Resveratrol, a potential mimetic of caloric restriction, as a treatment for neurodegenerative diseases

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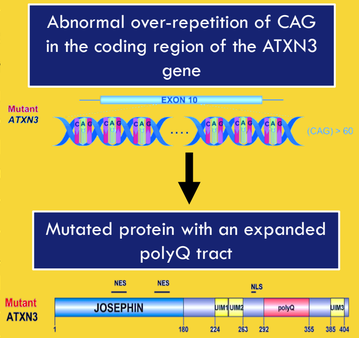
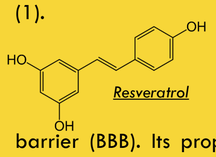
Introduction

Machado-Joseph disease (MJD) or Spinocerebellar ataxia type 3 (SCA3) is an autosomal dominant neurodegenerative that despite being rare, represents 56% of all the dominant ataxias in mainland Portugal, affecting 3.1 per 100,000 individuals. The disease is caused by an expansion of the CAG tri-nucleotide in the ATXN3 gene region, which translates into an abnormal tract of glutamines within the ataxin-3 protein. The accumulation of this protein in the cells induces neurotoxic effects and neurodegeneration of different brain regions, including cerebellum. MJD is characterized by progressive cerebellar ataxia, which results in motor dysfunction, speech and oculomotor abnormalities. Currently, there is no treatment available for this disease. (1).





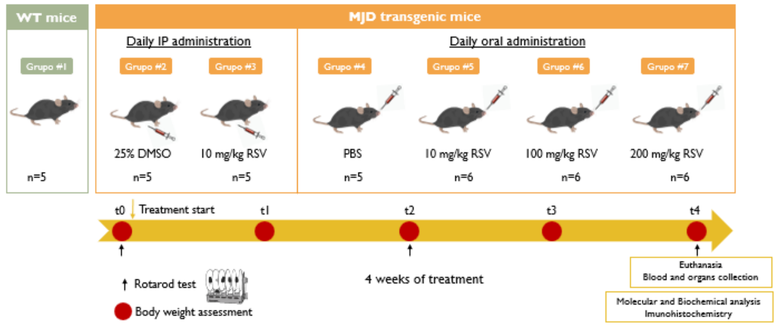
Resveratrol

Resveratrol (RSV) is a caloric restriction mimetic (CRM). It is a phytoalexin (a molecule produced by a plant) with numerous health benefits and acts in particular on the brain, passing the blood-brain barrier (BBB). Its properties are broad, ranging from anti-inflammatory, anti-oxidant, anti-apoptotic, and autophagy enhancing abilities (2). In a previous study from our lab, intraperitoneal administration of RSV drastically improved motor performance and neuropathology in animal models of disease, suggesting that it is highly promising to treat MJD patients (3).

Therefore, the main objetive of this study is to develop oral formulations able to conteract the low bioavalability profile of RSV and to evaluate their efficacy in a MJD animal model, opening the way for a future clinical trial.

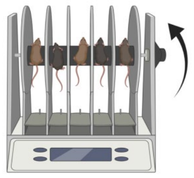
Materials and methods

The protocol is divided into two experiments, the first of which consists of daily intraperitoneal (IP) administration of 10 mg/kg and oral administration of 10, 100 and 200 mg/kg RSV to MJD transgenic mice. A control group for IP and an oral group are also present.



Regular motor tests (week 0, 2 and 4) are carried out on Rotarod in order to evaluate the motor capacities of the mice and their evolution. The weight of the mice is measured every week in order to adapt the treatment.

At the end of the experiment, the peripheral organs and the brain are perfused washed and collected. RNA and protein extraction is performed on the peripheral organs and part of the brain. The other part of the brain is fixed for immunohistochemical sections. The aim is to identify the different signaling pathways involved and the biomarkers by RT qPCR in order to evaluate the action of RSV in the cells and to observe the effects on the cells by immunohistochemistry.



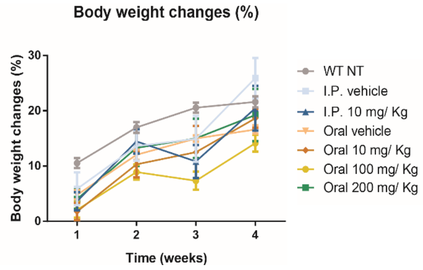
Rotarod

In parallel, another one-week experiment was carried out with control mice, divided into the same groups as the previous experiment. The protocol is the same but carried out over a week without motor tests. The aim is to evaluate the activity of resveratrol in the peripheral organs and brain of WT mice.

Results

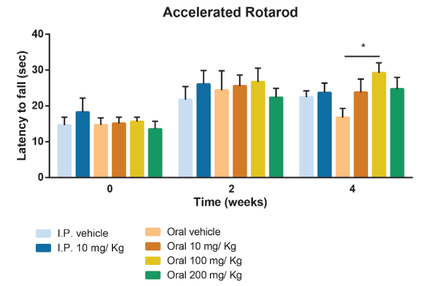
1. Body weigth assessment

Although the weight gain profile was variable between groups, with a tendency to decrease in the 10 mg/kg, intraperitoneal and 100 mg/kg, oral groups at 3 weeks of treatment, no significant differences were found at any of the time-points. These results indicate that the different doses and routes of administration tested have no impact on the body weight of the animals.



2. Motor coordination and balance assessment

In the accelerated speed rotarod test, animals administered orally with 100 mg/kg RSV showed a significantly increased latency to fall compared to animals administered with vehicle . To validate the optimal dose regimen, the analysis of neuropathology and molecular targets of RSV will be carried out in the same groups of animals.



Clinical perspectives

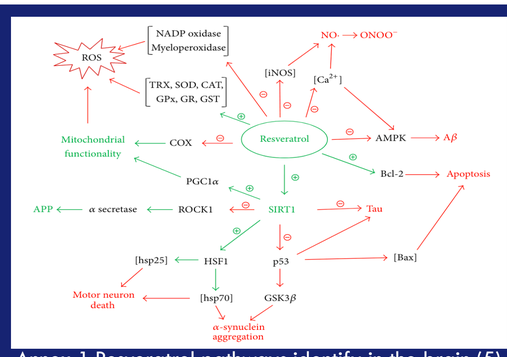
The aim of the study is to move towards to clinical application. RSV is already studied in other diseases and is considered a well-tolerated and safe molecule, which may facilitate a quick transition to the clinic.

The evaluation of efficient formulations for human use will be necessary to implement a resveratrol-based therapeutic approach for MJD in a clinical trial.

Future research avenues :

Our next goal is to evaluate the effect of RSV treatment in disease toxicity and neuropathology, by analysing the levels of mutant ataxin-3 and cerebellar atrophy in transgenic mice. Moreover, we intend to identiify biomarkers for resveratrol activity, by measuring the levels of sirtuin-1 and protein acetylation. These molecular analyses will allow us to identify the optimal dose of resveratrol for the treatment of this disease. In our preliminary results only one dose (100 mg/kg) showed a significant result on the motor tests, however a few animals were used in this study. It would be interesting to reproduce the experiment under the same conditions in order to increase the number of individuals n. A prolongation of the treatment over a longer period would also be interesting. These results open the way to future research perspectives, in particular to better understand the mode of action of resveratrol, by carrying out a more detailed study of the different signalling pathways impacted, by observing the effects on the polarisation of inflammation or again on studying the preventive effects of RSV on this disease. The potential co-administration of RSV with other CRMs, the development of a new formulation of RSV with improved bioavailability would also be viable avenues of research and many questions remain to be asked. Resveratrol could be evaluated with other treatment methods still in the research stage such as cell therapy using patient stem cells, for example, as RSV has already shown the ability to improve outcomes in cell transplantation (4).

As the experiment is still in progress, not all the results have been obtained, other avenues may be explored depending on the results.



Annex 1:Resveratrol pathways identify in the brain (5)

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(3) Cunha-Santos, J. *et al.* Caloric restriction blocks neuropathology and motor deficits in Machado-Joseph disease mouse models through SIRT1 pathway. *Nat Commun* 7, 11445 (2016)
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