ALZHEIMER'S DISEASE – ESTROGENS AND SELECTIVE ESTROGEN RECEPTOR MODULATORS, FRIENDS OR FOES?

L. Coman¹, H. Păunescu^{2,*}, R. Catană³, L.I. Coman⁵, Ș. Voiculescu⁴, O.A. Coman²

“Carol Davila” University of Medicine and Pharmacy, Faculty of Pharmacy, ¹Dept. of Physiology, ²Dept. of Pharmacology and Pharmacotherapy, ³Dept. of Pathophysiology and Immunology, ⁴Dept. of Surgery, ⁵Student, Bucharest, Romania

Abstract

Alzheimer's disease (AD) is the leading cause of dementia and is characterized by the presence of extensive plaque deposition and neurofibrillary pathology. The aim of the present study was to make an update regarding the influence of estrogens and SERMs on inflammation and on the resolution of inflammation, respectively, focusing on these most important features implicated in the pathophysiology of AD. Several hypothesised mechanisms of action of estrogens and SERM are exposed and also some relevant clinical studies on this subject are analysed. The analyzed studies have a high heterogeneity of preparations used, of administration routes, of the female population included and of the periods of time from the appearance/induction of menopause to the therapeutic intervention and also of follow-up periods of patients and of the means of evaluating their cognitive decline. One can say that all the ways of pharmacological influence on the membrane or intracellular signalling system associated to estrogens that may have clinical importance in the prevention and possibly in the treatment of AD have not been exhausted. Estrogens with selective ER α or G protein-coupled estrogen receptors (GPER1 or GqMER) effects could be used to influence the resolution of inflammation process, with positive effects on AD evolution.

Key words: Alzheimer's disease, estrogens, SERM, inflammation.

INTRODUCTION

Alzheimer's disease (AD) is a chronic progressive neurodegenerative disorder that is manifested by memory loss, impaired thinking and language, as well as changes in mood and behaviour. It has an insidious onset, with history of worsening of cognition and there is no evidence of substantial concomitant cerebrovascular disease. Alzheimer's disease is the leading cause of dementia in the elderly

and is characterized by the presence of extensive plaque deposition and neurofibrillary pathology. These “positive” lesions are accompanied by “negative” lesions such as neuronal and synaptic loss (1).

There is no curative treatment of Alzheimer's disease, the medicines used globally in its management being cholinesterase inhibitors and memantine, which are substances that have only symptomatic effects without influencing the disease progression. However, many treatments are investigated in clinical trials, with results more or less promising. Among them are to be mentioned estrogens or selective estrogen receptor modulators (SERM) used as hormone replacement therapy in early but also late post-menopause, non-steroidal antiinflammatory drugs (NSAIDs), especially coxibs, vitamin E, selegiline (2).

AIM

Taking into account that many studies showed that the use of estrogens after menopause in women could reduce the risk of developing AD, the aim of the present study was to make an update regarding the influence of estrogens and SERMs on chronic inflammation and on the resolution of inflammation, respectively, focusing on these two of most important features implicated in the pathophysiology of AD. Several hypothesised mechanisms of action of estrogens and SERM are exposed and also some relevant clinical studies on this subject are analysed.

Pathophysiology

The pathophysiology of Alzheimer's disease is not fully understood until the present days. Several factors have been incriminated in the development of this neurodegenerative disorder: amyloid- β (A β) deposition, tau proteins, mitochondrial dysfunction, oxidative stress, inflammation and resolution of

*Correspondence to: Horia Păunescu MD, 8 Eroilor Sanitari Av, Bucharest, Romania, E-mail: phpaunescu@yahoo.com

inflammation, microglial dysfunction, infection and autoimmune diseases.

Genetic causes have been identified for a small proportion of early-onset AD, which are autosomal dominant and are a consequence of mutations in the presenilin-1, presenilin-2 or the amyloid precursor protein genes. These mutations lead to an increased production of a neurotoxic form of the amyloid- β peptide and ultimately to neuron death through events that are not completely elucidated. Also, the presence of the E4 allele of the apolipoprotein E (APOE) gene is correlated with an increased risk of developing AD (3). However, the majority of cases of AD, especially late-onset AD, have an unknown cause.

One of the main theories regarding the pathogenesis of AD is the amyloid- β hypothesis. It states that the event that triggers the changes seen in AD is the formation of the amyloid plaque, an insoluble protein and other cellular material deposits outside the neurons, secondary to overproduction of beta-amyloid peptide (4). A β is a 38-42 amino acid peptide produced by cleavage of amyloid precursor protein (APP). It is believed that alterations in APP metabolism lead to excess formation of A β 42 and subsequent plaque production.

Other important protein incriminated in AD is the *tau* protein. It is a normal constituent of microtubules and stabilises their structure. However, in AD the *tau* protein is hyper-phosphorylated and deposits as neurofibrillary tangles (NFTs) in the cell body of medium and large pyramidal neurons. This may lead to alterations in microtubule integrity and ultimately death of the neurons. Indeed NFTs are a pathological hallmark in AD and can be seen initially in the hippocampus and temporal lobes of these patients (5).

Oxidative stress is caused by an excess amount of free oxygen reactive species (ROS) due to a combination of insufficient clearance and increased production. These can react with normal bio molecules of cells - proteins, nucleic acids, lipids - and impair their function. Numerous studies have found increased levels of ROS markers in the central nervous system of AD patients: protein oxidation markers such as 3-nitrotyrosine were significantly raised in the cerebrospinal fluid and various brain areas; reactive aldehydes, by-products of lipid peroxidation are also significantly elevated in the hippocampus of AD subjects in comparison with age-matched controls. Nucleic acids can also be damaged by ROS in this group of patients as shown by increased levels of DNA breaks in the cerebral cortex and hippocampus

and oxidized mRNA or rRNA (6). Furthermore, the clearance of ROS may be impaired in AD patients because of decreased levels of antioxidant molecules such as superoxide dismutase, glutathione peroxidase, heme oxygenase and others or a decrease in their activity (7).

The main cellular source of ROS are mitochondria which lead to the production of superoxide anion as a by-product of electron transfer. Therefore, dysfunction of this organelle may cause a redox imbalance through an efficient generation of ROS and impaired ATP production. Indeed, structurally altered mitochondria were found in neurons from biopsy specimens of AD patients (8). Other consequences of mitochondrial dysfunction involved in the pathogenesis of AD may be DNA damage, calcium mishandling and impaired energy metabolism (8, 9).

Another theory of AD pathogenesis is based on persistent inflammation in the common affected areas of the brain. The main cell involved is the microglia, which has an important physiological role in immune surveillance, growth factors secretion, maintenance of synapses and neurogenesis (10). Activated microglia secrete pro-inflammatory cytokines like tumour necrosis factor, IL-23, IL-12, nitric oxide and other mediators and trigger the inflammatory response (11). These cells also clear the excess of A β , however in AD this function is impaired leading to accumulation of plaques and subsequent inflammation (12). Furthermore, genetic disorders that alter microglial function can lead to neurodegenerative disorders or can increase the risk of AD (12, 13).

Recently, several infectious diseases that produce chronic inflammation like borreliosis and herpes simplex virus type 1 infection and autoimmune diseases have been significantly associated with AD (14, 15). Whether this is a result of persistent inflammation or another underlying mechanism is involved remains to be uncovered.

Once activated by pathological triggers, such as neuronal death or protein aggregates, microglia extend their processes to the site of injury, and migrate to the lesion, where they initiate an innate immune response (16).

Plasticity and functional polarization are main characteristics of the mononuclear phagocyte system including microglia. The different form of macrophages activation and polarization (M1, M2a, M2b, M2c) represents a continuum but these forms are strikingly different. M2a is an alternative activation phenotype that is linked to Th2 response, type 2 inflammation,

allergies and encapsulation of parasites (17). IL-4 and IL-13 induce the M2a phenotype of microglia responsible for the resolution of the inflammatory phase (18).

Estrogen mechanisms of action in AD

There are two types of estrogen receptors (ER) described:

- Nuclear receptors - ER α and ER β . Six splice variants of ER β were described in the brain and other tissues (19). ER β 1 isoform was proved to have a neuroprotector effect and ER β 2 isoform a tumoral suppressor effect (20, 21);

- G-Protein coupled receptors ER1 (GPER1) which are present in the brain and at periphery are situated on the membrane. They mediate the rapid non-genomic signalling actions of estrogens. GPER1 receptor is coupled with Gs-protein increasing the intracellular concentration of cAMP. The presence of ER α might be necessary to the effect and these two receptors might be coupled (22).

A new SERM, the diphenylacrylamide compound STX (2-(4-hydroxyphenyl)-3-phenylpent-2-enoic acid [4-(2-dimethylaminoethoxy) phenyl] amide, E-enantiomer), that exhibits the neuroprotective effects of estrogens without inducing oncogenic and thrombotic side effects, was identified. Unlike E2 and its functional analogs, STX does not bind nuclear estrogen receptors (ER α and ER β). Instead, STX binds the G protein-coupled estrogen receptor GqMER, located in the plasma membrane (23).

Due to the existence of these two types of receptors, estrogens play complex genomic and non-genomic actions. Also, they have pleiotropic actions due to their central and peripheral distribution. In brain there are ER in cognitive zones such as: amygdala, cerebral cortex, hippocampus, and basal forebrain (24).

A neuroprotective effect of estrogens is already well documented and may be a multifactorial combination of neurobiological and signaling actions. This neuroprotective effect plays an essential role in the prevention of cognitive impairment in AD and in improving the cognitive functions of patients with AD. The protective role of estrogens after brain injuries might be due to the estrogens synthesized in the central nervous system (CNS) (25, 26).

The anti-inflammatory actions of estrogens are the most important factors implicated in their neuroprotective effect. 17 β -estradiol (E2) plays a pivotal role in attenuating endothelial dysfunction and it has also a protective role on the blood brain barrier, modulating

its permeability by the action on ER α and ER β . It also inhibits the activation of nuclear factor kappaB (NF κ B) which induces the transcription of certain cytokines (tumour necrosis factor alpha, chemokine ligand 2-CCL2, interleukine 6) (27). E2 reduced expression of mRNA of proinflammatory endothelial molecules: e-selectin, ICAM-1, and VCAM-1 (28).

Another mechanism implicated in the anti-inflammatory effect of estrogens is scavenging of free radicals due to the interruption of the peroxidation chain of lipids (29). It is suggested that estrogens accelerate the inflammatory process, consequently promoting the natural resolution of inflammation that could be an important aspect which can be exploited in new hormone replacement therapies (19).

ER α selective agonists might represent an alternative to hormone replacement therapies in AD due to possible changing of the microglia phenotype to M2a that could initiate resolutive phase in neural tissue. This mechanism is suggested by the genetic ablation of ER α that is associated with a spontaneous reactive phenotype of microglia (30).

In the neuroprotective role of estrogens there are many other mechanisms implicated, such as:

- Repairing of the mitochondrial function inhibited by beta-amyloid oligomers (31);

- Direct antioxidant effect by enhancement of the reduced glutathione concentrations and decrease of oxidative DNA damage in mitochondria (32);

- Enhancement of the mnemonic function through an NMDA dependent mechanism - enhanced long term potentiation (33);

- Favouring of neurogenesis (formation of new neurons) especially in cornu ammonis 1 (CA1) region of the hippocampus (34);

- Raising the level of neurotrophines (nerve growth factor – NGF, brain derived neurotrophic factor – BDNF) and the level of mRNA in basal forebrain, frontal cortex and hippocampus (35);

- Influencing of the basal forebrain cholinergic system, known as very important for learning and memorizing and very susceptible at alterations due to aging. Some studies (36) show that 17 β -E2 but not E1 (estrone) raised the activity of choline acetyltransferase (ChAT) and also the number of ChAT immunoreactive cells in basal forebrain;

- Effects on other neurotransmitters: norepinephrine, serotonin;

- Effects on thrombosis and coagulation;

- Increased cerebral blood flow and glucose transport;

- Increased expression of apolipoprotein E in the brain;
- Decrease of formation of beta-amyloid (A β) and hyper phosphorylated tau protein;
- Effects on thyroid hormones and tetrahydrofolate metabolism (37-39).

Selective estrogen receptor modulators (SERM) mechanisms of action in AD

SERM includes a wide category of compounds which act both as partial agonists by selectively influencing a certain type of estrogen receptors and also as antagonists on other types of signaling systems (membranous/intracellular) associated normally to natural estrogens. Thus, clomiphene, tamoxifen, raloxifene, bazedoxifene, ospemifene were described as partial agonists of ER β (40). SERM category could also include ER α receptor agonists, agonists of membrane receptors GPR30, agonists of GqMER receptors (eg. STX) (23).

The benefits of SERM are their ER agonist properties in bone and on cholesterol metabolism and antagonist properties on the uterus and breast, having the advantage of reducing the risk of developing estrogen-dependent tumours. That is why, in the latest years, more and more experimental and clinical studies have been focused on the possible beneficial effects of SERM on reducing the risk of AD development and progression, comparing their properties with those of estrogens (E2 and so on) (19).

Their effects are common with those of estrogens, but off-target effects are also described:

- On the serotonin system - active on 5-HT₆ receptors (41);
- On the glutamatergic system, by stimulating the glutamate/aspartate transporter (GLAST), growth of the levels of mRNA for GLAST in astrocytes, increased expression of transforming growth factor beta 1, which is a modulator of the stimulatory effects of estrogens (E2) of SERMs (tamoxifen) on the dysfunctional transport of glutamate. This effect was mediated through the activation of signaling pathways: mitogen activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) and phosphoinositide 3-kinase (PI3K)/Akt. These pathways are active in neurodegenerative disorders characterized by altered glutamate homeostasis (42);
- Tamoxifen inhibits cyclin-dependent kinase 5 (CDK5) and P25 protein, effect which opens the way for tauopathies treatment (as AD) (43). Sublethal doses of tamoxifen or raloxifene induced neuroprotection

against beta-amyloid, hydrogen peroxide and glutamate-induced toxicity but did not promote significant estrogen-dependent memory (44);

- Raloxifene and E2 induced both the phosphorylation of telomerase catalytic subunit (TERT) at the level of transcription and post-transcription and the association of NF κ B. By these effects raloxifene and E2 can exert neuroprotective actions (45);

- Raloxifene had estrogen-like properties on hippocampal choline acetyltransferase (ChAT) *in vivo*, a known estrogen-responsive gene product, suggesting that SERMs may have beneficial effects on cholinergic transmission in brain (46).

Non-classical intracellular signaling associated with estrogen called ANGELS (activator of non-genomic estrogen-like signaling) might have a neuroprotective effect. Estrene, an estrogen derivative, causes a recovery of the losses in cortical cholinergic projections to the basal forebrain and an attenuation of learning deficit in a model of AD in mice obtained by injecting fragments of A β 1-42 (47).

Clinical trials using estrogens in AD

A relatively small number of clinical trials have suggested that treatment with estrogen or estrogen-progestogen associations could delay the onset of Alzheimer's disease, have a preventive effect or mitigate the evolution of this disease.

As reviewed by Henderson 2007 (studies considered were the ones of Barrett-Conor *et al.* 1993, Pagani-Hill *et al.* 1996, Tang *et al.* 1996, Kawas *et al.* 1997, Resnick *et al.* 1997, Jacobs *et al.* 1998, Baldereschi *et al.* 1998, Matthews *et al.* 1999, Waring *et al.* 1999, Slioter *et al.* 1999, Rice *et al.* 2000, Carlson *et al.* 2001, de Moraes *et al.* 2001, Binder *et al.* 2001, Grady *et al.* 2002, Zandi *et al.* 2002, Kang *et al.* 2004, Viscoli *et al.* 2005), the majority of case-control and cohort studies showed favorable effects of administering estrogen in reducing the cognitive decline in women using estrogen or estrogen-progestogen associations compared to women who did not use such associations (37). Reducing the incidence of cognitive decline was over 29%, as a result of two meta-analyses(48).

Cognitive decline cannot be stopped after onset of the disease, although there are studies showing that some benefit might be got. A study of Fillit *et al.*, 1986 showed that some women benefited from estrogen therapy after onset of Alzheimer's disease.

There are studies showing that in older ages there is technically any benefit of hormone replacement

therapy (Binder *et al.* 2001, Grady *et al.* 2002, Viscoli *et al.* 2005)(37).

Intervention studies of recent date which have shown a lack of preventive effect of estrogen on AD is WHIMS: Women's Health Initiative Memory Study (49, 50) and KEEPS: Kronos Early Estrogen Prevention Study (51).

Women in WHIMS had been assigned to conjugated equine estrogen (CEE) for those with prior hysterectomy (mean duration 7.1 years), CEE with medroxyprogesterone acetate (progestin) for those without prior hysterectomy (mean duration 5.4 years) or placebo. The study was halted early because of certain increased health risks for women. Among postmenopausal women aged 65 years or older, estrogen plus progestin did not improve cognitive function when compared with placebo and a small increased risk of cognitive decline occurred in the estrogen plus progestin group (49). In the follow-up, started at 6 years after discontinuation of the treatment, estrogen-based hormone therapy delivered near the time of menopause provided neither cognitive benefit nor detriment. If administered in older women, it resulted in small decrements in several cognitive domains that remained for many years (52).

In a cohort of women in the study KEEPS branch KEEPS-Cog (n = 662) administration of menopause hormone treatment (MHT) with oral CEE and progestin or transdermal E2 and progestin (first 12 days/month) (average 2.85 y, standard deviation [SD] = 0.49) did not alter cognitive functions. The study duration was four years and beyond. Because most of the women in the study were white, well-educated, and at low risk of cardiovascular disease, its results may not be applicable to the general postmenopausal population (51).

Hiroi *et al.* 2016 showed that just the estrogen preparation used in these studies - EEC might be ineffective or poorly effective in cognitive deterioration caused by AD. EEC is comprised of more than 10 different forms of estrogens, including estrone sulfate as the primary form of estrogen, and only a minimal level of E2. In humans and rats E2 (17beta estradiol) is more effective (53).

Nested case-control study conducted on the entire Finnish population between 2005-2011 which included 70700 persons with AD and pairs without AD (282.862). Out of these, 47.117 of those who developed dementia and 184.463 from the control group used HT (hormone therapy replacement). The conclusion of this study is that HT is an important determinant for the risk

of AD. The study is in agreement with the Cochrane metaanalysis 2017 (54).

A Finnish cohort study also published in 2017 with data from the register-based study Kupio Osteoporosis Risk Factors and Prevention found an incidence of 227 cases of Alzheimer's dementia from 8195 people who completed all questionnaires. The study was conducted between 1989 and 2009. After removing the confounding factors (socio-economic and lifestyle), neither register-based nor self-reported hormone therapy use was linked with a risk of AD, and there was no association between duration of its use and AD (55).

Clinical trials using SERMs in AD

SERMs have selective agonist or antagonist effects on estrogen responsive tissues. The biological effects of the SERMs are mediated by their increased affinity to the estrogen receptors with regulation of gene expression.

In 2015 Henderson VW *et al.* conducted a double blind randomized clinical trial on 42 women with mild to moderate AD who received high doses of raloxifene 120 mg orally. The results showed that raloxifene did not significantly affect ADAS-Cog (Alzheimer's Disease Assessment Scale) (56). This study was not strong enough to determine mild effects on cognitive performance.

For studies using raloxifene for the treatment of cognitive disorders and anxiety, depression, sleep, sexual and vasomotor disorders associated with menopause there is a review made in 2013 (57) which found seven trials which fitted the criteria of eligibility, including 3 studies which assessed the cognitive function. Two studies (Jacobsen *et al.* 2010 and Yaffe *et al.* 2005) showed that raloxifene in a dose of 60 mg improved verbal memory after 12 months of treatment and that raloxifene in a dose of 120 mg /day had a preventive effect which consisted in a reduction of AD risk and of the MCI by 33% after 36 months of treatment. The number of patients in those studies was very high (213 patients, mean age 73.5 years) for Jacobsen *et al.* 2010, 5386 respectively, mean age 66 at 19 years post-menopausal for Yaffe *et al.*, 2005. Jacobsen *et al.*, 2010 included in the study only healthy women without visual cognitive disorders. Yaffe *et al.*, 2005 did not have as primary outcome the cognitive impairment but parameters as bone density. Mild cognitive impairment (MCI) and dementia were secondary outcomes. Nickelsen *et al.*, 1999 did not show significant changes in cognition and affective

disorders after 1 year of treatment, with a trend toward increased cognitive performance for the dose of raloxifene of 120 mg/day. The group of patients was lower in this study (143 patients, mean age 68 years, post-menopause 22.5 years). Women in the study were healthy (no cognitive impairment).

In conclusion, the studies analyzed in the present paper have a high heterogeneity of preparations used, of administration routes, of the female population included and of the periods of time from the appearance / induction of menopause to the therapeutic intervention and also of follow-up periods of patients and of the means of evaluating their cognitive decline. One can say that all the ways of pharmacological influence on the membrane or intracellular signaling system associated to estrogens that may have clinical importance in the prevention and possibly in the treatment of Alzheimer's disease have not been exhausted.

A new SERM, namely STX, a GqMER agonist, reviewed above, that exhibits the neuroprotective effects of estrogens without inducing oncogenic and thrombotic side effects, was identified. Its peculiar effect on nuclear and membrane ER suggests that STX could provide a novel therapeutic strategy (23).

Estrogens with selective ER α or G protein-coupled estrogen receptors: GPER1 or GqMER effects could be used in the future to influence favourably the resolution of inflammation process, with positive effects in AD evolution (18).

Conflict of interest

The authors declare that they have no conflict of interest concerning this article.

References

- Serrano-Pozo A, Frosch MP, Masliah E, Hyman BT. Neuropathological alterations in Alzheimer disease. *Cold Spring Harb. Perspect. Med.* 2011; 1:a006189.
- Anand R, Gill KD, Mahdi AA. Therapeutics of Alzheimer's disease: Past, present and future. *Neuropharmacology* 2014; 76 Pt A:27–50.
- Finch CE, Morgan TE. Systemic inflammation, infection, ApoE alleles, and Alzheimer disease. a position paper. *Curr. Alzheimer Res.* 2007; 4:185–189.
- Hardy J, Allsop D. Amyloid deposition as the central event in the aetiology of Alzheimer's disease. *Trends Pharmacol. Sci.* 1991; 12:383–388.
- Morris M, Maeda S, Vossell K, Mucke L. The Many Faces of Tau. *Neuron* 2011; 70:410–426.
- Wang X, Wang W, Li L, Perry G, Lee H, Zhu X. Oxidative Stress and Mitochondrial Dysfunction in Alzheimer's Disease. *Biochim. Biophys. Acta* 2014; 1842:1240–1247.
- Venkateshappa C, Harish G, Mahadevan A, Srinivas Bharath MM, Shankar SK. Elevated oxidative stress and decreased antioxidant function in the human hippocampus and frontal cortex with increasing age: implications for neurodegeneration in Alzheimer's disease. *Neurochem. Res.* 2012; 37:1601–1614.
- Hirai K, Aliev G, Nunomura A, Fujioka H, Russell RL, Atwood CS, Johnson AB, Kress Y, Vinters HV, Tabaton M, Shimohama S, Cash AD, Siedlak SL, Harris PL, Jones PK, Petersen RB, Perry G, Smith MA. Mitochondrial abnormalities in Alzheimer's disease. *J. Neurosci. Off. J. Soc. Neurosci.* 2001; 21:3017–3023.
- Kruman I, Guo Q, Mattson MP. Calcium and reactive oxygen species mediate staurosporine-induced mitochondrial dysfunction and apoptosis in PC12 cells. *J. Neurosci. Res.* 1998; 51:293–308.
- Ransohoff RM. How neuroinflammation contributes to neurodegeneration. *Science* 2016; 353:777–783.
- Loane DJ, Byrnes KR. Role of microglia in neurotrauma. *Neurother. J. Am. Soc. Exp. Neurother.* 2010; 7:366–377.
- Mosher KI, Wyss-Coray T. Microglial Dysfunction in Brain Aging and Alzheimer's Disease. *Biochem. Pharmacol.* 2014; 88:594–604.
- Zhang B, Gaiteri C, Bodea L-G, Wang Z, McElwee J, Podtelezchnikov AA, Zhang C, Xie T, Tran L, Dobrin R, Fluder E, Clurman B, Melquist S, Narayanan M, Suver C, Shah H, Mahajan M, Gillis T, Mysore J, MacDonald ME, Lamb JR, Bennett DA, Molony C, Stone DJ, Gudnason V, Myers AJ, Schadt EE, Neumann H, Zhu J, Emilsson V. Integrated systems approach identifies genetic nodes and networks in late-onset Alzheimer's disease. *Cell* 2013; 153:707–720.
- Harris SA, Harris EA. Herpes Simplex Virus Type 1 and Other Pathogens are Key Causative Factors in Sporadic Alzheimer's Disease. *J. Alzheimers Dis.* 2015; 48:319–353.
- Wotton CJ, Goldacre MJ. Associations between specific autoimmune diseases and subsequent dementia: retrospective record-linkage cohort study, UK. *J. Epidemiol. Community Health* 2017; in press.
- Heneka MT, Carson MJ, El Khoury J, Landreth GE, Brosseron F, Feinstein DL, Jacobs AH, Wyss-Coray T, Vitorica J, Ransohoff RM, Herrup K, Frautschy SA, Finsen B, Brown GC, Verkhatsky A, Yamanaka K, Koistinaho J, Latz E, Halle A, Petzold GC, Town T, Morgan D, Shinohara ML, Perry VH, Holmes C, Bazan NG, Brooks DJ, Hunot S, Joseph B, Deigendesch N, Garaschuk O, Boddeke E, Dinarello CA, Breitner JC, Cole GM, Golenbock DT, Kummer MP. Neuroinflammation in Alzheimer's disease. *Lancet Neurol.* 2015; 14:388–405.
- Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M. The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol.* 2004; 25:677–686.
- Villa A, Rizzi N, Vegeto E, Ciana P, Maggi A. Estrogen accelerates the resolution of inflammation in macrophagic cells. *Sci. Rep.* 2015; 5:15224.
- Engler-Chiurazzi EB, Brown CM, Povroznik JM, Simpkins JW. Estrogens as neuroprotectants: Estrogenic actions in the context of cognitive aging and brain injury. *Prog. Neurobiol.* 2016; in press.
- Böttner M, Thelen P, Jarry H. Estrogen receptor beta: tissue distribution and the still largely enigmatic physiological function. *J. Steroid Biochem. Mol. Biol.* 2014; 139:245–251.
- Dey P, Velazquez-Villegas LA, Faria M, Turner A, Jonsson P, Webb P, Williams C, Gustafsson J-Å, Ström AM. Estrogen Receptor β Induces Hypoxia Signature of Gene Expression by Stabilizing HIF-1 α in Prostate Cancer. *PLoS One* 2015; 10:e0128239.
- Alexander A, Irving AJ, Harvey J. Emerging roles for the novel estrogen-sensing receptor GPER1 in the CNS. *Neuropharmacology* 2017; 113:652–660.
- Gray NE, Zweig JA, Kawamoto C, Quinn JF, Copenhaver PF. STX, a Novel Membrane Estrogen Receptor Ligand, Protects Against Amyloid- β Toxicity. *J. Alzheimers Dis. JAD* 2016; 51:391–403.
- Gillies GE, McArthur S. Estrogen Actions in the Brain and the Basis for Differential Action in Men and Women: A Case for Sex-Specific Medicines. *Pharmacol. Rev.* 2010; 62:155–198.
- Arevalo M-A, Azcoitia I, Garcia-Segura LM. The neuroprotective actions of oestradiol and oestrogen receptors. *Nat. Rev. Neurosci.* 2015; 16:17–29.
- Zhang Q-G, Wang R, Tang H, Dong Y, Chan A, Sareddy GR,

- Vadlamudi RK, Brann DW. Brain-derived estrogen exerts anti-inflammatory and neuroprotective actions in the rat hippocampus. *Mol. Cell. Endocrinol.* 2014; 389:84–91.
27. Vegeto E, Benedusi V, Maggi A. Estrogen anti-inflammatory activity in brain: a therapeutic opportunity for menopause and neurodegenerative diseases. *Front. Neuroendocrinol.* 2008; 29:507–519.
28. Nakagami F, Nakagami H, Osako MK, Iwabayashi M, Taniyama Y, Doi T, Shimizu H, Shimamura M, Rakugi H, Morishita R. Estrogen attenuates vascular remodeling in Lp(a) transgenic mice. *Atherosclerosis* 2010; 211:41–47.
29. Grimm A, Lim Y-A, Mensah-Nyagan AG, Götz J, Eckert A. Alzheimer's disease, oestrogen and mitochondria: an ambiguous relationship. *Mol. Neurobiol.* 2012; 46:151–160.
30. Vegeto E, Belcredito S, Etteri S, Ghisletti S, Brusadelli A, Meda C, Krust A, Dupont S, Ciana P, Chambon P, Maggi A. Estrogen receptor- α mediates the brain antiinflammatory activity of estradiol. *Proc. Natl. Acad. Sci. U. S. A.* 2003; 100:9614–9619.
31. Sarkar S, Jun S, Simpkins JW. Estrogen amelioration of $A\beta$ -induced defects in mitochondria is mediated by mitochondrial signaling pathway involving ER β , AKAP and Drp1. *Brain Res.* 2015; 1616:101–111.
32. Guerriero F, Sgarlata C, Francis M, Maurizi N, Faragli A, Perna S, Rondanelli M, Rollone M, Ricevuti G. Neuroinflammation, immune system and Alzheimer disease: searching for the missing link. *Aging Clin. Exp. Res.* 2016; in press.
33. Brinton RD. Cellular and molecular mechanisms of estrogen regulation of memory function and neuroprotection against Alzheimer's disease: recent insights and remaining challenges. *Learn. Mem. Cold Spring Harb. N* 2001; 8:121–133.
34. Woolley CS, Gould E, Frankfurt M, McEwen BS. Naturally occurring fluctuation in dendritic spine density on adult hippocampal pyramidal neurons. *J. Neurosci. Off. J. Soc. Neurosci.* 1990; 10:4035–4039.
35. Bimonte-Nelson HA, Nelson ME, Granholm A-CE. Progesterone counteracts estrogen-induced increases in neurotrophins in the aged female rat brain. *Neuroreport* 2004; 15:2659–2663.
36. Gibbs RB. Effects of estrogen on basal forebrain cholinergic neurons vary as a function of dose and duration of treatment. *Brain Res.* 1997; 757:10–16.
37. Henderson VW Chapter 23. The role of sex steroids in Alzheimer's Disease: Prevention and Treatment in Lobo RA. *Treatment of the Postmenopausal Woman*. Third Edition. pp 295-306.
38. Persa OD, Joanta AE, Miclutia I, Decca N, Balaj R, Clichici S, Szentagotai L. Correlations Between Thyroid Stimulating Hormone (TSH) and Oxidative Stress in Mixed Dementia. *Acta Endocrinologica-Bucharest* 2013; 9(2):161-169.
39. Ren H, Tan S, Zhang Y, Lin Z, Peng D, Liu W, Huang T, Hu J. The C677T Methylenetetrahydrofolate Reductase Mutation and Alzheimer's Disease: A Meta-Analysis of 33 Studies. *Acta Endocrinologica-Bucharest* 2014; 10(3):443-456.
40. Maximov PY, Lee TM, Jordan VC. The discovery and development of selective estrogen receptor modulators (SERMs) for clinical practice. *Curr. Clin. Pharmacol.* 2013; 8:135–155.
41. Hajjo R, Setola V, Roth BL, Tropsha A. Chemocentric informatics approach to drug discovery: identification and experimental validation of selective estrogen receptor modulators as ligands of 5-hydroxytryptamine-6 receptors and as potential cognition enhancers. *J. Med. Chem.* 2012; 55:5704–5719.
42. Lee E-SY, Sidoryk M, Jiang H, Yin Z, Aschner M. Estrogen and tamoxifen reverse manganese-induced glutamate transporter impairment in astrocytes. *J. Neurochem.* 2009; 110:530–544.
43. Corbel C, Zhang B, Le Parc A, Baratte B, Colas P, Couturier C, Kosik KS, Landrieu I, Le Tilly V, Bach S. Tamoxifen inhibits CDK5 kinase activity by interacting with p35/p25 and modulates the pattern of tau phosphorylation. *Chem. Biol.* 2015; 22:472–482.
44. O'Neill K, Chen S, Brinton RD. Impact of the selective estrogen receptor modulator, raloxifene, on neuronal survival and outgrowth following toxic insults associated with aging and Alzheimer's disease. *Exp. Neurol.* 2004; 185:63–80.
45. Du B, Ohmichi M, Takahashi K, Kawagoe J, Ohshima C, Igarashi H, Mori-Abe A, Saitoh M, Ohta T, Ohishi A, Doshida M, Tezuka N, Takahashi T, Kurachi H. Both estrogen and raloxifene protect against beta-amyloid-induced neurotoxicity in estrogen receptor α -transfected PC12 cells by activation of telomerase activity via Akt cascade. *J. Endocrinol.* 2004; 183:605–615.
46. Wu X, Glinn MA, Ostrowski NL, Su Y, Ni B, Cole HW, Bryant HU, Paul SM. Raloxifene and estradiol benzoate both fully restore hippocampal choline acetyltransferase activity in ovariectomized rats. *Brain Res.* 1999; 847:98–104.
47. Kwakowsky A, Milne MR, Waldvogel HJ, Faull RL. Effect of Estradiol on Neurotrophin Receptors in Basal Forebrain Cholinergic Neurons: Relevance for Alzheimer's Disease. *Int. J. Mol. Sci.* 2016; 17 in press.
48. Brann DW, Dhandapani K, Wakade C, Mahesh VB, Khan MM. Neurotrophic and neuroprotective actions of estrogen: basic mechanisms and clinical implications. *Steroids* 2007; 72:381–405.
49. Rapp SR, Espeland MA, Shumaker SA, Henderson VW, Brunner RL, Manson JE, Gass MLS, Stefanick ML, Lane DS, Hays J, Johnson KC, Coker LH, Dailey M, Bowen D, WHIMS Investigators. Effect of estrogen plus progestin on global cognitive function in postmenopausal women: the Women's Health Initiative Memory Study. a randomized controlled trial. *JAMA* 2003; 289:2663–2672.
50. Espeland MA, Rapp SR, Shumaker SA, Brunner R, Manson JE, Sherwin BB, Hsia J, Margolis KL, Hogan PE, Wallace R, Dailey M, Freeman R, Hays J, Women's Health Initiative Memory Study. Conjugated equine estrogens and global cognitive function in postmenopausal women: Women's Health Initiative Memory Study. *JAMA* 2004; 291:2959–2968.
51. Gleason CE, Dowling NM, Wharton W, Manson JE, Miller VM, Atwood CS, Brinton EA, Cedars MI, Lobo RA, Merriam GR, Neal-Perry G, Santoro NF, Taylor HS, Black DM, Budoff MJ, Hodis HN, Naftolin F, Harman SM, Asthana S. Effects of Hormone Therapy on Cognition and Mood in Recently Postmenopausal Women: Findings from the Randomized, Controlled KEEPS-Cognitive and Affective Study. *PLoS Med.* 2015; 12:e1001833; discussion e1001833.
52. Espeland MA, Rapp SR, Manson JE, Goveas JS, Shumaker SA, Hayden KM, Weitlauf JC, Gaussoin SA, Baker LD, Padula CB, Hou L, Resnick SM, WHIMSY and WHIMS-ECHO Study Groups. Long-term Effects on Cognitive Trajectories of Postmenopausal Hormone Therapy in Two Age Groups. *J. Gerontol. A. Biol. Sci. Med. Sci.* 2016; in press.
53. Hiroi R, Weyrich G, Koebele SV, Mennenga SE, Talboom JS, Hewitt LT, Lavery CN, Mendoza P, Jordan A, Bimonte-Nelson HA. Benefits of Hormone Therapy Estrogens Depend on Estrogen Type. 17 β -Estradiol and Conjugated Equine Estrogens Have Differential Effects on Cognitive, Anxiety-Like, and Depressive-Like Behaviors and Increase Tryptophan Hydroxylase-2 mRNA Levels in Dorsal Raphe Nucleus Subregions. *Front. Neurosci.* 2016; 10:517.
54. Imtiaz B, Taipale H, Tanskanen A, Tiihonen M, Kivipelto M, Heikkinen A-M, Tiihonen J, Soininen H, Hartikainen S, Tolppanen A-M. Risk of Alzheimer's disease among users of postmenopausal hormone therapy: A nationwide case-control study. *Maturitas* 2017; 98:7–13.
55. Imtiaz B, Tuppurainen M, Rikkinen T, Kivipelto M, Soininen H, Kröger H, Tolppanen A-M. Postmenopausal hormone therapy and Alzheimer disease: A prospective cohort study. *Neurology* 2017; in press.
56. Henderson VW, Ala T, Sainani KL, Bernstein AL, Stephenson BS, Rosen AC, Farlow MR. Raloxifene for women with Alzheimer disease: A randomized controlled pilot trial. *Neurology* 2015; 85:1937–1944.
57. Yang ZD, Yu J, Zhang Q. Effects of raloxifene on cognition, mental health, sleep and sexual function in menopausal women: a systematic review of randomized controlled trials. *Maturitas* 2013; 75:341–348.