CrossMark

Methionine and methionine sulfoxide treatment induces M1/classical macrophage polarization and modulates oxidative stress and purinergic signaling parameters

Lien M. dos Santos[1] · Tatiane M. da Silva[2] · Juliana H. Azambuja[1,4] · Priscila T. Ramos[1] ·
Pathise S. Oliveira[2] · Elita F. da Silveira[3] · Nathalia S. Pedra[1] · Kennia Galdino[1] ·
Carlus A. T. do Couto[1] · Mayara S. P. Soares[1] · Rejane G. Tavares[2] · Roselia M. Spanevello[1] ·
Francieli M. Stefanello[2] · Elizandra Braganhol[4]

Abstract Methionine is an essential amino acid involved in critical metabolic process, and regulation of methionine flux through metabolism is important to supply this amino acid for cell needs. Elevation in plasma methionine commonly occurs due to mutations in methionine-metabolizing enzymes, such as methionine adenosyltransferase. Hypermethioninemic patients exhibit clinical manifestations, including neuronal and liver disorders involving inflammation and tissue injury, which pathophysiology is not completely established. Here, we hypothesize that alterations in macrophage inflammatory response may contribute to deleterious effects of hypermethioninemia. To this end, macrophage primary cultures were exposed to methionine (1 mM) and/or its metabolite methionine sulfoxide (0.5 mM), and M1/proinflammatory or M2/anti-inflammatory macrophage polarization was evaluated. In addition, inflammation-related pathways including oxidative stress parameters, as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) activities; reactive oxygen species (ROS) production, and purinergic signaling, as ATP/ADP/AMPase activities, were investigated. Methionine and/or methionine sulfoxide induced M1/classical macrophage activation, which is related to proinflammatory responses characterized by increased iNOS activity and TNF-α release. Further experiments showed that treatments promoted alterations on redox state of macrophages by differentially modulated SOD and CAT activities and ROS levels. Finally, methionine and/or methionine sulfoxide treatment also altered the extracellular nucleotide metabolism, promoting an increase of ATPase/ADPase activities in macrophages. In conclusion, these findings contribute to better understand the participation of proinflammatory responses in cell injury observed in hypermethioninemic patients.

Keywords Methionine · Methionine sulfoxide · Macrophage polarization · Oxidative stress · Ectonucleotidases

✉ Francieli M. Stefanello
  fmstefanello@gmail.com

✉ Elizandra Braganhol
  ebraganhol@ufcspa.edu.br

1 Laboratório de Neuroquímica, Inflamação e Câncer, Centro de Ciências Químicas Farmacêuticas e de Alimentos, Universidade Federal de Pelotas (UFPel), Pelotas, RS, Brazil

2 Laboratório de Biomarcadores, Centro de Ciências Químicas Farmacêuticas e de Alimentos, Universidade Federal de Pelotas (UFPel), Campus Universitário s/n, Capão do Leão, Pelotas, RS 96160-000, Brazil

3 Departamento de Morfologia, Instituto de Ciências Biológicas, Universidade Federal de Rio Grande (FURG), Rio Grande, RS, Brazil

4 Departamento de Ciências Básicas da Saúde, Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA), Rua Sarmento Leite, 245 - Anexo I - sala 303, Porto Alegre, RS 90050-170, Brazil

Introduction

Hypermethioninemia is an inborn error of amino acid metabolism commonly related to methionine adenosyl-transferase (MAT) deficiency [1, 2]. As a result of altered methionine metabolism, a persistent increase of this amino acid and its metabolite methionine sulfoxide is found in blood and tissues of patients [2], leading to oxidative stress and tissue damage with inflammatory response associated [2, 3]. Hypermethioninemic patients exhibit clinical manifestations, including cognitive impairment, cerebral

edema, and demyelination, as well as liver disorders involving inflammation and tissue injury, which pathophysiology is not completely established [2, 4, 5].

Macrophages are key components of innate immune response, acting in almost all aspects of inflammatory processes, through its abundance, distribution, mobility, and responsiveness [6]. Additionally, these cells are characterized by a marked heterogeneity and its activation spectrum is modulated by microenvironment [7]. M1/classical macrophage activation (proinflammatory phenotype) is characterized by proinflammatory cytokine production, release of cytotoxic mediators as reactive oxygen and nitrogen species (ROS/RNS), increased phagocytic and microbicidal activities, and ability to initiate an adaptive immune response. In contrast, M2/alternative macrophage activation (anti-inflammatory phenotype) is characterized by an anti-inflammatory activity, remodeling function, wound healing, and tissue repair, which indicate the resolution of inflammatory response [7–12].

Macrophages are activated in response to danger signals, such as microbial products, cytokines, chemokines, and mediators, released by damaged cells, such as nucleotides [13, 14]. Indeed, ATP and adenosine are emerging as important mediators of inflammatory and immune responses via purinergic receptor activation [15, 16]. ATP acts as a proinflammatory molecule by stimulating the recruitment of immune cells to damage tissue and by inducing the release of IL-1β, IL-6, and TNF-α [15, 17, 18]. In contrast, adenosine, the breakdown product of ATP hydrolysis, has opposite effects, acting as an anti-inflammatory and immunosuppressive molecule. For example, this nucleoside has been known to affect TNF-α secretion and increases IL-10 production in macrophages [19, 20]. Purinergic receptor activation is controlled by ectonucleotidases, including members of the ectonucleoside triphosphate diphosphohydrolase (E-NTPDase) family and the ecto-5′-nucleotidase/CD73 (ecto-5′-NT/CD73), which efficiently hydrolyze extracellular nucleotides to respective nucleosides in the extracellular space [21, 22].

Although inflammatory complications have been observed in hypermethioninemic patients, little is known about the mechanisms involved in the pathophysiology of this disorder. Here, we hypothesize that methionine and/or methionine sulfoxide induce a proinflammatory/M1 macrophage polarization, which in turn may contribute to deleterious effects of hypermethioninemia. Therefore, we investigated the in vitro effect of methionine and/or methionine sulfoxide on mouse macrophage phenotype and evaluated some inflammatory parameters associated as oxidative stress and ectonucleotidase activity. The potential contribution of methionine and/or methionine sulfoxide-activated macrophages in the pathophysiology of hypermethioninemia is further discussed.

Materials and methods

Chemicals

Roswell Park Memorial Institute (RPMI1640) medium and fetal bovine serum (FBS) were obtained from Gibco (Gibco BRL, Carlsbad, CA, USA). Nucleotides (ATP, ADP, AMP), methionine and methionine sulfoxide were purchased from Sigma Chemical (St. Louis, MO, USA). Cytokine kits were provided by R&D Systems (Minneapolis, MN, USA). All other chemicals and solvents used were of analytical or pharmaceutical grade.

Animals

Swiss male mice (8 weeks-old) were maintained under a standard dark–light cycle (lights on between 7:00 a.m. and 7:00 p.m.) at room-controlled temperature (22 ± 2 °C). Mice had free access to standard laboratory chow and water. All procedures used in the present study followed the "Principles of Laboratory Animal Care" of the National Institute of Health and were approved by the Ethical Committee of UFPel (protocol number 9221).

Macrophage cultures and differential macrophage phenotype activation

Macrophages were collected by lavage of peritoneal cavity with 5 mL of sterile RPMI1640/FBS-free culture medium. Cells were washed twice with sterile PBS, suspended in RPMI1640/FBS-free medium, transferred to 6 or 48 multiwell plates, and allowed to attach for 30 min in cell incubator (37 °C and 5 % CO_2 atmosphere). Unattached cells were washed out with RPMI1640/FBS-free medium. Attached cells, mainly peritoneal macrophages, were used for the experiments thereafter. To evaluate differential macrophage phenotype activation, obtained macrophages were treated for 18 h with methionine (1 mM) and/or methionine sulfoxide (0.5 mM) in complete medium (RPMI1640/10%FBS). Macrophages stimulated with lipopolysaccharides (LPS) (10 ng/mL) or IL-4 (10 ng/mL) were applied as positive controls of M1/classically or M2/alternative macrophage activation, respectively [8]. Macrophages exposed to RPMI1640/10%FBS were applied as control. Macrophage culture purity was evaluated by images captured using a digital camera connected to an inverted microscope (Olympus IX71, Japan).

Cell viability assay

Macrophages were seeded (1×10^6 cells/well) in 96 multiwell plates, and cells were exposed to methionine

and/or methionine sulfoxide as described above. Following 18 h of treatment, cell viability was assessed by 3(4,5-dimethyl)-2,5-diphenyl tetrazolium bromide (MTT) assay. This method is based on the ability of viable cells to reduce MTT and form a blue formazan product. MTT solution was added to culture medium at a final concentration of 0.5 mg/mL. After 90 min of incubation, medium was removed and plates were shaken with DMSO. Optical density of each well was measured at 492 nm. Results were expressed as percentage of control (untreated cells).

Cell proliferation assay

Macrophage cultures were prepared and treated as described above. Following 18 h of treatment, cells were washed and fixed in trichloroacetic acid (TCA) 50 % for 45 min at 4 °C. Then, TCA was removed, cells were washed 5 times with distilled water, and 0.4 % sulforhodamine B (SRB) solution in 1 % acetic acid was added. Following 30 min incubation to stain proteins, SRB was removed and cells were washed 5 times with 1 % acetic acid for complete removal of uncomplexed protein dye. Finally, SRB was eluded in Tris solution (10 mM). Optical density of each well was measured at 530 nm. Results were expressed as percentage of control (untreated cells).

Nitrite and arginase assays

Nitrite concentrations were measured using the Griess reaction [23]. In brief, 200 μL of tested cell medium were incubated with 100 μL of 1 % sulfanilamide and 100 μL of 0.3 % N-1-naphthylethylenediamine dihydrochloride at room temperature for 5 min. Nitrite was quantified by spectrophotometry at 540 nm using sodium nitrite as standard. Results were expressed as μM per mg of protein.

Arginase activity in cell lysates was measured based on the conversion of L-arginine to L-ornithine and urea according to the technique described by Corraliza et al. [24] with minor modifications. Briefly, cells were lysed for 30 min with 40 μL of 0.1 % Triton X-100. Thirty microliters of 25 mM Tris–HCl (pH 7.4) and 10 μL of 10 mM MnCl$_2$ were added and the enzyme was heat-activated for 10 min at 56 °C. Similar amounts of samples (40 μL) and 0.5 M L-arginine (pH 9.7) were mixed and incubated for 1 h at 37 °C. The reaction was stopped by adding 400 μL of H$_2$SO$_4$ (96 %), H$_3$PO$_4$ (85 %), H$_2$O (1/3/7, v/v/v). The urea concentration was measured at 540 nm after the addition of 8 μL of 6 % α-isonitrosopropiophenone, followed by heating at 95 °C for 30 min. Values were compared with a standard curve of urea concentration, and they were expressed as nmol urea per min per mg protein.

Cytokine release determination

TNF-α and IL-10 secreted by macrophage cultures were quantified in the conditioned media of these cells by enzyme-linked immunosorbent assay (ELISA), according to the manufacturer's instructions (R&D Systems). Mouse recombinant TNF-α or IL-10 was used as a standard. Results were expressed as ng of cytokine per mL.

Determination of SOD, CAT, and GPx activities and ROS production

Catalase (CAT) activity was assayed according to Aebi [25] based on the decomposition of H$_2$O$_2$ monitored at 240 nm at room temperature. CAT activity was reported as percentage of control (untreated cells).

Superoxide dismutase (SOD) activity was measured by the method described by Misra and Fridovich [26]. This method is based on the inhibition of superoxide-dependent adrenaline auto-oxidation in a spectrophotometer adjusted at 480 nm. SOD activity was reported as percentage of control (untreated cells).

Glutathione peroxidase (GPx) activity was measured according to the method described by Wendel [27], using *tert*-butyl hydroperoxide as substrate. NADPH disappearance was monitored at 340 nm in a medium containing 2 mM glutathione, 0.15 U/mL glutathione reductase, 0.4 mM azide, 0.5 mM *tert*-butyl hydroperoxide, and 0.1 mM NADPH. GPx activity was reported as percentage of control (untreated cells).

Intracellular generation of ROS was determined by DCFH-DA (2′,7′-dichlorodihydrofluorescein diacetate) assay. DCF-DA reacts with reactive oxygen species emitting fluorescence. Macrophages were incubated with 1 μM DCFH-DA in serum-free medium for 30 min. Then, cells were washed with PBS and fluorescence was determined in multiwell plate reader (485/520 nm). ROS production was reported as percentage of control (untreated cells).

Ectonucleotidase assay

ATPase, ADPase, and AMPase activities were evaluated in 48 well plates containing macrophages that were washed three times with phosphate-free incubation medium in the absence of nucleotides. The enzymatic reaction was started by the addition of 200 μL of incubation medium containing 2 mM CaCl$_2$ (2 mM MgCl$_2$ for AMPase assay), 120 mM NaCl, 5 mM KCl, 10 mM glucose, 20 mM HEPES (pH 7.4), and 2 mM ATP, ADP, or AMP as substrates. Following 10 min incubation at 37 °C, the reaction was stopped by transferring an aliquot of the incubation medium to a pre-chilled tube containing TCA (final concentration 5 % w/v). The release of inorganic phosphate (P$_i$) was measured by the malachite green method [28], using

KH_2PO_4 as a P_i standard. Controls to determine non-enzymatic P_i release were performed by incubating the cells in the absence of the substrate, or the substrate in the absence of the cells. All samples were run in triplicate. The protein concentration was measured by coomassie blue method using bovine serum albumin as standard [29]. Specific activity was expressed as nmol P_i released per min per mg of protein.

Statistical analysis

Data were expressed as mean ± SD and were subjected to one-way analysis of variance (ANOVA) followed by Tukey post hoc test (for multiple comparisons). Differences between mean values were considered significant when $P \leq 0.05$.

Results

Methionine and/or methionine sulfoxide alter macrophage cell shape and induce M1/classical macrophage polarization

Cell shape, biochemical profile, and cytokine changes have been associated to different functional states of M1/classical and M2/alternative macrophage polarization [7, 30–32]. To explore whether methionine and/or methionine sulfoxide treatment may play a role in macrophage phenotypic polarization, cells were cultured on multiwell plates and they were exposed to 1 mM methionine and/or 0.5 mM methionine sulfoxide, which represent the concentrations found in human blood with persistent hypermethioninemia [2, 33–36]. Macrophages exposed to LPS or IL-4 were applied as positive controls to M1/classical and M2/alternative macrophage polarization, respectively. Following 18 h of stimulation, macrophage polarization was evaluated by analysis of cell morphology, iNOS and arginase activities, and TNF-α and IL-10 cytokine release. As shown in Fig. 1, exposition of macrophages to methionine, methionine sulfoxide, or its combination (mix) promoted significant morphological alterations, making cells to flatten into a round, pancake-like shape (Fig. 1a). In addition, no significant changes in cell viability or proliferation were observed in macrophages exposed to treatments, as determined by MTT and SRB assays (Fig. 1b, c). Finally, methionine, methionine sulfoxide, or mix promoted an increase of 1.95, 2.2, and 1.82 times of iNOS activity, respectively (Fig. 2a) and 4.7, 3.7, and 2.6 times of TNF-α, respectively (Fig. 2c) when compared to controls, whereas arginase (Fig. 2b) and IL-10 secretion (Fig. 2d) were not significantly altered at same condition. Taken together, these data suggest that methionine and/or methionine sulfoxide treatment induces M1/classical macrophage polarization.
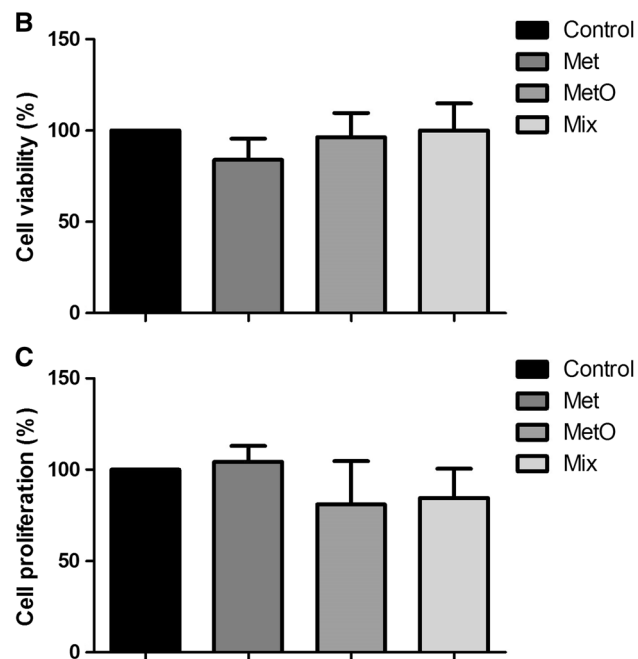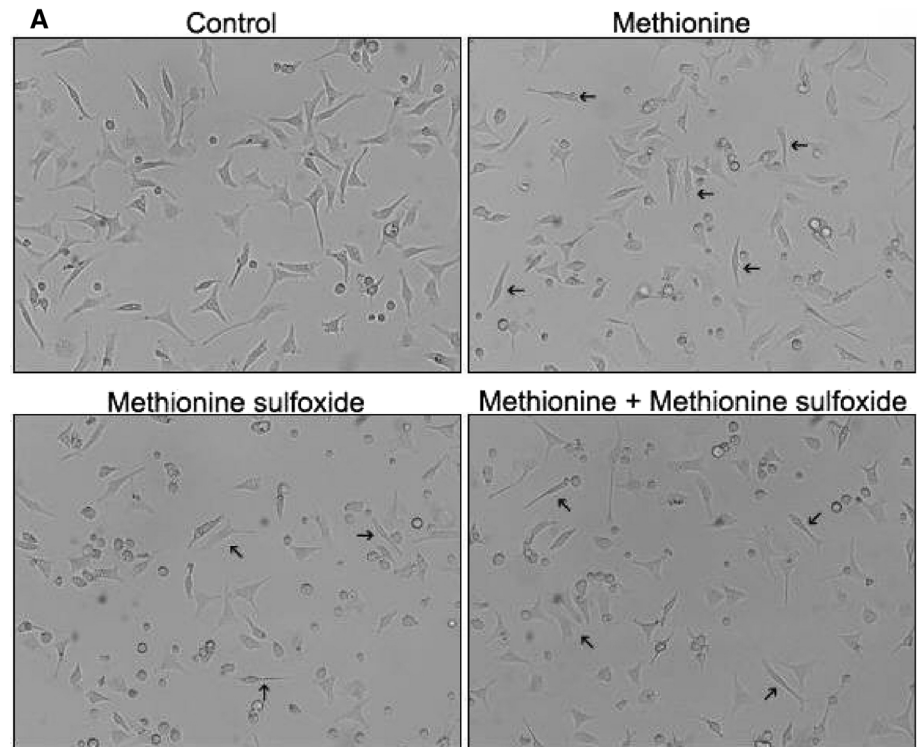
Methionine and/or methionine sulfoxide modulate antioxidant enzyme activity and ROS production in macrophage cultures

To better understand the potential of methionine and/or methionine sulfoxide to modulated M1/classical macrophage polarization, we examined the metabolism of reactive oxygen species in cultured macrophages, which has been implicated in proinflammatory signaling [6, 37–40]. Macrophages were exposed to methionine and/or methionine sulfoxide for 18 h as described above, and SOD, CAT, and GPx activities and ROS production were determined as described in "Materials and methods" section. As shown in Fig. 3, treatment with methionine promoted ∼89 % increase of SOD activity, while methionine sulfoxide alone or in combination with methionine reduced ∼70 % of its activity (Fig. 3a). In addition, CAT activity was inhibited 50, 20, and 45 % by methionine, methionine sulfoxide, or both in combination, respectively (Fig. 3b). On the other hand, GPx activity was not modified by treatments (Fig. 3c). Finally, all treatments reduced ∼35 % ROS production by macrophages (Fig. 3d). These results show that methionine and methionine sulfoxide differentially modulated the activity of antioxidant enzyme system and ROS production, which may favor a proinflammatory state.

Methionine and/or methionine sulfoxide alter extracellular nucleotide metabolism in macrophage cultures

Given that purinergic signaling play an important role in immune/inflammatory responses and that polarized macrophages are likely to be subjected to extracellular nucleotide modulation in in vivo microenvironment [41, 42], methionine and/or methionine sulfoxide effect on ectonucleotidase activities was evaluated (Fig. 4). Macrophages were cultured and exposed to methionine and/or methionine sulfoxide for 18 h and ATP, ADP, and AMP hydrolysis were determined. Macrophage stimulation with methionine sulfoxide alone or in combination with methionine promoted ∼57 and 60 % increase of ATP and ADP hydrolysis, respectively, when compared to control (Fig. 4a, b). On the other hand, AMP hydrolysis was not changed (Fig. 4c). This enzyme profile suggests that methionine sulfoxide and its combination with methionine modulates the activity of NTPDase1, which is the main ectonucleotidase expressed by macrophages and that, by hydrolyzing ATP and ADP, it regulates the purinergic receptor activation in activated macrophages [42].

Fig. 1 Representative images of macrophages exposed to methionine and/or methionine sulfoxide (**a**). Cell viability (**b**). Cell proliferation (**c**). Cell cultures were exposed to methionine (1 mM) and/or methionine sulfoxide (0.5 mM) and after 18 h of treatment phase-contrast microphotographs were taken using an Olympus inverted microscope (*arrows* indicate morphologic changes in macrophages following treatment; ×20 magnification); cell viability and proliferation were determined by MTT and SRB, respectively. Values represent mean ± SD of at least three independent experiments. Data were analyzed by ANOVA followed by post hoc comparisons (Tukey–Kramer test)

Discussion

The present work demonstrates a novel role of methionine and its metabolite methionine sulfoxide as modulators of macrophage phenotype and inflammatory process. First, we evaluated the effect of methionine and/or methionine sulfoxide on macrophage polarization. We observed that both molecules induced M1/classical macrophage activation, which is related to proinflammatory responses characterized by increased iNOS activity and TNF-α release. We further demonstrated that the treatment promoted alterations on redox state of macrophage cultures by differentially modulated SOD and CAT activities and ROS production. Finally, methionine and/or methionine sulfoxide treatment also altered the extracellular nucleotide
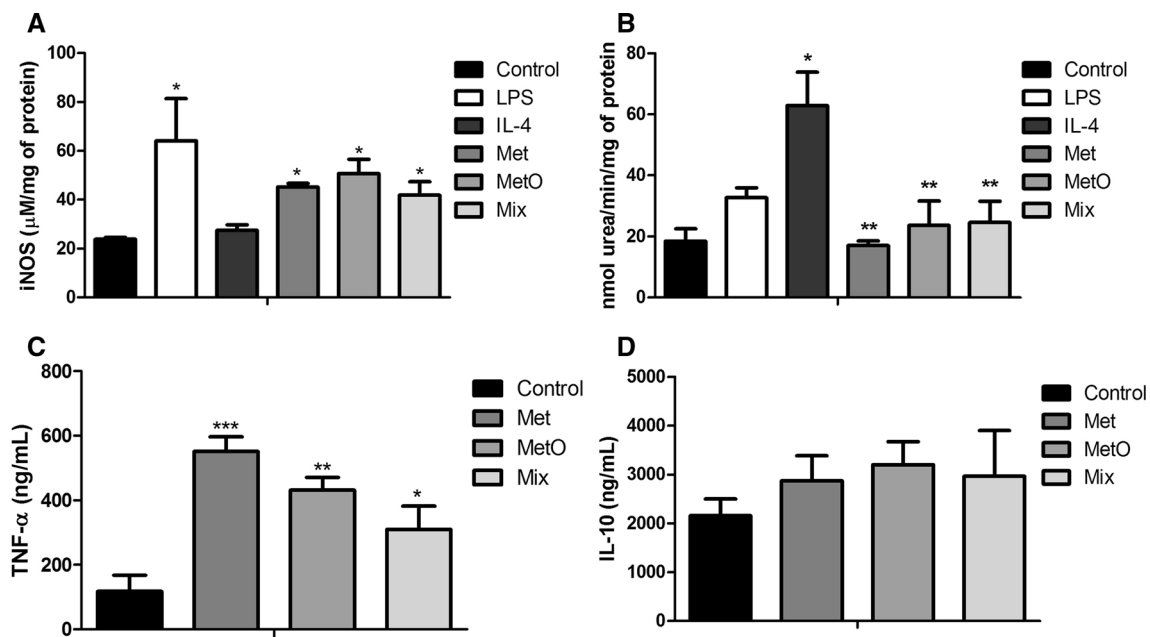
Fig. 2 Characterization of macrophage phenotype following methionine and/or methionine sulfoxide treatment. Macrophages were exposed to methionine (Met), methionine sulfoxide (MetO), or both (Mix) for 18 h and cell polarization was evaluated as follows: **a** iNOS and **b** arginase activities: iNOS was estimated by the NO^{2-} (nitrite) accumulation in the supernatant of cultured cells, and arginase activity was evaluated by measuring the formation of urea from arginine. **c** TNFα. **d** IL-10 cytokines were measured from supernatants of macrophage cultures by ELISA. Values represent mean ± SD of at least three independent experiments. Data were analyzed by ANOVA followed by post hoc comparisons (Tukey–Kramer test). *Significantly different from control cells ($P < 0.05$)
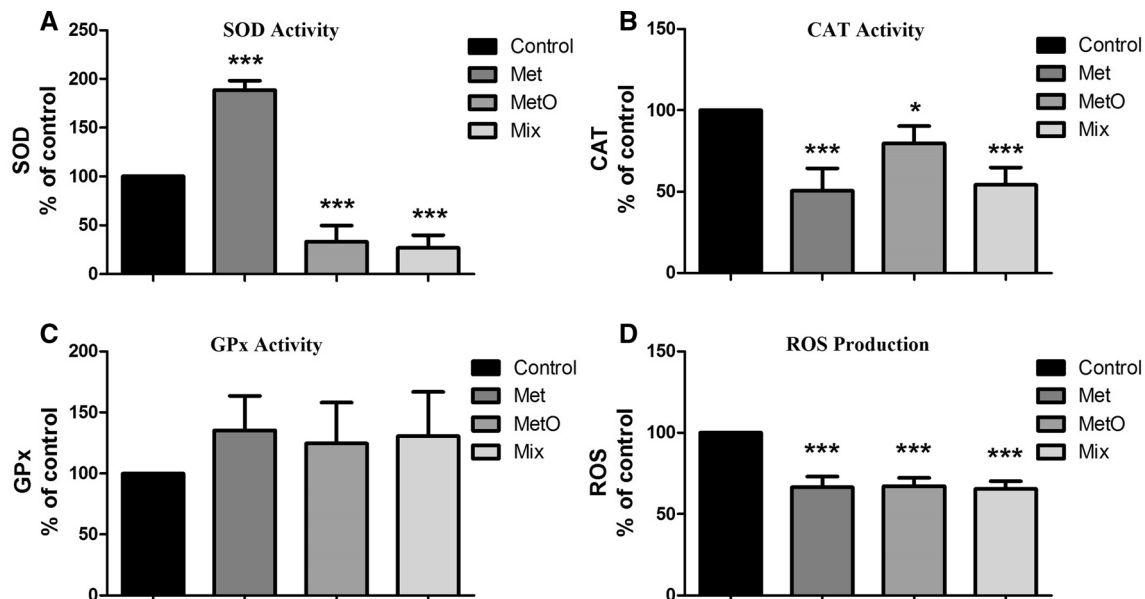

Fig. 3 Analysis of oxidative stress parameters in methionine and/or methionine sulfoxide-treated macrophages. Macrophages were exposed to methionine (Met), methionine sulfoxide (MetO), or both (Mix) as described above, and the activity of **a** superoxide dismutase (SOD). **b** Catalase (Cat). **c** GPx. ROS production (**d**) were evaluated as described in materials and methods. Values represent mean ± SD of at least three independent experiments. Data were analyzed by ANOVA followed by post hoc comparisons (Tukey–Kramer test). *Significantly different from control cells ($P < 0.05$)

metabolism, promoting an increase of ATP and ADP hydrolysis in macrophages. Such modifications may contribute to proinflammatory responses.

Methionine is an essential amino acid involved in critical metabolic process, including protein synthesis, sulfur metabolism, methylation, redox regulation, and signal
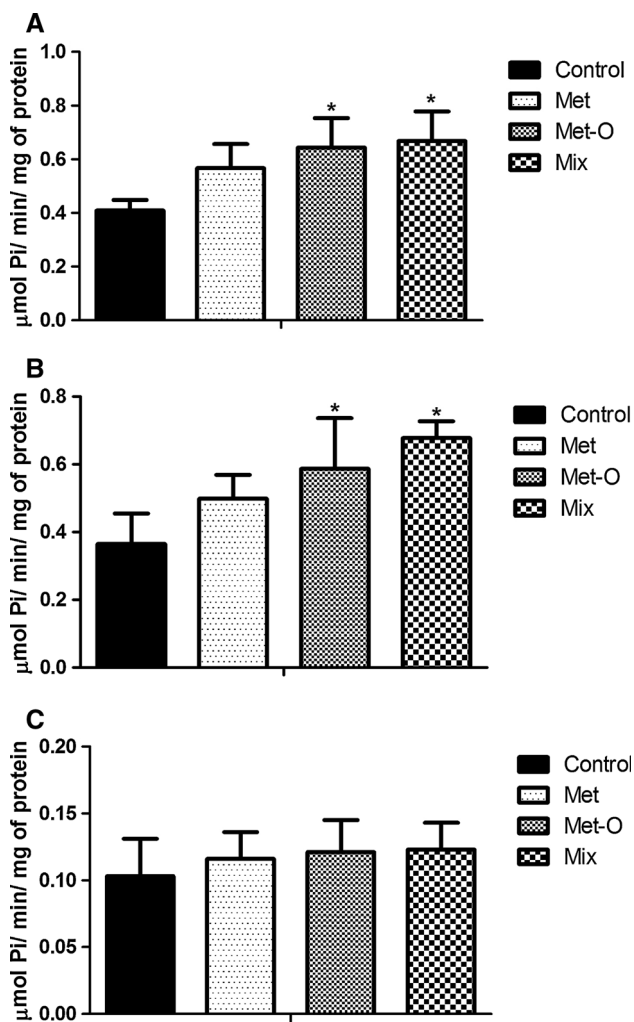
Fig. 4 Evaluation of ectonucleotidase activity in methionine and/or methionine sulfoxide-treated macrophages. Macrophages were exposed to methionine (Met), methionine sulfoxide (MetO), or both (Mix) as described above, and the hydrolysis of **a** ATP, **b** ADP, and **c** AMP were evaluated by malachite green method. Values represent mean ± SD of at least three independent experiments. Data were analyzed by ANOVA followed by post hoc comparisons (Tukey–Kramer test). *Significantly different from control cells ($P < 0.05$)

transduction [2]. However, mammals are not able to synthesize this amino acid, whose only sources are diet and recycling [2, 43]. Therefore, the regulation of methionine flux through the metabolism is important to supply this amino acid for cell needs. Elevation in plasma methionine commonly occurs due to mutations in genes encoding methionine-metabolizing enzymes, such as MAT [2, 5, 34, 44]. In this work, we hypothesize that methionine and/or methionine sulfoxide induce a proinflammatory/M1 macrophage polarization which may contribute to pathophysiology of hypermethioninemia. To this end, macrophage cultures were exposed to methionine and its metabolite methionine sulfoxide and macrophage polarization and inflammation-related pathways, as oxidative

stress and purinergic signaling, were evaluated. The treatment with methionine and/or methionine sulfoxide polarized macrophages to a M1/classical phenotype, which exhibit proinflammatory actions and in general, is associated to tissue injury and inflammation. Indeed, recently methionine was reported to affect immune status by improving innate immune response through inflammation [45]. In addition, these results are in accordance to symptoms exhibited by hypermethioninemic patients, and changes in macrophage activation may explain the diseases associated to high methionine and methionine sulfoxide levels as liver disorders [2, 5, 46], neurological abnormalities, and brain demyelization [2, 47, 48].

Regarding the enzymatic antioxidant system, methionine and/or methionine sulfoxide treatment differentially modulated SOD activity in macrophages. Methionine-induced SOD activity increases, while methionine sulfoxide alone or in combination with methionine had the opposite effect. Moreover, CAT activity was decreased by all treatments, while GPx activity remained unchanged. Although we cannot determine precisely the mechanisms by which the methionine and/or methionine sulfoxide modulated the activity of these enzymes, studies from literature point the differential expression and activity regulation of these enzymes. Indeed, there is a balance between SOD activity and H_2O_2-metabolizing enzymes, CAT and GPx [49]. SOD-increased activity is associated to a positive modulation of GPx as a compensatory mechanism to degrade H_2O_2 [49]. However, a compensatory CAT activity increase is frequently not found [50] and, in some situations, it is decreased [51]. In this regard, evidence suggest that, in contrast to the other antioxidant enzymes, CAT is not a redox-sensitive enzyme, but a regulator of cell processes, as inflammation for example [52]. The differential regulation of antioxidant enzymes may explain, at least in part, the opposite effects promoted by methionine in SOD and CAT activities of macrophages and the persistent CAT inhibition under methionine and/or methionine sulfoxide treatment. Interestingly, as SOD and CAT activity decreased, ROS levels were also found to decrease in all groups. Costa and collaborators [46] demonstrated a decrease of ROS levels in liver cells after methionine and/or methionine sulfoxide treatment which was associated to methionine-free radical activity scavenger [46]. Additionally, the production of *S*-adenosylmethionine (SAM) after treatment of macrophages with methionine may also contribute to decrease ROS levels. SAM is a product formed from methionine and ATP, which is described to suppress oxidative stress in pathological conditions [53–55]. Even though we can not explain the exact mechanism, other hypothesis for this result is that somehow macrophages are trying to control inflammatory environment, through ROS scavenger strategies.

Mounting evidence links the activation of extracellular nucleotide signaling and immune/inflammatory response [15]. ATP acts as a danger signal during inflammation and, in combination with LPS, trigger the IL-1β release in macrophages [56]. In addition, changes in the expression of ectonucleotidases during phenotypic differentiation allow the macrophages to adjust their functions during the inflammatory set [42]. Here, we observed that methionine sulfoxide alone or in combination with methionine increased the ATPase/ADPase activities in macrophages, while AMPase activity was not altered by the treatment. The enzyme activity profile is consistent with NTPDase1 expression, which has been described as the main NTPDase present in macrophages [42]. Since the ATP exocytosis is required for macrophage activation via P2Y$_{11}$ sensitization [57] but, on the other hand, high ATP concentration may induce cell death via P2X7 activation; we suggest that the increase of ATPase/ADPase activities is a compensatory mechanism to maintain macrophage activation and also to protect it from cell death. Indeed, NTPDase1 plays a key role in the control of P2X7-dependent macrophage responses [42]. In addition, ectonucleotidase activity of macrophages may be modulated by oxidative/nitrosative stress followed methionine and methionine sulfoxide treatment, as the ectonucleotidase activity can be altered by free radicals [58].

In summary, our results demonstrate that methionine and/or methionine sulfoxide induce M1/classical macrophage polarization. The proinflammatory macrophage phenotype involved the increase of iNOS activity and TNF-α release. In line with this, alterations in stress oxidative and purinergic signaling parameters are in according to proinflammatory environment of macrophages exposed to methionine and methionine sulfoxide. Although further studies are necessary to examine the mechanisms involved in macrophage polarization induced by methionine and methionine sulfoxide in an in vivo hypermethioninemia models, the data reported here reinforce the hypothesis that these molecules induce a proinflammatory response that could play an important role in cell injury observed in patients. Therefore, since the current therapy for hypermethioninemia is based on methionine intake restriction, novel therapeutic strategies taking into account the participation of inflammatory process in the pathophysiology of hypermethioninemia may be employed to offer alternatives to patients.

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

References

1. Finkelstein JD (2006) Inborn errors of sulfur-containing amino acid metabolism. J Nutr 136:1750S–1753S

2. Mudd SH, Levy HL, Kraus JP (2001) Disorders of transsulfuration. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic and molecular bases of inherited disease, 8th edn. McGraw-Hill, New York, pp 2007–2056

3. Avila MA, Berasain C, Torres L, Martín-Duce A, Corrales FJ, Yang H, Prieto J, Lu SC, Caballería J, Rodés J, Mato JM (2000) Reduced mRNA abundance of the main enzymes involved in methionine metabolism in human liver cirrhosis and hepatocellular carcinoma. J Hepatol 33:907–914

4. Lu SC, Alvarez L, Huang ZZ, Chen L, An W, Corrales FJ, Avila MA, Kanel G, Mato JM (2001) Methionine adenosyltransferase 1A knockout mice are predisposed to liver injury and exhibit increased expression of genes involved in proliferation. Proc Natl Acad Sci USA 98:5560–5565

5. Fernández-Irigoyen J, Santamaría E, Chien YH, Hwu WL, Korman SH, Faghfoury H, Schulzr A, Hoganson GE, Stable SP, Allen RH, Wagner C, Mudd SH, Corrales FJ (2010) Enzymatic activity of methionine adenosyltransferase variants identified in patients with persistent hypermethioninemia. Mol Genet Metab 101:172–177

6. Laskin DL, Laskin JD (2001) Role of macrophages and inflammatory mediators in chemically induced toxicity. Toxicology 160:111–118

7. Martinez FO, Gordon S (2014) The M1 and M2 paradigm of macrophage activation: time for reassessment. F1000Prime Rep 6:6–13

8. Gordon S (2003) Alternative activation of macrophages. Nat Rev Immunol 3:23–25

9. Mosser DM, Edwards JP (2008) Exploring the full spectrum of macrophages activation. Nat Rev Immunol 8:958–969

10. Mantovani A (2009) Orchestration of macrophage polarization. Blood 114:3135–3136

11. Martinez FO, Helming L, Gordon S (2009) Alternative activation of macrophages: an immunologic functional perspective. Annu Rev Immunol 27:451–483

12. Liu YC, Zou XB, Chai YF, Yao YM (2014) Macrophage polarization in inflammatory diseases. Int J Biol Sci 10:520–529

13. Ley S, Weigert A, Brune B (2010) Neuromediators in inflammation—a macrophage/nerve connection. Immunobiology 215:674–684

14. Di Virgilio F, Chiozzi P, Ferrari D, Falzoni S, Sanz JM, Morrelli A, Falzoni S, Sanz JM, Morelli A, Torboli M, Bolognesi G, Baricordi OR (2001) Nucleotide receptors: an emerging family of regulatory molecules in blood cells. Blood 97:587–600

15. Bours MJ, EL Swennen, Di Virgilio F, Cronstein BN, Dagnelie PC (2006) Adenosine 5′-triphosphate and adenosine as endogenous signaling molecules in immunity and inflammation. Pharmacol Ther 112:358–404

16. Desai BN, Leitinger N (2014) Purinergic and calcium signaling in macrophage function and plasticity. Front Immunol 5:580

17. La Sala A, Ferrari D, Di Virgilio F, Idzko M, Norgauer J, Girolomoni G (2003) Alerting and tuning the immune response by extracellular nucleotides. J Leukoc Biol 73:339–343

18. Lemaire I, Leduc N (2003) Purinergic P2X7 receptor function in lung alveolar macrophages: pharmacologic characterization and bidirectional regulation by Th1 and Th2 cytokines. Drug Dev Res 59:118–127

19. Antonioli L, Pacher P, Vizi ES, Hasko G (2013) CD39 and CD73 in immunity and inflammation. Trends Mol Med 19:355–367

20. Hasko G, Cronstein BN (2004) Adenosine: an endogenous regulator of innate immunity. Trends Immunol 25:33–39

21. Robson SC, Sévigny J, Zimmermann H (2006) The E-NTPDase family of ectonucleotidases: structure function relationships and pathophysiological significance. Purinergic Signal 2:409–430

22. Longhi MS, Robson SC, Bernstein SH, Serra S, Deaglio S (2013) Biological functions of ecto-enzymes in regulating extracellular adenosine levels in neoplastic and inflammatory disease states. J Mol Med 91(2):165–172

23. Stuehr DJ, Nathan CF (1989) Nitric oxide. A macrophage product responsible for cytostasis and respiratory inhibition in tumor target cells. J Exp Med 169:1543–1555

24. Corraliza IM, Campo ML, Soler G, Modolell M (1994) Determination of arginase activity in macrophages: a micromethod. J Immunol Methods 174:231–235

25. Aebi H (1984) Catalase in vitro. Methods Enzymol 105:121–126

26. Misra HP, Fridovich I (1972) The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 247:3170–3175

27. Wendel A (1981) Glutathione peroxidase. Methods Enzymol 77:325–333

28. Chan KM, Delfert D, Junger KD (1986) A direct colorimetric assay for Ca^{2+}-stimulated ATPase activity. Anal Biochem 157:375–380

29. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

30. Jay SM, Skokos E, Laiwalla F, Krady MM, Kyriakides TR (2007) Foreign body giant cell formation is preceded by lamellipodia formation and can be attenuated by inhibition of Rac1 activation. Am J Pathol 171:632–640

31. Waldo SW, Li Y, Buono C, Zhao B, Billings EM, Chang J, Kruth HS (2008) Heterogeneity of human macrophages in culture and in atherosclerotic plaques. Am J Pathol 172(4):1112–1126

32. McWhorter FY, Wang T, Nguyen P, Chung T, Liu WF (2013) Modulation of macrophage phenotype by cell shape. Proc Natl Acad Sci USA 110:17253–17258

33. Stefanello FM, Matté S, Scherer EB, Wannmacher CMD, Wajner M, Wyse ATS (2007) Chemically induced model of hypermethioninemia in rats. J Neurosci Methods 160:1–4

34. Stefanello FM, Matté C, Pederzolli CD, Kolling J, Mescka CP, Lamers ML, De Assis AM, Perry ML, Dos Santos MF, Dutra-Filho CS, Wyse AT (2009) Hypermethioninemia provokes oxidative damage and histological changes in liver of rats. Biochimie 91:961–968

35. Augoustides-Savvopoulou P, Luka Z, Karyda S, Stabler SP, Allen RH, Patsiaoura K, Wagner C, Mudd SH (2003) Glycine N-methyltransferase deficiency: a new patient with a novel mutation. J Inherit Metab Dis 26:745–759

36. Ozias MK, Schalinske KL (2003) All-trans-retinoic acid rapidly induces glycine N-methyltransferase in a dose-dependent manner and reduces circulating methionine and homocysteine levels in rats. J Nutr 133:4090–4094

37. Forman HJ, Torres M (2001) Redox signaling in macrophages. Mol Asp Med 22:189–216

38. Oliveira CC, Oliveira SM, Godoy LMF, Gabardo J, Buchi DF, Canova (2006) A Brazilian medical formulation, alters oxidative metabolism of mice macrophages. J Infect 52:420–432

39. Tyteca D, Nishino T, Debaix H, Van Der Smissen P, N'Kuli F, Hoffmann D, Cnops Y, Rabolli V, Loo GV, Beyaert R, Huaux F, Devuyst O, Courtoy PJ (2015) Regulation of macrophage motility by the water channel aquaporin-1: crucial role of M0/M2 phenotype switch. PloS One 10:2

40. Bohlson SS, O'Conner SD, Hulsebus HJ, Ho M-M, Fraser DA (2014) Complement, C1q, and C1q-related molecules regulate macrophage polarization. Front Immunol 5:402

41. Zanin RF, Braganhol E, Bergamin LS, Campesato LF, Filho AZ, Moreira JC, Morrone FB, Sévigny J, Schetinger MR, de Souza Wyse AT, Battastini AM (2012) Differential macrophage activation alters the expression profile of NTPDase and ecto-5′-nucleotidase. PLoS One 7:e31205

42. Lévesque SA, Kukulski F, Enjyoji K, Robson SC, Sévigny J (2010) NTPDase1 governs P2X7-dependent functions in murine macrophages. Eur J Immunol 40:1473–1485

43. Yamada H, Akahoshia N, Kamatad S, Hagiyad Y, Hishikia T, Nagahatac Y, Matsuurac T, Takanoc N, Morib M, Ishizakib Y, Izumib T, Kumagaie Y, Kasaharad T, Suematsua M, Ishii I (2012) Methionine excess in diet induces acute lethal hepatitis in mice lacking cystathionine γ-lyase, an animal model of cystathioninuria. Free Radic Biol Med 52:1716–1726

44. Hirabayashi K, Shiohara M, Yamada K, Sueki A, Ide Y, Takeuchi K, Hagimoto R, Kinoshita T, Yabuhara A, Mudd SH, Koike K (2013) Neurologically normal development of a patient with severe methionine adenosyltransferase I/III deficiency after continuing dietary methionine restriction. Gene 530:104–108

45. Machado M, Azeredo R, Díaz-Rosales P, Afonso A, Peres H, Oliva-Teles A, Costas B (2015) Dietary tryptophan and methionine as modulators of European seabass (Dicentrarchus labrax) immune status and inflammatory response. Fish Shellfish Immunol 42:353–362

46. Costa MZ, Da Silva TM, Flores NP, Schmitz F, Da Silva Scherer EB, Viau CM, Saffi J, Barschak AG, De Souza Wyse AT, Spanevello RM, Stefanello FM (2013) Methionine and methionine sulfoxide alter parameters of oxidative stress in the liver of young rats: in vitro and in vivo studies. Mol Cell Biochem 384:21–28

47. Chamberlin ME, Ubagai T, Mudd SH, Wilson WL, Leonard JV, Chou JY (1996) Demyelination of the brain is association with methionine adenosyltransferase I/III deficiency. J Clin Investig 98:1021–1027

48. Furujo M, Kinoshita M, Nagao M, Kubo T (2012) Methionine adenosyltransferase I/III deficiency: neurological manifestations and relevance of S-adenosylmethionine. Mol Genet Metab 107:253–256

49. Wang X, Hai C (2016) Novel insights into redox system and the mechanism of redox regulation. Mol Biol Rep 43(7):607–628

50. Keller JY, Kindy MS, Holtsberg FW, St. Clair DK, Yen HC, Germeyer A, Steiner SM, Bruce-Keller AJ, Hutchins JB, Mattson MP (1998) Mitochondrial manganese superoxide dismutase prevents neural apoptosis and reduces ischemic brain injury: suppression of peroxynitrite production, lipid peroxidation, and mitochondrial dysfunction. J Neurosci 18:687–697

51. Chovolou Y, Watjem W, Kampkotter A, Kahl R (2003) Resistance to tumor necrosis factor-α (TNF-α)-induced apoptosis in rat hepatoma cells expressing TNF-α is linked to low antioxidant enzyme expression. J Biol Chem 278:29626–29632

52. Pelletier M, Lepow TS, Billingham LK, Murphy MP, Siegel RM (2013) New tricks from an old dog: mitochondrial redox signaling in cellular inflammation. Semin Immunol 24:384–392

53. Ghorbani Z, Hajizadeh M, Hekmatdoost A (2016) Dietary supplementation in patients with alcoholic liver disease: a review on current evidence. Hepatobiliary Pancreat Dis Int 13:1–3

54. Stiuso P, Bagarolo ML, Ilisso CP, Vanacore D, Martino E, Caraglia M, Porcelli M, Cacciapuoti G (2016) Protective effect of tyrosol and S-adenosylmethionine against ethanol-induced oxidative stress of Hepg2 cells involves sirtuin 1, P53 and Erk1/2 signaling. Int J Mol Sci 17:622

55. Yoon SY, Hong GH, Kwon HS, Park S, Park SY, Shin B, Kim TB, Moon HB, Cho YS (2016) S-Adenosylmethionine reduces airway inflammation and fibrosis in a murine model of chronic severe asthma via suppression of oxidative stress. Exp Mol Med 48:236

56. Solle M, Labasi J, Perregaux DG, Stam E, Petrushova N, Koller BH, Griffiths RJ, Gabel CA (2001) Altered cytokine production in mice lacking P2X(7) receptors. J Biol Chem 276:125–132

57. Sakaki H, Tsukimoto M, Harada H, Moriyama Y, Kojima S (2013) Autocrine regulation of macrophage activation via exocytosis of ATP and activation of P2Y11 receptor. PLoS One 8:59778

58. Vuaden FC, Savio LE, Rico EP, Mussulini BH, Rosemberg DB, de Oliveira DL, Bogo MR, Bonan CD, Wyse AT (2016) Methionine exposure alters glutamate uptake and adenine nucleotide hydrolysis in the Zebrafish brain. Mol Neurobiol 53(1):200–209