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ScienceDirect
Nutrition Research 29 (2009) 70 – 74
Nutrition Research
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Dietary supplementation with a combination of α-lipoic acid, acetyl-L-carnitine, glycerophosphocoline, docosahexaenoic acid, and phosphatidylserine reduces oxidative damage to murine brain and improves cognitive performance

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Received 6 October 2008; revised 26 November 2008; accepted 26 November 2008

Abstract

Alzheimer disease has a complex etiology composed of nutritional and genetic risk factors and predispositions. Moreover, genetic risk factors for cognitive decline may remain latent pending age-related decline in nutrition, suggesting the potential importance of early nutritional intervention, including preventative approaches. We hypothesized that a combination of multiple nutritional additives may be able to provide neuroprotection. We demonstrate herein that dietary supplementation with a mixture of ALA, ALCAR, GPC, DHA, and PS reduced reactive oxygen species in normal mice by 57% and prevented the increase in reactive oxygen species normally observed in mice lacking murine ApoE when maintained on a vitamin-free, iron-enriched, oxidative-challenge diet. We further demonstrate that supplementation with these agents prevented the marked cognitive decline otherwise observed in normal mice maintained on this challenge diet. These findings add to the growing body of research indicating that key dietary supplementation may delay the progression of age-related cognitive decline.

Keywords: Nutritional supplement; Cognitive performance; Oxidative stress; Aging; Apolipoprotein E; Mouse

Abbreviations: AD, Alzheimer disease; ALA, α-lipoic acid; ALCAR, acetyl-L-carnitine; ApoE, apolipoprotein E; DHA, docosahexaenoic acid; GPC, glycerophosphocoline; PS, phosphatidylserine; TBARs, thiobarbituric acid reactive substances.

1. Introduction

A growing body of research indicates that nutritional deficiencies contribute to age-related cognitive decline, including that which accompanies Alzheimer disease (AD). Moreover, genetic risk factors for cognitive decline may remain latent pending age-related decline in nutrient intake [1-4]. Controlled studies with mice have demonstrated that cognitive performance is subject to dietary compromise [5] and that key dietary supplementation can alleviate and in some cases reverse the impact of dietary deficiencies on cognitive performance [6-8]. This suggests the potential importance of early nutritional intervention, including preventative approaches before definitive diagnosis [1,4].

Oxidative stress is a pivotal factor in AD and is evident before cytopathologic hallmarks of the disorder [9]. Antioxidants therefore represent a potential preventative approach [3,10,11]. The beneficial effects of nutritional supplementation, especially that of combinatorial supplementation, are supported by a number of preclinical and clinical studies [8,12-14]. Of interest, therefore, was to

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doi:10.1016/j.nutres.2008.11.004

Table 1
Composition of diets fed to mice for 4 weeks

Ingredients	Dietary groups			
	Complete	Complete + supplements	Deficient	Deficient + supplements
Treatment supplements				
ALA (mg/kg)	0	200	0	200
ALCAR (mg/kg)	0	500	0	500
DHA (g/kg)	0	6	0	6
GPC (mg/kg)	0	100	0	100
PS (mg/kg)	0	50	0	50
Vitamins				
Vitamin A (IU/g)	22.1	22.1	22.1	22.1
Vitamin D (IU/g)	2.2	2.2	2.2	2.2
Vitamin E (IU/g)	50.1	50.1	3	3
Vitamin K (ppm)	10.4	10.4	10.4	10.4
Thiamin HCl (ppm)	18	18	18	18
Riboflavin (ppm)	20	20	20	20
Niacin (ppm)	90	90	90	90
Pantothenic acid (ppm)	55	55	55	55
Folic acid (ppm)	4	4	0	0
Pyridoxine (ppm)	16.5	16.5	16.5	16.5
Biotin (ppm)	0.4	0.4	0.4	0.4
Vitamin B12 (μg/kg)	20	20	20	20
Choline chloride (ppm)	1400	1400	1400	1400
Minerals				
Calcium (%)	0.6	0.6	0.6	0.6
Phosphorus (%)	0.57	0.57	0.57	0.57
Potassium (%)	0.4	0.4	0.4	0.4
Magnesium (%)	0.07	0.07	0.07	0.07
Sodium (%)	0.24	0.24	0.24	0.24
Chloride (%)	0.3	0.3	0.3	0.3
Fluorine (ppm)	5	5	5	5
Iron (ppm)	3	3	188	188
Zinc (ppm)	27	27	27	27
Manganese (ppm)	65	65	65	65
Copper (ppm)	15	15	15	15
Cobalt (ppm)	3.2	3.2	3.2	3.2
Iodine (ppm)	0.57	0.57	0.57	0.57
Chromium (ppm)	3	3	3	3
Molybdenum (ppm)	0.82	0.82	0.82	0.82
Selenium (ppm)	0.23	0.23	0.23	0.23
Protein (%)				
Arginine	0.73	0.73	0.73	0.73
Histidine	0.54	0.54	0.54	0.54
Isoleucine	1	1	1	1
Leucine	1.82	1.82	1.82	1.82
Lysine	1.53	1.53	1.53	1.53
Methionine	0.69	0.69	0.69	0.69
Cystine	0.08	0.08	0.08	0.08
Phenylalanine	1	1	1	1
Tyrosine	1.06	1.06	1.06	1.06
Threonine	0.81	0.81	0.81	0.81
Tryptophan	0.23	0.23	0.23	0.23
Valine	1.2	1.2	1.2	1.2
Alanine	0.58	0.58	0.58	0.58
Aspartic acid	1.35	1.35	1.35	1.35
Glutamic acid	4.29	4.29	4.29	4.29
Glycine	0.41	0.41	0.41	0.41
Proline	2.47	2.47	2.47	2.47
Serine	1.16	1.16	1.16	1.16
Fat (ppm)				
Linoleic acid	3.27	3.27	3.27	3.27
Linolenic acid	0.07	0.07	0.07	0.07
Arachidonic acid	0.02	0.02	0.02	0.02
Omega 3 fatty acids	0.07	0.07	0.07	0.07
Total saturated fatty acids	2.77	2.77	2.77	2.77
Total monosaturated fatty acids	3.52	3.52	3.52	3.52
Polyunsaturated fatty acids	3.46	3.46	3.46	3.46

Note specific differences in folate, vitamin E, and iron content in our standard complete vs deficient diets [32]; treatment supplements: ALA, ALCAR, GPC, DHA, and PS were added to both the complete and deficient diets as indicated.

investigate the efficacy of additional combinatorial formulations on oxidative damage and cognitive function.

Laboratory studies demonstrate that the energetic cofactor ALA and ALCAR ameliorate mitochondrial aging in rats and may maintain cognitive performance [15,16]. Acetyl-L-carnitine, GPC, DHA, and PS provide mitochondrial support, modulate age-related changes in brain, and can maintain cholinergic receptors during aging [17-20]. In addition to the above preclinical investigations, clinical studies demonstrate that these agents provide cognitive and/or behavioral benefits in AD [21-29]. Our prior preclinical [5-8,12] and clinical [13,14] studies indicate that superior neuroprotection can be achieved by administration of multiple agents with overlapping and complimentary function vs that achieved by individual agents. We therefore hypothesized that treatment with a cocktail of the above agents (ALA, ALCAR, GPC, DHA, and PS) may exert superior neuroprotection than has been observed with any individual agents. We further hypothesized that this may be reflected by beneficial impact upon cognitive performance and levels of reactive oxidative species in normal mice and mice lacking murine ApoE (ApoE−/−mice), which display increased reactive oxidative species and are a useful model for age-related oxidative damage including that in AD [30-33].

2. Methods and materials

Three groups of 3 adult (9-12 months of age) C57B/6 mice from our colony that either express (“normal”) or lack murine ApoE (ApoE−/− mice) received a diet (AIN-76; Purina, St. Louis, MO/Mother Hubbard, Inc., Chelmsford, MA) either lacking folate and vitamin E (defined as the *deficient diet*) or supplemented with folic acid (4 mg/kg) and vitamin E (50 IU/kg total diet wet weight) (defined as the *complete diet*). The deficient diet was also supplemented in all cases with iron (50 g/500 g total diet) as a prooxidant [5,33,34]. Additional groups of mice received the above complete or deficient diets supplemented with 200 mg ALA, 500 mg ALCAR, 6 g DHA, 100 mg GPC, and 50 mg PS per kg of total diet dry weight; for simplicity of writing, this

combination of supplements is referred to as the *formulation* (Table 1). α-Lipoic acid and DHA were obtained from Suan Farma (Hackensack, NJ); ALA was obtained from Aceto Co (Lake Success, NY), and GPC and PS were obtained from Chemi Nutra (White Bear Lake, MN). Complete and deficient diets were prepared with and without these supplements by TestDiet (Richmond, IN).

Before and after the above feeding regimen, mice were analyzed in a standard Y maze described previously [5,7,8]. The pattern of exploration of the Y maze was recorded over 5-minute intervals; and the percentage of alternations was determined, which was defined as the *frequency* in which mice visited each of the 3 arms during any 3-arm visitation sequence.

After Y maze testing, mice were killed by cervical dislocation; and the frontal portion of the brain (encompassing cortex and hippocampus) was immediately removed and frozen for subsequent analyses. As in prior studies [33], oxidative species were monitored using a commercial kit to quantify TBARs as an index of total oxidative species in brain tissue. Thiobarbituric acid reactive substances were quantified in homogenates of frontal cortex using an assay kit according to the manufacturer's instructions (Cell Biolabs, San Diego, CA). Frontal cortex was homogenized in 50 mmol/L Tris containing 150 mmol/L NaCl and 1% Triton-X, supplemented with the EDTA-free Protease Inhibitor cocktail (Roche, Basel, Switzerland), and centrifuged (15 000*g* at 15 minutes); and the resulting supernatant was incubated for 60 minutes at 95°C using the supplied sodium dodecyl sulfate–lysis solution and TBA reagent. The TBARs were quantified spectrophotometrically at 532 nm. This regimen was completed 3 times, for a total of 9 mice per diet. All animal procedures were approved by the Institutional Animal Care and Use Committee.

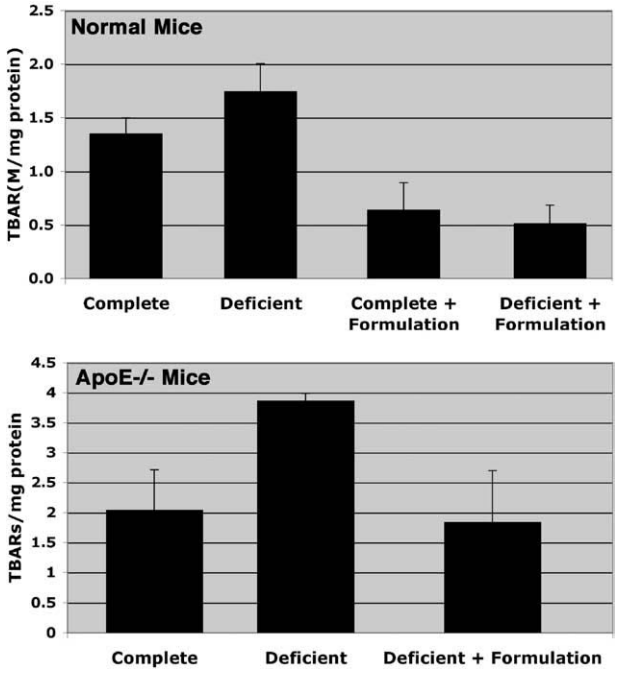

Fig. 1. Supplementation with a combination of ALA, ALCAR, GPC, DHA, and PS reduces oxidative species in brain tissue. Normal and ApoE−/− mice were maintained for 1 month on the indicated diets, after which brain tissue was examined for TBARs as described in "Methods and materials." Maintenance on the deficient diet increased TBARs in both mouse genotypes, with a more severe increase in ApoE−/− mice. Supplementation with a combination of ALA, ALCAR, GPC, DHA, and PS (treatment supplements) prevented these increases ($P < .05$) and furthermore significantly ($P < .01$) reduced basal levels of reactive oxygen species in normal mice. Values represent the means ± standard error of the mean for 3 groups of 3 mice of each genotype.

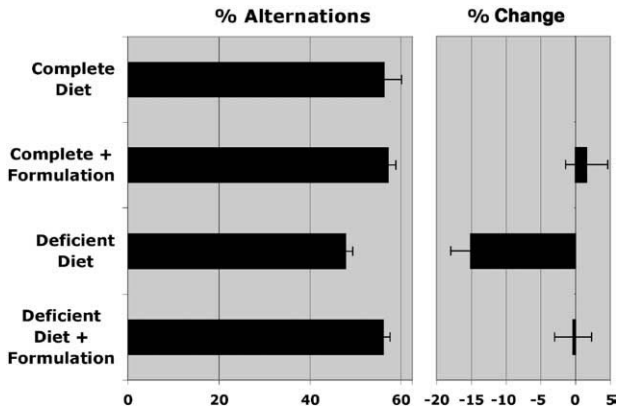

Fig. 2. Supplementation with a combination of ALA, ALCAR, GPC, DHA, and PS supports cognitive performance. Normal mice were maintained for 1 month on the indicated diets and subjected to Y maze analyses as described in "Methods and materials." Maintenance on the deficient diet induced a 15.1% ± 3% decrease in successful alternations, whereas supplementation with a combination of ALA, ALCAR, GPC, DHA, and PS (*formulation*) prevented this decrease. Values represent the means ± standard error of the mean for 3 groups of 3 mice.

3. Results and discussion

Consistent with prior studies, maintenance of normal mice on the challenge diet only slightly increased oxidative species in brain tissue; however, supplementation with the combinatorial formulation prevented this increase and significantly ($P < .01$, 2-tailed t test) reduced levels of oxidative species in brain tissue of mice maintained under either the complete or the deficient diet (Fig. 1). As shown previously [8], ApoE−/− mice displayed a larger increase when maintained on the deficient diet than did normal mice; however, supplementation with the formulation prevented the increase for ApoE−/− mice as well (Fig. 1).

Supplementation with this formulation also affected cognitive performance. Maintenance of normal mice on the deficient diet alone induced a moderate (15%) decline in performance that exhibited a trend toward significance ($P < .10$, 1-tailed t test vs performance on the complete diet) [5]. However, supplementation with the formulation prevented this decline (Fig. 2). By contrast, supplementation was not capable of preventing the decline in cognitive performance in ApoE−/− mice maintained on the deficient diet; ApoE−/−

mice on the deficient diet declined in percentage of successful alternations by 14.7% ± 2%, whereas those on the supplemented deficient diet declined by 15.5% ± 2%.

Based on prior preclinical and clinical studies, we tested the efficacy of a combination of ALA, ALCAR, GPC, DHA, and PS on oxidative damage to brain tissue and cognitive function in normal and ApoE−/− mice. To accomplish this, we subjected some mice to our vitamin-deficient, iron-enriched, oxidative-challenge diet, which we have shown to increase brain oxidative species and reduce cognitive performance [5,7,8,33]. Herein, we observed that this formulation prevented the increase in brain oxidative species that otherwise accompanies maintenance of mice on this challenge diet and furthermore reduced basal levels of oxidative species in normal mice.

This formulation prevented the decline in cognitive performance observed after maintenance of normal mice, but not ApoE−/− mice, on the deficient diet. This limitation underscores that dietary supplementation may be useful to delay the aging process but cannot necessarily be expected to compensate for all aspects of genetic predisposition toward neurodegeneration [2,35].

We have not examined the complete mechanism(s) by which these supplements provided the degree of neuroprotection observed herein. In addition to providing potential antioxidant protection to cell membranes and mitochondria [15-20], a portion of the efficacy of these agents may be due to membrane generation because DHA enzymatically combined with GPC and PS to form membrane phospholipids [36], whereas PS administration alone enhanced learning [37]. Notably, preclinical studies demonstrate that some of these compounds provide beneficial effects against hallmarks of AD; for example, ALA and ALCAR each provide neuroprotection against Abeta toxicity [38,39], whereas PS reduces levels of phospho-τ [40]. Further studies on the efficacy of these constituents are warranted.

Acknowledgment

This research was supported by Memacin, Inc.

References

[1] Burgener SC, Buettner L, Coen Buckwlater K, Beattie E, Bossen AL, Fick DM, et al. Evidence supporting nutritional interventions for persons in early-stage Alzheimer's disease. J Nutr Health Aging 2008; 12:18-21.

[2] Fusco D, Colloca G, Lo Monaco MR, Cesari M. Effects of antioxidant supplementation on the aging process. Clin Interv Aging 2007;2: 377-87.

[3] Mattson MM, Shea TB. Folate and homocysteine in neural plasticity and neurodegenerative disorders. Trends Neurosci 2002;26:137-46.

[4] Panza F, Solfrizzi V, Colacicco AM, D'Introno A, Capurso C, Torres F, et al. Mediterranean diet and cognitive decline. Public Health Nutr 2004;7:959-63.

[5] Mihalick SM, Ortiz D, Kumar R, Rogers E, Shea TB. Folate and vitamin E deficiency impair cognitive performance in mice subjected to oxidative stress: differential impact on normal mice and mice lacking apolipoprotein E. Neuromol Med 2004;4:197-202.

[6] Janson M. Orthomolecular medicine: the therapeutic use of dietary supplements for anti-aging. Clin Interv Aging 2006;1(3):261-5.

[7] Chan A, Shea TB. Supplementation with apple juice attenuates presenilin-1 overexpression during dietary and genetically-induced oxidative stress. J Alzheimers Dis 2006;10:353-8.

[8] Chan A, Tchantchou F, Graves V, Rozen R, Shea TB. Dietary and genetic compromise in folate availability reduces acetylcholine and cognitive performance: critical role of *S*-adenosylmethionine. J Health Nutr Aging 2008;12:252-61.

[9] Zhu X, Su B, Wang X, Smith MA, Perry G. Causes of oxidative stress in Alzheimer disease. Cell Mol Life Sci 2007;64:2202-10.

[10] Frank B, Gupta S. A review of antioxidants and Alzheimer's disease. Ann Clin Psychiatry 2005;17:269-86.

[11] Joseph JA, Denisova NA, Bielinski D, Fisher DR, Shukitt-Hale B. Oxidative stress protection and vulnerability in aging: putative nutritional implications for intervention. Mech Aging Dev 2000;116:141-53.

[12] Chan A, Shea TB. Improved cognitive performance and decreased aggression in normal and ApoE4 mice following dietary supplementation with *N*-acetyl cysteine, acetyl-L-carnitine and *S*-adenosyl methionine. Neuromol Med 2007;9:264-9.

[13] Chan A, Paskavitz J, Remington R, Shea TB. Efficacy of a vitamin/nutriceutical formulation for early-stage Alzheimer's disease: a one-year open-label pilot study with an 11-month extension. Am J Alzheimers Dis Other Demen 2008 [electronic publication ahead of print].

[14] Remington R, Chan A, Shea TB. Efficacy of a vitamin/nutriceutical formulation for moderate to late-stage Alzheimer's disease: a placebo-controlled pilot study. Am J Alzheimers Dis Other Demen 2008 [electronic publication ahead of print].

[15] Aliev G, Liu J, Shenk JC, Fischbach K, Pacheco GJ, Chen SG, et al. Neuronal mitochondrial amelioration by feeding acetyl-L-carnitine and lipoic acid to aged rats. J Cell Mol Med 2008 [electronic publication ahead of print].

[16] Liu J. The effects and mechanisms of mitochondrial nutrient alpha-lipoic acid on improving age-associated mitochondrial and cognitive dysfunction: an overview. Neurochem Res 2008;33:194-203.

[17] Amenta F, Franch F, Ricci A, Vega JA. Cholinergic neurotransmission in the hippocampus of aged rats: influence of L-alpha-glycerylphosphorylcholine treatment. Ann N Y Acad Sci 1993;695:311-3.

[18] Amenta F, Liu A, Zeng YC, Zaccheo D. Muscarinic cholinergic receptors in the hippocampus of aged rats: influence of choline alphoscerate treatment. Mech Ageing Dev 1994;76:49-64.

[19] Bazan NG. Omega-3 fatty acids, pro-inflammatory signaling and neuroprotection. Curr Opin Clin Nutr Metab Care 2007;10:136-41.

[20] Favrelière S, Perault MC, Huguet F, De Javel D, Bertrand N, Piriou A, et al. DHA-enriched phospholipid diets modulate age-related alterations in rat hippocampus. Neurobiol Aging 2003;24:233-43.

[21] Cucinotta D, Passeri M, Ventura S, et al. Multicenter clinical placebo-controlled study with acetyl-L-carnitine (LAC) in the treatment of mildly demented elderly patients. Int J Clin Pharmacol Res 1990;10: 75-9.

[22] Liu J, Head E, Gharib AM, Yuan W, Ingersoll RT, Hagen TM, et al. Memory loss in old rats is associated with brain mitochondrial decay and RNA/DNA oxidation: partial reversal by feeding acetyl-L-carnitine and/or R-alpha-lipoic acid. Proc Natl Acad Sci U S A 2002;99:2356-61.

[23] Hager K, Kenklies M, McAfoose J, Engel J, Münch G. Alpha-lipoic acid as a new treatment option for Alzheimer's disease—a 48 months follow-up analysis. J Neural Transm Suppl 2007;72:189-93.

[24] Heiss WD, Kessler J, Mielke R, Szelies B, Herholz K. Long-term effects of phosphatidylserine, pyritinol, and cognitive training in Alzheimer's disease. A neuropsychological, EEG, and PET investigation. Dementia 1994;5:88-98.

[25] Montgomery SA, Thal LJ, Amrein R. Meta-analysis of double blind randomized controlled clinical trials of acetyl-L- carnitine versus placebo in the treatment of mild cognitive impairment and mild Alzheimer's disease. Int Clin Psychopharmacol 2003;18:61-71.

[26] Parnetti L, Abate G, Bartorelli L, Cucinotta D, Cuzzupoli M, Maggioni M, et al. Multicentre study of L-alpha-glycerylphosphorylcholine vs

ST200 among patients with probable senile dementia of Alzheimer's type. Drugs Aging 1993;3:159-64.
[27] Pettegrew JW, Klunk WE, Panchalingam K, et al. Clinical and neurochemical effects of acetyl-L-carnitine in Alzheimer's disease. Neurobiol Aging 1995;16:1-4.
[28] Rai G, Wright G, Scott L, et al. Double-blind, placebo controlled study of acetyl-L-carnitine in patients with Alzheimer's dementia. Curr Med Res Opin 1990;11:638-47.
[29] Thal LJ, Carta A, Clarke WR, et al. A 1-year multicenter placebo-controlled study of acetyl-L-carnitine in patients with Alzheimer's disease. Neurology 1996;47:705-11.
[30] Huang GS, Yang SM, Hong MY, Yang PC, Liu YC. Differential gene expression of livers from ApoE deficient mice. Life Sci 2000;68: 19-28.
[31] Ramassamy C, Averill D, Beffert L, Throux L, Lussier-Cacan S, Cohn JS, et al. Oxidative damage and protection by antioxidants in the frontal cortex of Alzheimer's disease is related to the apolipoprotein E genotype. Free Rad Biol Med 1999;27:544-53.
[32] Ramassamy C, Kryzwkowski P, Averill D, Lussier-Cacan S, Throux L, Christen Y, et al. Impact of ApoE deficiency on oxidative insults and antioxidant levels in the brain. Mol Brain Res 2001;86:76-83.
[33] Shea TB, Rogers E. Folate quenches oxidative damage in brains of apolipoprotein E-deficient mice: augmentation by vitamin E. Mol Brain Res 2002;108:1-6.
[34] Tchantchou F, Graves M, Ashline D, Morin A, Pimenta A, Ortiz D, et al. Increased transcription and activity of glutathione synthase in response to deficiencies in folate, vitamin E and apolipoprotein E. J Neurosci Res 2004;75:508-15.
[35] Bragin V, Chemodanova M, Dzhafarova N, Bragin I, Czerniawski JL, Aliev G. Ann Clin Psychiatry 2005;17:269-86.
[36] Kidd PM. Neurodegeneration from mitochondrial insufficiency: nutrients, stem cells, growth factors, and prospects for brain rebuilding using integrative management. Altern Med Rev 2005;10:268-93.
[37] Kataoka-Kato A, Ukai M, Sakai M, Kudo S, Kameyama T. Enhanced learning of normal adult rodents by repeated oral administration of soybean transphosphatidylated phosphatidylserine. J Pharmacol Sci 2005;98:307-14.
[38] Akpinar D, Yargiçoglu P, Derin N, Alicigüzel Y, Agar A. The effect of lipoic acid on antioxidant status and lipid peroxidation in rats exposed to chronic restraint stress. Physiol Res 2007.
[39] Dhitavat S, Ortiz D, Rogers E, Rivera E, Shea TB. Folate, vitamin E and acetyl-L-carnitine provide synergistic protection against oxidative stress resulting from exposure of human neuroblastoma cells to amyloid beta. Brain Res 2005;1061:114-7.
[40] Shea TB. Phosphatidylserine alters tau antigenicity, phosphorylation, proteolysis and association with microtubules: implication for tau function under normal and degenerative conditions. J Neurosci Res 1997;50:114-22.