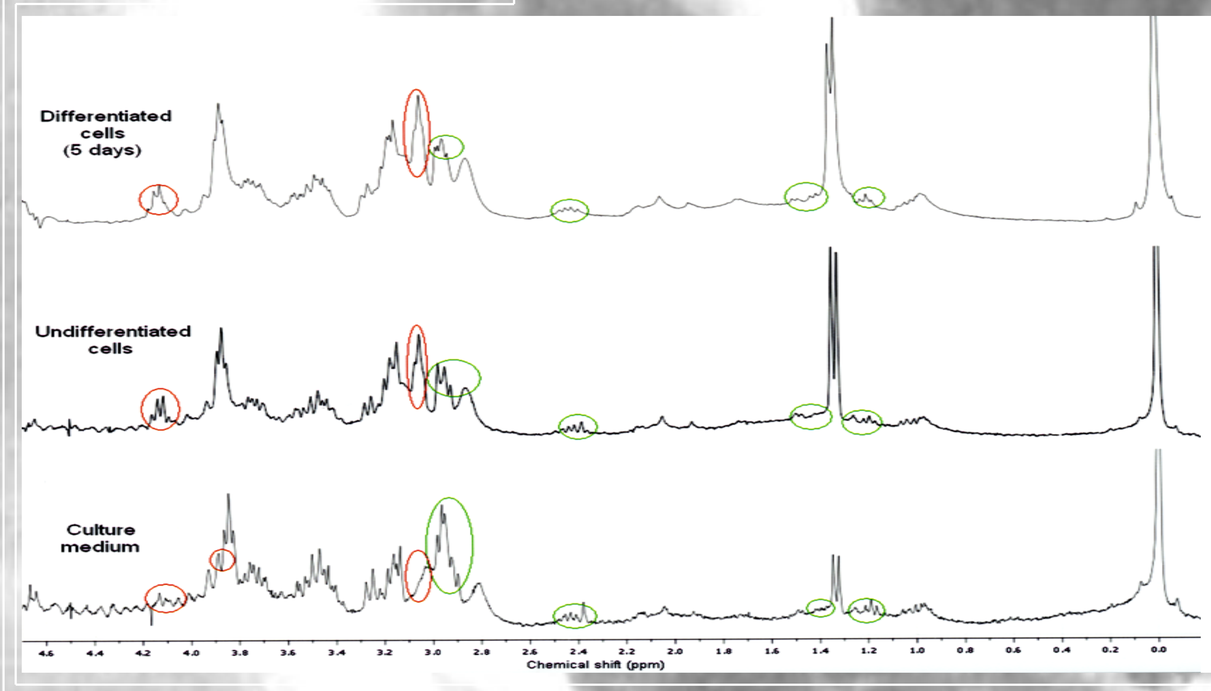


Figure 4. 1H-NMR spectra acquired at 300MHz (aliphatic region) of the media incubated with GTCs (differentiated cells) and TS cells (undifferentiated cells). Changes between the medium alone and the media incubated with cells are represented in red. Changes between TS's and GTC's media are represented in green. Unfortunately, further investigations at 500MHz and MS are needed to clearly identify these metabolites.

After setting up an extraction protocol of intracellular metabolites (M/C), we are going to proceed their analysis shortly with or without CTS in TS and GTCs.

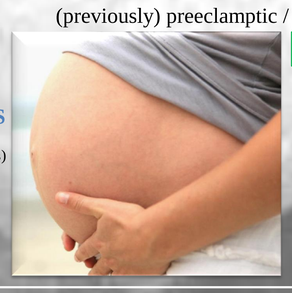
In vivo models

The second step of this PhD project is to evaluate *in vivo* the inhibition of Na/K-ATPase by CTS during pregnancy in mice. Morphological investigations will be combined with the analysis of biofluids by H-NMR and compared with pregnant, normal, preeclamptic and previously preeclamptic women.

Pregnant mice treated or not with CTS (IV).

Morphological analysis
Histochemistry
MALDI imaging MS
H-NMR (blood, urine, uterine mucus)
?early biomarkers of preeclampsia?

(previously) preeclamptic / pregnant / normal women



H-NMR (blood, urine)
? early biomarkers of preeclampsia ?
? predisposition for preeclampsia ?

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