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Redox modulation of cellular stress response and lipoxin A4 expression by *Coriolus versicolor* in rat brain: Relevance to Alzheimer's disease pathogenesis

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ABSTRACT

Increasing evidence supports the notion that oxidative stress-driven neuroinflammation is an early pathological feature in neurodegenerative diseases. As a prominent intracellular redox system involved in neuroprotection, the vitagenes system is emerging as a potential neurohormetic target for novel cytoprotective interventions. Vitagenes encode for cytoprotective heat shock proteins 70, heme oxygenase-1, thioredoxin and lipoxin A4. Emerging interest is now focusing on molecules capable of activating the vitagenes system as novel therapeutic targets to minimize deleterious consequences associated with free radical-induced cell damage, such as in neurodegeneration. Mushroom-derived lipoxin A4 (LXA4) is an emerging endogenous eicosanoid able to promote resolution of inflammation, acting as an endogenous “braking signal” in the inflammatory process. Mushrooms have long been used in traditional medicine for thousands of years, being now increasingly recognized as rich source of polysaccharopeptides endowed with significant antitumor, antioxidant, antiviral, antibacterial and cytoprotective effects, thereby capable of stimulating host immune responses. Here we provide evidence of a neuroprotective action of the *Coriolus* mushroom when administered orally to rat. Expression of LXA4 was measured in different brain regions after oral administration of a *Coriolus* biomass preparation, given for 30 days. LXA4 up-regulation was associated with an increased content of redox sensitive proteins involved in cellular stress response, such as Hsp72, heme oxygenase-1 and thioredoxin. In the brain of rats receiving *Coriolus*, maximum induction of LXA4 was observed in cortex and hippocampus. Hsps induction was associated with no significant changes in Ikb α , NFkB and COX-2 brain levels. Conceivably, activation of LXA4 signaling and modulation of stress-responsive vitagenes proteins could serve as a potential therapeutic target for AD-related inflammation and neurodegenerative damage.

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1. Introduction

Neuroinflammation, a specialized immune response that occurs in the nervous system, has been connected to the onset of certain chronic degenerative disorders of the central nervous system (CNS) characterized by gradual loss of neurons from specific regions of the CNS. This cell loss is believed to explain the cognitive and motor deficits suffered by patients with neurodegenerative disorders. Brain inflammation has been linked to many of these diseases, including amyotrophic lateral sclerosis (ALS), multiple

sclerosis (MS), Parkinson's disease (PD) and, particularly, Alzheimer's disease (AD) (Calabrese et al., 2009a, 2009b, 2009c, 2010; Pietrzak et al., 2015). Increasing evidence indicates that factors such as oxidative stress and disturbed protein metabolism, and their interaction in a vicious cycle, are central to AD pathogenesis (Blair et al., 2015; Calabrese et al., 2011; Edrey et al., 2014). To adapt to environmental changes and survive different types of injuries, brain cells have evolved networks of responses that detect and control diverse forms of stress (Calabrese et al., 2010; Zhang et al., 2011). Consistent with this notion, integrated survival responses exist in the brain, which are under control of redox-dependent genes, called vitagenes, including heat shock proteins (Hsps), sirtuins, thioredoxin and lipoxin A4. These proteins actively operate in detecting and controlling diverse forms of stress and

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neuronal injuries (Calabrese et al., 2009a, 2009b, 2009c, 2014b). LXA4, a metabolic product of arachidonic acid, is considered an endogenous “stop signal” for inflammation, and shows potent anti-inflammatory properties in many inflammatory disorders, such as nephritis, periodontitis, arthritis, inflammatory bowel disease (Medeiros et al., 2013). Chronic inflammation sustains the progression of Alzheimer’s disease (AD), however identification of mechanisms capable of resolving the pro-inflammatory environment stimulated by AD pathology remains an area of active investigation (Figueiredo-Pereira et al., 2015; Joshi and Praticò, 2015). Treatment with the pro-resolving mediator aspirin-triggered lipoxin A4 (ATL), resulted in improved cognition, reduced A β levels, and enhanced microglia phagocytic activity in Tg2576 transgenic AD mice (Dunn et al., 2015). Furthermore, LXA4 levels are reduced with age, a finding even more evident in 3xTg-AD mice (Gangemi et al., 2005). Moreover, aspirin-triggered lipoxin A4 upregulation enhanced cognitive performance of 3xTg-AD mice, an effect associated with reduction of A β and phosphorylated-tau (p-tau) levels, as well as microglial and astrocyte reactivity (Medeiros et al., 2013). Activation of LXA4 signaling could therefore serve as a potential therapeutic target for AD-related inflammation and cognitive dysfunction. LXA4 action is mediated by the membrane-bound LXA4 receptor (ALX), which is known as formyl-peptide receptorlike 1 (FPRL1) (Chen et al., 2013). Microglia play an essential role in innate immunity, homeostasis, and neurotropic support in the CNS. In Alzheimer disease (AD), these cells may affect disease progression by modulating the buildup of A β or by releasing proinflammatory cytokines and neurotoxic substances. Discovering agents capable of increasing Lipoxin A4 (LXA4) and consequently of increasing A β uptake by phagocytic cells is of potential therapeutic interest for AD. Lipoxin A4 (LXA4) as an endogenously produced eicosanoid, inhibits neutrophil recruitment and activation, reduces many cell responses evoked by pathogens and pro-inflammatory cytokines, blocks the generations of pro-inflammatory cytokines and toxic compounds including ROS, thereby promoting resolution of inflammation, and acts as an endogenous “braking signal” in the inflammatory process (Chen et al., 2013).

Mushrooms, from which LXA4 is derived, have been used in traditional medicine for thousands of years (Elsayed et al., 2014; El Enshasy et al., 2013). Many controlled studies have since investigated the long list of medicinal actions thought to be associated with extracts of these and other mushrooms, including antitumor, immunomodulatory, antioxidant, antiviral, antibacterial, and hepatoprotective effects (Paterson and Lima, 2014). Mushrooms are a rich source of these polysaccharides, and many have been shown to stimulate host immune responses (Komura et al., 2014). Indeed, the effects of polysaccharides on the immune system are by far the most exploited attribute of “medicinal mushrooms”. Some of the most potent immunostimulatory molecules derived from mushrooms are the β -glucans, which activate many types of immune cells and stimulate cytokine responses (da Silva et al., 2013; Lindequist et al., 2014; Wasser et al., 2014). Studies in rodents and humans suggest that polysaccharides derived from mushrooms can stimulate the immune system to attack malignant cells (Monro, 2003; Jeong et al., 2013), and several of these compounds are now used in East countries in association to radio and chemotherapy (Xu et al., 2012).

Medicinal effects have been demonstrated for many traditionally used mushrooms, including extracts of *Agaricus campestris*, *Pleurotus ostreatus* and *Coriolus versicolor* (Cui et al., 2007; Walton, 2014; Wang et al., 2012). Of the mushroom-derived therapeutics, polysaccharopeptides obtained from *C. versicolor* are commercially the best established. In addition to its medical applications, *C. versicolor* is widely used to degrade recalcitrant organic pollutants

such as pentachlorophenol (PCP) (Cui and Chisti, 2003). Taken all this into account and given the neuroinflammatory pathogenesis of neurodegenerative damage present in Alzheimer’s disease, the present study was conducted in order to gain insight into the possible neuroprotective role of *Coriolus* biomass preparation against the inflammatory process and to evaluate the impact of this intervention on cellular stress response mechanism operating in rat brain.

2. Materials and methods

2.1. Chemicals

All reagents were from Merck (Darmstadt, Germany) and of the highest grade available. The *C. versicolor* biomass powder containing both mycelium and primordia (young fruit body) was supplied by Mycology Research Laboratories Ltd., Luton, UK.

2.2. *Coriolus versicolor* biomass preparation

C. versicolor is found almost worldwide; however, its bioactivity varies with the habitat in which it grows. To eliminate these variations, the established CV-OH1 strain was used, a strain that demonstrates rapid and aggressive colonization. *C. versicolor* powder contains both mycelium and primordia (young fruit body) cultivated into a biomass that is grown on a sterilised (autoclaved) substrate. The production process involves the inoculation of sterile organic edible grain with spawn from the mother culture. The fungus is allowed to completely colonize the growth medium aseptically. At the correct stage of development, corresponding to the maximum bioavailability, the living biomass is aseptically air-dried, granulated, tested microbiologically and reduced to powder. In comparison to *Coriolus* extracts, biomass has the advantage of preserving all nutraceutical potential (usually reduced with extracts or concentrates), including during lyophilisation, and thus the activity of the product corresponds with the source mushroom, while being further intensified by utilizing the entire mycelium. The *Coriolus* biomass containing mycelium and primordia of the respective mushroom was used for experiments.

2.3. Animals

Male Sprague-Dawley rats (200 to 230 g; Harlan, Nossan, Italy) were given food and water ad libitum. The study was approved by the University of Messina Review Board for the care of animals in compliance with Italian regulations on the protection of animals used for experimental and other scientific purposes (DM116192) as well as with the relevant European Economic Community (EEC) regulations (OJ of EC L 358/1 12/18/1986).

2.4. Experimental groups and treatments

Mice were randomly allocated into the following groups:

Group 1: Sham + Veh = vehicle solution (saline) was administered orally for 30 days ($N = 10$); *Group 2*: Sham + *Coriolus* = same as the Sham + Veh group, but *Coriolus* biomass powder (200 mg/kg body weight, soluble in saline o.x.) was administered for the full experiment duration of 30 days ($N = 10$). At the specified time-points, animals were killed and brains were quickly removed and dissected into the cerebral cortex, hippocampus, septal area, and striatum (St), according to a standardized procedure, in a cold anatomical chamber and following a protocol that allows a maximum of 50 s time-variability for each sample across animals. Substantia nigra was dissected from the deepest part of the interpeduncular fossa. Samples from different brain areas, total

brain, and from liver and kidney were homogenized for 2 min in 0.05 mol/L Tris–HCl buffer, pH 7.4 (1:9).

2.5. Sampling and lymphocyte purification

Blood was collected via cardiac puncture and added to tubes containing EDTA. Aliquots (2 mL) were utilized for lymphocyte purification, which was accomplished by using the Ficoll Paque System following the procedure provided by the manufacturer (GEHealthcare, Piscataway, NJ, USA).

2.6. Western blot analysis

Inducible Hsp-70, HO-1, and Trx protein levels were estimated by Western blot analysis as previously described in [Cornelius et al. \(2013\)](#). Plasma samples were processed as such, while the isolated lymphocyte pellet as well as dissected brain regions brain were homogenized and centrifuged at $10,000 \times g$ for 10 min. The supernatant was then used for analysis after determination of protein content. Proteins extracted from each sample, at equal concentration (50 μ g), were boiled for 3 min in sample buffer (containing 40 mM Tris–HCl pH7.4, 2.5% SDS, 5% 2-mercaptoethanol, 5% glycerol, 0.025 mg/ml of bromophenol blue) and then separated on a precast 4–20% polyacrylamide mini gels (codNB10420 NuSept Ltd, Homebush, Australia). Separated proteins were transferred onto nitrocellulose membrane (BIO-RAD, Hercules, CA, USA) in transfer buffer containing (0.05% SDS, 25 mM Tris, 192 mM glycine and 20% v/v methanol). Protein transfer was confirmed by staining with Ponceau Red which was then removed by three washes with PBS (phosphate buffered saline) for 5 min each. Membranes were incubated for 1 h at RT in 20 mM Tris pH 7.4, 150 mM NaCl and Tween 20 (TBS-T) containing 2% milk powder and incubated overnight at 4 °C in TBS-T with appropriate primary anti-HSP-72, anti-HO-1, and anti-Trx polyclonal antibodies (Santa Cruz Biotech. Inc.). The same membrane was incubated with a goat polyclonal antibody anti-beta-actin (SC 1615 Santa Cruz Biotech Inc., Santa Cruz, CA, USA, dilution 1:1000) to verify that the concentration of protein loaded in the gel was the same in each sample. Excess unbound antibodies were removed by three washes with TBS-T for 5 min. After incubation with primary antibody, the membranes were washed three times for 5 min in TBS-T and then incubated for 1 h at RT with the secondary polyclonal antibody conjugated with horseradish peroxidase (dilution 1:500). The membranes were then washed three times with TBS-T for 5 min. Finally, the membranes were incubated for 3 min with Super Signal chemiluminescence detection system kit (Cod34080 Pierce Chemical Co, Rockford, IL, USA) to display the specific protein bands for each antibody. The immunoreactive bands were quantified by capturing the luminescence signal emitted from the membranes with the Gel Logic 2200 PRO (Bioscience) and analyzed with Molecular Imaging software for the complete analysis of regions of interest for measuring expression ratios. The molecular weight of proteins analyzed was determined using a standard curve prepared with standard protein molecular weight.

2.7. Western Blot analysis of I κ B- α , NF- κ B p65, iNOS and COX-2

To measure I κ B α , NF- κ B p65, iNOS and COX-2, tissue samples from total brain, kidney and liver were taken and suspended in extraction Buffer A containing 0.2 mM PMSF, 0.15 μ M pepstatin A, 20 μ M leupeptin, 1 mM sodium orthovanadate, before homogenization at the highest setting for 2 min, and centrifuged at $1000 \times g$ for 10 min at 4 °C. Supernatants represented the cytosolic fraction. The pellets, containing enriched nuclei, were resuspended in Buffer B containing 1% Triton X-100, 150 mM NaCl, 10 mM Tris–HCl pH 7.4, 1 mM EGTA, 1 mM EDTA, 0.2 mM PMSF, 20 mM leupeptin,

0.2 mM sodium orthovanadate. After centrifugation for 10 min at $12,000 \times g$ at 4 °C, the supernatants contain the nuclear protein. Samples were heated to 100 °C for 5 min, and equal amounts of protein were separated on 18% SDS-PAGE gel and transferred to nitrocellulose membrane. Specific primary antibody, anti-I κ B α (1:500; Santa Cruz Biotechnology), anti-NF- κ B p65 (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-COX2 (1:500; Cayman Chemical, Ann Arbor, MI), anti-iNOS (1:1000; BD transduction), were mixed in 1 \times PBS, 5% w/v nonfat dried milk, 0.1% Tween-20 (PMT) and incubated at 4 °C, overnight. Thereafter, membranes were incubated with peroxidase-conjugated bovine anti-mouse IgG secondary antibody or peroxidase-conjugated goat anti-rabbit IgG (1:2000, Jackson ImmunoResearch, Suffolk, UK) for 1 h at RT. Protein loading was controlled by probing the membranes with a β -actin antibody or laminin (1:1000; Sigma-Aldrich, Saint Louis, MO, USA). Signals were detected with enhanced chemiluminescence detection system reagent according to manufacturer's instructions (SuperSignal West Pico Chemiluminescent Substrate, Pierce, Madison, WI, USA). The relative expression of the protein bands was quantified by densitometry with BIORAD ChemiDocTM XRS-software and standardized to β -actin levels. Images of blot signals (8 bit/600 dpi resolution) were imported to analysis software (Image Quant TL, v2003, Hercules, CA, USA). A preparation of commercially available molecular weight markers made of proteins of molecular weight 10 to 250 kDa was used to define molecular weight positions and as reference concentrations for each molecular weight.

2.8. Quantification of lipoxin A4

Lipoxin A4 (LXA₄) was analyzed using an ELISA kit (CEB452Ge Cloud-Clone Corp). The assay was performed following the instructions of the manufacturer and measured by a microplate reader at 450 nm. For this assay, aliquots of plasma or tissues homogenates were weighed after rinsing with saline to remove excess blood thoroughly. Minced tissues were homogenized in ice-cold PBS (0.01 M, pH 7.2). The resulting suspension was sonicated and then centrifuged for 5 min at $5000 \times g$ at 4 °C, and supernatants collected. Aliquots of standard, blank, plasma or tissue homogenates containing equal amounts of proteins were brought to a final volume of 50 μ L and added into the appropriate wells, followed by addition of 50 μ L of detection reagent A to each well. The plate was shaken gently before incubation for 1 h at 37 °C. The solution was aspirated and the wells washed 3 times. The remaining liquid was removed completely by snapping the plate onto absorbent paper. 100 μ L of detection reagent B were then added to each well followed by incubation for 30 min at 37 °C. After repeated washing (5 times), 90 μ L of substrate solution was added to each well followed by incubation for 25 min at 37 °C. Finally, 50 μ L of stop solution was added to each well before measurement through reading at 450 nm.

2.9. Statistical analysis

All results were expressed as mean \pm standard deviation. Data were analyzed by ANOVA to compare the different groups and considered significant at $P < 0.05$. Each experiment was carried out in triplicate and repeated twice each time.

3. Results

3.1. Regional distribution of LXA4 expression in rat brain and the effect of treatment with *Coriolus*

Expression of Lipoxin A4, in control animals and after administration of *Coriolus* biomass, was investigated in different

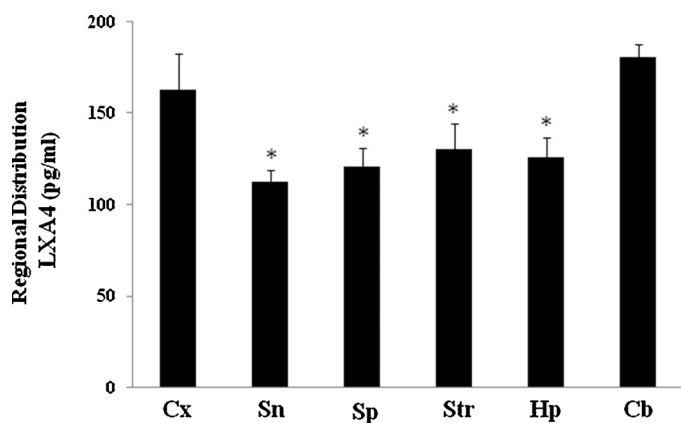


Fig. 1. Regional distribution of lipoxin A4 protein levels in different brain regions of rats. Data are expressed as mean \pm SEM of three independent analyses on 10 animals. * $P < 0.05$ vs cortex or cerebellum.

brain regions of rats, as well as in plasma and peripheral tissues, such as liver and kidney. As shown in Fig. 1, higher levels of LXA4 protein expression was observed in cortex and cerebellum, followed with a statistically significant difference by striatum, hippocampus and septum, whereas the lowest levels were found in the substantia nigra. Administration of *Coriolus* for 30 days at the oral daily dose of 200 mg/kg induced an increase in the protein level of LXA4 in all brain regions examined. This effect was significant ($P < 0.05$) in the cortex, hippocampus and in the total brain compared to control group, but not in the cerebellum (Fig. 2).

3.2. Mushroom-induced increase of LXA4 in peripheral tissues

In the group of animals receiving chronic administration of *Coriolus*, compared to untreated controls, brain changes in LXA4 protein were associated with a significant ($P < 0.05$) increase in plasma (Fig. 3A), lymphocytes and peripheral organs, such as liver and kidney (Fig. 3B).

3.3. Modulation of HO-1 and Hsp70 protein expression in rat brain after mushroom supplementation

As shown in Figs. 4 and 5, mushroom supplementation with *Coriolus* biomass resulted in up-regulation of brain cellular stress

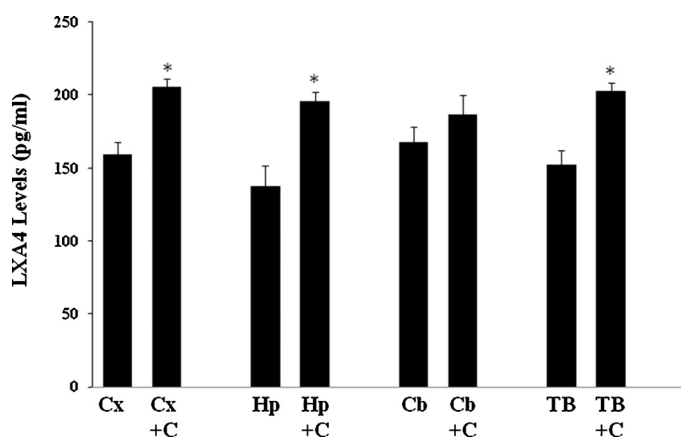


Fig. 2. Regional distribution of Lipoxin A4 protein levels in different brain regions and in total brain of control or *Coriolus*-fed rats. Values are expressed as mean \pm SEM of three independent analyses on 10 animals per group. CX: cortex; Hp: hippocampus; Cb: cerebellum; TB: total brain. C: *Coriolus*, given orally at the dose of 200 mg/kg for 30 days.

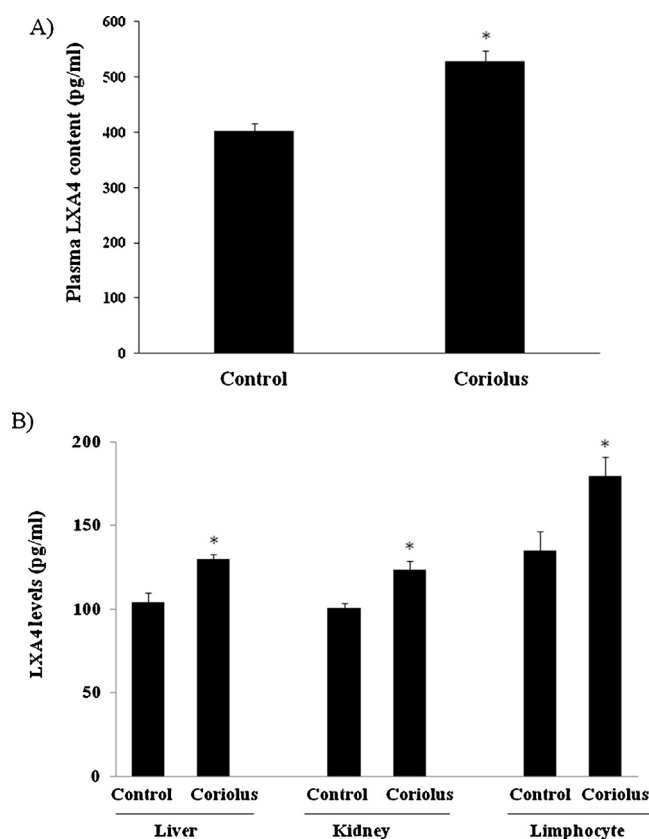


Fig. 3. (A) LXA4 levels in plasma from rats fed *Coriolus* biomass preparation as compared to the control group. Data are expressed as mean \pm SEM of 10 animals per group. * $P < 0.05$ vs controls; (B) LXA4 levels in liver, kidney and in lymphocytes from rats fed *Coriolus* biomass preparation as compared to control group. Data are expressed as mean \pm SEM of 10 animals per group. * $P < 0.05$ vs controls.

response proteins heme oxygenase-1 (HO-1) (Fig. 4), and the inducible isoform of Hsp70 (Fig. 5). A representative Western blot, obtained by probing total brain tissue homogenate with an antibody specific for the inducible isoform of heme oxygenase

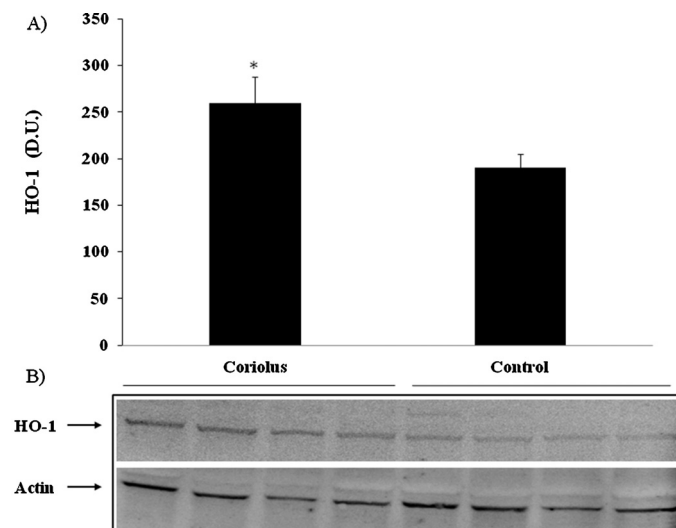


Fig. 4. Heme oxygenase-1 protein levels in the brain of rats fed *Coriolus* biomass preparation as compared to the control group. Total brain homogenates from control and mushroom-supplemented rats were assayed for HO-1 expression by Western blot. (A) Densitometric evaluation: the bar graph shows the values are expressed as mean standard error of mean of three independent analyses. * $P < 0.05$ vs control. (B) A representative immunoblot is shown. β -Actin has been used as loading control. D.U., densitometric units.

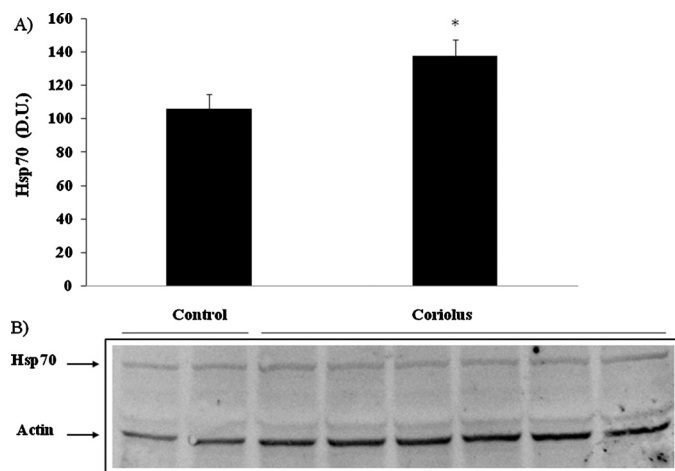


Fig. 5. Inducible Heat shock protein 70 protein levels in the brain of rats fed *Coriolus* biomass preparation as compared to control group. Total brain homogenates from control and mushroom supplemented rats were assayed for Hsp70 expression by Western blot. (A) Densitometric evaluation: the bar graph shows the values are expressed as mean standard error of mean of three independent analyses. $P < 0.05$ vs control. (B) A representative immunoblot is shown. β -Actin has been used as loading control. D.U., densitometric units.

protein, is shown in Fig. 4A. Western blot analysis of the inducible heat shock proteins 70 (Hsp72) revealed a significant increase in the brain of animals receiving *Coriolus* compared to control group. A representative blot is reported in Fig. 5B. These results were also confirmed when measuring HO-1 and Hsp70 protein expression in different brain regions of animals supplemented with *Coriolus*, as compared to the control group. Fig. 6 is a representative Western blot, obtained probing the different brain regions for HO-1 (Fig. 6A) and, respectively, Hsp70 (Fig. 6B) proteins, which show a significant increase of protein levels induced by this nutritional mushroom in the cortex, substantia nigra and hippocampus.

We then investigated whether administration of *Coriolus* affected the systemic stress response. As illustrated in Figs. 7A and 9A, a significant induction of HO-1 protein was observed in the plasma and, respectively, in lymphocytes of *Coriolus*-fed rats, as compared to the untreated rat group. Representative blots are reported in Fig. 7B and, respectively, Fig. 9B. A parallel increase in Hsp70 protein expression was found in lymphocytes (Fig. 10A and B) but not in the plasma (Fig. 8A and B) of *Coriolus*-fed animals.

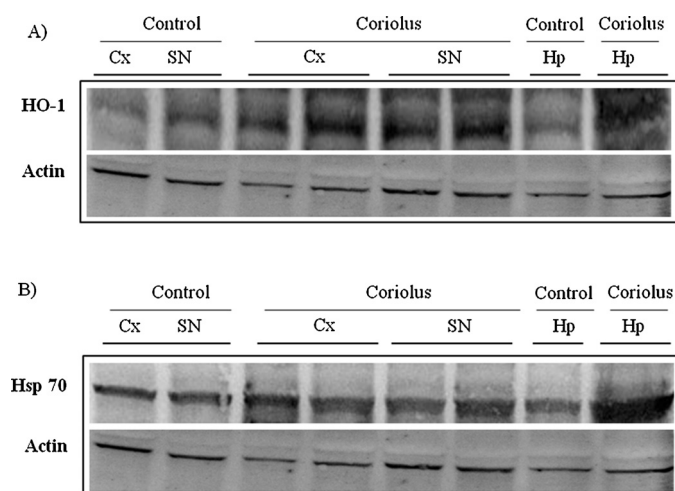


Fig. 6. Representative Western blots, obtained probing the different brain regions for HO-1 (A) and, respectively Hsp70 (B) proteins. CX: cortex; Hp: hippocampus; SN: substantia nigra.

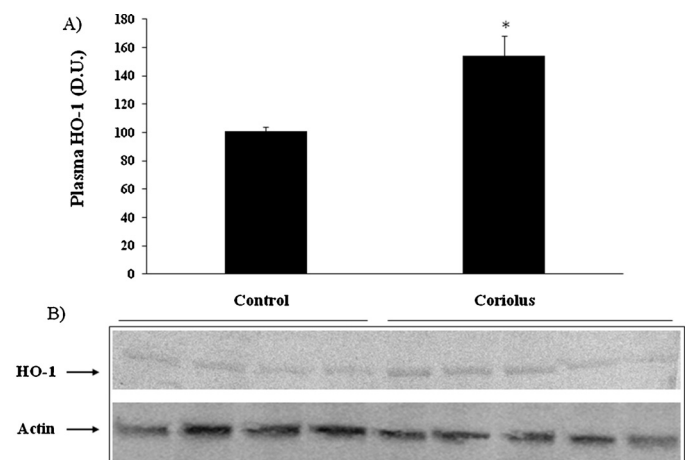


Fig. 7. Heme oxygenase-1 protein levels in plasma from rats fed *Coriolus* biomass preparation as compared to the control group. Samples from control and mushroom supplemented rats were assayed for HO-1 expression by Western blot. (A) Densitometric evaluation: the bar graph shows the values are expressed as mean standard error of mean of three independent analyses. $P < 0.05$ vs control. (B) A representative immunoblot is shown. β -Actin served as the loading control. D.U., densitometric units.

Consistent with the notion that mushroom supplementation modulates stress responsive vitagenes, we found an increased protein expression of redox-sensitive thioredoxin in total brain homogenate of *Coriolus*-fed rats, which was significantly higher relative to the basal expression of this redox protein measured in control, untreated animals (Fig. 11). A representative Western blot, obtained by probing total brain tissue homogenate with an antibody specific for the thioredoxin protein is shown in Fig. 11B.

3.4. Effect of *Coriolus* on $I\kappa B$ - α , NF- κB p65, iNOS and COX-2 expression

To determine if treatment with *Coriolus* induced inflammation in rats, we assessed by Western blot analysis the degradation of $I\kappa B$ - α and the nuclear translocation of NF- κB p65. A basal level of $I\kappa B$ - α was detected in all experimental groups and in all organs examined (Fig. 12A and C). Consequently, translocation of p65 in

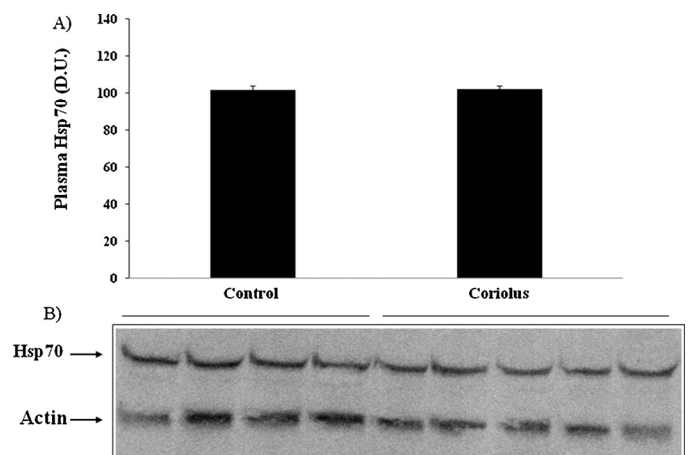


Fig. 8. Inducible Heat shock protein 70 protein levels in plasma from rats fed *Coriolus* biomass preparation as compared to the control group. Samples from control and mushroom-supplemented rats were assayed for Hsp70 expression by Western blot. (A) Densitometric evaluation: the bar graph shows the values are expressed as mean standard error of mean of three independent analyses. $P < 0.05$ vs control. (B) A representative immunoblot is shown. β -Actin has been used as loading control. D.U., densitometric units.

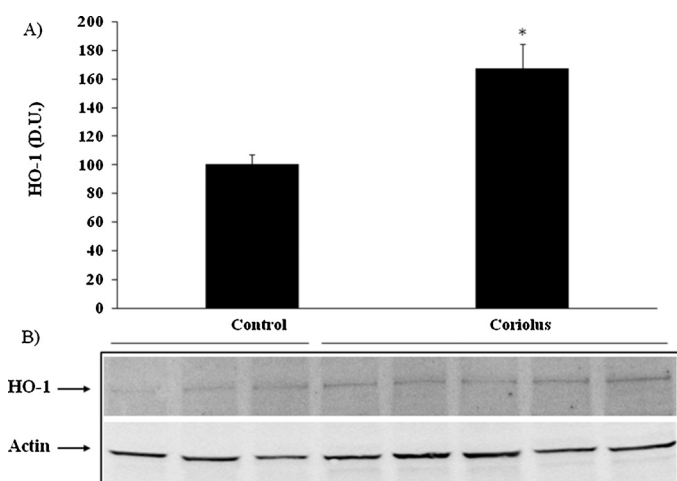


Fig. 9. Heme oxygenase-1 protein levels in lymphocytes from rats fed *Coriolus* biomass preparation as compared to the control group. Samples from control and mushroom supplemented rats were assayed for HO-1 expression by Western blot. (A) Densitometric evaluation: the bar graph shows the values are expressed as mean standard error of mean of three independent analyses. $P < 0.05$ vs control. (B) A representative immunoblot is shown. β -Actin has been used as loading control. D.U., densitometric units.

the nuclear extracts from total brain, liver and kidney homogenates was not observed in either the Sham group or in the groups treated with *Coriolus*. Furthermore, changes in both iNOS and COX-2 expression were not observed in rats treated with *Coriolus* compared to control group (data not shown).

4. Discussion

We provide evidence of the neuroprotective action of *Coriolus* when administered orally to rat. Expression of LXA4 was measured in different brain regions after oral administration of a biomass *Coriolus* preparation, given for 30 days. LXA4 up-regulation was associated with an increased content of redox-sensitive proteins involved in the cellular stress response, such as Hsp72, Heme oxygenase-1 and Thioredoxin. In the brain of rats receiving

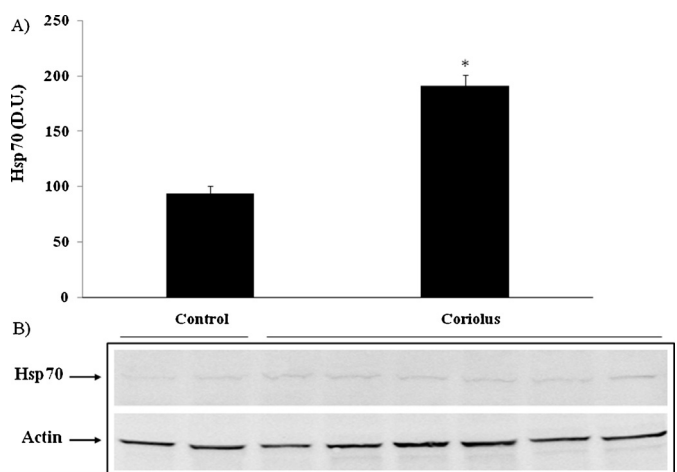


Fig. 10. Inducible Heat shock protein 70 protein levels in lymphocytes from rats fed *Coriolus* biomass preparation as compared to the control group. Samples from control and mushroom supplemented rats were assayed for Hsp70 expression by Western blot. (A) Densitometric evaluation: the bar graph shows the values are expressed as mean standard error of mean of three independent analyses. $P < 0.05$ vs control. (B) A representative immunoblot is shown. β -Actin served as the loading control. D.U., densitometric units.

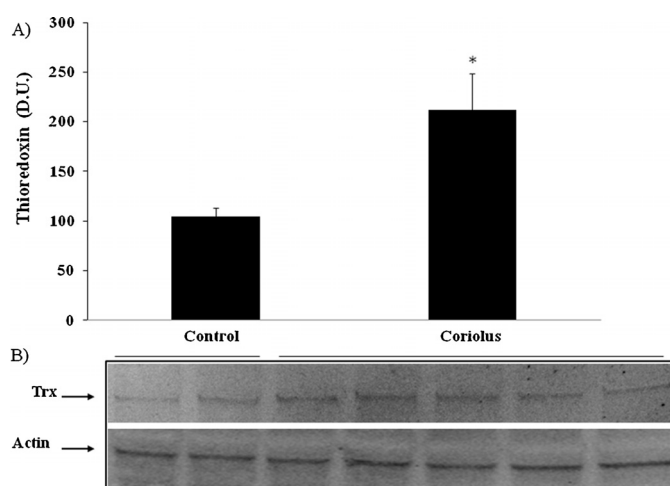


Fig. 11. Thioredoxin protein levels in the brain of rats fed *Coriolus* biomass preparation as compared to the control group. Total brain homogenates from control and mushroom-supplemented rats were assayed for thioredoxin (Trx) by Western blot. (A) Densitometric evaluation: the bar graph shows the values are expressed as mean standard error of mean of three independent analyses. $P < 0.05$ vs control. (B) A representative immunoblot is shown. β -Actin has been used as loading control. D.U., densitometric units.

Coriolus, maximum induction of LXA4 was observed in cortex and hippocampus. Hsps induction was associated with no significant changes in I κ B α , NF κ B and COX-2 brain levels.

This is the first study describing the distribution in the brain of a potential vitagene protein such as LXA4. We show that substantia nigra and septum are the brain regions with the lowest content of this neuroprotective agent. This finding is relevant to AD and PD pathogenesis, particularly to theories connecting aging and neuronal degeneration with oxidative damage. SN neurons are depleted during physiological aging and even more so in all neurodegenerative processes associated with parkinsonian symptoms (Uhl, 1998; Junn and Mouradian, 2001; Calabrese and Fariello, 1988; Calabrese et al., 2002; Calabrese et al., 2007). Furthermore, the septal region, a predominantly cholinergic structure, and cholinergic neurons are primarily affected in primary degenerative dementia of Alzheimer type (36) (Calabrese et al., 2004; Castellani et al., 2010; Calabrese et al., 2012). In addition, we demonstrate that *Coriolus* treatment resulted in a significant increase of LXA4 in most brain regions examined and in the modulation of the expression of cytoprotective proteins, such as heme oxygenase-1, Hsp70 and Thioredoxin. Our results are consistent with the notion that protein-bound polysaccharides or polysaccharopeptides produced by *C. versicolor* are effective immunopotentiators.

4.1. Relevance to Alzheimer's disease

AD is a progressive neurodegenerative disorder that represents the most common cause of dementia in the elderly, accounting for 50–60% of all cases in Western world (Buratti et al., 2015). Characterized by cognitive decline, this pathology affects more than 15 million people worldwide (Cornelius et al., 2014; Pietrzak et al., 2015). The pathological hallmarks of AD are amyloid plaques, containing amyloid- β peptide, derived from the transmembrane amyloid precursor protein, and neurofibrillary tangles, composed of hyperphosphorylated tau protein, in the medial temporal lobe structures and cortical areas of the brain together with neuronal death and synapses loss (Cornelius et al., 2013). Recently, the involvement of neuroinflammation and microglial activation in the pathogenesis of AD has been emphasized by compelling evidence

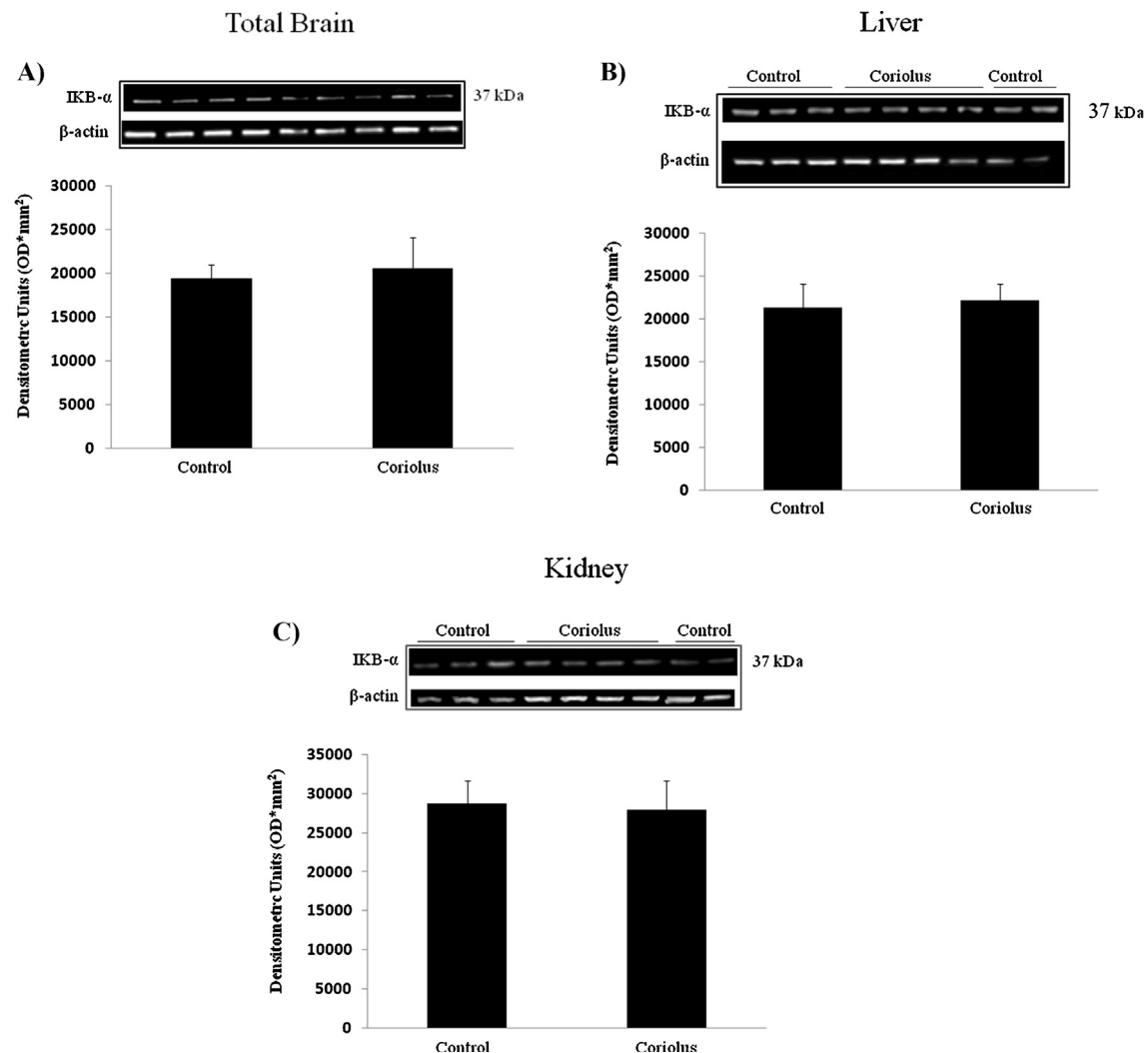


Fig. 12. Western blot analysis of IκB-α degradation in (A) total brain, (B) liver and (C) kidney of control and *Coriolus*-supplemented rats.

from basic and clinical research studies indicating that inflammation induced by Aβ is intimately associated with the development of AD neuropathology (Joshi and Praticò, 2015). Aβ activates microglia (Wu et al., 2011), the resident macrophages of the brain, and activated microglia may then promote neuronal injury through the release of proinflammatory and cytotoxic factors, including IL-1b, TNFα and NF-κB (Wu et al., 2011; Dunn et al., 2015). NF-κB, in particular, plays a key role in inflammation associated with neurodegenerative diseases such as AD, by sustaining further glial activation via a detrimental cycle where neuroinflammation and oxidative stress act in synergy, exacerbating the course of the disease (McGeer and McGeer, 2002; O'Neill and Kaltschmidt, 1997). In the quiescent state, NF-κB is sequestered in the cytoplasm by its inhibitor of NF-κB (IκB) molecules. Upon activation, IκB is phosphorylated by the IκB kinase (IKK) complex leading to its degradation, and subsequently, nuclear transport of NF-κB proteins initiates the downstream transcription of target genes (Liu et al., 2012). Furthermore, recent advances in knowledge of the mechanisms of inflammatory resolution have identified lipoxins as attractive therapeutic tools to treat diseases in which inflammation is involved (Hawkins et al., 2014). Lipoxin A4 (LXA4) is generated via the lipoxygenase pathway during cell-cell interactions in inflammatory conditions, whereas aspirin-triggered LXA4 (ATL), a molecule that displays the same anti-inflammatory activities as the native lipoxins, is generated after the acetylation of

cyclooxygenase-2 and is more resistant to metabolic inactivation (Yang et al., 2014). Lipoxins potentiate inflammatory resolution by means of potent agonistic actions at the G-protein-coupled receptor, termed the LXA4 receptor (ALX/FPR2). Activation of ALX by LXA4 impacts many endogenous processes, such as neutrophil and eosinophil recruitment and activation, leukocyte migration, NF-κB translocation, and chemokine and cytokine production (Hawkins et al., 2014). Likewise, evidence shows that LXA4 signaling primes macrophages for chemotaxis and enhances phagocytosis of microorganisms and apoptotic cells. In the CNS, LXA4 protects neurons against experimental stroke and Ab42 toxicity by modulating inflammation. In addition, lipoxins inhibit inflammatory pain processing through their actions on astrocytic activation in the spinal cord (Abdelmoaty et al., 2013). However, the ability of LXA4 signaling to modulate neuroinflammation and AD pathology in vivo has not been yet completely elucidated.

4.2. *Coriolus versicolor* bioactive principles

Several kinds of protein-bound polysaccharides with antitumor activity are produced by the white rot fungus, *C. versicolor* (Cui and Chisti, 2003; Xu et al., 2012). In the traditional medical practices of China and Japan, *C. versicolor* mushroom is harvested, dried, ground, and made into tea. The healing properties of *C. versicolor* extracts were first noticed by Chinese and Japanese scientists who

began controlled clinical research on *C. versicolor* extracts. Interestingly, the dose of the active polymers in traditional tea corresponds to that used in modern clinical practice. In nature, *C. versicolor* grows as a bracket or shelf mushroom; however, the fungus can be grown in submerged fermentation as mycelia biomass. The best known commercial polysaccharopeptide preparations of *C. versicolor* are polysaccharopeptide Krestin and polysaccharopeptide PSP (Cui and Chisti, 2003). In addition to clinically tested Krestin and PSP, numerous other extract preparations of *C. versicolor* are marketed as nutraceuticals and traditional medicines (Lee et al., 2012). Nutraceutical polysaccharopeptide preparations are sold worldwide in the form of capsules, ground biomass tablets, syrups, food additives, and teas.

Summary

Our results indicate that nutritional modulation of critical proteins involved in brain stress tolerance can be achieved via supplementation with a well characterized strain of *C. versicolor*. Our findings are relevant to those theories connecting faulty protein quality control mechanisms with age-associated neurodegenerative diseases. Consistent to this notion, in AD pathology, the accumulation of amyloid plaques (APs), composed of amyloid-beta peptide ($A\beta$) aggregates, and neurofibrillary tangles (NFTs), composed of misfolded Tau proteins, is associated with a deficit in those mechanisms participating in the induction of cytoprotective proteins (Hsps) or, more generally, cellular pathways of stress tolerance. In these conditions, it is conceivable that administration of *Coriolus* biomass, which increases the redox potential associated with induction of vitagenes, may help vulnerable neurons to resist proteotoxic insults and, hence, apoptotic neurodegeneration. Consistent with this idea is the finding that restoration of normal proteostasis is crucial for neuronal survival (Brehme et al., 2014; Sulistio and Heese, 2015). Our finding opens potential neuroprotective strategies in brain aging and neurodegenerative disease aimed at inducing the vitagene defense system mechanism, including thioredoxin and lipoxin A4.

Conflict of interest statement

There are no conflicts of interest.

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