

ISN-CAEN PROGRESS REPORT CATEGORY 1B – December 2016

PROJECT TITLE: Involvement of NLRP3 inflammasome-driven pathways in the behavioral and neurochemical modifications induced by chronic stress

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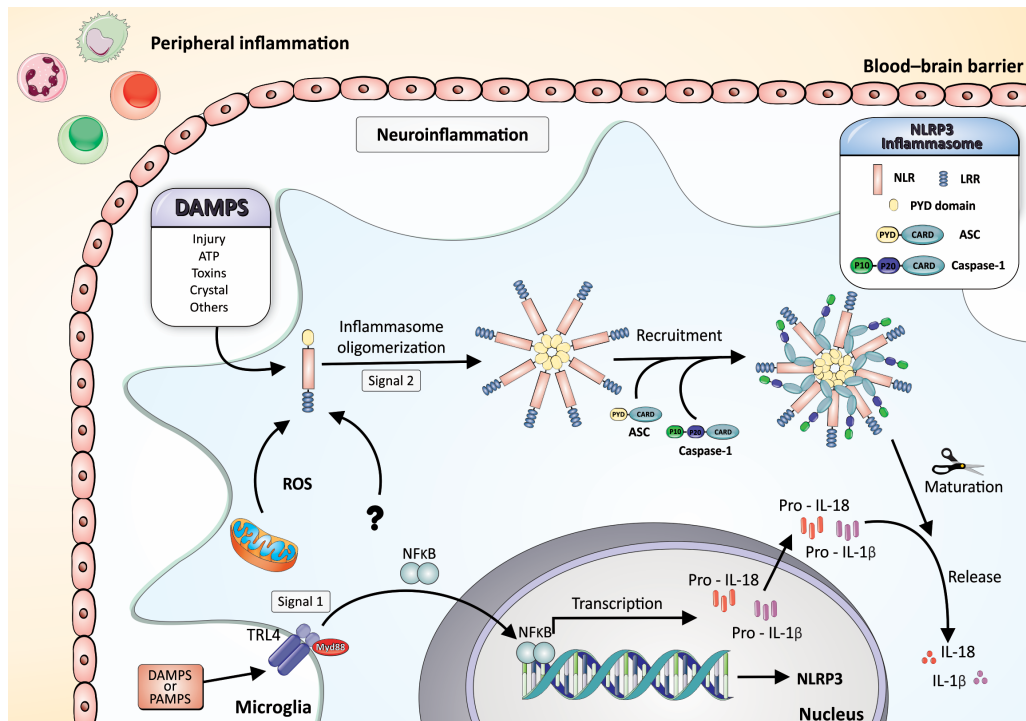
1. INTRODUCTION

Despite the reciprocal influence of both genetic and environmental factors, depressive symptoms are frequently associated with inflammatory diseases. Notably, peripheral increase on acute phase proteins, chemokines and inflammatory cytokines are frequently observed in MDD patients. These molecules have already been associated with the current mood episode, with the disease duration or severity, although some contradictory findings were reported (Maes, 1995; Miller et al., 2009). In some patients, the remission of clinical symptoms is accompanied by a normalization of inflammatory markers, while lack of response is associated with persistent elevated levels of inflammatory molecules (Lanquillon et al., 2000). In this context, pattern recognition receptors (PRRs) also seem to play a major role in the reciprocal interaction between the inflammatory responses and behavior. These receptors act through recognition of both foreign molecules and the associated cell damage, being activated by pathogen associated molecular patterns (PAMPs) and damage associated molecular patterns (DAMPs) (Martinon et al., 2002). PRRs are primarily expressed by immune cells, as well as microglia, astrocytes, oligodendrocytes and neurons and can be membrane bound (tolllike receptors) or cytosolic receptors (Nod-like receptors, NLRs) (Kigerl et al., 2014).

The activation of NLRs is involved in the assembly of cytosolic protein complexes known as inflammasomes. These multiprotein complexes are ultimately responsible for the activation of caspase-1, cleavage of precursor forms of pro-inflammatory cytokines interleukin-1 β (IL-1 β) and interleukin-18 (IL-18) into their active forms, promoting innate immune responses associated with

infection and inflammation (Fig. 1) (Singhal et al., 2014).

Fig. 1. Inflammasome signaling



Inflammasome activation is dependent on two signals. First, the recognition of DAMPs or PAMPs by TLR4 induces its intracellular signaling in microglial cells, which leads to activation and further translocation of the transcription factor NF- κ B to nucleus, where it induces transcription of pro-IL-1 β , pro-IL-18 and components of the NLRP3 inflammasome. A second signal, such as DAMPs, ROS and other unknown stimuli, is necessary to induce NLR oligomerization followed by recruitment of the ASC adaptor protein and the recruitment and activation of caspase-1. Once caspase-1 is cleaved into its active form (not shown), the NLRP3 inflammasome can process pro-IL-1 β and pro-IL-18 into mature interleukins that can be released by the cell and act as pro-inflammatory mediators. Abbreviations: Danger Associated Molecular Patterns (DAMPs); Pathogen Associated Molecular Patterns (PAMPs); Toll-like Receptor-4 (TLR4); Nuclear Factor Kappa B (NF κ B); Interleukin-1 β (IL-1 β); Interleukin-18 (IL-18); Reactive Oxygen Species (ROS); Nod-like Receptor (NLR). Kaufmann et al., 2017.

Caspase-1 and NLRP3 mRNA expression, as well as NLRP3 protein levels are increased in peripheral blood mononuclear cells of MDD patients when compared to nondepressed subjects. Besides that, inflammatory cytokines activated by the inflammasome complex, IL-1 β and IL-18, were also elevated in the serum of MDD patients and positively correlated with the Beck Depression

Inventory scores. These effects were reversed by amitriptyline treatment (Alcocer-Goméz et al., 2014).

Glibenclamide is a hypoglycemic medication used for treatment of diabetes mellitus and act through the inhibition of ATP-sensitive potassium channels (KATP) known as Sur1-Kir6.2 (Ashcroft, 2006). Lamkanfi and coworkers (2009) demonstrated that glibenclamide is able to inhibit the NLRP3 inflammasome and IL-1 β production in peripheral macrophages (Lamkanfi et al., 2009). It was already demonstrated that intracerebroventricular administration of glibenclamide reduces immobility time of mice submitted to the forced swimming test, an indicative of antidepressant-like effect (Kaster et al., 2007). However, the potential benefits of glibenclamide as a pharmacological strategy for counteract chronic and low-grade inflammatory processes associated with mood disorders needs to be further elucidated. In this context, the present work hypothesizes that, through NLRP3 inflammasome inhibition and normalization of inflammatory processes, systemic glibenclamide administration might prevent behavioral alterations in a model of depression induced by chronic unpredictable stress (CUS).

2. RESULTS

In a brief summary of our results we observed that chronic unpredictable stress (CUS) for 21 days, induced a reduction on body weight gain. However, no significant differences were found in the weight of stressed mice treated with glibenclamide versus control mice in any time point. These results further support the ability of CUS to impact the body weight gain and suggest that this effect might be minimized by gliburide treatment. Since glibenclamide is a hypoglycemic medication, we evaluated the effects of glibenclamide and CUS on serum glucose levels. No main effects were observed for stress, treatment or interaction between stress and treatment. In order to verify depressive-like behavior in mice submitted to CUS and the effects of systemic glibenclamide administration for 21 days we performed the TST and FST. The results suggest that mice submitted to stress and treated with vehicle had a significant increase in immobility time (both

in FST and in TST) when compared to control group ($p < 0.05$ using Bonferroni post hoc test) and this effect was prevented in the stress group treated with glibenclamide. No differences were found in the spontaneous locomotion of mice analyzed in the open-field test (OFT). The effects of CUS and glibenclamide treatment in anxiety-related behaviors evaluated by the percentage of time spent in the open arms of the elevated plus-maze. However, no effects were observed after CUS or CUS and glibenclamide treatment. On the other hand, CUS was able to reduce the learning index in the object recognition test, an effect that was prevented by glibenclamide treatment. No changes were observed in the expression of pro-caspase-1, caspase-1 and NLRP3 in the hippocampus or prefrontal cortex of mice submitted to CUS or submitted to CUS and treated with glibenclamide. We are still evaluating changes in the expression of other proteins related to the NLRP3 pathway and the production of IL-1 β . The following aims of our work will be to determine if the ability of glibenclamide treatment to prevent the depressive-like behavior and the cognitive deficits induced by chronic stress involves changes in other immune-related pathways and the blood brain barrier.

3. SCIENTIFIC PUBLICATIONS DERIVED FROM THE GRANT

The partial results of this project were presented in national and international meetings:

- 1) KAUFMANN, F. N.; BATISTA, P.; NEIS, V. B. ; COSTA, A. P. ; RODRIGUES, A. L. S. ; KASTER, M. P. . Glyburide treatment prevents depressive-like behavior induced by chronic unpredictable stress in mice. World Congress on Brain, Behavior and Emotions, Porto alegre - Brazil 2017.
- 2) KAUFMANN, F. N.; ROSA, P. B.; NEIS, V. B. ; RODRIGUES, A. L. S. ; KASTER, M. P. . Tratamento com Gliburide previne o comportamento tipo-depressivo induzido pelo estresse crônico imprevisível em camundongos. V Simpósio de Biotecnologia: O potencial da Integração Científica e I Mostra Científica, Pelotas, Brazil, 2017.

4. PURCHASES

We spent the funds of the CAEN grant in buying reagents, primary and secondary antibodies for western blot (NLRP3, caspase-1, TLR-4, claudin and occludin) and reagents for immunohistochemistry (antibody for Iba-1 and fluoromount).

5. REFERENCES

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