SOCIETÀ ITALIANA DI FARMACOLOGIA

MODELLO PER INVIO RELAZIONE DI METÀ E FINE PERIODO

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TIPOLOGIA DI BORSA RICEVUTA: ___Borsa di ricerca SIF per soggiorno all'estero_____

TIPOLOGIA DI RELAZIONE : ___Relazione finale_____

TITOLO DELLA RELAZIONE: __***In vitro*** **evaluation of anti-obesity effects of flavan-3-ol metabolites**

RELAZIONE:

Obesity is a multifactorial disorder primary caused by the imbalance of energy storage and energy expenditure. The adipose tissue can be considered as an organ organised in distinct anatomical depots and constituted of three different type of adipocytes (white, beige and brown) that have different functions. White adipose tissue (WAT) assure healthy storage of excess nutrients and their rapid mobilisation when necessary, whereas brown and beige adipocytes contribute to heat production through uncouple lipid oxidation. Brown depot is the main site of non-shivering thermogenesis in rodents and infants. Since, the recent confirmation that adult humans have active brown adipose tissue (BAT), increasing the mass and activation of BAT has been suggested as a new therapeutic approach against obesity (1).

Consumption of dietary products rich in flavan-3-ols ((epi)catechins and their oligomers, procyanidins), such as green tea, cacao, nuts and grapes has been associated with positive effects on cardiometabolic risk factors (2-4). Anti-obesity properties of either pure (epi)catechins or flavan-3-ol rich-food extracts have been widely investigated in rodents, confirming their possible efficacy in prevent obesity (5, 6). The beneficial anti-obesity effects of flavan-3-ols appear related, at least in part, with their capacity to enhance energy expenditure and non-shivering thermogenesis (7-9). However, after consumption, a considerable amount of (epi)catechins is not absorbed in the small intestine and reaches the colon where it is metabolized by the colonic microflora producing γ-valerolactone compounds, that can reach high concentration in the biological fluids (10).

In this context, my goal during my visiting period in the group of Professor Antonio Vidal-Puig under the supervision of Dr. Stefania Carobbio, at the University of Cambridge, was to investigate the *in vitro* bioactivity of three major γ-valerolactones compounds, 5-(3'-4'-Hydroxyphenyl)-γ-

valerolactone (M6-2OH), 5-(3'-Hydroxyphenyl)-γ-valerolactone-4'-sulfate (M6-1S), 5-(Phenyl)-γ-valerolactone-3'-4'-sulfate (M6-2S), on a model of brown adipocyte.

In particular we investigate:

1. The activity as PPARγ agonist;
2. The capacity to modulate the differentiation of brown pre-adipocytes in adipocytes;
3. The bioactivity in mature adipocytes (modulation of expression of key genes for brown cells functionality, mitochondrial biogenesis, lipolysis)

1-PPARγ agonist activity

PPAR-γ is a key driver of brown adipogenic programme. (1) Therefore, we evaluated the capacity of γ-valerolactones to act as PPARγ agonist. The assay were performed in HEK293 cells transfected with the luciferase gene under the control of a promoter containing repeated PPARγ response elements and an expression vector for PPARγ. None of the three γ-valerolactones compounds tested at the concentration of 10 µM could act as PPARγ agonist. Rosiglitazone (PPARγ agonist) was used as positive control at the concentration of 1 µM and increased the luciferase signals of 2.6 times ($p<0.0001$ ANOVA) (Figure 1).

2- Effect on brown adipocyte differentiation

C57 BAT derived pre-adipocytes were treated with M6-2OH, M6-1S, M6-2S 2-10 µM during all the differentiation (8 days) and gene expression of marker of differentiation were evaluated at different time point during the differentiation period (day 2-5-8). No changes of expression of the major transcription factors for brown adipocytes program (*pparγ2, prdm16, pgc1α, pgc1β*) nor of markers of brown adipocyte differentiation (*cidea, ucp1*) were induced by the treatment (figure2.A). Accordingly, treatments did not affect cellular mitochondrial content, evaluated through the incubation with a green-fluorescent mitochondrial stain (Figure2.B).

3-Bioactivity on differentiated adipocytes

Differentiated C57 BAT adipocyte were treated for 6 or 24h with M6-2OH, M6-1S, M6-2S (2-10 µM). Expression of key genes of brown adipocyte functionality were evaluated at the end of the incubation period. (Figure 3). Gene expression of *Pparγ2*, *Pgc1α* and *Ucp1* was unchanged following the treatment (Figure 3.A). In accordance, the mitochondrial content was unchanged after 48h incubation with the compounds (Figure 3.C).

A direct effect of γ-valerolactones on BAT function was evaluated by their ability to modulate norepinephrine-stimulated lipolysis. The release of glycerol in the culture medium was measured as an index of lipolysis. As expected, norepinephrine increased the glycerol release in a dose-dependent manner, plateauing at 10^{-5}M. Nevertheless, none of the treatment (γ-valerolactones,

10 µM) was able to modulate the basal and norepinephrine-stimulated lipolysis response after 8h or 24h (Figure 3.B).

A pathological increase in ROS, notably in a pro-inflammatory context, can result in BAT impairment (11). Interestingly, several polyphenolic compounds have been referred to as potent antioxidant (10). We evaluated ROS production in differentiated C57 BAT adipocytes treated for 24 h with phenyl-γ-valerolactones (2 and 10 µM), in basal or oxidative stress condition (H_2O_2, 2 h). While, none of the tested molecules modified ROS production under basal condition, M6-2OH, and for a lesser extend M6-1S reversed the increase in ROS caused by H_2O_2 treatment (Figure 3.D), suggesting an antioxidant role of these molecules.

Conclusions

To conclude, γ-valerolactone compounds, in our experimental conditions, did not affect brown adipocyte physiology, suggesting that the anti-obesity effect of flavan-3-ols are not dependent on the effects of their colonic metabolites on BAT. However, they display a potential protective role against oxidative stress. These results highlight the need for future studies aiming to explore the bioactivity of these compounds in a pathological framework such as inflammation related to obesity, in which increased ROS production may have a negative impact.

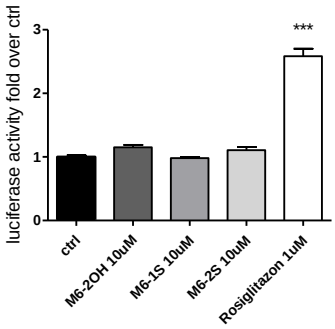

Figure 1: Luciferase activity.

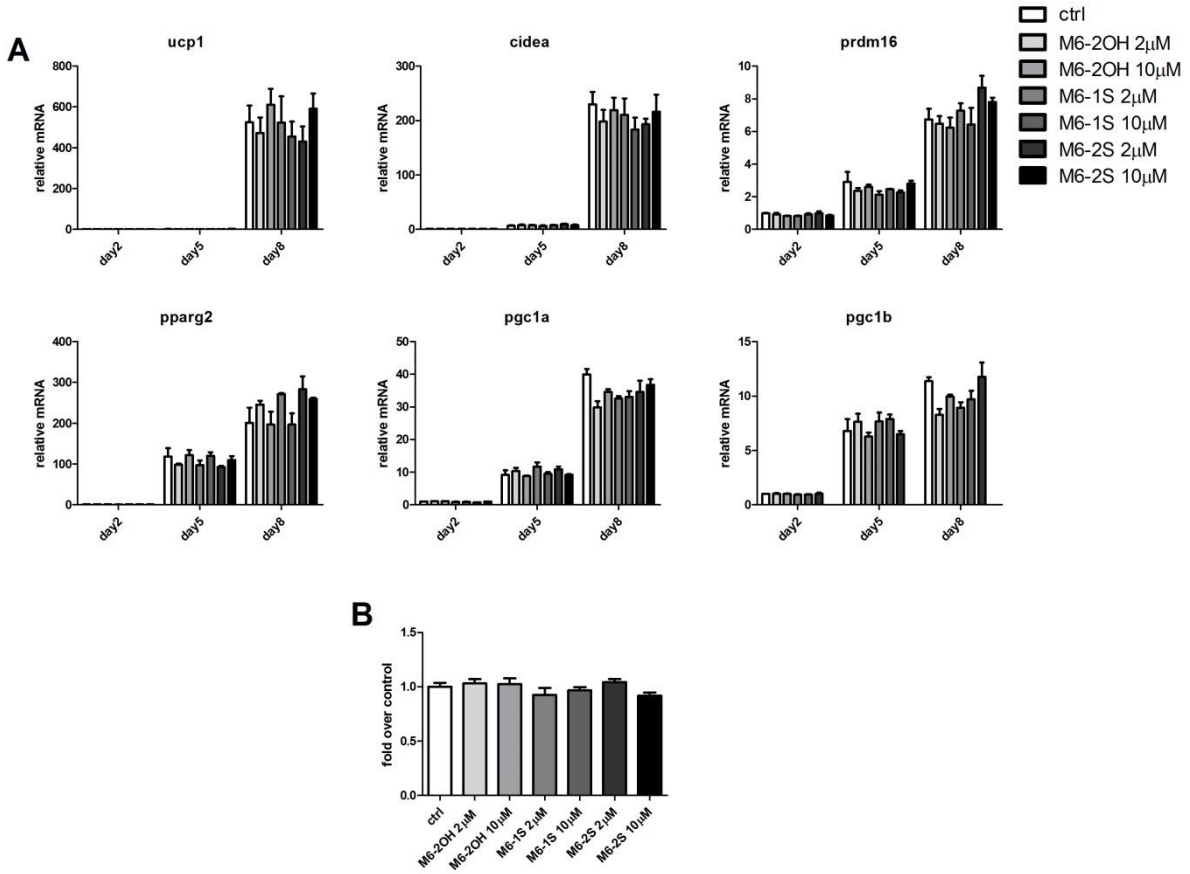

Figure 2: Effect on brown adipocyte differentiation.

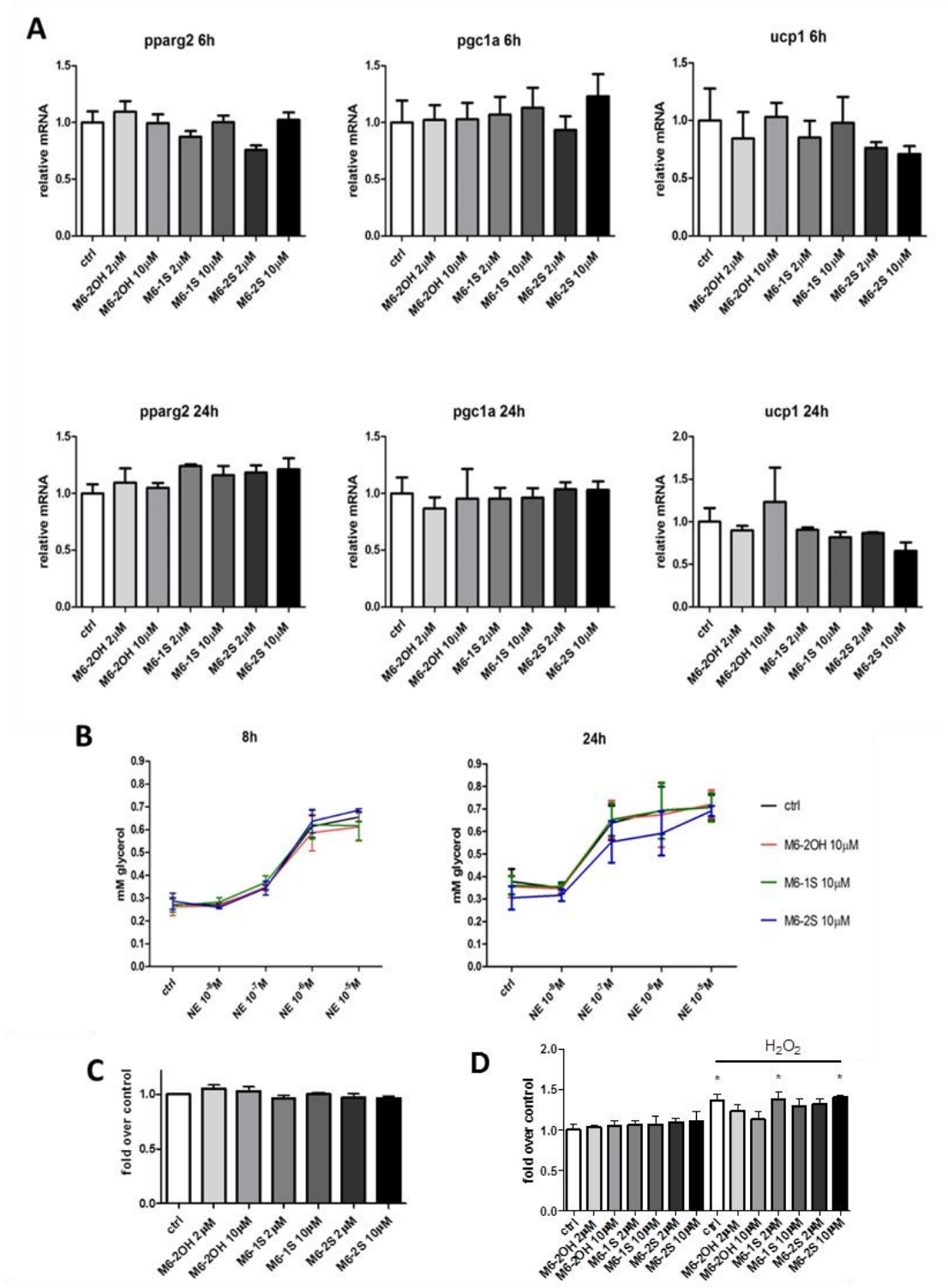

Figure 3: Bioactivity on differentiated adipocytes.

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Data, 23-04-2017

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