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Research Article

Ameliorative Effects of Intranasal AM-125b in AD

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- Y maze
- Spatial working reference memory

Abstract

Background: Alzheimer's disease (AD) is a global public health crisis. Currently, there are no treatments to prevent or halt the disease. Given the precedence of chronic neuroinflammation in triggering AD-like neurodegeneration and an observed upregulation of NFkB-driven miRs i.e. mir-125b positively correlating with AD, silencing specific micro-RNA with antisense-microRNA (antagomir) to miR-125b was studied to evaluate its efficacy in ameliorating AD-like neurobehavioral deficits in 5XFAD transgenic mice modeling AD.

Objective: This study evaluated therapeutic potential of intranasally (IN) delivered 2'-O-Methyl/locked nucleic acid (LNA)-modified antagomir 125b-5p (AM-125b) in correcting neurobehavioral outcomes in 5XFAD transgenic mice modeling AD.

Methods: 5XFAD mice were intranasally administered with AM-125b (8nMols/4µl/week) for 8 weeks. Controls were intranasally administered with equal volume of saline-vehicle containing equal length 2'-O-Methyl/LNA modified scrambled nucleotides for the same duration. After confirming miR-125b-5p blocking ability of AM-125b using QRTPCR, ameliorative efficacy of intranasal AM-125b in improving spatial reference working memory and in reducing cerebral levels of total and oligomeric Aß, total and phospho-tau, and key inflammatory markers were evaluated using Y-maze and ELISA.

Results: Results confirmed direct brain targeting of AM-125b after intranasal delivery. Intranasally delivered AM-125b significantly improved spatial reference working memory (52-55% increased alterations between three arms, $p<0.0001$) along with reduced cerebral levels of total and oligomeric Aß (2.1-2.5-fold reduction, all values, $p<0.0001$), reduced total and phospho-tau (2.4-2.5-fold reduction, all values, $p<0.0001$) and reduced inflammatory markers (2.2-2,3-fold reduction, all values, $p<0.0001$). Intranasally delivered AM-125b significantly improved neurobehavioral deficits in 5XFAD mice modeling AD.

Conclusions: This is one of the lead reports showing therapeutic efficacy of antagomiR-mediated silencing of microRNAs 125b-5p using a non-invasive nose-to-brain drug delivery method in ameliorating Alzheimer-like neurocognitive deficits.

ABBREVIATIONS

Aß: ß-amyloid; AD: Alzheimer's disease; ALOXI15: Arachidonate 15-lipoxygenase; AM: AntagomiR; APP: ß-amyloid Precursor Protein; BBB: Blood brain barrier; BCSFB: Blood cerebrospinal fluid barrier; DHA: Docosahexaenoic Acid; IL-1ß: Interleukin-1ß; IN: Intranasal; LNA: Locked nucleic acid; Lts: Littermates; mRNA: Messenger ribonucleic acid; MCI: Mild cognitive impairment; miR/miRs: Micro RNA(s); NFkB: Nuclear factor kappa B; Nucleotide: nt; NPD1: Neuroprotection D1; oAß: oligomeric ß-amyloid; 2'-O-ME: 2'-O-Methyl; PKR: Protein kinase ribonucleic acid activated protein; Phospho-tau: Phosphorylated tau protein; PSEN1: Presenilin1; QPCR: Quantitative Polymerase Chain Reaction; ROS: Reactive Oxygen Species; RNA: Ribonucleic Acid; SD: Standard deviation; TNFα: Tumor Necrosis Factor Alpha; Tgs: Transgenic Mice

INTRODUCTION

Alzheimer's disease (AD) is a global public health crisis currently afflicting ~6 million Americans (and ~40 million people worldwide). By the middle of the century, these numbers will escalate to ~16 million Americans (and ~150 million people worldwide) suffering from AD, if breakthrough disease-modifying treatments are not discovered [1,2]. Currently, there are no treatments to prevent or halt the disease. There is a growing consensus that Alzheimer's is a multifactorial disease involving an interplay of many deregulated "aging" factors occurring much earlier than the actual onset of the disease [3], among which neuroinflammation and oxidative damage [4-6] constitute the key prime factors resulting from high energy requirement of brain with its modest anti-oxidant defense, oxidative damage caused by reactive oxygen species (ROS) [7], and chronic inflammation [8-10], along with cholinergic dysfunction [11-13],

insulin resistance [14-16] and other factors. Recently, it has been implicated that increase in cerebral ß-amyloid (Aß) in the aging brain either due to reduced Aß clearance, influx of peripheral Aß due to blood brain barrier (BBB)/blood cerebrospinal fluid barrier (BCSFB) breach caused by age-related oxidative damage, inflammation-Protein kinase RNA activated protein (PKR) induced Aß formation [17], or Aß overproduction due to familial mutations, all result in cerebral Aß accumulation destroying synaptic integrity fundamental to cognitive decline observed in prodromal AD and/or mild cognitive impairment (MCI) [3]. Oxidative stress and chronic neuroinflammation constitute the earliest changes in triggering AD [7]. Emerging evidence indicates that these changes in AD are regulated by small non-coding microRNAs (miR/miRs) [18].

MicroRNAs (miR/miRs) are highly conserved ~22-nucleotide (nt) long non-coding RNAs that function as post-transcriptional regulators of gene expression [19,20]. MicroRNAs regulate gene expression by interfering with translation of their target messenger RNAs (mRNAs) via binding to the 3'-untranslated region (3'-UTR) of mRNAs to induce repression or degradation of target mRNA [21], thus blocking translation of mRNA into proteins [22,23]. The activity of any given miR can be experimentally inhibited by antisense oligonucleotides. In order to attain the in vivo stability, antisense oligonucleotides are chemically modified [24]. Among all, the "Locked" nucleic acid (LNA) conformation and 2'-O-Methyl nucleic acid modification [25] results in enhanced hybridization to target mRNA with increased resistance to degradation, sensitivity and selectivity [26,27].

Growing body of evidence indicates crucial role played by miRs in human health and diseases [28,29]. There are about >2600 miRs characterized in human brain, of which only selected ~50 miRs have been found to be enriched within selective region(s) of the brain [30]. Increasing number of studies indicate that the dysregulation of miRs is fundamental to the etiology of neurodegenerative diseases including AD [31,32]. Multiple studies on brain gene expression have indicated that in AD, about $1/3^{rd}$ of the genes are upregulated while the rest $2/3^{rd}$ of the genes are downregulated [33]. Interestingly, most of the upregulated pathogenic genes in AD are known to be under the transcriptional control of a pro-inflammatory mediator-nuclear factor kappa B (NF*k*B) [33] which are significantly upregulated in AD-specific anatomic brain regions [34]. Given the precedence of chronic neuroinflammation in triggering AD-like neurodegeneration and an observed significant upregulation of NF*k*B-driven miRs (i.e. mir-125b) positively correlating with AD progression [34-36], both in early and late onset AD [37-39], silencing miR-125b micro-RNA using anti-microRNA (antagomiR) is expected to improve AD-like neurobehavioral deficits. This study evaluated therapeutic potential of intranasally (IN) delivered 2'-O-Methyl locked nucleic acid (LNA)-stabilized antagomiR-125b (AM-125b) in ameliorating neurobehavioral deficits in 5XFAD transgenic mice modeling AD.

MATERIALS AND METHODS

Animals

All animal procedures were performed in accordance with the institutionally approved protocol for the care and use of animals. 5XFAD mice harboring ß-amyloid precursor protein (APP) and presenilin 1 (PSEN1) transgenes, originally obtained from Dr. Vassar (Northwestern University, Chicago, IL) were used for generating 5XFAD transgenic colony [40,41]. One set of 5XFAD transgenic mice (Tgs) were used for studying brain uptake of radiolabeled antagomir 125b (AM-125b) up to 24h after a single bolus intranasal (IN) administration. Experimental set of 5XFAD Tgs and non-transgenic littermates (Lts) were first used for assessing Y maze exploratory spatial reference working memory, and then euthanized to collect brain tissues, left hemisphere was used to isolate total proteins for ELISA measurements of total and oligomeric Aß, total and phospho-tau and key inflammatory markers i.e. tumor necrosis factor alpha (TNFα) and interleukin-1beta (IL-1ß). While the right hemisphere was used for quantitative reverse transcriptase polymerase chain reaction (QRTPCR) measurements of miR-125b-5p to confirm AM-125b mediated inhibition of miR-125b-5p.

Anti-MicroRNA (AM) Chemical Modification

The single-stranded 2'-O-ME/LNA modified antisense RNA mixmiR-125b-5p (AM-125b-5p) as well as scrambled sequence-control with equal number of 2'-O-ME/LNA modified nucleotides and similar GC content were designed based on mmu-miR-125b-5p sequence (Accession # MIMAT0000136) (www.mirbase.org) as follows: AM-125b-5p: (5'-**A*G*G*G*ACTC**tgggattga a*c*a*c*t-3') Scrambled Control: (5'-**A*A*C*A*GTGT**gcggcgatt *a*c*g*a-3') Capital bold letters represent LNA modifications, unbold lowercase letters represent 2"-O-ME modifications, and asterisks (*) represent phosphorothioate linkages (GenePharma, Shanghai, China).

Intranasal Administration and Brain Uptake of Intranasally delivered AM125b-5p

Brain Uptake Studies: 5XFAD mice were nasally administered with a single bolus injection of I^{125}-labeled AM-125b-5p (Iodobid, Pierce) [42] suspended in a saline vehicle 4nMols/2µl/naris ≡ 8nMols/4µl/animal/once (N=10). Controls were IN-administered only with equal volume of saline vehicle 2µl/naris/once (N=10). Mice were killed after 24h, brains harvested, homogenized, centrifuged at 100,000g (MTX Sorvall), and 100µl of homogenate equating to 100µg of brain tissue were recorded. The data were presented as cpm/100ug (Figure 1).

Behavioral Studies: Experimental set of 5XFAD Tgs and non-transgenic littermate (Lts) were intranasally injected with AM-125b suspended in saline vehicle (4nMols/2µl/naris≡8nMols/4µl/animal/week) for 8 weeks (Males/N=5, Females/N=5). Controls were intranasally injected with equal strength scrambled nucleotides suspended in saline vehicle (4nMols/2µl/naris≡8nMols/4µl/animal/week) for 8 weeks Tgs (Males/N=5, Females/N=5) and Lts (Males/N=5, Females/N=5). Efficacy of intranasally administered AM-125b-5p in improving spatial reference working memory in 5XFAD mice was evaluated using Y-maze (YM) performance [43,44].

Y-Maze (YM) Assessment for Evaluating Spatial Working Reference Memory

Y-Maze (YM) test for evaluating spontaneous alteration behavior and exploratory activity to assess the spatial working

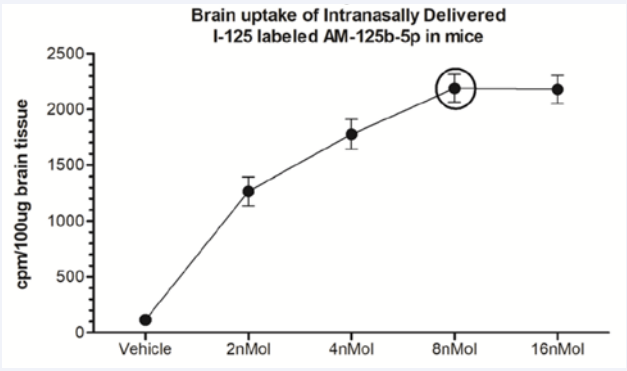

Figure 1 Brain Uptake after intranasal (IN) single bolus administration of different concentrations of I-125 labeled AntagomiR 125b (AM-125b) in mice, expressed as counts per minute (cpm)/100µg brain tissue presented as Mean ± standard deviation (SD). Note optimum uptake of AM-125b at the concentration of 8nMols.

reference memory that is stored temporarily and elicited actively during the completion of the task, a task known to involve hippocampus, septum, basal forebrain and pre-frontal cortex [45], will be performed as established [40,43,46]. The animals typically tend to explore a new arm of the maze rather than returning to one that was previously visited. Y maze is made up of dark grey acrylic material with three 40cms high, 21cm long and 4cm wide identical arms at a 120° angle from each other. Each animal is placed in the center zone. After introduction to the center of the maze, the animal is given free access to all three arms in a single trial of 6 min duration. If the animal chooses a different arm than the one it arrived from, this choice is called an alteration. Alterations and total number of entries in each arm and the sequence of entries are video-tracked and recorded (AnyMaze). The Y maze activity index as the number of entries in each arm and

percent alterations calculated as:

$$\% \text{ Alterations} = \frac{\text{Total Number of Alterations}}{\text{Total Number of Arms Entered}} \times 100$$

The data were expressed as Mean ± standard deviation (SD) and presented (Figure 2).

Quantitative Reverse Transcriptase Polymerase Chain Reaction (QRTPCR)

The mRNA expression of miR-125b-5p was quantitated by QRT-PCR as follows. Brain RNA was isolated using TRIzol reagent (Invitrogen) and treated with DNase I (Thermo Scientific) to destroy possible DNA contamination. The quality and quantity of RNA was measured by Nanodrop Lite (Thermo Scientific) and RNA agarose gels. High Capacity cDNA Reverse Transcription Kit (Applied Biosystems™) was used to convert 2ug of isolated RNA into single-stranded cDNA which was used for QRT-PCR using QuantStudio-3 (Applied Bio-systems™). The expression of miR-125b-5p mRNA was quantitated using Taqman mRNA-specific assays using FastStart universal SYBR green master-mix (Rox) (Life Technologies). U6 mRNA was used as endogenous control to normalize the expression data of miR-125b. Specific primer pairs used:

U6-RT:
5′ctcaactggtgtcgtggagtcggcaattcagttgagaaaaatatggaacgct-3′

U6-F: 5′-ctggtagggtgctcgcttcggcag-3′; U6- R:
5′-caactggtgtcgtggagtcggc-3′

miR-125b- RT:
5′-ctcaactggtgtcgtggagtcggcaattcagttgagtacaa-3′

miR-125b -F:5′-cgcgctccctgagaccctaac-3′; miR-125b- R:
5′-tggtgtcgtggagtcg-3′

The expression of miR-125b-5p target mRNA was calculated relative to the endogenous U6 mRNA control. Comparative CT (ΔΔCT) method was used to quantitate differential mRNA expression and fold change, analyzed and plotted (Figure 3).

Enzyme-Linked Immunosorbent Assay (ELISA)

After behavioral studies, mice were euthanized, brain tissue lysates subjected to ELISA measurement of cerebral levels of total and oligomeric Aß (BioSource), total and phospho-tau (BioSource), and inflammatory markers i.e. tumor necrosis factor alpha (TNFα) and interleukin-1-beta (IL-1ß) (R&D Systems) using commercial kits as established [46-48]. The data were presented as Mean ± standard deviation (SD) and plotted (Figure 4).

Statistical Analysis

Data were subjected to column statistics to obtain respective group means with standard deviation (SD). The data were further subjected to analysis of variance (ANOVA), followed by Tukey post hoc test. A value of $p<0.05$ was considered statistically significant.

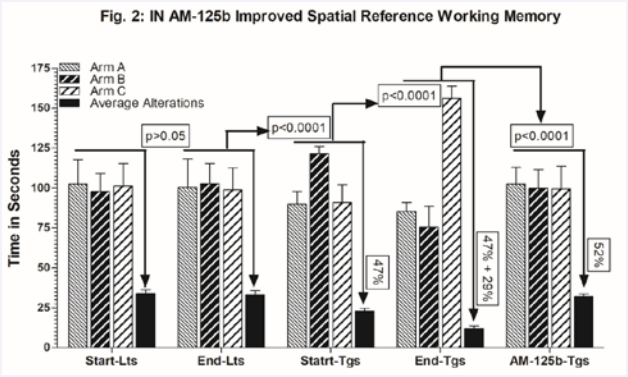

Figure 2 Y maze spontaneous alteration and exploration test to assess spatial working reference memory in mice after intranasal AM-125b treatment in 5XFAD mice. Data recorded as time spent in each arm in seconds and expressed as Mean ± standard deviation (SD). Note unequal alterations/explorations recorded for untreated Tgs compared to littermate (Lts) controls, indicating deterioration in Y maze performance in untreated Tgs at the start ($p<0.05$) and in the age-matched untreated Tgs at the end of the treatment ($p<0.004$). Note significantly improved Y maze performance in AM-125b treated Tgs showing almost equal alteration and exploration in all A, B and C arms of Y maze indicating improved spatial reference working memory, compared to untreated Tgs.

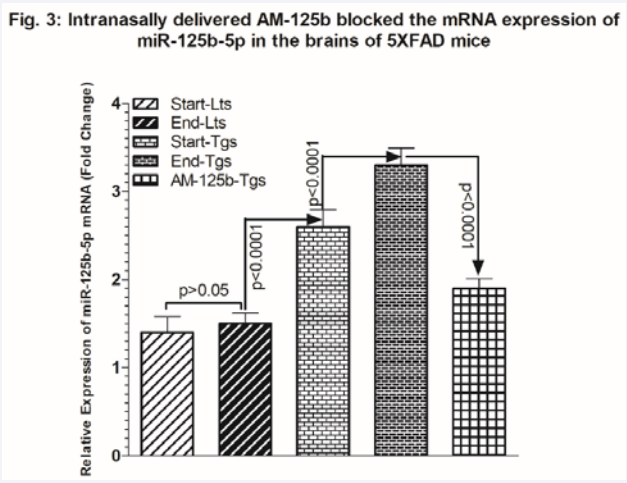

Figure 3 Effect of intranasally delivered AM-125b on the relative expression of miR-125b-5p mRNA in the brains of 5XFAD mice presented as group mean ± standard deviation (SD). Note non-significant (p>0.05) difference in the basal expression levels of miR-125b-5p mRNA between start- and end-Lts, which were significantly increased by 1.9-fold (p<0.0001) in start-Tgs and further increased by 2.4-fold (p<0.0001) in end-Tgs. Intranasally delivered AM-125b significantly decreased the levels of miR-125-5p mRNA by 1.8-fold (p<0.0001) in 5XFAD mice.

RESULTS

Intranasally Administered AM-125b Exhibited Efficient Brain Targeting

Consistent with previous findings [42], current study showed dose-dependent brain uptake of radiolabeled AM-125b after a single bolus intranasal (IN) delivery. The results showed linear increase in the brain uptake of IN delivered AM-125b from 2nMol up to 8nMol, however, the next level concentration of 16nMol did not exhibit significant increase in brain uptake (Figure 1), indicating 8nMols as the optimum concentration beyond which there was no further linear increase in the brain uptake. Therefore, 8nMols/single bolus IN concentration was concluded to be optimum which was used in current studies.

Intranasal AM-125b Improved Spatial Working Reference Memory

The results showed that Y maze spontaneous alteration in littermate controls (Lts) IN administered only with saline vehicle did not change significantly from 8 weeks of age (Start-Lts) up to 16 weeks of age (End-Lts) (p>0.05) (Figure 2). Compared to Start/End-Lts, start-Tgs at 8 weeks of age administered with saline vehicle only, exhibited unequal and restricted arm entries, more time spent in arm B, and 47% reduced alterations between A/B/C arms (p<0.0001) (Figure 2). Compared to 8-week-old start-Tgs, Y maze spontaneous exploration in 16-week-old Tgs IN administered only with saline vehicle was further deteriorated by 29% (p<0.0001), showing restricted exploration in A/B/C arms and more time spent in arm C (Figure 2). Intranasal administration of AM-125b significantly improved Y maze spontaneous exploratory behavior, as evidenced by almost equal number of entries in all arms A/B/C and 52% increased alterations between 3 arms in AM-125b-treated Tgs (p<0.0001), which was observed to be normalized. Given the fact that Y Maze test assesses damage to limbic and non-limbic brain regions, quantifying spatial working reference memory that is stored temporarily and elicited actively during the completion of the task [45], equal exploration and entry in all arms suggests normal spontaneous exploratory behavior observed in Lts. By contrast, unequal time spent in all arms with relatively more time spent in randomly selected arm showing restricted arm exploration and reduced number of entries between these arms are indicative of impaired spatial working memory in untreated Tgs. antagomir-mediated blockade of miR-125b significantly improved Y maze exploration in AM-125b treated Tgs.

Intranasal AM-125b Successfully Blocked Cerebral Expression of miR-125b

The results showed efficient blockade of miR-125b-5p after intranasal administration of AM-125b in 5XFAD mice. There was non-significant (p>0.05) difference in the basal expression levels of miR-125b-5p mRNA between start-Lts vs end-Lts (p>0.05). This basal expression of miR-125b-5p mRNA was significantly increased in 8-week-old Tgs at the treatment start-point by 1.9-fold (p<0.004) (Start/End-Lts vs 8-week-old Tgs). In 16-week-old Tgs, there was observed further increase in the expression of miR-125b-5p mRNA by 2.4-fold (p<0.003) (Start-Lts vs 16-week-old Tgs). Intranasal administration of AM-125b successfully blocked the expression of miR-125b-5p mRNA, as evidenced by significantly decreased the levels of miR-125-5p mRNA by 1.8-fold (p<0.0001) (16-week-old untreated Tgs vs 16-week-old AM-125b treated Tgs) in Tgs IN injected with AM-125b (Figure 3).

Intranasal AM-125b Reduced Cerebral Inflammation, Amyloid and Tau

It was interesting to see that AM-125b-mediated silencing of miR-125b resulted in reducing cerebral levels of total Aß, oAß, Tau, Phospho-Tau, TNFα and IL-1ß in AM-125b treated 5XFAD mice. In all these parameters studied, there was no significant difference start-Lts vs end-Lts (p>0.05). In general, basal cerebral levels of total Aß, oAß ,Tau, Phospho-Tau, TNFα and IL-1ß in start/end Lts were increased age-dependently in 8-week-old (p<0.0001) (Start/End-Lts vs 8-week-old Tgs) and 16-week-old (p<0.0001) (Start/End-Lts vs 16-week-old Tgs) untreated Tgs. Intranasal administration of AM-125b resulted in significant reductions of cerebral levels of total Aß, oAß, Tau, Phospho-Tau, TNFα and IL-1ß (p<0.0001) (16-week-old untreated Tgs vs 16-week-old AM-125b treated Tgs) in AM-125b treated 5XFAD mice (Figure 4, Table 1).

DISCUSSION

Early upregulation of neuroinflammation in AD and its persistence during the disease process in AD is characterized with the upregulation of a dimeric DNA binding protein NF*k*B as p50/p65 complex that has emerged as a ubiquitous transcription factor controlling diverse biological functions predominantly including inflammatory and immune functions [49]. NF*k*B activation and binding to the promoters of NF*k*B-sensitive genes via microRNAs, facilitates transcriptions of many pathogenic genes altered in many neurodegenerative conditions including

AD [34]. MicroRNAs (miRs) bind to the complementary RNA sequences in the 3'-UTR on mRNA and thereby repress the expression of target mRNA [34]. Upregulated miRs are generally accepted to predominantly decrease the expression/levels of target mRNA, thus down-regulating the protein translated by that target mRNA, and vice versa [34].

NF*k*B regulated miRs have been shown to be significantly elevated in AD brain, among which common to aging brain and AD brain are significant upregulation of miR-125b [34]. Bioinformatics and multiple analytical techniques including RT-PCR, DNA-Array, Western blots, etc. have confirmed that miR-125b targets the 3'-UTR of several AD-related mRNAs [33,34]. Micro-RNA 125b was first shown to be upregulated in both stressed and differentiating mouse and human neurons, and has been implicated in neuronal development, cell-signaling and neurodegeneration[50]. NF*k*B-regulated miR-125b has been shown to be induced by neurotoxic aluminum sulfate that generates oxidative stress and ROS in human brain cells [51]. Consistent upregulation of miR-125b is associated with deregulated astroglial proliferation and linked to astrogliosis in various neurodegenerative conditions including AD [52].

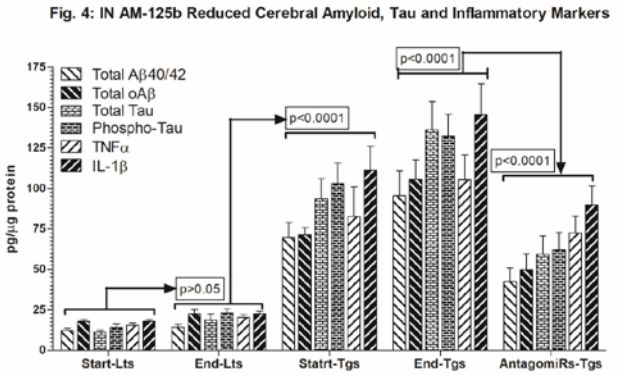

Figure 4 Effect of intranasally delivered AM-125b on the levels of total Aβ, oAβ, total Tau, Phospho-Tau, TNFα and IL-1β in the brains of 5XFAD mice, expressed as pg/µg protein and presented as group mean ± standard deviation (SD). Note non-significant (p>0.05) difference in the levels of total Aβ, oAβ, total Tau, Phospho-Tau, TNFα and IL-1β between start- and end-Lts, which were significantly increased by 5-6 fold (p<0.005) in start-Tgs and further increased by 7-8 fold (p<0.003) in end-Tgs. Intranasally delivered AM-125b significantly decreased the levels of total Aβ, oAβ, total Tau, Phospho-Tau, TNFα and IL-1β by 2-3 fold (p<0.0001) in 5XFAD mice.

MicroRNA-125b is known to regulate neuronal synaptic functions, synaptic vesicle trafficking and neurotransmitter release, which when impaired in conditions such as AD, is reported to impair synaptic signaling and neurotransmitter release [53]. In addition, miR-125b is known to regulate cell cycle arrest and arachidonate 15-lipoxygenase (ALOX15) essential for conversion of docosahexaenoic acid (DHA) to neuroprotection D1 (NPD1), and therefore dysregulation of miR-125b leads to the down-regulation of cell cycle control and deficits in neurotrophic omega-3 fatty acids in the brain which in turn upregulates ß-secretase, prevents neurotrophic cleavage of ß-amyloid precursor protein (ßAPP) and increases Aß production [54]. In summary, upregulation of brain miR-125b is associated with glial cell proliferation (gliosis in AD), down-regulates synaptic vesicle-associated neurotransmitter release (synaptic degeneration in AD), conversion of omega fatty acids into neuroprotective DHA (DHA deficits in AD), stimulation of inflammatory response (neuroinflammation in AD), a shift from non-amyloidogenic to amyloidogenic processing of ßAPP leading to excessive Aß production (Aß toxicity at subtle levels in early AD and Aß aggravation at advanced stages of AD).

Observed age-dependent upregulation of miR-125b in untreated 5XFAD Tgs corroborates with previously reported role played by miR-125b in aggravating AD-like changes in 5XFAD mice. Furthermore, the role played by miR-125b in aggravating AD-like neurobehavioral deficits is confirmed and reinforced by currently observed impairment if Y maze spatial reference working memory, and AD-characteristic increased cerebral levels of total Aß, oAß, Tau, Phospho-Tau, TNFα and IL-1ß. Current findings showed successful inhibition of miR-125b after AM-125b-mediated silencing of miR-125b mRNA. Furthermore, AM-125b-mediated silencing of miR-125b mRNA resulted in ameliorating AD-like neurobehavioral deficits. Thus, current finding on silencing miR-125b as a preventive and therapeutic strategy to treat AD, has a great potential of clinical translation.

Table 1: Effect of Silencing miR-125b on Cerebral Levels of total Aß, oAß, Total Tau, Phospho-Tau, TNFα and IL-1ß in 5XFAD mice.

Groups	Total Aß	oAß	Total Tau	Phospho-Tau	TNFα	IL-1ß
Untreated Lts Vs 8-week-old	↑5.2-fold	↑3.8-fold	↑6.4-fold	↑5.8-fold	↑4.1-fold	↑4.7-fold
Untreated Tgs Untreated Lts vs 16-week-old	↑7.6-fold	↑5.5-fold	↑8.6-fold	↑6.3-fold	↑5.7-fold	↑5.6-fold
Untreated Tgs 16-week-old Untreated Tgs vs 16-week-old AM-125b-treated Tgs	↓2.1-fold	↓2.5-fold	↓2.4-fold	↓2.5-fold	↓2.3-fold	↓2.2-fold

Abbreviations: ß-amyloid (Aß), oligomeric ß-amyloid (oAß), tumor necrosis factor alpha (TNFα), interleukin-1beta (IL-1ß), Non-transgenic littermate controls (Lts), Transgenic mice (Tgs), Increase (↑), Decrease (↓).

CONCLUSION

This is one of the lead reports showing therapeutic efficacy of silencing microRNAs using a non-invasive nose-to-brain drug delivery method in ameliorating Alzheimer-like neurocognitive deficits.

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