Specific Triazine Herbicides Induce Amyloid-β42 Production

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Accepted 15 July 2016

Abstract. Proteolytic cleavage of the amyloid-β protein precursor (AβPP) by secretases leads to extracellular release of amyloid-β (Aβ) peptides. Increased production of Aβ42 over Aβ40 and aggregation into oligomers and plaques constitute an Alzheimer’s disease (AD) hallmark. Identifying products of the ‘human chemical exposome’ (HCE) able to induce Aβ42 production may be a key to understanding some of the initiating causes of AD and to generate non-genetic, chemically-induced AD animal models. A cell model was used to screen HCE libraries for Aβ42 inducers. Out of 3500+ compounds, six triazine herbicides were found that induced a β- and γ-secretases-dependent, 2–10 fold increase in the production of extracellular Aβ42 in various cell lines, primary neuronal cells, and neurons differentiated from human-induced pluripotent stem cells.

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INTRODUCTION

Proteolytic processing of amyloid-β protein precursor (AβPP) by β- and γ-secretases leads to the production of various Aβ peptides, including the 42-amino acid form (Aβ42) which plays a crucial role in AD [1–3]. The action of β-secretase first leads to a soluble fragment (sAβPP) and a membrane-bound fragment (βCTF, β-carboxyl-terminal fragment). γ-Secretase then cleaves the βCTF, leading to the generation of Aβ peptides of various lengths. Aβ peptides tend to aggregate as extracellular oligomers and ultimately as plaques, one of the clinical hallmarks of AD.

Aβ40 is the most abundantly produced Aβ peptide. Considerable data indicates that generation of the aggregation-prone Aβ42 strongly correlates with the onset and development of AD. In early-onset AD (EOAD) (<1% of all cases), mutations in AβPP, or the γ-secretase subunits PSEN1 & PSEN2 (reviewed in [4]), all lead to enhanced Aβ42 production and/or increased Aβ42/Aβ40 ratio, a critical factor in AD pathology initiation [5]. Increased Aβ42/Aβ40 ratio is also found in brain tissue in late-onset AD (LOAD) (>99% of AD cases). Aβ42 is more toxic than Aβ40, a consequence of its higher stability and strong tendency to oligomerize and to aggregate in plaques [6–8]. Aβ43 is also enriched in the brain of AD patients and has been reported as a toxic, aggregation-prone amyloid, inducing strong AD phenotypes in mice [9–12].

We recently reported that some tri-substituted purines, the afins (Amyloid β Forty-Two Inducers), trigger a robust, secretases-dependent increase in extracellular Aβ42 production in cultured cells [13, 14]. Under these conditions Aβ39 levels dropped while Aβ40 remained relatively stable. These results suggest that (1) such molecules might constitute new pharmacological tools to investigate the mechanisms underlying the increased Aβ42/Aβ40 ratio observed in AD, (2) these molecules might contribute to generate a chemically induced animal model of AD [15] and (3) some simple, low molecular weight (LMW) products in our environment might shift the Aβ42/Aβ40 ratio similarly to what is seen in AD patients and might thus contribute to the development, acceleration or even initiation of LOAD.

We therefore screened for potential Aβ42-inducing molecules in libraries of HCE products [16–21]. We here report that a subset of the widely used triazine herbicides is able to shift Aβ production towards longer, aggregation-prone amyloid peptides (Aβ42/Aβ43) at the expense of shorter variants (Aβ37, Aβ38, Aβ40). In addition, production of the shorter Aβ1–16 and Aβ1–17 peptides, the generation of which is dependent on β- and γ-secretase activities [22, 23], was also enhanced. This effect is observed in various cell lines, primary neuron cultures and neurons differentiated from iPSCs obtained from healthy controls or AD patients. Triazines shift the cleavage pattern of alcadeinα, another γ-secretase substrate [24–28], in a way similar to the AβPP cleavage shift, suggesting a direct effect on γ-secretase rather than on its substrates. Altogether these data support our hypothesis that the HCE contains products able to modulate γ-secretase activity towards the production of high MW, aggregation-prone, AD-associated amyloids. Such products could be qualified as potential “Alzheimerogens” (name by analogy with “carcinogens”). Their identification and regulation of human exposure might constitute a key step in AD prevention.

MATERIAL AND METHODS

Material and methods are described in full in the Supplementary Material. They include: 1) Triazines and other reagents; 2) Cell cultures: cell lines, primary neuron cultures, human iPSCs-derived neurons, and HEK293-alcadeinα cells; 3) Transient transfec-
Fig. 1. Some triazines trigger β- and γ-secretase dependent production of extracellular Aβ42. A) Effect of 37 triazines on extracellular Aβ42 production by N2a-APP695 and CHO-7PA2-APPsw cells. Cells were treated with 100 μM of each compound for 18 h and cell supernatants were collected for Aβ42 levels measurement by ELISA. Aftin-5 was used as a positive control and the corresponding volume of DMSO as a negative control. Levels are expressed as fold change, average ± SEM of triplicate values, of Aβ42 levels over those of control, vehicle-treated cells (representative of four independent experiments). Horizontal dotted lines indicate levels for 1 and 3 fold changes in Aβ42 concentration.

B) Structure of the six active triazines and of aftin-5. C) Extracellular Aβ42 production induced by triazines is inhibited by γ-secretase inhibitors DAPT & BMS 29897 and γ-secretase modulator "Torrey Pines" compound. N2a-APP695 cells were exposed to 10 μM of each inhibitor. 1.5 h later, cells were treated with 100 μM of each active triazine or 50 μM aftin-5. Extracellular Aβ42 levels were measured after 18 h. Average ± SEM of triplicate values (representative of two independent experiments). D) Triazines trigger Aβ42 production in primary rat neuron cultures. Cells were exposed to DMSO, 100 μM of each triazine or aftin-5 for 18 h. Cell supernatants were collected and the levels of Aβ38, Aβ40, and Aβ42 (bottom panel) were determined by ELISA assays (average ± SEM of triplicate values). The Aβ42/Aβ40 ratios are shown in the top panel. The horizontal dotted line refers to the basal ratio in control cells.

ions with AβPP truncation mutants; 4) Amyloids assays: ELISA capture assays, absolute quantification of amyloids by tandem mass spectrometry (MS/MS), amyloids profile analysis by immunoprecipitation/mass spectrometry (IP-MS); 5) Cell viability assay.

RESULTS

Screening the HCE reveals triazines as Aβ42 inducers

A library of 3500+ LMW (<1000 daltons) products representative of the HCE was assembled. All compounds were tested for their ability to trigger extracellular Aβ42 production by N2a cells stably expressing AβPP695 (N2a-APP695), at 1, 10 and 100 μM (data not shown. A dose-dependent effect was often seen with the active compounds). In parallel, cell viability assays were run to assess cell survival at these concentrations. The vast majority of products were unable to induce Aβ42 production. Among the few active products, we identified several triazines, a class of products which are widely used as herbicides, anti-fouling agents or flame retardants (reviewed in [29]). We next tested a library of 37 triazines representing the most produced triazines worldwide (1–37, Supplementary Table 1), along
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Fig. 2. Absolute quantification of A\(\beta\)38, A\(\beta\)40, and A\(\beta\)42 using LC-MS/MS. Levels of the three amyloid peptides were determined by mass spectrometry in supernatants of N2a-APP695 (A) and CHO-7PA2-APPSw (B) cells following 18 h treatment with DMSO, 100 \(\mu\)M of each triazine or aftin-5. Amyloid levels are expressed as percentage of levels in vehicle-treated cells (bottom panels; average ± SEM of triplicate values; absolute values in control cell supernatants are indicated under the bottom panels). A\(\beta\)42/A\(\beta\)38 ratios are shown in the top panels (horizontal dotted lines refer to the basal ratios in control cells).

with aftin-5 (38) as a positive control, on both N2a-APP695 and CHO-7PA2 cells stably expressing A\(\beta\)PP751 (CHO-7PA2-APP751), for their ability to trigger A\(\beta\)42 production at 1, 10, and 100 \(\mu\)M (Supplementary Table 2) (Fig. 1A).

Six triazines were found to induce more than a 3-fold change in A\(\beta\)42 levels (Fig. 1A, B): ametryn, prometryn, dipropetryn, terbutryn, cybutryne, and dimethametryn. As observed with aftins [13, 14], A\(\beta\)42 production was strongly inhibited by \(\beta\)- (inhibitor IV) and \(\gamma\)-secretes (BMS 299897, DAPT) inhibitors and by a \(\gamma\)-secretes modulator (‘Torrey Pines’ compound) (the latter induces a dose-dependent increase in A\(\beta\)38 and a decrease in A\(\beta\)42 and A\(\beta\)40) [30, 31] (Fig. 1C). Similarly, A\(\beta\)38 production was strongly reduced, while A\(\beta\)40 levels were only modestly affected as measured by ELISA (data not shown) and by mass spectrometry (Fig. 2). Most of the triazines are metabolized in the environment and by organisms. We thus tested some of the cybutryne/terbutryn metabolites (39–44) (Supplementary Figure 1) for their ability to trigger A\(\beta\)42 production in N2a-APP695 and CHO-7PA2 cells. None of the tested metabolites was active as an inducer of A\(\beta\)42 production (data not shown). We next tested a library of 236 triazines that had been synthesized as affinity chromatography reagents, for their ability to induce A\(\beta\)42 production [32, 33]. Twenty-one of these (45–65) showed significant enhancement of A\(\beta\)42 production (Supplementary Table 3), showing that A\(\beta\)42 induction is an intrinsic property of some triazines. Affinity chromatography attempts with immobilized triazines did not allow us to purify specific targets, probably because of unselective hydrophobic interactions and low-level expression of the specific targets (data not shown).

Results were confirmed with HEK293 stably expressing A\(\beta\)PPsw (data not shown) and neurons derived from human iPSCs (see below). We also analyzed the effects of triazines on primary neuronal cultures prepared from E18 OFA rat embryo brains. Neurons were exposed to 100 \(\mu\)M of each triazine for 18 h, and the supernatants were collected for A\(\beta\) determination by ELISA assays. The triazines also induce an increase in A\(\beta\)42 production by primary neurons and in the A\(\beta\)42/A\(\beta\)38 ratios (Fig. 1D). A\(\beta\)40 production remained relatively stable, while that of A\(\beta\)38 was reduced by all triazines (Fig. 1D).
Fig. 3. Pattern of amyloid peptides produced by CHO-7PA2-APPsw cells exposed to triazines. Cells were treated for 18 h with DMSO, 100 µM of each triazine or aftin-5. Cell supernatants were collected and analyzed as described. A) Example spectra of supernatants amyloid profiles from CHO-7PA2-APPsw cells exposed to DMSO, aftin-5 or terbutryn. B) Quantification of all amyloid peptides in CHO-7PA2-APPsw cell supernatants (Log of fold change in triazine or aftin-5 treated cells over control, DMSO-treated cells).

**Mass spectrometric quantification and profile analysis of induced amyloids**

Aβ_{38}, Aβ_{40}, and Aβ_{42} were quantified in the supernatants of N2a-APP695 (Fig. 2A) and CHO-7PA2-APP751 (Fig. 2B) using LC-MS/MS [34, 35]. Like Aftins [13, 14], triazines induced a reduction in Aβ_{38} levels, a slight increase or modest decrease in Aβ_{40} levels and a strong increase in Aβ_{42} levels (Fig. 2, bottom). Aβ_{42}/Aβ_{40} ratios were strongly increased (Fig. 2, top).

We next analyzed, by IP-MS, the range of Aβ peptides produced by both cell lines exposed to each of the six triazines and aftin-5. Cell supernatants were
collected and Aβ peptides were immunoprecipitated and analyzed using MALDI TOF/TOF [36]. Examples of spectra for cells exposed to terbutryn, aftin-5 and DMSO are provided in Fig. 3 and Supplementary Figure 2. Exposure to triazines increased the production of Aβ1-17, Aβ1-42, Aβ5-42, and Aβ1-42, while the production of Aβ1-19, Aβ1-27, Aβ1-33, Aβ1-38, and Aβ1-39 was reduced (Fig. 3). Other amyloid peptides (including Aβ1-40) showed only modest changes. Aβ1-43, a highly neurotoxic amyloid [9–12], was undetectable in supernatants of control cells, but strongly induced in aftin-5 and triazine-treated cells.

**Neurons differentiated from human iPSCs of AD patient and healthy control**

We next tested the effects of aftin-5 and the active triazines on neurons differentiated from human iPSCs derived from a healthy individual (AβPP WT, wild-type) or from a patient with familial AD (AβPP K724N mutation) [37, 38] (Fig. 4). Neurons from the healthy control were first differentiated for either 4 or 10 weeks before 24 h exposure to 100 μM aftin-5 or terbutryn (Fig. 4A). Treatment resulted in a 2-3-fold increase in Aβ42 levels compared to neurons exposed to DMSO. Aβ40 levels remained essentially unchanged. All six triazines were next tested on neurons derived from the control and the AD patient differentiated for 4 weeks [38, 39] (Fig. 4B). AβPP K724N neurons produced more Aβ42 versus Aβ40 compared to AβPP WT neurons. Addition of aftin-5 or any of the six active triazines resulted in further increase in Aβ42 production, in both AβPP WT and AβPP K724N neurons.

**AβPP sequence requirements for Aβ42 induction by triazines**

To investigate the molecular mechanisms and possible ε cleavage sites requirement for the induced Aβ42 production, we generated six AβPP truncations (T1–T6) and expressed them transiently in N2a cells (Fig. 5A). Cells expressing these truncations were then exposed to 100 μM aftin-5 and Aβ42 production was measured (Fig. 5B). Full-length (FL) AβPP and the first three truncations displayed enhanced Aβ42 production when cells were exposed to aftin-5. Cells expressing FL AβPP and truncations T1, T3, T4 were next exposed to 100 μM of each triazine (Fig. 5C). Although T3 allowed stimulation of Aβ42 production, T4 did not. These results reveal a strong AβPP structural requirement for enhanced Aβ42 production induced by aftin-5 and triazines, which seems to correspond to the ε cleavage sites of AβPP by γ-secretase. At least 10 residues downstream of the Aβ42 cleavage site are required for the full effect of aftin-5 and triazines.
Fig. 5. Effect of AβPP C-terminal truncations on triazines’ efficacy. A) Only the C-terminal aa sequences of AβPP full length (WT) and C-terminal truncations mutants (T1-T6) are shown. The γ and ε cleavage sites are indicated in orange (left arrows) and blue (right arrows) respectively. Numbers indicate the position of the residues involved in those cleavages and refer to the α cleavage site. B) Mutants T1 to T6 were expressed transiently in N2a cells which were exposed to DMSO or aftin-5 (100 μM) for 24 h and the levels of released Aβ42 was measured by ELISA (average ± SEM of triplicate values). C) Mutants T1, T3, and T4 expressing N2a cells were exposed for 24 h to DMSO (D), aftin-5 or the six triazines (100 μM). Aβ42 level were measured and are expressed as fold-increase versus untreated cells (average ± SEM of triplicate values).

Triazines and Aftin-5 shift the cleavage pattern of the γ-secretase substrates alcadeins

Like AβPP, alcadeins/calsyntenins are sequentially cleaved by secretases, first by α-secretase, leading to N- and C-terminal fragments, the latter being then cleaved by γ-secretase to an intracellular domain and the p3-Alcs peptide, in a way similar to AβPP [25, 27] (Fig. 6A, Supplementary Figure 3). We used HEK293 cells stably expressing full-length alcadeinα to investigate the effects of triazines on alcadein cleavage. Alcadeinα is first cleaved on the N-terminal side (two possible sites) followed by cleavage by γ-secretase leading to p3-Alcα35 and p3-Alcα 2N+35, the latter representing the major peptide in cultured cells (Fig. 6A). Cleavage at nearby sites (blue arrows) leads to other less abundant peptides. HEK293-alcadeinα cells were grown till 60% confluence and treated with 100 μM aftin-5 or triazines for 24 h. The secreted p3-Alcα peptides were recovered and analyzed by MALDI TOF MS (Fig. 6B).

Quantification of p3-Alcα peptides showed that, compared to the p3-Alcα peptide profile in vehicle treated cells, concentrations of the main alcadeinα peptide (p3-Alcα 2N+35) and p3-Alcα 2N+37 peptide remained stable. In contrast, both p3-Alcα 2N+34 and p3-Alcα 2N+36 concentrations dropped by ~50% and p3-Alcα 2N+38 peptide concentration increased massively (up to 28.1 fold for dimethametryn; 16.8 fold for aftin-5) (Fig. 6C). These results show that, like for AβPP, triazines and aftins induce a shift in the cleavage pattern of Alcadeinα, another
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A

Alcadein $\alpha$

\[ ...HMAQPQFVHPEHSFDLGSNHANPHFAVPSTAVIVVCSFL... \]

B

IP-MS p3-Al$\alpha$ from Alcadein$\alpha$.

C

Fold change of p3-Al$\alpha$ peptide ratios (vs. control, DMSO-treated) for Triazines or Aftin-5.
γ-secretase substrate, suggesting that these products are more likely to interact with γ-secretase rather than with its substrates.

**DISCUSSION**

**Induction of Aβ42 production, increase in Aβ42/Aβ40 ratio**

Various drugs (fenofibrate, celecoxib, indomethacin, isoprenoids) [40], DAPT under certain conditions [41, 42], steroids [43], ceramide analogs [44], and SIN-1 (a peroxynitrite donor) [45] have been shown to increase the Aβ42/Aβ40 ratio, mostly by increasing Aβ42 production, though never to the high level seen with aftins [13, 14]. We here show that some, but not all, widely used (though mostly banned nowadays) triazine herbicides induce a massive production of the AD-associated Aβ42 peptide in a variety of cell types. Consequently, the Aβ42/Aβ40 ratio is increased, as observed in both familial EOAD (genetic origin) and LOAD (environmental, epigenetic origin). Detailed analysis of a variety of Aβ peptides reveals a pattern clearly associated with AD onset, such as increased Aβ1-16/17 [22, 23], Aβ1/5-11-42, and Aβ1-43 [9–12], and decreased Aβ1-33/37/38, which have been shown previously to be affected in EOAD subjects [46, 47]. While the underlying molecular mechanisms for the observed chemically-induced shift of AβPP cleavage pattern remain unclear, several remarks can be made:

1. there is a clear structure/activity relationship within triazines, as also observed with aftins: not all products of the chemical class are active. This suggests specific molecular interactions rather than unspecific effects such as detergent, hydrophobic, membrane or protein structure disrupting actions.

2. the mechanism of action is more likely to involve an effect on γ-secretase and/or its micro-environment rather than an interaction with its substrates, as shown by the fact that aftins and triazines also induce a shift in the cleavage pattern of alcadeins, another γ-secretase substrate. The AβPP truncation experiments clearly suggest a very specific molecular requirement rather than a global, non-selective effect.

(3) despite extensive proteomics analyses using the isobaric stable isotope labeling quantitative analysis approach over the reported exposure time (data not shown), we were unable to detect major/significant modifications in protein expression that might be linked to the AβPP cleavage shift induced by triazines, suggesting that RNA or protein synthesis alterations are unlikely involved in the induction of Aβ42 production. We were also unable to identify a specific target of triazines through affinity chromatography/proteomics approaches, suggesting that either the lipid raft comprising the γ-secretase or rather hydrophobic domains of γ-secretase might constitute the real targets of triazines (and aftins).

“Alzheimerogens” in the HCE?

The Chemical Abstracts Service (CAS) registry, the world’s largest chemical database, contains more than 101 million organic and non-organic substances. About 15,000 novel substances are registered every day (on average one new substance every 2.5 min for the last 50 years!) (www.cas.org). The US EPA Toxic Substances Control Act lists over 84,000 chemicals that are manufactured or imported at levels >10 tons per year, not including pesticides, cosmetics, food stuffs and food additives which are covered by other legislations (www.epa.gov). It is estimated that man is exposed to over 85,000 products. The European REACH initiative aims at regulating all products which are produced/imported at >100 tons/year (>1

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**Fig. 6.** Triazines alter the cleavage pattern of Alcadeina, leading to increased p3-Alco38 production. A) Schematic representation of the production of p3-Alco peptides from Alcadeina. The full length protein is cleaved primarily by α-secretase at His814 or Ala816 (purple, 2 left arrows). It is then cleaved by γ-secretase at Thr851 (orange arrow) leading to the two main Alcadeina peptides p3-Alco35 and p3-Alco2N+Alco35 (‘2N’ denotes the two additional, N-terminal amino acids). Alternative cleavage sites (blue, right arrows) generate additional p3-Alco peptides of different sizes. B) Immunoprecipitation/mass spectrometry spectra of p3-Alco peptides produced by HEK-Alcadein α cells exposed to various triazines, aftin-5, or DMSO. Cells were treated for 24 h with 100 μM of each reagent and p3-Alc peptides were analyzed by MALDI-TOF/MS. Representative profiles for each product (top) and zoom on the p3-Alco2N+34, p3-Alco2N+35, and p3-Alco2N+38 peaks (bottom). C) Relative quantification of p3-Alco peptides produced by cells exposed to all triazines and aftin-5. Levels of each peptide are presented as fold change of ratios over p3-Alco2N+35 versus corresponding peptide ratios for DMSO-treated cells (average ± SEM of triplicate values). Horizontal dotted lines indicate levels for 1-fold change in p3-Alco/p3-Alco35 ratio in treated versus control cell supernatant. Note the change of scale for p3-Alco2N+38/p3-Alco2N+35 treated/control ratio.
obtained with aftin-4 [15] and celecoxib or FT-1 [40].

The impact of environment on health has been known since antiquity. It is therefore no surprise that a small number of products may enter the human body, cross the blood-brain barrier (BBB), alter specific molecular pathways in some of the human brain $10^{11}$ neurons and $10^{12}$ glia cells and thereby induce or contribute to specific diseases affecting the central nervous system. Identification of environmental factors involved in neurodegeneration and neurodegenerative diseases is surprisingly still in its infancy (reviews in [50–54]). The nervous system may be exposed to neurotoxic agents acutely (hours, days) or chronically (weeks, years, decades) before disease symptoms appear. Epidemiology studies are particularly difficult for neurodegenerative diseases since causes and effects are often separated by decades. These studies have, therefore, provided only few examples of environmental agents linked to the onset of neurodegenerative diseases. Pesticides, organic solvents, metals and some natural toxins (cyanobacteria) constitute the most frequently proposed neurotoxic agents. Two recently published books [55, 56] review the impact of early age and even in utero exposure to environmental chemical entities on brain development and cognitive abilities.

AD is one of the most prevalent and worrisome neurodegenerative diseases [57]. EOAD is clearly a genetic disease due to specific AβPP or PSEN1/2 mutations leading to overproduction of Aβ$_{42}$ over Aβ$_{40}$. The origin of LOAD (sporadic AD) (>99% of all AD cases) remains a mystery which epidemiological or genome-wide association studies have not solved, the latter having only revealed a few low impact genetic risk factors [58]. The most prominent risk alleles, ApoE ε4 and clusterin/Apol, link AD to lipid metabolism, and aging, together with several environmental factors, also impose an increased risk. Exposure to numerous industrial and agricultural chemicals correlates with neurotoxicity [50–54, 59, 60]. Elevated serum pesticides levels, in particular DDE, the major DDT metabolite, are associated with increased risk for AD [61]. DDT increases Aβ levels [62]. There are epidemiological links between exposure to pesticides and AD [63].

Continuous subcutaneous injection of aftin-5 in mice triggers robust dose-dependent increase in brain Aβ$_{42}$ levels (unpublished data). Similar results were obtained with aftin-4 [15] and celecoxib or FT-1 [40]. Although orally administered triazines readily cross the BBB, their short half-life in mice prevented any accumulation in vivo (data not shown).

Based on results obtained with products belonging to various chemical classes, we can suspect the presence, in the HCE, of products able to increase the production of the AD-associated Aβ$_{42}$/Aβ$_{43}$ peptides. Such products might be classified as potential “Alzheimerogens” if long-term exposure, slow turnover, low elimination, and/or metabolism into stable Aβ$_{42}$ inducers and BBB permeability allow accumulation in the brain and long-term action on brain cells. It is difficult to predict whether long-term, daily exposures of humans to the widely used triazines described here might have resulted in sustained increase in Aβ$_{42}$ production. We are now investigating other Aβ$_{42}$ inducers which have a longer half-life both in the environment and in man, which accumulate in adipose tissues and which cross the BBB. We believe that such products may contribute to the onset, development and acceleration of sporadic LOAD. It is intriguing that both aftin-5 and triazines were able to stimulate Aβ$_{42}$ production in human cells displaying a pathological AβPP mutation and already showing enhanced Aβ$_{42}$ production. This suggests that environmental factors may synergize with genetic/epigenetic factors in enhancing Aβ$_{42}$ production and triggering AD. Identification of such potential “Alzheimerogens” in the HCE and regulation of human exposure to them should open the way to innovative AD prevention strategies. In addition, some of these products might be turned into pharmacological tools to develop a chemically-induced animal model of AD, with a fundamental and applied potential similar to the MPTP-induced Parkinsonism model [64].

**ACKNOWLEDGMENTS**

This work was supported by ‘Fonds Unique Interministériel’ PHARMASEA/TRIAD projects (LM, HG), Fondation Jérôme Lejeune, ANSES (LM, HG) and CRITT-Santé Bretagne/FEDER (LM) and the Alzheimer’s Foundation, Sweden (EP, JP, KB, HZ). This research was also partly supported by an FP7-KBBE-2012 grant (BlueGenics) to LM. MC is CIFRE recipient. KB is a Torsten Söderberg Professor. HZ is a Wallenberg Academy Fellow. SDG & AM’s work was supported by the Deanship of Scientific Research, Prolific Research Group Program (PRG-1436-15), Vice-Rectorate for Graduate Stud-
ies and Scientific Research and the Visiting Professor Program of King Saud University, Riyadh, Saudi Arabia. This work was supported in part by Grant-in-aid for Scientific Research 26293010 (TS) and 24790062 (SH) from the Ministry of Education, Culture, Sports, Science and Technology in Japan.

Authors’ disclosures available online (http://j-alz.com/manuscript-disclosures/16-0310r2).

SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: http://dx.doi.org/10.3233/JAD-160310.

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