

IN VITRO MODULATION OF HUMAN ADIPOCYTE DIFFERENTIATION BY GLAUCINE: ENZYMATIC, MORPHOLOGICAL AND FUNCTIONAL EVIDENCE OF CELL-TYPE REVERSION

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INTRODUCTION

The prevalence of obesity is a major preoccupation for our public health institutions. Research in understanding the processes that lead to fat accumulation reaches even into the cosmetic field.

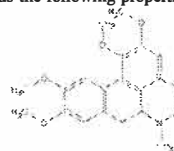
The exact embryological source of adipocytes is unknown: precursor cells of various lines may differentiate *in vivo* into mature adipocytes (bone marrow cells, chondrocytes, subcutaneous fibroblasts). In the absence of sufficient data on the adipocyte precursor cell, it is thus appropriate to study the differentiation process.

Some natural regulators of differentiation are known, but they are not as such useful in topical application. For instance, TNF α is a differentiation inhibitor for human, rat and mouse adipocytes. In the presence of TNF α , adipocytes lose the activity of the enzymes involved in lipid metabolism; they lose their spheroid morphology very fast. Another regulating factor is the calcium ion concentration whose complex role in cell differentiation has been well documented. Calcium initially plays an inhibitory role: it blocks mitosis by maintaining the c-myc regulator.

OBJECTIVE OF THE STUDY:

We screened non-controversial natural plant derived substances for those able to interfere with the major pathways of lipogenesis and cell differentiation. Regulation of the calcium antagonism pathway was an initial screening criterion. Among various aporphine structures, the molecule glaucine (1,2,9,10 tetramethoxyaporphine) turned out to be of particular interest. This substance was first identified in *Glaucium flavum*, a native plant of the Mediterranean coast, also called yellow horn poppy. Scientific literature on glaucine indicated that it might be able to interfere with adipocyte maturation as the following properties of glaucine have been described in various models:

- inhibition of phosphodiesterase PDE4
- action on calcium channels
- α 1-adrenergic antagonist
- inhibitor of membrane protein translocation
- decoupling of mitochondrial phosphorylation



METHODS:

3T3L1 as well as human pre-adipocytes were cultivated and induced to differentiation/maturation with appropriate media. Glaucine was then added to the culture for various lengths of time. Glycerine secretion, G3PDH activity, morphological changes and collagen gel contraction capacity were monitored in dose dependent manner (10-100 μ M). Clinical tests on panels of female subjects analyzing skin surface were conducted using interference fringe topometry (FOITS method). In parallel, the water retention of the adipose tissue using a dual-probe operating at a frequency of 300 MHz (Moister Meter D) and skin elasticity parameters (cutometry) were measured.

RESULTS:

Glaucine induced, in both matured 3T3 L1 and human adipocytes, a significant, dose dependent, increase (+60 to +80%) in lipolysis (glycerol production, table 1), a decrease in G3PDH activity (50-90% depending on concentration: fig. 1), and prevented full differentiation and lipogenesis in more than 90% of the adipocytes (fig. 2).

Glaucine concentration	% glycerol released by adipocytes 3T3-L1 vs. control	Significance vs. control	% glycerol released by human adipocytes	Significance vs. control
8 ppm \approx 24 μ M	+25.3	p < 0.01	+46	p = 0.07
10 ppm \approx 30 μ M	+35.8	p < 0.01	+65	p < 0.01
15 ppm \approx 45 μ M	+65.2	p < 0.01	-	-
25 ppm \approx 75 μ M	+86.5	p < 0.01	+66	p = 0.08

Morphology: The lipid droplets in adipocytes may be visually observed: A mature adipocyte population (= differentiated cells) forms a dense cell layer that is strongly colored by red oil. Immature cells do not take up this stain. Thus, cells at the end of the differentiation stage were fixed and stained with red oil. In parallel, quantification was conducted by image analysis of the area of the cell layer stained red. The images obtained (fig. 2) confirmed the results obtained by the assay: incubation with glaucine prevented differentiation up to 91% (at 25ppm).

Fig2: adipocytes stained with red oil: 72 h of incubation with or without glaucine



Photograph 1:
Control 3T3-L1 culture

Photograph 3: 15 ppm
Glaucine: -65% lipids

and having reverted to fibroblast-like appearance (right picture) are capable of contracting the collagen gel in similar manner as dermal fibroblasts (not shown).

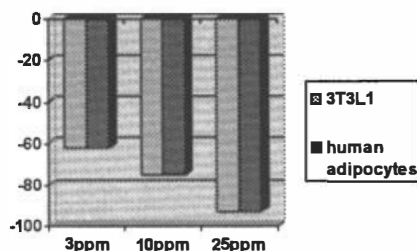
CLINICAL STUDIES:

Three *in vivo* efficacy studies of glaucine containing lotions were conducted on various panels of female subjects. The volunteers applied twice daily on one thigh an emulsion containing 25 ppm glaucine for 28 and 56 days. Mean surface roughness ("cellulite" appearance) decreased by 17% ($p < 0.01$). The decrease in water retention by the fat deposits, as measured by the propagation and re-emission of high frequency waves on steatometry sites (Moisture meter, Delfin technology) showed a 6.4% increase in the lower dermis and a 7.5% increase in the hypodermis after treatment with 25 ppm of glaucine. This increase is to be interpreted as a release of captive water initially trapped between the fat deposits and between those deposits and the superficial dermis by compression. A cutometer study showed increased firmness, tonicity of up to 20% on the treated skin (data not shown).

CONCLUSION:

Glaucine has many documented activities interfering with biochemical pathways: inhibition of phosphodiesterase, calcium and $\alpha 1$ adrenergic receptor inhibition, inhibition of translocation of membrane proteins and decoupling of mitochondrial phosphorylation. All of these may play a role in the observed effects in adipocyte differentiation and morphological reversion. Three clinical studies on the use of glaucine in cosmetic body care applications indicate beneficial effects on cellulite appearance, on water retention and skin firmness parameters, in line with expectations from the observed *in vitro* effects.

Figure 1: G3PDH activity after incubation with increasing concentrations of Glaucine in the culture medium



Reversion of phenotype: In a further experiment, fully differentiated adipocytes were incubated with glaucine; within 72h the cells that initially appear well filled with lipids (coloration red oil), progressively shrink, leaving only an empty cell envelope after 3 days (not shown). This intriguing observation led us to extend the incubation time to 6 days: after this period, we find that 75 μ M of glaucine induces the entire population of differentiated, lipid filled adipocytes to revert to mesenchymateous fibroblast-like cells (fig.3).

Finally, whereas matured differentiated adipocytes are not able to contract a collagen gel in the classical "wound contraction model", those treated with glaucine

Fig 3: left: matured adipocytes; red oil stain after 72 h; right: idem, in presence of 75 μ M glaucine

