Mifepristone Alters Amyloid Precursor Protein Processing to Preclude Amyloid Beta and Also Reduces Tau Pathology

David Baglietto-Vargas, Rodrigo Medeiros, Hilda Martinez-Coria, Frank M. LaFerla, and Kim N. Green

Background: Increased circulating glucocorticoids are features of both aging and Alzheimer's disease (AD), and increased glucocorticoids accelerate the accumulation of AD pathologies. Here, we analyzed the effects of the glucocorticoid receptor antagonist mifepristone (RU486) in the 3xTg-AD mouse model at an age where hippocampal damage leads to high circulating corticosterone levels.

Methods: The effects of mifepristone were investigated in 3xTg-AD mice using a combination of biochemical, histological, and behavior analyses.

Results: Mifepristone treatment rescues the pathologically induced cognitive impairments and markedly reduces amyloid beta (Aβ)-load and levels, as well as tau pathologies. Analysis of amyloid precursor protein (APP) processing revealed concomitant decreases in both APP C-terminal fragments C99 and C83 and the appearance of a larger 17-kDa C-terminal fragment. Hence, mifepristone induces a novel C-terminal cleavage of APP that prevents it being cleaved by α- or β-secretase, thereby precluding Aβ generation in the central nervous system; this cleavage and the production of the 17-kDa APP fragment was generated by a calcium-dependent cysteine protease. In addition, mifepristone treatment also reduced the phosphorylation and accumulation of tau, concomitant with reductions in p25. Notably, deficits in cyclic-AMP response element-binding protein signaling were restored with the treatment.

Conclusions: These preclinical results point to a potential therapeutic role for mifepristone as an effective treatment for AD and further highlight the impact the glucocorticoid system has as a regulator of Aβ generation.

Key Words: Amyloid beta, Alzheimer's disease, glucocorticoids, mifepristone, tau, 3xTg-AD

Alzheimer's disease (AD) is the most prevalent cause of dementia in the elderly, with only palliative treatments available at this time. It has been shown that AD patients display significant elevated levels of the glucocorticoid hormone cortisol in plasma and cerebrospinal fluid, as well as hypothalamic-pituitary-adrenal (HPA) axis dysfunction (1–3).

We previously showed that glucocorticoids (GCs) (cortisol in humans and corticosterone in rodents) could drive the formation of AD hallmark pathologies through increased production of the amyloid beta (Aβ) peptide and accumulation of somatodendritic tau (4). In addition, both stress and increased GC exposure have shown to induce cognitive impairments, trigger amyloid precursor protein (APP) misprocessing, reduce Aβ clearance by decreasing activity of insulin degrading enzyme (IDE), and stimulating tau hyperphosphorylation (5–9), together demonstrating a key role for GCs in the progression of AD pathology and cognitive decline. Glucocorticoids are key stress hormones secreted by the adrenal gland and controlled by the HPA axis, which mediate their effects in the different brain areas through two types of receptors: mineralocorticoid type I receptors and glucocorticoid type II receptors (GR) (10). Recently, the GR have been implicated in epigenetic mechanisms of neurodegeneration via histone deacetylase 2 (11).

The current study investigates the therapeutic potential of the glucocorticoid receptor antagonist mifepristone (RU486) on both cognitive and pathological outcomes in the 3xTg-AD mouse model of AD. 3xTg-AD mice show increased circulating levels of GCs from 9 months of age, when hippocampal pathology is advanced (4). Hence, we sought to evaluate if blocking the effects of GCs could help reduce pathology and cognitive decline in animals of this age. Here, we report that the GR blocker, mifepristone, has unparalleled effects on both outcomes, leading to robust reductions in Aβ levels and plaques through the induction of a 17-kDa cleavage of APP, precluding Aβ generation. In addition, it restores cyclic-AMP response element-binding protein (CREB) signaling and reduces tau hyperphosphorylation via reductions in p25 levels. Hence, our results show that compounds targeting the glucocorticoid system could be useful for the treatment of AD, in part through novel disease-modifying effects on Aβ generation.

Methods and Materials

Transgenic Mice and Treatment

All animal procedures were performed in accordance with National Institutes of Health and University of California guidelines and Use Committee at the University of California, Irvine. The characterization of 3xTg-AD mice has been described previously (12). In this study, 12-month-old homozygous non-transgenic (Ntg) and 3xTg-AD mice, 8 to 10 per group (male animals) were anesthetized with isoflurane and drug pellets containing the glucocorticoid receptor antagonist mifepristone (17b-hydroxy-11a-(4-dimethylaminophenyl)-17a-(1-propynyl)-

From the Department of Neurobiology and Behavior and Institute for Memory Impairments and Neurological Disorders, University of California, Irvine, Irvine, California.

Address correspondence to Kim N. Green, Ph.D., University of California, Irvine, Department of Neurobiology and Behavior, Institute for Memory Impairments and Neurological Disorders, 3208 Biological Sciences 3, Irvine, CA 92697-4545; E-mail: kngreen@uci.edu.

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estra-4,9-dien-3-one) (Innovative Research of America, Sarasota, Florida) or vehicle pellets were implanted subcutaneous according to the manufacturer's instructions. In addition, 10-month-old homozygous 3xTg-AD mice, 10 per group (male animals) were anesthetized with isoflurane and drug pellets containing the selective glucocorticoid antagonist Cort-108297 ((R)-4a-Ethoxymethyl-1-(4-fluorophenyl)-6-(4-trifluoromethyl-benzenesulfonyl0-4,4a,5,6,7,8-hexahydro-1H-1,2,6-triaza-cyclopenta[b]naphthalene) (Corcept Therapeutics, Menlo Park, California) or vehicle pellets were implanted subcutaneous. The animals were treated for 21 days with 25.2 mg pellets that released a continuous flow of the drug at 1.2 mg per day (13–15).

Behavior Testing

Novel context, place, and object were conducted as described previously (16). Open field was performed as previously described (16). Hidden Morris water maze (MWM) tests were conducted as described previously (17,18). The passive inhibitory avoidance task was performed as described previously (19,20).

Tissue Preparation

Tissue preparation was performed as previously described (17,18). For protease analyses, whole brains were homogenized with/without complete protease inhibitor cocktail tablets (Roche Diagnostics GmbH, Mannheim, Germany) or specific families of protease inhibitor (leupeptin: 10 mg/mL; E64: 10 μmol/L; aprotinin: 10 mg/mL; pepstatin: 1 μmol/L; phosphoramidon: 10 μmol/L [Sigma–Aldrich, St. Louis, Missouri]), in the presence or absence of calcium in assay buffer (assay buffer: 135 mmol/L sodium chloride, 5 mmol/L potassium chloride, 1.2 mmol/L magnesium sulfate, 5 mmol/L N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid, 10 mmol/L glucose, and 2.5 mmol/L calcium chloride). The samples were incubated 20 minutes at 37°C with the differences protease inhibitors and then immunoblotted for APP fragments using C-terminal antibody CT20 (Calbiochem, San Diego, California).

Immunohistochemistry

Immunohistochemistry for light microscopy was performed as we described previously (21). The following antibodies were used: anti-6E10 (1:1000; Covance Research Products, Denver, Pennsylvania) and PHF1 (1:1000; Pierce Biotechnology). The specificity of the immune reactions was controlled by omitting the primary antibody.

Total Aβ Loading

Quantification of total Aβ content was performed as previously described (21).

Immunoblotting

Immunoblot analyses were performed as described (4). The following primary antibodies were used: anti-CREB (1:1000; Cell Signaling), anti-phospho-CREB (ser133) (1:1000; Cell Signaling), anti-CTF20 (1:5000; Calbiochem) for C99 and C83, anti-HT7 (1:5000; Pierce Biotechnology), anti-AT8 (1:1000; Pierce Biotechnology), anti-AT180 (1:1000; Pierce Biotechnology), anti-AT270 (1:1000; Pierce Biotechnology), anti-PHF1 (Dr. Peter Davies, Albert Einstein College of Medicine, Manhasset, New York), anti-ADAM10 (1:1000; Calbiochem), anti-BACE (1:1000; Calbiochem), anti-IDE (1:1000; Chemicon), anti-total glycogen synthase kinase 3 (GSK3) α and β (1:5000; Calbiochem), anti-pGSK3β (ser9) (1:3000; Cell Signaling), anti-cyclin-dependent kinase 5 (Cdk5) (1:1000; Calbiochem), anti-C′-term p35 (1:200; Santa Cruz Biotechnology, Santa Cruz, California) for p25 and p35, anti-PP2A (1:1000; Santa Cruz Biotechnology), and anti-glyceraldehyde 3-phosphate dehydrogenase (1:5000; Santa Cruz Biotechnology).

Enzyme-Linked Immunosorbent Assay for $A\beta_{40}$ and $A\beta_{42}$

$A\beta_{1-40}$ and $A\beta_{1-42}$ were measured using a sensitive sandwich enzyme-linked immunosorbent assay system as previously described (4).

Mass Spectrometry of Mifepristone

Plasma (30 uL) was vortexed with 200 uL of dichloromethane, and the dichloromethane fraction was taken and air-dried. The precipitate was dissolved in 50 uL of 50% acetonitrile and then separated on an Acquity UltraPerformance LC (Waters Corporation, Milford, Massachusetts) and then analyzed on a Quatro Premier XE. Mifepristone standards showed fragments of molecular weight 134 and 370, which were positively identified in the plasma samples.

Quantitative and Statistical Analyses

All immunoblot data were quantitatively analyzed using ImageJ 1.4 software (Center for Information Technology National Institutes of Health, Bethesda, Maryland). Statistical evaluation of the results was performed using Student *t* test comparison to compare two groups or two-way analysis of variance (ANOVA). After significant analyses of variance, multiple post hoc comparisons were performed using Bonferroni's test. Analysis of MWM acquisition was evaluated via repeated measure of variance. The significance was set at 95% of confidence. All values are presented as mean ± SEM. All tests were performed using Graphpad Prism software (Graphpad Prism Inc., San Diego, California).

Results

Mifepristone Rescues Cognitive Deficits in Aged 3xTg-AD Mice

Male 3xTg-AD mice (12-month-old) were implanted with subcutaneous pellets containing 15 mg mifepristone or vehicle pellets designed to release a continuous flow of 10 μg per hour for 60 days. This concentration of mifepristone has proven effective to inhibit GR activity in mice (22,23). To determine whether mifepristone treatment could rescue cognitive deficits, 3xTg-AD mice were tested on a battery of behavioral tasks. Testing occurred during the last 2 weeks of treatment and probed cortical, hippocampal, and amygdala-dependent memory tasks, brain areas that are most severely affected by AD-like pathology in the 3xTg-AD (12,24).

Treated and vehicle-treated 3xTg-AD and Ntg mice were first tested on novel context/place/object tasks. These tasks are mainly hippocampal and cortical dependent, respectively. Vehicle-treated 3xTg-AD mice were significantly impaired compared with Ntg mice only on the novel object task, spending 50.17 ± 12.99% of their time exploring the out-of-context object, 47.09 ± 7.56% of their time exploring the out-of-place object, and 44.53 ± 9.87% of their time exploring the new object (Figure 1A–C), compared with Ntg mice. In contrast, mifepristone-treated 3xTg-AD mice showed a significant improvement in performance, spending 71.08 ± 11.30% of their time exploring the new object and 70.92 ± 7.70% of their time exploring the out-of-context object (Figure 1A,C). No differences