CHAPTER V

NON-PHARMACOLOGICAL AND MITOCHONDRIAL THERAPIES FOR ALZHEIMER'S DISEASE: THE GAP AMONG ANIMAL AND PATIENTS' STUDIES

TERAPIAS NÃO-FARMACOLÓGICAS E MITOCONDRIAIS PARA A DOENÇA DE ALZHEIMER: A LACUNA ENTRE OS ESTUDOS EM ANIMAIS E OS ESTUDOS COM PACIENTES

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ABSTRACT

Alzheimer's disease (AD) is the most common neurodegenerative disorder that leads to dementia development and does not have a cure. Neuronal loss, formation of plaques and tangles, inflammation, and diminished neurogenesis are some of the main features in AD. It is a strong scientific interest that the development of AD's animal models occurs to investigate the pathology and molecular mechanisms, identify potential biomarkers, and to assess the safety of therapeutic intervention prior to human studies. However, several research are using different approaches, like non-pharmacological (i.e., exercise, nutrition, etc) and pharmacological interventions, have been applied to a great diversity of animal models of AD and often showing success in treating and completely reversing the AD-like pathology, but the same has not been seen in clinical trials. Therefore, are the animal models of AD exercising too much or is the drug dosage administered absurd to reverse the negative outcomes of this disease? Here, we review and analyze possible directions towards the development of new therapies for AD that could be translated from animal models to patients.

Keywords: Cognitive function. Dementia. Memory. Mitochondria. Neuroinflammation.

RESUMO

A doença de Alzheimer (DA) é o distúrbio neurodegenerativo mais comum que leva ao desenvolvimento de demência e não tem cura. Perda neuronal, formação de placas e emaranhados neurofibrilares, inflamação e diminuição da neurogênese são algumas das principais características da DA. É de forte interesse científico que o desenvolvimento de modelos animais de DA ocorra para investigar a patologia e os mecanismos moleculares, identificar potenciais biomarcadores e avaliar a segurança da intervenção terapêutica antes dos estudos em humanos. Várias pesquisas estão usando abordagens diferentes, como intervenções não farmacológicas (ou seja, exercício, nutrição, etc.) e farmacológicas, que foram aplicadas a uma grande diversidade de modelos animais de DA e muitas vezes mostram sucesso no tratamento e na reversão completa da DA, mas o mesmo não foi visto em ensaios clínicos. Portanto, estariam os modelos animais de DA se exercitando demais ou a dosagem do medicamento administrado seria absurda para reverter os resultados negativos desta doença? Aqui, revisamos e analisamos possíveis direções para o desenvolvimento de novas terapias para a DA que possam ser traduzidas de modelos animais para pacientes.

Palavras-chave: Função cognitiva. Demência. Memória. Mitocôndria. Neuroinflamação.

1 INTRODUÇÃO

Alzheimer's disease (AD) is the most common cause of dementia and does not have a cure. AD patients show increased neuronal loss, formation of plaques and tangles and diminished neurogenesis, with neuroinflammation playing a fundamental role at all stages of this disease (De Sousa et al. 2020). The molecular mechanisms involving AD present different theories that are linked in-between, like deficits in acetylcholine (Magdesian et al. 2005), hyperphosphorylation of Tau protein (Mandelkow and Mandelkow 1998), and accumulation of amyloid-beta (Aβ) peptide, which are also called Aβ oligomers (Lacor et al. 2007), with insulin resistance being usually present in this disease (De Sousa et al. 2020).

Intringuily, it has been shown that independently of peripheral metabolic status bad life style habits such as the consumption of a high-fat diet (Takalo et al. 2014) will favor the enhancement of Tau expression with these effects being greater in sedentary animals (Jeong and Kang 2018). It seems that some non-pharmacological mechanisms, such as dietary imbalance, might contribute to epigenetic changes that lead to the development of AD. The usage of non-pharmacological interventions like dietary management (Arem et al. 2013) have shown positive results in people with different pathologies, such as diabetes and cancer, respectively. However, the amazing results in reversing AD in animal models of this pathology have not been seen in humans yet. Here, we review and analyze possible directions towards the development of new therapies for AD that could be translated from animal models to patients.

2 DIETARY INTERVENTIONS

Among the non-pharmacological interventions indicated to diminish the risk or treat AD is controlling what you eat (Scarmeas et al. 2009). But can healthy diets really postpone the progression of AD in elderly individuals (George and Hemachandra Reddy 2019)? Kadish et al. showed that dietary composition will accelerate or not the development of cognitive decline transgenic (Tg) AD mice (Kadish et al. 2016). The results of this study revealed that long-term feeding with balanced diets having similar caloric content, but with significant changes in the source of calories, induces cognitive impairment when compared to the control diet, and this effect is much more significant in Tg animals with AD pathology.

It is known that changes in dietary balance can affect neuronal and glial cells, and also induce behavioral alterations (Freeman et al. 2011; de Sousa et al. 2019). The usage of high-fat diet, for example, leads to impairment in sensorimotor gating (Labouesse et al. 2013), and affects memory in rodents (Lee et al. 2016). It is known that chronic administration of high-fat diet, starting at the time of weaning, is enough to induce the cognitive impairment and formation of Aβ oligomers in mice with 6months of age (Petrov et al. 2015).

Petrov et al. also showed that these changes are followed by a significantly reduced activity of the mitochondrial oxidative phosphorylation system (OXPHOS) indicating that mitochondria complexes play a pivotal role in memory formation and retention in both the AD-like rodents which were treated with high-fat diet (Petrov et al. 2015). A vast number of proteins related to AD (APP, Aβ 1-40, Aβ 1-42, Tau, and p Ser^{404}Tau) pathology, insulin resistance (pSer^{612}-IRS1, IRS-1, pSer^{723}-IRS2, IRS-2, IGF-1, IGF-1R, INS-1, INSR), inflammation (pSer473-AKT, AKT pTyr^{216}-GSK3β, GSK3β, pThr^{183}/pTyr^{185}-JNK1, JNK1, pThr^{202}/pTyr^{204}- ERK1/2, ERK1/2, pTyr^{15}-CDK5, CDK5) and OXPHOS (Prkaa1, Prkaa2 Pparg, PGC-1α, mitochondrial complex I (MCI), 2, 3, 4, 5) were analyzed. Their results indicate that just the usage of high-fat diet in control mice can trigger some of the features of the AD mouse model APP-PS1.

Another dietary intervention that has been extensively studied is caloric restriction because it can be used as treatment or prevention to AD (Dias et al. 2020). A few studies have shown that a long-term caloric restriction preserves cardiac function with an improvement in cardiomyocyte function and enhanced autophagy (Han et al. 2012; Melo et al. 2013). Not only a moderate caloric restriction, but also a severe caloric restriction presented improvement in several physiological aspects and delayed the onset of age-related pathologies (Colman et al. 2009; Melo et al. 2016). Nevertheless, Wahl et al. revealed that a calorie restriction and *ad libitum* low-protein, and high-carbohydrate diets improve cardiometabolic health in mice of both sexes (Wahl et al. 2018). The authors also showed that these diets changed hippocampal gene expression with caloric restriction being associated to better performance in the behavioral tasks related to cognitive function and memory mostly in female mice, but with male mice showing improvement at 13 months-age on 10% protein diets. The positive effects of caloric restriction were partially mediated by enhanced expression of silent information regulator 1 (SIRT1), the nuclear transcriptional co-activator peroxisome proliferator-activated receptor-γ co-activator-1-α (PGC-1α), and greater

BDNF levels, especially in the hippocampus. Another research showed that caloric restriction is also capable to reduce Aβ 1-40 and Aβ 1-42 in a mouse model (Dhurandhar et al. 2013). Nevertheless, there is another non-pharmalocogical intervention, physical exercise, that has been applied successfully in reversing AD in several animal models of the disease.

3 PHYSICAL EXERCISE

A recent systematic review evaluated different protocol of physical exercise applied in several animal models of AD (De Sousa et al. 2021). In this study, the authors identified that running on treadmill is the most common adopted protocol to Tg APP/PS1ΔE9 mice or to animals that received intracerebroventricular (i.c.v) infusion of Aβ oligomers. The exercise intensity most evaluated in the studies analyzed in this systematic review was moderate being performed 60 min/day, 5 days/week during 3 to 4 weeks. All studies evaluated in this study significantly diminished the main AD outcomes. It was also identified that there are no studies that have evaluated the outcomes of different resistance training protocols in animal models of AD. Finally, the authors suggested that physical exercise protocols must be adapted to the specie of the animal, its lineage and life span.

PGC-1α/FNDC5/Irisin pathway has been also pointed as a possible therapeutic target for mitochondrial biogenesis in neurodegenerative disorders (Jamwal et al. 2021), such as AD. Therefore, Irisin induces several neuroprotective effects in AD, such as reduction of plaques and enhancement of the brain derived neuro factor (BDNF). Nevertheless, Sirtuin 1 also plays a pivotal role on the PGC-1α/FNDC5/Irisin pathway (Oliveira et al. 2014; Koo et al. 2017). Therefore, physical exercise and caloric restriction seem to be an excellent non-pharmacological tool to prevent and fight AD (Figure 1).

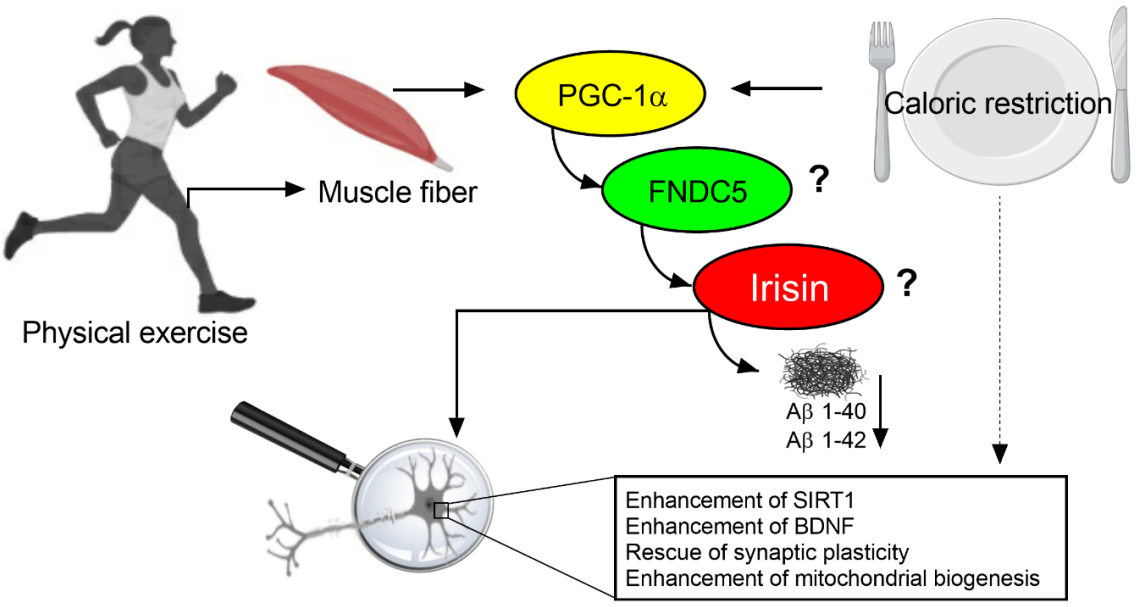

Figure 1. Physical exercise and caloric restriction induce neuroprotective effects in Alzheimer's disease.

The practice of physical exercise enhances the expression of PGC-1α and FNDC5, which is cleaved and generates Irisin. The production of Irisin occurs in muscle fibers leads to the enhancement of SIRT1 and BDNF levels, improvement of mitochondrial biogenesis, regeneration of synaptic plasticity and reduces the soluble and insoluble Aβ 1-40 and 1-42. Caloric restriction also leads to reduction of amyloid plaques, rescue of synaptic plasticity, enhancement of SIRT1 and BDNF levels and mitochondrial biogenesis. However, it is still unknown if caloric restriction would lead to significant changes in FNDC5 and Irisin, which are supposed to be significantly improved just by physical exercise.

4 NEW DIRECTIONS FOR TRANSLATIONAL THERAPIES TO ALZHEIMER'S DISEASE

Until now, animal models of AD have had total success reversing AD pathology entirely, while, after so many years of research, we are still far from finding a cure to this devastating disease. However, there are new therapeutic strategies for AD by improving, maintaining or rescuing brain energetics (Cunnane et al. 2021). Therefore, mitochondrial dysfunction is an important event during AD development and mouse models are able to provide important tools to better understand the molecular mechanisms involved in AD.

A recent review highlighted various aspects of changes in mitochondrial dynamics and function in AD. The authors described that morphological alterations in mitochondrial dynamics are regulated and balanced by a variety of proteins, which are known as fission and fusion proteins. Dynamin GTPase regulators Optic Atrophy 1 (OPA1), Mitofusin 1 (MFN1), Mitofusin 2 (MFN2) are fusion proteins, while dynamin-like GTPase regulator Dynamin related protein 1 (DRP1) and several receptor/adaptor proteins are considered to be fission proteins. Fusion proteins are enhanced by physical exercise, while fission proteins are usually reduced (Marques-Aleixo et al. 2015). These proteins act at outer mitochondrial membrane and in the inner mitochondrial membrane to produce the necessary morphophysiological changes to these cells. This study also shows that axonal transport mediates the mitochondria transportation within neurons. Thus, it is expected that non-pharmacological (i.e. caloric restriction and exercise) and pharmacological interventions will improve mitochondria function and help to find new targets to inhibit, delay or stop the progression of AD.

A promising target therapeutic related to mitochondria dysfunction is 5' adenosine monophosphate-activated protein (AMPK), which is activated when the cellular (AMP+ADP)/ATP ratio grows and also works as a detector of fuel deficiency (McCarty 2014). AMPK regulates autophagy processes through the the enhancement of UNC-51-like kinase 1 (ULK1) signaling and management of the mechanistic target of rapamycin (mTOR); inhibits nuclear factor kappa B (NF-κB) pathway, therefore, suppressing inflammation; and stimulates forkhead box protein O (FoxO)/dauer formation protein-16 (DAF-16) – a pro-longevity pathway; induces the stimulation of nuclear factor erythroid-derived 2 (Nrf2)/skinhead family member 1 (SKN-1) – responsible for cell defense against oxidative stress; and stimulates SIRT1signaling pathways, which improves cellular stress resistance (Salminen and Kaarniranta 2012). Mitochondrial SIRT3 is also known for providing neuroprotection (Barbato et al. 2015). It seems that the most important AD features are associated with mitochondria dysfunction and targeting mitochondrial complexes, especially MCI, is a promisor pathway to the development of new and efficient therapies. Thus, targeting AMPK and MCI to the development of new therapies can lead to a scientific breakthrough on the prevention and treatment of AD (Figure 2).

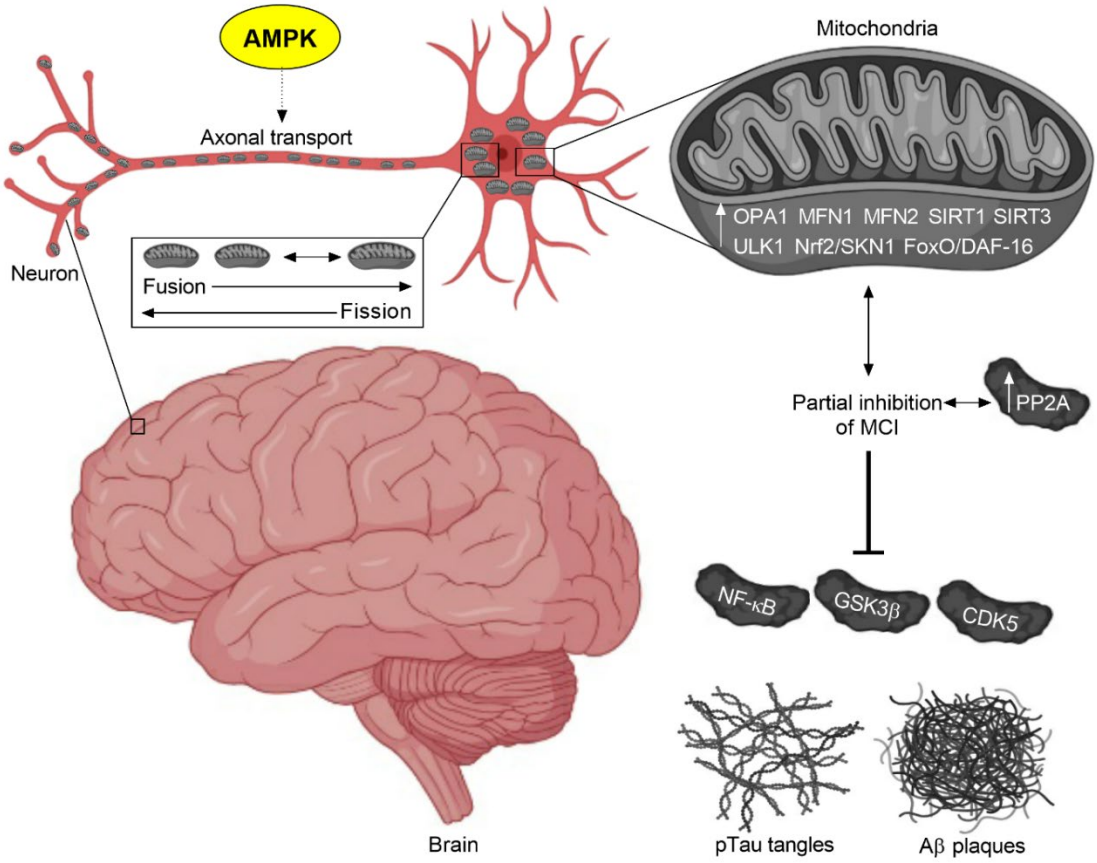

Figure 2. Targeting mitochondrial cellular and molecular mechanisms as a possible therapeutic to attenuate the consequences of Alzheimer's disease.

Targeting changes in mitochondrial dynamics and function has shown some benefits. A pivotal protein in this therapeutic is AMPK, which induces the enhancement of fusion(OPA1, MFN1, MFN2), ULK1, SIRT1, and to greater activation of FoxO/DAR-16,andNrf2/SKN1signalingpathways. Mitochondrial SIRT3 is also enhanced to provide neuroprotection. Partial inhibition of MCI will favor greater expression of OPA1, MFN1, MFN2, ULK1, SIRT1, SIRT3, FoxO/DAR-16, Nrf2/SKN1 and PP2A with concomitant inhibition of NF-B, GSK3 and CDK5, and contribute to the reduction of the formation of plaques and tangles, which is a remarkable feature of AD. Therefore, targeting AMPK and MCI can lead to the development of new therapies in AD.

A recent theory has claimed that not only mitochondrial dysfunction, but also inflammation, and oxidative stress that could contribute to cognitive decline, mood disorders, and the development of neurodegenerative diseases would be mediated by ferroptosis, excess of iron, in the neuronal and glial cells.

There are many questions involving the successful usage of pharmacological and non-pharmacological interventions that have not got to be reproducible in humans:

i) Are the dose-time interventions appropriate in the animal models of AD?; ii) Are the physiological differences between species/lineages/sex-gender not undertaken when applying the inventions to both animal models and humans?; iii) Are the studies involving animal models of AD designed to be translational to humans or are the authors just worried in getting a publication in high-impact journals without really carrying about the possible translational mechanisms? The reproducibility of animal studies is also something questionable. If the studies were designed to be possibly translated to humans it would help to reduce bias and it would improve the quality of the published papers too.

5 CONCLUSION

Animal models of AD have been established to serve as a testing stage prior to human clinical trials. It is a scientific interest that the development of AD's animal models to investigate the pathology and molecular mechanisms, identify potential biomarkers, and to assess the safety of therapeutic intervention prior to human studies. Using different animal models and interventions can lead to the development of new therapies that inhibits partially or totally the development and progression of AD. Non-pharmacological and mitochondrial therapies for AD are a promissory field of exploration.

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