Experimental Neurology 311 (2019) 88–105

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Research paper

Early intrathecal infusion of everolimus restores cognitive function and mood in a murine model of Alzheimer's disease

Tommaso Cassano[a,*], Alessandro Magini[b], Stefano Giovagnoli[c], Alice Polchi[b], Silvio Calcagnini[d], Lorenzo Pace[a], Michele Angelo Lavecchia[d], Caterina Scuderi[d], Maria Rosanna Bronzuoli[d], Loredana Ruggeri[e], Maria Pia Gentileschi[f], Adele Romano[d], Silvana Gaetani[d], Federico De Marco[f], Carla Emiliani[b], Diego Dolcetta[f,**]

[a] Department of Clinical and Experimental Medicine, Medical School, University of Foggia, 71100 Foggia, Italy
[b] Department of Chemistry, Biology and Biotechnology, University of Perugia, 06126 Perugia, Italy
[c] Department of Pharmaceutical Sciences, University of Perugia, 06123 Perugia, Italy
[d] Department of Physiology and Pharmacology "V. Erspamer", Sapienza University of Rome, 00185 Rome, Italy
[e] Division of Hematology and Clinical Immunology and Bone Marrow Transplant Program, Department of Medicine, University of Perugia, 06132 Perugia, Italy
[f] UOSD SAFU, RiDAIT Dept, The Regina Elena National Cancer Institute, 00144 Rome, Italy

ARTICLE INFO

Keywords:
Intrathecal administration
Everolimus
Mammalian target of rapamycin
Alzheimer's disease
Depression
Learning and memory
3 × Tg-AD mice

ABSTRACT

The discovery that mammalian target of rapamycin (mTOR) inhibition increases lifespan in mice and restores/delays many aging phenotypes has led to the identification of a novel potential therapeutic target for the treatment of Alzheimer's disease (AD). Among mTOR inhibitors, everolimus, which has been developed to improve the pharmacokinetic characteristics of rapamycin, has been extensively profiled in preclinical and clinical studies as anticancer and immunosuppressive agent, but no information is available about its potential effects on neurodegenerative disorders. Using a reliable mouse model of AD (3 × Tg-AD mice), we explored whether short-term treatment with everolimus injected directly into the brain by osmotic pumps was able to modify AD-like pathology with low impact on peripheral organs.

We first established in non-transgenic mice the stability of everolimus at 37 °C in comparison with rapamycin and, then, evaluated its pharmacokinetics and pharmacodynamics profiles through either a single peripheral (i.p.) or central (i.c.v.) route of administration. Finally, 6-month-old (symptomatic phase) 3 × Tg-AD mice were treated with continuous infusion of either vehicle or everolimus (0.167 μg/μl/day, i.c.v.) using the osmotic pumps. Four weeks after the beginning of infusion, we tested our hypothesis following an integrated approach, including behavioral (tests for cognitive and depressive-like alterations), biochemical and immunohistochemical analyses.

Everolimus (i) showed higher stability than rapamycin at 37 °C, (ii) poorly crossed the blood-brain barrier after i.p. injection, (iii) was slowly metabolized in the brain due to a longer $t_{1/2}$ in the brain compared to blood, and (iv) was more effective in the CNS when administered centrally compared to a peripheral route. Moreover, the everolimus-induced mTOR inhibition reduced human APP/Aβ and human tau levels and improved cognitive function and depressive-like phenotype in the 3 × Tg-AD mice.

The intrathecal infusion of everolimus may be effective to treat early stages of AD-pathology through a short and cyclic administration regimen, with short-term outcomes and a low impact on peripheral organs.

1. Introduction

Alzheimer's disease (AD) is an age-related neurodegenerative disorder clinically characterized by a progressive deterioration of cognitive function. AD is defined pathologically by the presence of amyloid plaques, composed of aggregated amyloid β-peptides (Aβ), and neurofibrillary tangles (NFTs), composed of hyperphosphorylated and aggregated tau protein. Despite recent advances in treatment strategies, AD remains incurable and new therapeutic targets are needed.

The mammalian target of rapamycin (mTOR) signaling pathway has

* Correspondence to: T. Cassano, Department of Clinical and Experimental Medicine, University of Foggia, Viale Luigi Pinto 1, 71100 Foggia, Italy.
** Correspondence to: D. Dolcetta, UOSD SAFU, RiDAIT Department, The Regina Elena National Cancer Institute, via Elio Chianesi 53, 00144 Rome, Italy.
E-mail addresses: tommaso.cassano@unifg.it (T. Cassano), diego.dolcetta@gmail.com (D. Dolcetta).

https://doi.org/10.1016/j.expneurol.2018.09.011
Received 23 March 2018; Received in revised form 13 August 2018; Accepted 17 September 2018
Available online 20 September 2018
0014-4886/ © 2018 Elsevier Inc. All rights reserved.

received much attention for its role in the aging process (Powers et al., 2006; Vellai et al., 2003). In fact, the inhibition of mTOR leads to a number of downstream events including the inhibition of cellular proliferation and the stimulation of autophagy, an intracellular degradation pathway essential for cellular and energy homoeostasis (Harrison et al., 2009; Powers et al., 2006; Vellai et al., 2003). Since neurodegeneration-associated aggregation-prone proteins are predominantly degraded by autophagy (Kim et al., 2011), emerging evidence indicates that compromised autophagy can lead to the accumulation of mutant proteins and cellular toxicity. Enhancing this process exerts beneficial effects in a wide range of transgenic disease models (Harrison et al., 2009; Majumder et al., 2011, 2012).

In particular, genetic and pharmacological reduction of mTOR activity has been shown to significantly increase the lifespan in different organisms including yeast, Drosophila and mammals (Harrison et al., 2009; Majumder et al., 2012; Powers et al., 2006; Vellai et al., 2003). Moreover, mTOR signaling was reported to be up-regulated in Down's syndrome patients and in selected neurons of AD brains that are predicted to develop tau pathology suggesting that chronic high levels of mTOR signaling may exert detrimental effects in both Down's syndrome and AD brains (An et al., 2003; Iyer et al., 2014). Furthermore, the neuropathological alterations and learning and memory deficits in animal models of AD and tuberous sclerosis were associated with an increase in mTOR signaling (Caccamo et al., 2010, 2011; Ehninger et al., 2008; Magini et al., 2017).

Rapamycin, an immune-modulator approved by the U.S. Food and Drug Administration (FDA) for clinical use as an immunosuppressant, rescues cognitive deficits and ameliorates Aβ and tau pathology by increasing autophagy (Caccamo et al., 2010). Inhibition of mTOR signaling also has beneficial effects in murine models of Apolipoprotein E4-related and Down's syndrome-related AD (Andrade-Talavera et al., 2015; Lin et al., 2017).

In all these studies, the drug was chronically administered orally for 2 to 6 months (Andrade-Talavera et al., 2015; Caccamo et al., 2010; Lin et al., 2017). However, more recent studies have demonstrated that long-term treatment with mTOR inhibitors resulted in mTOR complex 1 (mTORC1) resistance and the development of autophagy inhibition (Kurdi et al., 2016), due to an uncoupling of the mTORC1 substrate, the unc-51-like autophagy-activating kinase 1 (ULK1). This suggests that the long-term use of mTOR inhibitors as a treatment for AD may have limited value. ULK1 is the main effector by which mTORC1 is able to initiate autophagy (Kim et al., 2011). Moreover, the chronic administration of rapamycin is associated with detrimental effects on metabolism, including hyperglycemia, hyperlipidemia, and insulin resistance in a murine model of nutrition-dependent type-2 diabetes (Fraenkel et al., 2008), and similar metabolic changes have been shown in patients treated with rapamycin (Stallone et al., 2009).

One potential new approach to administer novel experimental therapies with the beneficial effects of mTOR inhibition but fewer systemic side effects is to administer local intracerebroventricular (i.c.v.) infusion of the mTOR inhibitor directly into the brain. Intracerebroventricular access is routinely used in any event that might impede normal cerebrospinal fluid outflow (Fried et al., 2016). The same route can also operate in the opposite direction and serve as an efficient route for drug delivery into the ventricular space (Peyrl et al., 2014). This route of administration is currently exploited in case of meningitis that are sensitive to antibiotics that do not readily cross the blood-brain barrier (Arnell et al., 2007), or in primary brain lymphoma cases that are sensitive to high doses of anticancer drugs, which are only achievable locally without causing intolerable side effects (Korfel and Schlegel, 2013).

Previous studies have demonstrated that i.c.v. infusion is reliable in humans (Arnell et al., 2007; Korfel and Schlegel, 2013; Peyrl et al., 2014), therefore in this study we examined the efficacy of administering everolimus, a synthetic analogue of rapamycin into the cerebroventricular space of a triple transgenic model of AD (3 × Tg-AD). These mice develop age-dependent and region-specific Aβ and tau aggregations that closely mimic the disease progression seen in humans (Bellanti et al., 2017; Cassano et al., 2011, 2012; Oddo et al., 2003; Romano et al., 2014).

Although the antitumor properties of everolimus have been extensively profiled in preclinical (Mabuchi et al., 2007) and clinical studies (Motzer et al., 2008), little has been reported about its pharmacokinetics and potential effects on neurodegenerative disorders.

Therefore, we initially performed an exploratory study on non-transgenic (Non-Tg) mice and investigated the pharmacokinetic behavior of everolimus administered through different routes (i.p. *vs* intrathecal). We, then, investigated the effects of a short-term intrathecal infusion of everolimus on cognitive and non-cognitive components of behavior, as well as the disease-modifying potential (*i.e.*, ability to reduce the brain levels of Aβ and hyperphosphorylated tau) in young/adult 3 × Tg-AD mice.

Briefly, our results suggest that intrathecal everolimus treatment enhances cognitive function and exerts antidepressant-like effects in young/adult 3 × Tg-AD mice. In addition, our data demonstrate that everolimus reduces both human APP/Aβ and human tau levels in 3 × Tg-AD mice.

2. Material and methods

2.1. Experimental chemicals and reagents

Everolimus was purchased from Selleckchem (> 99%, cat. N. S1120, Munich, Germany), rapamycin from Ningbo Heyreal Import & Export Co., Ltd. (Zhejiang, China), carboxymethylcellulose sodium salt and polysorbate-80 were from Sigma-Aldrich (> 99%, Milan, Italy). HPLC-grade methanol, ammonium formate and acetonitrile were from J. T. Baker (Milan, Italy), formic acid was from Sigma-Aldrich (> 98%, Milan, Italy). Zinc sulfate was from Carlo Erba (> 99%, Milan, Italy). HPLC-grade water was obtained from inverse-osmosis MilliQ system. The osmotic pumps (model 1002) were purchased from Alzet (Cupertino, CA, USA).

2.2. Evaluation of everolimus and rapamycin stability in artificial cerebrospinal fluid

In order to evaluate the stability of everolimus during its delivery by osmotic pumps in animal models, everolimus and its natural analogue rapamycin were dissolved at a concentration of 10 μg/ml in a vehicle solution consisting of 10% (v/v) DMSO in artificial cerebrospinal fluid (aCSF) containing, in mM, NaCl 145, KCl 2.7, $MgCl_2$ 1, $CaCl_2$ 2.4, and Na_2HPO_4 2, buffered at pH 7.4. Samples were then incubated at 37 °C and aliquots were withdrawn at established time intervals and submitted to HPLC-UV analysis. The concentrations of everolimus and rapamycin were then measured over time and were expressed as a percentage of the initial value, which was set to 100%.

2.3. Everolimus quantification by HPLC-UV method

The UV method was based on previously published work (Giovagnoli et al., 2015). The instrument used was a Portlab STAYER HPLC system equipped with UV detection, a parallel pump and Triathlon autosampler (Portlab, Rome, Italy). The conditions employed were the following: isocratic mode with acetonitrile:water (60:40, v/v) eluted at 0.9 ml/min and a Zorbax sb-300 C18 column (Agilent, Milan, Italy) equilibrated at 60 °C. UV detection was performed at 278 nm and calibration was performed between 25 and 200 ng/ml (r^2 = 0.99957).

2.4. Evaluation of everolimus and rapamycin stability in cells

Everolimus and rapamycin stability were determined by evaluating cell proliferation after drug treatment (Polchi et al., 2016). Both

Download English Version:

https://daneshyari.com/en/article/11025560

Download Persian Version:

https://daneshyari.com/article/11025560

Daneshyari.com