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Alzheimer's disease therapeutics targeted to the control of amyloid precursor protein translation: Maintenance of brain iron homeostasis

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Abstract

The neurotoxicity of amyloid beta (A β), a major cleavage product of the amyloid precursor protein (APP), is enhanced by iron, as found in the amyloid plaques of Alzheimer's disease (AD) patients. By contrast, the long-known neuroprotective activity of APP is evident after α -secretase cleavage of the precursor to release sAPP α , and depends on the iron export actions of APP itself. The latter underlie its neurotrophic and protective effects in facilitating the homeostatic actions of ferroportin mediated-iron export. Thus APP-dependent iron export may alleviate oxidative stress by minimizing labile iron thus protecting neurons from iron overload during stroke and hemorrhage. Consistent with this, altered phosphorylation of iron-regulatory protein-1 (IRP1) and its signaling processes play a critical role in modulating APP translation via the 5' untranslated region (5'UTR) of its transcript. The APP 5'UTR region encodes a functional iron-responsive element (IRE) RNA stem loop that represents a potential target for modulating APP production. Targeted regulation of APP gene expression via the modulation of 5'UTR sequence function represents a novel approach for the potential treatment of AD since altering APP translation can be used to improve both the protective brain iron balance and provide anti-amyloid efficacy. Approved drugs including paroxetine and desferrioxamine and several novel compounds have been identified that suppress abnormal metal-promoted A β accumulation with a subset of these acting via APP 5'UTR-dependent mechanisms to modulate APP translation and cleavage to generate the nontoxic sAPP α .

Keywords

Alzheimer's disease; Amyloid precursor protein; Brain iron homeostasis

1. Introduction

Extracellular amyloid beta (A β) plaques and intraneuronal neurofibrillary tangles are the predominant histopathological hallmark lesions of Alzheimer's disease (AD), which is the

most common form of dementiating disorder, predicted to affect approximately 65 million individuals by 2030 [1,2]. The “amyloid hypothesis” of AD specifies a deposition of pre-plaque A β in the brain as a primary factor in AD causality. Briefly, generation of A β is dependent on the processing of amyloid precursor protein (APP), the pivotal protein involved in AD pathology [3]. APP (predominant 695, 751, 770 amino acid isoforms in the brain) is post-translationally processed via amyloidogenic or non-amyloidogenic pathways that involve pathological and physiological functions, respectively [4].

Key factors underlying A β pathogenicity through dysregulated processing of APP include the switch from the non-amyloidogenic to the amyloidogenic pathway, where β -secretase (BACE) cleaves APP at the 1st or 11th residue of the A β peptide sequence (Fig. 1), resulting in sAPP β and a C-terminal 99mer (C99). The γ -secretase, composed of presenilins (PS), cleaves the β -stub to generate the APP intracellular domain (AICD) and amyloidogenic A β 40 and A β 42 [5] that can form neurotoxic amyloid fibrils in the brain. The alternative pathway prevents formation of A β in the event of cleavage by an α -secretase at the 17th amino acid within 40–42 amino acid ‘A β ’ to release neurotrophic sAPP α and the 83mer C terminal fragment (CTF) α [6]. CTF α is further cleaved by γ -secretase to generate the non-amyloidogenic P3 peptide and AICD, both of which are internalized and degraded.

In the present review, novel RNA-based approaches are described that have the potential to limit amyloid production and thus may represent a unique approach to AD therapy. This strategy is an alternative to current amyloid targeted drugs, that while showing promise in animal models, have repeatedly failed in clinical trials [7].

A β cannot always be regarded as a purely deleterious entity. Thus A β 40 and A β 42 were found to attenuate paralyzing brain inflammation in models of experimental autoimmune encephalomyelitis (EAE), that included models of chronic progressive disease, relapsing remitting disease, adoptive Th1 transfer, and adoptive Th17 transfer [8]. A β 40 and A β 42 suppressed the proliferation capacity and cytokine secretion of activated lymphocytes that penetrated and damaged the CNS during EAE, reducing inflammation in lymphoid tissues and CNS parenchyma indicating that the beneficial and pathological roles of A β are dependent on whether the inflammation arises from secondary lymphoid tissues or the glial-rich microenvironment surrounding senile plaques [8].

The failure of anti-amyloid therapies that target γ -secretase [9] and A β vaccine in clinical trials has generated considerable interest in discovering safer and more effective alternatives [10]. The work described below is focused on the development of RNA targeting approaches to block APP translation. In the present review strategies to assess the interaction of compounds with APP 5' untranslated region (APP 5'UTR), that reduce amyloid production and may optimize brain iron balance, a result of the iron export protein actions of APP [11], are described in the context of the control of APP translation by iron-regulatory protein 1 (IRP1) [12].

2. Iron and inflammation in AD and translational control of the APP transcript

2.1. Iron and inflammation promote A β toxicity

Several neurodegenerative disorders including AD [13], Parkinson's disease (PD) [14], Neurodegeneration with brain iron accumulation type I, prion-related disease [15] and amyotrophic lateral sclerosis (ALS) [16] exhibit a linked brain neuronal loss to perturbation of iron metabolism. In AD, brain iron is present at a concentration of approximately 1 mM surrounding amyloid plaques where it appears to correlate with AD pathogenesis [17]. Iron availability can modulate APP processing, and at high levels inhibits maturation of APP without hindering the formation of immature APP; its chelation reduces APP production [5].

Mechanistically, iron is a pathogenic regulator of both amyloid toxicity and APP translation. As described below and summarized in Fig. 1, APP mRNA encodes a functional iron-responsive Element (IRE) RNA stem loop that contains a unique CAGA box, 'amyloid' (+83/+86), in the 146 nt-5'-untranslated region (5'UTR) of APP mRNA (+51 to +94 from the 5'-cap site) that binds to the IRE-binding protein IRP1 [12]. IRPs are sufficient to regulate intracellular iron homeostasis by modulating mRNA translation and stability of mRNAs for the iron-associated proteins, APP, ferritin and transferrin-receptor, respectively [18]. The IRE RNA stem loop in APP mRNA (Figs. 1 and 2) is the focus of current efforts in RNA-based anti-amyloid therapy. Thus, a change in cellular iron levels can deregulate IRP1 binding to IRE RNA stem loops, affect transferrin-ferritin homeostasis [19] and that of APP, which is also an iron export protein (see below).

2.2. Beneficial iron export role of the amyloid precursor protein: The role of ferritin

In health, the natural function of ferritin is iron storage. An intrinsic ferroxidase activity imparted by the H-subunit of ferritin protects the endothelium from heme-aggravated oxidative stress such as that which occurs during stroke [20]. Likewise the secreted ectodomain of the membrane associated APP, APP(s), can protect neurons during conditions of heme release during hemorrhagic stroke [21,22] a neuroprotective effect mediated via the RERMS sequence domain immediately downstream of the Kunitz protease inhibitor (KPI) domain of secreted APP [23].

Secretion of the APP can be increased as the result of IL-1 mediated stress responses by translational up-regulation of APP mRNA translation concurrently with the induction of α -secretase activity to generated the 90 kDa ectodomain of APP, APP(s) [24]. These findings are consistent with data that APP can provide protection against catalyzed oxidative stress, e.g. from mini hemorrhagic lesions during AD pathogenesis and from ischemic lesions and reperfusion after stroke [22]. Soluble APP and A β fragments can also promote neurogenesis and protect neurons from toxic protofibrils or oligomers via their antioxidant properties [25,26].

Although controversial, APP has been reported to encode an REXXE ferroxidase consensus motif, similar to that found in H-ferritin but absent in APP-like proteins [11]. It remains to be confirmed whether this APP REXXE site represents the mechanism involving conversion

of Fe²⁺ to Fe³ to enhance the capacity of APP to facilitate ferroportin (FPN1)-dependent toxic iron export [11,27] (Fig. 3). FPN1 interacts with APP, which then exports iron at the plasma membrane of neurons, microglia, and astrocytes [11], detoxifying iron-burdened neurons to prevent iron-catalyzed free radical damage and cell death [28].

In addition to the iron export capacity of APP, α -secretase processing of the precursor can be neuroprotective. Incomplete α -secretase processing of APP stimulates necrosis, apoptosis and astrogliosis in the hippocampus and cortex, resulting in behavioral abnormalities, hyperactivity, seizures and premature death [6]. The disintegrin metalloproteases, ADAM-10 (a disintegrin and metalloproteinase domain-containing protein 10), ADAM-17/ tumor necrosis factor α -convertase (TACE), and ADAM-9, that have a consensus zinc binding motif, HEXXH, in their catalytic domain [29], fulfill some of the criteria required of α -secretases [6]. ADAM-10 interacts with synapse-associated protein-97 (SAP97) to prevent amyloidogenesis [30–32], decreases generation of toxic A β peptides, reduces senile plaques and alleviates learning deficits [33] in human APP transgenic mice (ADAM10 $\times$ APP[V717I]) [34].

2.3. APP mRNA translation control by iron and interleukin-1

The 3 kb APP transcript binds to many RNA binding proteins (Fig. 1) that physiologically contribute to controlling precursor expression at the post-transcriptional level. This includes proteins that interact with the AU rich stability element in the 3'UTR region of the transcript [12,35]. For example, AU-rich binding AUF protein interacts with the 29-nucleotide element in the APP 3'UTR as an mRNA destabilizer whose function can be inhibited by inclusion of cell growth media supplemented with epidermal, basic fibroblast, insulin-like, and vascular endothelial growth factors. The poly G quartet region of the coding region of the APP transcript binds to fragile X protein (FMRP) such that post-synaptic FMRP can bind and regulate the translation of APP mRNA via metabotropic glutamate receptor activation [36]. Indeed, G-quartet RNA sequences appear to have a role in the RNA-based pathology of frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS). In this case, the culprit C9orf72 gene exhibits a GGGGCC expansion associated with internal non-ATG translation (RAN translation) of the C9orf72 mRNA, which then synthesizes dipeptide inclusion proteins [37]. These patients show dipeptide aggregates in the hippocampus and frontotemporal neocortex in addition to TDP-43 pathology [38].

As observed for the C9orf72 mRNA, internal translation events that may generate an increase of A β peptide levels have yet to be demonstrated to occur in the APP transcript, as associated with AD pathology. An IRE-like RNA stem loop sequence was hypothesized to exist near an internal AUG codon immediately in front of the A β domain in the coding region of APP mRNA (Fig. 2) [39]. However, this RNA sequence was found not to bind either IRP1 or IRP2 [12,40,41], an event that might be predicted to promote internal entry of ribosomes at this site. In Fig. 1, the APP transcript is present as a ribonucleoprotein complex where IRP1 binds preferentially compared to IRP-2 at its 5'UTR site.

In terms of AD pathology, the APP IRE RNA secondary structure may be disrupted in the presence of an adjacent 5'UTR specific single nucleotide polymorphism (SNP) that has been genetically linked to increased risk for spontaneous AD [42]. This is consistent with the fact

that the 3 kb APP mRNA sequence encodes two RNA stem-loops within two 35 nucleotide sequences that are related to the canonical IREs RNA stem-loop in ferritin mRNA. However transfection studies only validated the presence of the fully functional IRE (APP IRE-1) to APP 5'UTR sequences [40,43].

Further homologies to the IREs in the L- and H-ferritin and APP mRNAs has been observed in the 5'UTR of the α -synuclein transcript [44–46], although the mechanism by which iron controls alpha-synuclein expression pertinent to AD and Parkinson's disease has yet to be elucidated. Additionally, a potential IRE can be computer-predicted to be present in the 5'UTR of mRNA encoding C9orf72 such that this RNA structure may post-transcriptionally regulate this key ALS-linked gene in response to iron homeostasis. Thus, as is the case for APP expression during the progression of AD, a potential mechanistic link may exist between iron control of levels of C9orf72 to worsen ALS pathology. Previously reported alterations in iron and ferritin levels were reported in patients with ALS [47].

2.4. Translational control of APP mRNA by IRP-1: Ferritin model

IRP1 and IRP2 control both APP and ferritin mRNA translation and TfR mRNA stability [48]. IRP1 and IRP2 bind at high affinity ($K_d = 40\text{--}100\text{ pM}$) to the highly conserved IRE RNA stem-loops at the 5' cap sites of L- and H-mRNAs and the 3'UTRs of TfR mRNA. Other iron-specific transcripts also interact with IRP1 and IRP2 [49], including that encoding erythroid aminolevulinate synthase (eALAS) protein (heme biosynthesis) [50]. Thus, IRP1 exhibits a dual role as an RNA repressor protein in the absence of iron (binding tightly to IREs and preventing ribosome access to the 5' cap sites of ferritin mRNAs), whereas iron promotes the formation of an Fe4S4 cluster in IRP1, which then acts as a cytoplasmic *cis*-aconitase and no longer binds to IREs (see [39]). IRP2 is destabilized in conditions of iron influx, whereas IRP2 avidly binds canonical IREs in ferritin and TfR mRNAs when intracellular iron is chelated [39]. Iron influx increases ferritin mRNA translation by releasing IRP1/IRP2 binding to the 5' cap site IRE stem loop, and the same IRP1/IRP2 interactions control TfR mRNA stability by 3'UTR specific IREs [49].

The role of IRP1 to regulate APP translation may be prominent as part of a protective response to spare brain neurons recovering from ischemic stroke and hemorrhage. [21]. Acute protective upregulation of APP in defined regions of the brain can be linked to the key finding that APP has an integral role in ferroportin-dependent iron export and neuronal iron efflux [11]. Iron induced expression of APP is controlled at the translational level in response to cellular iron levels by related, but distinct, pathways to those governing ferritin translation [12]. IRP2 had long-been considered as being the prime iron-dependent mediator of the post-transcriptional regulation of intracellular ferritin and TfR levels [51], and only IRP2 knockout mice exhibited a disruption in iron metabolism resulting in ataxia associated with neurodegeneration [52].

By contrast, IRP1 was shown to be more critical as an oxygen sensor for dietary iron transport and iron influx to promote erythropoiesis by controlling rates of translation of IRE-dependent Hif-2- α mRNA [53]. The APP IRE preferentially interacts with IRP1 consistent with our report of RNA binding assays that showed the presence of high affinity binding of IRP1, not IRP2, to biotinylated APP IRE probes using SH-SY5Y cells [12,40].

This observation was reproduced by the discovery of the same IRP1 interaction with APP mRNA in human brain (with an increase in the relative interaction in AD compared to control subjects) [12]. Thus, it may be proposed that translational control by IRP1 can be a component of the pathway governing the role of APP in regulating blood–brain barrier transport of iron by transferrin [54], the divalent metal ion transporter (DMT) [55], and promote iron efflux from the brain by ferroportin [56] (Fig. 3). In this regard, iron can regulate DMT-1-dependent iron import and ferroportin dependent iron export from various cell-types via IRE/IRP1 modulated pathways [57–59].

In addition to Fe, IL-1, an inflammatory mediator of anemia in chronic disease, can regulate translational control of both ferritin L- and H-subunits via distinct acute box domain sequences downstream of the IREs in 5'UTR domains [60]. As a model for inflammation to exacerbate amyloidosis in AD, the 5' UTR of APP mRNA [61] is also responsive to IL-1 via a related acute box domain [60]. In the case of ferritin, IL-1 stimulates H-subunit gene expression at the level of message translation via pyrimidine tract RNA binding proteins (CP-1 and CP-2), operating through the GC rich IL-1 responsive acute box domain in the H-ferritin mRNA 5'UTR which is 105 nt downstream from the 5' cap specific IRE in H-mRNA [60]. The APP mRNA acute box domain, like H-ferritin, is located immediately upstream of the start codon and may well interact with the poly [C] binding proteins, CP-1 and CP-2 (Fig. 4).

3. AD therapeutics linked to APP mRNA translation

3.1. APP over-expression and amyloidosis in Down syndrome (DS) and familial AD with trisomic precursor gene dosage: Rationale to pharmacologically inhibit APP translation

APP overexpression can be linked to clinical amyloidosis in DS patients over the age of 40, and also to a small subset of familial AD patients supporting a potential therapeutic strategy to limit APP expression [62–64]. There have also been several reports where sporadic, late-onset AD (LOAD) displays amyloidosis as a consequence of spontaneous non-disjunction of chromosome 21 with APP overexpression that increases brain amyloid burden [65–67].

Additional evidence to connect APP over-expression with amyloidosis and dementia stems from the finding that known transcription mutations in the APP gene promoter may be critical to increase risk of AD pathology [68] Transcription factors like PUF-9 may contribute to increasing APP levels by generating sufficient template to alter the balance toward increased A β levels and amyloidosis [69], observations that are supported by the fact that most cases of familial AD result from APP processing by presenilins and BACE [70,71].

Supporting an approach to selectively limit APP mRNA translation, restoration of trisomic APP gene copy number to diploid levels in a DS mouse model can attenuate the cognitive decline associated with enhanced APP gene expression [72]. Limiting APP expression can limit amyloidosis in vivo and restore blocked trophic retrograde nerve growth factor transport [72,73]. The use of translation blockers directed to the APP-5'UTR therefore represents a pharmacological equivalent of this effect [74] (Fig. 2). Additionally, an increased APP dose, like that occurring in DS, may perturb brain iron metabolism. Thus

another major rationale for inhibiting APP translation is that this therapy may reset iron dys-homeostasis, an effect that may occur as a consequence of APP over-expression in DS syndrome individuals exhibiting features of AD [72,75,76].

3.2. Iron chelators and APP translation

Functionally, iron chelators may mechanistically target and facilitate IRP1 to repress IRE dependent translation via the APP 5'UTR to thus limit APP expression [40,77] (Figs. 2 and 4). Chelation-based therapy also targets A β pathology post-translationally by addressing cerebral metal dyshomeostasis of APP/A β metal-redox complexes, ROS production and further A β generation. Administration of iron and the Al-chelator, desferrioxamine (DFO) can slow dementia in AD patients [48]. However it is unstable, expensive quickly metabolized with poor BBB permeability [5]. Clioquinol (CQ), a Cu/Zn-chelator that can cross the BBB clinically attenuates cognitive loss and lowers plasma A β levels in moderate-to-severe AD patients [78] but is associated with myelinopathies [79].

A second-generation 8-hydroxyquinoline analog metal chaperone, PBT2 lacking serious adverse events [80,81] was superior to CQ in terms of sequestering metal ions from A β . PBT2 improved cognition in aged C57Bl/6 mice [82] and APP transgenic Tg2576 mice, effects associated with decreased interstitial A β [83]. A Phase II trial with PBT2 in 78 AD patients showed a reduction in CSF A β levels of CSF and enhanced performance metrics. The Cu²⁺ chelator, tetrathiomolybdate, that was efficacious in animal models of AD [5] is in Phase II/III trials [84]. Other metal chelators, including the bi-functional molecules XH-1 (amyloid-binding and metal-chelating moieties), VK28 (Fe³⁺ chelator), HLA20 and M30 (Fe³⁺ chelator with *N*-propargylamine-like properties) suppressed APP holoprotein expression and lower A β secretion [5]. However, additional studies are required to establish their efficacy and safety in targeting amyloidogenesis and to associate their effects with inhibition of APP translation.

3.3. Targeting APP expression at the level of translation of its transcript

APP expression can be inhibited by selective RNA targeting of APP translation via the uniquely folded 5' untranslated region of the precursor transcript [18] offering a novel target to complement existing strategies. Utilizing knowledge of the translational control circuits through which iron regulates translation of the iron storage protein ferritin and APP via interactions with their respective 5'UTRs, each of which encodes different versions of IRE [12], provides a strategy to identify and characterize inhibitors of this process. Indeed, to justify use of high throughput screening (HTS) approaches, known drugs and clinical candidates have been identified that limit APP translation and display in vivo anti-amyloid efficacy with improved cognitive performance [85,86]. For example, the cholinesterase inhibitor phenserine and its (+)-enantiomer, posiphen, both reduced APP 5'UTR dependent translation while limiting brain amyloid A β production in vivo [86–88]. Thus, an HTS benzimidazole that was designated as BRD-K24025829 or 'BL-1' was recently identified from the Broad Institute Molecular Library using the strategy shown in Fig. 2. BL-1 was shown to act as an inhibitor of APP translation that may have potential to limit amyloidogenic neurodegeneration while improving iron balance to offset cognitive decline.

3.4. APP translation blockers as selective anti-amyloid agents formulated to improve brain iron balance

3.4.1. Background—The strategy to modulate APP levels by limiting APP expression can be employed not only to reduce brain amyloid burden but also to counterbalance potential perturbations in brain iron (see model in Fig. 3). This is supported by the fact that APP itself can bind to the central iron export protein, FPN1, and thus serve to shuttle iron from overloaded neurons [11].

3.4.2. APP translation blockers to maintain iron homeostasis—The canonical IRE stem loops that control translation of L- and H-ferritin mRNAs (iron storage) bind equally to IRP1 and IRP-2, the two known RNA binding proteins that regulate cellular iron homeostasis by their inducible interaction with the IREs to control ferritin mRNA translation and transferrin receptor mRNA stability (iron homeostasis) [41,49]. In RNA gel-shift assays and functional RNAi knockout studies, it was shown that iron modulated the interaction between IRP1 and the APP 5'UTR to directly control neuronal APP levels [40,89]. Consistent with this finding, APP expression levels directly facilitated iron efflux from toxically overloaded neurons where the induced extra neuronal presence of APP(s) actively accelerated iron export by FPN1 [11,12]. APP mRNA encodes a highly active IRE that binds with a different RNA binding specificity to IRP-1 relative to the ferritin IRE [12] thus supporting the concept that the APP 5'UTR offers specificity as a target to identify small molecules to suppress APP and A β production [90]. Consistent with the role of an active IRE in the APP 5'UTR [12], the iron chelator, DFO, limited APP expression [40] while also improving cognition in the clinic [91]. The model in Fig. 3 links APP expression by 5'UTR-directed translational modulators to the role of the precursor as a controller of iron export by FPN1. Thus assays can be established to identify APP translation blockers that act as anti-amyloid agents while also ensuring maintenance of iron homeostasis and neuronal viability [18].

3.4.3. Approved drugs and clinical candidates can act as APP mRNA 5' untranslated region (5'UTR) inhibitors—The APP 5'UTR, that is responsive to both metals and IL-1, drives APP translation and ultimately A β -peptide generation [12,24,61]. Based on this concept, screening a library of 1,200 FDA approved drugs and clinical candidates in SH-SY5Y transfected cells [77,92,93] resulted to 17 “hits”, classified as antibiotics, statins, metal chelators, mutagens, detergents and blockers of receptor-ligand interactions that suppressed greater than 95% translation of the APP 5'UTR-driven luciferase transcript [77,93]. Of these 17 hits, paroxetine (selective serotonin reuptake inhibitor), dimercaptopropanol, DFO (metal chelator), *N*-acetyl cysteine (NAC, antioxidant), erythromycin (macrolide antibiotic), and phenserine (a discontinued anticholinesterase) suppressed APP holoprotein expression without affecting amyloid-like protein expression [6,93]. Azithromycin also suppressed luciferase reporter mRNA translation via the 146 nt APP 5'UTR sequence without secretase activation [5,48]. The strategy of targeting APP 5'UTR to reduce amyloid expression was validated when paroxetine and NAC were shown to limit amyloid levels in the TgCRND9 mouse model of AD [85].

Table 1 shows these above compounds that selectively reduced luciferase reporter gene expression driven by the APP 5'UTR while maintaining co-expression of a dicistronic green fluorescent protein reporter, translated via an internal viral internal ribosome entry site (IRES) [77,93]. Proof of concept was established when dietary NAC lowered cortical A β levels in TgCRND8 transgenic mice [85]. Phenserine and posiphen, were also found to be bona fide APP 5'UTR directed translation blockers that limited A β production in vivo and improving cognition and have entered clinical trials [94].

3.5. High throughput screen (HTS) APP translation blockers

High throughput RNA directed screening efforts to limit expression of toxic neurodegenerative disease transcripts are outlined in Figs. 2 and 3. Collectively, 300,000 chemical probes were screened from the molecular libraries network collection of the NIH. Potent translation blockers were reported as being specific to repress α -synuclein (SNCA mRNA) [95]. The lead structures from these screens have been cataloged on PUBCHEM (AID: 1285) and may represent promising leads in the pipeline for AD and DS therapeutics.

3.6. Potent high throughput screened APP 5'UTR translation blockers

In addition to identifying FDA approved drugs active as APP 5'UTR directed inhibitors, high throughput screen of this RNA target also identified other compounds as APP 5'UTR blockers including a series of benzimidazoles [90]. The lead, designated as JTR-009, limited neuronal levels of both APP and amyloid at picomolar concentrations [18] (Fig. 3). JTR-009 acted as an intercalator that prevented ribosome attachment to the precursor transcript and thus inhibited APP expression with no change to iron homeostasis in SH-SY5Y neuroblastoma cells and primary neurons [18]. The anti-amyloid efficacy of BL-1 was superior to that of JTR-009 [18]. In this screening campaign the PrP-mRNA 5'UTR was originally employed as the primary target and APP 5'UTR was to confirm selectivity in transfection bases experiments (Fig. 3). However, BL-1 inhibited both APP and PrP 5'UTR conferred translation, potentially the result of similarities in both 5' untranslated region sequences. Interestingly, both APP and prion proteins transport iron from neurons [11,96] where APP is a facilitator of FPN1 mediated iron export [11].

The models in Fig. 3 propose a link between APP, as an iron- and IRP1-dependent translationally controlled protein, and its action as an iron exporter. APP exports iron by FPN1. Thus, any 5'UTR translation blocker would be anticipated to increase iron retention in treated neurons. To achieve this, drugs would have to be formulated to find conditions for administration in order to achieve optimal brain iron homeostasis at the same as ensuring potent anti-amyloid efficacy in vivo. Perls' Prussian blue, calcein staining and FlouroJADE staining techniques can be used check for optimized iron homeostasis and neuronal viability while ELISA and immunohistochemical stains are readily available to measure amyloid burden. APP translation blockers can be predicted to offset potential micro-anemia in neurons in FAD patients that overexpress APP [64] (Fig. 3), a scenario similar to non-disjunction of the APP gene in trisomic DS patients, who at later age express features of AD [72].

3.7. Future combination therapies for AD employing both RNA and protein based approaches

3.7.1. Ongoing clinical trials—The clinical failure of γ -secretase inhibitors, e.g. semagacestat and A β vaccines and passive immunity, each of which demonstrated activity in preclinical models suggested an urgent need for alternative approaches to amyloid modulation [9,97–101]. This raises the issue of what the normal role(s) of APP and amyloid fragments actually are [98] with recent studies showing that administration of A β in TH1 and TH17 versions of EAE encephalomyelitis actually reversed paralysis and reduced inflammation in this model of multiple sclerosis [8].

3.7.2. Future prospects for combining RNA-based APP translation blockers with existing protein based AD therapies—RNA sequence directed translation blockers that reduce brain amyloid production, and improve brain iron homeostasis, can be considered as candidates for eventual clinical assessment for AD therapy. As a precedent, APP 5'UTR directed inhibitors could well be administered in combination therapy with APP protein targeting agents as has been the practice to treat for human acquired immunodeficiency virus (HIV) with patients [102]. For example, cocktails comprising two nucleoside-analog reverse transcriptase inhibitors (RTI) and non-nucleoside-analogue RTIs or protease inhibitor are considered standard therapies for HIV [103]. In conclusion, this approach must be viewed in the knowledge that APP knockout mice exhibit only mild iron/copper mis-homeostasis and gliosis that, if left unchallenged by hemorrhage, are non toxic and allow the mice to live a full lifespan [104,105].

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Fig. 1.

RNA regulatory domains in the APP transcript. The 3 kb APP transcript is controlled at the level of message translation by the action of 5'UTR regulatory domains that are responsive to IL-1 and iron. The 3' untranslated region is alternatively polyadenylated, and the longer APP transcript is translated more efficiently than the shorter transcript. A 29 nt RNA destabilizing element was mapped to the 3'UTR of APP mRNA. A second IRE-like RNA sequence is depicted in the A β domain of the APP coding region, and the Fragile X mental retardation protein (FMRP) is a cytoplasmic mRNA binding protein that binds to downstream segment of the coding region of APP mRNA at a guanine-rich, G-quartet-like sequence. IL-1 co-induces APP mRNA translation and α -secretase cleavage in the APP A β domain where this primary inflammatory cytokine induces the non-amyloidogenic secretion of APP(s) from astrocytes.

Fig. 2.

RNA stem loop target in 5'UTR of APP mRNA ($G = -54$ kCal/mol. Left Panel: The canonical iron-responsive elements (IREs) in the L- and H-chain mRNA s of the iron-storage multimer ferritin harbors 75% homology with the RNA stem loop folded from the APP 5'UTR (shown). This IRE confers iron-responsive translation to APP and its unique RNA structure is a drug target to suppress APP expression. Right panel: technique to screen molecular libraries using pIRES(APP)-Luc transfectants for APP 5'UTR directed compounds that limit translation of the luciferase reporter gene driven by APP 5'UTR, where drug selectivity is internally monitored for maintenance of the dicistronic green fluorescent (GFP) gene expression.

Fig. 3.

Model for cooperative translation of APP by IRP1 and facilitated ferroportin dependent export. Panel A: It has been previously reported that translation of APP mRNA is repressed by IRP1, which binds to the IRE loop in the 5'UTR of the APP mRNA thus preventing translation. When iron levels rise intracellularly, iron displaces IRP1 from the mRNA. Iron-IRP1 dissociates and translation of APP protein proceeds. APP is a transmembrane protein that acts together with ferroportin to export iron out of cells for loading onto transferrin (Tf; [11]). Panel B: APP translation blockers that are proven to be anti-amyloid would be predicted to either intercalate into APP-5'UTR RNA stem loop (TR-009) or cause IRP1 dependent super-repression (posiphen). In (B), we suggest that the APP translation blocker- (JTR-009) can be formulated and dosed to generate conditions for added neuronal iron retention to potentially optimize brain iron homeostasis as an added efficacy to its anti-amyloid therapy.

Table 1

Alternatively positioned therapeutic FDA and clinical trial candidate drugs targeted to the 5' untranslated region of the amyloid precursor protein mRNA. Phenserine is a withdrawn anticholinesterase drug candidate that underwent a phase 3 clinical trials for AD. Posiphen is its + enantiomer in phase 2 human clinical trials.

Drug	Activity	APP inhibition (IC ₅₀) (μM)	Citation of Aβ inhibition (IC ₅₀) in vitro/in vivo
<i>N</i> -Acetyl-cysteine	Antioxidant/Fe ²⁺ -chelator	5	~1–10 μM [93]; Oral: 5 mg/kg/day for 3 month [85]
Phenserine/PS	Anti-cholinesterase	5	~1 μM [86]
Posiphen	(+) Enantiomer of PS	5	~1 μM [106]
Strophanthidine	Plant glycoside	0.5	ND [107]
Desferrioxamine	Iron chelator	10	~10 μM [12] and [91]
Dimercaptopropanol	Hg chelator	0.1–1	10 μM [93]
Paroxetine	Serotonin reuptake-inhibitor	1–10	~1–10 μM [93]; Oral: 5 mg/kg/day for 3 month [85]
Erythromycin	Macrolide antibiotic	1–10	[85]
Azithromycin	Macrolide antibiotic	1–10	[93]
Mycophenolic acid	Immunosuppressant, Fe chelator activity?	10	[107]
Tetrathiomolybdate	Copper chelator	1–10	[108]