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Evaluation of the Mitochondria-Related Redox and Bioenergetics Effects of Gastrodin in SH-SY5Y Cells Exposed to Hydrogen Peroxide

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Abstract

Mitochondrion is the main site of ATP production in animal cells and also orchestrates signaling pathways associated with cell survival and death. Mitochondrial dysfunction has been linked to bioenergetics and redox impairment in human diseases, such as neurodegeneration and cardiovascular disease. Protective agents able to attenuate mitochondrial impairment are of pharmacological interest. Gastrodin (GAS; 4-hydroxybenzyl alcohol 4-O-beta-D-glucoside) is a phenolic glucoside obtained from the Chinese herbal medicine *Gastrodia elata* Blume and exhibits antioxidant, anti-inflammatory, and antiapoptotic effects in several cell types. GAS is able to cross the blood-brain barrier, reducing the impact of different stressors on the cognition of experimental animals. In the present work, we investigated whether GAS would protect mitochondria of human SH-SY5Y neuroblastoma cells against an exposure to a pro-oxidant agent. The cells were treated with GAS at 25 μM for 30 min before the administration of hydrogen peroxide (H_2O_2) at 300 μM for an additional 3 or 24 h, depending on the assay. We evaluated both mitochondrial redox state and function parameters and analyzed the mechanism by which GAS protected mitochondria in this experimental model. Silencing of the nuclear factor erythroid 2-related factor 2 (Nrf2) transcription factor suppressed the GAS-induced mitochondrial protection seen here. Moreover, Nrf2 knockdown abrogated the effects of GAS on cell viability, indicating a potential role for Nrf2 in both mitochondrial and cellular protection promoted by GAS. Further research would be necessary to investigate whether GAS would be able to induce similar effects in in vivo experimental models.

Keywords Gastrodin · Mitochondria · Bioenergetics · Redox impairment · Nrf2

Introduction

Mitochondria are double-membrane organelles in which the oxidative phosphorylation (OXPHOS) system is the major source of ATP in mammalian cells (Arnold 2012; Jonckheere et al. 2012). The tricarboxylic acid (TCA) cycle occurs in the mitochondrial matrix and generates reducing power (mainly in the form of NADH and $FADH_2$) that is utilized by the respiratory chain, which is located in the inner mitochondrial membrane (Robinson and Srere 1985). The

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flux of electrons between the respiratory chain complexes I–IV generates an electrochemical gradient across the inner mitochondrial membrane that is utilized by complex V (ATP synthase/ATPase) to produce ATP from ADP and Pi (Flippo and Strack 2017; Letts and Sazanov 2017). Oxygen (O_2) is the final acceptor of electrons in the respiratory chain, and inhibition of O_2 utilization by mitochondria leads to general cellular impairment causing, for example, cell death (Enríquez 2016). The integrity of mitochondrial membranes is crucial for the maintenance of the OXPHOS function (Letts and Sazanov 2017). Redox impairment in the mitochondria, for example, causes loss of membrane integrity in the organelles and affects OXPHOS directly (de Oliveira et al. 2012; de Oliveira 2015). In this context, mitochondria are a major source of reactive species in virtually any cell type in mammals (Chong et al. 2014; Sies et al. 2017). Mitochondria-related redox disruption and OXPHOS dysfunction have been seen during the intrinsic apoptotic pathway, which is dependent on the release of the mitochondria-located electron transfer cytochrome c (Green et al. 2014). Oxidation of cardiolipin, which is a lipid

responsible for the association of cytochrome c in the inner mitochondrial membrane, leads to cytochrome c release from the organelle and consequent activation of the apoptosome complex in the cytosol (Ott et al. 2007). The exposure of mammalian cells to certain toxicants leads to mitochondrial dysfunction, redox impairment, and cell death, as previously reported (Oliveira 2015; de Oliveira 2016a; de Oliveira and Jardim 2016). Moreover, mitochondrial dysfunction has been observed during neurodegeneration and cardiovascular disease (Witte et al. 2010; Peixoto et al. 2012; Tocchi et al. 2015; Anandhan et al. 2017; Erpapazoglou et al. 2017; Kanaan and Harper 2017).

There is evidence showing that natural and synthetic compounds can protect mitochondria against a myriad of toxicants and also during the progression of some types of diseases (de Oliveira et al. 2015a, 2016a, b, c; de Oliveira 2016b; Picard et al. 2016; Jardim et al. 2017). Gastrodin (GAS; 4-hydroxybenzyl alcohol 4-O-bata-D-glucoside; $C_{13}H_{18}O_7$) is a phenolic glucoside found in the Chinese herbal medicine *Gastrodia elata* Blume (Yang et al. 2007). GAS exerts antioxidant, antiapoptotic, and anti-inflammatory actions in mammalian cells (Peng et al. 2013; Xiao et al. 2016; Chen et al. 2017a). Recently, Jiang et al. (2014) demonstrated that GAS suppressed the mitochondria-related triggering of cell death in the human SH-SY5Y neuroblastoma cells by activating the p38/nuclear factor erythroid 2-related factor 2 (Nrf2) axis. Similar antioxidative and antiapoptotic effects were seen in in vivo experimental models, as shown by Peng et al. (2015). Moreover, Chen et al. (2017b) published that GAS alleviated seizures induced by pentylenetetrazole in C57BL/6 mice. GAS also exhibited antidepressant effects in experimental animals, as demonstrated by Lee et al. (2016) and Chen et al. (2016). As previously reported by Wang et al. (2008), GAS crosses the blood-brain barrier (BBB) and was found in several rat brain regions. Thus, GAS may serve as a potential neuroprotective agent.

Nrf2 is a major modulator of the redox environment in mammals (Lu 2013; Costa et al. 2016; Kim and Keum 2016). Nrf2, after being released from the Nrf2-Kelch-like ECH-associated protein 1 (Keap1) complex in the cytosol, migrates to the cell nucleus and mediates the transcription of genes involved in both antioxidant defense and metabolism of xenobiotics and takes a role in controlling the expression of genes related to mitochondria-associated bioenergetics, by binding to the antioxidant responsive element (ARE) found in these genes (Nguyen et al. 2009). Disruption in the Nrf2-mediated signaling is associated with generalized cell dysfunction, as observed experimentally and in human diseases (Jin et al. 2013; Sachdeva et al. 2014).

Even though efforts have been made aiming to elucidate how GAS would cause cytoprotective effects in several experimental models, it remains to be demonstrated exactly how this phenolic glucoside promotes mitochondrial protection in mammalian cells. Therefore, we investigated here whether and how GAS would prevent mitochondria-related bioenergetics and redox impairment in SH-SY5Y cells exposed to the pro-oxidant agent hydrogen peroxide (H_2O_2). This reactive species is produced by mitochondria and by other reactions occurring in several cell types and also plays a role during the progression of neurodegeneration, as previously reported (Coombes et al. 2011; Koppenhöfer et al. 2015). Furthermore, we analyzed whether Nrf2 would take a role in the protection elicited by GAS in this experimental model.

Materials and Methods

Materials

The cell culture-related plastic materials were obtained from Corning, Inc. (NY, USA) and Beckton Dickson (NJ, USA). Culture analytical grade chemicals were purchased from Sigma (MO, USA). The other chemicals and assay kits used in the present work were acquired as described.

Cell Culture and Chemical Treatment

The human neuroblastoma SH-SY5Y cell line was acquired from the American Type Culture Collection (Manassas, VA, USA) and cultured in Dulbecco's modified Eagle's medium (DMEM)/F-12 HAM nutrient medium (1:1 mixture; supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 1000 units/mL penicillin, 1000 μg/mL streptomycin, and 2.5 μg/mL amphotericin B) in a 5% CO_2-humidified incubator at 37 °C, as previously described (de Oliveira et al. 2015b, 2016d).

Cytotoxicity and mitochondrial dysfunction were induced by exposing the SH-SY5Y cells to H_2O_2 at 300 μM for 24 h, as previously described by our research group (de Oliveira et al. 2017a). The cells were treated with gastrodin at 5–25 μM for 30 min before exposure to H_2O_2 for an additional 3 or 24 h, as described in details according to each experiment.

Cell Viability and Cytotoxicity Analyses

Cell viability was analyzed through the 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Mosmann 1983). The release of the lactate dehydrogenase (LDH) enzyme was utilized as an index of cytotoxicity, and we performed this assay based on the protocol of the manufacturer of the kit (CytoTox 96-Non-Radioactive Cytotoxicity Assay, Promega).
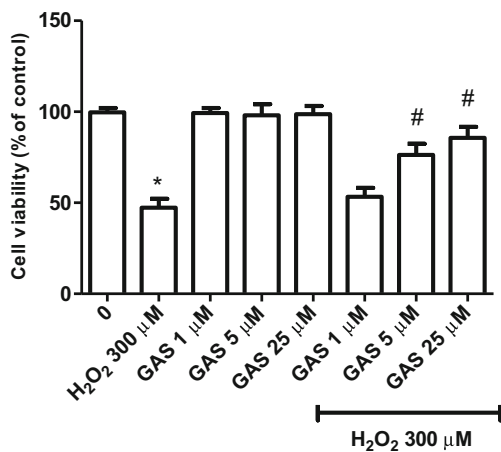
Fig. 1 The effect of GAS on the viability of SH-SY5Y cells challenged with H_2O_2. The cells were treated with GAS at 1–25 μM during 30 min before exposure to H_2O_2 at 300 μM for additional 24 h. Data are shown as the mean ± SEM of three or five independent experiments each done in triplicate. One-way ANOVA followed by the post hoc Tukey's test, *$p < 0.05$ different from the control group; # different from H_2O_2-treated group

Malondialdehyde, Protein Carbonyl, and 8-Oxo-dG Level Measurement

The levels of MDA and protein carbonyl, as well as the amounts of nuclear 8-oxo-dG, were measured by using commercial kits following the instructions of the manufacturer (Abcam, MA, USA), as previously published by us (de Oliveira et al. 2017b).
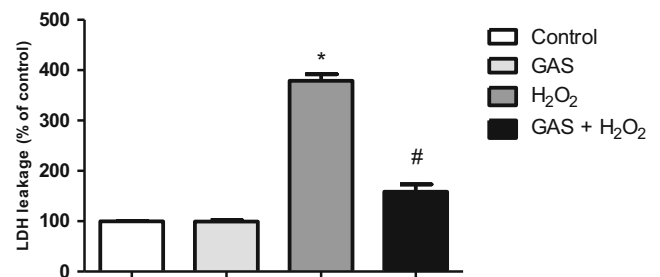

Fig. 2 The effect of GAS on the H_2O_2-induced cytotoxicity in SH-SY5Y cells. The cells were treated with GAS at 25 μM during 30 min before exposure to H_2O_2 at 300 μM for an additional 24 h. Data are shown as the mean ± SEM of three or five independent experiments each done in triplicate. One-way ANOVA followed by the post hoc Tukey's test, *$p < 0.05$ different from the control group; # different from the H_2O_2-treated group

3-Nitrotyrosine Level Quantification

We evaluated the levels of 3-nitrotyrosine by utilizing a polyclonal antibody to 3-nitrotyrosine (Calbiochem, Germany) in an indirect ELISA assay, as previously described (de Oliveira et al. 2015b, 2016d).

Mitochondrial Isolation

We isolated mitochondria from the human SH-SY5Y neuroblastoma cells by using a previously published protocol (Wang et al. 2014). Briefly, the cells were washed and re-
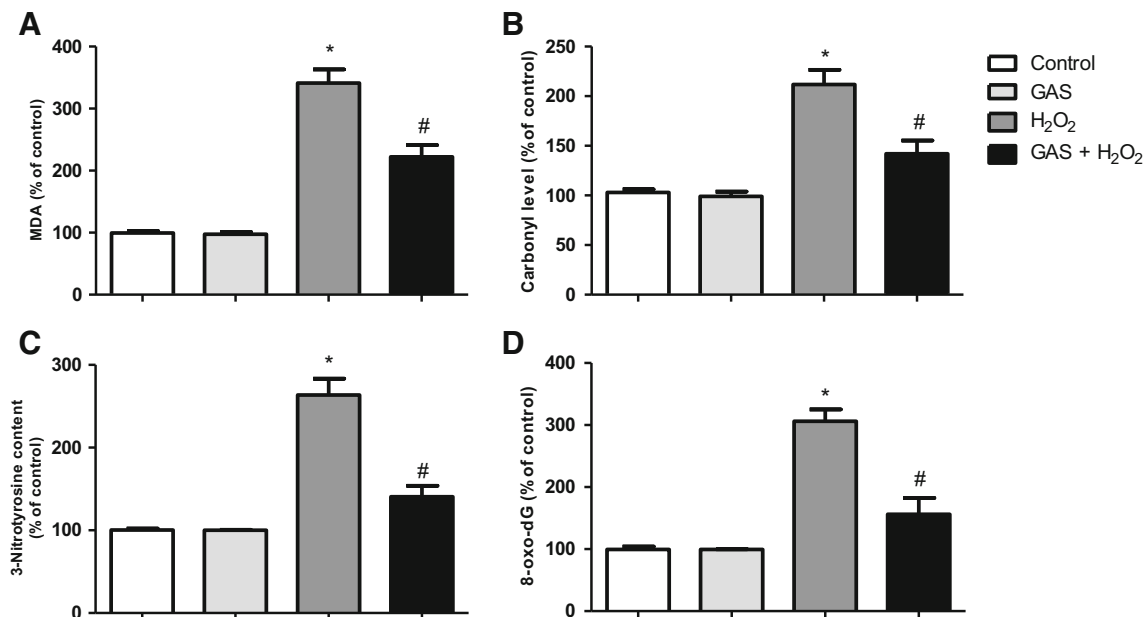

Fig. 3 The effects of GAS on the total levels of lipid peroxidation (**a**), protein carbonylation (**b**), 3-nitrotyrosine (**c**), and 8-oxo-dG (**d**) in SH-SY5Y cells exposed to H_2O_2. The cells were treated with GAS at 25 μM during 30 min before exposure to H_2O_2 at 300 μM for additional 24 h.

Data are shown as the mean ± SEM of three or five independent experiments each done in triplicate. One-way ANOVA followed by the post hoc Tukey's test, *$p < 0.05$ different from the control group; # different from the H_2O_2-treated group

suspended in a buffer containing 250 mM sucrose, 10 mM KCl, 1 mM EGTA, 1 mM EDTA, 1 mM MgCl$_2$, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 1 mM benzamidine, 1 mM pepstatin A, 10 mg/mL leupeptin, 2 mg/mL aprotinin, and 20 mM HEPES (pH 7.4). After several differential centrifugations, samples containing purified mitochondria were obtained and used in posterior assays.

Submitochondrial Particle Isolation

In order to isolate SMP from SH-SY5Y cells, the mitochondria obtained by using the abovementioned protocol were frozen and thawed (three times), causing rupture of mitochondrial membranes and leakage of mitochondrial matrix-located enzymes, such as Mn-superoxide dismutase. The samples containing SMP were washed (twice) with a buffer containing 140 mM KCl, 20 mM Tris-HCl (pH 7.4), leading to the complete leakage of Mn-superoxide dismutase leakage from the organelles. This enzyme would interfere in the quantification of the radical anion superoxide ($O_2^{-\bullet}$) produced by the organelle. Therefore, we used this protocol to verify the $O_2^{-\bullet}$ production by mitochondria and to study the redox-related effects of H_2O_2 and/or gastrodin in mitochondrial membranes (Poderoso et al. 1996).

Intracellular Reactive Oxygen Species Production Measurement

We investigated the production of intracellular ROS by using the nonpolar compound 2′-7′-dichlorodihydrofluorescein diacetate (DCFH-DA) assay, as reported (LeBel et al. 1992).
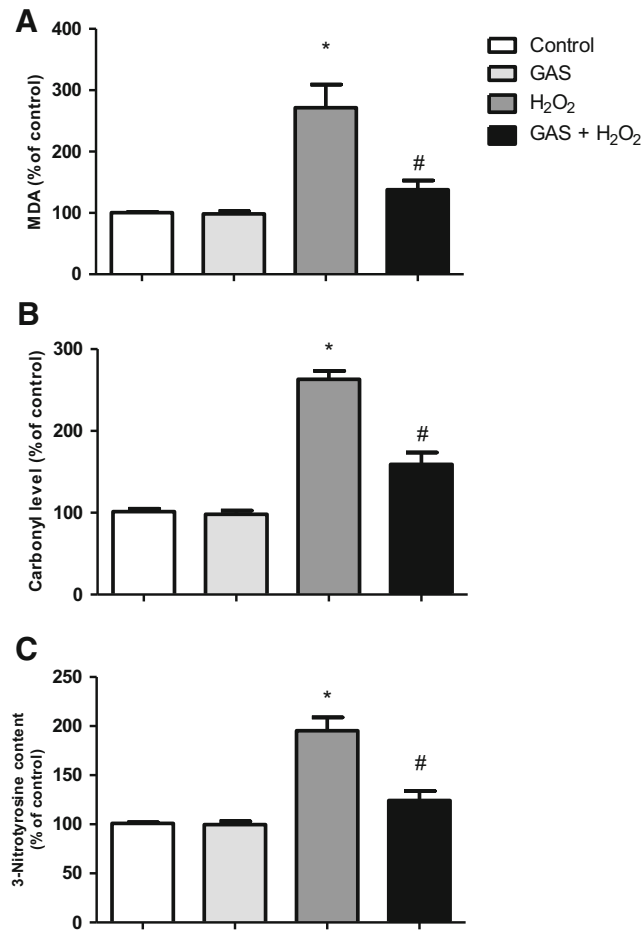

Fig. 4 The effects of GAS on levels of lipid peroxidation (**a**), protein carbonylation (**b**), and 3-nitrotyrosine (**c**) in mitochondrial membranes obtained from cultured SH-SY5Y cells exposed to H_2O_2. The cells were treated with GAS at 25 μM during 30 min before exposure to H_2O_2 at 300 μM for an additional 24 h. Data are shown as the mean ± SEM of three or five independent experiments each done in triplicate. One-way ANOVA followed by the post hoc Tukey's test, *$p < 0.05$ different from the control group; # different from the H_2O_2-treated group
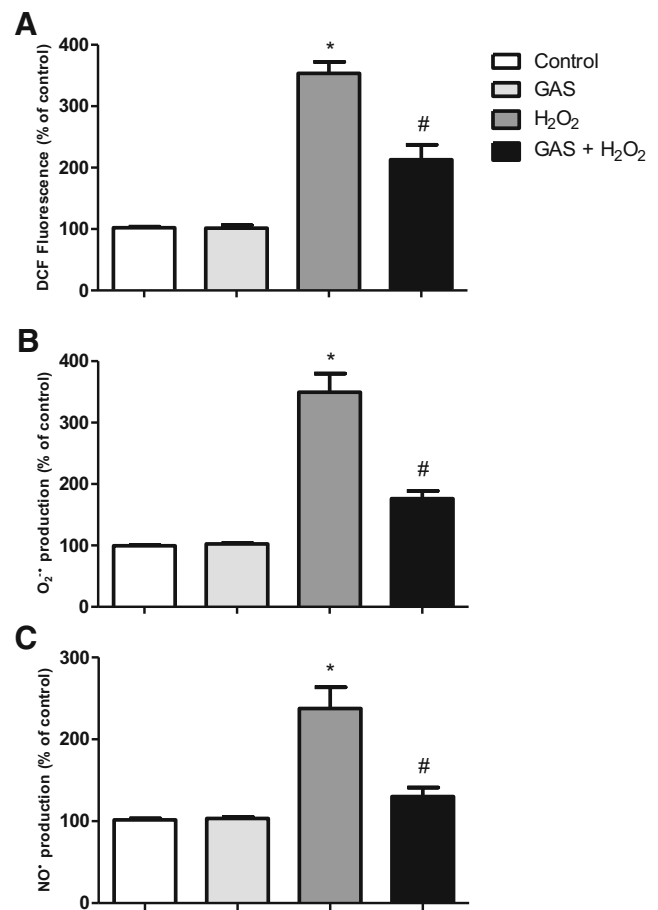

Fig. 5 The effects of GAS on the production of reactive species (**a**), $O_2^{-\bullet}$ (**b**), and NO$^\bullet$ in cultured SH-SY5Y cells challenged with H_2O_2. The cells were treated with GAS at 25 μM during 30 min before exposure to H_2O_2 at 300 μM for an additional 3 h. Data are shown as the mean ± SEM of three or five independent experiments each done in triplicate. One-way ANOVA followed by the post hoc Tukey's test, *$p < 0.05$ different from the control group; # different from the H_2O_2-treated group

$O_2^{-\bullet}$ Production Measurement

The production of $O_2^{-\bullet}$ by mitochondria was studied by measuring the auto-oxidation of adrenaline to adrenochrome in a plate reader (Molecular Devices, CA, USA) at 480 nm at 32 °C, as described by others (Poderoso et al. 1996).

Nitric Oxide Production Evaluation

We measured the production of $NO^{\bullet}$ by utilizing an assay kit based on the instructions of the manufacturer (Abcam, MA, USA).

Enzyme Activity Analyses

The activity of the mitochondria-located enzymes aconitase, α-ketoglutarate dehydrogenase (α-KGDH), succinate dehydrogenase (SDH), complex I, and complex V were quantified through the utilization of commercial kits according to the instructions of the manufacturer (Abcam, MA, USA).

ATP Level Measurement

The levels of ATP were measured by utilizing a commercial kit based on the instructions of the manufacturer (Abcam, MA, USA).
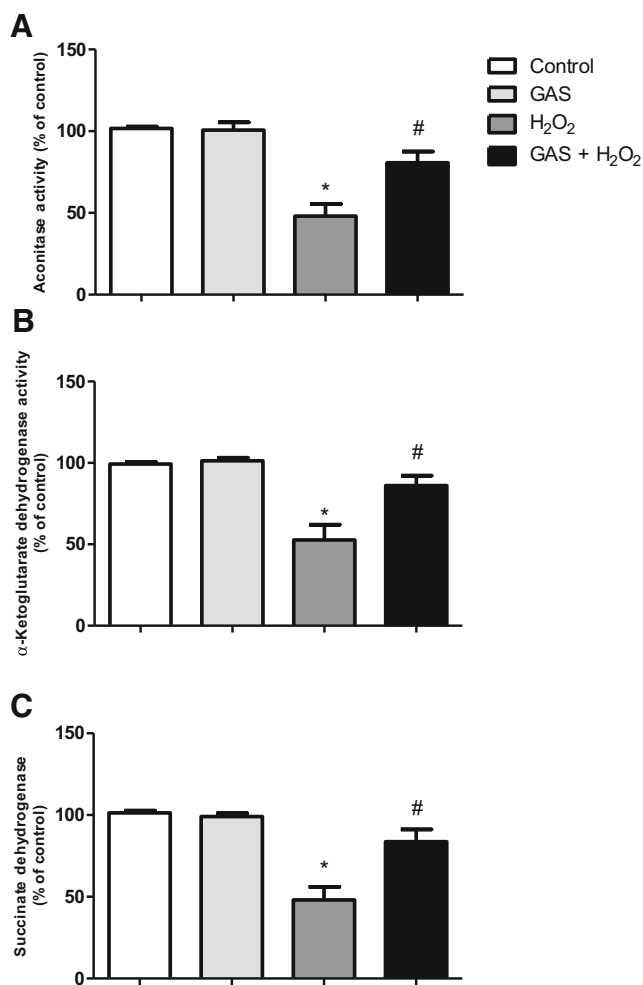

Fig. 6 The effects of GAS on the activity of enzymes of the tricarboxylic acid cycle aconitase (**a**), α-ketoglutarate dehydrogenase (**b**), and succinate dehydrogenase (**c**) in H_2O_2-treated SH-SY5Y cells. The cells were treated with GAS at 25 μM during 30 min before exposure to H_2O_2 at 300 μM for additional 24 h. Data are shown as the mean ± SEM of three or five independent experiments each done in triplicate. One-way ANOVA followed by the post hoc Tukey's test, *$p < 0.05$ different from the control group; # different from the H_2O_2-treated group

Fig. 7 The effects of GAS on the activity of the oxidative phosphorylation system complex I (**a**) and complex V (**b**) and on the levels of ATP (**c**). The cells were treated with GAS at 25 μM during 30 min before exposure to H_2O_2 at 300 μM for an additional 24 h. Data are shown as the mean ± SEM of three or five independent experiments each done in triplicate. One-way ANOVA followed by the post hoc Tukey's test, *$p < 0.05$ different from the control group; # different from the H_2O_2-treated group
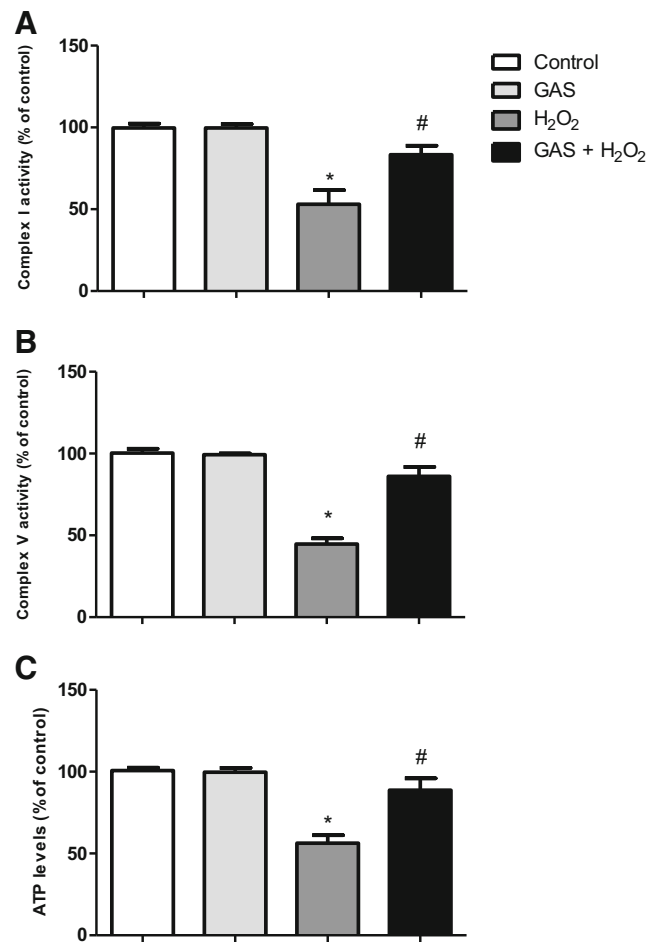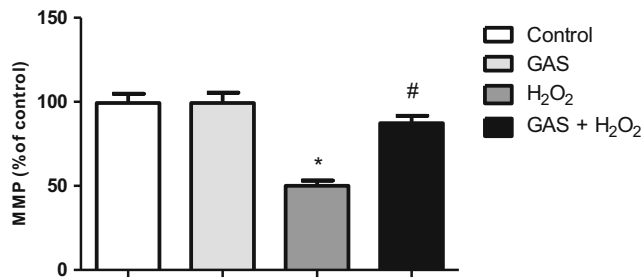
Fig. 8 The effect of GAS on the mitochondrial membrane potential (MMP) in SH-SY5Y cells exposed to H_2O_2. The cells were treated with GAS at 25 μM during 30 min before exposure to H_2O_2 at 300 μM for additional 24 h. Data are shown as the mean ± SEM of three or five independent experiments each done in triplicate. One-way ANOVA followed by the post hoc Tukey's test, $*p < 0.05$ different from the control group; # different from the H_2O_2-treated group

MMP Determination

MMP was investigated by using a commercial kit applying the tetraethylbenzimidazolylcarbocyanide iodine (JC-1) according to the instructions of the manufacturer (Abcam, MA, USA).

Nrf2 Silencing

The silencing of the transcription factor Nrf2 was obtained by performing transient transfection of SH-SY5Y cells with

Fig. 9 The effects of Nrf2 knockdown (for 48 h) on the activity of complex I (**a**) and complex V (**b**) in SH-SY5Y cells treated with GAS and/or H_2O_2. Data are shown as the mean ± SEM of three or five independent experiments each done in triplicate. One-way ANOVA followed by the post hoc Tukey's test, $*p < 0.05$ different from the cells transfected with scrambled control (NC) siRNA and treated with GAS and H_2O_2

siRNA targeting Nrf2 based on the recommendations of the manufacturer (Santa Cruz, CA, USA) and as previously described (Quesada et al. 2011; Jin et al. 2015).

Statistical Analyses

We utilized the GraphPad 5.0 software in order to perform statistical analyses in the herein presented work. Data are demonstrated as the mean ± standard error of the mean (S.E.M.) of three or five independent experiments each done in triplicate; p values were considered significant when $p < 0.05$. The differences between the experimental groups were checked by one-way ANOVA followed by the post hoc Tukey's test.

Results

GAS Attenuated Loss of Cell Viability and Cytotoxicity in SH-SY5Y Cells Treated with H_2O_2

As depicted in Fig. 1, GAS pretreatment for 30 min at 5 or 25 μM reduced the effect of H_2O_2 on the viability of SH-SY5Y cells ($p < 0.05$). Thus, we next investigated whether GAS would prevent H_2O_2-induced cytotoxicity in SH-SY5Y cells. We found that GAS pretreatment at
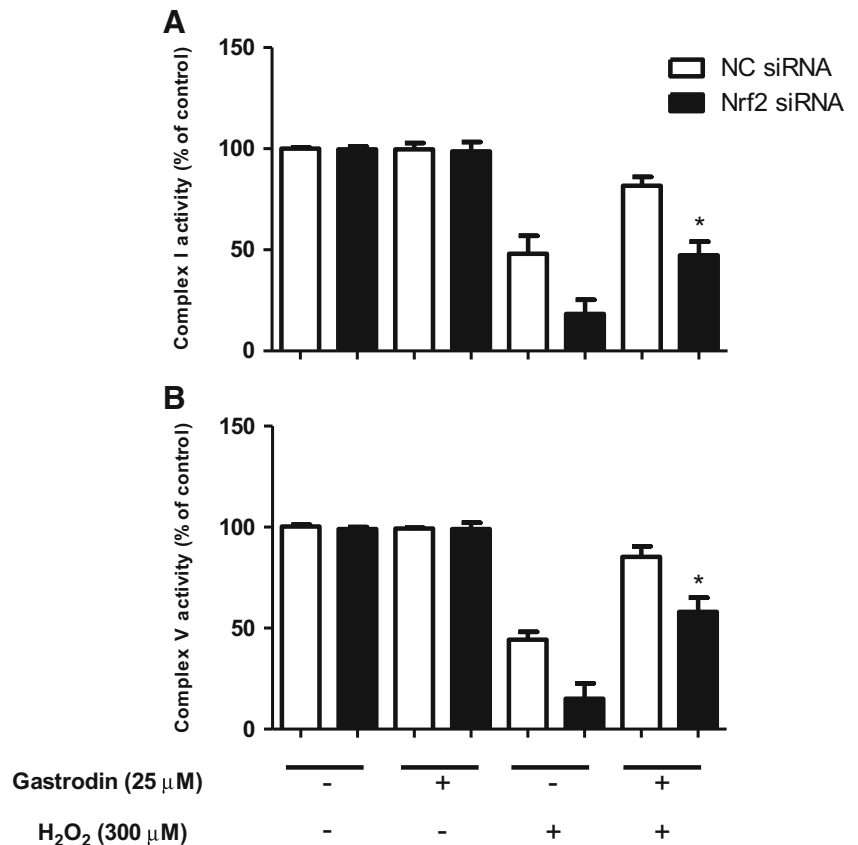
25 μM abrogated cytotoxicity elicited by H_2O_2 in this experimental model (Fig. 2).

GAS Exerted an Antioxidant Effect in SH-SY5Y Cells Exposed to H_2O_2

Based on the data demonstrating the cytoprotective effect induced by GAS in SH-SY5Y cells, we examined whether gastrodin would affect redox-related parameters in cells treated with the pro-oxidant agent H_2O_2. As may be viewed in Fig. 3, GAS pretreatment for 30 min at 25 μM decreased lipid peroxidation (Fig. 3a; $p < 0.05$), protein carbonylation (Fig. 3b; $p < 0.05$), protein nitration (Fig. 3c; $p < 0.05$), and DNA oxidation (Fig. 3d; $p < 0.05$) in H_2O_2-treated SH-SY5Y cells.

GAS Promoted Mitochondria-Related Antioxidant Effects in H_2O_2-Treated SH-SY5Y Cells

We next evaluated whether GAS would be able to protect mitochondria of SH-SY5Y cells exposed to H_2O_2. GAS pretreatment for 30 min at 25 μM significantly reduced the levels of lipid peroxidation (Fig. 4a; $p < 0.05$), protein carbonylation (Fig. 4b; $p < 0.05$), and protein nitration (Fig. 4c; $p < 0.05$) in the membranes of mitochondria obtained from SH-SY5Y cells.

GAS Alleviated the Production of Reactive Species in SH-SY5Y Cells Treated with H_2O_2

As demonstrated in Fig. 5a, GAS pretreatment for 30 min at 25 μM alleviated the production of reactive species in SH-SY5Y cells treated with H_2O_2. Based on this finding, we investigated whether GAS would exert an effect on the production of specific reactive species. GAS reduced the production of $O_2^{-\bullet}$ (Fig. 5a; $p < 0.05$) and $NO^\bullet$ (Fig. 5c; $p < 0.05$) in H_2O_2-treated SH-SY5Y cells.

GAS Prevented H_2O_2-Induced Mitochondria-Related Bioenergetics Effects in SH-SY5Y Cells

Since GAS induced an antioxidant effect in the mitochondria obtained from SH-SY5Y cells exposed to a pro-oxidant agent, we evaluated whether GAS would promote a benefit regarding the function of mitochondria. As may be observed in Fig. 6, GAS pretreatment for 30 min at 25 μM reduced the H_2O_2-induced effects on the activity of aconitase (Fig. 6a; $p < 0.05$), α-KGDH (Fig. 6b; $p < 0.05$), and SDH (Fig. 6c; $p < 0.05$).

Furthermore, GAS prevented the H_2O_2-elicited inhibition in the activities of the OXPHOS complexes I (Fig. 7a; $p < 0.05$) and V (Fig. 7b; $p < 0.05$) and prevented the reduction in the levels of ATP in SH-SY5Y cells (Fig. 7c; $p < 0.05$).
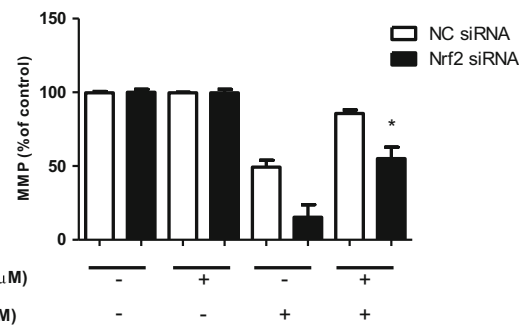

Fig. 10 The effects of Nrf2 knockdown (for 48 h) on the mitochondrial membrane potential (MMP) in SH-SY5Y cells treated with GAS and/or H_2O_2. Data are shown as the mean ± SEM of three or five independent experiments each done in triplicate. One-way ANOVA followed by the post hoc Tukey's test, *$p < 0.05$ different from the cells transfected with scrambled control (NC) siRNA and treated with GAS and H_2O_2

GAS also blocked the H_2O_2-induced loss of MMP in this experimental model, as depicted in Fig. 8 ($p < 0.05$).

GAS Exerted Mitochondrial Protection by an Nrf2-Related Mechanism

In order to analyze the mechanism underlying the mitochondria-related benefits elicited by GAS in SH-SY5Y cells, we silenced Nrf2 in these cells by using siRNA targeting Nrf2. We observed that knockdown of Nrf2 attenuated the effects of GAS pretreatment on the activity of the mitochondrial complexes I (Fig. 9a; $p < 0.05$) and V (Fig. 9; $p < 0.05$). Moreover, the protective effect elicited by GAS regarding MMP was alleviated by Nrf2 silencing in the cells exposed to H_2O_2 (Fig. 10; $p < 0.05$).

GAS Promoted Cytoprotection by a Mechanism Involving Nrf2

Finally, we evaluated the role of Nrf2 in GAS-treated cells exposed to H_2O_2. We found that Nrf2 silencing abrogated
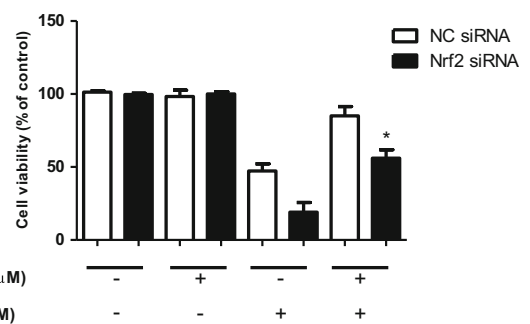

Fig. 11 The effects of Nrf2 knockdown (for 48 h) on the viability of SH-SY5Y cells treated with GAS and/or H_2O_2. Data are shown as the mean ± SEM of three or five independent experiments each done in triplicate. One-way ANOVA followed by the post hoc Tukey's test, *$p < 0.05$ different from the cells transfected with scrambled control (NC) siRNA and treated with GAS and H_2O_2

the cytoprotection induced by a pretreatment (for 30 min) with GAS (at 25 μM) in H_2O_2-treated SH-SY5Y cells (Fig. 11; $p < 0.05$).

Discussion

In the presented work, we demonstrate that a pretreatment with GAS attenuated the H_2O_2-induced mitochondrial dysfunction in SH-SY5Y cells. GAS exerted cytoprotective and antioxidative effects by reducing the levels of markers of lipid peroxidation and protein carbonylation and nitration in the mitochondrial membranes obtained from cultured SH-SY5Y cells. GAS also attenuated the production of reactive species in H_2O_2-treated cells. Moreover, GAS suppressed the effects of H_2O_2 on the activity of mitochondria-located enzymes involved in the maintenance of TCA and OXPHOS system. GAS caused mitochondrial protection, at least in part, by a mechanism associated with Nrf2, since the silencing of this transcription factor abrogated the GAS-elicited benefits regarding mitochondrial function. This is the first work demonstrating a direct role between Nrf2 and mitochondrial protection (involving redox state and function of the organelle) in GAS-treated cells.

Nrf2, in addition to the modulation of the redox environment, takes a role in the bioenergetics homeostasis in mammalian cells, as indicated by an increasing body of evidence (Kim et al. 2011; Ludtmann et al. 2014). Nrf2 modulates the expression of genes whose products are involved in the consumption of reactive species, such as Mn-SOD and glutathione peroxidase (GPx), and coordinates the expression of enzymes that participate in the metabolism of xenobiotics, such as glutathione-S-transferase (GST) (Ma 2013). Additionally, Nrf2 activation has been linked to anti-inflammatory effects induced by natural compounds, as evidenced in several experimental models (Ahmed et al. 2017). Nrf2 also demonstrated the ability to modulate cell fate by preventing death of cells exposed to some toxicants (de Oliveira et al. 2016e, 2017c). Therefore, there is increasing interest in investigating natural and synthetic agents able to promote Nrf2 activation in mammalian cells.

The maintenance of mitochondrial function is necessary physiologically and specially during exposure of cells to chemical or physical stressors (Broadley and Hartl 2008). During a mitochondria-dependent cell death event, for example, it is important to maintain some mitochondria-producing ATP in order to preserve the function of the apoptosome and, consequently, the activation of the caspases, which execute reactions that lead to the formation of apoptotic bodies (Green et al. 2014). Otherwise, absence of ATP at sufficient levels may block the activation of caspases and cell death would occur by necrosis, causing inflammation (Elmore 2007). Thus,

mitochondria play a pivotal role in both triggering and sustaining the intrinsic apoptotic pathway in mammals. Mitochondria exposed to some types of toxicants may present increased levels of protein carbonylation and nitration and augmented lipid peroxidation, in their membranes (de Oliveira and Moreira 2007; de Oliveira et al. 2009, 2011). Redox impairment in the organelles may be associated with morphological alterations and impaired mitochondrial dynamics (Klamt et al. 2005; Ito and Di Polo 2017).

In this context, GAS efficiently prevented H_2O_2-induced mitochondrial dysfunction regarding redox state and bioenergetics by a mechanism associated with the transcription factor Nrf2. Further research would be necessary in order to evaluate whether GAS would act in a similar way in in vivo experimental models.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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