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Short Communication

# Inhibition of 11β-HSD1, a key enzyme in the stress management, improves cognition by RL-118 drug treatment

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In recent years, stress and stress-coping mechanisms constitute a growing public healthcare issue concerning modern society. Experiencing stress engenders a great complex mechanism named stress response, which consists of a rapid release of catecholamines by the sympathetic nervous system, followed by a slower response in which hormones, mainly glucocorticoids (GCs), are synthesized and released to the bloodstream. Once the stressful stimulus is perceived, the hypothalamus secretes the corticotropin-releasing hormone (CRH), which acts on the pituitary gland, activating the release of adrenocorticotropic hormone (ACTH) that binds to the adrenal glands, promoting GC secretion and conforming the hypothalamus-hypophysis-adrenal (HPA) axis. Under normal conditions, GC secretion follows a robust circadian oscillation with a peak around the onset of the active period of the day, i.e., about 1 hour before arising [1]. This basal level of GC secretion is important in exerting tonic effects upon metabolic, immune and neuronal pathways, involving gluconeogenesis stimulation, protein degradation and lipolysis increase, priming of neural regions involved in sensory processing, attention and adaptive responding, as well as accounting for immunosuppressive and anti-inflammatory actions [2]. However, when stressful exposure is prolonged, the HPA axis deregulates and GC secretion is exacerbated. This excessive GC concentration leads to several metabolic, neurological and behavioral alterations, notably cognitive impairment and affective dysfunctions. GC activity is regulated by 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) enzyme, which inhibition has been proved to restore metabolic and behavioral alterations, as well as enhance cognitive abilities. In fact, cortisol, the main active GC in humans, has been postulated as a potential biomarker for neurodegenerative disorders [3], like Alzheimer's disease (AD) in which aging is the major risk factor. Although it is completely assumed that stress directly influences the frailty phenotype in aged people, there are strikingly few measures to restrain stressful lifestyles in order to reduce the progression of pathological towards successful aging. Therefore, the study of stress effects on cognition and its relationship with aging is of the utmost importance to unveil what challenged we might have to cope with as a society in a not so far future.

In consequence, and considering that aging leads to gradual decline of cognitive abilities and is the primary risk factor for AD, we firmly believe that it is necessary taking a step forward in the study of the molecular mechanisms underlying neurodegeneration and find a different approach of the disease to achieve an appropriate treatment. Additionally, it has been described that chronic stress in midlife exerts persisting effects leading to cognitive and affective dysfunctions in old age via mechanisms that depend, at least in part, on brain GCs generated locally by 11β-HSD1 [4]. Thus, 11β-HSD1 may be an essential factor in the regulation of the HPA axis and may itself be relevant to age-related diseases susceptibility, severity or outcome [5]. In line with this, elderly who exhibit learning and memory decline showed high GC levels and correlated with greater hippocampal atrophy [6]. Moreover, a recent clinic study published a positive correlation between increased brain 11β-HSD1 expression with advancing age [7]. Not only in humans, but also in rodents its expression was increased in aged mice and its overexpression accelerated age-related cognitive decline, while 11β-HSD1-knockout mice resisted age-dependent cognitive loss [8-10].

Another type of chronic stress exposure is metabolic stress induced by a high-fat diet (HFD). In recent years, interest in the impact of nutrition in health has grown since obesity is one of the

features of modern society. Mainly, obesity constitutes a risk factor for many disorders, including diabetes, hypertension, cardiovascular alterations, mild cognitive impairment and AD. As mentioned, prolonged GC overexposure has been linked to metabolic disturbances development and 11β-overexpression in rodents displays a phenotype mimicking human metabolic syndrome, which can be prevented by its inhibition, proposing that intracellular metabolism of GCs by 11β-HSD1 is critical to the development of insulin resistance rather than the circulating GCs [11-13].

Notwithstanding, it has been suggested a causal role of stress in the onset and progression of age-related cognitive decline and neurodegenerative disorders like AD, as sustained GC overexposure has been related to amyloid-β (Aβ) formation increase and hyperphosphorylated tau accumulation - hallmarks of AD - and adversely affect behavior [7,14-16]. Accordingly, in clinics, cortisol levels were inversely correlated with cognitive performance and hippocampal volume [17], and a rare single nucleotide polymorphism in the 11β-HSD1 gene has been reported to increase sporadic AD risk [18], supporting that local tissue levels of GCs may be a significant risk factor for AD development.

Taking into account all the detrimental effects of excessive GCs and the essential role of 11β-HSD1 mediating them, 11β-HSD1 inhibitors have been identified and developed. Despite, in the recent years there has been a focus of attention upon targeted 11β-HSD1 inhibition in the context of metabolic disease, involving insulin resistance and type 2 diabetes mellitus (T2DM), its interest for neurodegenerative diseases treatment has increased. Early clinical studies demonstrated that a 11β-HSD1 inhibitor (UE2343) is well tolerated and is, therefore, a suitable candidate to improve memory in patients with AD [19,20]. In view of these results, Leiva et al. [21] synthesized a new family of potent 11β-HSD1 inhibitors, featuring unexplored pyrrolidine-based polycyclic structure. The most potent compounds were characterized in terms of cellular potency, isoenzyme selectivity, human microsomal stability and predicted brain penetration to select a candidate, which was named RL-118 and was selected for in vivo experiments in an animal model of aging, senescence-accelerated mouse prone 8 (SAMP8) (Table 1).

In consequence, we designed and performed different experimental approaches with the aim of determining RL-118 potential beneficial effects in SAMP8 animal, evaluating the underlying mechanisms of RL-118 modulating 11β-HSD1 action in different pathways underpinning neurodegeneration, and assessing its role in preventing the negative impact of chronic stress, induced by HFD feeding.

In an attempt to address this issue, different projects were established. In particular, 3 animal studies using SAMP8 mice. On the one hand, although the 11β-HSD1 inhibition in the human embryonic kidney (HEK) 293 cells by RL-118 drug was determined, we assessed whether the drug could decrease GCs levels in an animal model. In rodents, the main active GC is corticosterone. Therefore, SAMP8 were divided into 2 groups, control and treated with RL-118, and 2 hours after acute treatment, animals that received RL-118 drug showed lower corticosterone levels in blood and brain tissues, so that, effectively, RL-118 was able to cross the blood-brain barrier (BBB) and exert its activity reducing GC action [22].

Afterwards, we designed a study to investigate whether the drug might modulate cognitive abilities to enhance age-related cognitive decline. To address that proof of concept, we used aged SAMP8 animals, divided them into control and under RL-118 treatment, treated them for 4 weeks and submitted several behavioral tests. Particularly, we conducted the open field and the object location tests (OFT and OLT, respectively). Behavioral results showed that RL-118 drug decreased recklessness and anxiety-like behavior, as well as increased spatial memory, meaning that the drug had a neuroprotective effect. In addition, the mechanisms underlying neuroprotection were studied in the hippocampal tissue. Interestingly, we found that autophagy was promoted with RL-118 treatment, indicating that the drug favored the cellular mechanism to clean harmful material, which positively correlated with behavioral improvement and negatively correlated with pro-inflammatory and oxidative stress (OS) mediators. Not only neuroinflammation and OS were reduced after RL-118 treatment, but also AD hallmarks [23].

Considering the positive effect of the present drug in reverting age-related cognitive decline, our aim was to evaluate in adult mice whether RL-118 had a neuroprotective effect on cognition when animals were exposed to chronic stress situation, represented by HFD-induced metabolic stress approach. 4 groups were established: control fed with normal diet (ND), ND-fed treated with RL-118, HFD-fed and HFD-fed treated with RL-118. Since weaning, mice consumed their dietary condition. At the age of 4 months, drug treatment started for 4 weeks and afterwards, behavioral tests assessing behavioral and cognitive abilities were performed: the three-chamber test (TCT), the novel object recognition test

| Characteristic | RL-118 |
|---|---|
| **HEK human $HSD_1$ inhibition at 10μM (%)** | 100 |
| **Human $HSD_1$ $IC_{50}$ (μM)** | 0.03 |
| **Human $HSD_2$ $IC_{50}$ (μM)** | <0.1 |
| **HLM parent (%)** | 94 |
| **PAMPA-BBB $P_e$ ($10^{-6}$cm $s^{-1}$)** | <30 (CNS+) |

**Table 1:** Biological profile of RL-118 drug. Percentage inhibition was determined compared to a non-inhibitor control. HEK293 cells transfected with the full-length gene coding for human either 11β-HSD1/2 was used. The microsomal stability was determined using human liver microsomes (HLM) and central nervous system (CNS) + predicted positive blood-brain barrier (BBB) penetration by parallel artificial membrane permeability assay (PAMPA) [21].

(NORT) and the Morris water maze (MWM).

Briefly, our results highlight an improvement in metabolic parameters, such as glucose intolerance and triglyceride concentration, as well as activation of metabolic pathways related to energy sensing like sirtuin 1 (SIRT1)/ peroxisome proliferator-activated receptor gamma coactivator (PGC) 1α/ AMP-activated protein kinase α(AMPKα) signaling route. SIRT1/PGC1α/AMPKα axis is regulated by fibroblast growth factor 21 (FGF21) expression. Hormones just as FGF21 and GCs play crucial roles in coordinating the adaptive starvation response. Explicitly, it has been demonstrated that FGF21 modulates energy homeostasis of glucose and lipid through activation of SIRT1/PGC1α/AMPKα axis, mainly through liver kinase B1 (LKB1) activation, resulting in enhanced mitochondrial activity [24,25]. In addition, SIRT1 reduces fat accumulation, decreases the risk of visceral obesity and, noteworthy, it has been described that reduces the activity of GC receptors (GR), thus attenuating GCs role in metabolic disorders [26]. Consistently, our results determined that HFD feeding decreased their expression, although treatment with RL-118, thus inhibiting the 11β-HSD1 enzyme, significantly increased FGF21 and LKB1 protein levels under both dietary conditions [27,28]. In line with these results, it has been described that while aging, FGF21 expression increases, but cells apparently become insensitive to it [29]. Herein, we hypothesized that RL-118 treatment could reverse FGF21 resistance, as its protein levels were increased in treated with HFD and RL-118, as well as some of its downstream mediators like AMPKα phosphorylation, suggesting an improvement in nutrient sensing and mitochondrial function. Accordingly, mice that received HFD pronouncedly increased their body weight gain and consumed more calories than ND-fed mice, thus supporting that HFD contributes to glucose tolerance impairment. Despite the studies affirming the use of 11β-HSD1 inhibitors for the treatment of metabolic syndrome [13] although in accordance with previous results, in our project RL-118 drug did not improve glucose metabolism under normal conditions, though it did in mice receiving concomitant HFD. In addition, these results could be explained by a significant reduction in 11β-HSD1 and GR protein levels in the groups that received drug treatment, indicating that when GCs exposure is normalized, metabolic disturbances improve. These results are in line with reports describing a reduction in 11β-HSD1 and GR gene expression in diet-induced obese mice after treatment with carbenoxolone, a 11β-HSD1 inhibitor [30].

In agreement with previous projects, OS and neuroinflammation were increased in HFD animals [31] but attenuated after RL-118 treatment. Of note, RL-118 treatment not only decreases ROS concentration, but also favors the ER stress response, which is involved in increasing antioxidant defense mechanisms through the nuclear erythroid-related factor 2 (NRF2). In reference to neuroinflammation, RL-118 drug reduced nuclear factor κB (NF-κB) protein levels, which is involved in pro-inflammatory cytokine expression. Accordingly, pro-inflammatory mediators gene expression was reduced after RL-118 treatment as well as microglia activation, assessed by Iba-1 expression, in the mice hippocampus. Interestingly, 11β-HSD1 inhibitor treatment enhancement of these markers was notably higher in animals that also received HFD feeding, demonstrating that when SAMP8 mice are under stress conditions, the drug can exert its beneficial effects.

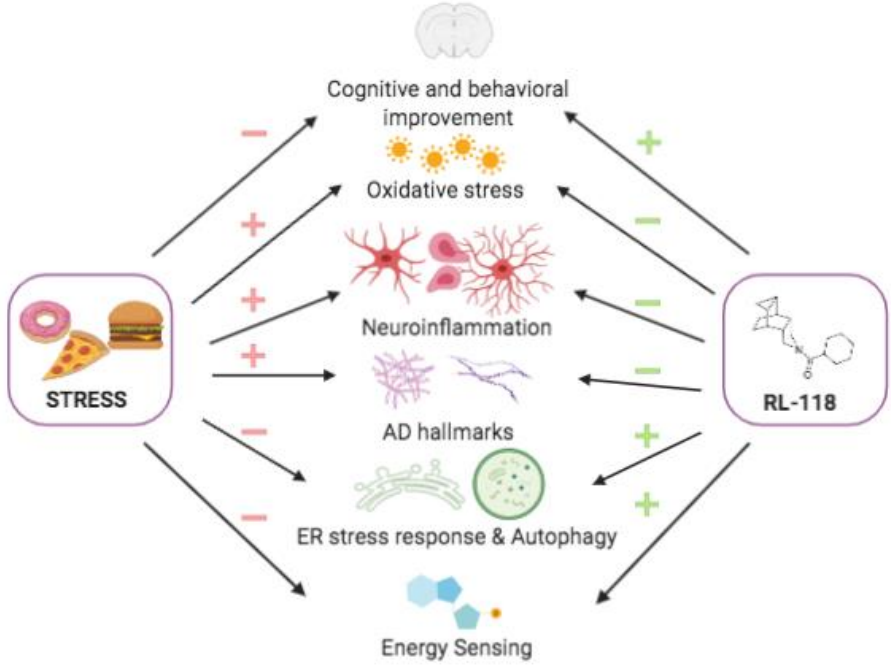


**Figure 1:** Schematic representation of the molecular pathways affected after HFD feeding and the protective role of RL-118 drug. Chronic stress has an aversive impact on cognitive and behavioral abilities, increases OS and neuroinflammatory markers, promotes AD hallmarks as well as attenuates the UPR and energy-sensing mechanism. By contrast, 11β-HSD1 inhibition by RL-118 boosts the opposite actions (in red it can be observed the unfavorable effects, whereas in green the beneficial. A positive sign indicates activation or increment, while a negative sign inhibition or decrease, and both signs changes related to both, increases and reductions).

Remarkably, drug treatment altered endoplasmic reticulum (ER) stress response in both dietary groups, especially in the HFD mice. In accordance, HFD-fed treated with RL-118 group showed increased Beclin1 protein levels, thus promoting autophagy [23]. ER stress and autophagy jointly form the unfolded protein response (UPR) to react against misfolded proteins and promote cellular clearance through different mechanisms.

Also, RL-118 treatment induced a reduction of tau hyperphosphorylation and Aβ formation regardless of the diet [7,23,32]. In reference to AD hallmarks, not only Aβ formation and tau hyperphosphorylation markers have been evaluated but also others controlling them, like glycogen synthase kinase 3 (GSK3-β). It has been reported that GC treatment reduces GSK3-βexpression and function [33]. Consistent with this, it regulates Aβproduction by down-regulating the activity of the α-secretase and interfering with the γ-secretase activity, thus resulting in Aβ-induced neurotoxicity reduction [34]. Additionally, GSK3-β participates in tau phosphorylation and on the contrary, growing evidence indicates that hyperphosphorylated tau activates it, through an increase in OS, neuroinflammation and apoptosis [35]. In consequence, beneficial effects on social behavior and cognitive performance were found in treated mice, supporting the therapeutic strategy that GC excess attenuation by selective 11β-HSD1 inhibition for the treatment of age-related cognitive decline and AD through improving metabolic and eventually cognitive disturbances caused by HFD [22].

In conclusion, stress modulates a broad constellation of cellular mechanisms involved in aging and neurodegeneration. Under stressful situations, GCs release is increased, generating an adaptive response. However, when stress is constant, the synthesis and release of GCs, which in physiological conditions is regulated by strict control of the HPA axis, become altered in such a way that large amounts of GCs are released and produce detrimental effects, in particular, on cognition. The metabolic stress induced by HFD has contributed to increasing the deregulation of the HPA axis and thus, GC excess and detrimental molecular mechanisms underpinning neurodegeneration. However, this alteration does not occur only under stressful situations, but also as we age. It is widely recognized that as we age, the body's ability to adapt decreases. Taking into consideration all these reasons, the implication of 11β-HSD1 in the senescence demonstrated. RL-118 treatment, inhibiting 11β-HSD1 and therefore reducing GCs exposure, led to cognitive improvement and decreased OS, neuroinflammation and AD neurodegeneration markers. By contrast, RL-118 increased the UPR response, energy-sensing mechanisms and synaptic plasticity markers assessed; therefore, providing a protective cellular and effect (Figure 1). Of note, RL-118 treatment was able to restore most of the deleterious effects produced by HFD. Consequently, 11β-HSD1 could be a feasible target to fight against cognitive decline in age-related pathologies.

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