ELSEVIER

Neurobiology of Aging 23 (2002) 633–638

NEUROBIOLOGY OF AGING

www.elsevier.com/locate/neuaging

Dietary melatonin selectively reverses age-related changes in cortical cytokine mRNA levels, and their responses to an inflammatory stimulus

Kaizhi G. Sharman, Edward H. Sharman, Ellen Yang, Stephen C. Bondy*

Center for Occupational and Environmental Health, Department of Community and Environmental Medicine, University of California, Irvine, CA 92697-1825, USA

15 May 2001; revised 28 September 2001; accepted 03 October 2001

Abstract

The basal levels of expression of mRNA of cytokines, interleukin-6 (IL-6) and tumor necrosis factor (TNF-α), in the cerebral cortex of 5 and 26 month-old male B6C3F1 mice have been compared. In addition, the responsivity of animals of differing age to an inflammatory stimulus (lipopolysaccharide, LPS) has been studied. Basal levels of both of these cytokine mRNAs were elevated in aged animals relative to the younger group. However LPS administration led to a robust increase in cytokine mRNA levels in the younger animals but in aged mice, there was either an unchanged (IL-6) or a depressed (TNF-α) response. Administration of dietary melatonin (200 ppm) to aged mice for 6 weeks prior to sacrifice, resulted in reduction of basal levels of cytokine mRNA to values found in the younger animals. Furthermore, following administration of LPS to melatonin fed animals, cerebral cytokine mRNA levels were significantly elevated rather than being unchanged or depressed. Taken together these findings reflect a trend in the cortices of melatonin-treated aged mice, to more closely approximate the status of younger mice. For comparative purposes, parallel studies were carried out using an immunologically active organ (spleen) and a non-neural organ with a low rate of cell turnover (heart muscle). In both these tissues, basal levels of cytokine mRNAs of animals of either age were very low, and there was a marked positive response to LPS. Dietary melatonin had no effect on the responses of TNF-α mRNA to LPS but attenuated the reaction of splenic IL-6 mRNA, thus bringing the response closer to that of the younger mice.

Keywords: brain aging; melatonin; mRNA; cytokines; spleen; heart; inflammation

1. Introduction

There is evidence that the immune system may be compromised during aging [12]. In the central nervous system, part of this impairment may be expressed as an inappropriate and prolonged response to inflammatory stimuli [7]. Microglial cells also become progressively active with normal aging [39,45]. Lipopolysaccharide-induced chronic inflammation can cause extensive astrogliosis in the temporal lobe regions of the rat brain [51]. Such activation of the astroglial cells is associated with changes, which mirror those seen in the AD brain [21,51]. Age-related declines of function have also been associated with higher levels of oxidative damage [10,23,44]. Various antioxidant treatments have been proposed in an attempt to retard deleterious changes associated with senescence [16,18,20,40]. However, many antioxidants such as α-tocopherol and coenzyme Q [27] have limited access to the brain [50].

In view of its combination of lipophilic and hydrophilic properties, melatonin can readily cross the blood-brain barrier and has been proposed as a potentially neuroprotective agent [36]. Melatonin has been reported to have antioxidant activity [25,26]. It can act directly by scavenging reactive species such as hydroxyl [47], H_2O_2 [46], NO [48], and peroxynitrite [6,56]; or it can act indirectly by inducing antioxidant enzymes [25,38]. Extended deprivation of melatonin can lead to elevated levels of oxidative damage [37]. This agent can reduce the extent of injury to the CNS caused by a wide range of neurotoxicants including iron-induced necrosis [25], mercury [30], kainate [28], cyanide [52] and paraquat [29]. Melatonin levels decrease with age [33]. Since melatonin can prolong survival time of mice [2,31] – albeit associated with increased spontaneous tumor incidence according to one report [2] – this is likely to be relevant to the aging process [36], even if the exact mechanism is incompletely understood [54]. We have found

* Corresponding author. Tel.: +1-949-824-8077; fax: +1-949-824-2793.

E-mail address: scbondy@uci.edu (S. C. Bondy).

0197-4580/02/$ – see front matter
PII: S0197-4580(01)00329-3

dietary melatonin can prevent age-related changes in both cerebral mitochondrial function [42] and in levels of production of nitric oxide and reactive oxygen species (ROS) [9]. This agent has also been reported to reverse age-related behavioral changes [4]. However, melatonin may not be a direct antioxidant [26], but may act by enhancing gene expression of antioxidant enzymes [3].

A relation between oxidative stress and inflammatory events within nervous tissues has often been described [8,18]. In the current study, the effect of an inflammatory stimulus (LPS) on cortical levels of mRNAs for cytokines IL-6 and TNF-α, was studied in mice of two ages. Melatonin was shown to wholly or partially reverse age-related changes in both resting levels and inflammation-induced fluxes of these parameters.

2. Materials and methods

2.1. Animal treatment

Male B6C3F1 mice, a hybrid between C57BL/6 and C3H from Harlan Labs (Indianapolis, IN), aged 5 months (young group) and 26 months (old group), were housed six per cage and were maintained on a 12 h light/dark cycle in a temperature controlled (20 $\pm$ 1°C) room. Food and water were provided ad *lib*. to 3 animals per group. All food was in the form of pellets manufactured by compressing thoroughly mixed, finely powdered ingredients (Dyets Inc., Bethlehem, PA). Food was stored at 4°C in sealed, plastic bags as received from the manufacturer. The minimal basal diet (Dyets #101101) consisted of 50% sucrose and 26% casein (w/w) as well as a minimal salt and vitamin mix. For One group of mice was fed basal diet supplemented with 200-ppm (w/w) melatonin (Dyets #101475; melatonin from Sigma, St. Louis, Mo) for 6 weeks; based on measured food consumption, this provided an average melatonin dosage of 29 mg/kg/day for the young animals and 23 mg/kg/day for the old. Control groups were fed unsupplemented basal diet.

2.2. RNA extraction

Mice were killed by cervical dislocation; tissues were excised quickly, immediately frozen on dry ice (brains) or in liquid nitrogen (other organs) and stored at –70°C. Total RNA was extracted using the TRI REAGENT Kit (Molecular Research Center, Inc., Cincinnati, OH), following the manufacturer's protocol. RNA concentrations were determined by absorption at 260-nm wavelength. Purity was monitored by measuring the ratio of absorbance at 260 nm to that at 280 nm.

2.3. Preparation of cDNA probes

A rat IL-6 cDNA-containing plasmid (gift from Drs. Wolfgang Northemann and Georg Fey, Scripps Research Foundation, La Jolla, CA) was transformed into JM101 cells and then digested with *Pst*I and *Bam*HI restriction enzymes to obtain the insert for hybridization.

A murine TNF-α cDNA-containing plasmid (gift from George N. Davetelis) was transformed into JM101 cells. The TNF cDNA was inserted in the pUC9 plasmid at the Pst I and Bam HI restriction sites. For the purpose of hybridization, however, Pst I and Eco RI were used as restriction enzymes for the purification of the insert from the vector because Eco RI cleaves off a portion of the 3′-untranslated region of the insert that contains a TTATTTATT consensus sequence that is also common to IL-1 and other cytokines [7] and might result in unwanted cross-hybridization.

2.4. Northern blot analysis

Aliquots of total RNA (10 μg each, as determined from absorbance at 260 nm wavelength and verified by gel ethidium bromide fluorescence intensity) were denatured with formaldehyde and formamide, electrophoresed on 1.2% agarose gel containing 6% formaldehyde and transferred onto nylon Zeta-probe blotting membranes (Bio-Rad Laboratories, Hercules, CA). This standardization was selected in preference to use of housekeeping genes, actin-γ or glyceraldehyde phosphate dehydrogenase (GAPDH). This was because these genes were responsive to varying age and to melatonin supplementation (data not shown). Membrane-bound RNA was then hybridized with one of the cDNA probes labeled with [^{32}P]dCTP using the RTS Radprime System (Life Technologies, Rockville, MD) to yield a specific activity of approximately 10^9 cpm/μg. The membranes were autoradiographed for periods varying from 8 h to 7 days at –70°C on x-ray film (X-OMAT AR, Kodak, and Rochester, NY). A densitometer (Eagle Eye image-processor combined with DNA Scan signal analysis software, Stratagene, San Diego, CA) was used to quantify the signals as area-integrated optical density.

2.5. Statistical analyses

Differences between groups were assessed by one-way Analysis of Variance followed by Gabriel's Test. The acceptance level of significance was $P < 0.05$ using a two-tailed distribution.

3. Results

IL-6 and TNF-α mRNA expression levels were measured in brain and also, for comparative purposes, parallel studies were carried out using an immunologically active tissue (spleen) and a non-neural tissue with a similarly low rate of cell turnover (heart muscle).

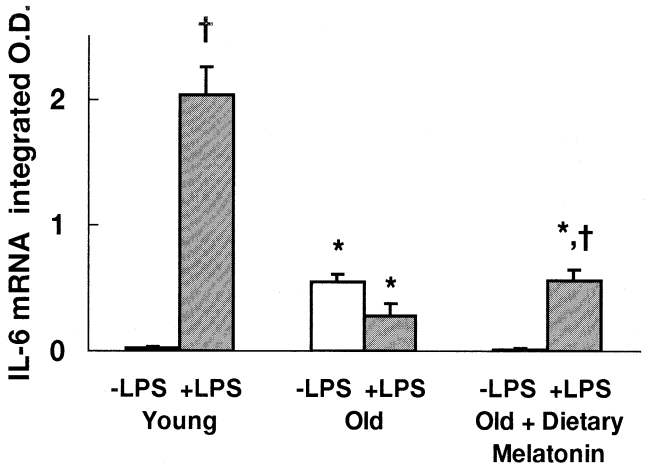

Fig. 1. Levels of IL-6 mRNA in cerebral cortex of 5 and 26 month old mice. Values were densitometrically quantitated and are expressed as units of integrated optical density ± standard error (n = 3). Some animals received a prior injection of lipopolysaccharide (LPS) while two groups of older animals were fed a melatonin-containing diet. * = differs significantly from corresponding value for 5 month-old mice. † = differs significantly from value for corresponding group not treated with LPS.

3.1. Basal levels

Unstimulated levels of both of these cytokine mRNAs were elevated in the brains of aged animals relative to the younger group (Figs. 1, 2). This was not the case for heart (Fig. 3) and spleen (Fig. 4).

3.2. Lipopolysaccharide (LPS) treatment

LPS administration led to a robust increase in cerebral cytokine mRNA levels in the younger animals, but not in aged mice, where LPS resulted in either an unchanged (IL-6, Fig. 1) or a depressed cytokine mRNA level (TNF-α, Fig. 2). In both heart and spleen of animals of either age, levels of both cytokine mRNAs were greatly elevated following LPS injection (Figs. 3, 4).

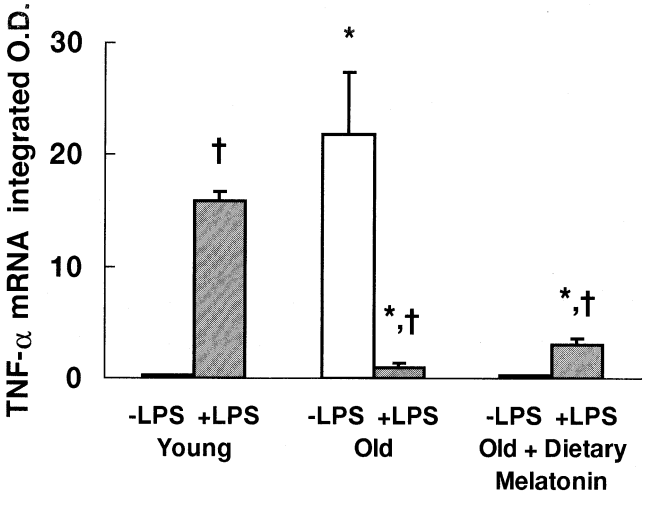

Fig. 2. Levels of TNF-α mRNA in cerebral cortex of 5 and 26 month old mice. Values were densitometrically quantitated and are expressed as units of integrated optical density ± standard error (n = 3). Some animals received a prior injection of lipopolysaccharide (LPS) while two groups of older animals were fed a melatonin-containing diet. * = differs significantly from corresponding value for 5 month-old mice. † = differs significantly from value for corresponding group not treated with LPS.

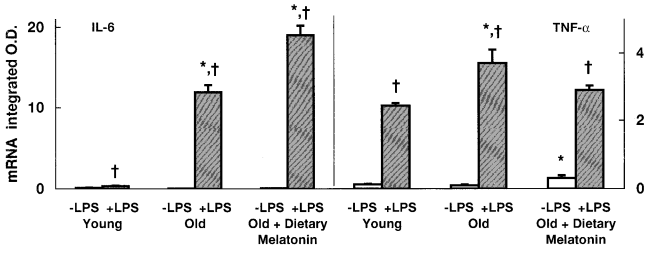

Fig. 3. Levels of IL-6 mRNA and TNF-α in heart of 5 and 26 month old mice. Values were densitometrically quantitated and are expressed as units of integrated optical density ± standard error (n = 3). Some animals received a prior injection of lipopolysaccharide (LPS) while two groups of older animals were fed a melatonin-containing diet. * = differs significantly from corresponding value for 5 month-old mice. † = differs significantly from value for corresponding group not treated with LPS.

3.3. Dietary melatonin

Administration of dietary melatonin (200 ppm) to aged mice for 6 weeks prior to sacrifice, resulted in restoration of basal levels of cerebral cytokine mRNA to values found in the younger animals. Furthermore, in melatonin-treated older mice, cerebral cytokine mRNA levels were significantly elevated by administration of LPS rather than being unchanged or depressed (Figs. 1, 2). In heart and spleen, dietary melatonin had no effect on the responses of TNF-α mRNA to LPS (Figs. 3, 4). The reaction of splenic IL-6 mRNA to LPS, was attenuated in melatonin-treated aged mice, thus bringing the response closer to that of the younger mice (Fig. 4).

4. Discussion

In all tissues studied, basal levels of cytokine mRNAs of young animals were very low, but in the brain, were ele-

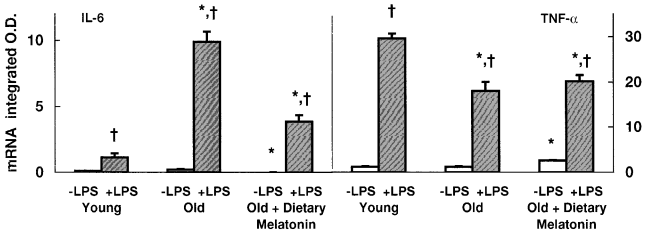

Fig. 4. Levels of IL-6 mRNA and TNF-α mRNA in spleen of 5 and 26 month old mice. Values were densitometrically quantitated and are expressed as units of integrated optical density ± standard error (n = 3). Some animals received a prior injection of lipopolysaccharide (LPS) while two groups of older animals were fed a melatonin-containing diet. * = differs significantly from corresponding value for 5 month-old mice. † = differs significantly from value for corresponding group not treated with LPS.

vated with age. In line with the current study, there are several other reports of a modest elevation of non-stimulated IL-6 levels within the aging brain [19,53]. There is previous*ly published* evidence that systemically administered LPS is able to rapidly and directly target the CNS [21, 15, 22, 49].

In tissues from young animals there was a marked positive response of TNF-α expression to LPS, and this was maintained with age in heart and spleen. Our young-animal results parallel reported changes in serum TNF-α protein levels, which were increased five-fold by LPS administration [5]. However, we found this reactivity to be absent in the cortex of aged mice. Our finding of a weaker response to LPS in the cortex of older animals is in accord with the reported ability of LPS to increase the number of activated microglia and impair behavior in young, but not old rats [17].

In aged mice, a large response of IL-6 mRNA expression to LPS treatment occurred in heart and spleen tissue. This heightened reaction has also been described in several other tissues including lung and kidney [43]. In contrast, cerebral IL-6 mRNA levels of aged mice following LPS exposure, were greatly attenuated relative to the young. This suggests that, while hyper-reactivity of immune responses may characterize many aged organ systems, this is not the case for the central nervous system. It appears that in the brain, aging may be associated with high basal levels of cytokine mRNAs, rather than increased susceptibility to induction. This high background and attenuated response implies a reduced signal to noise ratio, which may have adverse physiological consequences.

Melatonin had no pronounced effect on the basal or induced levels of mRNAs for cytokines of the non-neural tissues studied. However, in the cortices of melatonin-treated aged mice, levels of both cytokine mRNAs were depressed to values paralleling those present in younger mice.

There is evidence that melatonin can enhance immune processes in cells derived from aged animals [24,55]. Chronic melatonin administration led to an increased IL-1 and TNF-α production and enhanced T-cell-promoted antigen presentation by splenic macrophages [32]. This sensitization of circulating components of the immune system by melatonin was not paralleled in spleen tissue but was reflected within the CNS following melatonin treatment by enhanced positive reactivity to an inflammatory stimulus (Figs I and 2).

The effect of melatonin upon the levels of cerebral cytokine peptides has not yet been investigated but is an obvious and necessary sequel to the current work. Although ROS play a significant role in inflammation and melatonin can clearly scavenge ROS [34], the regional specificity of the findings described here suggests that antioxidant effects did not directly mediate the effects observed.

It is likely but not proven, that restoration of inflammatory responses within the brains of aged animals, to those found in younger animals, is a beneficial event. This is supported by the report that normal age-related increases in circulating TNF-α and IL-6 may be exacerbated with neurological disease [13].

An optimal situation may be low intrinsic levels of cytokines accompanied by a rapid ability to respond to a stimulus. However, a response that is excessively prolonged, may be a component of several neurodegenerative disorders associated with aging, notably Alzheimer's disease [7]. Therefore, the temporal nature of the effect of melatonin upon induction of inflammatory cytokines is being investigated in isolated cell lines of neural origin.

Acknowledgments

This work was supported in part by grants from the National Institutes of Health (ES 7992, AG 16794 and AG 14882).

References

[1] Akiyama H, Barger S, Barnum S, Bradt B, Bauer J, Cole GM, Cooper NR, Eikelenboom P, Emmerling M, Fiebich, BL, Finch CE, Frautschy S, Griffin WS, Hampel H, Hull M, Landreth G, Lue L, Mrak R, Mackenzie IR, McGeer PL, O'Banion MK, Pachter J, Pasinetti G, Plata-Salaman C, Rogers J, Rydel R, Shen Y, Streit W, Strohmeyer R, Tooyoma I, Van Muiswinkel FL, Veerhuis R, Walker D, Webster S, Wegrzyniak B, Wenk G, Wyss-Coray T. Inflammation and Alzheimer's disease. Neurobiol Aging 2000;21:383–421.

[2] Anisimov VN, Zavarzina Niu, Zabezhinskii MA, Popovich IG, Anikin IV, Zimina OA, Solov'ev MV, Shtylik AV, Arutiunian AV, Oparina TI, Prokopenko VM, Khavinson VKh. The effect of melatonin on the indices of biologic age, on longevity and on the development of spontaneous tumors in mice. Voprosy Onkologii 2000; 46(3):311–9.

[3] Antolin I, Rodriguez C, Sainz RM, Mayo JC, Uria H, Kotler MM, Rodriguez-Colunga HA, Toliver D, Menendez-Palaez A. Neurohormone melatonin prevents cell damage: effect on gene expression for antioxidant enzymes. FASEB J 1996;10:882–890.

[4] Araghi-Niknam M, Lane L, Watson RR. Modification of physical movement in old C57BL/6 mice by DHEA and melatonin supplementation. Proc Soc Exptl Med Biol 1999;221:193–7.

[5] Ben-Nathan D, Maestroni GJM, Conti A. The protective effect of melatonin in viral and bacterial infections. In: Maestroni GJM, Conti A, Reiter RJ, editors. Therapeutic potential of melatonin. Basel: Karger, 1997. pp. 72–80.

[6] Blanchard B, Pompon D, Ducrocq C. Nitrosation of melatonin by nitric oxide and peroxynitrite. Journ of Pineal Res 2000;(3):184–92.

[7] Bondy SC, Campbell A. Initiation of futile pathways common to many neurological diseases. In: Marwah J, Lo E, editors. Neuroprotection. Scottsdale: Prominent Press, 2002. In press.

[8] Bondy SC, Campbell A. Oxidative and inflammatory properties of aluminum: possible relevance in Alzheimer's disease. In: Exley C, editor. Aluminum in Alzheimer's Disease. New York: Elsevier Press, 2001;311–21.

[9] Bondy SC, Yang YE, Walsh TJ, Gie YW, Lahiri DK. Dietary modulation of age-related changes in cerebral pro-oxidant status. Neurochem Int 2002;40:123–30.

[10] Butterfield DA, Howard BJ, Yatin S, Allen KL, Carney JM. Free radical oxidation of human proteins in accelerated senescence and its

modulation by N-tert-butyl-α-phenylnitrone. Proc Nat Acad Sci 1997;94:674–8.
[11] Caput D, Beutler B, Hartog K, Thayer R, Brown-Shimer S, Cerami A. Identification of a common nucleoside sequence in the 3′-untranslated region of mRNA molecules specifying inflammatory mediators. Proc Nat Acad Sci 1986;83:1670–4.
[12] Chakravarti B, Abraham GN. Aging and T-cell-mediated immunity. Mech Ageing Develop 1999;108:183–206.
[13] Dobbs RJ, Charlett A, Purkiss AG, Dobbs SM, Weller C, Peterson DW. Association of circulating TNF-α and IL-6 with ageing and parkinsonism. Acta Neurol Scand 1999;100:34–41.
[14] Floyd RA, Hensley K. Nitrone inhibition of age-associated oxidative damage. Ann NY Acad Sci 2000;899:222–37.
[15] Gabellec MM, Griffais R, Fillion G, Haour F. Expression of interleukin 1 alpha, interleukin 1 beta and interleukin 1 receptor antagonist mRNA in mouse brain: regulation by bacterial lipopolysaccharide (LPS) treatment. Brain Research. Mol Brain Res 1995;(1–2):122–30.
[16] Hagen TM, Ingersoll RT, Lykkesfeldt, Lui J, Wehr CM, Vinasksky V, Bartholomew JC, Ames BN. (R)-α-lipoic acid-supplemented old rats have improved mitochondrial function, decreased oxidative damage and increased metabolic rate. FASEB J 1999;13:441–8.
[17] Hauss-Wegrzyniak B, Vraniak P, Wenk GL. The effects of a novel NSAID on chronic neuroinflammation are age dependent. Neurobiol Aging 1999;20:305–13.
[18] Hensley K, Pye QN, Tabatabaie T, Stewart CA, Floyd RA. Reactive oxygen involvement in neurodegenerative pathways. In: Wood PL, editor. Neuroinflammation. New Jersey: Humana Press, 1998. pp. 265–80.
[19] Hull M, Strauss S, Berger M, Volk B, Bauer J. The participation of IL-6, a stress-inducible cytokine, in the pathogenesis of Alzheimer's disease. Behav Brain Res 1996;78:37–41.
[20] Joseph JA, Shukitt-Hale B, Denisova NV, Prior RL, Cao G, Martin A. Long term dietary strawberry, spinach or vitamin E supplementation retards the onset of age-related neuronal signal-transduction and cognitive behavioral deficits. J Neurosci 1998;18:8047–55.
[21] Lacroix S, Feinstein D, Rivest R. The bacterial endotoxin lipopolysaccharide has the ability to target the brain in upregulating its membrane CD14 receptor within specific cellular populations. Brain Pathol 1998;8:625–40.
[22] Laye S, Bluthe RM, Kent S, Combe C, Medina C, Parnet P, Kelley K, Dantzer R. Subdiaphragmatic vagotomy blocks induction of IL-1 beta mRNA in mice brain in response to peripheral LPS. Am Journ Phys 1995;268(5 Pt 2):R1327–R1331.
[23] LeBel CP, Bondy SC. Oxidative damage and cerebral aging. Prog Neurobiol 1992;38:601–2.
[24] Liebmann PM, Wolfer A, Felsner P, Hofer D, Schauenstein K. Melatonin and the immune system. Int Arch Allergy Immunol 1997;112: 203–11.
[25] Lin AMY, Ho LT. Melatonin suppresses iron-induced neurodegeneration in rat brain. Free Rad Biol Med 2000;28:904–11.
[26] Longoni B, Salgo MG, Pryor WA, Marchiafava PL. Effect of melatonin on lipid peroxidation induced by oxygen radicals. Life Sci 1998;62:853–9.
[27] Matthews RT, Yong L, Browne S, Baik M, Beal MF. Coenzyme Q10 administration increases brain mitochondrial concentrations and exerts neuroprotective effects. Proc Nat Acad Sci 1998;95:8892.
[28] Melchiorri D, Reiter RJ, Chen LD, Sewerynek E, Nistico G. Melatonin affords protection against kainate-induced in vitro lipid peroxidation in brain. Europ J Pharmacol 1996a;305:239–42.
[29] Melchiorri D, Reiter RJ, Sewerynek E, Hasa M, Chen L, Nistico, G. Paraquat toxicity and oxidative damage. Reduction by melatonin. Biochem Pharmacol 1996b;51:1095–9.
[30] Oliveri G, Brack G, Muller-Spahn F, Stahelin HB, Hermann M, Renard P, Brockhaus M, Hock C. Mercury induces cell cytotoxicity and oxidative stress and increases β-amyloid secretion and tau phosphorylation in 5H5Y5Y neuroblastoma cells. J Neurochem 2000;74: 231–6.
[31] Pierpaoli W, Regelson W. Pineal control of aging: effect of melatonin and pineal grafting on aged mice. Proc Nat Acad Sci 1994;91:787–91.
[32] Pioli C, Caroleo MC, Nistico G, Doria G. Melatonin increases antigen presentation and amplifies specific and non-specific signals for T-cell proliferation. Int J Immunopharmacol 1993;15:463–8.
[33] Reiter RJ. Oxidative damage in the nervous system: protection by melatonin. Prog Neurobiol 1998;56:359–84.
[34] Reiter RJ, Calvo JR, Karbownik M, Qi W, Tan DX. Melatonin and its relation to the immune system and inflammation. In: Conti A, Maestroni GJM, McCann SM, Sternberg EM, Lipton JM, Smith CC, editors. Neuroimmunomodulation: perspectives at the new millennium. Ann NY Acad Sci 2000;917:376–386.
[35] Reiter RJ, Craft CM, Johnson JE, King TS, Richardson BA, Vaughn GM, Vaughn MK. Age-associated reduction in nocturnal pineal melatonin levels in female rats. Endocrinology 1981;109:1295–7.
[36] Reiter RJ, Guerreo JM, Garcia JJ, Acuña-Castroviejo D. Reactive oxygen intermediates, molecular damage and aging; relation to melatonin. Ann NY Acad Sci 1998;854:410–24.
[37] Reiter RJ, Tan D, Kim SJ, Manchester LC, Qi W, Garcia JJ, Cabrera JC, El-Sokkary G, Rouvier-Garay V. Augmentation of indices of oxidative damage in life-long melatonin deficient rats. Mech Aging Develop 1999;110:157–73.
[38] Reiter RJ, Tang L, Garcia JJ, Muñoz-Hoyos A. Pharmacological actions of melatonin in oxygen radical pathophysiology. Life Sciences 1997;60(25): 2255–71
[39] Rozovsky I, Finch CE, Morgan TE. Age-related activation of microglia and astrocytes: in vitro studies show persistent phenotypes of aging, increased proliferation, and resistance to down-regulation. Neurobiol Aging 1998;19:97–103.
[40] Sack CA, Socci D, Crandall BM, Arendash GW. Antioxidant treatment with phenyl-α-tert-butyl nitrone (PBN) improves the cognitive performance and survival of aging rats. Neurosci Lett 1996;205:181–4.
[41] Sewerynek E, Ortiz GG, Reiter RJ, Pablos MI, Melchiorri D, Daniels WMU. Lipopolysaccharide-induced DNA damage is greatly reduced in rats treated with the pineal hormone melatonin. Mol Cell Endocrinol 1996;117:183–8.
[42] Sharman E, Bondy SC. Effects of dietary antioxidants and age on cortical electron transport chain activity. Neurobiol Aging, 2001;22: 629–34.
[43] Sharman K, Sharman E, Campbell A, Bondy SC. Reduced basal levels and enhanced LPS response of IL-6 mRNA in aged mice. Journal of Gerontology: Biologic Sciences 2001;56:8520–8523.
[44] Sohal RS. Role of mitochondria and oxidative stress in the aging process. In: Beal MF, Howell N, Bodis-Wollner I, editors. Mitochondria, Free Radicals, and Neurodegenerative Disease. New York: Wiley-Liss, 1997. pp. 91–107.
[45] Streit WJ, Walter SA, Pennell NA. Reactive microgliosis. Prog Neurobiol 1999;57:563–581.
[46] Tan D.-X, Manchester LC, Reiter RJ, Plummer BF, Limson J, Weintraub ST, Qi W. Melatonin directly scavenges hydrogen peroxide: a potentially new metabolic pathway of melatonin biotransformation. Free Rad Biol Med 2000;29(11):1177–85.
[47] Tan DX, Manchester LC, Reiter RJ, Plummer BF, Hardies LJ, Weintraub ST, Vijayalaxmi, Shepherd AM. A novel melatonin metabolite, cyclic 3-hydroxymelatonin: a biomarker of in vivo hydroxyl radical generation. Biochem Biophys Res Comm 1998;253(3):614–20.
[48] Turjanski AG, Leonik F, Estrin DA, Rosenstein RE, Doctorovich F. Scavenging of NO by melatonin. J Am Chem Soc 2000;122:10468–9.
[49] van Dam AM, Poole S, Schultzberg M, Zavala F, Tilders FJ. Effects of peripheral administration of LPS on the expression of immunoreactive IL-1 alpha, beta, and receptor antagonist in rat brain. Ann NY Acad Sci 1998; 840:128–8.
[50] Vatassery GT, Fahn S, Kuskowski MA. Alpha-tocopherol in cerebrospinal fluid of subjects on high dose vitamin E in the DATATOP study. Neur 1998;50:1900–2.

[51] Wegrzyniak BH, Dobrzanski P, Stoehr JD, Wenk GL. Chronic neuroinflammation in rats reproduces components of the neurobiology of Alzheimer's disease. Brain Res 1998;780:294–303.
[52] Yamamoto H, Hai-wang T. Antagonistic effect of melatonin against cyanide-induced seizures and acute lethality in mice. Toxicol Lett 1996;87:19–24.
[53] Ye SM, Johnson RW. Increased IL-6 expression by microglia from brain of aged mice. J Neuroimmunology 1999;93:139–48.
[54] Yu BP. Approaches to anti-aging intervention: the promises and the uncertainties. Mech Age Dev 1999;111:73–87.
[55] Zhang Z, Inserra PF, Liang B, Ardestani SK, Elliott KK, Molitor M, Watson RR. Melatonin, immune modulation and aging. Autoimmun 1997;26:43–53.
[56] Zhang, H, Squadrito GL. Uppu R, Pryor WA. Reaction of peroxynitrite with melatonin: A mechanistic study. Chem Res Tox 1999;12: 526–34.