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RESEARCH ARTICLE

Efficacy of probiotic supplementation on quality of life and pulmonary symptoms due to sulfur mustard exposure: a randomized double-blind placebo-controlled trial

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Abstract

Objective: This study investigated the efficacy and safety of supplementation with probiotics in improving chronic pulmonary symptoms due to sulfur mustard (SM) exposure. *Methods*: In a randomized double-blind placebo-controlled study, 65 subjects suffering from chronic pulmonary complications of SM were assigned to one probiotic capsule (1×10^9 CFU containing seven strains of lactic acid-producing bacteria) every 12 h or an identical placebo for six weeks. Serum high-sensitivity C-reactive protein (CRP) concentrations, pulmonary function tests (FEV1, FEV1/FVC and MMEF 25–75%) and COPD assessment test (CAT) were assessed at baseline and at the end of trial. *Results*: The groups were comparable in baseline characteristics. There were significant improvements in FEV1/FVC in the probiotic but not in placebo group. CAT scores were decreased in both study groups. However, between-group comparison of changes in the assessed parameters reached statistical significance only for CAT score ($p < 0.001$). There was no report of adverse events during the course of trial. *Conclusions*: Findings of the present trial favor the efficacy of probiotic supplementation in improving the pulmonary symptoms of SM-exposed subjects.

Keywords

Probiotics, sulfur mustard, respiratory function, inflammation, quality of life, randomized controlled trial

History

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Introduction

Sulfur mustard, bis (2-chloroethyl) sulfide (SM), is an alkylating agent and the most widely used chemical weapon among over 70 different chemical warfare agents (Malhotra et al., 1999; Shadboorestan, 2012). Respiratory, skin and ocular injuries are the most common late complication of SM exposure (Ghanei & Harandi, 2011; Ghanei, 2010). Pulmonary complications due to SM often manifest in the form of bronchiolitis obliterans (BO) and chronic obstructive pulmonary disease (COPD) (Ghanei & Harandi, 2007). Cough, sputum and shortness of breath have been reported in 80% of Iranian patients after exposure to SM. Hemoptysis, feeling of pressure in the thorax, chest pain and nocturnal dyspnea are other common complications (Emami, 2014; Ghanei, 2010; Ghanei & Harandi, 2007). Treatment options for chronic SM-induced complications are mainly limited to bronchodilators and inhaled corticosteroids, thus developing curative treatments for these complications is a major priority.

Cellular and molecular findings have shown that lipid peroxidation, protein alkylation, DNA alkylation and mutation, and immune system activation are potential mechanisms that underlie SM injury (Chiarugi & Moskowitz, 2002; Ghanei & Harandi, 2011; Mansour Razavi, 2012). The flux of reactive oxygen and nitrogen species, along with inflammation are other factors that exacerbate SM-induced pathologic changes (Brigati, 2010). Following SM exposure, cellular and molecular alterations lead to the apoptosis of airway epithelial cells and immunological imbalances (Emad & Rezaian, 1999; Ray, 2008). Several inflammatory mediators can be detected in patient's serum and tissues, such results confirm inflammatory role of SM in chronic phase (Ghanei & Harandi, 2011; Ghanei & Harandi, 2007; Pourfarzam, 2009; Panahi, 2013a). Pro-inflammation factors such as interleukin (IL)-1α, -8, -6, -13 (Ghazanfari, 2009), tumor necrosis factor (TNF)-α (Ghazanfari, 2013), interferon (IFN)-α (Ghazanfari, 2009; Ghanei & Vosoghi, 2002), heat shock proteins (HSPs) 27, 70 and 90 (Malaviya, 2010), SWI/SNF, inducible nitric oxide synthase (iNOS), macrophage inflammatory protein-1 (MIP-1), and granulocyte-macrophage colony-stimulating factor (GM-CSF) (Emad & Emad, 2007) are traced in serum and tissue samples of SM patients. On the other hand, intestinal inflammation is another typical feature of SM, mainly due to

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constant exposure to wide spectrum of antibiotics and different medications (Chang, 2014; Ghasemi, 2009; Panahi, 2014).

Recent studies have suggested beneficial effects of lactic acid-producing bacteria (LABs) in the prevention and treatment of inflammatory and immune diseases (Cong, 2003; Tamboli, 2003). Most probiotics contain bacteria such as *Lactobacillus*, *Streptococcus*, *Bifidobacterium* and *Enterococcus* spp., or nonpathogenic yeasts such as *Saccharomyces boulardii*. Probiotics can modulate immune response in the lung (Karen, 2010; Mortaz, 2013) and may also revert the T-regulatory response in the airways (Sudo, 2002).

Changes in the balance of TH17/Treg cell populations, activation of pro-inflammatory NK cells and/or macrophages (Harata, 2010; Izumo, 2010), alterations in macrophage function resulting in reduced allergic responses (Salva et al., 2010), and induction of anti-inflammatory cytokine expression are important immune response modifications induced by probiotic LABs during pulmonary inflammatory diseases. There is evidence that probiotic supplementation decreases the rate of pulmonary exacerbation and hospital admission in patients with pulmonary inflammatory diseases (Morimoto, 2005; Panahi, 2012a; Rao, 2009). To the best of our knowledge, the effects of supplementation with probiotic LABs on circulating levels of inflammatory mediators in SM-exposed veterans have not been studied.

Owing to the presence of chronic lung inflammation in SM-exposed subjects, the immunoregulatory and anti-inflammatory effects of probiotics may improve the symptoms of mustard lung disease. The current study investigated the effects of a commercially-available multi-microbial probiotic supplementation on the severity of clinical symptoms, spirometric function and serum C-reactive protein (CRP) concentrations of SM-exposed individuals suffering from chronic pulmonary complications. CRP was chosen as an efficacy measure owing to the evidence on the role of inflammation in the pathogenesis of chronic respiratory complications due to SM (Ghabili, 2011). In addition, several lines of evidence have suggested the prognostic and causal association between elevated CRP productions and COPD (Aksu, 2013), a condition that has several shared features with mustard lung disease (Sahebkar, 2015).

Methods

Subjects and study design

The study was designed as a randomized double-blind placebo-controlled and parallel-group trial, and was conducted from June 2011 to January 2012 in the Baqiyatallah Hospital, Tehran, Iran. This hospital is a referral and university affiliated hospital that provides medical care for SM-exposed Iraq–Iran war veterans and maintains medical records of these individuals. Volunteer patients with long-term obstructive respiratory disease compatible with the pattern of BO, following definite exposure to SM were enrolled. Exclusion criteria were acute bronchiolitis and/or pneumonia, history of pulmonary tuberculosis or resection of one or more lobes, exposure to other toxins, history of hypersensitivity to probiotic products, having contraindication for spirometry, presence of acute infection in the upper or lower respiratory tract, anemia, polycythemia and coagulative diseases, malignancies, asthma, heart failure, renal and hepatic diseases, and participating in simultaneous clinical trials. Patients were prohibited from smoking during the course of study.

The study protocol and advantages and disadvantages of the administered supplement were explained for patients and written informed consent form was obtained from all participants before recruitment. The consent form included a statement on the right of participants to leave the study at any time. This study was registered and confirmed in the scientific and ethical committee of the Baqiyatallah University (the code and date of ethical approval: 3491). The present study was conducted according to the guidelines laid down in the Declaration of Helsinki. A standard protocol was used for the study. Patients were selected using convenient sampling method from the whole population with documents of chronic pulmonary diseases due to SM. All subjects were on standard respiratory medications (salbutamol and fluticasone) plus pulmonary rehabilitation (30 min, twice weekly), and randomized to adjunctive therapy with probiotics (one capsule each 12 h, $n = 40$) or placebo ($n = 25$). The patients were treated for six weeks and were visited every 15 days to record any clinical adverse effects. The side effects of supplement during the course of trial were recorded.

Probiotics

The probiotic capsule Lactocare® used in this study was obtained from Zist Takhmir Co. (Tehran, Iran) and prepared in accordance with manufacturer directions. Each capsule contained 10^9 colony-forming units (CFU) of a total of seven strains: *Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus rhamnosus, Lactobacillus casei, Bifidobacterium breve, Bifidobacterium longum* and *Streptococcus thermophilusthe*.

Assessment of symptoms

The severity of symptoms and the impact of disease on subjects' health were assessed using COPD assessment test (CAT) at baseline and at study end. The CAT questionnaire has eight items and raises questions about symptoms, energy, sleep and activity. The total CAT score ranges between 0 and 40, with higher scores indicative of a more severe impact of disease on health. The scores were classified as: low impact (score range 0–10); medium impact (score range 11–20), high impact (score range 21–30) and very high impact (score range >30).

Biochemical analysis

At the start of the study and after completion of the six-week treatment period, blood samples (5 mL) were collected after a 12-h fast. After isolation of serum, high-sensitivity CRP concentrations were measured using a BT-3000 Chemistry Automated Analyzer (Biotecnica Instruments, Rome, Italy).

Statistical analysis

All data are shown as mean ± SD or median (interquartile range) for normally and non-normally distributed data,

respectively. Kolmogorov–Smirnov test was performed to test for normality of the data. Within-groups (pre-treatment versus post-treatment) comparisons were performed using paired-samples *t*-test (for normally distributed data) or Wilcoxon signed-rank test (for non-normally distributed data). Between-group comparison of the magnitude of changes in the evaluated parameters was performed using independent-samples *t*-test (for normally distributed data) or Mann–Whitney *U* test (for non-normally distributed data). Comparisons were performed using a per protocol (completer) approach. In all comparisons, a two-sided p value of <0.05 was considered as statistically significant. Statistical tests were performed using SPSS v21.0 software (SPSS Inc., Chicago, IL).

Results

A total of 83 subjects were initially screened for participation in this trial. Eleven subjects (13.2%) were excluded due to lack of eligibility criteria and seven subjects (8.4%) refused to give written informed consent. The remaining 65 patients were entered to the trial and randomized to the above-mentioned groups. The date range of exposure to SM was between 1981 and 1988, and the mean time from exposure in the population was 29 years. All subjects in the probiotics group completed the trial. In the placebo group, five subjects were dropped out because of not referring to the study center for medical examinations and blood sampling, and dissatisfaction with the efficacy of supplement (lack of improvement in the symptoms) (Figure 1).

Study groups were comparable in age, gender, body mass index (BMI), systolic and diastolic blood pressures, and history of diabetes and myocardial infarction. Furthermore, exposure to SM was confirmed by using the medical records in the Veterans and Martyrs Affair Foundation. There was no difference between the groups in terms of duration of their disease. A summary of baseline characteristics of patient and control subjects is presented in Table 1.

There was no significant difference between the probiotics and placebo groups in terms of spirometric indices (FEV_1, FEV_1/FVC and MMEF 25–75%), CAT score and serum CRP concentrations at baseline (Table 2). Probiotic supplementation significantly improved FEV_1/FVC ($p=0.029$) and reduced CAT score ($p<0.001$), but had no effect on serum CRP concentrations and other spirometric indices (FEV_1 and MMEF 25–75%) compared with baseline. None of these parameters significantly changed in the placebo group ($p>0.05$), apart from a reduction in CAT score ($p=0.028$) (Table 3). Between-group comparison of the magnitude of changes in the evaluated efficacy measures revealed a greater

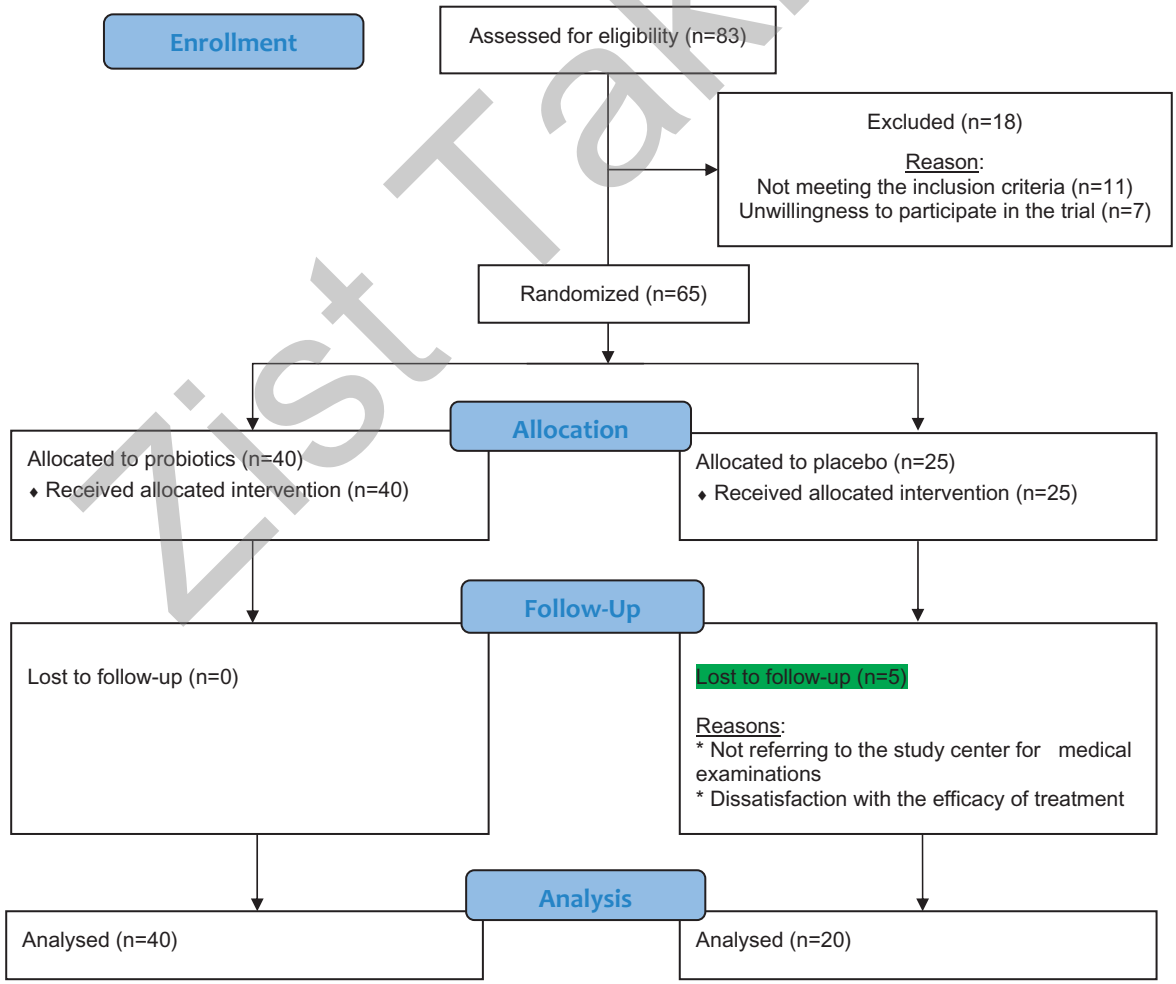

Figure 1. Flow chart of the study.

effect of probiotics versus placebo in reducing the CAT score ($p < 0.001$). Nevertheless, the difference in spirometric indices and serum CRP concentrations did not reach statistical significance (Table 4). When the subjects were categorized into different risk groups according to the CRP levels (low risk: hs-CRP < 1 mg/L, intermediate risk: 1 mg/L ≤ hs-CRP < 3 mg/L and high risk: hs-CRP ≥ 3 mg/L), no significant difference in the hs-CRP risk categories was found between the study groups either at baseline or at the study end ($p > 0.05$). The proportion of subjects whose hs-CRP-based risk group was improved during the study was also comparable between the groups (11.4% versus 7.1% in probiotic and placebo groups, respectively) ($p > 0.05$).

In the present study, probiotic supplement was safe and well tolerated. There was no report of adverse events related to the study supplement.

Table 1. Demographic and baseline characteristics of the study groups.

Variables	Probiotics group ($n = 40$)	Placebo group ($n = 20$)	p[a]
Gender (M/F)	40/0	20/0	1.00
Age (y)	42.22 ± 7.19	41.10 ± 4.67	0.58
Duration of disease (y)	28.80 ± 5.21	29.30 ± 3.75	0.29
BMI (kg/m^2)	23.10 ± 2.30	21.70 ± 2.60	0.34
Smoker (%)	23	13	0.83
SBP (mm Hg)	133.40 ± 9.10	132.10 ± 10.20	0.71
DBP (mm Hg)	84.40 ± 3.40	85.30 ± 2.40	0.43
Diabetes mellitus (%)	12	7	0.16
Previous MI (%)	9	6	0.28

All data expressed as mean ± SD.
BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; MI: myocardial infarction; FVC: forced vital capacity; FEV_1: forced expiratory volume in one second; MMEF: maximal mid-expiratory flow rate.
[a]No significant difference between the probiotics and placebo groups ($p > 0.05$).

Discussion

In this study, we investigated the effects of short-term multimicrobial probiotic supplementation on pulmonary function, severity of symptoms and serum CRP concentrations in SM-exposed subjects. The results revealed an improvement of FEV_1/FVC and CRP following probiotic supplementation, however, the difference between probiotic and placebo groups reached statistical significance only for the CAT, suggesting a reduction in disease severity.

Previous studies have shown that LABs may be beneficial in patients with inflammatory diseases such as coronary artery disease and also decrease CRP concentrations in COPD patients (Cong, 2003; Forsythe et al., 2007). Moreover, numerous studies have shown that probiotics can enhance the immune system, an effect that justifies the therapeutic use of these supplements in inflammatory and immunological diseases (Behnsen, 2013; Standen, 2013; Vitetta, 2014). Recent studies have suggested the regulatory role of LABs in the immunopathogenesis of inflammatory lung diseases such as COPD and idiopathic pulmonary fibrosis (IPF). For instance, the involvement of Th17 and Treg cells in lung disease has become clear. These cells play a critical role in clearing

Table 2. Comparison of efficacy measures between the study groups at weeks 0 and 6.

	0 week			6 weeks		
Variables	Probiotics group	Placebo group	p[a]	Probiotics group	Placebo group	p[a]
FEV_1 (L)	55.25 ± 15.30	58.17 ± 15.70	0.508	56.83 ± 16.30	59.27 ± 17.69	0.120
FEV_1/FVC	67.70 ± 13.20	72.89 ± 13.62	0.178	71.18 ± 13.20	70.93 ± 11.97	0.254
MMEF 25–75% (L.s^{-1})	44.75 ± 21.76	51.94 ± 29.42	0.302	47.30 ± 26.1	50.33 ± 27.00	0.063
CAT score	31.55 ± 5.05	32.40 ± 4.21	0.520	23.45 ± 7.12	31.00 ± 4.68	0.042
CRP (mg/L)	3.25 (1.95, 5.72)	4.00 (2.60, 5.00)	0.827	4.10 (2.10, 5.90)	3.10 (2.00, 4.00)	0.103

All data are expressed as mean ± SD apart from CRP which is expressed as median (interquartile range).
FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; MMEF: maximal mid-expiratory flow rate; CAT: COPD assessment test; CRP: C-reactive proteins.
[a]Independent-samples Student's *t*-test (or Mann–Whitney's *U*-test for non-normally distributed data) comparing values between the two groups at each time point (weeks 0 and 6).

Table 3. Within-group comparison of the efficacy measures.

	Probiotics group ($n = 40$)			Placebo group ($n = 20$)		
Variables	0 Week	6 Week	p[a]	0 Week	6 Week	p[a]
FEV_1 (L)	55.25 ± 15.30	56.83 ± 16.31	0.471	56.83 ± 16.30	59.27 ± 17.69	0.670
FEV_1/FVC	67.70 ± 13.20	71.18 ± 13.61	0.029	71.73 ± 13.91	70.93 ± 11.97	0.697
MMEF 25–75% (L.s^{-1})	44.75 ± 21.76	47.30 ± 26.08	0.328	51.67 ± 30.7	51.33 ± 28.10	0.942
CAT score	31.55 ± 5.05	23.45 ± 7.19	< 0.001	32.40 ± 4.21	31.00 ± 4.68	0.028
CRP (mg/L)	3.25 (1.95, 5.72)	4.10 (2.10, 5.90)	0.773	4.00 (2.60, 5.00)	3.10 (2.00, 4.00)	0.266

All data are expressed as mean ± SD apart from CRP which is expressed as median (interquartile range).
FVC: forced vital capacity; FEV_1: forced expiratory volume in one second; MMEF: maximal mid-expiratory flow rate; CAT: COPD assessment test; CRP: C-reactive proteins.
[a]Paired-samples Student's *t*-test (or Wilcoxon signed-rank test for non-normally distributed data) comparing baseline versus end-trial values in each group.

Table 4. Between-group comparison of the efficacy measures.

Variables	Probiotics group ($n = 40$)	Placebo group ($n = 20$)	p[a]
ΔFEV_1 (L)	1.58 ± 1.36	1.20 ± 1.06	0.310
$\Delta FEV_1/FVC$	3.47 ± 1.97	-0.80 ± 0.79	0.856
ΔMMEF 25–75% ($L.s^{-1}$)	2.55 ± 1.62	-0.34 ± 1.73	0.890
ΔCAT score	-8.01 ± 0.68	-1.41 ± 0.26	<0.001
ΔCRP (mg/L)	0.10 (−3.20, 3.00)	−1.00 (−1.00, 0.12)	0.307

All data are expressed as mean ± SD apart from CRP which is expressed as median (interquartile range).
FVC: forced vital capacity; FEV_1: forced expiratory volume in one second; MMEF: maximal mid-expiratory flow rate; CAT: COPD assessment test; CRP: C-reactive portions.
[a]Independent-sample Student's *t*-test (or Mann–Whitney's *U*-test for non-normally distributed data) for comparison of change values between the study groups.

pathogens during host defense reactions and inducing tissue inflammation in autoimmune diseases (Panahi, 2013a,b; Wang, 2014). Probiotic supplementation has been shown to regulate the balance between different subpopulations of T cells including Th1, Th2, Treg and Th17 (27). In addition, the antimicrobial activity of probiotics has been reported to decrease secondary infections and therefore reduce the production of diverse pro-inflammatory mediators and activated leukocytes in acute necrotizing pancreatitis (Morrow et al., 2012; Rydzewska, 2009).

Inflammation is integral to the destructive reactions in the lung tissue of SM-exposed subjects (Panahi, 2012b, 2014). Several pathophysiological studies on SM-exposed patient have shown significant imbalances in inflammatory mediators (Ghanei & Harandi, 2011; Ghanei & Harandi, 2007; Pourfarzam, 2009; Panahi, 2013a). In the present study, between-group difference of CRP changes did not reach statistical significance. Nevertheless, the effect of probiotics on circulating CRP concentrations in SM-exposed subjects needs to be verified in larger populations, as there is evidence on the CRP-lowering effect of these supplements in other groups of people (Asemi, 2013; Shadnoush, 2013). In addition, since the mean baseline CRP level of subjects recruited for this study was <10 mg/L (thus being at high risk of cardiovascular disease (Bogaty, 2005)), it will be required to confirm the present results in subjects with upper normal hs-CRP levels at baseline. In addition, it is necessary to look at changes in other inflammatory mediators that could be implicated in the pathogenesis of respiratory complications following SM exposure. The impact of probiotics on IL-2, IL-6, IL-10, IL-17, TNF-α and IFN-γ as inflammatory drivers of COPD, a condition with several similarities to mustard lung disease (Sahebkar, 2015), has already been reported (Mortaz, 2013).

In conclusion, findings of the present trial, being the first of its kind, suggested a reduction in the severity of clinical symptoms (based on the CAT score) of subjects suffering from chronic SM-induced pulmonary complications following short-term supplementation with probiotics. The immunomodulatory and possible anti-inflammatory properties of probiotics appear to be mechanisms involved in the therapeutic effects of this supplement in subjects suffering from chronic SM-induced respiratory complications. Owing to the lack of any improvement in the spirometric indices, the present results do not favor any bronchodilatory activity of probiotics but this needs to be confirmed in studies with longer-term supplementation periods. Future studies are warranted to confirm the present results in larger populations, and also ascertain the impact of treatment duration and probiotic dose on the observed beneficial effects. Future studies are also warranted to clarify if there is any differential effect among different strains of probiotic bacteria in terms of improving clinical symptoms of SM-exposed subjects. Finally, assessment of changes in other inflammatory mediators and oxidative parameters both in serum and bronchoalveolar lavage fluid of probiotic-supplemented SM-exposed individuals is recommended.

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Declaration of interest

The authors have no financial interests related to the material in the manuscript. The authors declare that there are no conflicts of interest.

References

Aksu F, Capan N, Aksu K, et al. (2013). C-reactive protein levels are raised in stable chronic obstructive pulmonary disease patients independent of smoking behavior and biomass exposure. J Thorac Dis 5:414–421.

Asemi Z, Zare Z, Shakeri H, et al. (2013). Effect of multispecies probiotic supplements on metabolic profiles, hs-CRP, and oxidative stress in patients with type 2 diabetes. Ann Nutr Metab 63:1–9.

Behnsen J, Deriu E, Sassone-Corsi M, Raffatellu M. (2013). Probiotics: properties, examples, and specific applications. Cold Spring Harb Perspect Med 3:a010074.

Bogaty P, Brophy JM, Boyer L, et al. (2005). Fluctuating inflammatory markers in patients with stable ischemic heart disease. Arch Intern Med 165:221–226.

Brigati C, Banelli B, di Vinci A, et al. (2010). Inflammation, HIF-1, and the epigenetics that follows. Mediators Inflamm 2010:263914.

Chang YC, Wang JD, Hahn RA, et al. (2014). Therapeutic potential of a non-steroidal bifunctional anti-inflammatory and anti-cholinergic agent against skin injury induced by sulfur mustard. Toxicol Appl Pharmacol 280:236–244.

Chiarugi A, Moskowitz MA. (2002). Cell biology. PARP-1-a perpetrator of apoptotic cell death? Science 297:200–201.

Cong Y, Konrad A, Iqbal N, Elson CO. (2003). Probiotics and immune regulation of inflammatory bowel diseases. Curr Drug Targets Inflamm Allergy 2:145–154.

Emad A, Emad Y. (2007). Increased granulocyte-colony stimulating factor (G-CSF) and granulocyte-macrophage colony stimulating factor (GM-CSF) levels in BAL fluid from patients with sulfur mustard gas-induced pulmonary fibrosis. J Aerosol Med 20:352–360.

Emad A, Rezaian GR. (1999). Immunoglobulins and cellular constituents of the BAL fluid of patients with sulfur mustard gas-induced pulmonary fibrosis. Chest 115:1346–1351.

Emami MH, Talaei M, Panahi Y, et al. (2014). Efficacy of omeprazole on cough, pulmonary function and quality of life of patients with sulfur mustard lung injury: a placebo-control, cross-over clinical trial study. J Res Med Sci 19:1027–1033.

Forsythe P, Inman MD, Bienenstock J. (2007). Oral treatment with live *Lactobacillus reuteri* inhibits the allergic airway response in mice. Am J Respir Crit Care Med 175:561–569.

Ghabili K, Agutter PS, Ghanei M, et al. (2011). Sulfur mustard toxicity: history, chemistry, pharmacokinetics, and pharmacodynamics. Crit Rev Toxicol 41:384–403.

Ghanei M, Poursaleh Z, Harandi AA, et al. (2010). Acute and chronic effects of sulfur mustard on the skin: a comprehensive review. Cutan Ocul Toxicol 29:269–277.

Ghanei M, Harandi AA. (2007). Long term consequences from exposure to sulfur mustard: a review. Inhal Toxicol 19:451–456.

Ghanei M, Harandi AA. (2011). Molecular and cellular mechanism of lung injuries due to exposure to sulfur mustard: a review. Inhal Toxicol 23:363–371.

Ghanei M, Vosoghi AA. (2002). An epidemiologic study to screen for chronic myelocytic leukemia in war victims exposed to mustard gas. Environ Health Perspect 110:519–521.

Ghasemi H, Ghazanfari T, Yaraee R, et al. (2009). Evaluation of relationship between the serum levels of inflammatory mediators and ocular injuries induced by sulfur mustard: Sardasht-Iran Cohort Study. Int Immunopharmacol 9:1494–1498.

Ghazanfari T, Faghihzadeh S, Aragizadeh H, et al. (2009). Sardasht-Iran cohort study of chemical warfare victims: design and methods. Arch Iran Med 12:5–14.

Ghazanfari T, Kariminia A, Yaraee R, et al. (2013). Long term impact of sulfur mustard exposure on peripheral blood mononuclear subpopulations – Sardasht-Iran Cohort Study (SICS). Int Immunopharmacol 2013 17:931–935.

Harata G, He F, Hiruta N, et al. (2010). Intranasal administration of *Lactobacillus rhamnosus* GG protects mice from H1N1 influenza virus infection by regulating respiratory immune responses. Lett Appl Microbiol 50:597–602.

Izumo T, Maekawa T, Ida M, et al. (2010). Effect of intranasal administration of Lactobacillus pentosus S-PT84 on influenza virus infection in mice. Int Immunopharmacol 10:1101–1106.

Karen M, Yuksel O, Akyurek N, et al. (2010). Probiotic agent *Saccharomyces boulardii* reduces the incidence of lung injury in acute necrotizing pancreatitis induced rats. J Surg Res 160:139–144.

Malaviya R, Sunil VR, Cervelli J, et al. (2010). Inflammatory effects of inhaled sulfur mustard in rat lung. Toxicol Appl Pharmacol 248: 89–99.

Malhotra R, Ganesan K, Sugendran K, Swamy R. (1999). Chemistry and toxicology of sulphur mustard: a review. Defence Sci J 49:97–116.

Mansour Razavi S, Salamati P, Saghafinia M, Abdollahi M. (2012). A review on delayed toxic effects of sulfur mustard in Iranian veterans. Daru 20:51.

Morimoto K, Takeshita T, Nanno M, et al. (2005). Modulation of natural killer cell activity by supplementation of fermented milk containing *Lactobacillus casei* in habitual smokers. Prev Med 40:589–594.

Morrow LE, Gogineni V, Malesker MA. (2012). Probiotic, prebiotic, and synbiotic use in critically ill patients. Curr Opin Crit Care 18: 186–191.

Mortaz E, Adcock IM, Folkerts G, et al. (2013). Probiotics in the management of lung diseases. Mediators Inflamm 2013:751068.

Panahi Y, Davoudi SM, Sahebkar A, et al. (2012a). Efficacy of *Aloe vera*/olive oil cream versus betamethasone cream for chronic skin lesions following sulfur mustard exposure: a randomized double-blind clinical trial. Cutan Ocul Toxicol 31:95–103.

Panahi Y, Sarayani A, Beiraghdar F, et al. (2012b). Management of sulfur mustard-induced chronic pruritus: a review of clinical trials. Cutan Ocul Toxicol 31:220–225.

Panahi Y, Ghanei M, Ghabili K, et al. (2013b). Acute and chronic pathological effects of sulfur mustard on genitourinary system and male fertility. Urol J 10:837–846.

Panahi Y, Davoudi SM, Beiraghdar F, et al. (2013a). Serum levels of interleukins 2, 4, 6, and 10 in veterans with chronic sulfur mustard-induced pruritus: a cross-sectional study. Skinmed 11:205–209.

Panahi Y, Ghanei M, Hajhashemi A, Sahebkar A. (2014). Effects of curcuminoids-piperine combination on systemic oxidative stress, clinical symptoms and quality of life in subjects with chronic pulmonary complications due to sulfur mustard: a randomized controlled trial. J Diet Suppl 13:93–105.

Pourfarzam S, Ghazanfari T, Yaraee R, et al. (2009). Serum levels of IL-8 and IL-6 in the long term pulmonary complications induced by sulfur mustard: Sardasht-Iran Cohort Study. Int Immunopharmacol 9: 1482–1488.

Rao AV, Bested AC, Beaulne TM, et al. (2009). A randomized, double-blind, placebo-controlled pilot study of a probiotic in emotional symptoms of chronic fatigue syndrome. Gut Pathog 1:6.

Ray R, Keyser B, Benton B, et al. (2008). Sulfur mustard induces apoptosis in cultured normal human airway epithelial cells: evidence of a dominant caspase-8-mediated pathway and differential cellular responses. Drug Chem Toxicol 31:137–148.

Rydzewska G. (2009). New trends in prognosis and treatment of acute pancreatitis. Pol Merkur Lekarski 26:550–553.

Sahebkar A, Antonelli-Incalzi R, Panahi Y, et al. (2015). Mustard lung and COPD: common features and treatment? Lancet Respir Med 3: 747–748.

Salva S, Villena J, Alvarez S. (2010). Immunomodulatory activity of *Lactobacillus rhamnosus* strains isolated from goat milk: impact on intestinal and respiratory infections. Int J Food Microbiol 141:82–89.

Shadboorestan A. (2012). Commentary on: a review on delayed toxic effects of sulfur mustard in Iranian veterans. Daru 20:99.

Shadnoush M, Shaker Hosseini R, Mehrabi Y, et al. (2013). Probiotic yogurt affects pro- and anti-inflammatory factors in patients with inflammatory bowel disease. Iran J Pharm Res 12:929–936.

Standen BT, Rawling MD, Davies SJ, et al. (2013). Probiotic *Pediococcus acidilactici* modulates both localised intestinal- and peripheral-immunity in tilapia (Oreochromis niloticus). Fish Shellfish Immunol 35:1097–1104.

Sudo N, Yu XN, Aiba Y, et al. (2002). An oral introduction of intestinal bacteria prevents the development of a long-term Th2-skewed immunological memory induced by neonatal antibiotic treatment in mice. Clin Exp Allergy 32:1112–1116.

Tamboli CP, Caucheteux C, Cortot A, et al. (2003). Probiotics in inflammatory bowel disease: a critical review. Best Pract Res Clin Gastroenterol 17:805–820.

Vitetta L, Briskey D, Alford H, et al. (2014). Probiotics, prebiotics and the gastrointestinal tract in health and disease. Inflammopharmacology 22:135–154.

Wang C, Liu X, Peng Q, et al. (2014). Role of Foxp3/Treg and RORgammat/Th17 cell imbalance in rat model of chronic obstructive pulmonary disease. Zhonghua Wei Zhong Bing Ji Jiu Yi Xue 26: 860–864.