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BRIEF COMMUNICATION

Cortisol and IgA are Involved in the Progression of Alzheimer's Disease. A Pilot Study

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Abstract

It is known that stress and immune systems are related with Alzheimer's disease (AD). However, the relationship of both systems in the progression of disease is not clearly demonstrated. Hair cortisol and salivary immunoglobulin A (IgA) were quantified in 49 patients with mild, moderate, and severe AD. A significant change was seen in both molecules as AD progressed from mild to moderate and severe. Low levels of cortisol were observed in mild AD patients compared with moderate and severe. However, IgA showed a contrary pattern. High levels were observed in mild AD patientes but low in moderate and severe AD subjects. The secretion of cortisol and IgA seems to be very different at the start compared with posterior development of AD suggesting that neuroinflammation can be involved. Both molecules could be used as possible therapeutical tools.

Keywords Alzheimer's disease · Stress · Immune system · Cortisol · Immunoglobulin A · Neuroinflammation · Progression

Abbreviations

AD	Alzheimer's disease
AFAV	Asociacion Familiares Alzheimer Valencia
CSF	Cerebrospinal fluid
ELISA	Enzyme-linked immunosorbent
IgA	Immunoglobulin A
MMSE	Mini Mental State Examination

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Introduction

Alzheimer's disease (AD) is the most common form of dementia and at the moment does not have a cure. The study of etiology and associated molecules are essential in the development of new pharmacological therapies. In this sense, it is known that a mineralocorticoid cortisol and an incorrect function of hypothalamic–pituitary–adrenal axis (HPA) are associated with different neurotoxic effects and neuropathologies (Gómez-Gallego and Gómez-García 2018; Rodríguez-Arias et al. 2013). For example, in major depression or Parkinson disease a possible increased cortisol levels could be the cause (Furtado and Katzman 2015; Herrero et al. 2015). Regarding AD, it has been demonstrated that chronically elevated levels of cortisol induced damage, especially in the hippocampus, observing a decrease of neurogenesis and synaptic plasticity and postulating that stress exposure could induce neuroinflammation (Ricci et al. 2012; Bisht et al. 2018).

It is known that the brain receives and responds to signals from the immune system and an incorrect balance of this communication might induce psychiatric diseases (Ashraf et al. 2018). In fact, different studies evidenced the relationship between neurodegeneration and alterations in microglia function (Song and Colonna 2018) and neuroinflammation (Katsumoto et al. 2018). Concretely, using animal models, it seems that chemokines and their receptors affect amyloid-β (Aβ) and tau pathologies (Guedes et al. 2018). However, this inflammatory response does not only occur at brain level, but also at peripheral level, which produces an increase in serum of molecules which are related to an increased risk of developing AD (Tan et al., 2007). Via disturbed blood–brain barrier (BBB), peripheral immune cells access to the central nervous system and are activated observing inflamed brain damage or amyloid plaques (Goldeck et al. 2016). In fact, some studies evidenced increased BBB permeability in AD (Elovaara et al. 1985). Between these elements of the immune system peripherally altered, it is known that immunoglobulin A (IgA) is linked to a process of inflammation (Monteiro 2010) and appears increased in patients who suffer from Alzheimer's (Leblhuber etal. 1998; De la Rubia Ortí et al. 2017).

Previous studies in our laboratory have demonstrated the relationship between the secretion of cortisol and IgA in mild AD, showing that the immune system and cortisol levels are modified in these patients (De la Rubia Ortí et al. 2017). Nevertheless, the relationship between stress and the immune system on the progression of AD has not already been studied. For this reason, the main objectives of this study are to (1) compare levels of hormone stress and immunological competence by measuring cortisol and IgA of people with AD who are at different stages of the disease, and (2) establish a possible relationship in the production of these two markers in each state, in order to test their possible use for the clinical management of these patients.

Materials and Methods

Design

This study follows a quantitative approach. It is an exploratory, observational, prospective, transversal, and analytic study.

Sample

The sample used in the study was composed of participants registered at the Center of the "Asociacion Familiares Alzheimer Valencia, Spain" (AFAV). The diagnosis of Alzheimer was performed by the neurologist's center. The inclusion criteria were patients with AD diagnosis performed by a professional. The exclusion criteria were patients affected by cerebrovascular co-morbidity or other comorbidities like kidney disease, Cushing's disease, selective IgA deficiency, and major depression; or patients that took drugs that could influence the results of the study, concretely, corticosteroids and immunomodulators. The age, with an average of 69.6 years old (total sample), was equivalent across the diagnostic groups. All the participants had a high level of education and high socioeconomic status. Two days prior to collection of the sample described below, multiple psychologists tested the participants with the commonly used method to study cognitive impairment, Mini Mental State Examination (MMSE) test (Folstein et al. 1975) as a common used method to study cognitive impairment. After the application of inclusion and exclusion criteria, 49 patients were used in the study, 20 suffered from mild, 15 suffered from moderate, and 14 suffered from severe.

Sample Collection

Saliva—2 ml in sterile tubes—was obtained from all patients voluntarily at 12 p.m. in order to determine the level of IgA (Francis et al. 2005). A sample of hair—100 strands of hair of at least 3 cm in length, incubated with 1 ml of methanol at a temperature of 34 °C and shaken lightly for 36 h—was also obtained using fine scissors from the base of the nape, in order to determine the level of cortisol produced in the last months of the patient's life (Feller et al. 2014).

IgA was quantified by enzyme-linked immunosorbent (ELISA), using the salivary IgA ELISA SLV-4636 kit (Tecles et al. 2014); cortisol was quantified using the salivary cortisol ELISA SLV-2930 kit (DRG International, Inc.) (Feller et al. 2014).

Ethical Considerations

All basic principles of biomedical research described in the Declaration of Helsinki are considered in this study. The study protocol is approved by the Ethics Committee of the Universidad de Valencia. Statement on informed consent was obtained from the parents/LAR of participants (Alzheimer's patients). An attached Spanish version of this document is provided in Annexed 1.

Data Analysis

After checking normality using Kolmogorov–Smirnov test and homogeneity using Levene test through the SPSS statistical program, the data were analyzed with a one-way ANOVA with a one between-subjects variable "State" with three levels (mild, moderate, and severe) in both cases cortisol and IgA levels. IgA data did not show a normal distribution and for this reason, they were transformed into a

logarithmic function for analysis. Two subjects were discarded by presenting outlier data in IgA and cortisol levels. All post hoc comparisons were performed with Bonferroni test. To analyze the correlations between cortisol and IgA in each state, Pearson coefficient was used.

Results

Level of Cortisol and IgA in Different States of AD

Data about of cortisol and IgA levels in different states of AD are shown in Table 1 and Fig. 1. The ANOVA showed that the variable "State" [$F(2,46) = 12{,}899$; $p < 0.000$] was significant in cortisol levels. Post hoc comparisons revealed that there are differences between mild and moderate states ($p < 0.001$) and mild and severe states ($p < 0.01$). Also, the ANOVA showed that the variable "State" [$F(2,46) = 6059$; $p < 0.01$] was significant in LogIgA levels. Post hoc comparisons revealed that there are differences between mild and moderate states ($p < 0.05$) and mild and severe states ($p < 0.05$).

Correlation Between IgA and Cortisol

Data about of cortisol and LogIgA correlation in different states of AD are shown in Fig. 2. Regarding the correlation between the production of IgA and cortisol, the results

Table 1 Mean values ± SD of cortisol in hair and LogIgA in saliva of patients with mild, moderate, and severe AD

Group	Cortisol (pg/mg)	LogIgA (mg/dl)
Participants with mild AD	4.64 ± 2.51	1.95 ± 0.29
Participants with moderate AD	9.85 ± 4.02***	1.63 ± 0.37*
Participants with severe AD	8.79 ± 2.91**	1.61 ± 0.28*

***$p < 0.00$, a significant difference in cortisol levels between mild and moderate states. **$p < 0.01$, a significant difference in cortisol levels between mild and severe states. *$p < 0.05$, a significant difference in LogIgA levels between mild–moderate and mild–severe states

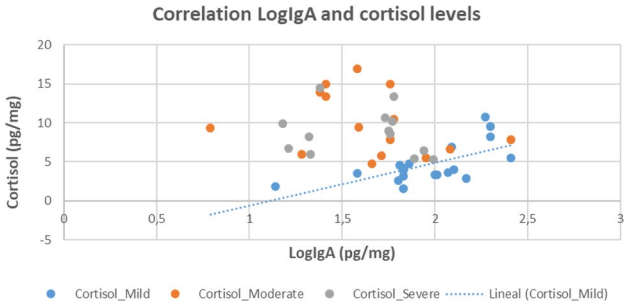

Fig. 2 Correlation of cortisol and IgA in patients with mild, moderate, and severe AD. Correlation ($r = 0.641$) was found in mild state

obtained show that there is a correlation between the two variables in mild state (see Fig. 1). It can be seen that in patients with mild AD, there were more IgA levels in saliva, and more cortisol hormones in hair ($p < 0.01$; $r = 0641$). However, in patients with moderate or severe AD no correlation was found ($p > 0.05$, Fig. 1).

Discussion

The present study demonstrated for the first time the involvement of cortisol and IgA on the progression of AD. Concretely, we showed that cortisol in first states of AD was lower but higher in posterior states. Curiously, the IgA acts in opposite form observing higher concentration in the mild state and lower in moderate and severe. Moreover, we observed that in the early state of the disease both systems are correlated.

In the first part of the experiment, we confirmed that cortisol and IgA are modified in patients with AD (De la Rubia Ortí et al. 2017). These results agree with those that observed that stress/cortisol could induce cognitive problems or AD (Tortosa-Martínez et al. 2018; Justice 2018). In fact, it has been proposed that high cerebrospinal fluid (CSF) levels of cortisol in AD patients may cause damage in the hippocampus (Huang et al. 2009). Using animal

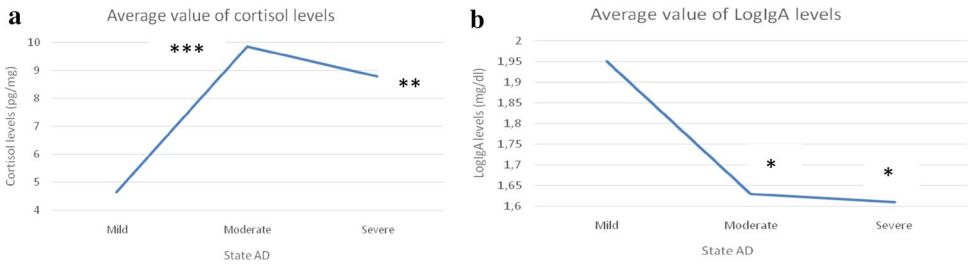

Fig. 1 **a** and **b** Average value of cortisol in hair and LogIgA in saliva of patients with mild, moderate, and severe AD. ***$p < 0.00$, a significant difference in cortisol levels between mild and moderate states. **$p < 0.01$, a significant difference in cortisol levels between mild and severe states. *$p < 0.05$, a significant difference in LogIgA levels between mild–moderate and mild–severe states

studies, concretely Tg2576 mice were observed that chronic mild stress accelerates the onset and progression of AD phenotype (Cuadrado-Tejedor et al. 2012). On the other hand, in agreement with other studies, high concentrations of IgA molecule have been showed in AD patients (Leblhuber et al. 1998; De la Rubia Ortí et al. 2017) and IgA/IgM antibodies are produced in significantly higher concentrations in patients with dementia than in other participants of the same age (Doss et al. 2014).

A possible explanation of the differences between states could be that both cortisol and IgA induce neuroinflammation that modified the progression of the disease indicating that microglial action plays a critical role in AD progression (Guerreiro et al. 2013; Jonsson et al. 2013; Jay et al. 2017). Maybe, the patient in early stages of AD goes from an immune system hyperactivity stage particularly due to the action of the proinflammatory cytokine IL-1 (which results in the formation of senile plaques and neurofibrillary tangles), to a stage of lower but chronic immunological activation (moderate and severe stages), associated to aging: 'inflammaging' (Franceschi et al. 2000). It seems that the immunocompetence is very effective in the first states but when the patient shows several states it decreases and cortisol is involved on the evolution of the neuroinflammation, since a global reduction in the capacity to cope with a variety of stressors has been observed in aging process according to the described "network theory of aging" term (Franceschi et al. 2000). It seems that the change observed from low to high levels of cortisol on the progression of the disease are associated with a disturbed of HPA axis balance and it can be associated again to this neuroinflammation (Notarianni 2013).

In the second part of the experiment, we observed that cortisol and IgA levels are correlated. However, this association is only demonstrated in the mild state and not in moderate–severe state. Although any work has previously studied the correlation between both molecules in different states of AD, these data agree with those observed in positive correlation in mild state (De la Rubia Ortí et al. 2017). This effect can be interpreted again by a different operation of both systems along the progression of the disease.

In conclusion, our results showed that cortisol and IgA concentrations, as well as the relationship that may be established between them, clearly vary from the mild to the moderate/severe stages of AD. Futures studies could observe the neurotoxicity in brain areas involved in AD, for example, different sections of hippocampus, after stress or immune system deficits. We propose the measurement of both molecules could be used as possible tools for the clinical management of these patients, due to their correlation with cognitive deterioration, and consequently the progression of the disease.

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Consent for Publication This manuscript has been read and approved by all authors, has not been previously published, and is not under simultaneous consideration by another journal. The authors give consent for publication in Molecular Neurodegeneration.

Availability of Data and Materials Materials and/or datasets used/generated are included in the manuscript or available upon reasonable request.

Declarations The authors declare that this work is original and has not been published elsewhere nor is it currently under consideration for publication elsewhere.

Author Contributions JERO and MPGP conceived and designed the experiments and were responsible for the interpretation of the results; Mariano JR performed, analyzed the data, and made the figure of the results; SSC performed the ELISA technique. VPG and FJR wrote the first draft of the paper and JERO and MPGP wrote the final version of the manuscript.

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Compliance with Ethical Standards

Conflict of interest The authors of this manuscript declare that they have no competing interests.

Ethics Approval The study was approved by the Ethics Committee of the Universidad de Valencia and all participants signed informed consent.

Annexed 1: Declaración de Consentimiento Informado

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D. /Dña ……., de …. años de edad y con DNI nº ……., manifiesta que ha sido informado/a de que el proyecto sigue la normativa de la Declaración de Helsinki de 1964 y de sus posteriores actualizaciones (la más reciente hecha en Brasil, octubre de 2013), así mismo ha sido informado sobre los beneficios psicológicos y de salud fisica que podría suponer la participación en el Proyecto "Estudio longitudinal del estado neurofisiológico y cognitivo en pacientes institucionalizados con demencia tipo Alzheimer. Aplicación de terapias no farmacológicas en la mejora de la enfermedad" para las áreas personal, social y familiar del paciente y para la investigación en psicología. Así mismo manifiesta que ha sido informado/a del tipo de pruebas y procedimientos que se le aplicarán a su familiar

y de los objetivos del proyecto, y de que se participa sin ánimo de lucro.

Manifiesta que también ha sido informado/a que sus datos personales y los de su familiar serán protegidos e incluidos en un fichero que deberá estar sometido a y con las garantías de la ley 15/1999 de 13 de diciembre.

Tomando ello en consideración, OTORGO mi CONSENTIMIENTO a participar en esta investigación.

Fecha:

Firma del participante (o representante legal):

Firma de los investigadores:

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