Jornal Brasileiro de Patologia e Medicina Laboratorial
ISSN: 1676-2444
jbpml@sbpc.org.br,adagmar.andriolo@gmail.com
Sociedade Brasileira de Patologia Clínica/Medicina Laboratorial

Obalski de Mello, Ricardo; Lunardelli, Adroaldo; Caberlon, Eduardo; Machado Bragança de Moraes, Cristina; Christ Vianna Santos, Roberto; Lorini da Costa, Vinícius; Viegas da Silva, Gabriela; da Silva Scherer, Patrícia; Coimbra Buaes, Luiz Eduardo; Fagundes Donadio, Márcio Vinícius; Bordignon Nunes, Fernanda; Rodrigues de Oliveira, Jarbas
N-acetylcysteine and fructose-1,6-bisphosphate: immunomodulatory effects on mononuclear cell culture
Jornal Brasileiro de Patologia e Medicina Laboratorial, vol. 48, núm. 2, abril, 2012, pp. 109-115
Sociedade Brasileira de Patologia Clínica/Medicina Laboratorial
Rio de Janeiro, Brasil

N-acetylcysteine and fructose-1,6-bisphosphate: immunomodulatory effects on mononuclear cell culture

Primeira submissão em 16/08/11
Última submissão em 07/12/11
Aceito para publicação em 12/12/11
Publicado em 20/04/12

N-acetilcisteína e frutose-1,6-bisfosfato: efeito imunomodulador em cultura de células mononucleares

Ricardo Obalski de Mello[1]; Adroaldo Lunardelli[2]; Eduardo Caberlon[3]; Cristina Machado Bragança de Moraes[4]; Roberto Christ Vianna Santos[5]; Vinícius Lorini da Costa[6]; Gabriela Viegas da Silva[7]; Patrícia da Silva Scherer[8]; Luiz Eduardo Coimbra Buaes[9]; Márcio Vinícius Fagundes Donadio[10]; Fernanda Bordignon Nunes[11]; Jarbas Rodrigues de Oliveira[12]

key words

Fructose-1,6-bisphosphate

N-acetylcysteine

T-lymphocytes

Interleukin-1β

MCP-1

abstract

Introduction: Sepsis is a complex syndrome caused by an uncontrolled systemic inflammatory response. Inflammatory cytokines play a pivotal role in septic shock pathogenesis. Therapeutic strategies have been tested in order to modulate the excessive generation or function of sepsis mediators. **Objective:** The objective of the present study was to investigate the therapeutic effect of N-acetylcysteine (NAC) and its association with fructose-1,6-bisphosphate (FBP) on T-lymphocytes proliferation, interleukin-1β (IL-1β) and monocyte chemotactic protein-1 (MCP-1) levels. **Material and methods:** Peripheral blood mononuclear cell samples were isolated from healthy individuals. T-lymphocytes were stimulated with phytohemagglutinin for 96 hours and submitted to different concentrations of NAC or NAC associated with FBP. Results: NAC (10 and 15 mM) and NAC (15 mM) associated with FBP reduced T-lymphocytes proliferation. IL-1β levels rose in the presence of both NAC (15 mM) and NAC with FBP (1.25 mM). MCP-1 levels were reduced only by NAC (15 mM) associated with FBP (1.25 mM). **Conclusion:** The results suggest that both NAC itself and NAC associated with FBP inhibit cellular proliferation, acting as potent immunomodulatory agents, which corroborates its use in the treatment of inflammatory diseases.

resumo

Introdução: *A sepse é uma síndrome complexa causada pela resposta inflamatória sistêmica descontrolada. As citocinas inflamatórias representam papel central na patogênese do choque séptico. Têm sido testadas estratégias terapêuticas a fim de modular a geração ou a função excessiva de mediadores na sepse.* ***Objetivo:*** *O objetivo deste estudo foi investigar o efeito terapêutico da N-acetilcisteína (NAC) e sua associação com a frutose-1,6-bisfosfato (FBP) sobre a proliferação de linfócitos T e a geração de interleucina-1β (IL-1β) e proteína quimiotática de monócitos 1 (MCP-1) em cultura celular.* ***Material e métodos:*** *Foram isoladas células mononucleares de sangue periférico de indivíduos saudáveis. Os linfócitos T foram estimulados por 96 horas com fitohemaglutinina e submetidos a diferentes concentrações de NAC ou NAC associada à FBP (1,25 mM).* ***Resultados:*** *O tratamento com NAC (10 e 15 mM) ou NAC (15 mM) associado à FBP reduziu a proliferação celular. Os níveis de IL-1β aumentaram com a presença de NAC (15 mM) e NAC + FBP. A concentração de MCP-1 mostrou-se reduzida apenas no grupo tratado com NAC associada à FBP.* ***Conclusão:*** *Os resultados sugerem que tanto a NAC quanto a NAC associada à FBP são capazes de inibir a proliferação celular, atuando como potentes agentes imunomoduladores, sugerindo seu uso em doenças inflamatórias.*

unitermos

Frutose-1,6-bisfosfato

N-acetilcisteína

Linfócitos T

Interleucina 1β

MCP-1

1. Mestre em Biologia Celular e Molecular pela Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS); pós-graduando do Laboratório de Pesquisa em Biofísica Celular e Inflamação da PUCRS.
2. Mestre em Biologia Celular e Molecular pela PUCRS; bioquímico do Serviço de Hemoterapia da Irmandade Santa Casa de Misericórdia de Porto Alegre (ISCMPA).
3. Mestre em Biociências pela PUCRS; laboratorista do Laboratório de Pesquisa em Biofísica Celular e Inflamação da PUCRS.
4. Doutora em Biologia Celular e Molecular pela PUCRS; professora do Centro Universitário Franciscano (UNIFRA).
5. Doutor em Biologia Celular e Molecular pela PUCRS; professor da UNIFRA.
6. Biólogo pela PUCRS.
7. Acadêmica do curso de Farmácia da PUCRS; estagiária do Laboratório de Pesquisa em Biofísica Celular e Inflamação da PUCRS.
8. Acadêmica do curso de Farmácia da PUCRS; estagiária do Laboratório de Pesquisa em Biofísica Celular e Inflamação PUCRS.
9. Acadêmico do curso de Farmácia da PUCRS; estagiário do Laboratório de Pesquisa em Biofísica Celular e Inflamação da PUCRS.
10. Doutor em Ciências Biológicas pela Universidade Federal do Rio Grande do Sul (UFRGS); professor da PUCRS.
11. Doutora em Medicina e Ciências Médicas pela UFRGS; professora da PUCRS.
12. Doutor em Metodologias Atuais em Ciências Biomédicas pela Universidade de Barcelona; professor coordenador do Laboratório de Pesquisa em Biofísica Celular e Inflamação da PUCRS.

Introduction

Sepsis is a complex syndrome caused by an uncontrolled systemic inflammatory response of the individual, with an infectious origin and characterized by multiple manifestations that can determine dysfunction or even failure of one or more organs[4, 19]. The main pathophysiological factors include the local of the infection and the coagulation, fibrinolytic and inflammatory systems are determinants on its evolution[3]. Sepsis has been considered a serious epidemiological problem for health systems all over the world, both in an economical as well as social point of view. Previous study conducted in the USA showed that the incidence of sepsis increased from 82.7 to 240.4/100 thousand inhabitants[17]. In Brazil, the mortality rate caused by sepsis and its consequences varies between 40% to 45%[33].

The septic shock represents an example of the increased inflammatory response. Its systemic effects are caused by an excessive production of inflammatory mediators (endogenous cytokines), as well as for the intense activity of inflammatory cells, resulting in a metabolic disequilibrium[3]. The shock complications are mainly linked with the release of bacterial wall components. Endotoxins (lipopolysaccharides) from gram-negative microorganisms (lipid A, mainly) and the teichoic acid from Gram-positives microorganisms, indirectly induce an inflammatory cascade by increasing the production of cytokines by macrophages and monocytes. Their activation sequentially produces tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), interleukin-6 (IL-6) and interleukin-8 (IL-8), that interacts with other cells and cellular elements (polymorphonuclear, endothelia cells, fibroblast cells, platelets and monocytes), inducing the production and release of secondary mediators, contributing to a delayed inflammatory response[34]. However, the overproduction or inappropriate expression of these factors can lead to a variety of pathological conditions, including systemic toxicity and septic shock[2, 35].

Therefore, therapeutical strategies in order to modulate the excessive generation or function of sepsis mediators have been tested. The intervention on any step of the pathophysiological event sequence that characterize the systemic inflammatory sepsis response, in order to modify/modulate the host reaction, seems to be the most likely therapeutical approach to change its evolution in the sepsis therapy. Unfortunately, the clinical use of individual mediator blockers have failed to reduce the mortality associated to sepsis. However, the interruption of the pathophysiological response sequence, in many levels, seems to be the best chance of reducing the high mortality of this pathology[5].

The fructose-1,6-bisphosphate (FBP) is a high-energy glycolytic metabolite that is believed to have a protective effect against toxic agents based on its ability to inhibit the production of inflammatory mediators[16]. In a recent study, it has been demonstrated that FBP in concentrations of 1.2 to 10 mM reduced the interleukin-2 (IL-2) soluble receptor levels, suggesting that FBP has an immunomodulatory effect[23]. The possible mechanism involved could be related to the interaction of FBP with cellular membranes, leading to changes in the ionic permeability. In the presence of FBP, there is a reduced K^+ efflux by passive and active channels in hepatocytes[30]. The K^+ conductance through the cell membrane is the main determinant for the T-lymphocyte electric potential, where changes can lead to mitogenesis in these cells[12].

The N-acetylcysteine (NAC), a thiol-compound, has been therapeutically used due to its property of being a glutathione precursor[27], assuming a key-role in the cellular homeostasis, since the glutathione depletion can cause cellular death due to lipidic peroxidation and decrease the thiol-protein levels[29]. NAC has been widely used both *in vitro* and *in vivo* as an antioxidant[6]. It is also efficient in the treatment of some pharmacological overdoses, as in the one caused by paracetamol[28]. NAC can also act in the mitochondrial metabolism influencing the oxidative phosphorylation through two mechanisms: protecting oxidative phosphorylation proteins from the oxidative damage through the maintenance of the thiols groups, which are essential for the enzymatic activity, and preventing the lipidic peroxidation of mitochondrial membranes, that could reduce the mitochondrial respiratory chain activity[22]. Wan *et al.*[37] showed that NAC inhibits the hydroxyl radical formation after d-amphetamine infusion (d-AMPH) in rats, suggesting that NAC could protect against induced oxidative stress.

Thus, the objective of the present study is to evaluate the use of alternative drugs, such as NAC and its association with FBP in the T-lymphocytes proliferation and in the IL-1β and monocyte chemotactic protein-1 (MCP-1) levels, which are involved in the process that can induce the septic shock.

Material and methods

The study experimental protocol was approved by the Ethics Research Committee of Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS).

Peripheral blood mononuclear cells preparation

The peripheral blood mononuclear cells (PBMCs) were isolated from the blood of healthy humans by gradient centrifugation on Ficoll-Paque (Sigma) and re-suspended in RPMI 1640 (Invitrogen) supplemented with 0.15% garamicin (Schering-Plough) and 20% homologous serum at a final cell density of 1.6×10^6 ml^{-1}. Platelet contamination of these preparations was < 1%[26]. The viability, measured by Trypan Blue dye exclusion, was uniformly greater than or equal to 90%. All human subjects read and signed an informed consent.

Lymphoproliferation

Phytohemagglutinin (PHA) (Invitrogen) was used to induce T-lymphocyte proliferation. The NAC and FBP solutions used were dissolved in supplemented RPMI 1640 medium. PBMCs (1.6×10^5 cell/well) were plated in 96-well microtiter bottomed flat plates (Nunc) with either different isolated NAC concentrations (1 mM; 5 mM; 10 mM; 15 mM) or associated with FBP (1.25 mM) at 37ºC in a 5% CO_2-humidified incubator for 96 hours (n = 6 for each concentration). PHA (10 μg/ml) was added and T-lymphocyte proliferation was determined by MTT (3-(4-5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Invitrogen) assay[20]. In brief, MTT was dissolved in RPMI 1640 (5 mg/ml) and added to all assay wells. Plates were incubated at 37ºC for 4 hours. Dimethyl sulfoxide (Sigma) was added to all wells and mixed thoroughly to dissolve the dark blue crystals. After a few minutes, the plates were read on a Hyperion MicroReader, using a test wavelength of 540 nm and a reference wavelength of 650 nm. The results are presented as optical density ± standard error about the mean (SEM) . The experiments were performed on different days and each experiment was done in triplicate.

Cytotoxicity analysis

NAC and FBP was dispensed in RPMI 1640 and added directly to PBMCs (1.6×10^5 cell/well), which were incubated in 96-well microtiter bottomed flat plates at 37ºC in a 5% CO_2 humidified incubator. The cells were treated with either isolated NAC concentrations (1 mM; 5 mM; 10 mM; 15 mM) or associated with FBP (1.25 mM) (n = 6 for each concentration). The cellular viability was performed by Trypan Blue dye exclusion after 96 hours of incubation[26, 32]. The results are presented as percentage ± SEM. The experiments were performed on different days and each experiment was done in triplicate.

Pro-inflammatory cytokine analysis

Cytokines production was evaluated in the supernatants of PBMCs (1.6×10^5 cells/well) incubated in a culture medium for 96 hours in 5% CO_2 at 37ºC. The cells were incubated (n = 6) in 96 wells plate and received the following treatments: a) not stimulated; b) stimulated with PHA; c) stimulated with PHA plus a 15 mM NAC solution; d) stimulated with PHA plus a 15 mM NAC solution associated with a 1.25 mM FBP solution; e) not stimulated, but treated with a 15 mM NAC solution; f) not stimulated, but treated with a 15 mM NAC solution associated with a 1.25 mM FBP solution. These concentrations were chosen based in the suppressive effect demonstrated in the lymphoproliferation assay. After the incubation period, the plates were centrifuged 900 G for 20 minutes and the supernatants were removed and stored at -70ºC until processed. Commercial enzyme linked immunosorbent assay (ELISA) kits (Biosource) were used to measure IL-1β and MCP-1 concentrations. All readings were performed in the ELISA (Bio-Rad) reader using a 570 nm wave length and a reference filter of 650 nm. All samples and standards were tested in triplicate. The results were expressed in pg/ml.

Statistical analysis

Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) version 16.0 software. Data were analyzed for significance using one-way analysis of variance (ANOVA). The Bonferroni's post-hoc test was used for the multiple group comparisons and significance was established when $p < 0.05$. Data are presented as mean ± SEM.

Results

NAC and FBP immunomodulatory effect in PHA stimulated T-lymphocytes

The immunomodulatory effects of NAC and FBP in PHA (10 mg/ml) stimulated T-lymphocytes were evaluated. **Figure 1** shows that NAC in concentrations of 10 mM and 15 mM significantly reduced ($p < 0.05$) the lymphocytic proliferation. On the other hand, when associated with FBP (1.25 mM), NAC significantly reduced the lymphocytic proliferation in concentrations of 1 mM and 15 mM ($p < 0.05$), as demonstrated in Figure 1.

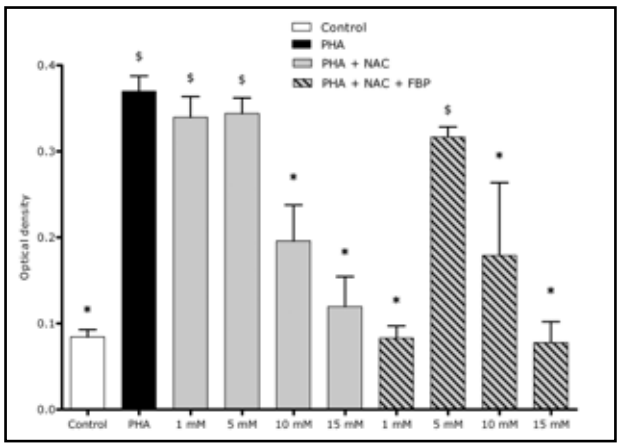

Figure 1 – *NAC alone and associated with FBP (1,25 mM) immunomodulatory effects of in PHA stimulated T-lymphocytes. The results were evaluated by optical density in triplicate cultures and expressed as mean ± SEM*

*NAC: N-acetylcysteine; FBP: fructose-1,6-bisphosphate; PHA: phytohemagglutinin; SEM: standard error about the mean ; $: a significant difference compared to the control group (p < 0.05); *: a significant difference compared to the PHA group (p < 0.05).*

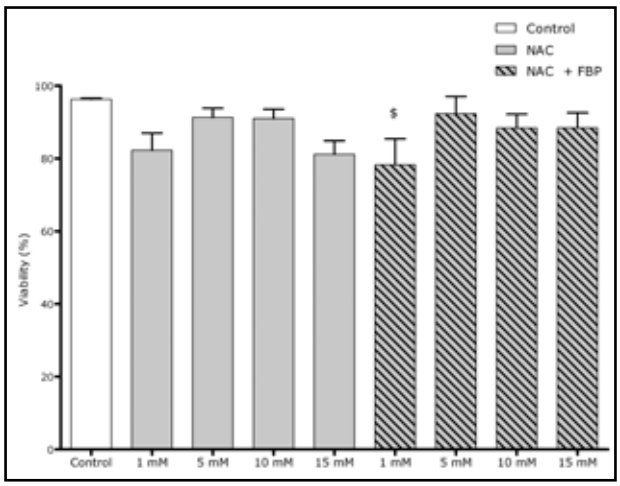

Figure 2 – *NAC alone and associated with FBP (1.25 mM) cytotoxic effects in PBMCs. The results are presented in absolute values in triplicate cultures and expressed as mean ± SEM*

NAC: N-acetylcysteine; FBP: fructose-1,6-bisphosphate; PBMCs: peripheral blood mononuclear cells; SEM: standard error about the mean ; $: a significant difference compared to the control group (p < 0.05).

NAC and FBP cytotoxic effects in PBMCs

In order to evaluate if the lymphoproliferative inhibitory effect of NAC alone and associated with FBP could be a result of cellular death, which would be a toxic effect, the cellular viability was also evaluated. **Figure 2** shows that when the same concentrations of NAC alone were used, no cellular cytotoxicity was demonstrated. However, when 1.25 mM of FBP was associated with 1 mM of NAC, a significant reduction ($p < 0.05$) in the cellular viability was demonstrated (Figure 2), excluding its use in the lymphoproliferative evaluation.

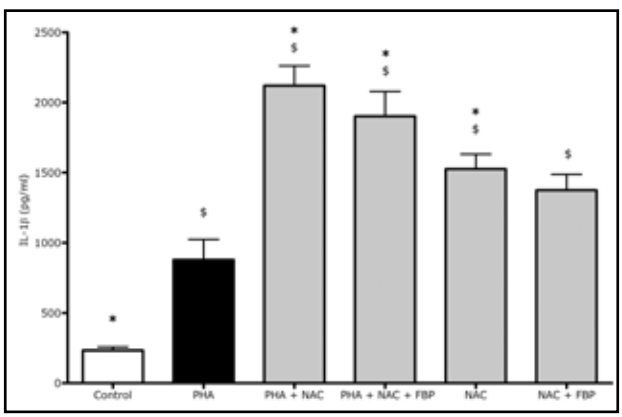

Figure 3 – *Effects of NAC (15 mM) and FBP (1.25 mM) in the IL-1β levels. The results are presented in absolute values in triplicate cultures and expressed as mean ± SEM*

*NAC: N-acetylcysteine; FBP: fructose-1,6-bisphosphate; IL-1β: interleukin-1β; SEM: standard error about the mean ; PHA: phytohemagglutinin; $: a significant difference compared to the control group (p < 0.05); *: a significant difference compared to the PHA group (p < 0.05).*

Evaluation of IL-1β and MCP-1 levels

In order to evaluate a possible mechanism for the NAC (15 mM) immunomodulatory effects, either isolated or associated with 1.25 mM FBP, the levels of IL-1β and MCP-1 were measured with or without PHA stimulation. As showed in **Figure 3**, both NAC used isolated or associated with FBP, significantly increased IL-1β levels with or without PHA stimulation ($p < 0.05$). When the MCP-1 levels were evaluated, a significant reduction ($p < 0.05$) was demonstrated when NAC associated with FBP, but without PHA stimulation, was tested **(Figure 4**).

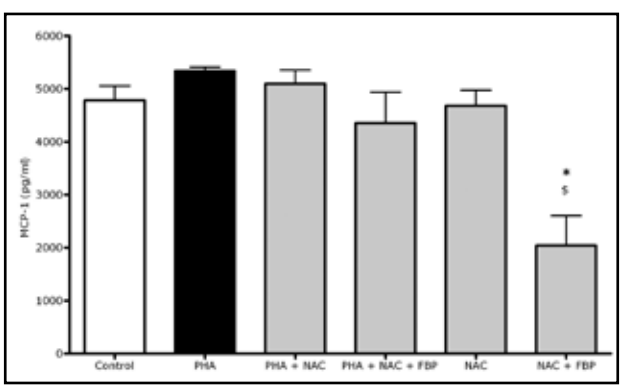

Figure 4 – *Effects of NAC (15 mM) and FBP (1.25 mM) in MCP-1 levels. The results are presented in absolute values in triplicate cultures and expressed as mean ± SEM*

*NAC: N-acetylcysteine; FBP: fructose-1,6-bisphosphate; MCP-1: monocyte chemotactic protein-1; IL-1β: interleukin-1β; SEM: standard error about the mean ; PHA: phytohemagglutinin; $: a significant difference compared to the control group (p < 0.05); *: a significant difference compared to the PHA group (p < 0.05).*

Discussion

The objective of the present study was to investigate the possible effects of both NAC alone or associated with FBP,

that already has a well known immunomodulatory effect, on the proliferation of T-lymphocytes, which could help in the development of alternative drugs for the septic shock treatment. Moreover, it also aims at explaining possible immunomodulatory mechanisms, through the evaluation of pro-inflammatory cytokine (IL-1β and MCP-1).

Our results demonstrate that 10 and 15 mM NAC administrated alone and 15 mM associated with 1.25 mM FBP presented an important immunomodulatory effect. However, Hadzic *et al.*[13] showed that in an inhibited lymphocyte proliferation model induced by a glutathione (GSH) blockage, NAC induced an increase in the T-lymphocytes proliferation, suggesting that the thiol groups would participate in the T-lymphocytes proliferation regulation. On the other hand, 1 mM NAC associated with FBP significantly reduces the cellular viability, suggesting that this association would have a cellular toxic effect. Furthermore, when NAC concentrations are increased, either isolated or associated with FBP, a proportional reduction in the T-lymphocytes proliferation was seen. Karlsson *et al.*[14] indicates that NAC may have dual and opposing effects on immunity, depending on the dose and kinetics, showing that PBMCs exposed to high concentrations of NAC are not proliferative with a primary stimulation, but respond strongly when re-stimulated in the absence of the drug.

Previous studies *in vivo* of our group showed a reduction in mortality of animals subjected to experimental sepsis when treated with FBP[24] and NAC[21], confirming an important role of both drugs in the treatment of sepsis. On the other hand, when rats were treated with combination of NAC and FBP, there was not improvement of the picture[21]. Extensive evidence in the literature demonstrates a correlation between the increased proinflammatory cytokines production and the sepsis mortality rate, both in humans and experimental models, being the IL-1 and IL-6 cytokines suggested to play a key role. Therefore, many recent clinical therapies are evaluating the security and effectiveness of the anti-cytokine use either isolated or in association with other compounds[8].

IL-1 is formed by two different molecules: IL-1α and IL-1β. It induces an increase in the concentration of colony-stimulating factors, IL-6, MCP-1, acute phase hepatic proteins, bone reabsorption, collagen synthesis and lipoprotein lipase inhibition[9, 31]. The IL-1 natural mechanism of inhibition involves blocking the receptor binding trough the use of cytokines receptor antagonists, as IL-1 receptor antagonist (IL-1Ra) . IL-1Ra is a protein from the interleukins family, originally described as a molecule secreted by monocytes and macrophages, that modulate many immune and inflammatory responses related to the IL-1[1, 15]. The IL-1 participation in the sepsis physiopathology was mostly studied through the use of this antagonist (IL-1Ra), which reduces the mortality caused by endotoxin administration[25] or *E. coli*[36]. Present study shows an increase in the IL-1β levels, either when isolated NAC or in association with FBP were used with or without PHA stimulation, suggesting that the NAC (and/or its association with FBP) protective role may be due to its immunomodulatory effect and not to its anti-inflammatory effect. However, some studies in the literature, have shown a reduction in the IL-1β levels after NAC treatment[7, 11].

MCP-1 has the ability to attract circulating monocytes to become macrophages in the adipose tissue. These macrophages are a source of cytokines with inflammatory activity. Pre-adipocytes and adipocytes produce MCP-1 (among others) in response to several stimuli: nitric oxide (NO) and TNF-α, IL-1β, IL-4 and interferon-γ (IFN-γ)[10]. Our study demonstrates, in spite of the IL-1β increase, a MCP-1 reduction when NAC was used in association with FBP without PHA stimulation, suggesting that this would probably be a FBP effect, since when NAC was administered alone no significant differences were demonstrated. However, Maruno *et al.*[18] showed a reduction in the MCP-1 levels when NAC treatment was used after vascular endothelial growth factor induction, indicating that NAC itself may have an effect on this cytokine production.

Taken together, our results suggest that either NAC alone or in association with FBP can inhibit the cellular proliferation, acting as major immunomodulatory agents and suggesting their use in inflammatory processes, including sepsis. The IL-1β and MCP-1 production does not explain the immunomodulatory action in T-lymphocytes cell cultures stimulated with PHA, since no reductions in their levels were seen. On the other hand, the NAC and its association with FBP in T-lymphocytes cell cultures without PHA stimulation reduced the MCP-1 levels, suggesting that there is a modulation in the interleukins synthesis and that this response can be related to its immunomodulatory effect.

References

1. AREND, W. P.; GUTHRIDGE, C. J. Biological role of interleukin 1 receptor antagonist isoforms. *Annals of the Rheumatic Diseases*, v. 59, p. 60-4, 2000.
2. BEUTLER, B.; CERAMI, A. Tumor necrosis, cachexia, shock, and inflammation: a common mediator. *Annu Rev Biochem*, v. 57, p. 505-18, 1988.
3. BONE, R. C. The pathogenesis of sepsis. *Ann Intern Med*, v. 115, n. 6, p. 457-69, 1991.
4. BONE, R. C.; GRODZIN, C. G.; BALK, R. A. Sepsis: a new hypothesis for pathogenesis of the disease process. *Chest*, v. 112, n. 1, p. 235-43, 1997.
5. CARVALHO, P. R. A.; TROTTA, E. A. Avanços no diagnóstico e tratamento da sepsis. *Jornal de Pediatria*, v. 79, n. 2, p. S195-204, 2003.
6. CETINKAYA, A. *et al*. Beneficial effects of N-acetylcysteine on acetic acid-induced colitis in rats. *Tohoku J Exp Med*, v. 206, p. 131-9, 2005.
7. CHEN, G. *et al*. Inhibitory effect on cerebral inflammatory response following traumatic brain injury in rats: a potential neuroprotective mechanism of N-acetylcysteine. *Mediators of Inflammation*, v. 10, p. 1155-63, 2008.
8. DINARELLO, C. A. The proinflammatory cytokines interleukin-1 and tumor necrosis factor and treatment of the septic shock syndrome. *J Infect Dis*, v. 163, p. 1177-84, 1991.
9. FISCHER, E. *et al*. Comparison between effects of interleukin-1 alpha administration and sublethal endotoxemia in primates. *Am J Physiol*, v. 261, p. R442-52, 1991.
10. FRETER, R. R. *et al*. Platelet-derived growth factor induction of the immediate-early gene MCP-1 is mediated by NF-kB and a 90-kDa phosphoprotein coactivator. *J Biol Chem*, v. 271, p. 17417-24, 1996.
11. GEUDENS, N. *et al*. N-acetylcysteine pre-treatment attenuates inflammatory changes in the warm ischemic murine lung. *The Journal of Heart and Lung Transplantation*, v. 26, n. 12, p. 1326-32, 2007.
12. GRINSTEIN, S. *et al*. Volume-induced increase of anion permeability in human lymphocytes. *J Gen Physiol*, v. 80, p. 801-23, 1982.
13. HADZIC, T. *et al*. The role of low molecular weight thiols in T lymphocyte proliferation and IL-2 secretion. *J Immunol*, v. 175, p. 7965-72, 2005.
14. KARLSSON, H. *et al*. N-acetyl-L-cysteine increases acute graft-*versus*-host disease and promotes T-cell-mediated immunity *in vitro*. *European Journal of Immunology*, v. 41, p. 1143-53, 2011.
15. KONDERA-ANASZ, Z. *et al*. Concentrations of interleukin (IL)-1alpha, IL-1 soluble receptor type II (IL-1sRII) and IL-1 receptor antagonist (IL-1 Ra) in the peritoneal fluid and serum of infertile women with endometriosis. *Eur J Obstet Gynecol Reprod Biol*, v. 123. n. 2, p. 198-203, 2005.
16. LUNARDELLI, A. *et al*. Extract of the bristles of *Dirphia sp.* increases nitric oxide in a rat pleurisy model. *Inflamm Res*, v. 55, p. 129-35, 2006.
17. MARTIN, G. S. *et al*. The epidemiology of sepsis in the United States from 1979 through 2000. *N Eng J Med*, v. 348, p. 1546-54, 2003.
18. MARUMO, T.; SCHINI-KERTH, V. B.; BUSSE, R. Vascular endothelial growth factor activates nuclear factor-kb and induces monocyte chemoattractant protein-1 in bovine retinal endothelial cells. *Diabetes*, v. 48, p. 1131-7, 1999.
19. MATOT, I.; SPRUNG, C. L. Definition of sepsis. *Intensive Care Medicine*, v. 27, p. S3-9, 2001.
20. MELLO, K. F. *et al*. Immunomodulatory effects of oral antidiabetic drugs in lymphocyte cultures from patients with type 2 diabetes. *JBPML*, v. 47, n. 1, p. 41-6, 2011.
21. MELLO, R. O.; LUNARDELLI, A.; CABERLON, E. Effect of N-acetylcysteine and fructose-1,6-bisphosphate in the treatment of experimental sepsis. *Inflammation*, v. 34, n. 6, p. 539-50, 2011.
22. MIQUEL, J. *et al*. N-acetylcysteine protects against age-related decline of oxidative phosphorylation in liver mitochondria. *European Journal of Pharmacology*, v. 292, p. 333-5, 1995.
23. NUNES, F. B. *et al*. Immunomodulatory effect of fructose-1,6-bisphosphate on T-lymphocytes. *Int Immunopharmacology*, v. 3, p. 267-72, 2003.
24. NUNES, F. B. *et al*. Physiopathological studies in septic rats and the use of fructose-1,6-bisphosphate as cellular protection. *Critical Care Medicine*, v. 30, p. 2069-74, 2002.
25. OHLSSON, K. *et al*. Interleukin-1 receptor antagonist reduces mortality from endotoxin shock. *Nature*, v. 348, p. 550-2, 1990.
26. PAUL, E. L. *et al*. Anti-inflammatory and immunomodulatory effects of *Baccharis trimera* aqueous extract on induced pleurisy in rats and lymphoproliferation *in vitro*. *Inflammation*, v. 32, n. 6, p. 419-25, 2009.
27. PINHO, R. A. *et al*. N-acetylcysteine and deferoxamine reduce pulmonary oxidative stress and inflammation in rats after coal dust exposure. *Environ Res*, v. 99, p. 355-60, 2005.
28. PRESCOTT, L. F. *et at*. Intravenous N-acetylcysteine: the treatment of choice for paracetamol poisoning. *Br Med J*, v. 2, p. 1097-100, 1979.
29. REED, D. J.; FARISS, M. W. Glutathione depletion and susceptibility. *Pharmacological Reviews*, v. 36, p. 25S-33S, 1984.
30. ROIG, T.; BARTRONS, R.; BERMÚDEZ, J. Exogenous fructose-1,6-bisphosphate reduces K^+ permeability in isolated rat hepatocytes. *Am J Physiol*, v. 273, p. C473-8, 1997.
31. ROSSI, M. *et al*. Identification of genes regulated by interleukin-1beta in human endometrial stromal cells. *Reproduction*, v. 130, n. 5, p. 721-9, 2005.
32. SANTOS, R. C. V. *et al*. Anti-inflammatory and immunomodulatory effects of *Ulomoides dermestoides* on induced pleurisy in rats and lymphoproliferation *in vitro*. *Inflammation*, v. 33, n. 3, p. 173-9, 2010.

33. SILVA, E.; PEDRO, M. A.; SOGAYAR, A. C. Brazilian Sepsis Epidemiological Study. *Crit Care*, v. 8, n. 4, p. 251-60, 2004.
34. THIJS, L. G.; HACK, C. E. Time course of citokine levels in sepsis. *Intensive Care Med*, v. 21, p. S258-63, 1995.
35. VILCEK, J.; LEE, T. H. Tumor necrosis factor. New insights into the molecular mechanisms of its multiple actions. *J Biol Chem*, v. 266, n. 12, p. 7313-6, 1991.
36. WAKABAYASHI, G. *et al*. A specific receptor antagonist for interleukin 1 prevents *Escherichia coli*-induced shock in rabbits. *FASEB J*, v. 5, p. 338-43, 1991.
37. WAN, F. J. *et al*. Effects of alpha-phenyl-N-tert-butyl nitrone and N-acetylcysteine on hydroxyl radical formation and dopamine depletion in the rat striatum produced by d-amphetamine. *Eur Neuropsychopharmacol*, v. 16, p. 147-53, 2006.

Mailing address

Jarbas Rodrigues de Oliveira
Laboratório de Pesquisa em Biofísica
Celular e Inflamação da PUCRS
Av. Ipiranga, 6.681/ Prédio 12C/ Sala 263
CEP: 90619-900 – Porto Alegre-RS
Tel.: (51) 3353-4147
e-mail: jarbas@pucrs.br