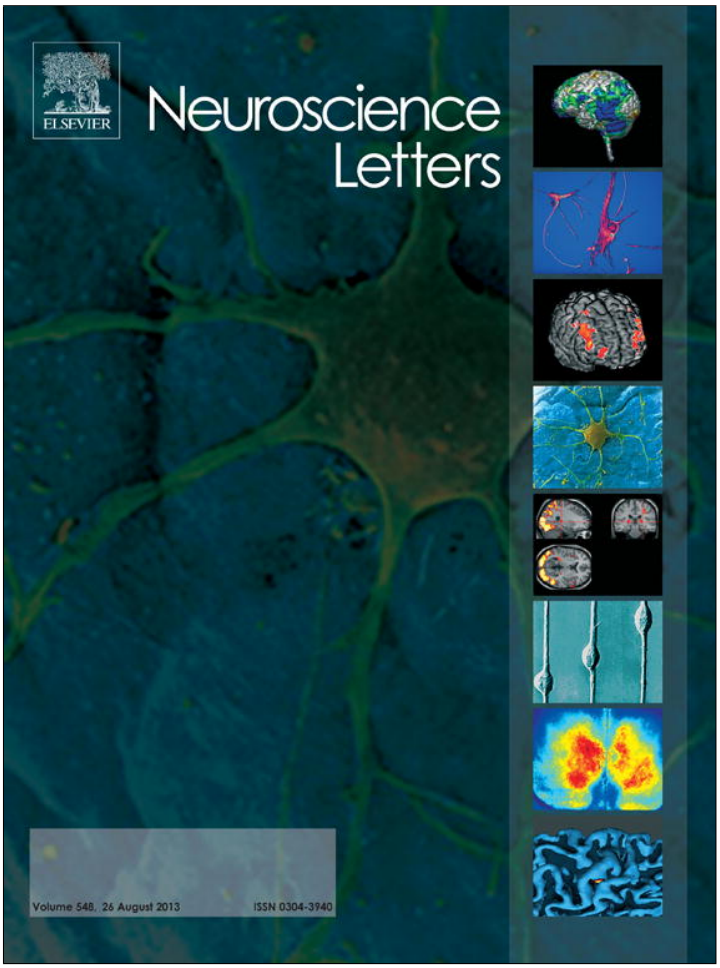

Neuroscience Letters 548 (2013) 170–175

Contents lists available at SciVerse ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Intraperitoneal melatonin is not neuroprotective in the G93ASOD1 transgenic mouse model of familial ALS and may exacerbate neurodegeneration

Efthimios Dardiotis[a], Elena Panayiotou[a], Marianne L. Feldman[a], Andreas Hadjisavvas[a], Stavros Malas[a], Ilia Vonta[b], Georgios Hadjigeorgiou[c], Kyriakos Kyriakou[a], Theodoros Kyriakides[a,*]

[a] The Cyprus Institute of Neurology and Genetics, 1683 Nicosia, Cyprus
[b] Department of Mathematics, National Technical University of Athens, 15780 Athens, Greece
[c] Institute for Biomedical Technology (BIOMED), Centre for Research and Technology-Thessaly (CERETETH), 41222 Larissa, Greece

HIGHLIGHTS

- Melatonin was found to have detrimental effects on phenotype and motoneuron survival.
- Melatonin treated animals were associated with increased oxidative stress.
- Melatonin was found to upregulate the neurotoxic mutant SOD1 in the G93ASOD1 mouse.
- G93ASOD1 mouse may not be ideal for assessing the beneficial effects of melatonin.

ARTICLE INFO

Article history:
Received 11 February 2013
Received in revised form 11 May 2013
Accepted 18 May 2013

Keywords:
Amyotrophic lateral sclerosis
G93ASOD1 transgenic mouse model
Melatonin
Neuroprotection
Oxidative stress

ABSTRACT

In amyotrophic lateral sclerosis (ALS) reactive oxygen species and apoptosis are implicated in disease pathogenesis. Melatonin with its anti-oxidant and anti-apoptotic properties is expected to ameliorate disease phenotype. The aim of this study was to assess possible neuroprotection of melatonin in the G93A-copper/zinc superoxide dismutase (G93ASOD1) transgenic mouse model of ALS. Four groups of mice, 14 animals each, were injected intraperitoneally with 0 mg/kg, 0.5 mg/kg, 2.5 mg/kg and 50 mg/kg of melatonin from age 40 days. The primary end points were; disease onset, disease duration, survival and rotarod performance. No statistically significant difference in disease onset between the four groups was found. Survival was significantly reduced with the 0.5 mg/kg and 50 mg/kg doses and tended to be reduced with the 2.5 mg/kg dose. Histological analysis of spinal cords revealed increased motoneuron loss in melatonin treated mice. Melatonin treated animals were associated with increased oxidative stress as assessed with 4-hydroxynonenal (4-HNE), a marker of lipid peroxidation. Histochemistry and Western blot data of spinal cord from melatonin treated mice revealed upregulation of human SOD1 compared to untreated mice. In addition, real-time PCR revealed a dose dependent upregulation of human SOD1 in melatonin treated animals. Thus, intraperitoneal melatonin, at the doses used, does not ameliorate and perhaps exacerbates phenotype in the G93ASOD1 mouse ALS model. This is probably due to melatonin's effect on upregulating gene expression of human toxic SOD1. This action presumably overrides any of its direct anti-oxidant and anti-apoptotic properties.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder characterized by selective loss of motor neurons in the brain and spinal cord leading to progressive weakness and muscle atrophy [5]. Approximately 5–10% of all ALS cases are familial, 20% of which are caused by mutations in the Cu/Zn superoxide dismutase gene (SOD1). Familial ALS is clinically and pathologically indistinguishable from sporadic ALS. Similarly, the G93ASOD1

Abbreviations: ALS, amyotrophic lateral sclerosis; SOD1, superoxide dismutase gene.

* Corresponding author at: The Cyprus Institute of Neurology and Genetics, PO Box 23462, 1683 Nicosia, Cyprus. Tel.: +357 22358600; fax: +357 22392786.
E-mail address: theodore@cing.ac.cy (T. Kyriakides).

0304-3940/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neulet.2013.05.058

transgenic mice exhibit pathology and phenotype reminiscent of human ALS [3].

There is both human and animal data supporting several pathogenic mechanisms in ALS including glutamate excitotoxicity, generation of free radicals, cytoplasmic protein misfolding, mitochondrial dysfunction, neuroinflammation and disruption of axonal transport through neurofilament disorganization [2].

Melatonin, an endogenous neurohormone, possesses powerful anti-oxidant properties by directly scavenging reactive oxygen species and by up regulating anti-oxidant enzymes [7,14]. Melatonin has been proposed as a candidate compound for neuroprotection in ALS and high tolerability of the oral/rectal drug has been demonstrated in ALS patients [4,20]. Oral administration of 57–88 mg/kg/day of melatonin to G93ASOD1 transgenic mice was found to delay disease onset, prolong disease progression and extend survival, while lower doses did not seem to be effective [20]. However, the exact neuroprotective mechanism of melatonin in the G93ASOD1 transgenic mice remains obscure. Western blot analysis of spinal cord homogenates did not detect any differences either in the mutant or endogenous SOD1 protein levels or in the total amount or phosphorylation status of AKT and ERK1/2 molecules of survival signaling pathways which were previously found to mediate the antiapoptotic effects of melatonin in other animal models [20].

In the present study we examined the effects of low (0.5–2.5 mg/kg/day) and high (50 mg/kg/day) doses of melatonin on the G93ASOD1 mouse model of ALS administered by the intraperitoneal route. Such doses have been previously demonstrated to be biologically neuroprotective in vivo [9,12]. Moreover, in order to gain some understanding of the possible neuroprotective mechanisms of melatonin in the G93ASOD1 animal model we investigated the effect of melatonin on oxidative stress and the expression of mutant and endogenous superoxide dismutases by PCR and Western blot.

2. Materials and methods

2.1. SOD1 G93A transgenic mice

Mice carrying a high copy human SOD1 G93A transgene (TgN(SOD1-G93A)1 GUR) were obtained from Jackson Laboratories. The experiments were conducted under the local Cyprus Institute of Neurology animal use guidelines and were approved by the Animal Care Committee of the Cyprus Government. Animal housing conditions: temperature: 21 °C, humidity: 50–60%, number of animals per cage: max 4 animals/cage, time of lights on: 6 am lights on and 6 pm lights off (circadian rhythm), air changes: 20/h, air centrally supplied and HEPA filtered.

2.2. Melatonin treatment protocol

The experiments were done in groups of 14 animals each (7 females and 7 males) and included intraperitoneal injection of 0 mg/kg (vehicle), 0.5 mg/kg, 2.5 mg/kg and 50 mg/kg. The vehicle used was ethanol–saline to accommodate the higher concentration of melatonin. Melatonin was administered daily from day 40 during the first part of photophase. Four animals of each group (two males, two females) were sacrificed at ages of 90 and 120 days to perform pathological and other analyses.

2.3. Disease endpoints

The disease endpoints were derived by blind clinical observation and by various rotarod parameters.

Onset of the disease was defined as the age in days when tremor appeared in both hind limbs, together with the inability of the mouse to extend and paddle at least one hind paw, when suspended in the air by its tail. This test was performed 3 times a week from day 70 onwards. Survival was defined as the age in days when mice could no longer ride themselves when gently prodded for approximately 10 s and/or the appearance of severe eye infection. Disease duration was defined as the time between disease onset and survival.

The rotarod performance test was done using a Rotarod (Columbus Instruments, Columbus, OH), which has a rotating rod starting with a speed 20 rpm and accelerated 1 rpm every 10 s. The rotarod performance test measured the time it took for each mouse to fall off the rotarod. The rotarod test was used to obtain two endpoints: rotarod onset defined as the age in days after which the mouse fell consistently below 80% of average performance and rotarod survival as the age in days after which the mouse fell consistently below 20% of average performance.

2.4. Spinal cord motor neuron count

The effect of melatonin on the number of motor neurons in lumbar spinal cord sections was evaluated in two mice from each group at the age of 120 days. Sections were incubated with primary antibody (Mouse monoclonal [SMI-32] to 200KD neurofilament heavy chain (ab28029) abcam. Both large (α-motor neurons) and small (γ-motor neurons) were counted in the anterior horns, anterior to a line drawn through the central canal.

2.5. SOD1 immunocytochemistry

SOD1 immunocytochemistry of motor neurons from lumbar cord was performed using rabbit anti-human-Cu/Zn superoxide dismutase polyclonal antibody (product#: SOD-101, Stressgen) and biotinylated polyclonal goat anti-rabbit immunoglobulin (code No. E0432, Dakocytomation).

2.6. Western blot of SOD1

A semi quantitative measurement of 4-HNE, human SOD1 and murine SOD2 with Western blot in spinal cord homogenates was performed comparing melatonin treated animals and controls. Beta-tubulin was used as a loading marker. Proteins were detected by incubating with the following primary antibodies directed against: human SOD1 with anti-Cu/Zn SOD (SOD-101) (Stressgen Bioreagents), murine SOD2 with anti-Mn SOD (SOD-110) (Stressgen Bioreagents), 4-hydroxynonenal (4-HNE) with anti-4-HNE antibody (MHN-100P) (JaICA, Nikken SEIL Co) and beta-tubulin (Sigma).

2.7. Real time PCR of mouse SOD2 and human SOD1

Expression of the SOD1 gene was measured in mice treated with different doses of melatonin, by real-time PCR using TaqMan® Gene Expression Assays and the 7900HT Real-Time PCR System (Applied Biosystems, Foster City, CA).

2.8. Statistical analyses

A one-tailed Student's *t*-test and the Wilcoxon rank sum test were used to analyze the significance of difference in onset of the disease, survival and disease duration. A 2-tailed Student's *t*-test was used regarding rotarod performance of each group. The number of neurons among groups was compared using the non-parametric Kruskal–Wallis test as well as the one-way ANOVA using Bonferonni, Scheffe and Tukey multiple comparisons.

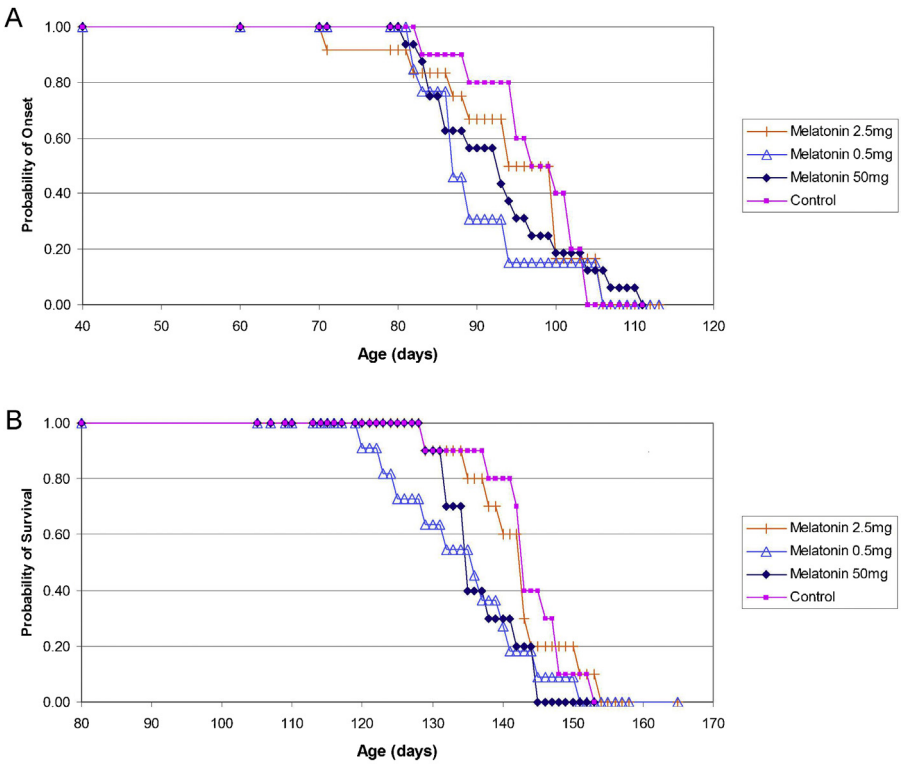

Fig. 1. (A) Disease onset curves in controls and melatonin treated animals. (B) Survival curves in controls and melatonin treated animals.

3. Results

No gender differences were observed with respect to disease onset, motor function, and disease progression between male and female G93ASOD1 mice ($p > 0.05$). Therefore, male and female mice were pooled together and were used as a group in the following analyses.

3.1. Disease onset

Mean age of disease onset of controls and melatonin (0.5, 2.5 and 50 mg/kg) treated mice were 97.1 ± 6.9, 92.5 ± 10.8, 94.6 ± 11.3 and 95.7 ± 9.7 days, respectively (Fig. 1A). One-sided *t*-tests showed no significant difference between controls and each one of the three treatment groups (0.5 mg/kg, 2.5 mg/kg and 50 mg/kg, *p*-values 0.13, 0.27 and 0.35). When all melatonin injected animals were grouped together and compared with controls no statistically significant difference was found in terms of disease onset (two-sided *t*-test *p*-value 0.42, nonparametric Mann–Whitney test *p*-value = 0.33).

3.2. Survival

Mean survival of the controls and melatonin (0.5, 2.5 and 50 mg/kg) treated mice were 143.3 ± 6.5, 134.2 ± 10.1, 142 ± 7.2 and 136.8 ± 5.6 days, respectively (Fig. 1B). An one sided *t*-test comparing the mean survival of controls versus injected animals showed a shorter survival for the 0.5 and 50 mg/kg groups with *p* values of 0.014 and 0.013, respectively. For the 2.5 mg/kg group the *p*-value was 0.34. Nonparametric two-sided Mann–Whitney tests showed a significant difference between the 0.5 mg/kg group and controls (*p*-value 0.029) and between the 50 mg/kg group and controls (*p*-value 0.029). For the 2.5 mg/kg group the *p*-value was 0.63. When all melatonin injected animals were grouped together and compared with controls there was a significant shorter survival versus controls with a *p*-value of 0.05 obtained through the Kruskal–Wallis test.

3.3. Disease duration

Mean duration of disease for controls and melatonin (0.5, 2.5 and 50 mg/kg) treated mice were 46.2 ± 7.8, 41.7 ± 14.9, 47.4 ± 12.9 and 41.1 ± 12.9 days, respectively. An one sided *t*-test comparing the mean disease duration for the 0.5, 2.5 and 50 mg/kg injected animals versus controls did not show a significantly faster disease progression in melatonin treated animals with *p* values of 0.2, 0.4 and 0.15, respectively.

3.4. Rotarod endpoints

Age at rotarod onset was not significantly different in the treated groups compared to controls (one sided *t*-test, $p > 0.05$). However, survival was significantly shorter in the 0.5 mg/kg injected group (one sided *t*-test, $p = 0.03$) and tended to be shorter in the 2.5 and 50 mg/kg injected groups (one sided *t*-test, $p = 0.13$ and $p = 0.065$).

3.5. Motor neuron loss

Mean motor neuronal count per section at 120 days for controls and melatonin (0.5, 2.5 and 50 mg/kg) treated mice were 18.7 ± 3.4, 16.4 ± 6.7, 12.4 ± 2.1 and 10.1 ± 3.5, respectively (supplementary Fig. 3). The Tukey multiple comparisons revealed that the number of neurons was significantly higher among controls as compared to the 2.5 and 50 mg/kg groups (*p*-value = 0.016 and *p*-value = 0.001, respectively). No difference was observed among controls and the group of 0.5 mg/kg melatonin treated animals (*p*-value = 0.640). Of note, there was a significant difference in the number of neurons between the 0.5 and the 50 mg/kg groups (*p*-value = 0.018).

Supplementary material related to this article found, in the online version, at http://dx.doi.org/10.1016/j.neulet.2013.05.058.

3.6. SOD1 immunocytochemistry

To try and explain the lack of expected neuroprotection of melatonin we investigated the possibility whether this was mediated by upregulation of the toxic human G93ASOD1. We performed anti-human SOD1 immunocytochemistry on spinal cord sections and qualitative comparisons were made as shown in Supplementary Figs. 1 and 2. Motor neurons exposed to melatonin demonstrated increased cytoplasmic SOD1 staining while those exposed to vehicle injections only appear as ghost cells. The results were compatible with up regulation of SOD1 in mice exposed to melatonin.

Supplementary material related to this article found, in the online version, at http://dx.doi.org/10.1016/j.neulet.2013.05.058.

3.7. Western blot

The Western blot data were consistent with up regulation of human SOD1 (Fig. 2A) and mouse SOD2 (Fig. 2B) in the experimental animals exposed to melatonin. Up regulation of both the transgenic human SOD1 and the endogenous SOD2 takes place in melatonin treated animals at age 90 days onwards. Similarly, the 4-HNE marker of lipid peroxidation was increased in melatonin treated animals especially at approximately age 90 days (Fig. 2C).

3.8. Real time PCR of human SOD1 and murine SOD2

We proceeded to independently verify melatonin induced up regulation of human SOD1 and murine SOD2 using real time PCR, measuring RNA of both the endogenous murine SOD2 and the transgenic human SOD1. As can be seen in Table 1 there is a dose dependent upregulation of both superoxide dismutases. Interestingly the upregulation of the transgenic human SOD1 was greater at approximately 90 days, the same as the highest oxidative stress.

4. Discussion

In the present study intraperitoneal melatonin at doses of 0.5, 2.5 and 50 mg/kg did not appear to ameliorate disease phenotype in the G93ASOD1 mouse model of ALS. On the contrary, treated animals at 0.5 and 50 mg/kg doses showed a shorter mean survival time. Histological analysis of spinal cords at the age of 120 days revealed significant motoneuron loss in melatonin treated mice in a dose dependant manner. Melatonin treated animals were associated with increased oxidative stress at both 90 and 120 days, as assessed by a marker of lipid peroxidation. In the 0.5 mg group, mice start to die off at about 120 days while in the control group and the other two melatonin doses this occurs at about 130 days. This earlier onset of attrition may perhaps be partly accounted for by the higher levels of oxidative stress shown in the 0.5 mg group at round both the 90 day and 120 day time points.

The lack of neuroprotection by melatonin may due to the up regulation of toxic human SOD1 which may have counteracted any direct antioxidant and radical scavenging activity of melatonin. Similar doses of melatonin had been previously shown to be neuroprotective in other models of neuronal injury due to the direct and indirect anti-oxidant activities of melatonin [11,12,17]. These models however do not have an inherent toxic gain of function oxidative stressor. The G93ASOD1 mouse has several copies of a mutated hSOD1 which has been demonstrated to exhibit a toxic gain of function. Therefore the net result of melatonin's upregulation of antioxidant enzymes would be to create a net excess of hydrogen peroxide which cannot be handled by the other antioxidant enzymes even if upregulated.

In a previous study of Weishaupt et al. [20] oral administration of 57–88 mg/kg/day of melatonin to G93ASOD1 transgenic mice

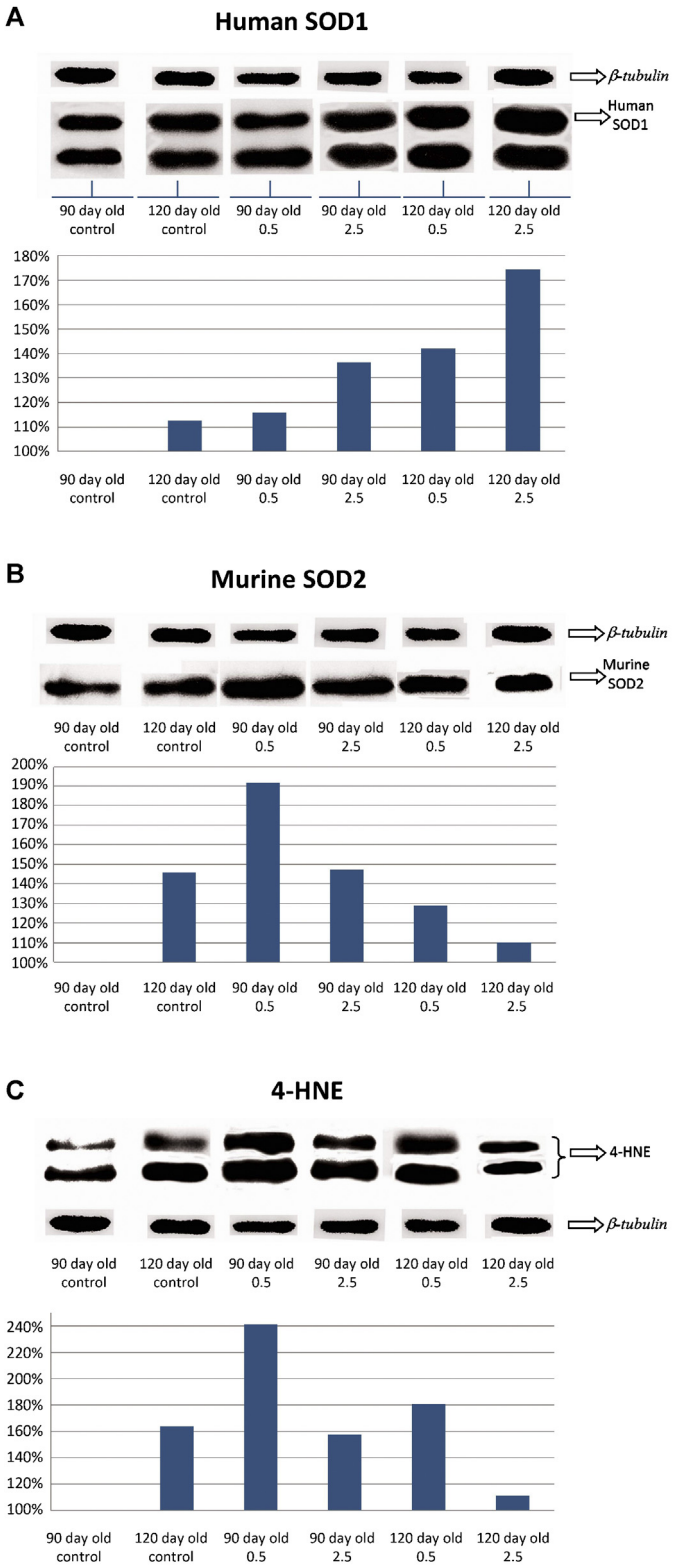

Fig. 2. Western blot of (A) human SOD1, (B) murine SOD2, and (C) 4-HNE.

was found to extend mean survival time and prolong disease progression. The protective effects of melatonin were lost at lower oral doses (≈ 9 mg/kg/day) or when treatment was delayed until the onset of disease symptoms (≈at age of 90 days). It is possible that at high doses, melatonin's direct anti-oxidant properties may over compensate for the upregulation of toxic hSOD1. Evidence to that effect comes from the observation that while hSOD1

Table 1
Real time PCR, measuring RNA of murine SOD2 and transgenic human SOD1.

Sacrificed time (days)	Treatment	RNA (ng/μl)	Relative quantity	
			mSOD2	hSOD1
90	Vehicle	308	0	0
91	Melatonin 2.5 mg/kg	199	0.38	0.35
90	Melatonin 50 mg/kg	124	0.62	5.74
120	Vehicle	133	0	0
121	Melatonin 2.5 mg/kg	237	0.36	0.25
120	Melatonin 50 mg/kg	127	0.61	3.94

upregulation at both 90 and 120 days was higher with 2.5 mg rather than 0.5 mg of melatonin the net level of oxidative stress was actually less with 2.5 mg versus the 0.5 dose (Fig. 2). There are also a number of methodological differences between the two studies that could partly account for the different results. We used daily, intraperitoneal injection of melatonin, during the first part of photophase while Weishaupt et al. [20] administered melatonin orally which was presumably drank by the mice under more physiological conditions, i.e. at night, during the active phase of the animals. In our study melatonin administration was started from day 40 of age, while in the study of Weishaupt et al. [20] melatonin was added in drinking water from day 28. It is possible that the stress of intraperitoneal injections per se could also have potentially acted in a detrimental way to the well being of the mice.

A relevant finding in our study, be it in the small number of animals examined, was the almost dose dependant motoneuron loss in the spinal cords of melatonin treated mice compared with untreated controls. In the study of Weishaupt et al. [20] no morphometric data on lumbar cord motor neurons in treated and untreated animals were provided and so we do not know the pathological substrate of the prolonged survival provided by high dose oral melatonin. Was prolonged survival the result of motor neuron preservation or was it due to other effects of melatonin? Melatonin been shown to improve motor performance of rats in an experimental model of depression, possibly through an interaction with central serotonin neurotransmission [10].

In the current study, melatonin treated G93ASOD1 animals were found to have increased levels of 4-HNE, a marker of oxidative stress, compared to vehicle animals. On the contrary, in a mouse model of cerebral ischemia–reperfusion injury, melatonin was found to reduce oxidative damage assessed by of 4-HNE [8]. Also, previous studies have shown increased levels of 4-HNE in the spinal cord of G93A SOD-1 transgenic mice [6,13] while effective treatment significantly suppressed 4-HNE immunoreactivity [16] suggesting a link between 4-HNE and motor neuron survival.

We have demonstrated, using immunocytochemistry, Western blot and real time PCR that melatonin up regulates toxic human SOD1 in the G93ASOD1 mice. This is in agreement with a study showing that administration of melatonin increases the expression of SOD1 in the lumbar spinal cord of neonatal rats [15].

Up regulation of mutant SOD1 in the G93ASOD1 ALS mouse model raises the possibility that this animal model may not be appropriate for assessing the neuroprotective properties of melatonin in ALS. Similarly in a rotenone model of Parkinson's disease, melatonin was proved detrimental by potentiating rotenone toxicity [19].

Admittedly there is no strict concordance between melatonin associated neuronal loss and shortened survival. This might be the result of the complex effects of melatonin on the behavior and biology of mice, its ability to up regulate anti oxidative enzymes, its direct anti-oxidant and anti-apoptotic properties or simply be due to the relatively small number of mice used. Furthermore, U-shaped effects of melatonin have been reported in other animal models [1,18]. Admittedly our results need confirmation in a larger number of animals.

5. Conclusions

We conclude that intraperitoneal melatonin at doses 0.5, 2.5 and 50.0 mg/kg does not appear to be neuroprotective and may possibly be detrimental to motor neurons in the G93ASOD1 mouse model of ALS. Our results can probably be explained by melatonin's unique dual mode of action. Our data imply that G93ASOD1 mouse model may not be an ideal model for assessing the neuroprotective properties of melatonin or other molecules with complex anti-oxidative properties.

Conflict of interest statement

The authors report no conflicts of interest.

Author contributions

All authors have approved the final article.

Participation of authors: Concept/design was done by T. Kyriakides. Acquisition of data was done by T. Kyriakides, E. Panayiotou, S. Malas, M. Feldman and A. Hadjisavvas. Analysis/interpretation was done by T. Kyriakides, E. Dardiotis, G. Hadjigeorgiou and I. Vonta. Drafting of the manuscript was done by E. Dardiotis, T. Kyriakides. E. Dardiotis and T. Kyriakides did critical revision of the manuscript and approved of the article.

Acknowledgement

The study was supported by a Cyprus TELETHON Grant.

References

[1] S.M. Beni, R. Kohen, R.J. Reiter, D.X. Tan, E. Shohami, Melatonin-induced neuroprotection after closed head injury is associated with increased brain antioxidants and attenuated late-phase activation of NF-kappaB and AP-1, FASEB Journal 18 (2004) 149–151.
[2] D.W. Cleveland, J.D. Rothstein, From Charcot to Lou Gehrig: deciphering selective motor neuron death in ALS, Nature Reviews Neuroscience 2 (2001) 806–819.
[3] M.E. Gurney, H. Pu, A.Y. Chiu, M.C. Dal Canto, C.Y. Polchow, D.D. Alexander, J. Caliendo, A. Hentati, Y.W. Kwon, H.X. Deng, W. Chen, P. Zhai, R.L. Sufit, T. Siddique, Motor neuron degeneration in mice that express a human Cu, Zn superoxide dismutase mutation, Science 264 (1994) 1772–1775.
[4] S. Jacob, B. Poeggeler, J.H. Weishaupt, A.L. Siren, R. Hardeland, M. Bahr, H. Ehrenreich, Melatonin as a candidate compound for neuroprotection in amyotrophic lateral sclerosis (ALS): high tolerability of daily oral melatonin administration in ALS patients, Journal of Pineal Research 33 (2002) 186–187.
[5] M.C. Kiernan, S. Vucic, B.C. Cheah, M.R. Turner, A. Eisen, O. Hardiman, J.R. Burrell, M.C. Zoing, Amyotrophic lateral sclerosis, Lancet 377 (2011) 942–955.
[6] J. Kim, T.Y. Kim, J.J. Hwang, J.Y. Lee, J.H. Shin, B.J. Gwag, J.Y. Koh, Accumulation of labile zinc in neurons and astrocytes in the spinal cords of G93A SOD-1 transgenic mice, Neurobiology of Disease 34 (2009) 221–229.
[7] A. Korkmaz, R.J. Reiter, T. Topal, L.C. Manchester, S. Oter, D.X. Tan, Melatonin: an established antioxidant worthy of use in clinical trials, Molecular Medicine 15 (2009) 43–50.

[8] E.J. Lee, M.Y. Lee, H.Y. Chen, Y.S. Hsu, T.S. Wu, S.T. Chen, G.L. Chang, Melatonin attenuates gray and white matter damage in a mouse model of transient focal cerebral ischemia, Journal of Pineal Research 38 (2005) 42–52.
[9] J.B. Liu, T.S. Tang, H.L. Yang, D.S. Xiao, Antioxidation of melatonin against spinal cord injury in rats, Chinese Medical Journal 117 (2004) 571–575.
[10] V. Micale, A. Arezzi, L. Rampello, F. Drago, Melatonin affects the immobility time of rats in the forced swim test: the role of serotonin neurotransmission, European Neuropsychopharmacology 16 (2006) 538–545.
[11] D. Ozdemir, K. Tugyan, N. Uysal, U. Sonmez, A. Sonmez, O. Acikgoz, N. Ozdemir, M. Duman, H. Ozkan, Protective effect of melatonin against head trauma-induced hippocampal damage and spatial memory deficits in immature rats, Neuroscience Letters 385 (2005) 234–239.
[12] Z. Pei, S.F. Pang, R.T. Cheung, Administration of melatonin after onset of ischemia reduces the volume of cerebral infarction in a rat middle cerebral artery occlusion stroke model, Stroke 34 (2003) 770–775.
[13] M. Perluigi, H. Fai Poon, K. Hensley, W.M. Pierce, J.B. Klein, V. Calabrese, C. De Marco, D.A. Butterfield, Proteomic analysis of 4-hydroxy-2-nonenal-modified proteins in G93A-SOD1 transgenic mice—a model of familial amyotrophic lateral sclerosis, Free Radical Biology and Medicine 38 (2005) 960–968.
[14] R.J. Reiter, D.X. Tan, L.C. Manchester, S. Lopez-Burillo, R.M. Sainz, J.C. Mayo, Melatonin: detoxification of oxygen and nitrogen-based toxic reactants, Advances in Experimental Medicine and Biology 527 (2003) 539–548.
[15] F. Rogerio, S.A. Teixeira, A.C. de Rezende, R.C. de Sa, L. de Souza Queiroz, G. De Nucci, M.N. Muscara, F. Langone, Superoxide dismutase isoforms 1 and 2 in lumbar spinal cord of neonatal rats after sciatic nerve transection and melatonin treatment, Brain Research. Developmental Brain Research 154 (2005) 217–225.
[16] J.S. Seo, I.S. Baek, Y.H. Leem, T.K. Kim, Y. Cho, S.M. Lee, Y.H. Park, P.L. Han, SK-PC-B70M alleviates neurologic symptoms in G93A-SOD1 amyotrophic lateral sclerosis mice, Brain Research 1368 (2011) 299–307.
[17] G. Shokouhi, R.S. Tubbs, M.M. Shoja, S. Hadidchi, A. Ghorbanihaghjo, L. Roshangar, R.M. Farahani, M. Mesgari, W.J. Oakes, Neuroprotective effects of high-dose vs low-dose melatonin after blunt sciatic nerve injury, Childs Nervous System 24 (2008) 111–117.
[18] S.H. Tai, Y.C. Hung, E.J. Lee, A.C. Lee, T.Y. Chen, C.C. Shen, H.Y. Chen, M.Y. Lee, S.Y. Huang, T.S. Wu, Melatonin protects against transient focal cerebral ischemia in both reproductively active and estrogen-deficient female rats: the impact of circulating estrogen on its hormetic dose–response, Journal of Pineal Research 50 (2011) 292–303.
[19] V. Tapias, J.R. Cannon, J.T. Greenamyre, Melatonin treatment potentiates neurodegeneration in a rat rotenone Parkinson's disease model, Journal of Neuroscience Research 88 (2010) 420–427.
[20] J.H. Weishaupt, C. Bartels, E. Polking, J. Dietrich, G. Rohde, B. Poeggeler, N. Mertens, S. Sperling, M. Bohn, G. Huther, A. Schneider, A. Bach, A.L. Siren, R. Hardeland, M. Bahr, K.A. Nave, H. Ehrenreich, Reduced oxidative damage in ALS by high-dose enteral melatonin treatment, Journal of Pineal Research 41 (2006) 313–323.