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PERSPECTIVE

Anticholinergic drugs and dementia risk: Using stem cell–based studies to complement pharmacoepidemiology

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Abstract

BACKGROUND: Anticholinergic (AC) use remains common in older adults despite evidence of safety risks, including increased risk in dementia. Pharmacoepidemiology studies from various populations report associations between specific anticholinergic classes – antidepressants and bladder antimuscarinics – and increased dementia incidence. However, it is difficult to determine whether these associations are directly caused by the neurotoxic effects of anticholinergic drugs or by the underlying health conditions which the medications are taken for, known as confounding by indication. Here, we leverage human induced pluripotent stem cells-derived-neurons (hiPSC-Ns) to complement the pharmacoepidemiology studies by directly examining the effects of various anticholinergic classes on dementia-related cellular phenotypes.

METHODS: We treated human induced pluripotent stem cell (hiPSC)–derived neurons with eight drugs representing different AC medication classes, including antidepressants, bladder antimuscarinics, antihistamines, and antispasmodics. We analyzed these neurons for cytotoxicity, amyloid beta (Aβ) peptide levels in the conditioned medium, and the level of intracellular phosphorylated tau from these cultures.

RESULTS: We observed that antidepressants and bladder antimuscarinics were consistently cytotoxic, whereas antihistamines and antispasmodics did not show overt cytotoxicity at the times and concentrations that we tested. Some of the cytotoxic medications altered the amounts of Aβ1-42 peptides, but there were no significant differences in the intracellular ratio of phosphorylated tau/total tau between AC drug treatments.

CONCLUSIONS: These results corroborate population-based studies and suggest a molecular basis for the differences in dementia risk observed according to AC class. This warrants future work examining the effect of AC medications on hiPSC-derived cells from multiple subjects and examining other molecular outcomes including synaptic function and neuroinflammation in hiPSC-based models.

Tiara A. Schwarze-Taufiq and Inez K. A. Pranoto contributed equally to this work.

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KEYWORDS
Alzheimer's disease, anticholinergics, cytotoxicity, human induced pluripotent stem cells, pharmacoepidemiology

Highlights

- Certain classes of anticholinergic (AC) medications are linked to dementia.
- Human-induced pluripotent stem cell (hiPSC) models are used to directly test the cytotoxicity of AC medications.
- AC classes that are associated with dementia are more neurotoxic.

1 | INTRODUCTION

Medications with anticholinergic (AC) activity (known as anticholinergics) are widely used in older adults to manage diverse health conditions such as overactive bladder, seasonal allergies, and depression.[1,2] AC medications prevent acetylcholine from binding to muscarinic acetylcholine receptors (mAChRs) and/or nicotinic receptors expressed throughout the body. There are five types of mAChRs: M1–M5. In the central nervous system (CNS), the primary role of the mACh pathway in neurons is modulation of neuronal excitability, synaptic plasticity, and feedback regulation.[3]

Given the wide distribution of muscarinic receptors, AC medications that specifically target these receptors are associated with several adverse drug events, such as constipation, blurred vision, xerostomia, delirium, and impaired coordination and memory. Older adults are especially susceptible to these events because of age- and disease-related changes in pharmacokinetics and pharmacodynamics. It is well established that ACs cause temporary short-term impairment in cognition, including attention and reaction time, and increasing evidence suggests that ACs may have more permanent effects on cognition.[4,5] Prescribing guidelines recommend avoiding the use of ACs in most older adults, especially for those with cognitive impairment because of the potential for worsened cognition and delirium.[6–8]

Over the past decade, mounting evidence suggests that overall AC exposure may be associated with increased dementia risk.[5,9–11] Risk is greater for those with higher overall exposure.[9–11] A 2020 meta-analysis of seven observational studies estimated a 20% greater incidence, on average, of dementia associated with the use of drugs with definite AC activity (pooled odds ratio [OR] 1.20, 95% confidence interval [CI] 1.09–1.32), with risk greatest with longer duration of use.[5] The authors concluded that most included studies had serious bias, and it is likely that confounding by indication, residual confounding, and reverse causation bias may be present.

2 | CHALLENGES IN THE UNDERSTANDING OF AC–DEMENTIA RISK IN OBSERVATIONAL STUDIES

2.1 | Overall AC burden or AC medication classes

Because many medications have AC properties, considerable effort has been focused on developing tools that capture overall AC burden from a person's medication list.[12] The central premise has been that the additive effects of all AC medications are important to quantify when examining cognitive outcomes, even for medications with mild effects. However, recent studies have assessed AC exposure according to pharmacological or therapeutic class in addition to overall burden. Unexpectedly, findings suggest inconsistent associations across these AC classes.[10,11] Specifically, antidepressants,[10,11] anti-Parkinson's,[10,11] bladder antimuscarinics,[10,11] and antiemetics[11] were significantly associated with higher risk of dementia, whereas antihistamines[10,11] and muscle relaxants[10,11] were not. Mixed findings were found for antipsychotics and gastrointestinal drugs.[10,11] Although these findings need to be replicated in other samples, it is unclear why only *some* of the strong AC medication classes would be associated with dementia risk. These findings are inconsistent with an AC-specific causal link and contradict the hypothesis that overall AC burden is central for adverse cognitive outcomes.

2.2 | Bias in observational studies

Observational studies inherently suffer to varying degrees from methodological challenges and bias. Different findings according to medication class may be due to confounding by indication, which arises when the clinical indication for a drug is associated with an outcome rather than the drug itself. Although statistical models attempt to account for this bias, it can be difficult if information about the severity of disease is not available in administrative databases or if data in the confounders were not captured at the correct time relative to the AC use. Most studies do not account for reverse causation bias, which occurs when a drug is prescribed as a treatment for early symptoms of an outcome. For instance, ACs could be prescribed for mood or sleep disorders which are early symptoms of dementia. In this type of bias, ACs would be related to dementia, but there would not be a causal relationship between ACs and dementia. Older adults often take several medications (i.e., polypharmacy) and have multimorbidity, with high prevalence in those with the highest AC exposure[9] and residual confounding may occur, despite adjustment for these factors. Finally, studies rarely adjust for other medication classes and therefore we know little about the effects of ACs in isolation from other types of medication.

3 | BIOLOGICAL PLAUSIBILITY FOR THE AC–DEMENTIA RISK ASSOCIATION

There are mixed findings surrounding the biological association between AC medications and dementia risk. On one hand, molecular and animal studies in vitro have shown a direct biological association between cholinergic modulators and AD pathology. On the other, these studies have not translated reliably to humans, specifically as they pertain to neuropathologic features and neuroimaging data.

There is some evidence suggesting that cholinergic signaling and, by extension, cholinergic antagonism is associated with AD pathology through several pathways. Cholinergic signaling plays a key role in learning and memory, primarily dependent on the neurotransmitter acetylcholine.[13] Blocking central cholinergic activity with scopolamine in young human subjects produces memory deficits that were recovered by the cholinergic agonist physostigmine.[14] Stimulation of cholinergic receptors with muscarinic agonists was shown to shift amyloid precursor protein (APP) processing toward non-amyloidogenic cleavage.[15] Human embryonic kidney (HEK)-293 cells treated with carbachol resulted in a significant increase in the amount of preexisting APP derivatives released from the cells, a process blocked by staurosporine, suggesting that a kinase-dependent mechanism could provide a direct link between cholinergic stimulation and amyloid beta (Aβ) processing.[15] This is further supported by studies demonstrating that mAChRs regulate several downstream targets including protein kinase C, extracellular-signal-regulated kinase (ERK), cyclic adenosine monophosphate (AMP), and protein kinase A. These targets have all been implicated in alpha-secretase cleavage of APP as well as tau phosphorylation, both central disease processes in AD.[16–18]

Although relatively few animal studies have investigated the role of cholinergic antagonism in Alzheimer's disease pathology, some evidence from mouse studies suggests that muscarinic antagonism could be linked to tau pathology. In M1 mAChR knockout mice, M1 receptor loss-of-function led to higher amyloidogenic APP processing in neurons through lower agonist-regulated shedding of the neuroprotective sAPPα.[19] Similarly, M2 mAChR knockout mice have deficits in working memory and synaptic plasticity, as well as less short- and long-term potentiation in hippocampal neurons.[20] In a neurodegenerative tauopathy (P301S) model, administration of trihexyphenidyl, a predominantly CNS-acting AC, exacerbated tau pathology, microglial proliferation and activation, and neuroinflammation.[21] In a transgenic mouse model of AD (the 3XFAD model) a selective M1 agonist, AF267B, reduced AD pathology and improved cognition, whereas an M1 antagonist, dicyclomine, exacerbated pathology.[22]

Although molecular and animal studies suggest that cholinergic antagonism exacerbates AD pathology, the effect on hallmark neuropathological features of AD in humans is less clear. Specifically, the association between AC exposure and neuroimaging (i.e., brain structure, white matter hyperintensities, volume) appears to be region dependent, and there is limited evidence to suggest an association between AC exposure and AD pathologic features, such as amyloid plaques. In some studies, AC exposure was associated with lower hippocampus volume[23] and temporal ventricular volume,[24] whereas associations were not found with basal forebrain cholinergic system.[23] AC exposure was associated with lower cortical volume in one study,[24] but another study did not find an association for those who used definite anticholinergics.[25,26] Information about AC exposure is limited in these studies.[23,24] In the largest study to date involving 17,000 MRIs in the UK Biobank, AC exposure measured from electronic pharmacy data was not associated with overall brain atrophy or in 82 brain regions.[27] In a study also using electronic pharmacy data to ascertain AC exposure, higher exposure was related to higher white matter hyperintensities in participants who underwent a scan as part of routine medical care.[28] Two studies have examined anticholinergic exposure in general populations, and neither found an association between AC exposure and amyloid plaques and neurofibrillary tangles.[26,29]

4 | BRIDGING THE GAP BETWEEN EPIDEMIOLOGY AND MECHANISM: HUMAN STEM CELL MODELS

Human induced pluripotent stem cells (hiPSCs), which can be differentiated into a nearly endless supply of human brain cells, can help to fill the gap between population studies, neuroimaging, and neuropathological studies when it comes to understanding dementia risk associated with medications (Figure 1A). hiPSC models for AD and related disorders have existed since 2011.[30] Although initially these models were criticized for the study of age-related disorders due to the fetal nature of the cells, over the past decade, multiple studies have uncovered significant AD-related cellular phenotypes using hiPSC-derived neural cells.[31,32] For example, a recent large study using hiPSCs derived from participants in the Religious Orders Study and the Memory and Aging Project (ROS-MAP) detailed positive correlations between Aβ and phosphorylated tau (p-tau) levels in vitro with matched brain tissue from the donors.[33]

5 | AC DRUG CLASSES SHOW DIFFERENT LEVELS OF CYTOTOXICITY AND AD CELLULAR PHENOTYPES IN hiPSC-DERIVED NEURONS

Our goal for this *Perspective* was to test whether an hiPSC-based model could overcome confounding biases in traditional epidemiological studies. We hypothesized that AC medications associated with dementia would be more neurotoxic than those not associated with dementia. This neurotoxicity may be due to either the on-target or off-target effects of the drugs. As a proof-of-principle experiment, we tested different AC drug classes on hiPSC-derived neurons from two independent clones of a well-characterized hiPSC line.[34–38] This cell line is from a living individual without dementia but whose sequenced genome harbors an apolipoprotein E (*APOE*) ε4 allele and is therefore representative of a genetic background at risk for sporadic AD (sAD).[39] We selected eight AC medications that block mAChRs across four different classes based on medication classes examined in observational studies discussed above and the prevalence of use in older adults (Table 1).[40,41]

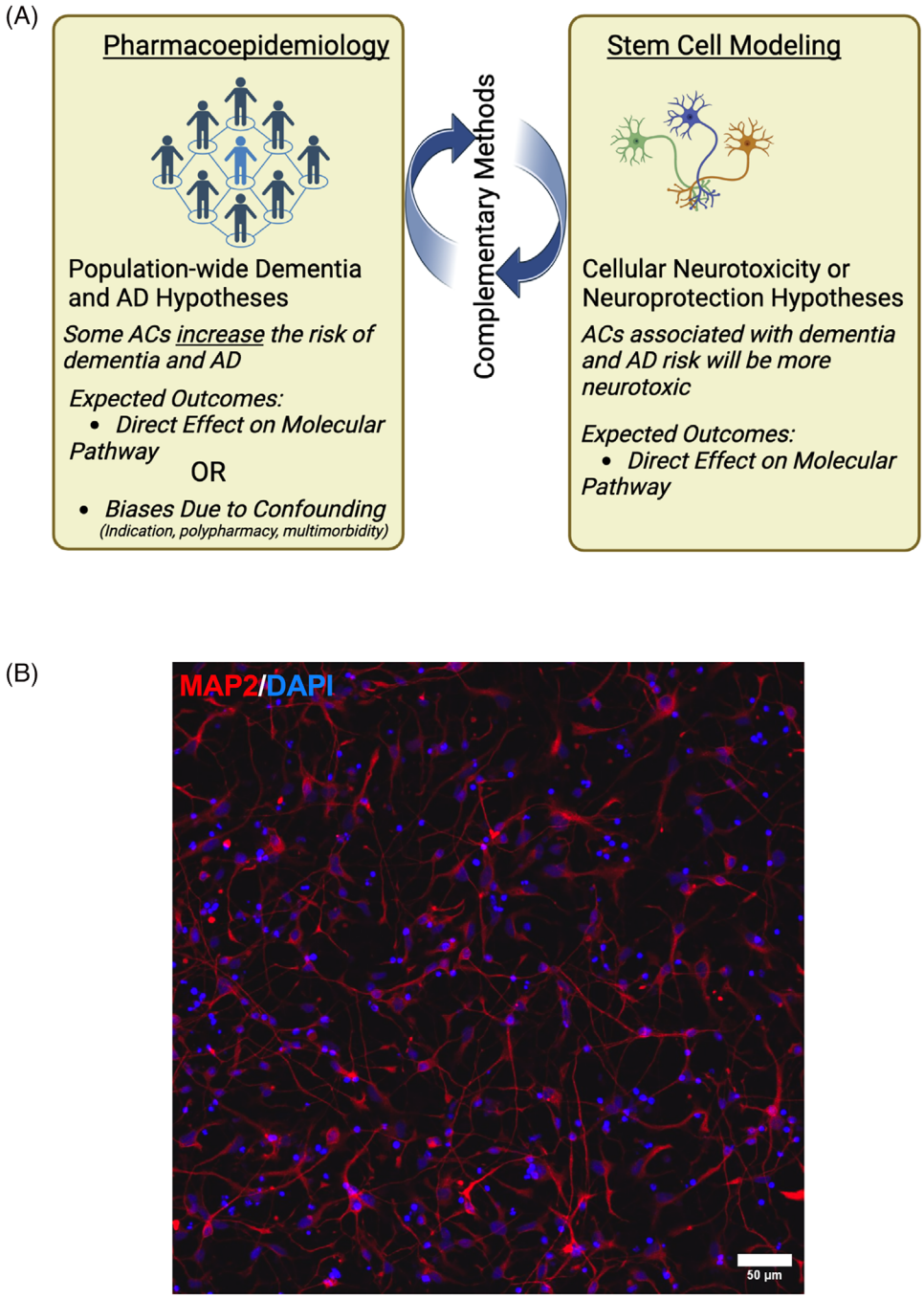

FIGURE 1 Stem cell modeling complements pharmacoepidemiology. (A) Bias is difficult to completely eliminate from pharmacoepidemiology studies. Stem cells from selected individuals from the population can be used to make clonal populations of cells without extraneous factors such as chronic disease. These cells allow us to test molecular drug mechanisms in a way that rules out biases from population-based studies. (B) Representative immunofluorescent image of hiPSC-derived cortical neurons used in this study. Microtubule-associated protein 2 (MAP2) is a marker of neuronal processes. 4′,6-diamidino-2-phenylindole (DAPI) is a marker for cell nuclei.

To look at the potential neurotoxicity of AC medications, we treated hiPSC-derived cortical neurons with three doses of each AC medication for two time spans, chosen based on comparable literature.[42–49] Cells were also treated with a cholinergic agonist, carbachol, and a vehicle control, dimethyl sulfoxide (DMSO). We measured three outcomes: cytotoxicity, Aβ peptides, and tau phosphorylation.

5.1 | Cortical neuron differentiation and characterization

We followed a standard and published protocol[34–36,50–53] to generate cortical neurons from hiPSCs. These cells are >95% neurons as indicated by microtubule-associated protein 2 (MAP2)

TABLE 1 List of anticholinergic medications tested in this study.

	Association with dementia in observational studies (Richardson et al. 2020; Coupland et al. 2019; Grey et al. 2015)	Cytotoxicity in stem cell–derived neurons	Change in Aβ1-42/Aβ1-40 ratio	Change in p-tau/t-tau ratio
Antidepressants	Yes; positive			
Amitriptyline		Dose-dependent	Slight increase	None
Doxepin		Dose- and time-dependent	None	None
Paroxetine		Dose- and time-dependent	None	None
Antihistamines	No			
Diphenhydramine		None	None	None
Chlorpheniramine		None	None	None
Bladder antimuscarinics	Yes; positive			
Oxybutynin		Dose- and time-dependent	Strong increase	None
Tolterodine		None	None	None
Antispasmodics	No			
Atropine		None	None	None

immunostaining Figure 1B and are karyotypically normal (Figure S1A). Neurons generated through this protocol are generally forebrain, cortical neurons. We confirmed the expression of muscarinic receptors *CHRM1-3* in these neurons using quantitative reverse transcriptase-quantitativepolymerase chain reaction (RT-qPCR) (Figure S1B).

5.2 | Treatment of hiPSC-derived neurons with AC medications

We chose eight AC medications based on their associations with dementia.[9,11] These medications are atropine, amitriptyline, doxepin, paroxetine, chlorpheniramine, diphenhydramine, oxybutynin, and tolterodine (Table 1). Based on the previous literature, we treated neurons with three concentrations of AC medications ranging from 10–100 μM[42,44–46,49] for two time spans (24–48 h).[42–44] To more accurately contextualize our in vitro findings with the physiologically relevant conditions, we normalized the doses in vitro to the Cmax (maximal dose in patient blood plasma) obtained from U.S. Food and Drug Administration (FDA) datasheets or the available literature. Although the concentrations of AC medications applied to our cells are higher than what is likely found in vivo, there is not a positive correlation between the drug concentration/Cmax ratio and cytotoxicity (Figure S1C,D). This suggests that the cytotoxicity we observed for specific drugs is not due solely to high in vitro concentration.

5.3 | Cytotoxicity after treatment with AC medications

Cytotoxicity was measured using a lactate dehydrogenase (LDH) assay, where LDH release into the culture medium is an indicator of membrane damage and cell death. We found evidence of dose- and time-dependent relationships between drug treatment and cytotoxicity compared to the control (Figure 2A–E). Notably, drugs belonging to the antidepressant and bladder antimuscarinic classes demonstrated higher levels of cellular toxicity, whereas antihistamine and antispasmodic drugs did not. We tested three concentrations (10, 50, and 100 μM) of AC medications for 24 h and observed dose-dependent toxicity of paroxetine and amitriptyline (Figure 2A–C). At 48 h the extent of cell death in samples treated with 100 μM paroxetine and amitriptyline was nearly 100%, so we focused our studies on the lower concentrations (10 and 50 μM) and continued to observe toxicity of antidepressants: amitriptyline, paroxetine, and doxepin, and one of the bladder antimuscarinics: oxybutynin (Figure 2D,E). Because LDH is a general measure of cell death, we tested whether the cytotoxic drugs were inducing neuronal apoptosis. Using assays for cleaved caspase-3/7 and cleaved poly (ADP-ribose) polymerase-1 (c-PARP), signature markers of apoptosis,[54,55] we observed significant induction of neuronal apoptosis in neurons after 24 h of treatment in the antidepressants (Figure 2F, Figure S1G). The bladder antimuscarinic oxybutynin did not show a significant increase in apoptotic markers, suggesting either an alternative mechanism of cell death or a different timing of neuronal apoptosis.

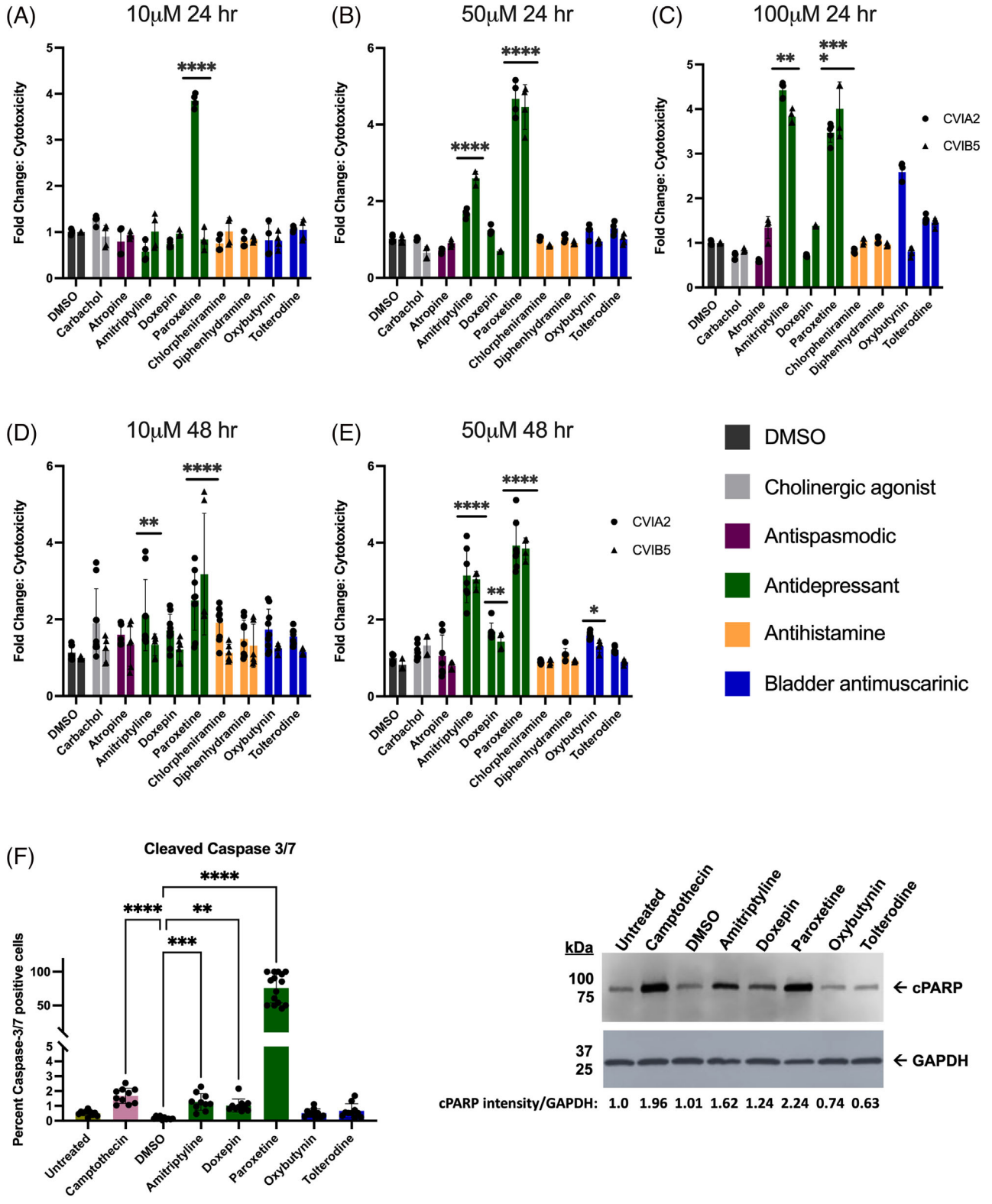

FIGURE 2 Cytotoxicity of anticholinergic medications. (A–C): 10, 50, and 100 μM treatment of neurons with eight AC medications at 24 h. (D–E): 10 and 50 μM treatment of neurons with eight AC medications at 48 h. (F) Cleaved Caspase-3/7 and cleaved PARP expression in neurons treated with cytotoxic AC medications at 50 μM for 24 h with camptothecin as a positive control for apoptosis. Overall, amitriptyline, doxepin, paroxetine, oxybutynin, and tolterodine show consistent cytotoxicity. Amitriptyline, doxepin, and paroxetine induce neuronal apoptosis. Cleaved PARP is normalized to GAPDH. For (A–E) Statistics were performed by two-way ANOVA. For (F) statistics were performed by one-way ANOVA. $^{*}p \leq 0.05$; $^{**}p < 0.01$; $^{***}p \leq 0.001$; $^{****}p \leq 0.0001$. Closed circles indicate hiPSC clone CVIA2; closed triangles indicate hiPSC clone CVIB5. AC, anticholinergic; ANOVA, analysis of variance; DMSO, dimethyl sulfoxide; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; hiPSCs; human induced pluripotent stem cells; c-PARP, cleaved poly (ADP-ribose) polymerase.

5.4 | AD cellular phenotypes after treatment with AC medications

Two main cellular phenotypes routinely measured in hiPSC-neuronal AD models are secreted Aβ peptides and intracellular levels of p-tau protein.[33,52,56] We treated hiPSC-cortical neurons with the lowest dose of AC medications and measured secreted Aβ peptides using chemiluminescent enzyme-linked immunosorbent assay (ELISA). We calculated the Aβ42:Aβ40 ratio after 48 h of treatment. Across all AC classes, oxybutynin showed the strongest difference in the Aβ42:Aβ40 ratio (Figure 3A). When individual peptides were measured across all AC treatments, oxybutynin treatment consistently was associated with higher levels of Aβ1-42 peptides, whereas Aβ1-40 peptides were not altered across different AC classes (Figure 3B,C). We then tested multiple doses (3.2–50 μM) of oxybutynin at two time points, 24 and 48 h after treatment, and documented consistently higher levels of Aβ1-42 peptides. Of interest, the increase in the Aβ42:Aβ40 ratio with oxybutynin appears to be due to a slight decrease in Aβ1-40 peptides and a strong increase in Aβ1-42 peptides at higher doses. This effect is stronger after 48 h of treatment (Figure 3 D–I). While these neurons were not positive for apoptotic markers at the timepoints tested in this study, this increase in amyloidogenic APP processing after oxybutynin treatment may contribute to neurotoxicity and its link to increased dementia risk.

We measured p-tau (Thr 231) and total tau (t-tau) levels also using chemiluminescent ELISA. We did not observe significant changes in the p-tau/t-tau ratio at two doses (10 and 100 μM) after a 24-h treatment (Figure S1E,F). It may be that changes in tau phosphorylation take longer than 24 h to occur; however, at longer time points, the medications that were the most cytotoxic (antidepressants and bladder antimuscarinics) showed non-detectable tau readings, likely due to the cytotoxicity of the medications.

5.5 | Summary

These results support the utility of in vitro studies not only to fill in the gaps of understanding from observational studies but to provide information on individual drugs that could inform investigations of cellular mechanisms. The differential cytotoxicity observed with different drug treatments in the absence of confounding factors suggests that these drugs display toxicity independent of their clinical indications. However, future studies are needed to determine if this toxicity is due to "on-target" effects of blocking cholinergic signaling or due to "off-target" effects that are independent of cholinergic pathways. By examining time and dose dependence of ACs, we may be able to identify nuances in how these drugs exert effects on AD phenotypes. For example, molecular pathways that lead to cytotoxicity or changes in APP processing may occur at different doses or at different exposure periods to a particular compound, enabling us to gain a better understanding of both the drugs and their targets as they relate to the development of dementia and AD in an aging population.

6 | OPPORTUNITIES FOR USING hiPSC-DERIVED CELLS FOR MECHANISMS OF DEMENTIA RISK FROM COMMON MEDICATIONS

As highlighted in this *Perspective*, hiPSC-derived neurons proved a unique opportunity to complement findings from observational studies. By directly applying drugs to cultures that are "disease free," we can examine the effects of the drugs without considering the clinical indication the drugs are used to treat (i.e., confounding by indication). Likewise, we can examine the effects of the drugs in isolation of health status and polypharmacy (i.e., residual confounding) (Figure 1A). Our approach is easily adaptable to further investigate acute versus chronic drug exposures or combinations of drugs that may be commonly taken together.

Using hiPSC-derived neurons from subjects of various genetic backgrounds, our approach also enables us to glean unique insights on gene–environment interactions that are otherwise difficult to determine using animal models or population studies. In the future we plan to apply this methodology to cell lines from human donors who have available complete data on several variables of interest, including disease status, neuropathology, *APOE* alleles, and polygenic risk. This will enable us to generate novel conclusions that could contribute to personalized medicine by inferring differential risks of these medications in different populations.

7 | LIMITATIONS FOR USING hiPSC-DERIVED CELLS FOR MECHANISMS OF DEMENTIA RISK FROM COMMON MEDICATIONS

Despite the opportunities described above, there remain several limitations to this approach.

1. **Dose**. One of the most significant limitations of this approach is that we cannot directly translate the exact concentration of a medication dose present in a person's brain to the doses used to screen drugs in our in vitro cultures. It is also difficult to accurately model time points representative of long-term exposures. Other groups have utilized methods of normalizing Lethal Dose 50 (LD50) values of drugs observed in vitro to the Cmax values representing the maximum blood plasma concentration of each drug in patients, allowing for a reasonable extrapolation of cytotoxicity from hiPSC-derived models,[57] and we have performed a similar analysis here (Figure S1C,D). Animal models also face this limitation. Mice and rats exhibit tolerance around 4.5–100 times that of humans to many drugs, and mice differ substantially from humans in their physiology.[58,59]
2. **Potential bioactive metabolites**. As is common in many in vitro studies, we cannot rule out if this is due to off target effects or effects of bioactive metabolites. For the antidepressants, potential metabolites include nortriptyline and nordoxepin. For antihistamines, potential metabolites include mono- and di-desmethyl

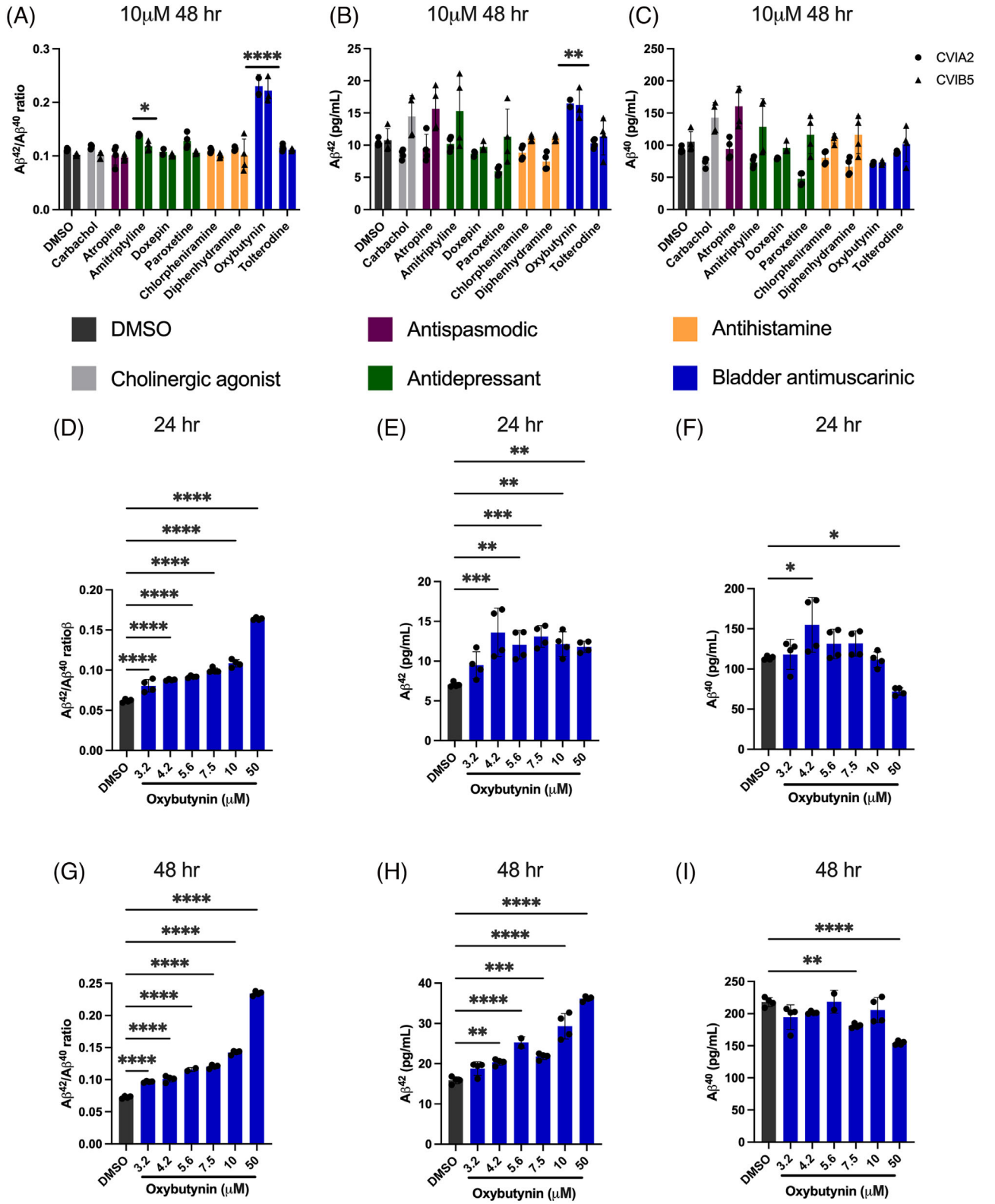

FIGURE 3 Oxybutynin increases the Aβ42:Aβ40 ratio in neurons by increasing Aβ1-42. (A) Aβ 42:Aβ40 ratio after 10 μM treatment with eight AC medications at 48 h. (B) Aβ1-42 peptides measured after treatment with eight AC medications at 48 h. (C) Aβ1-40 peptides measured after treatment with eight AC medications at 48 h. (D) Aβ42:Aβ40 ratio at varying doses of oxybutynin after 24 h. (E) Aβ1-42 peptides secreted at varying doses of oxybutynin (3.2–50 μM) after 24 h. (F) Aβ1-40 peptides secreted at varying doses of oxybutynin (3.2–50 μM) after 48 h. (G) Aβ42:Aβ40 ratio at varying doses of oxybutynin after 48 h. (H) Aβ1-42 peptides secreted at varying doses of oxybutynin (3.2–50 μM) after 48 h. (I):Aβ1-40 peptides secreted at varying doses of oxybutynin (3.2–50 μM) after 48 h. All statistics were performed by two-way ANOVA. $^{*}p \leq 0.05$; $^{**}p \leq 0.01$; $^{***}p \leq 0.001$; $^{****}p \leq 0.0001$. Closed circles indicate hiPSC clone CVIA2; closed triangles indicate hiPSC clone CVIB5. AC, Anticholinergic; DMSO, dimethyl sulfoxide; hiPSCs; human induced pluripotent stem cells.

compounds. For bladder antimuscarinics, potential metabolites include N-desethyloxybutynin and 5-hydroxylmethyl metabolites.

3. **Fetal state**. A common critique of hiPSC-derived neuronal cultures for studying neurodegenerative diseases is that they do not accurately represent the research participants from whom they are derived. This is because in the process of pluripotency induction hiPSCs reset to a prenatal epigenetic age, effectively erasing many age-related transcriptomic signatures. Similarly, hiPSC-derived neurons may be functionally immature. This raises concerns regarding the applicability of findings generated in hiPSC studies to older patients. However, despite these limitations, many studies have shown that patient phenotypes are well recapitulated in in vitro models across various metrics, including drug-treatment response.[60] For instance, in a study that examined the effect of lithium on neurons derived from hiPSCs of individuals with bipolar disorder who did and did not respond to lithium treatment, the investigators found that hyperexcitability in neurons was only rescued by lithium in neurons derived from patients who also responded to lithium treatment.[61] In terms of age-related diseases specifically, AD patient-derived hiPSCs show signs of AD pathology early on in their differentiation, including those derived from patients with sporadic AD.[31]
4. **Cell types**. Alzheimer's disease is due to dysfunction of multiple cell types of the central nervous system. In this study, we have focused primarily on neuronal cell responses. Experiments testing the effects of AC medication on other cell types, such as astrocytes, microglia, and oligodendrocytes are warranted.

8 | CONCLUSION

Understanding cellular mechanisms for how drugs affect AD risk is necessary for understanding results from epidemiologic studies. The incorporation of cellular models in these studies will help to address some challenges in current pharmacoepidemiologic research as well as potentially allow for repurposing of existing drugs for dementia prevention to identify novel potential AD drug targets.

9 | METHODS

9.1 | Cell culture

Cortical neurons were differentiated from hiPSCs of normal karyotype using the dual-SMAD (Suppressor of Mothers against Decapentaplegic) inhibition technique to generate neural progenitor cells (NPCs) and matured to cortical neurons following our published protocol.[50] All cell lines were routinely tested for mycoplasma. Mature cortical cultures were plated at 500,000 neurons/well in 24-well plates after 21 days of differentiation. After being given 72 h to recover, cells were treated with 10, 50, and 100 μM of drug (Table 1), carbachol (a canonical muscarinic agonist) or DMSO. Conditioned media was collected from each well after 24 or 48 h and stored at −80^{0}C until use for the cytotoxicity or Aβ assays. Lysates were also collected at the same time frames and stored at −80^{0}C for p-tau assays. The data presented represent three independent differentiations of two hiPSC clones.

9.2 | Cytotoxicity assay

To assess cytotoxicity, we measured LDH secreted in the culture media using the ProMega LDH-Glo Cytotoxicity Assay. Conditioned media was diluted 1:5 into LDH storage buffer, and the assay was conducted according to manufacturer instructions.

9.3 | Cleaved Caspase 3/7 assay (CellEvent)

Cortical neurons were seeded at a density of 400,000 cells per well of a 24-well plate on glass coverslips coated with Geltrex. Seven days after replating, cells were treated with various AC drugs at 10 μM for 48 h. To observe apoptotic events, we used CellEvent Caspase 3/7 Green Detection Reagent (Thermo Fisher Scientific; C10423) according to the manufacturer's protocol. Fluorescence images were acquired using a Nikon Live Cell Widefield microscope with a 20× objective lens. National Institutes of Health (NIH) ImageJ software was used for further adjustment and analysis of the acquired images.

9.4 | Cleaved Caspase 3 Immunostaining

Cortical neurons were seeded at a density of 400,000 cells per well of a 24-well plate on glass coverslips coated with Geltrex. After 7 days in culture, cells were treated with various AC drugs at 50 μM for 24 h. Cells were fixed in 4% paraformaldehyde (PFA, Santa Cruz) for 10 min at room temperature and washed 3x with phosphate-buffered saline (PBS). Cells were incubated in blocking buffer containing 2.5% bovine serum albumin, 5% normal goat serum, and 0.1% Triton X-100 (Sigma Aldrich, St Louis, MO, USA) for 1 h at room temperature followed with an overnight incubation at 4^{0}C in primary antibody solution containing cleaved caspase 3 (1:200; Cell Signaling #9661; rabbit) and neuronal marker MAP2 (1:2000; Abcam, ab92434, chicken) in blocking buffer. Cells were washed 3x with PBS + 0.1% Triton X-100 and incubated with secondary antibody solution in blocking buffer supplemented with 4′,6-diamidino-2-phenylindole (DAPI) (1:4000) for 2 h at room temperature. Cells were washed 3x in PBS + 0.1% Triton X-100 and mounted on glass slides with ProLong Gold Antifade mounting reagent (Thermo Fisher Scientific; P36930). Fluorescence images were acquired using a Leica SP8 laser scanning confocal microscope with a 20× objective lens. NIH ImageJ software was used for further adjustment and assembly of the acquired images.

9.5 | qRT-PCR

We isolated total RNA from 500,000 cells with TRIzol (Invitrogen, Cat# 15596026). We used 1 μg of RNA to produce cDNA with iScript

Reverse Transcription Supermix (Bio-Rad, Cat#1725120). The cDNA was subjected to quantitative real-time PCR with iTaq Universal SYBR Green Supermix (Bio-Rad, Cat#1708840) and CFX-384 (Bio-Rad). The fold-changes in RNA transcript levels were normalized against housekeeping genes, *RPL27* and *CYC1*. Primer sequences are the following: *RPL27* AAACCGCAGTTTCTGGAAGA, TGGATATCCCCTTGGACAAA; *CYC1* AGCCTACAAGAAAGTTTGCCTAT, TCTTCTTCCGGTAGTGGATCTTGGC; *CHRM1* CTGGCTGGTTTCCTTTGTGCTC, GGAGAGGAACTGGATGTAGCAC (OriGene, # HP200684); *CHRM2* TGCTGTCACCTTTGGTACGGCT, TGGTTGGCAACAGGCTCCTTCT (OriGene, # HP200685); *CHRM3* ACGAGAGCCATCTACTCCATCG, TGTCGGCTTTCCTCTCCAAGTC (OriGene, # HP200686).

9.6 | Western blot analysis

We isolated protein lysate from 500,000 cells with 100 µL of radioimmunoprecipitation assay (RIPA) buffer supplemented with a 100X protease inhibitor cocktail (Millipore; #535142) and 100X Halt phosphatase inhibitors (Thermo Fisher Scientific; #78427). Cell lysates were run on 4%–15% Mini-PROTEAN TGX Precast Protein Gels (Bio-Rad; #4561084) and transferred to polyvinylidene fluoride (PVDF) membranes (Bio-Rad; #1620177). The membrane was blocked in 5% milk diluted in tris-buffered saline (TBS) buffer + 0.05% Tween-20 (Fisher Scientific; BP337) for 1 h at room temperature and probed with primary antibodies diluted in 5% milk overnight at 4^0C. The membrane was washed 3x with TBS buffer + 0.05% Tween-20 followed by a 2-h incubation in a secondary antibody solution diluted in 5% milk. Membrane imaging was performed with a BioRad ChemiDoc system. Primary antibodies: cleaved poly (ADP-ribose) polymerase (c-PARP) antibody (1:1000; Cell Signaling; #9541; rabbit) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody (1:5000; GeneTex; #GTX627408; mouse). Secondary antibodies: anti-rabbit antibody (1:7500; Invitrogen; #616520) and anti-mouse antibody (1:5000; Invitrogen; #31430). Blots were analyzed in ImageJ and c-PARP signal was normalized to GAPDH.

9.7 | Amyloid beta assay

Conditioned media collected from the mixed cortical cultures after drug treatment were frozen at −80°C until use. The media was run on an Aβ Triplex ELISA plate (Meso Scale Discovery; K151200E-2). Two technical replicates were run for each biological replicate.

9.8 | P-tau and t-tau assays

We isolated protein lysate from 500,000 cells with 100 uL of MSD buffer (150 mM NaCl, 20 mM Tris pH 7. mM EGTA, 1 mM EDTA, 1% Triton X-100) supplemented with 100X protease inhibitor cocktail (Millipore; #535142) and 100X Halt phosphatase inhibitors (Thermo Fisher Scientific; #78427). The lysates were frozen at -80^0C until ready to use. The lysates were run on a phospho tau (Thr231)/total tau ELISA kit (Meso Scale Discovery; K15121D-2) according to the manufacturer's protocol. Two technical replicates were run for each biological replicate.

9.9 | Cmax calculations

Cmax values were obtained from datasheets released by the FDA as well as published literature. To calculate the ratio of the drug concentrations administered in our in vitro model to the Cmax, we divided our doses (10 and 50 µM) by the Cmax values. This was in order to obtain an approximation of the discrepancy between the doses we administered and the maximum amount of drug circulating through a patient's bloodstream shortly after administration, not accounting for chronic use.

AUTHOR CONTRIBUTIONS

Conception and design of study: Shelly L. Gray, Paul K. Crane, and Jessica E. Young. *Experimental procedures*: Tiara A. Schwarze-Taufiq, Inez K. A. Pranoto, Katherine W. Hui. *Writing, first draft*: Shelly L. Gray, Tiara A. Schwarze-Taufiq, and Jessica E. Young. *Writing, reviewing, and editing*: Shelly L. Gray, Tiara A. Schwarze-Taufiq, Inez K. A. Pranoto, Onchee Yu, Paul K. Crane, and Jessica E. Young. *Funding acquisition*: Shelly L. Gray, Paul K. Crane, and Jessica E. Young. All authors contributed to manuscript revision and read and approved the submitted version.

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CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. Author disclosures are available in the Supporting Information.

DATA AVAILABILITY STATEMENT

The data sets in this study will be shared upon request from the corresponding authors.

CONSENT STATEMENT

No informed consent was necessary for this study.

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REFERENCES

1. Naples JG, Marcum ZA, Perera S, et al. Concordance between anticholinergic burden scales. *J Am Geriatr Soc*. 2015;63(10):2120-2124.

2. Kachru N, Carnahan RM, Johnson ML, Aparasu RR. Potentially inappropriate anticholinergic medication use in community-dwelling older adults: a national cross-sectional study. *Drugs Aging*. 2015;32(5):379-389.
3. Picciotto MR, Higley MJ, Mineur YS. Acetylcholine as a neuromodulator: cholinergic signaling shapes nervous system function and behavior. *Neuron*. 2012;76(1):116-129.
4. Tannenbaum C, Paquette A, Hilmer S, Holroyd-Leduc J, Carnahan R. A systematic review of amnestic and non-amnestic mild cognitive impairment induced by anticholinergic, antihistamine, GABAergic and opioid drugs. *Drugs Aging*. 2012;29(8):639-658.
5. Pieper NT, Grossi CM, Chan W-Y, et al. Anticholinergic drugs and incident dementia, mild cognitive impairment and cognitive decline: a meta-analysis. *Age Ageing*. 2020;49(6):939-947.
6. American Geriatrics Society 2019. Updated AGS beers criteria® for potentially inappropriate medication use in older adults. *J Am Geriatr Soc*. 2019;67(4):674-694.
7. Dengler KL, High RA, Moga DC, et al. Overactive bladder and cognitive impairment: the American Urogynecologic Society and pelvic floor disorders research foundation state-of-the-science conference summary report. *Urogynecology*. 2023;29(1S Suppl 1):S1-S19.
8. O'mahony D, O'sullivan D, Byrne S, O'connor MN, Ryan C, Gallagher P. STOPP/START criteria for potentially inappropriate prescribing in older people: version 2. *Age Ageing*. 2015;44(2):213-218.
9. Gray SL, Anderson ML, Dublin S, et al. Cumulative use of strong anticholinergics and incident dementia: a prospective cohort study. *JAMA Intern Med*. 2015;175(3):401-407.
10. Richardson K, Fox C, Maidment I, et al. Anticholinergic drugs and risk of dementia: case-control study. *BMJ*. 2018;361:k1315.
11. Coupland CAC, Hill T, Dening T, Morriss R, Moore M, Hippisley-Cox J. Anticholinergic drug exposure and the risk of dementia: a nested case-control study. *JAMA Intern Med*. 2019;179(8):1084-1093.
12. Lozano-Ortega G, Johnston KM, Cheung A, et al. A review of published anticholinergic scales and measures and their applicability in database analyses. *Arch Gerontol Geriatr*. 2020;87:103885.
13. Chen Z-Ru, Huang J-B, Yang S-L, Hong F-F. Role of cholinergic signaling in Alzheimer's disease. *Molecules*. 2022;27(6).
14. Prohovnik I, Arnold SE, Smith G, Lucas LR. Physostigmine reversal of scopolamine-induced hypofrontality. *J Cereb Blood Flow Metab*. 1997;17(2):220-228.
15. Nitsch RM, Slack BE, Wurtman RJ, Growdon JH. Release of Alzheimer amyloid precursor derivatives stimulated by activation of muscarinic acetylcholine receptors. *Science*. 1992;258(5080):304-307.
16. May LG, Johnson S, Krebs S, Newman A, Aronstam RS. Involvement of protein kinase C and protein kinase A in the muscarinic receptor signalling pathways mediating phospholipase C activation, arachidonic acid release and calcium mobilisation. *Cell Signal*. 1999;11(3):179-187.
17. Morozova V, Cohen LS, Makki AE-H, et al. Normal and pathological tau uptake mediated by M1/M3 muscarinic receptors promotes opposite neuronal changes. *Front Cell Neurosci*. 2019;13:403.
18. Fisher A. M1 muscarinic agonists target major hallmarks of Alzheimer's disease—an update. *Curr Alzheimer Res*. 2007;4(5):577-580.
19. Davis AA, Fritz JJ, Wess J, Lah JJ, Levey AI. Deletion of M1 muscarinic acetylcholine receptors increases amyloid pathology in vitro and in vivo. *J Neurosci*. 2010;30(12):4190-4196.
20. Seeger T, Fedorova I, Zheng F, et al. M2 muscarinic acetylcholine receptor knock-out mice show deficits in behavioral flexibility, working memory, and hippocampal plasticity. *J Neurosci*. 2004;24(45):10117-10127.
21. Yoshiyama Y, Kojima A, Itoh K, Uchiyama T, Arai K. Anticholinergics boost the pathological process of neurodegeneration with increased inflammation in a tauopathy mouse model. *Neurobiol Dis*. 2012;45(1):329-336.
22. Caccamo A, Oddo S, Billings LM, et al. M1 receptors play a central role in modulating AD-like pathology in transgenic mice. *Neuron*. 2006;49(5):671-682.
23. Kilimann I, Wucherer D, Ittermann T, et al. Inverse association between the anticholinergic burden and hippocampus volume in a population-based cohort across the entire adult age range. *Geroscience*. 2022;44(3):1715-1726.
24. Risacher SL, Mcdonald BC, Tallman EF, et al. Association between anticholinergic medication use and cognition, brain metabolism, and brain atrophy in cognitively normal older adults. *JAMA Neurol*. 2016;73(6):721-732.
25. Chuang Y-F, Elango P, Gonzalez CE, Thambisetty M. Midlife anticholinergic drug use, risk of Alzheimer's disease, and brain atrophy in community-dwelling older adults. *Alzheimers Dement*. 2017;3(3):471-479.
26. Richardson K, Wharton SB, Grossi CM, et al. Neuropathological correlates of cumulative benzodiazepine and anticholinergic drug use. *J Alzheimers Dis*. 2020;74(3):999-1009.
27. Mur J, Marioni RE, Russ TC, Muniz-Terrera G, Cox SR. Anticholinergic burden in middle and older age is associated with lower cognitive function, but not with brain atrophy. *Br J Clin Pharmacol*. 2023;89:2224-2235.
28. Li KH, Krakauer C, Nelson JC, et al. Cumulative anticholinergic exposure and white matter hyperintensity burden in community-dwelling older adults. *J Am Geriatr Soc*. 2024; in press. doi:10.1111/jgs.19325
29. Gray SL, Anderson ML, Hanlon JT, et al. Exposure to strong anticholinergic medications and dementia-related neuropathology in a community-based autopsy cohort. *J Alzheimers Dis*. 2018;65(2):607-616.
30. Yagi T, Ito D, Okada Y, et al. Modeling familial Alzheimer's disease with induced pluripotent stem cells. *Hum Mol Genet*. 2011;20(23):4530-4539.
31. Penney J, Ralvenius WT, Tsai LH. Modeling Alzheimer's disease with iPSC-derived brain cells. *Mol Psychiatry*. 2020;25(1):148-167.
32. Young JE, Goldstein LSB. Human-induced pluripotent stem cell (hiPSC)-derived neurons and glia for the elucidation of pathogenic mechanisms in Alzheimer's disease. *Methods Mol Biol*. 2023;2561:105-133.
33. Lagomarsino VN, Pearse RV, Liu L, et al. Stem cell-derived neurons reflect features of protein networks, neuropathology, and cognitive outcome of their aged human donors. *Neuron*. 2021;109(21):3402-3420 e9.
34. Knupp A, Mishra S, Martinez R, et al. Depletion of the AD risk gene SORL1 selectively impairs neuronal endosomal traffic independent of amyloidogenic APP processing. *Cell Rep*. 2020;31(9):107719.
35. Young JE, Boulanger-Weill J, Williams DA, et al. Elucidating molecular phenotypes caused by the SORL1 Alzheimer's disease genetic risk factor using human induced pluripotent stem cells. *Cell Stem Cell*. 2015;16(4):373-385.
36. Young JE, Fong LK, Frankowski H, Petsko GA, Small SA, Goldstein LSB. Stabilizing the retromer complex in a human stem cell model of Alzheimer's disease reduces TAU phosphorylation independently of amyloid precursor protein. *Stem Cell Reports*. 2018;10(3):1046-1058.
37. Gore A, Li Z, Fung H-L, et al. Somatic coding mutations in human induced pluripotent stem cells. *Nature*. 2011;471(7336):63-67.
38. Woodruff G, Young JE, Martinez FJ, et al. The presenilin-1 DeltaE9 mutation results in reduced gamma-secretase activity, but not total loss of PS1 function, in isogenic human stem cells. *Cell Rep*. 2013;5(4):974-985.
39. Levy S, Sutton G, Ng PC, et al. The diploid genome sequence of an individual human. *PLoS Biol*. 2007;5(10):e254.
40. Brown JB, Wess J. Muscarinic receptor agonists and antagonists. In: Brunton L.L., ed. *Goodman and Gilman's: The Pharmacological Basis of Therapeutics*. 14th ed. McGraw-Hill Education; 2023.

41. Nishtala PS, Salahudeen MS, Hilmer SN. Anticholinergics: theoretical and clinical overview. *Expert Opin Drug Saf*. 2016;15(6):753-768.
42. Chen C, Li J, Wang J, Zhang M, Zhang L, Lin Z. Oxybutynin ameliorates LPS-induced inflammatory response in human bladder epithelial cells. *J Biochem Mol Toxicol*. 2024;38(1):e23584.
43. Tran NQV, Nguyen AN, Takabe K, Yamagata Z, Miyake K. Pretreatment with amitriptyline causes epigenetic up-regulation of neuroprotection-associated genes and has anti-apoptotic effects in mouse neuronal cells. *Neurotoxicol Teratol*. 2017;62:1-12.
44. Wheeler HE, Wing C, Delaney SM, Komatsu M, Dolan ME. Modeling chemotherapeutic neurotoxicity with human induced pluripotent stem cell-derived neuronal cells. *PLoS One*. 2015;10(2):e0118020.
45. Zhong X, Harris G, Smirnova L, et al. Antidepressant paroxetine exerts developmental neurotoxicity in an iPSC-derived 3D human brain model. *Front Cell Neurosci*. 2020;14:25.
46. Woehrling EK, Parri HR, Tse EHY, et al. A predictive in vitro model of the impact of drugs with anticholinergic properties on human neuronal and astrocytic systems. *PLoS One*. 2015;10(3):e0118786.
47. Rudd KM, Raehl CL, Bond CA, Abbruscato TJ, Stenhouse AC. Methods for assessing drug-related anticholinergic activity. *Pharmacotherapy*. 2005;25(11):1592-1601.
48. Orzechowski RF, Currie DS, Valancius CA. Comparative anticholinergic activities of 10 histamine H1 receptor antagonists in two functional models. *Eur J Pharmacol*. 2005;506(3):257-264.
49. Kwan C-Y, Zhang W-B, Kwan TK, Sakai Y. In vitro relaxation of vascular smooth muscle by atropine: involvement of K+ channels and endothelium. *Naunyn Schmiedebergs Arch Pharmacol*. 2003;368(1):1-9.
50. Mishra S, Knupp A, Szabo MP, et al. The Alzheimer's gene SORL1 is a regulator of endosomal traffic and recycling in human neurons. *Cell Mol Life Sci*. 2022;79(3):162.
51. Yuan SH, Martin J, Elia J, et al. Cell-surface marker signatures for the isolation of neural stem cells, glia and neurons derived from human pluripotent stem cells. *PLoS One*. 2011;6(3):e17540.
52. Mishra S, Knupp A, Kinoshita C, et al. Pharmacologic enhancement of retromer rescues endosomal pathology induced by defects in the Alzheimer's gene SORL1. *Stem Cell Reports*. 2023;18(12):2434-2450.
53. Rose SE, Frankowski H, Knupp A, et al. Leptomeninges-derived induced pluripotent stem cells and directly converted neurons from autopsy cases with varying neuropathologic backgrounds. *J Neuropathol Exp Neurol*. 2018;77(5):353-360.
54. Chaitanya GV, Alexander JS, Babu PP. PARP-1 cleavage fragments: signatures of cell-death proteases in neurodegeneration. *Cell Commun Signal*. 2010;8:31.
55. Lakhani SA, Masud A, Kuida K, et al. Caspases 3 and 7: key mediators of mitochondrial events of apoptosis. *Science*. 2006;311(5762):847-851.
56. Ochalek A, Mihalik B, Avci HX, et al. Neurons derived from sporadic Alzheimer's disease iPSCs reveal elevated TAU hyperphosphorylation, increased amyloid levels, and GSK3B activation. *Alzheimers Res Ther*. 2017;9(1):90.
57. Sharma A, Burridge PW, Mckeithan WL, et al. High-throughput screening of tyrosine kinase inhibitor cardiotoxicity with human induced pluripotent stem cells. *Sci Transl Med*. 2017;9(377):eaaf2584.
58. Cota-Coronado A, Ramírez-Rodríguez PB, Padilla-Camberos E, et al. Implications of human induced pluripotent stem cells in metabolic disorders: from drug discovery toward precision medicine. *Drug Discov Today*. 2019;24(1):334-341.
59. Sala L, Bellin M, Mummery CL. Integrating cardiomyocytes from human pluripotent stem cells in safety pharmacology: has the time come? *Br J Pharmacol*. 2017;174(21):3749-3765.
60. Farkhondeh A, Li R, Gorshkov K, et al. Induced pluripotent stem cells for neural drug discovery. *Drug Discov Today*. 2019;24(4):992-999.
61. Mertens J, Wang Q-W, Kim Y, et al. Differential responses to lithium in hyperexcitable neurons from patients with bipolar disorder. *Nature*. 2015;527(7576):95-99.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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