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Brief communication

Amalaki Rasayana improved memory and neuronal metabolic activity in AβPP-PS1 mouse model of Alzheimer's disease

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Alzheimer's disease (AD) is the most common neurodegenerative disorder characterized by progressive loss of memory and cognitive function. The cerebral metabolic rate of glucose oxidation has been shown to be reduced in AD. The present study evaluated efficacy of dietary Amalaki Rasayana (AR), an Ayurvedic formulation used in Indian traditional system, in AβPP-PS1 mouse model of AD in ameliorating memory and neurometabolism, and compared with donepezil, a standard FDA approved drug for AD. The memory of mice was measured using Morris Water Maze analysis. The cerebral metabolism was followed by ^{13}C labelling of brain amino acids in tissue extracts *ex vivo* using ^{1}H-[^{13}C]-NMR spectroscopy together with a short time infusion of [1,6-$^{13}C_2$]glucose to mice. The intervention with Amalaki Rasayana showed improved learning and memory in AβPP-PS1 mice. The ^{13}C labelings of Glu_{C4}, $GABA_{C2}$ and Gln_{C4} were reduced in AβPP-PS1 mice when compared with wild-type controls. Intervention of AR increased the ^{13}C labelling of amino acids suggesting a significant enhancement in glutamatergic and GABAergic metabolic activity in AβPP-PS1 mice similar to that observed with donepezil treatment. These data suggest that AR has potential to improve memory and cognitive function in AD.

Keywords. Alzheimer's disease; ^{13}C nuclear magnetic; GABA; glutamate; neurotransmitter cycle; resonance spectroscopy

1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder associated with gradual deterioration of cognitive functions, personality and memory (Goedert and Spillantini 2006). The cause and pathogenesis of AD remains complex, and has been shown to be associated with gray matter atrophy, formation of neurofibrillary tangles and disruption of neuronal function in the isocortex (Braak and Braak 1996; Delacourte *et al.* 1999). The disordered degradation of amyloid precursor protein (APP) is believed to be the leading cause of AD (Selkoe 1998; Hardy and Selkoe 2002). Deficit in different neurotransmitters are shown to be increased with progress of disease pathology (Selkoe 1998; Hardy and Selkoe 2002). Analysis in postmortem tissue has suggested 25–35% loss in synapses in AD brain (DeFelipe and Farinas 1992). Metabolic analysis using Positron Emission Tomography has indicated glucose hypometabolism in AD brain (Rabinovici *et al.* 2010). The reduced rates of neuronal glucose oxidation was also shown at early age in AβPP-PS1 mouse model of AD (Tiwari and Patel 2012). Glutamate and GABA are the major excitatory and inhibitory neurotransmitters, respectively, in the mature central nervous system (Mattson and Kater 1989). Majority of brain energy is utilized to sustain the processes associated with glutamate and GABA neurotransmitter pathways (Ottersen and Storm-Mathisen 1986; Schmidt *et al.* 1992).

Although AD was discovered more than a century ago, the definite diagnosis of AD is only possible by detection of β-amyloid plaques and neurofibrillary tangles in postmortem brain tissues. Hence, early diagnosis and sensitive treatment are the major challenges which hamper effective management of disease. Ayurveda, the traditional medicine system of India, is being extensively practiced uninterruptedly at least since the beginning of the Buddhist period in India. In recent time, there has been increased interest in using plant products and traditional remedies for alleviating symptoms of AD and other neural disorders because of the holistic and

generally side-effect free actions of the traditional medicines. Ayurveda claims to facilitate 'healthy aging' and thus has the possibility to alleviate the suffering from neurodegenerative disorders (Lakhotia 2013). Amalaki Rasayana (AR), a preparation derived from Indian gooseberry (*Emblica officinalis*) fruit, has been used as part of the rejuvenating therapy. It is believed that AR promotes long life with enhanced physical and mental strength so that age-related disorders are minimized (Singh *et al.* 2009; Sarkar and Chaudhary 2010). The aging-associated DNA damage in neurons and astrocytes has been shown to be reduced in rats supplemented with AR (Swain *et al.* 2012). The dietary supplementation of AR has been shown to improve life span and stress tolerance in *Drosophila* (Dwivedi *et al.* 2012). Moreover, feeding AR to larvae suppresses neurodegeneration in *Drosophila* models of Alzheimer's and Huntington's diseases without any adverse consequence (Dwivedi *et al.* 2013), suggesting the potential of AR intervention in improvement of memory and energy metabolism in AD condition.

The present study assessed effects of AR supplementation on memory and neurometabolic activities in AβPP-PS1 mice, a humanized transgenic mouse model of AD, and compared with those treated with donepezil, a standard AD drug. AβPP-PS1 mice have been developed by inserting mutants of amyloid precursor protein and presenilin at the single locus under the control of mouse prion promoter (Jankowsky *et al.* 2004). These mice exhibit severe plaque loading in cerebral cortex and hippocampus at the age of 12 months. Our results indicate AR intervention improved memory and neuro-metabolic activity in AβPP-PS1 mice.

2. Materials and methods

All the experimental procedures with mice were approved by the Institutional Animals Ethics Committee of Centre for Cellular and Molecular Biology (CCMB), Hyderabad, India. Since estrous cycle dependent fluctuations in hormonal levels in females may perturb their neuronal activity and cognitive functions (Epperson *et al.* 2002), we used only male AβPP-PS1 (12 months old) and age matched wild-type (WT) male mice in this study. Mice were divided into following six groups: Group A: WT + normal saline (NS, n=4); Group B: AβPP-PS1 + NS (n=4); Group C: WT + AR (n=5); Group D: AβPP-PS1 + AR (n=5); Group E: WT + donepezil (DP, n=4); Group F: AβPP-PS1 + DP (n=5) to assess the effects of different interventions. Mice in Groups C and D received AR (2 g/kg, i.g.), and those in Groups E and F, DP (2 mg/kg, i.p.) between 10 and 11 a.m. for 30 days. Groups A and B mice were administered normal saline for the same period. AR was mixed in NS and administered intra-gastrically, while donepezil was dissolved in NS and delivered intraperitoneally to the animals. AR, prepared following the traditional procedure (Dwivedi *et al.* 2012), was obtained from the Arya Vaidyasala, Kottakal (India).

2.1 *Evaluation of learning and memory in AD mouse*

Learning and memory of animals were evaluated using Morris Water Maze (MWM) test (Vorhees and Williams 2006). Animal performance in MWM has been associated with long-term potentiation and NMDA receptor function (Morris *et al.* 1986, 1998), because of which it is commonly used as an essential test for assessing hippocampal circuitry. A typical MWM consists of a circular tank, which is virtually divided into four equal quadrants with different clues provided on the wall for spatial map of the pool. The pool was filled with water to a depth of 30 cm, and an escape platform was submerged 0.5 cm under water level in the fourth quadrant. In MWM test, mice were trained for 4 days from four different quadrants to locate the platform. The path of movement of animals was video recorded and analyzed by the Ethovision software. Memory of mice was evaluated on 7th and 8th day with and without the platform. The latency time to reach the platform, and frequency of crossing over the platform zone were measured.

2.2 *Infusion of [1,6-$^{13}C_2$]glucose*

Metabolic analysis was carried out by following the ^{13}C labelling of amino acids with an infusion of [1,6-$^{13}C_2$]glucose. For metabolic analysis, mice were fasted for 8–10 h to reduce the endogenous blood glucose level. Animals were anesthetized with urethane (1.5 g/kg, i.p.), and a catheter was placed in tail vein for infusion of ^{13}C-labelled glucose. The core body temperature of mice was maintained at 37°C using a heated pad and temperature regulated water bath. [1,6-$^{13}C_2$]glucose was infused for 10 min in mice using bolus variable infusion rate protocol (Fitzpatrick *et al.* 1990; Tiwari *et al.* 2013). Blood was collected from the retro-orbital sinus artery during the last minute of the experiment, and centrifuged to separate plasma. At the end of the experiment, animal head was frozen with liquid nitrogen.

2.3 *Preparation of brain extract*

Brain was removed from head under frozen condition, and dissected at −20°C to isolate the cerebral cortex, hippocampus and striatum. Cerebral metabolites were extracted from frozen tissue using the protocol described previously (Patel *et al.* 2001). The frozen weighed tissues were powdered with 0.1 N HCl in methanol (1:2 w/v) in a dry ice/

ethanol bath. [2-^{13}C]Glycine (0.1 μmol) was added for concentration reference. The powdered tissue was homogenized with ethanol, and centrifuged at 20,000*g*. The supernatant was lyophilized, and powder dissolved in deuterium oxide containing sodium 3-trimethylsilyl[2,2,3,3-D4]-propionate (TSP) for NMR analysis.

2.4 *NMR analysis of brain extract and plasma*

^{1}H-[^{13}C]-NMR spectra of brain extracts were recorded at 600 MHz (Bruker Biospin, Germany) spectrometer (Fitzpatrick *et al.* 1990; de Graaf *et al.* 2003). The concentrations of brain metabolites were determined relative to [2-^{13}C]glycine added during extract preparation. The percentage ^{13}C enrichment of different brain metabolites was determined as the ratio of the peak areas in the ^{1}H-[^{13}C]-NMR difference spectrum ($2\times{}^{13}$C only) to the non-edited spectrum (^{12}C+^{13}C), and was corrected for the natural abundance (1.1%) of ^{13}C.

Blood plasma was mixed with deuterium oxide containing sodium formate, and passed through a centrifugal filter to remove macromolecules. The concentrations and ^{13}C labelling of plasma glucose were measured using ^{1}H NMR spectroscopy using formate as reference. The percent ^{13}C labelling of glucose-C1α centered at 5.2 ppm was calculated by dividing the intensity of the ^{13}C with the total (^{12}C+^{13}C).

2.5 *Determination of cerebral metabolic rate of glucose oxidation*

The cerebral metabolic rates of glucose oxidation were estimated from ^{13}C labelling of brain amino acids from a short time (10 min) infusion of [1,6-$^{13}C_2$]glucose. The metabolic rates of glucose oxidation by different cell types were estimated by accounting the ^{13}C label trapped into different amino acids from [1,6-$^{13}C_2$]glucose (Patel *et al.* 2005). The cerebral metabolic rate of glucose oxidation is calculated as following:

$$CMR_{Glc(Ox)} = (1/10) \times \{[Glu](Glu_{C4} + 2Glu_{C3}) + [GABA](GABA_{C2} + GABA_{C2}) + [Asp](2Asp_{C3})\} \quad (1)$$

where Glu_{C4}, Glu_{C3}, $GABA_{C2}$, $GABA_{C3}$, and Asp_{C3} represent fractional ^{13}C enrichment of glutamate, GABA and aspartate at specific carbon position.

The cerebral metabolic rate of glucose oxidation by glutamatergic neurons is determined by:

$$CMR_{Glc(Glu)} = (1/10) \times \{0.82[Glu](Glu_{C4} + 2Glu_{C3}) + 0.42[Asp](2Asp_{C3})\} \quad (2)$$

The cerebral metabolic rate of glucose oxidation by GABAergic neurons is calculated as following:

$$CMR_{Glc(GABA)} = (1/10) \times \{0.02[Glu](Glu_{C4} + 2Glu_{C3}) + [GABA](GABA_{C2} + GABA_{C2}) + 0.42[Asp](2Asp_{C3})\} \quad (3)$$

2.6 *Statistics*

One-way ANOVA was carried out to find the significance of differences in the concentrations and metabolic rates among different groups. The *post hoc* Tukey honest test was performed to identify the significance of difference between groups.

3. Results

3.1 *Effects of Amalaki Rasayana and donepezil on learning and memory*

Learning and memory in mice were assessed using Morris Water Maze test. Wild-type (WT) mice showed a good learning pattern during 4 days of training but AβPP-PS1 mice treated with normal saline could not locate the platform even after 4 days of intense training (figure 1b). Memory tests carried out two days after the training revealed that wild-type mice had good memory retention and could locate the platform in 47±11 s. In contrast, the escape latency in AβPP-PS1 mice was more than 90 s suggesting impaired memory in these mice (figure 1d). Interestingly, AβPP-PS1 mice treated with Amalaki Rasayana (AR) learnt to locate the platform during the training period (figure 1c), and reached the hidden platform in ∼65 s on the 4th day of training. The AβPP-PS1 mice treated with donepezil could locate the platform in ∼51 s (data not shown).

Memory tests performed on 7th day revealed that AβPP-PS1 mice treated with normal saline could not locate the platform during 90 s of test period, while those treated with AR could locate the platform in ∼66±7 s (figure 1d). The AβPP-PS1 mice treated with donepezil also showed improved memory, and reached the platform in ∼42±11 s (figure 1d). Memory retention analysis revealed that wild-type mice treated with either AR or donepezil or normal saline crossed the platform zone three times during the 90 s of test period (figure 1e). In contrast, AβPP-PS1 mice treated with normal saline did not make any cross over the platform zone. Interestingly, treatment with AR (∼1 cross) and donepezil (∼2 cross) increased the crossing frequency of AβPP-PS1 mice (figure 1e).

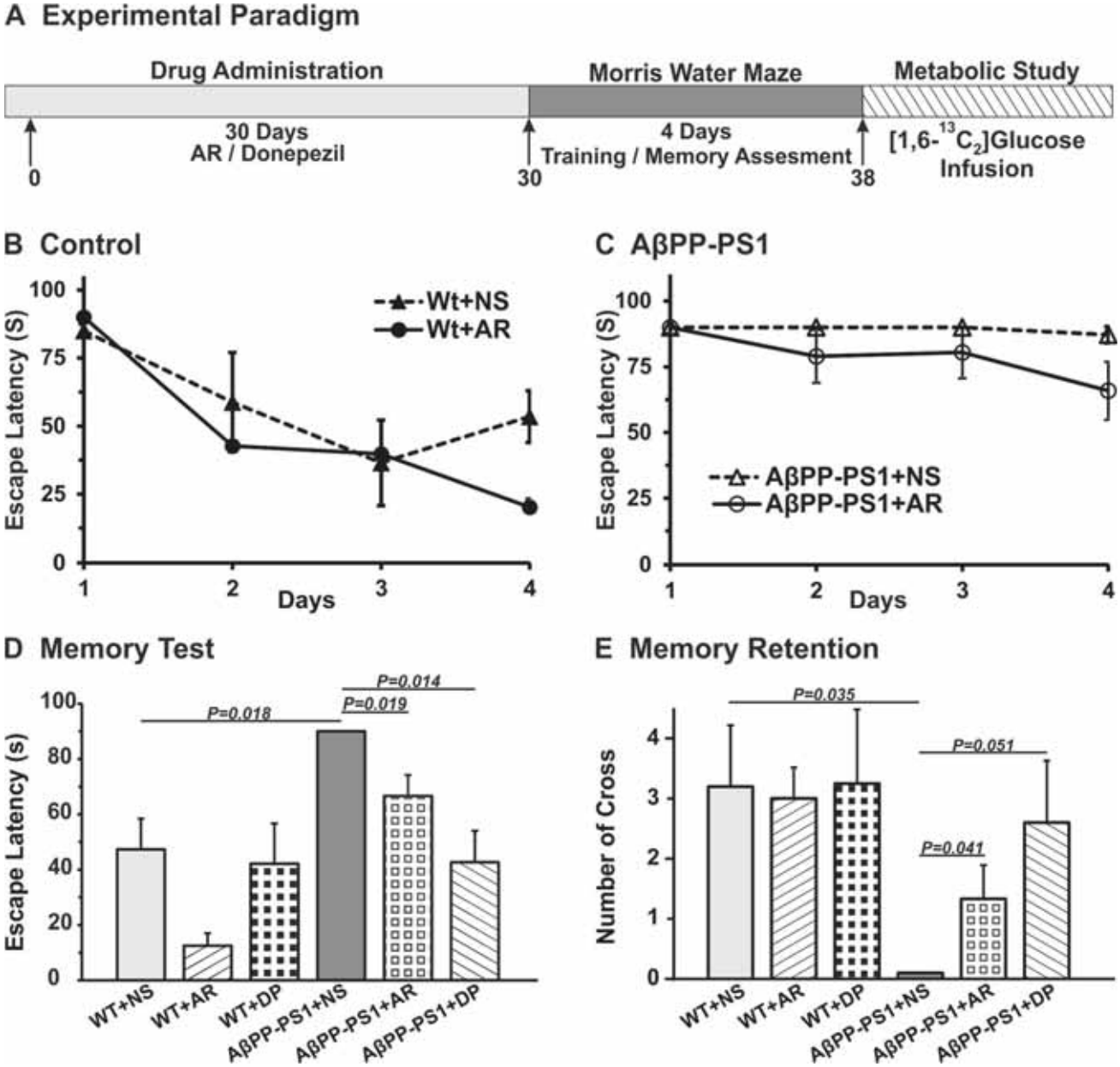

Figure 1. **a** Schematic depiction of timeline for different interventions. Learning of Control (WT) and AβPP-PS1 mice after **b** normal saline, **c** AR interventions. **d** Memory (escape latency to locate the platform), and **e** Retention of memory (frequency of crossing to platform zone) in AβPP-PS1 and WT mice after different interventions. Learning and memory were assessed using MWM test. The statistical significance for difference between groups was determined by two tailed Student's *t* test.

3.2 *Effects of AR and donepezil interventions on brain energy metabolism in AβPP-PS1 mice*

In order to evaluate effects of AR and donepezil interventions on brain energy metabolism, ^{13}C labelling of brain metabolites from [1,6-$^{13}C_2$]glucose was measured following 10 min infusion experiment. A typical ^{1}H-[^{13}C]-NMR spectrum depicting the ^{13}C labelling of hippocampal metabolites in AβPP-PS1 is presented in figure 2. The ^{13}C labelling of Glu_{C4}, $GABA_{C2}$ and Gln_{C4} could be seen. The concentration of these amino acids was quantified relative to [2-^{13}C]glycine added during the extraction.

The ^{13}C concentrations of hippocampal Glu_{C4} (WT+NS 1.68±0.14 μmol/g, AβPP-PS1+NS 0.94±0.08 μmol/g), $GABA_{C2}$ (WT+NS 0.23±0.01 μmol/g, AβPP-PS1+NS 0.15±0.02 μmol/g), and Gln_{C4} (WT+NS 0.23±0.04 μmol/g, AβPP-PS1+NS 0.14±0.02 μmol/g) were found to be significantly ($p \leq 0.015$) lower in the AβPP-PS1 mice as compared

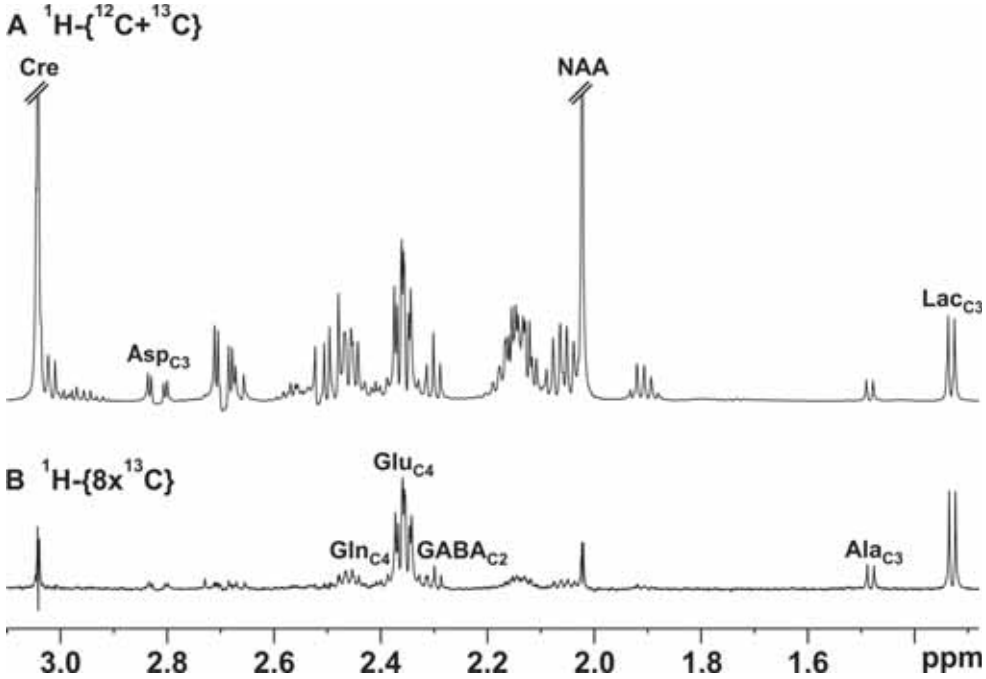

Figure 2. ^{1}H-[^{13}C]-NMR spectrum depicting ^{13}C labelling of hippocampal amino acids from [1,6-$^{13}C_2$]glucose in AβPP-PS1 mice. Mice were infused with [1,6-$^{13}C_2$]glucose for 10 min. Metabolites were extracted from frozen hippocampal tissue, and ^{1}H-[^{13}C]-NMR spectrum of hippocampal extracts was recorded at 600 MHz NMR spectrometer.

with controls (table 1). The AR treatment in AβPP-PS1 mice increased the ^{13}C labelling of Glu_{C4} (1.36±0.12 μmol/g) significantly (p<0.01) as compared with the normal saline treated mice (0.94±0.08 μmol/g). Additionally, there was significant (p<0.05) increase in labelling of $GABA_{C2}$ in AβPP-PS1 mice treated with AR (0.22±0.04 μmol/g) as compared with the normal saline treated mice (0.15±0.2 μmol/g). The labelling of Gln_{C4} from [1,6-$^{13}C_2$]glucose, which is an indicator of neurotransmitter cycling flux, also increased significantly (p<0.01) in AβPP-PS1 mice upon AR treatment (AβPP-PS1+NS 0.14±0.02 μmol/g; AβPP-PS1+AR 0.20±0.03 μmol/g). Moreover, Gln_{C4} labelling in AβPP-PS1 mice treated with AR is not significantly (p=0.74) different than the controls mice (0.23±0.04 μmol/g). These data suggest that AR improves energy metabolism in hippocampal region of AβPP-PS1 mice.

The AR intervention in AβPP-PS1 mice increased the cortical ^{13}C labelling of Glu_{C4} (AβPP-PS1+AR 1.48±0.09 μmol/g, WT+NS 2.06±0.18 μmol/g; p<0.5) as compared with the normal saline treated AβPP-PS1 (1.16±0.03 μmol/g). However, there was no significant (p=0.63) increase in $GABA_{C2}$ labelling in AβPP-PS1 mice treated with AR (0.14±0.02 μmol/g) when compared with the normal saline treated AβPP-PS1 mice (0.12±0.02 μmol/g). The labelling of Gln_{C4} in AβPP-PS1 was increased upon AR intervention (0.17±0.02 μmol/g) as compared with the normal saline treated (0.13±0.02 μmol/g) AβPP-PS1 mice. Similar results were observed in striatal regions (table 1).

Donepezil intervention in AβPP-PS1 mice increased the labelling of hippocampal Glu_{C4} (1.39±0.15 vs. 0.94±0.08 μmol/g), $GABA_{C2}$ (0.21±0.05 vs. 0.15±0.02 μmol/g), and Gln_{C4} (0.20±0.02 vs. 0.14±0.02 μmol/g) when compared with normal saline-treated AβPP-PS1. Moreover, donepezil intervention increased the ^{13}C labelling of amino acids in the striatum of AβPP-PS1 mice. The effects of donepezil on the cortical metabolism were minimal.

3.3 *Cerebral metabolic rate of glucose oxidation upon AR and donepezil intervention*

AβPP-PS1 mice exhibited reduced $CMR_{Glc(Ox)}$ in hippocampal, and striatal regions (figure 3). The AR intervention led to significant increase in $CMR_{Glc(Glu)}$ (AβPP-PS1+NS 0.113±0.007; AβPP-PS1+AR 0.143±0.009 μmol/g/min, p<0.01), and $CMR_{Glc(GABA)}$ (AβPP-PS1+NS 0.022±0.004; AβPP-PS1+AR 0.026±0.003 μmol/g/min, p<0.05) in hippocampal region of AβPP-PS1 mice when compared with NS treated AβPP-PS1 mice (figure 3a). Moreover, striatal region showed significant increase in oxidative glucose metabolism of glutamatergic and GABAergic neurons with AR intervention (figure 3b). It is noteworthy that AR intervention did not affect CMR_{Glc} in

Table 1. Concentration (μmol/g) of ^{13}C-labelled amino acids from [1,6-$^{13}C_2$]glucose in AR and donepezil-treated mice

Brain region	Treatment group	Glu_{C4}	$GABA_{C2}$	Gln_{C4}
Cerebral Cortex	Control + NS	2.06 ± 0.18	0.20 ± 0.02	0.25 ± 0.02
	AβPP-PS1 + NS	1.15 ± 0.09**	0.12 ± 0.02**	0.13 ± 0.02**
	Control + AR	2.05 ± 0.12	0.17 ± 0.03	0.23 ± 0.05
	AβPP-PS1 + AR	1.48 ± 0.09$^{\#}$	0.14 ± 0.02	0.17 ± 0.02
	Control + DP	1.70 ± 0.22	0.16 ± 0.03	0.19 ± 0.01
	AβPP-PS1 + DP	1.04 ± 0.16	0.09 ± 0.02	0.05 ± 0.02$^{\$}$
Hippocampus	Control + NS	1.68 ± 0.14	0.23 ± 0.01	0.23 ± 0.04
	AβPP-PS1 + NS	0.94 ± 0.08**	0.15 ± 0.02*	0.14 ± 0.02**
	Control + AR	1.78 ± 0.12	0.30 ± 0.02	0.26 ± 0.02
	AβPP-PS1 + AR	1.36 ± 0.12$^{\#\#}$	0.22 ± 0.04$^{\#}$	0.20 ± 0.03$^{\#\#}$
	Control + DP	1.66 ± 0.14	0.25 ± 0.05	0.24 ± 0.02
	AβPP-PS1 + DP	1.39 ± 0.15$^{\$\$}$	0.21 ± 0.05	0.22 ± 0.02$^{\$\$}$
Striatum	Control + NS	1.77 ± 0.08	0.33 ± 0.04	0.27 ± 0.01
	AβPP-PS1 + NS	1.16 ± 0.05**	0.20 ± 0.04*	0.18 ± 0.03*
	Control + AR	1.83 ± 0.08	0.32 ± 0.02	0.27 ± 0.01
	AβPP-PS1 + AR	1.44 ± 0.13$^{\#}$	0.22 ± 0.03	0.20 ± 0.03
	Control + DP	1.87 ± 0.10	0.28 ± 0.08	0.24 ± 0.06
	AβPP-PS1 + DP	1.48 ± 0.17$^{\$\$}$	0.27 ± 0.09	0.20 ± 0.05

Mice were infused with [1,6-$^{13}C_2$]glucose for 10 min. The concentrations of ^{13}C-labelled amino acids were measured in tissue extracts in ^{13}C edited spectrum using glycine as standard. Values are presented as mean ± SD

AR Amalaki rasayana, *DP* donepezil, *NS* normal saline

*$p< 0.05$; **$p< 0.01$ when AβPP-PS1 + NS mice were compared with Control + NS
$^{\#}p < 0.05$; $^{\#\#}p< 0.01$ when AβPP-PS1 + AR group was compared with AβPP-PS1 + NS
$^{\$}p< 0.05$; $^{\$\$}p< 0.01$ when AβPP-PS1 + DP group was compared with AβPP-PS1 + NS

control mice. Donepezil intervention in AβPP-PS1 mice also improved neuronal glucose oxidation associated with glutamatergic and GABAergic neurons in hippocampus, cerebral cortex and striatum when compared with NS treated AβPP-PS1 mice (figure 3).

4. Discussion

Present study evaluates the potential of Amalaki Rasayana for alleviation of AD symptoms, and compares its efficacy with donepezil, the standard AD drug. The data presented here indicate that neuronal function is decreased in AD mice at the stage of high plaque loading. Most interestingly, AR medication improved memory and brain energy metabolism in AD mice much like that seen with donepezil.

Biologically active compounds from natural sources have always been of great interest for researchers around the world. Following an earlier report (Dwivedi *et al.* 2013) that AR can substantially suppress AD symptoms in the *Drosophila* model, we examined its efficacy in the mouse AD model, and compared it with donepezil, a cholinesterase inhibitor (Birks and Harvey 2006; Hansen *et al.* 2008) and the first line AD drug approved by FDA. A most interesting finding of present study is that, like donepezil, AR intervention enhanced learning and memory in AβPP-PS1 mice, and significantly increased oxidative glucose metabolism of glutamatergic and GABAergic neurons in hippocampal region without any detectable side-effects. The $CMR_{Glc(Glu)}$ improved towards control values upon AR as well as donepezil intervention in AβPP-PS1 mice across brain, suggesting that AR improved the function of excitatory neurons. Though AR treatment in AβPP-PS1 did not increase $CMR_{Glc(GABA)}$ in the cortical region, it improved $CMR_{Glc(GABA)}$ in hippocampus region. Moreover, AR treatment in AβPP-PS1 mice increased glutamine labelling in AβPP-PS1 mice suggesting that AR intervention enhanced neurotransmission in AD. Since the neuronal glucose oxidation and neurotransmitter cycling rates are shown to be stoichiometrically coupled (Sibson *et al.* 1998; Patel *et al.* 2004; Hyder *et al.* 2006), the improved neurometabolism in AβPP-PS1 mice following AR intervention is suggestive of enhanced synaptic transmission, which explains the improved memory in AβPP-PS1 mice.

Our observations on alleviation of AD symptoms in the mouse model following AR supplement are in agreement with an earlier report (Dwivedi *et al.* 2013) that dietary supplementation of AR substantially suppressed neurodegeneration in Huntington disease (HD) and AD fly models. Dietary supplement of AR in fly model is known to prolong life span and to improve tolerance to thermal, starvation, crowding and oxidative stresses (Dwivedi *et al.* 2012,

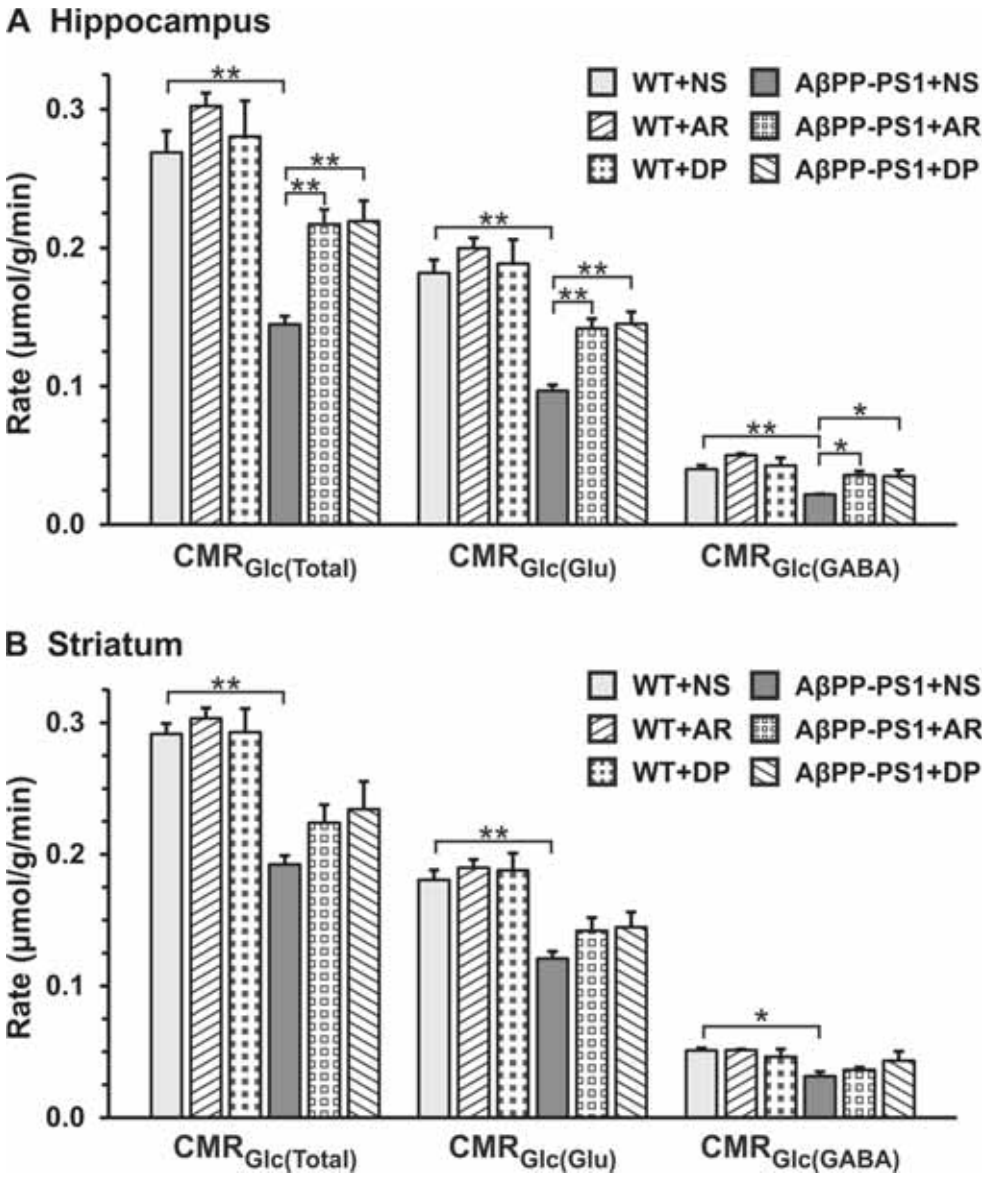

Figure 3. Cerebral metabolic rate of glucose oxidation ($CMR_{Glc(Ox)}$) oxidation in **a** hippocampus; **b** striatum of WT and AβPP-PS1 mice upon different interventions. The $CMR_{Glc(ox)}$ associated with glutamatergic and GABAergic neurons was calculated using Eqs. 2 and 3, respectively. Values represent Mean ± SD. *$p < 0.05$; **$p < 0.01$.

Dwivedi and Lakhotia 2016). AR supplement has also been shown to significantly reduce the accumulation of inclusion bodies/neurofibrillary tangles in fly models of HD and AD disorders (Dwivedi *et al.* 2013). The Amalaki (Phyllanthus e*mblica*) fruits are known to be rich in antioxidants (Carlsen *et al.* 2010), and therefore, as shown recently *in vivo* fly model, AR supplement permits better management of oxidative stress (Dwivedi and Lakhotia 2016), which is one of the major factors in aging as well as AD pathology (Butterfield *et al.* 2001). AR supplement seems to improve physiology and homeostasis in the fly model through enhanced levels of key regulatory molecules like different hnRNPs and CBP300 (histone acetyl transferase), better proteasomal activity and improved oxidative stress tolerance (Dwivedi *et al.* 2012, 2013; Dwivedi and Lakhotia 2016). Furthermore, it has been found that apoptotic cell death induced by various environmental insults, including the accumulation of inclusion bodies in HD and AD conditions, is largely suppressed by AR intervention in flies (Dwivedi et al 2015). This is also likely to contribute to the restoration of memory and learning by preventing neuronal loss in AD brain. AR-administration has been shown to reduce aging

induced DNA damage in neurons and astrocytes in rats (Swain *et al*. 2012), which may also provide improved brain functions. Together, these data suggest that AR has a strong potential to manage memory and cognitive functions in AD condition. In order to understand the mechanism of action of AR in APP-PS1 mice, future studies would assess the level of Aβ1-40/Aβ1-42 peptides, amyloid plaques/neurofirbillary tangles and oxidative stress in AR treated mice.

5. Conclusion

Metabolic analysis indicates neuronal glucose oxidation was impaired across the brain in AβPP-PS1 mice. The most interesting finding of the present study is that intervention with the Amalaki Rasayana improved memory and neurometabolic activity in AβPP-PS1 mice nearly comparable to that of donepezil treated AβPP-PS1 mice, suggesting that Amalaki Rasayana has a good potential to improve cognitive functions in AD.

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