Mol Neurobiol (2009) 39:101–106
DOI 10.1007/s12035-009-8056-1

Cytoprotective Effect of Acetyl-L-Carnitine Evidenced by Analysis of Gene Expression in the Rat Brain

Giovanna Traina · Giuseppe Federighi · Marcello Brunelli · Rossana Scuri

Received: 26 November 2008 / Accepted: 22 January 2009 / Published online: 7 February 2009

Abstract Acetyl-L-carnitine (ALC), the acetyl ester of L-carnitine, is a naturally occurring substance that when administered at supraphysiological concentrations is neuroprotective. ALC plays an essential role in intermediary and mitochondrial metabolism. It has also neurotrophic and antioxidant actions. ALC has demonstrated efficacy and high tolerability in the treatment of neuropathies of various etiologies, and it is a molecule of considerable interest for its clinical application in various neural disorders, such as Alzheimer's disease and painful neuropathies, although little is known regarding the effects of ALC on gene expression. Suppression subtractive hybridization methodology was used for the generation of subtracted complementary DNA libraries and the subsequent identification of differentially expressed transcripts in the rat brain after a chronic ALC treatment. In the present paper, we provide evidences for the up-regulation of the expression of prostaglandin D_2 synthase, brain-specific Na^+-dependent inorganic phosphate transporter, and cytochrome b oxidase, bc1 complex induced in the rat brain by ALC. On the contrary, ALC treatment down-regulates the expression of the gene of *ferritin-H.* Altogether, these results suggest that ALC might play a cytoprotective role against various brain stressors.

G. Traina (✉)
Department of Internal Medicine, University of Perugia,
Via San Costanzo,
06126 Perugia, Italy
e-mail: traina@unipg.it

G. Federighi · M. Brunelli · R. Scuri
Department of Biology, Unit of General Physiology,
University of Pisa,
Pisa, Italy

Keywords Acetyl-L-carnitine · Rat · Brain · Suppression subtractive hybridization · Cytoprotection

Introduction

Acetyl-L-carnitine (ALC) is the acetyl ester of the trimethylated amino acid L-carnitine (LA) that plays an essential role in energy production as "shuttles" of long-chain fatty acids between the cytosol and the mitochondria for subsequent β oxidation [1–3]. Together with LA, ALC is involved in the control of mitochondrial acyl-CoA/CoA ratio and peroxisomal oxidation of fatty acids [4]. ALC exerts cytoprotective, antioxidant, and anti-apoptotic activity, and many studies have focused on the neurotrophic effects of ALC in the nervous system [5], since it is more widely used than its closer analog LA because it enters cells and crosses more efficiently the blood brain barrier [6]. In addition, ALC improves different aspects of the neuronal metabolism [7–10] and has wide neuromodulatory effects [4, 11–13]. ALC is involved in cognitive functions and neuronal bioenergetics mechanisms with both short- and long-term treatments in rats [14], and there is some experimental evidence that ALC might also have anti-aging effects and cardioprotective activity [11, 15, 16], restoring the age-associated decline of learning and memory in old animals [17]. Several studies have also indicated that ALC potentiates the cholinergic transmission and provides a possible tool for Alzheimer's disease (AD) treatment [18]. A recent study suggests that supplementation with ALC improves spatial working memory deficits, reduces oxidative stress, and inhibits apoptotic cascade induced by hypoxia [19]. Recent evidences show that ALC treatment produces lasting effects, suggesting that ALC might modulate protein synthesis through qualitative and/or

quantitative changes of gene expression, but the mechanisms underlying this role have never been characterized at cellular and molecular level. We worked out an extensive investigation in the brain of rats subjected to a chronic ALC treatment in order to evaluate the possibility that ALC might affect the gene expression. We carried out a comprehensive analysis of all the genes that are up- or down-regulated after long-lasting ALC treatment using polymerase chain reaction (PCR)-based suppressive subtractive hybridization (SSH). The method combines normalization and subtraction of complementary DNAs (cDNAs) in a single procedure and allows enrichment of rare differentially expressed sequences and generates an equalized representation of differentially expressed genes irrespective of their relative abundance. It is an excellent technology to search for differentially expressed genes in the tissues and, in particular, for rare transcripts and unknown genes. Two different messenger RNA (mRNA) populations are compared so as to obtain clones of genes that are differentially expressed [20]. Previously, the construction of forward and reverse cDNA libraries allows us to identify genes that are up-regulated by ALC, such as *subunit γ of 14,3,3 protein*, *heat shock protein 72*, *voltage-dependent anion channel*, *lysosomal H^+/ATPase*, *V1 subunit D*, and *kinesin 1 light chain*, and down-regulated genes such as *ATP synthase lipid-binding protein*, *subunit c*, and *myelin basic protein* (*MBP*) [21–24].

In the present paper, we completed our analysis through the identification of some differentially expressed genes which might be responsible of a cytoprotective effect of ALC.

Materials and Methods

Dissection of the Brain

Fifty-day-old male Wistar rats weighting approximately 200–250 g from the same litter bred, housed at a temperature of 22°C, were intraperitoneally injected daily for 21 days either with ALC (100 mg/kg body weight Sigma-Tau Laboratories, Pomezia, Italy, treated group) [25] or saline (control group) as previously described [21]. The animals were anesthetized with ether and then killed by decapitation. Brains were rapidly removed, fresh frozen, and stored at −80°C until use. Animal procedures were carried out in conformity with the principles expressed in the Helsinki Declaration.

Subtracted cDNA Library Construction

Total RNA was isolated from rat brains of four control and four ALC-treated animals as previously described [21]. Briefly, 2 mg of poly $(A)^+$ RNAs were purified from the pools of total RNAs of brain (except cerebellum and spinal cord) of both control and treated rats using the PolyATtract mRNA isolation system (Promega Corp., Madison, WI, USA). ALC-modulated cDNA was generated by SSH using the PCR-Select cDNA subtraction kit (BD Biosciences Clontech, Palo Alto, CA, USA) according to the manufacturer's protocol. Poly(A) $^+$mRNA samples were used to synthesize tester and driver cDNA pools [21]. For forward subtraction, tester cDNA was synthesized from poly(A) $^+$mRNA isolated from treated rat brains and driver cDNA was produced from poly(A) $^+$mRNA isolated from control rat brains; reverse subtraction was performed using control cDNA as tester and treated cDNA as driver.

Cloning, Differential Screening, and Sequence Analysis

Amplified cDNA sequences from the forward and reverse subtraction were directly inserted into a T/A cloning vector using the TOPO TA cloning kit (Invitrogen, Carlsbad, CA, USA) as reported in Traina et al. [21]. A differential screening was performed for all collected clones using the cDNA Array approach (Clontech PCR-Select differential screening kit, BD Biosciences Clontech). The positive clones were sequenced by the dideoxy chain termination method with an automated DNA sequencer and the sequences were analyzed with BLAST and FASTA algorithms at the National Center of Biotechnology Information and European Bioinformatics Institute servers.

Semi-quantitative RT-PCR

Reverse transcriptions were carried out with total RNA (4 μg) isolated from treated and control rat brains using the SuperScript™ II RNase H^- reverse transcriptase (Invitrogen) and with the $Oligo(dT)_{12–18}$ primer (Invitrogen) according to the manufacturer's instructions as reported in Traina et al. [22]. One microliter of first-strand cDNAs was used for each PCR amplification. PCRs were performed in 50 μl of 1× reaction buffer containing 250 μM of each dNTP, 1.7 mM $MgCl_2$, 0.4 μM of each primer, and 2.5 U EuroTaq (EuroClone, Milano, Italy). Amplifications were carried out according to the following temperature profile: 94°C for 4 min, then 24 cycles each consisting of 30 s at 94°C, 30 s at 59°C, 30 s at 72°C, and with a final extension of 7 min at 72°C. The relative amounts of each PCR product were quantified by direct scanning of ethidium-bromide-stained 2% TAE-agarose gels with a UVP Image Store 5000 (Ultra Violet Product Ltd., Cambridge, England) equipped with the UVP GelBase-GelBlot TM Windows software. To equalize the amounts of the total RNA and the efficiency of cDNA synthesis from various tissue samples, the band intensities were normalized with the average

intensity of the co-amplified housekeeping G3PDH fragment across the samples investigated. The relative expression levels have been calculated as ratio of each analyzed transcript with respect to the G3PDH product level from three independent experiments performed for each transcript. The statistical analysis was done with the Mann–Whitney *U* test. All data are expressed as mean values± SEM.

Northern Blot Analysis

For Northern blot analysis, total RNA was isolated from brains of control and treated ALC rats, separated on agarose gel containing formaldehyde according to Sambrook et al. [26], and transferred by vacuum blotting to a positively charged nylon membrane (Roche, Mannheim, Germany). The amplified products of the differentially isolated clones, obtained using the nested primers and labeled with DIG DNA labeling kit (Roche), were used as probes. As quantitative control, blots were stripped and hybridized with the housekeeping gene for G3PDH.

Results

Identification of Differentially Expressed Genes

In order to identify the differentially expressed genes in the rat brain in response to ALC treatment, gene expression was compared at the mRNA level using the suppression subtractive hybridization. After the construction of forward and reverse cDNA libraries, the screening of the positive clones was carried out by dot blot screening. Following comparison with the sequences in the Genbank nucleotide database, we identified four clones as true positive, corresponding to four differentially expressed genes after ALC treatment. The up- or down-regulation of gene expression has been confirmed by relative RT-PCR analysis, as shown by comparative analysis with *G3PDH* gene or, in some cases, with Northern blot analysis. ALC treatment up-regulates the expression of the following clones: 5BG11, corresponding to *prostaglandin D_2 synthase* gene (accession no. RNPGS; similarity, 100%; *E* value, 1.8e−20; Fig. 1a); 5BE1, corresponding to *brain-specific Na^+-*

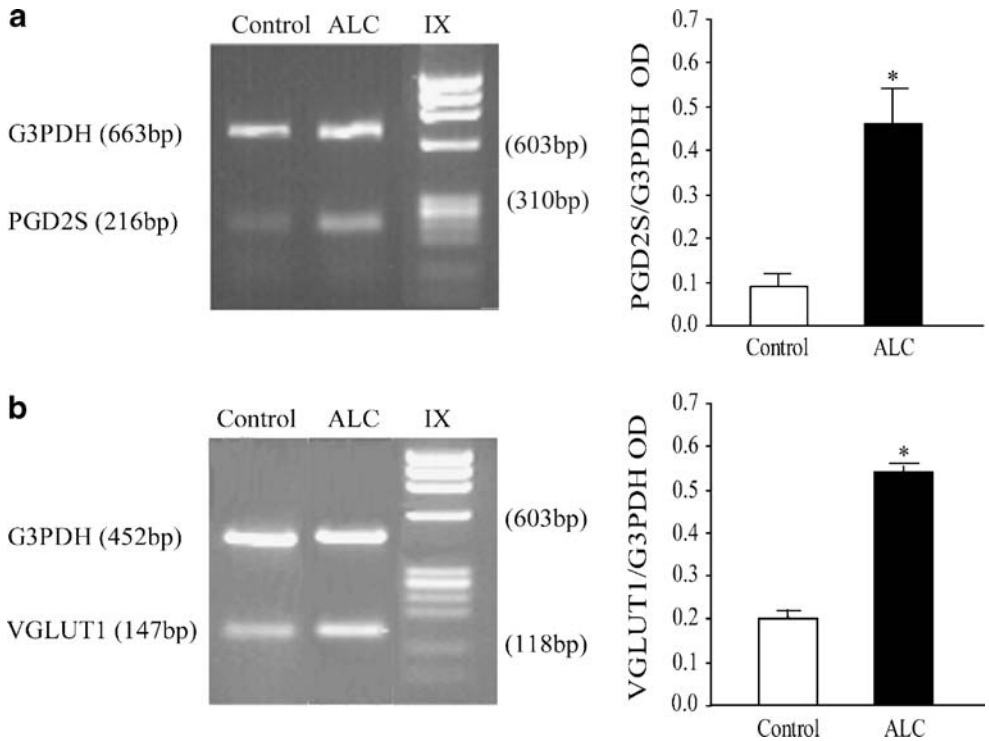

Fig. 1 **a** Representative relative RT-PCR products corresponding to PGD2S or G3PDH (*left*) and relative expression levels (*right*) are shown. The relative expression levels have been calculated as ratio of each analyzed transcripts with respect to the *G3PDH* housekeeping product level. The expression of the *PGD2S* transcript was higher in ALC-treated samples than in control samples (control, 0.082±0.0342; ALC, 0.452±0.0754; Mann–Whitney *U* test, $P=0.002$). **b** Representative relative RT-PCR products corresponding to VGLUT1 or G3PDH (*left*) and relative expression levels (*right*) as in **a**. Also, the expression of the VGLUT1 transcript resulted higher in ALC-treated samples than in control samples (control, 0.188±0.0023; ALC, 0.518±0.0092; Mann–Whitney *U* test, $P<0.001$). *Asterisks* indicate statistically significance. *Lane IX*, marker IX

dependent inorganic phosphate transporter (VGLUT1) gene (accession no. BC028938; similarity 96%; *E* value, 1e−20; Fig. 1b); 1BE11, corresponding to *cytochrome b oxidase, bc1 complex* gene (accession no. RNY17319; similarity, 100%; *E* value, 8.9e−51; Fig. 2b). ALC treatment down-regulates the expression of the following clone: 2BC9, corresponding to *ferritin-H* gene (accession no. RNU58829; similarity, 100%; *E* value, 3.7e−156; Fig 2a).

Figure 1a illustrates a representative result with primers for *PGD2S* and *G3PDH* housekeeping genes. The relative expression levels of *PGD2S* transcripts were estimated by measuring the staining of the PCR products resolved by gel electrophoresis. Figure 1b shows a representative result with primers for *VGLUT1* and *G3PDH* housekeeping genes. Also, in this case, the relative expression levels of *VGLUT1* transcripts were estimated by measuring the staining of the PCR products resolved by gel electrophoresis.

Northern blot analysis evidenced that the expression of *3BC9*, corresponding to *ferritin-H* gene expression, and *1BE11*, corresponding to cytochrome b oxidase cDNAs, was modulated by ALC treatment. The accuracy of the results was demonstrated by hybridization after stripping the filter with the *G3PDH* housekeeping gene (Fig. 2).

Discussion

New studies in rats have shown that chronic ALC treatment increases life span, improves both cognitive behavior and long-term memory performance in aged animals [27], and prevents age-related changes in mitochondrial respiration.

In our studies, we proved that a chronic ALC treatment modulate different gene expression in the rat brain and that the majority of detected clones are clones involved in the neuroprotection or in neuromodulation [21–24]. In the present paper, we have completed the analysis of differentially expressed genes induced in the rat brain by ALC, and we provide evidences that ALC might play a cytoprotective role against various brain stressors.

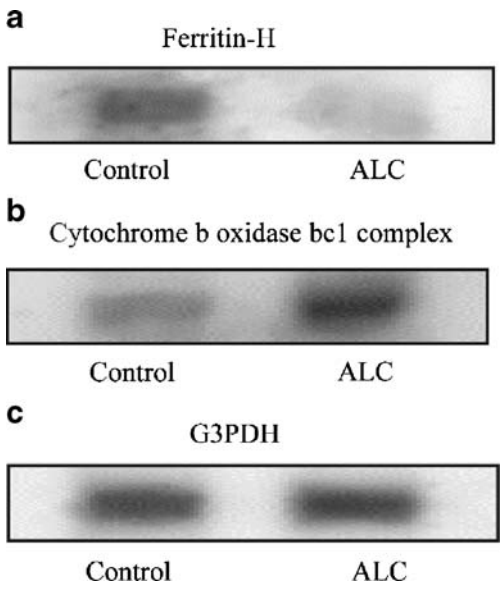

Fig. 2 Northern blot analysis of RNA isolated from control (*left lane*) and ALC-treated (*right lane*) rats. **a** The blot shows down-regulation of *ferritin-H* gene after the treatment with ALC. **b** The blot shows up-regulation of *cytochrome b* oxidase gene after ALC treatment. **c** *G3PDH* gene housekeeping as standard

Prostaglandin D_2 Synthase (*PGD2S*)

ALC up-regulates the *PGD2S* gene expression. Liang et al. [28] showed that in the brain, the activation of DP1, a receptor of PGD_2, can prevent neuronal injury in paradigms of acute excitotoxicity. More recently, Lin et al. [29] indicated that PGJ2, a non-enzymatic product of PGD_2, exhibits anti-inflammatory properties through activation of the gamma peroxisome proliferator activated receptor. These data support an emerging and neuronal protective role for prostaglandins that may represent new therapeutic targets in neurological diseases.

Na^+-Dependent Inorganic Phosphate Co-transporter (*VGLUT1*)

ALC up-regulates the expression of brain-specific Na^+-dependent inorganic phosphate transporter gene. This transporter is expressed in different neural populations predominantly in glutamatergic neurons of the rat diencephalon. For this reason, it is also named *VGLUT1*. It is localized to synaptic vesicles, suggesting a role in the transport of glutamate into synaptic vesicles for regulating exocytotic release [30]. The up-regulation of *VGLUT1* gene expression by ALC is consistent with studies which have ascribed to *VGLUT1* a role in the protection against excitotoxic injury [31].

Ferritin-H

Ferritin represents the major iron storage in the brain. Iron utilization is important for normal brain development, myelination, electron transport, and the activity of a number of enzymes responsible for neurotransmitter synthesis [32, 33]. Ferritin is composed of different ratios of two types of subunits termed H (high chain) and L (light chain). The H-rich form is found mainly in tissue in which iron is more rapidly utilized, such as the heart or the brain. It has mainly two functions: first, it provides the iron necessary for the oxidative metabolism or neurotransmitter synthesis because some enzymes require iron as a cofactor [33]; second, it

acts as a cytoprotectant against oxidant-mediated injury [34]. The content of cytoplasmic ferritin is regulated by the translation of *ferritin H* and L mRNAs in response to an intracellular pool of "chelatable" or "labile" iron. Thus, when iron levels are low, ferritin synthesis is decreased; conversely, when iron levels are high, ferritin synthesis increases.

The regulatory response of ferritin to iron is largely posttranscriptional and is due to the recruitment of stored mRNA from monosomes to polysomes in the presence of iron. However, reports indicate that elevated serum ferritin level, especially of the H subunit, accompany many clinical malignancies [35]. In several neurodegenerative disorders, such as AD and Parkinson's disease, ferritin-H expression, and brain iron homeostasis, might be altered. Iron encrustation of blood vessels is a common observation in AD brain [36]. In Parkinson's disease, iron accumulation in the substantia nigra occurs and this is considered part of the pathogenesis of the disease [37]. In addition, ferritin levels were elevated in all multiple sclerosis lesion stages [38].

It has been suggested that a relationship might exist between ferritin and cancer [39]. Several conditions can modify the transcriptional expression of the ferritin-H mRNA, factors controlling cell growth and differentiation such as tumor necrosis factor [40]. Our finding that ALC down-regulates the *ferritin-H* gene expression acquires interest with regard to the observation that the *ferritin-H* over-expression during tumor development is phenotypically correlated with tumor initiation and/or progression.

Several studies suggest that multiple independent pathways exist which converge in the augmentation of ferritin synthesis in response to various forms of oxidative insult. Ferritin, with its ability to oxidize and sequester intracellular iron in an internal mineral core, limits the levels of catalytically available iron, owing to the generation of free radicals, as a critical cytoprotective protein that constitutes an integral part of the antioxidant response. A recent study reported that ALC exerts antioxidant effect and reverses iron-induced oxidative stress in human fibroblast [41]. It is possible that ALC might reduce available iron by reducing *ferritin-H* expression.

Cytochrome b Oxidase

This study shows that ALC up-regulates *cytochrome b oxidase*, complex bc1 gene expression.

ALC increases the energy status of the cell by increasing the activities of cytochrome b oxidase, thus maintaining energy levels of the cells and stabilizing mitochondrial activity [42]. This evidence confirms the ALC cytoprotective role.

Concluding Remarks

Overall, our results provide an important support for cytoprotective role of ALC, as molecule capable of potentiating cellular stress response pathways, by modulating the redox status. This cytoprotective action of ALC acquires considerable importance in order to plan possible therapeutic intervention, corroborating ALC as a novel approach for some pathophysiological conditions in which it might delay the onset of age-associated alterations.

We cannot exclude that the effect of ALC might be posttranscriptional. It is known that the control of mRNA stability is modulated by several physiological stimuli, including cytokines, growth factors, hormones, and hypoxia. Furthermore, a clinical relevance of posttranscriptional gene regulation by mRNA stability is highlighted by various pathologies whose occurrence tightly correlates with mutations in the *cis*-regulatory regions responsible for mRNA decay or by a dysregulation of trans-acting proteins specifically binding to these regulatory elements.

Therefore, it is not possible to exclude that ALC might modulate mRNA stability. Further studies will be aimed at clarifying if a similar situation is concerning ALC action.

Acknowledgments The authors thank Sigma-Tau Laboratories, Pomezia, Roma, for providing with acetyl-L-carnitine and for partially supporting the work.

References

1. Bieber LL (1988) Carnitine. Annu Rev Biochem 57:261–283
2. Nalecz KA, Miecz D, Berezowski V, Cecchelli R (2004) Carnitine: transport and physiological functions in the brain. Mol Aspects Med 25:551–567
3. Shea TB (2007) Effects of dietary supplementation with *N*-acetyl cysteine, acetyl-L-carnitine and *S*-adenosyl methionine on cognitive performance and aggression in normal mice and mice expressing human ApoE4. Neuromolecular Med 9(3):264–269
4. Virmani A, Binienda Z (2004) Role of carnitine esters in brain neuropathology. Mol Aspects Med 25:533–549
5. Bodis-Wollner I (1990) Physiological effects of acetyl levo carnitine in the central nervous system. Int J Clin Pharmacol Res 10:109–114
6. Kidd PM (1999) A review of nutrients and botanicals in the integrative management of cognitive dysfunctions. Altitude Med Rev 4:144–161
7. Calvani M, Arrigoni-Martelli E (1999) Attenuation by acetyl-L-carnitine of neurological damage and biochemical derangement following brain ischemia and reperfusion. Int J Tissue React 21:1–6
8. Ori C, Freo U, Pizzolato G, Dam M (2002) Effects of acetyl-L-carnitine on regional cerebral glucose metabolism in awake rats. Brain Res 951:330–335
9. McDaniel MA, Maier SF, Einstein GO (2003) "Brain-specific" nutrients: a memory cure? Nutrition 19:957–975
10. Tanaka M, Nakamura F, Mizokawa S, Matsumura A, Matsumura K, Yasuyoshi W (2003) Role of acetyl-L-carnitine in the brain: revealed by bioradiography. Biochem Biophys Res Commun 306:1064–1069

11. Pettegrew JW, Levine J, McClure RJ (2000) Acetyl-L-carnitine physical-chemical, metabolic, and therapeutic properties: relevance for its mode of action in Alzheimer's disease and geriatric depression. Mol Psychiatry 5:616–632
12. Bertoni-Freddari C, Fattoretti P, Casoli T, Spagna C, Casell U (1994) Dynamic morphology of the synaptic junctional areas during aging: the effect of chronic acetyl-L-carnitine administration. Brain Res 656:359–366
13. Castorina M, Ferraris L (1994) Acetyl-L-carnitine affects aged brain receptorial system in rodents. Life Sci 54:1205–1214
14. Ando S, Tadenuma T, Tanaka Y, Fukui F, Kobayashi S, Ohashi Y, Kawabata TJ (2001) Enhancement of learning capacity and cholinergic synaptic function by carnitine in aging rats. Neurosci Res 66:266–271
15. Calò LA, Pagnin E, Davis PA, Semplicioni A, Nicolai R, Calvani M, Pessina AC (2006) Antioxidant effect of L-carnitine and its short chain esters relevance for the protection from oxidative stress related cardiovascular damage. Int J Cardiol 107:54–60
16. Manfridi A, Forloni GL, Arrigoni-Martelli E, Mancia M (1992) Culture of dorsal root ganglion neurons from aged rats: effects of acetyl-L-carnitine and NGF. Int J Dev Neurosci 10:321–329
17. Seidman MD, Khan MJ, Bai U, Shirwany N, Quirk WS (2000) Biologic activity of mitochondrial metabolites on aging and age-related hearing loss. Am J Otol 21:161–167
18. White HL, Scates PW (1990) Acetyl L-carnitine as a precursor of acetylcholine. Neurochem Res 15:597–601
19. Barhwal K, Singh SB, Hota SK, Jayalakshmi K, Ilavazhagan G (2007) Acetyl-L-carnitine ameliorates hypobaric hypoxic impairment and spatial memory deficits in rats. Eur J Pharmacol 570(1–3):97–107
20. Diatchenko L, Lau YF, Campbell AP, Chenchik A, Moqadam F, Huang B, Lukyanov S, Lukyanov K, Gurskaya N, Sverdlov ED, Siebert PD (1996) Suppression subtractive hybridization: a method for generating differentially regulated or tissue-specific cDNA probes and libraries. Proc Natl Acad Sci U S A 93 (12):6025–6030
21. Traina G, Valleggi S, Bernardi R, Rizzo M, Calvani M, Nicolai R, Mosconi L, Durante M, Brunelli M (2004) Identification of differentially expressed genes induced in the rat brain by acetyl-L-carnitine as evidenced by suppression subtractive hybridisation. Mol Brain Res 132:57–63
22. Traina G, Bernardi R, Cataldo E, Macchi M, Durante M, Brunelli M (2008) In the rat brain acetil-L-carnitine treatment modulates the expression of gene involved in neuronal ceroid lipofuscinosis. Mol Neurobiol 38(2):146–152
23. Traina G, Federighi G, Brunelli M (2008) Up-regulation of kinesin light-chain 1 gene expression by acetyl-L carnitine: therapeutic possibility in Alzheimer's disease. Neurochem Int 53:244–247
24. Traina G, Bernardi R, Rizzo M, Calvani M, Durante M, Brunelli M (2006) Acetyl-L-carnitine up-regulates expression of voltage-dependent anion channel in the rat brain. Neurochem Int 48:673–678
25. Jin HW, Flatters SJ, Xiao WH, Mulhern HL, Bennett GJ (2008) Prevention of paclitaxel-evoked painful peripheral neuropathy by acetyl-L-carnitine: effects on axonal mitochondria, sensory nerve fiber terminal arbors, and cutaneous Langerhans cells. Exp Neurol 210:229–237
26. Sambrook J, Fristch EF, Maniatis T (1989) Extraction and purification of RNA, molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor laboratory Press, New York, pp 7.3–7.83
27. Calabrese V, Cornelius C, Mancuso C, Pennisi G, Calafato S, Bellia F, Bates TE, Giuffrida Stella AM, Schapira T, Dinkova Kostova AT, Rizzarelli E (2008) Cellular stress response: a novel target for chemoprevention and nutritional neuroprotection in aging, neurodegenerative disorders and longevity. Neurochem Res 33(12):2444–2471
28. Liang X, Liejun W, Tracey H, Katrin A (2005) Prostaglandin D_2 mediates neuronal protection via the DP1 receptor. J Neurochem 92(3):477–486
29. Lin TN, Cheung WM, Wu JS, Chen JJ, Lin H, Chen JJ, Liou JY, Shyue SK, Wu KK (2006) 15d-prostaglandin J2 protects brain from ischemia–reperfusion injury. Arterioscler Thromb Vasc Biol 26:481–487
30. Bellocchio EE, Reimer RJ, Fremeau RT Jr, Edwards RH (2000) Uptake of glutamate into synaptic vesicles by an inorganic phosphate transporter. Science 289(5481):957–60
31. Glinn M, Ni B, Irwin RP, Kelley SW, Lin SZ, Paul SM (1998) Inorganic Pi increases neuronal survival in the acute early phase following excitotoxic/oxidative insults. J Neurochem 70(5):1850–1858
32. Raush WD, Hirada Y, Nagatsu T, Riederer P, Jellinger K (1988) Tyrosine hydroxylase activity in caudate nucleus from Parkinson's disease: effect of iron and phosphorylating agents. J Neurochem 50:202–208
33. Wrigglesworth JM, Baum H (1988) Iron-dependent enzymes in the brain. In: Youdim MBH (ed) The brain iron: neurochemical and behavioral aspects. Taylor and Francis, Philadelphia, pp 25–66
34. Nappi AJ, Vass E (2000) Iron, metalloenzymes and cytotoxic reactions. Cell Mol Biol 46(3):637–647
35. Wu CG, Groenink M, Bosma A, Reitsma PH, vanDeventer SJH, Chamuleau RAFM (1997) Rat ferritin-H: cDNA cloning, differential expression and localization during hepatocarcinogenesis. Carcinogenesis 18:47–52
36. Connor JR, Menzies SL (1995) Cellular management of iron in the brain. J Neurol Sci 134:33–44
37. Sofic E, Riederer P, Heinsen H, Beckmann H, Reynolds GP, Hebenstreit G, Youdim MB (1988) Increased iron (III) and total iron content in post mortem substantia nigra of parkinsonian brain. J Neural Transm 74(3):199–205
38. Petzold A, Eikelenboom J, Gveric D, Keir G, Chapman M, Lazeron RHC, Cuzner ML, Polman CH, Uitdehaag BMJ, Thompson EJ, Giovannoni G (2002) Marker for different glial cell responses in multiple sclerosis: clinical and pathological correlation. Brain 125:1462–1463
39. Worwood M (1980) Serum ferritin and iron deficiency anaemia in hospital patients. Lancet 1:375–376
40. Sanyal B, Polak PE, Szuchet S (1996) Differential expression of the heavy-chain ferritin gene in non-adhered and adhered oligodendrocytes. J Neurosci Res 46:187–197
41. Lal A, Atamna W, Killilea DW, Suh JH, Ames BN (2008) Lipoic acid and acetyl-carnitine reverse iron-induced oxidative stress in human fibroblasts. Redox Rep 13(1):2–10
42. Quereshi MY (1998) Stability of some hypothesized psychological determinants of fertility control. Psychol Rep 82(2):657–658