Study of inflammation mediated by fatty acids in a microglia cell model – the obesity perspective

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Introduction

Obesity has currently reached a worldwide pandemic level, being responsible for the development of non-communicable diseases. The strategies for body weight control have been targeting mainly adipose tissue. Nevertheless, several investigations suggested that brain plays a major role in obesity development: saturated fatty acids (SFAs) bind to a specific receptor (TLR4) in the hypothalamus, triggering inflammatory processes resulting in overconsumption and food addiction. Some concerns arise here since the current western diet, associated with high-fat and - fructose consumption, often provides considerable amounts of SFAs.

On the other hand, omega 3 from fish oils were reported as having anti-inflammatory properties in brain and revert the diet-induced obesity effects. Such processes involve the inhibition of the NFkB pathway (one of the most important inflammatory pathways). In addition, both CLA and CLNA (punicic acid) fatty acids isomers have shown to reduce body fat mass in animal models and possess anti-inflammatory properties.

Objectives

Despite promising results regarding a potential beneficial effect of CLA and CLNA isomers in obesity, namely on the peripheral tissues, few studies have specifically targeted the antiobesogenic effect of these isomers in hypothalamus inflammation.

Microglia is the main cellular component of the brain innate immune system and a key player in both regulation and protection of the central nervous system homeostasis. Therefore, excessive activation of microglia and inflammation-mediated neurotoxicity are implicated in the progression of several neurological disorders.

This work aimed to analyze a human microglia cell line activation by a solution mimicking the western pattern diet (palmitic acid, a SFA, and fructose), specifically targeting the NFkB pathway and other important processes such as reactive oxygen species (ROS) production and LynSrc activation, which is an important neurotoxicity indicator. Furthermore, the prevention of such effects by omega-3, CLA and CLNA was evaluated.

Methods

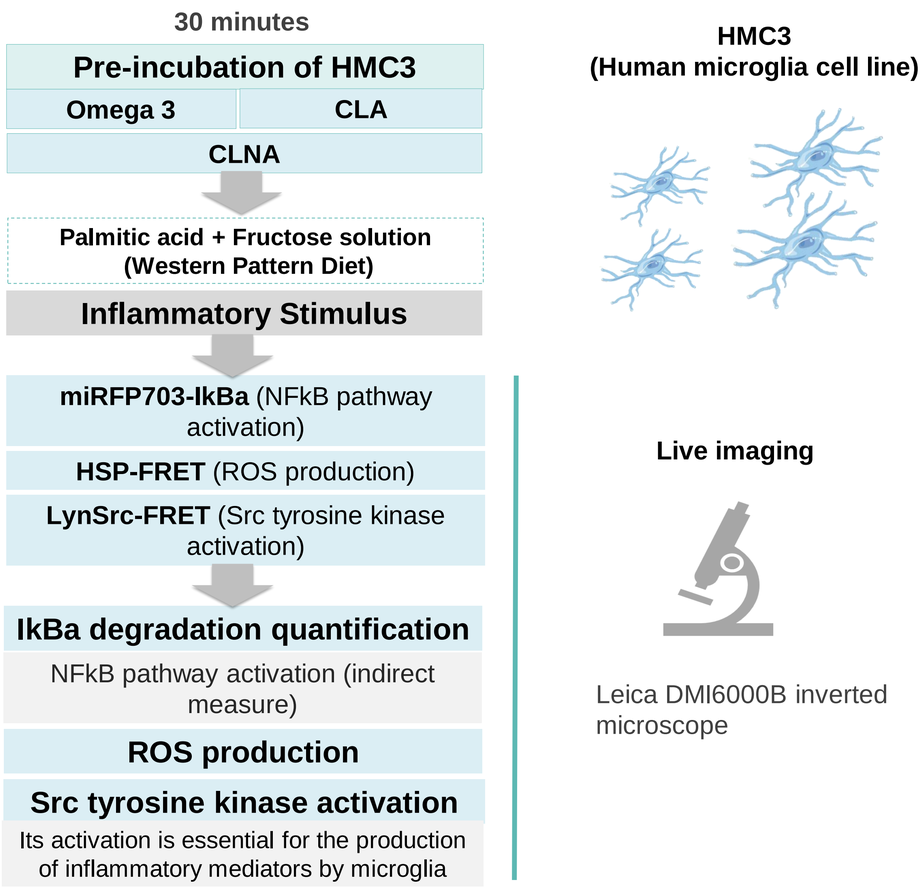

Figure 1. Experimental design schematic representation.

Table 1. Composition of the tested Fatty acids solutions.

Solution ID	Fatty acids	Other Components	Proportion	Final concentration/ solution
Stimulus (western pattern diet)	Palmitic Acid (C16:0)	Fructose	2:1	**100 µM**
Omega 3	EPA (C20:5 n-3) DHA (C22:6 n-3)	- -	1:1	
CLA	Rumenic acid (C18:2 *c*9*t*11) C18:2 *t*10*c*12 CLA isomer	- -	1:1	
CLNA	Punicic Acid C18:3 *c*9*t*11*c*13	-	-	

Results

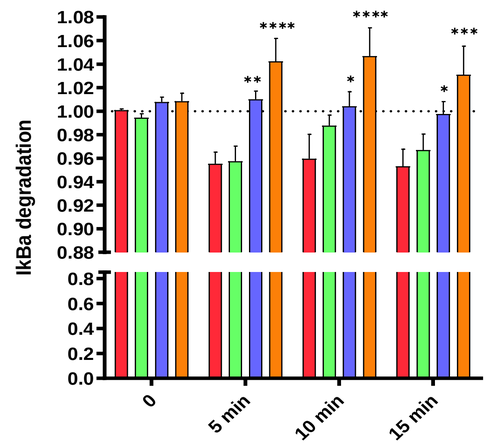

Figure 2. CLNA and CLA fatty acids prevent NFkB pathway activation by Fructose and Palmitic acid. Results of the quantification of human microglia expressing the miRFP703-IkBa sensor for the selected time points (0, 5, 10 and 15 minutes). A decreased signal means a bigger IkBa degradation and higher NFkB pathway activation. Error bar represents the SEM calculated from >15 cells from three independent cultures. Two-way ANOVA in relation to Frut+PA, where no pre-incubation with the selected fatty acids was performed. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

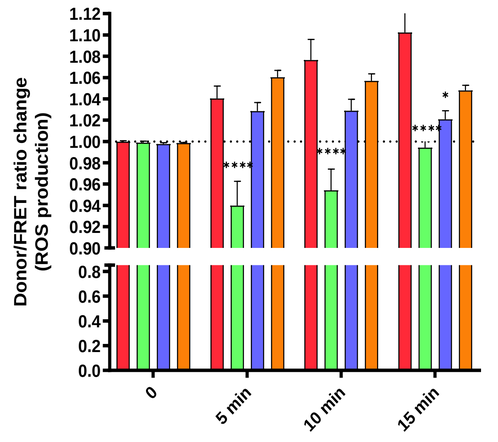

Figure 3. CLNA, CLA and omega 3 fatty acids prevent ROS production by Fructose and Palmitic acid. Results of the quantification of human microglia expressing the HSP-FRET sensor for the selected time points (0, 5, 10 and 15 minutes). Error bar represents the SEM calculated from >15 cells from three independent cultures. Two-way ANOVA in relation to Frut+PA, where no pre-incubation with the selected fatty acids was performed. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

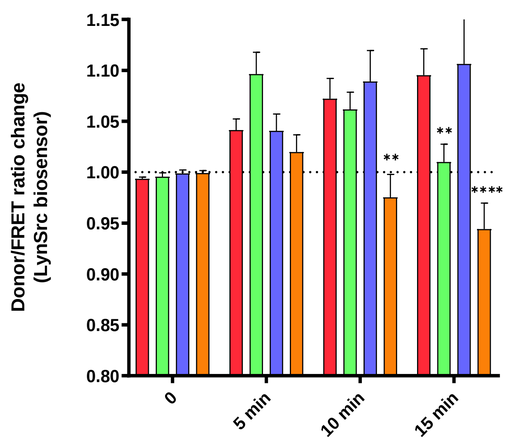

Figure 4. CLNA prevents Src Tyrosine kinase activation by Fructose and Palmitic acid. Results of the quantification of human microglia expressing the LynSrc-FRET sensor for the selected time points (0, 5, 10 and 15 minutes). Error bar represents the SEM calculated from >15 cells from three independent cultures. Two-way ANOVA in relation to Frut+PA, where no pre-incubation with the selected fatty acids was performed. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Conclusions

The incubation of the human microglia cell line cultures with the solution mimicking the western pattern diet (Fructose+Palmitic acid) showed an activation of the NFkB pathway (Figure 2) by a significative IkBa degradation (decreased signal), an increased ROS production (Figure 3) and LynSrc activation (Figure 4), an importante neurotoxicity indicator. By pre-incubating the cells with individual omega 3, CLA and CLNA solutions, the IkBa degradation was significantly lowered (increased signal), ROS production was decreased and LynSrc activation was suppressed in comparison to cells where no pre-incubation was performed. Thus, these results point out to an anti-inflammatory, antioxidante and inhibition effect of LynSrc activation of CLNA, CLA and omega 3 fatty acids under the studied conditions.

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