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Research paper

Incorporation in polymeric nanocapsules improves the antioxidant effect of melatonin against lipid peroxidation in mice brain and liver

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Abstract

It has been recently shown that the association of melatonin with polymeric nanoparticles causes a significant increase of the in vitro effect against lipid peroxidation. Hence, the aim of the present study was to compare the in vivo acute antioxidant effect of intraperitoneal administration of melatonin-loaded polysorbate 80-coated nanocapsules with that of melatonin aqueous solution in mice brain (frontal cortex and hippocampus) and liver. The lipid peroxidation through thiobarbituric acid reactive substance levels, the total antioxidant reactivity (luminol-enhanced chemiluminescence) and the free radical levels (formed dichlorofluorescein) has been carried out. Our results show that a single melatonin aqueous solution injection exerted no antioxidant activity in the evaluated range, while the administration of the melatonin-loaded polysorbate 80-coated nanocapsules caused a marked reduction on lipid peroxidation levels in all studied tissues. No differences on free radical content were found in the tissues. The melatonin-loaded nanocapsules also increased the total antioxidant reactivity in the hippocampus. These in vivo results are in accordance with our previous in vitro findings and confirm the hypothesis that polymeric nanocapsules improve the antioxidant effect of melatonin against lipid peroxidation.

Keywords: Melatonin; Polymeric nanocapsules; Lipid peroxidation; Total antioxidant reactivity; Brain; Liver

1. Introduction

Melatonin, a neurohormone that is synthesized mainly in the pineal gland and derived from serotonin, has many effects on a wide range of physiopathological functions; its secretion is controlled by the suprachiasmatic nucleus through the photoperiod [1,2]. Melatonin regulates the sleep/wake cycle, other circadian and seasonal rhythms, and acts as an immunostimulator and cytoprotective agent [3,4]. In addition, in both cultured cells and organs, as well as in in vivo systems, it has proved its capacity as a potent antioxidant [4,5].

Melatonin is a free radical scavenger, since it directly neutralizes toxic oxygen- and nitrogen-based reactants. It is effective in protecting nuclear DNA and membrane lipids from oxidative damage (lipid peroxidation), in upregulating antioxidant enzyme activities [6–8] and in downregulating pro-oxidant enzymes [4], so improving the total antioxidant defense capacity of the organism. Melatonin favorably influences the production and regeneration of glutathione, a major intracellular antioxidant [9]. It also increases the efficiency of the electron transport chain in the inner mitochondrial membrane with a consequent reduction of free radical generation [9,10]. Within cell

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membranes, melatonin can scavenge free radicals in both the lipid and aqueous environments of the cell [9], and shows stabilizing action, protecting them against the oxidative stress reduction in the fluidity [7].

Melatonin has also been shown to be potentially effective in ameliorating or preventing several induced oxidative stress conditions, exerting hepatic protective effect, as tissue damage and fibrosis associated with thioacetamide-induced liver cirrhosis [11], liver injury in streptozotocin-induced diabetic [12] and carbon tetrachloride-induced hepatic fibrogenesis [13].

The efficacy of melatonin in inhibiting oxidative damage has been tested in a variety of neurological disease models, in which free radicals are believed to play a causative role on the condition [7]. It also presents neuroprotective actions [14] against a variety of neural toxins, hyperbaric hyperoxia, traumatic brain injury [7], ischemia/reperfusion injury [15] and age-associated neurodegenerative diseases [4,14], such as Alzheimer's [7,10,16], Parkinson's [7,10], and Huntington's diseases [4,10].

Over the past few decades, there has been considerable interest in developing polymeric nanoparticles (nanocapsules and nanospheres) as effective drug delivery devices. In these submicron systems (<1 μm), the drug is dissolved, entrapped, encapsulated and/or adsorbed or attached to the polymeric particles [17,18]. Nanocapsules are vesicular systems composed of an oil-filled cavity surrounded by a polymeric wall, while nanospheres consist of a polymer matrix. Both nanoparticles present narrow particle size distribution and are stabilized by surfactants at the particle/water interface [18,19]. The advantages of nanoparticles include the improvement of therapeutic index [20], the delivery of poorly water-soluble compounds [21], the increase of bioavailability [22] and the protection of mucosa from drug toxicity [23,24]. As regards to antioxidant substances, their encapsulation [25–27] improves in vitro antioxidant drug effects [28–30]. According to Palumbo and co-workers [28], the improvement of antioxidant effect of idebenone-loaded polymeric nanocapsules is probably due to the adherence of nanostructure to the cells, allowing the lipophilic drug associated to diffuse into cellular and subcellular structures. In our previous work, the presence of polymeric nanostructures was important for increasing the melatonin protection against lipid peroxidation [29]. The ability of nanosphere-encapsulated vitamin E to quench Abeta-induced reactive oxygen species (ROS), even when applied up to an hour following Abeta exposure, suggests that vitamin E is able to quench cytosolic ROS following nanosphere-mediated delivery [30].

Nanoparticles have also been studied as drug carriers for brain delivery due to their ability to provide drug access across the blood–brain barrier (BBB) for non-transportable drugs by masking their physicochemical characteristics as a result of nanoencapsulation [18]. Nanoparticles can also be used as parenteral controlled release systems to prolong the CNS availability of drugs that freely penetrate the BBB but have a short duration of action [31]. In order to achieve a significant transport across the BBB, the hydrophobic surfaces of the nanoparticles must be coated with hydrophilic molecules or macromolecules. The nanoparticles become hydrophilic enough to escape, at least partially, from the adsorption of the plasmatic proteins (opsonization) and consequent recognition by the mononuclear phagocyte system, as well as by the macrophages of the liver and spleen [18,32]. Hence, polymeric nanoparticle surface coating has been employed for brain targeting either using hydrophilic surfactants as polysorbate 80 [31,33–41] or by direct chemical bound with poly(ethylene glycol) (block copolymers) [42,43].

We have recently developed new drug delivery systems for melatonin based on its encapsulation in polymeric nanocapsules or nanospheres [29,44]. Melatonin-loaded polysorbate 80-coated Eudragit S100®-nanoparticles provided an important increase in the in vitro effect of melatonin against lipid peroxidation, in comparison with the drug aqueous solution [29]. Given this, the aim of this study was to evaluate the in vivo acute antioxidant capacity (lipid peroxidation, total antioxidant reactivity and free radical levels) of melatonin-loaded polysorbate 80-coated nanocapsules in the brain and liver of mice as compared to the drug aqueous solution. Brain tissue (frontal cortex and hippocampus) was evaluated because of the promising neuroprotective effect of melatonin and the applicability of nanoparticles for brain drug delivery. Hence, the hypothesis considers that the in vivo brain antioxidant activity of melatonin can be increased by its encapsulation in polysorbate 80-coated nanocapsules.

2. Materials and methods

2.1. Chemicals

Melatonin and Eudragit S100® [poly(methacrylic acid-co-methyl methacrylate)] were obtained from Acros Organics (Belgic) and Röhm GmbH (Germany), respectively. Caprylic/capric triglyceride was obtained from Brasquim (Brazil) and sorbitan monooleate (Span 80®) was supplied by Sigma (USA). Polysorbate 80 (Tween 80®) was acquired from Delaware (Brazil). Thiobarbituric acid and Trolox were obtained from Merck, 2,2′-azo-bis-(2-amidinopropane) dihydrochloride (ABAP) was obtained from Wako Chemicals USA, Inc., 2′,7′-dichlorofluorescein diacetate (DCFH-DA), 2′,7′-dichlorofluorescein (DCF), trichloroacetic acid (TCA), 5-amino-2,3-dihydro-1,4-phtalazinedione (luminol) were purchased from Sigma Chemical Co. All other chemicals and solvents used were of pharmaceutical grade; all reagents were used as received.

2.2. Animals

Forty-five days old (CF-1) male mice (average body weight 34 g), obtained from FEPPS (Fundação Estadual de Produção e Pesquisa em Saúde, Porto Alegre, RS), were maintained in a colony under constant room temperature

(22 ± 2 °C) on a 12-h light-dark cycle and with food and water ad libitum. The experiments were conducted using healthy animals, without any previous treatment for induction of the oxidative stress. The Animal Care Committee approved all handling and experimental conditions.

2.3. Preparation of nanocapsule suspensions and melatonin aqueous solution

Nanocapsules containing melatonin were prepared by interfacial deposition of preformed polymer, as described by Fessi et al. [45]. Briefly, the organic solution consisted of 0.8 mL of oil (caprylic/capric triglyceride), 250 mg of polymer (Eudragit S100®), melatonin (25 mg), 192 mg of surfactant (Span 80®) and 67 mL of acetone. This organic phase was added under magnetic stirring into an aqueous solution (133 mL) containing 192 mg of surfactant (Tween 80®). Acetone was removed and the suspension concentrated by evaporation under reduced pressure and the final volume of suspension was adjusted to a total volume of 25 mL [29]. Melatonin aqueous solution (1 mg/mL) was prepared using 0.77 % (w/v) of polysorbate 80.

2.4. Physicochemical characterization of the nanocapsules

2.4.1. Particle size

The mean size and polydispersity of nanocapsules were determined by photon correlation spectroscopy (PCS). For PCS measurements, 20 μL of each suspension was diluted to 10 mL in MilliQ® water [46]. Measurements were made at room temperature (20 °C) and fixed angle of 90°, using a Brookheaven Instruments standard setup (BI-200M goniometer, BI-9000AT digital correlator and a BI9863 detection system). A Spectra Physics He–Ne laser (model 127, $\lambda_0 = 632.8$ nm) was used as light source.

2.4.2. Zeta potentials

The zeta potentials were determined after dilution of the samples in 1 mmol L^{-1} NaCl aqueous solution using Zetasizer® Nano Series (ZEN 3600 model, Malvern Instruments).

2.4.3. Transmission electron microscopy

The morphological analysis was conducted by transmission electron microscopy (TEM; Jeol, JEM 1200 Exll, Centro de Microscopia Eletrônica UFRGS) operating at 80 kV. For this analysis, the diluted suspension was deposited in Formvar-Carbon support film on specimen grid (Electron Microscopy Sciences) and negatively stained with 2% (w/v) uranyl acetate solution [44].

2.4.4. Determination of associated melatonin

Melatonin was assayed by high-performance liquid chromatography (Waters, USA; pump 600 controller; 2487 Dual λ absorbance detector; 717 plus autosampler). A Merck column LiChrospher® 100 RP-18 (5 μm; 250 × 4 mm, Germany) was employed. The mobile phase consisted of acetonitrile/water (55:45, v/v). The total sample volume injected was 20 μL and melatonin was detected at 229 nm [44].

Free melatonin (non-associated) was determined in the ultrafiltrate after separation of the nanocapsules by ultrafiltration–centrifugation technique (Ultrafree-MC® 10,000 MW, Millipore), at 15,300*g* for 10 min. The total content of melatonin in the nanocapsule suspension was determined using HPLC after dissolution by acetonitrile. The associated melatonin with the nanocapsules was calculated from the difference between the total and the free drug concentrations determined in the nanocapsule suspension and in the ultrafiltrate, respectively [29,44].

2.5. In vivo experiments

Melatonin (1 or 10 mg/kg) associated with polysorbate 80-coated nanocapsules or in aqueous solution was intraperitoneally administered to mice ($n = 6$). Unloaded polysorbate 80-coated nanocapsules (without drug) and an aqueous solution containing 0.77% (w/v) polysorbate 80 were used as controls. The experiments were conducted between 11.00 am and 3.00 pm. One hour after the administration, animals were decapitated and the brain regions (frontal cortex and hippocampus), as well as the liver, were removed and instantaneously placed in liquid nitrogen and stored at −70 °C until biochemical assays. At the day of measurements, the tissues were homogenized in ice-cold pH 7.4 phosphate buffer solution (100 mmol L^{-1}) containing 140 mmol L^{-1} KCl and 1 mmol L^{-1} EDTA, using a Teflon-glass homogenizer. The homogenates were centrifuged (960*g* for 10 min at 4 °C) and supernatants were used in the assays.

2.6. Assay of lipid peroxidation

Lipid peroxidation was evaluated by thiobarbituric acid reactive substances (TBARS) test [47]. Aliquots of samples were incubated with 10% (w/v) trichloroacetic acid (TCA) and 0.67% (w/v) thiobarbituric acid (TBA). The mixture was heated (30 min) using a boiling water bath. Then, *n*-butanol was added and the mixture was centrifuged. The organic phase was collected to measure fluorescence at excitation and emission wavelengths of 515 and 553 nm, respectively, using a fluorescence spectrophotometer Hitachi F-2000 [48]. 1,1,3,3-Tetramethoxypropane, which is converted to malondialdehyde (MDA), was used as standard.

2.7. Total antioxidant reactivity assay

The reaction mixture consisted of ABAP (2 mmol L^{-1}) and luminol (6 mmol L^{-1}) in glycine buffer. Total antioxidant reactivity (TAR) values were determined by assessing the initial decrease of luminescence calculated as the ratio I_0/I, where I_0 is the chemiluminescence (CL) in the absence of additives, and I is the CL after addition either

of 20 nmol L^{-1} Trolox or of the samples (5 μL) [49,50]. The CL generated was measured in a scintillation counter Beckman [48]. TAR values were expressed as equivalents of Trolox concentration per milligram of protein.

2.8. Free radical content

To assess the free radical contents, we used 2′,7′-dichlorofluorescein diacetate (DCFH-DA) as probe. An aliquot of the sample was incubated with DCFH-DA (100 μM) at 37 °C for 30 min. The reaction was terminated by chilling the reaction mixture in ice. The formation of the oxidized fluorescent derivative (DCF) was monitored at excitation and emission wavelengths of 488 and 525 nm, respectively [51,52], using a fluorescence spectrophotometer Hitachi F-2000 [48]. The free radical contents were quantified using a DCF standard curve and results were expressed as picomoles of DCF formed per milligram of protein. All procedures were performed in the dark, and blanks containing only DCFH-DA (no homogenate) were processed to determine the autofluorescence [51,52].

2.9. Protein determination

The total protein concentration was determined using the method described by Lowry et al. [53] with serum albumin as standard.

2.10. Statistical analysis

Data are expressed as means ± standard deviation. The statistical analysis of the data was carried out by a two-way analysis of variance (ANOVA) followed by the Tukey's test when appropriate. Analysis was performed using the Statistical Package for the Social Sciences (SPSS) software. A difference was considered significant when $p < 0.05$.

3. Results

3.1. Characterization of nanoparticle suspensions

The drug-loaded and drug-unloaded nanocapsule suspensions presented macroscopic homogeneous aspect, as well as submicronic sizes (241 ± 55 nm; 207 ± 44 nm), low polydispersity (0.01; 0.09), negative zeta potentials (−33 ± 0.3 mV; −35 ± 1.1 mV) and acid pH values (4.2; 4.3), respectively. The total content of melatonin was 0.996 mg/mL and the associated melatonin was 50%. TEM analysis carried out for the melatonin-loaded nanocapsules showed homogeneous particles (Fig. 1).

3.2. Lipid peroxidation

Fig. 2 shows the lipid peroxidation in mice frontal cortex and hippocampus after 1 h of intraperitoneal administration of the melatonin-loaded nanocapsules and of the drug solution. ANOVA revealed significant effects ($p < 0.05$) of the type of the formulation (melatonin-loaded nanocapsules or drug solution) and melatonin doses against lipid peroxidation in the frontal cortex. Moreover, there was a significant interaction between dose and formulation. The melatonin-loaded nanocapsules significantly decreased lipid peroxidation in the frontal cortex when administered in doses of 1 and 10 mg/kg (Fig. 2A).

However, a significant effect of 10 mg/kg of melatonin-loaded nanocapsules against lipid peroxidation in the hippocampus was observed without any significant differences between formulations (Fig. 2B).

Furthermore, significant effects of the type of the formulation (melatonin-loaded nanocapsules or drug solution) and melatonin doses were observed against lipid peroxidation in the liver (Fig. 3), whereas no significant interaction effect between formulation and dose was verified. The melatonin-loaded nanocapsules significantly decreased lipid

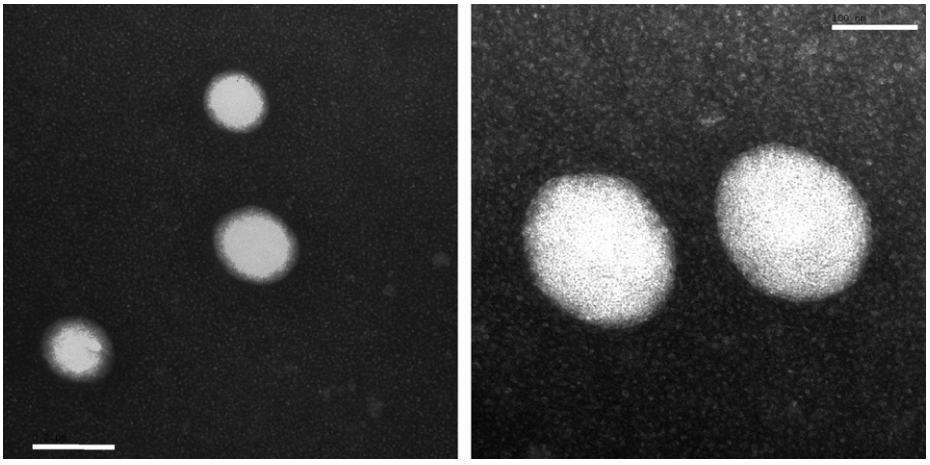

Fig. 1. Transmission electron microscopy images of melatonin-loaded polysorbate 80-coated Eudragit S100®-nanocapsules [image on the left: bar = 200 nm (150,000×); image on the right: bar = 100 nm (250,000×)].

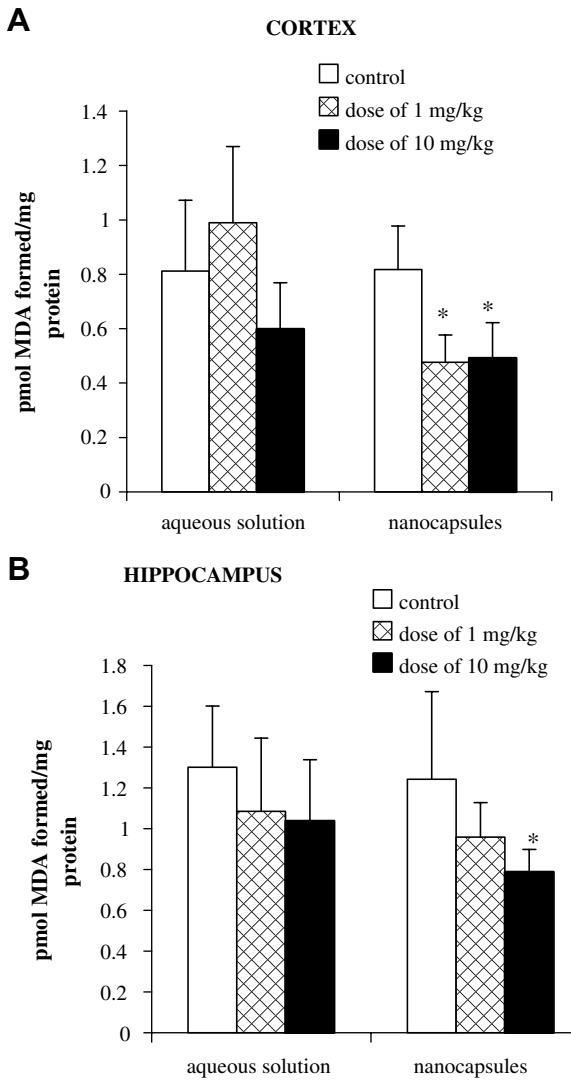

Fig. 2. Antioxidant effect of melatonin-loaded nanocapsules or melatonin solution against lipid peroxidation in brain structures. *Significantly different values ($p < 0.05$) from those of control group as determined by ANOVA followed by Tukey's test. Control for aqueous solutions: 0.77% (w/v) polysorbate 80 aqueous solution; control for nanocapsule suspensions: drug unloaded polysorbate 80-coated nanocapsules. Results are expressed as mean ± standard deviation.

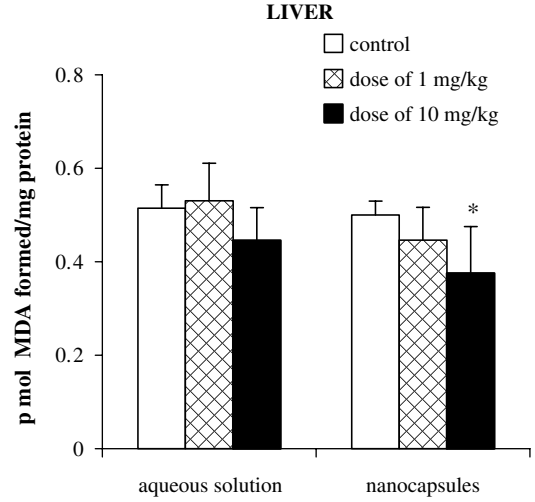

Fig. 3. Antioxidant effect of melatonin-loaded nanocapsules or melatonin solution against lipid peroxidation in liver. *Significantly different values ($p < 0.05$) from those of control group as determined by ANOVA followed by Tukey's test. Control for aqueous solutions: 0.77% (w/v) polysorbate 80 aqueous solution; control for nanocapsule suspensions: drug unloaded polysorbate 80-coated nanocapsules. Results are expressed as means ± standard deviation.

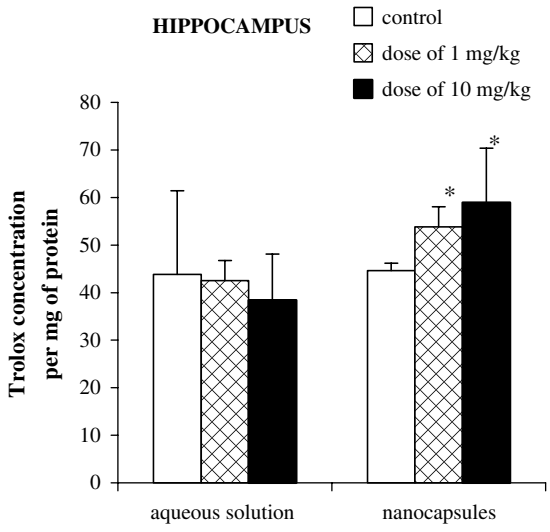

Fig. 4. The total antioxidant reactivity in hippocampus, after 1 h of intraperitoneal administration in mice. *Significantly different values ($p < 0.05$) from those of control group as determined by ANOVA. Control for aqueous solutions: 0.77% (w/v) polysorbate 80 aqueous solution; control for nanocapsule suspensions: drug unloaded polysorbate 80-coated nanocapsules. Results are expressed as means ± standard deviation.

peroxidation in the liver when administered in the dose of 10 mg/kg.

3.3. Free radical levels and total antioxidant reactivity

Both drug solution and nanoencapsulated melatonin administered in a single acute dose (1 or 10 mg/kg) did not significantly ($p < 0.05$) affect the free radical levels in the frontal cortex, hippocampus and liver, as well as the total antioxidant reactivity in the frontal cortex and in the liver in comparison with the controls (data not shown). On the other hand, a significant effect of the formulation on the total antioxidant reactivity was observed: the melatonin-loaded nanocapsules administered in doses of 1 and 10 mg/kg significantly increased the total antioxidant reactivity in the hippocampus (Fig. 4).

4. Discussion

The nanocapsule suspensions presented a homogeneous shape (Fig. 1) with particle mean sizes lower than 300 nm. These values are in agreement with the diameters usually

observed for nanocapsules prepared with preformed polymers using the interfacial deposition method (from 100 nm to 500 nm) [20]. The low polydispersity indicates homogeneous particle distribution for the nanocapsules. The zeta potentials were negative (−33 and −35 mV) indicating stable suspensions as a result of the repulsion between particles avoiding their aggregation [20], while the total content of melatonin was near 100%, and the associated melatonin was 50%, in agreement with our previous results [29,44].

It was observed that the lipid peroxidation has significantly decreased in the cortex and in the hippocampus when melatonin-loaded nanocapsules were administered in a single dose of 10 mg/kg (Fig. 2). On the contrary, melatonin aqueous solution did not exert any significant activity against lipid peroxidation in these tissues, in the dose range studied. In cortex, the dose of 1 mg/kg was also effective when melatonin was associated to nanocapsules. Present results suggest that lipids in cortex and hippocampus were partially protected by melatonin-loaded polysorbate 80-coated nanocapsules, probably due to a higher accumulation of the drug in brain tissue consequent to the nanocapsule targeting.

Melatonin is widely distributed within organisms because it easily crosses morphological barriers and it readily enters any cell, compartment or body fluid, including the BBB due to its small molecular weight and amphiphilic nature (highly lipophilic and somewhat soluble in aqueous media) [4,9]. According to results here presented, melatonin-loaded polysorbate-80 coated nanocapsules probably promoted a better biodistribution of the drug inside the brain, as compared to the aqueous drug solution. However, the mechanism behind the translocation of polymeric nanoparticles into the brain remains to be precisely determined [18].

Tröster et al. [54] showed that polysorbate 80, when used as coating for poly(methyl methacrylate) nanoparticles, effectively increased the brain levels of nanoparticles; it also increases MRZ 2/576 anticonvulsive activity when used as coating material on MRZ 2/576-loaded poly(butyl cyanoacrylate) nanoparticles [31]. According to Sun et al. [40], a partial coverage was enough for polysorbate 80 coating to play a specific role in the brain targeting, and it seemed that brain targeting of poly(lactic acid) nanoparticles was related to the interaction between the polysorbate 80 coating and the brain micro-vessel endothelial cells. Polysorbate 80-coated poly(butyl cyanoacrylate) nanoparticles have also significantly improved the methotrexate level in brain tissue and cerebrospinal fluid compared to uncoated nanoparticles and simple drug solution [41]. There have been proposed several possible mechanisms for the nanoparticle-mediated drug transport into the brain; all these mechanisms could also work in different combinations of the following factors: (a) the binding of nanoparticles to the inner endothelial lining of the brain capillaries improving passive diffusion [18,32]; (b) a general surfactant effect that would lead to membrane fluidisation and to an enhanced drug permeability through the BBB; (c) the nanoparticles could open the tight junctions between the endothelial cells; (d) the nanoparticles could be endocytosed by the endothelial cells or transcytosed through the endothelial cell layer [32,55]; (e) the polysorbate 80 could act as an anchor between nanoparticles and apolipoproteins and then taken up via receptor-mediated endocytosis [18]; (f) the polysorbate 80 used as the coating agent could inhibit the efflux system, specially P-glycoprotein [32,55]. Nanoparticle formulations can also act as controlled drug delivery systems [56] by their lymphatic targeting after intraperitoneal administration [57]. In our study, the intraperitoneal route was chosen because it is easily achieved, especially in small laboratory animals [56].

In the liver, a significant effect against lipid peroxidation was only observed after the administration of the melatonin-loaded nanocapsules (10 mg/kg), while aqueous drug solutions were ineffective (Fig. 3). This result suggests that nanocapsules were also partially taken up by the macrophages in the liver, generating higher melatonin concentration inside the organ.

Treatments with melatonin solutions have been proved effective against lipid peroxidation under different oxidative stress conditions in cerebral cortex [58–61] and in the hippocampus [58,60–63], as well as in the liver [13,64–67]. Therefore, the targeting of melatonin to the brain and to the liver can be potentially useful to improve its therapeutic efficacy against oxidative damage of those tissues.

The partial encapsulation of melatonin within the nanocapsules (50%) was able to improve the in vivo antioxidant capacity of this drug against lipid peroxidation in the brain and in the liver, using a single dose. The lipids of the hippocampus were significantly protected, in 37%, by the administration of 10 mg/kg melatonin-loaded nanocapsules compared to the control, while in the frontal cortex the protection was 42% and 40%, respectively, for 1 and 10 mg/kg melatonin-loaded nanocapsules (Fig. 2). In the liver, the lipids were significantly protected in 25% after the administration of 10 mg/kg melatonin-loaded nanocapsules compared to the control (Fig. 3).

In a previous work about the in vitro lipid peroxidation induced by the free radical ascorbyl [29], the lipids were protected depending on the melatonin dose, on the type of the lipid substrate (phosphatidylcholine liposomes or liver microsomes) and on the type of drug formulations. When microsomes and a melatonin dose of 400 μM were used, only the formulations containing polymer [polysorbate 80-coated nanocapsules ($36 \pm 3\%$) and polysorbate 80-coated nanospheres ($40 \pm 7\%$)] showed significant increase in the melatonin protection against lipid peroxidation in comparison with the drug solution ($16 \pm 1\%$), whereas the polysorbate 80-coated nanoemulsion (without polymer) presented protection ($19 \pm 2\%$) similar to the drug solution.

Summarizing, the in vivo presented results confirm the increase in the antioxidant effect of the melatonin-loaded nanoparticles against lipid peroxidation previously

observed in the vitro experiments [29]. In addition, only the melatonin-loaded nanocapsules caused a significant improvement in the total antioxidant reactivity in the hippocampus (1 mg/kg, 21%; 10 mg/kg, 32%; see Fig. 4) as compared to the control. Altogether, results confirm the working hypothesis that antioxidant activity of melatonin increased due to its nanoencapsulation in polysorbate 80-coated nanocapsules.

5. Conclusions

It was shown that a single administration of 10 mg/kg melatonin-loaded nanocapsules showed a significant antioxidant activity against lipid peroxidation in mice brain and liver, while the aqueous solution of the same dose was ineffective. To our knowledge, this is the first demonstration of the potential use of in vivo melatonin-loaded polymeric nanocapsules against lipid peroxidation; this supports that polymeric nanocapsules can be a promising technological platform for the melatonin delivery to the brain and/or to the liver due to the potential application of this substance in reducing the oxidative damage.

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References

[1] J. Barrenetxe, P. Delagrange, J.A. Martínez, Physiological and metabolic functions of melatonin, J. Physiol. Biochem. 60 (2004) 61–72.
[2] J.A. Boutin, V. Audinot, G. Ferry, P. Delagrange, Molecular tools to study melatonin pathways and actions, Trends Pharmacol. Sci. 26 (2005) 412–419.
[3] F.H. Epstein, Mechanisms of disease – melatonin in humans, N. Engl. J. Med. 336 (1997) 186–195.
[4] R. Hardeland, S.R. Pandi-Perumal, D.P. Cardinali, Molecules in focus, Int. J. Biochem. Cell Biol. 38 (2006) 313–316.
[5] R.J. Reiter, C.-S. Oh, O. Fujimori, Melatonin: its intracellular and genomic actions, Trends Endocrinol. Metab. 7 (1996) 22–27.
[6] R.J. Reiter, L. Tang, J.J. Garcia, A. Muñoz-Hoyos, Pharmacological actions of melatonin in oxygen radical pathophysiology, Life Sci. 60 (1997) 2255–2271.
[7] R.J. Reiter, Oxidative damage in the cerebral nervous system: protection by melatonin, Prog. Neurobiol. 56 (1998) 359–384.
[8] R.J. Reiter, Melatonin: clinical relevance, Best Pract. Res. Clin. Endocrinol. Metab. 17 (2003) 273–285.
[9] M. Karbownik, A. Lewinski, R.J. Reiter, Anticarcinogenic actions of melatonin which involve antioxidative process: comparison with other antioxidants, Int. J. Biochem. Cell Biol. 33 (2001) 735–753.
[10] R.J. Reiter, D.-X. Tan, S. Burkhardt, Reactive oxygen and nitrogen species and cellular and organismal decline: amelioration with melatonin, Mech. Ageing Dev. 123 (2002) 1007–1019.
[11] A. Cruz, F.J. Padillo, E. Torres, C.M. Navarrete, J.R. Muñoz-Castañeda, F.J. Caballero, J. Briceño, T. Marchal, I. Túnez, P. Montilla, C. Pera, J. Muntané, Melatonin prevents experimental liver cirrhosis induced by thioacetamide in rats, J. Pineal Res. 39 (2005) 143–150.
[12] A. Guven, O. Yavuz, M. Cam, F. Ercan, N. Bukan, C. Comunoglu, F. Gokce, Effects of melatonin on streptozotocin-induced diabetic liver injury in rats, Acta Histochem. 108 (2006) 85–93.
[13] H. Wang, W. Wei, N.-P. Wang, S.-Y. Gui, L. Wu, W.-Y. Sun, S.-Y. Xu, Melatonin ameliorates carbon tetrachloride-induced hepatic fibrogenesis in rats via inhibition of oxidative stress, Life Sci. 77 (2005) 1902–1915.
[14] A. Altun, B. Ugur-Altun, Melatonin: therapeutic and clinic utilization, Int. J. Clin. Pract. 61 (2007) 835–845.
[15] R.T.F. Cheung, The utility of melatonin in reducing cerebral damage resulting from ischemia and reperfusion, J. Pineal Res. 34 (2003) 153–160.
[16] E. Matsubara, M. Shoji, T. Murakami, T. Kawarabayashi, K. Abe, Alzheimer's disease and melatonin, Int. Congr. Ser. 1252 (2003) 395–398.
[17] S.K. Sahoo, V. Labhasetwar, Nanotech approaches to drug delivery and imaging, Drug Discov. Today 8 (2003) 1112–1120.
[18] E. Garcia-Garcia, K. Andrieux, S. Gil, P. Couvreur, Colloidal carriers and blood–brain barrier (BBB) translocation: a way to deliver drugs to the brain? Int. J. Pharm. 298 (2005) 274–292.
[19] S.R. Schaffazick, S.S. Guterres, L. Lucca-Freitas, A.R. Pohlmann, Caracterização e estabilidade físico-química de sistemas poliméricos nanoparticulados para administração de fármacos, Quim. Nova 26 (2003) 726–737.
[20] P. Couvreur, G. Barratt, E. Fattal, P. Legrand, C. Vauthier, Nanocapsule technology: a review, Crit. Rev. Ther. Drug Carrier Syst. 19 (2002) 99–134.
[21] M. Teixeira, M.J. Alonso, M.M.M. Pinto, C.M. Barbosa, Development and characterization of PLGA nanospheres and nanocapsules containing xanthone and 3-methoxyxanthone, Eur. J. Pharm. Biopharm. 59 (2005) 491–500.
[22] E. Allémann, J.-C. Leroux, R. Gurny, Polymeric nano- and microparticles for the oral delivery of peptides and peptidomimetics, Adv. Drug Deliv. Rev. 34 (1998) 171–189.
[23] S.S. Guterres, C.R. Muller, C.B. Michalowski, A.R. Pohlmann, T. Dalla Costa, Gastro-intestinal tolerance following oral administration of spray-dried diclofenac-loaded nanocapsules and nanospheres, S.T.P. Pharma Sci. 11 (2001) 229–233.
[24] S.R. Schaffazick, A.R. Pohlmann, T. Dalla-Costa, S.S. Guterres, Freeze-drying polymeric colloidal suspensions: nanocapsules, nanospheres and nanodispersion. A comparative study, Eur. J. Pharm. Biopharm. 56 (2003) 501–505.
[25] S.S. Kwon, Y.S. Nam, J.S. Lee, B.S. Ku, S.H. Han, J.Y. Lee, I.S. Chang, Preparation and characterization of coenzyme Q_{10}-loaded PMMA nanoparticles by a new emulsification process based on microfluidization, Colloids Surf. A Physicochem. Eng. Asp. 210 (2002) 95–104.
[26] D.V. Ratnam, D.D. Ankola, V. Bhardwaj, D.K. Sahana, M.N.V. Ravi Kumar, Role of antioxidants in prophylaxis and therapy: a pharmaceutical perspective, J. Control. Release 113 (2006) 189–207.
[27] I. Bala, V. Bhardwaj, S. Hariharan, S.V. Kharade, N. Roy, M.N.V.R. Kumar, Sustained release nanoparticulate formulation containing antioxidant-ellagic acid as potential prophylaxis system for oral administration, J. Drug Target. 14 (2006) 27–34.
[28] M. Palumbo, A. Russo, V. Cardile, M. Renis, D. Paolino, G. Puglisi, M. Fresta, Improved antioxidant effect of idebenone-loaded polyethyl-2 cyanoacrylate nanocapsules tested on human fibroblasts, Pharm. Res. 19 (2002) 71–78.
[29] S.R. Schaffazick, A.R. Pohlmann, C.A.S. de Cordova, T.B. Creczynski-Pasa, S.S. Guterres, Protective properties of melatonin-loaded

nanoparticles against lipid peroxidation, Int. J. Pharm. 289 (2005) 209–213.
[30] T.B. Shea, D. Ortiz, R.J. Nicolosi, R. Kumar, A.C. Watterson, Nanosphere-mediated delivery of vitamin E increases its efficacy against oxidative stress resulting from exposure to amyloid beta, J. Alzheimers Dis. 7 (2005) 297–301.
[31] A. Friese, E. Seiller, G. Quack, B. Lorenz, J. Kreuter, Increase of the duration of the anticonvulsive activity of a novel NMDA receptor antagonist using poly(butylcyanoacrylate) nanoparticles as a parenteral controlled release system, Eur. J. Pharm. Biopharm. 49 (2000) 103–109.
[32] J. Kreuter, Application of nanoparticles for the delivery of drugs to the brain, Int. Congr. Ser. 1277 (2005) 85–94.
[33] U. Schröder, B.A. Sabel, Nanoparticles, a drug carrier system to pass the blood–brain barrier, permit central analgesic effects of i.v. dalargin injections, Brain Res. 710 (1996) 121–124.
[34] J. Kreuter, V.E. Petrov, D.A. Kharkevich, R.N. Alyautdin, Influence of the type of surfactant on the analgesic effects induced by the peptide dalargin after its delivery across the blood–brain barrier using surfactant-coated nanoparticles, J. Control. Release 49 (1997) 81–87.
[35] A.E. Gulyaev, S.E. Gelperina, I.N. Skidan, A.S. Antropov, G.Ya. Kivman, J. Kreuter, Significant transport of doxorubicin into the brain with polysorbate 80-coated nanoparticles, Pharm. Res. 16 (1999) 1564–1569.
[36] J.-C. Olivier, L. Fenart, R. Chauvet, C. Pariat, R. Cecchelli, W. Couet, Indirect evidence that drug brain targeting using polysorbate-80-coated polybutylcyanoacrylate nanoparticles is related to toxicity, Pharm. Res. 16 (1999) 1836–1842.
[37] U. Schroeder, H. Schroeder, B.A. Sabel, Body distribution of ^{3}H-labelled dalargin bound to poly(butyl cyanoacrylate) nanoparticles after i.v. injections to mice, Life Sci. 66 (2000) 495–502.
[38] S.E. Gelperina, A.S. Khalansky, I.N. Skidan, Z.S. Smirnova, A.I. Bobruskin, S.E. Severin, B. Turowski, F.E. Zanella, J. Kreuter, Toxicological studies of doxorubicin bound to polysorbate 80-coated poly(butyl cyanoacrylate) nanoparticles in healthy rats and rats with intracranial glioblastoma, Toxicol. Lett. 126 (2002) 131–141.
[39] J. Kreuter, P. Ramge, V. Petrov, S. Hamm, S.E. Gelperina, B. Engelhardt, R. Alyautdin, H. von Briesen, D.J. Begley, Direct Evidence that polysorbate-80-coated poly(butylcyanoacrylate) nanoparticles deliver drugs to the CNS via specific mechanisms requiring prior binding of drug to the nanoparticles, Pharm. Res. 20 (2003) 409–416.
[40] W. Sun, C. Xie, H. Wang, Y. Hu, Specific role of polysorbate 80 coating on the targeting of nanoparticles to the brain, Biomaterials 25 (2004) 3065–3071.
[41] K. Gao, X. Jiang, Influence of particle size on transport of methotrexate across blood brain barrier by polysorbate 80-coated polybutylcyanoacrylate nanoparticles, Int. Pharm. 310 (2006) 213–219.
[42] P. Calvo, B. Gouritin, H. Chacun, D. Desmaële, J. D'Angelo, J.-P. Noel, D. Georgin, E. Fatal, J.P. Andreux, P. Couvreur, Long-circulation PEGylated polycyanoacrylate nanoparticles as new drug carrier for brain delivery, Pharm. Res. 18 (2001) 1157–1166.
[43] E. Garcia-Garcia, K. Andrieux, S. Gil, H.R. Kim, T.L. Doan, D. Desmaële, J. d'Angelo, F. Taran, D. Georgin, P. Couvreur, A methodology to study intracellular distribution of nanoparticles in brain endothelial cells, Int. J. Pharm. 298 (2005) 310–314.
[44] S.R. Schaffazick, A.R. Pohlmann, G. Mezzalira, S.S. Guterres, Development of nanocapsule suspensions and nanocapsule spray-dried powders containing melatonin, J. Braz. Chem. Soc. 17 (2006) 562–569.
[45] H. Fessi, F. Puisieux, J.-P. Devissaguet, N. Ammoury, S. Benita, Nanocapsule formation by interfacial polymer deposition following solvent displacement, Int. J. Pharm. 55 (1989) r1–r4.
[46] A.R. Pohlmann, V. Weiss, O. Mertins, N. Pesce da Silveira, S.S. Guterres, Spray-dried indometacin-loaded polyester nanocapsules and nanospheres: development, stability evaluation and nanostructure models, Eur. J. Pharm. Sci. 16 (2002) 305–312.
[47] J.A. Buege, S.D. Aust, Microssomal lipid peroxidation, Methods Enzymol. 52 (1978) 302–310.
[48] I.R. Siqueira, C. Fochesatto, I.L. da Silva Torres, C. Dalmaz, C.A. Netto, Aging affects oxidative state in hippocampus, hypothalamus and adrenal glands of Wistar rats, Life Sci. 78 (2005) 271–278.
[49] E. Lissi, C. Pascual, M.D. del Castillo, Luminol luminescence induced by 2,2′-Azo-bis(2-amidinopropane) thermolysis, Free Radic. Res. Commun. 17 (1992) 299–311.
[50] E. Lissi, M. Salim-Hanna, C. Pascual, M.D. del Castillo, Evaluation of total antioxidant potential (TRAP) and total antioxidant reactivity from luminol-enhanced chemiluminescence measurements, Free Radic. Biol. Med. 18 (1995) 153–158.
[51] A.S. Driver, P.R.S. Kodavanti, W.R. Mundy, Age-related in reactive oxygen species production in rat brain homogenates, Neurotoxicol. Teratol. 22 (2000) 175–181.
[52] K. Sriram, K.S. Pai, M.R. Boyd, V. Ravindranath, Evidence for generation of oxidative stress in brain by MPTP: in vitro and in vivo studies in mice, Brain Res. 749 (1997) 44–52.
[53] O.H. Lowry, N.J. Rosenbrough, A.L. Farr, R.J. Randall, Protein measurement with the Folin phenol reagent, J. Biol. Chem. 193 (1951) 265–275.
[54] S.D. Tröster, U. Müller, J. Kreuter, Modification on the body distribution of poly(methylmethacrylate) nanoparticles in rats by coating with surfactants, Int. J. Pharm. 61 (1990) 85–100.
[55] J. Kreuter, Nanoparticulate systems for brain delivery of drugs, Adv. Drug Deliv. Rev. 47 (2001) 65–81.
[56] M.A. Radwan, I.Y. Zaghloul, Z.H. Aly, In vivo performance of parenteral theophylline-loaded polyisobutylcyanoacrylate nanoparticles in rats, Eur. J. Pharm. Sci. 8 (1999) 95–98.
[57] P. Maincent, P. Thouvenot, C. Amicabile, M. Hoffman, J. Kreuter, P. Couvreur, J.P. Devissaguet, Lymphatic targeting of polymeric nanoparticles after intraperitoneal administration in rats, Pharm. Res. 9 (1992) 1534–1539.
[58] G. Baydas, S. Kutlu, M. Naziroglu, S. Canpolat, S. Sandal, M. Ozcan, H. Kelestimur, Inhibitory effects of melatonin on neural lipid peroxidation induced by intracerebroventricularly administered homocysteine, J. Pineal Res. 34 (2003) 36–39.
[59] J.L. Esparza, M. Gómez, M.R. Nogués, L. Paternain, J. Mallol, J.L. Domingo, Melatonin reduces oxidative stress and increases gene expression in the cerebral cortex and cerebellum of aluminum-exposed rats, J. Pineal Res. 39 (2005) 129–136.
[60] G. Baydas, M. Tuzcu, Protective effects of melatonin against ethanol-induced reactive gliosis in hippocampus and cortex of young and aged rats, Exp. Neurol. 194 (2005) 175–181.
[61] G. Baydas, A. Yasar, M. Tuzcu, Comparison of the impact of melatonin on chronic ethanol-induced learning and memory impairment between young and aged rats, J. Pineal Res. 39 (2005) 346–352.
[62] D. Acuña-Castroviejo, A. Coto-Montes, M.G. Monti, G.G. Ortiz, R.J. Reiter, Melatonin is protective against MPTP-induced striatal and hippocampal lesions, Life Sci. 60 (1997) 23–29.
[63] R.C.G. Carneiro, R.J. Reiter, Melatonin protects against lipid peroxidation induced by δ-aminolevulinic acid in rat cerebellum, cortex and hippocampus, Neuroscience 82 (1998) 293–299.
[64] R. Rezzani, B. Buffoli, L. Rodella, A. Stacchiotti, R. Bianchi, Protective role of melatonin in cyclosporine A-induced oxidative stress in rat liver, Int. Immunopharmacol. 5 (2005) 1397–1405.
[65] G. Contuk, F. Ercan, S. Çetinel, E. Çikler, G. Sener, Role of melatonin in reducing water avoidance stress-induced degeneration of the liver, Dig. Dis. Sci. 50 (2005) 738–744.
[66] I. Túnez, M.C. Muñoz, M.A. Villavicencio, F.J. Medina, E.P. de Prado, I. Espejo, M. Barcos, M. Salcedo, M. Feijóo, P. Montilla, Hepato- and neurotoxicity by thiocetamide: protective effects of melatonin and dimethylsulfoxide, Pharmacol. Res. 52 (2005) 223–228.
[67] F. Sigala, S. Theocharis, K. Sigalas, S. Markantonis-Kyroudis, E. Papalabros, A. Triantafyllou, G. Kostopanagiotou, I. Andreadou, Therapeutic value of melatonin in an experimental model of liver injury and regeneration, J. Pineal Res. 40 (2006) 270–279.