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ORIGINAL INVESTIGATION

Long-term flaxseed oil supplementation diet protects BALB/c mice against *Streptococcus pneumoniae* infection

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Abstract Intense host immune response to infection contributes significantly to the pathology of pneumococcal pneumonia. Therefore, the regulation of host immune response is critical for the successful outcome of pneumonia in such patients. The aim of the present study was to investigate the effect of n-3 PUFA, i.e. flaxseed oil supplementation for short (4 weeks) as well as long (9 weeks) term, on the course of *S. pneumoniae* D39 serotype 2 infection in mice. The efficacy of flaxseed oil supplementation was investigated in terms of survival of animals and production of various inflammatory molecules (malondialdehyde, myeloperoxidase, nitric oxide) in the lung homogenate of animals. This was correlated with bacteriological and histopathological parameters. The immunomodulation was studied in terms of cytokines in the lungs following infection with *Streptococcus pneumoniae*. Results suggest that long-term flaxseed supplementation protected the mice against bacterial colonization of lungs with *Streptococcus pneumoniae* with reduced histopathological involvement of lung tissue. Moderate pneumonia was observed in supplemented, infected mice compared to severe pneumonia seen in control mice. This was accompanied by decreased inflammatory markers (malondialdehyde, myeloperoxidase, nitric oxide) as the disease progressed. In addition, difference in the levels of pro-inflammatory (TNF-α and IL-1β) and anti-inflammatory (IL-10) cytokines was observed in the flaxseed fed animals. On the contrary, short-term supplementation did not show such an effect on lung colonization.

Keywords Dietary supplementation · Flaxseed oil · ω-3 PUFA · Pneumonia · *Streptococcus pneumoniae*

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Introduction

Streptococcus pneumoniae is one of the leading causes of morbidity and mortality in children. This ubiquitous organism, which often colonizes the human nasopharynx, causes a variety of localized, noninvasive (e.g., sinusitis, otitis media) and invasive (e.g., meningitis, bacteremia) infections [1]. Despite the availability of vaccines and antibiotic treatments, mortality rates remain high. The pathology of pneumococcal pneumonia results from an exaggerated host inflammatory response to bacterial components. This overwhelming inflammation contributes to tissue injury and shock [2]. Immunity to pneumococcal pneumonia has long been assumed to depend on the humoral arm of the immune system but it is increasingly recognized that innate immunity plays a critical role in the progression of pneumonia [3].

The recognition, that long-chain n-3 PUFA have the potential to inhibit (excessive) inflammatory responses, has led to a large number of clinical investigations with these fatty acids in inflammatory conditions as well as in healthy subjects. And, the results indicate that dietary n-3 PUFA supplementation has a beneficial effect in various diseases and conditions such as atherosclerosis, coronary heart disease, arrhythmias and in allergic conditions like asthma. A recent meta-analysis of data from these human clinical trials using "immunonutrition" suggests that giving an (n-3)

PUFA–supplemented enteral nutrition product (e.g., Impact, Sandoz Nutrition, Minneapolis, MN) may reduce nosocomial infection and sepsis rates in some patient populations [4]. Unfortunately, because of the combination of nutrients [i.e., arginine, nucleotides and (n-3) PUFA] in the enteral formula used in all of these studies, it is difficult to discern how much of the reported effects are attributable to (n-3) PUFA alone. Nutritional recommendations have recently promoted the increased need to consume omega-3 fatty acids. The most common way to consume omega-3 fatty acids has been in the form of marine oils like fish. Recently, flaxseed has been identified as a significant alternative source of omega-3 (n-3) fatty acids [5, 6]. Flaxseed is one of the richest sources of α-linolenic acid (ALA). ALA has been identified in several epidemiological trials as having significant beneficial effects. Inclusion of flaxseed in the diet in animal studies has shown that flaxseed can inhibit arrhythmogenesis during ischemia–reperfusion [7], inhibit atherogenesis [8, 9], and protect against vascular dysfunction during hypercholesterolemic conditions [10]. Hence, in this study we have evaluated the effects of flaxseed oil supplementation on the course of experimental pneumonia by *Streptococcus pneumoniae* in an animal model.

Materials and methods

Bacterial strain

Streptococcus pneumoniae strain D39 serotype 2 obtained from Dr. Dong Kwon Rhee, Prof. of Microbiology from College of Pharmacy, Sungkyunkwan University, South Korea and maintained in the laboratory, was used in this study. Organism was maintained on blood agar plates containing 5% defibrinated blood.

Animals

Pathogen-free BALB/c mice of 6–8-weeks old, weighing 20–25 g were procured from the central animal house of Panjab University, Chandigarh. Animals were kept in clean propylene cages and fed on standard antibiotic-free diet (JBD agencies Pvt. Ltd., India) and water ad libitum. Mice were exposed to a standardized 12 h light/dark cycle. Weight and health status were monitored before the induction of infection as well as at the time of death. The study was conducted after approval from the animal ethics committee of Panjab University, Chandigarh, India.

Experimental protocol

Animals of either sex weighing 25–30 g were divided in 3 different groups consisting of 30 mice each. One group served as control in which mice were fed on normal standard lab chow diet. Another group of mice was fed on standard lab chow diet along with daily supplementation of 0.5 ml flaxseed oil (Flax Oil, Nature's Bounty, U.S.A.) providing 2,000 mg/kg body weight/day of n-3 PUFA, administered orally with a feeding catheter (Romsons Ltd., India). One group was fed for short (4 weeks) and other for long (9 weeks) term, followed by infection with *Streptococcus pneumoniae* D39.

Pneumococcal pneumonia model

S. pneumoniae was passaged through mice, and aliquots were stored at −70°C with no significant loss of viability. When required, suspension was thawed at room temperature and used to seed fresh blood agar plate and was incubated overnight at 37°C in a 5% CO_2 atmosphere. The cultures were centrifuged, washed, and resuspended in sterile buffer saline (PBS) to obtain appropriate concentration for infecting the animals via intratracheal (i.t.) route as described earlier [11]. Briefly, mice were anesthetized with propofol (3 mg/ml/ip). The trachea was exposed, and 50 µl of inoculum containing 10^6 CFU of *S. pneumoniae* suspended in PBS saline was administered via sterile 26-gauge needle. The skin incision was closed using surgical staples.

Survival studies

Mice that were provided with flaxseed oil supplementation or standard lab chow diet for different time intervals and intratrachealy inoculated with *Streptococcus pneumoniae* were observed daily, and mortality records were kept for 5 days. On this basis, the percent survival and mean survival time were calculated.

Quantitation of bacteria in lungs

Mice were killed at different time intervals, and lungs were removed aseptically and then homogenized in one milliliter of normal saline with the help of glass hand-held tissue homogenizer. Bacteria were quantitated by spreading serial dilutions of homogenized lung tissue on 5% defibrinated blood agar plates, *S. pneumoniae* was counted and expressed as CFU per ml after overnight incubation at 37°C.

The tissue homogenate from each mouse was processed for the following parameters as well:

Inflammatory cells

Pulmonary neutrophil infiltration in lung tissue was quantified through the measurement of myeloperoxidase (MPO), as previously described [12]. Briefly, lungs were removed and homogenized in 2 ml of solution containing 50 mM

PBS (pH 6.0) with 5% hexadecyl trimethyl ammonium bromide and 5 mM EDTA. The homogenate was sonicated and centrifuged at 15,000× *g* for 15 min. The supernatant was mixed at a ratio of 1:15 in assay buffer (50 mM PBS, pH 6.0; 0.167 mg/ml o-diansidine dihydrochloride; 0.0005% H_2O_2). MPO activity was assayed by measuring the change in absorbance at 450 nm.

Biochemistry

Injuries to cell membranes were monitored through the measurement of malondialdehyde (MDA), a metabolite resulting from lipid peroxidation, which was detected by the method of Ohkawa et al. [13]. Briefly, lungs were isolated, homogenized, and 0.1 ml of the homogenate was mixed with 0.2 ml of the 8.1% sodium dodecyl sulfate (SDS), 1.5 ml of 20% acetic acid (pH 3.5), and 1.5 ml of 0.8% thiobarbituric acid (TBA). The mixture was heated at 100°C for 1 h, and then 1.0 ml of distilled water and 5.0 ml of the mixture of n-butanol and pyridine (15:1; v/v) were added. After centrifugation at 1500*g* for 10 min, the absorbance of the upper organic layer was measured at 532 nm. MDA concentration was expressed as nanomole/mg protein.

Inflammatory mediators

The release of nitric oxide (NO) in the lung homogenate was evaluated through the measurement of its oxidized nitrite and nitrate metabolites, according to the colorimetric method of Griess, as described previously [14].

IL-1 β, TNF-α and IL-10 levels were detected in the supernatants of lung homogenates of control and supplemented infected animals. Samples were subjected to a mouse sandwich enzyme-linked immunosorbent assay to quantify all the above cytokines (IL-1 β, TNF-α and IL-10 kits; BD Biosciences, Pharmingen, C.A., USA.) following the manufacturer's instruction provided along with kit.

Histopathology

At different time points, the lungs were fixed in formaldehyde, embedded in paraffin, and processed for microscopy as previously described [15]. Tissue sections were stained with hematoxylin and eosin. Tissue was assessed on a semiquantitative scale of 0–3 as shown in Table 1.

Statistical analysis

Data were analyzed for statistically significant differences by two-way ANOVA between animals. Individual group comparisons were made by two-tailed Student's t test. Statistical significance was accepted at $p < 0.005$.

Table 1 Semiquantitative scoring of lung tissue

Tissue	Histological changes	Score
Alveoli	No change	0
	Edema	+1
	Inflammatory cells in alveolar lumina	+2
	Inflammatory destruction of alveoli (with lung abscess)	+3
Bronchioles	No change	0
	Mild inflammation in the wall (without lumina slough)	+1
	Severe inflammation in the wall (with lumina slough)	+2
	Severe inflammation with luminal slough and peribronchial inflammation	+3

Results

Short-term dietary supplementation with flaxseed oil did not show any improvement on the survival as well as on the establishment of *Streptococcus pneumoniae* D39 serotype 2 in the lungs. The lung bacterial count in infected mice was 6.23 CFU/ml and in flaxseed oil fed infected mice was 6.07 CFU/ml, respectively ($p > 0.05$). Results indicated that short-term (4 weeks) supplementation with flaxseed oil did not provide protection against challenge with *S. pneumoniae*. However, flaxseed oil supplementation for 9 weeks led to improvement in the survival of mice after pulmonary *S. pneumoniae* infection. Figure 1 shows the survival rates

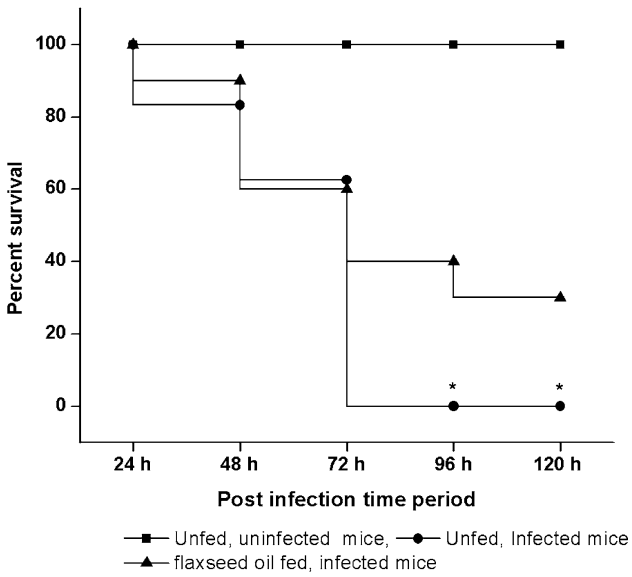

Fig. 1 The survival of mice infected with *S. pneumoniae* D39 serotype 2 intratrachealy after feeding with flaxseed oil-supplemented diet for 9 weeks. Each group consisted of 10 mice. *, p was < 0.05 when compared to infected control

in the flaxseed oil-supplemented groups following infection with *S. pneumoniae*. In the flaxseed oil-supplemented group, 60% mortality was observed on 4th post infection day when compared to 100% mortality in normal saline fed group (control).

Effect of flaxseed oil supplementation on bacterial growth in lungs

The effect of flaxseed oil supplementation on lung bacterial colonization was next investigated, and the results are presented in Fig. 2a. During initial stage of infection, bacterial burden in the flaxseed oil-supplemented and normal saline fed control group was similar. No significant decrease in bacterial load in flaxseed oil-supplemented group was seen till 48 h. However, significant decrease was observed in this group at 72 h post infection ($p < 0.05$).

Histopathologic assessment of lung tissue

Lung tissue was examined at 4, 24, 48, and 72 h after intratracheal inoculation of *S. pneumoniae* in different groups of mice. No pathological changes in the lung could be found at 4 h post inoculation (Fig. 3), whereas severe pneumonia (severity score 3+/3+) was observed at 72 h after infection in the control group. In flaxseed oil-supplemented group, the severity of histopathological involvement was much less (severity score 1+/2+).

Effect of supplementation of flaxseed oil on neutrophil infiltration

Bacterial burden in infected animals is dependent on a balance between the bacterial proliferation and clearance by host's innate immune system. To investigate whether neutrophil migration was responsible for decreased bacterial load and severity of infection seen in flaxseed oil-supplemented mice, the neutrophil migration was compared in the lungs at various time points in flaxseed fed and normal saline fed mice. In the normal saline fed group, pneumococcal pneumonia induced a rapid, time-dependent accumulation of neutrophils and monocytes/macrophages in the lungs. Neutrophil numbers in control animals increased with time after infection and were highest at 48 h post infection (Fig. 2b). In the flaxseed oil-supplemented group, a sudden increase in MPO content was observed at 24 h indicating early infiltration of lungs with neutrophils.

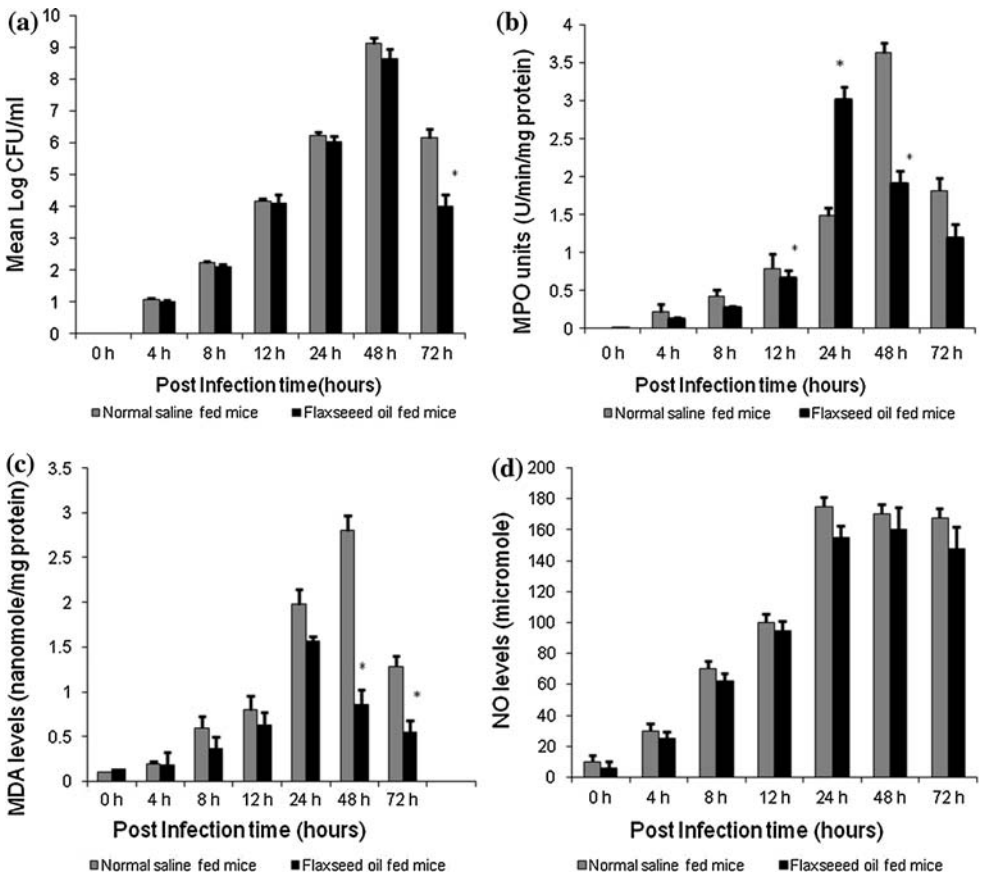

Fig. 2 Evaluation of various parameters in the lung homogenate of mice infected with *S. pneumoniae*. Before challenge, animals were fed on flaxseed oil for 9 weeks. **a** Mean log bacterial counts **b** Myeloperoxidase (MPO) units **c** Malondialdehyde (MDA) levels **d** Nitric oxide (NO) levels. Each group consisted of 30 mice, and 6 mice were killed at each time point. *, p was < 0.05 compared to that for the infected control. And, *error bars* indicate standard errors of the mean

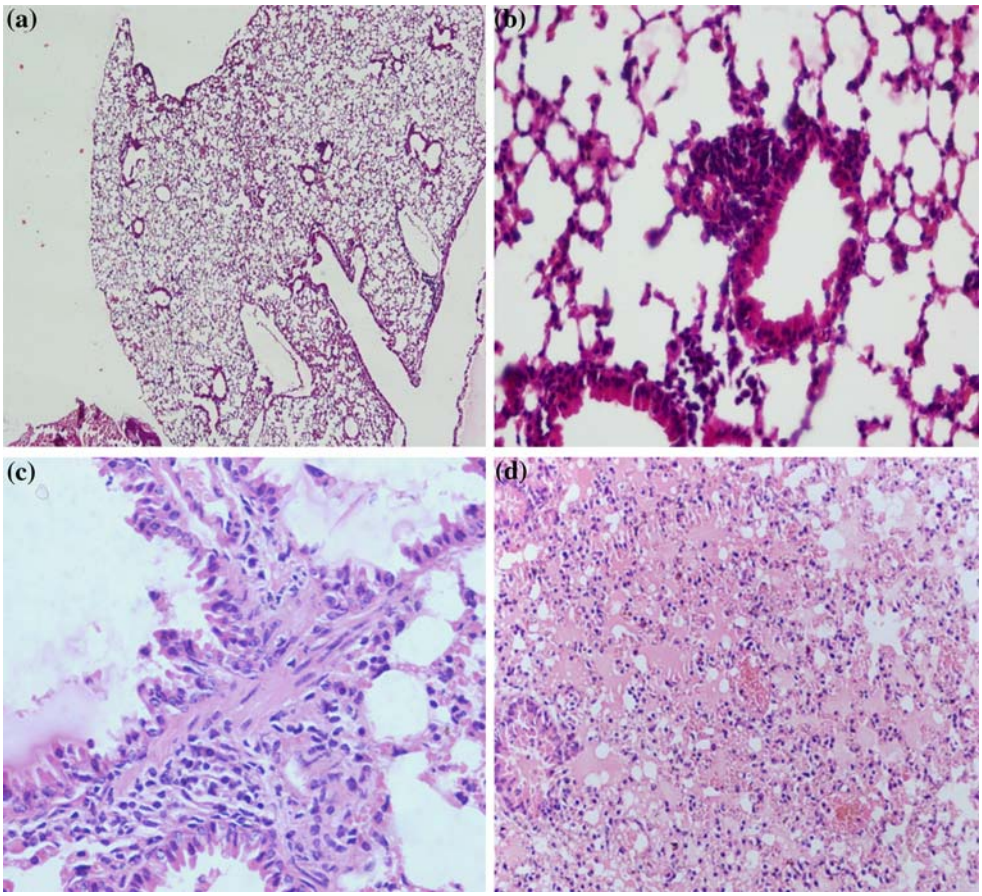

Fig. 3 Representative photomicrographs of lung tissue (hematoxylin & eosin) from mice after intratracheal instillation of *S. pneumoniae.* **a** Photomicrograph showing lung tissue of normal mice, bronchii is normal, no infiltration is seen around the alveoli or in the bronchii (HE X 100). **b** Photomicrograph of lung tissue of flaxseed oil fed mice at 4 h post infection. Lymphoid tissue is normal along with bronchii (HE × 200). **c** Photomicrograph showing lung tissue of unsupplemented infected mice at 72 h post infection. Thick interalveolar septum with hyperplasia of pneumocytes was observed with severity score +3 (HE × 200). **d** Photomicrograph showing lung tissue of flaxseed oil-supplemented mice at 72 h post infection. Eosinophilic edema fluid in the alveoli and congested vessels in septal wall with a severity score +1 (HE × 200) was seen

Effect of flaxseed oil supplementation on MDA levels in lung homogenates

The MDA estimation in the lung homogenates of mice was carried out, and the results are presented in Fig. 2c. Since MDA is an indicator of tissue damage following the infection, no abrupt increase in MDA level was observed in the lung homogenates of mice fed on flaxseed oil. The difference was found to be significant as MDA levels in flaxseed oil fed mice were significantly less when compared to control animal at 48 and 72 h post infection.

Effect of flaxseed oil supplementation on NO levels in lung homogenates

NO levels in lung homogenates of flaxseed oil fed mice followed by infection with *Streptococcus pneumoniae* are presented in Fig. 2d. Decrease in nitric oxide level in the lung homogenates of flaxseed oil fed mice was observed at all time points. However, this difference in the NO levels at all time points was not found to be significant ($p > 0.05$) when compared to its level in control mice, at different time intervals.

Effect of flaxseed oil supplementation on cytokine levels

Levels of pro-inflammatory cytokines (IL1-β and TNF-α) were evaluated in the lung homogenates of mice fed on flaxseed oil for 9 weeks and subsequently infected with *S. pneumoniae*. The results presented in Fig. 4a show that IL1-β level in flaxseed oil-supplemented mice was highest during the early phase of infection. At 24 h, a sharp decline in IL1-β level was observed while no such decline was seen in control infected mice. Increase in TNF-α level was observed at all time points in flaxseed oil-supplemented mice when compared to normal saline fed mice Fig. 4b. However, the difference seen in the TNF-α level in the lung homogenates of both the groups was insignificant. Results

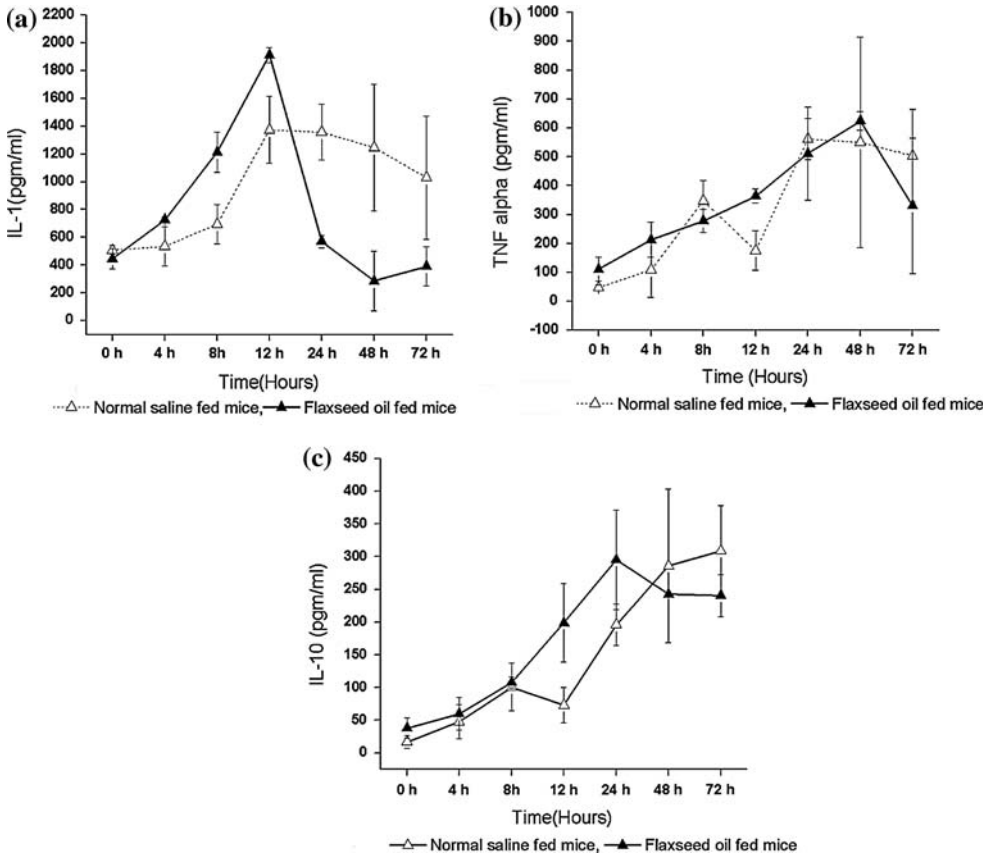

Fig. 4 Levels of cytokines IL-1α (**a**), TNF-α (**b**) and IL-10 (**c**) in the lung homogenates of mice at different time intervals. Before challenge with *S. pneumoniae*, mice were given flaxseed oil orally for 9 weeks. Each group consisted of 30 mice, and 6 mice were killed at each time point

presented in Fig. 4c show the level of anti-inflammatory cytokine (IL-10) in the lung homogenates of mice. Flaxseed oil-supplemented mice showed peak production of IL-10 at 24 h followed by a decline at 48 and 72 h.

Discussion

Dietary supplements are becoming a part of conventional or complementary and alternative medicine as these play an important role in maintaining health. In the present study, the group fed on flaxseed oil for 4 weeks followed by infection with *S. pneumoniae* was not able to survive the infection. Our findings corroborate the observation made by Thors et al. [16], who demonstrated the beneficial effect of dietary fish oil (10% w/w of the diet) on the survival of mice following induction of experimental pneumonia with *Klebsiella pneumoniae*, but not after infection with *Streptococcus pneumoniae* serotype 3 when supplemented for 6 weeks [16]. Interestingly, the group fed on flaxseed oil for 9 weeks showed improved survival after infection with *S. pneumoniae*. Bacterial load assessment showed significant decrease in lung bacterial colonization when compared to control. Pathological evaluation of lung tissue also supported bacteriological observation as supplementation of diet with flaxseed oil in the animals for 9 weeks, brought down the infection from severe pneumonia to moderate pneumonia. These results suggest that the duration of feeding is a crucial factor for obtaining a positive effect of dietary oil supplementation.

There are reports available in literature on feeding of dietary fish oil followed by an in vivo challenge with an infectious agent [17–19]. Nearly an equal number of papers published on this subject report an adverse effect of these oils on host's ability to resist infectious disease whereas others show neither beneficial effect nor any other effect [17, 20–22]. Most studies with the ex vivo and in vitro data suggest that dietary intake of these oils could impair host defense against infectious agents. However, such in vitro data may not be predictive of actual clinical outcome when a host is challenged with a pathogen [23]. Although survival is clearly the most important clinical end point, it may not necessarily be a complete indicator of host's immunity. The underlying mechanism(s) by which these essential oils affect infectious disease resistance therefore will be of interest. Thus, the present study was carried out to improve our understanding as to how vegetable source of n-3 PUFA i.e. flaxseed oil supplementation may affect infectious disease resistance.

Neutrophil migration from the vascular space to the air space is an early and prominent component of the host innate response to pneumococcal invasion [24]. Following

initial tissue injury, neutrophils migrate to the area, become activated and initiate a series of events. In fatal cases of pneumococcal pneumonia there are often no viable bacteria, and a persistent neutrophilic infiltrate in the lung is frequently the only finding [25]. A dysregulated inflammatory response and excessive production of microbicidal factors contribute to mortality. The traditional paradigm suggests that the neutrophils producing ROS are important to bacterial clearance but excessive neutrophil recruitment is harmful. In the present study, delayed MPO production along with decrease in MPO levels, as the disease progressed, points toward beneficial effects of flaxseed oil supplementation. The intense inflammatory neutrophilic infiltrate that accompanies pneumococcal pneumonia is also responsible for significant host tissue damage due to acute release of oxygen free radicals from activated neutrophils [26]. MDA is an end product of lipid peroxidation induced by free oxygen radicals and gives information on the magnitude of tissue damage. In the present study, decrease in tissue MDA levels suggests protective effect of flaxseed supplementation.

Nitric oxide (NO) production during inflammation is an essential element of antimicrobial immunity but it can also contribute to host-induced tissue damage [27]. Under the conditions of pneumococcal pneumonia, large amounts of NO are produced, causing hypotension, a critical pathological feature of septic shock. n-3 PUFA have been shown to inhibit immune cell NO production [27]. Results of this previous study are in accord with our results as decrease in nitric oxide levels was observed in the lung homogenates of flaxseed oil fed mice compared to control infected mice.

Cytokines play an important role in host defense as well as detrimental effect which in turn under certain conditions may contribute toward the manifestations of septic shock, such as multiple organ failure and death. TNF-α prevents outgrowth of bacteria in lungs and the onset of bacteraemia, thereby playing a protective role in pneumococcal pneumonia [28].The role of TNF-α is a double-edged sword, as it is required in the lung in the early stage of disease but is detrimental in the latter part of infection process. A number of studies have demonstrated enhanced TNF secretion in the group of mice fed on fish oil [19, 29]. Previous results support our findings as in the early stage of infection, increased TNF-α resulted in decreased bacterial growth in the lungs when compared to control. However, at later stages in the infection process, decrease in TNF activity was seen in case of flaxseed oil fed mice while no decrease was seen in control infected mice. It seems that PUFA helps in regulating cytokine production which might be protecting the host. Similarly, increase in interleukin-1 (IL-1) levels was seen during early infection followed by a decrease. TNF-α, along with interleukin-1 (IL-1), can greatly increase the production of many acute-phase proteins [30]. These proteins are known to play an important role in the defense against *Streptococcus pneumoniae*. It potentiates the bactericidal properties of neutrophils and it also upregulates vascular and neutrophil adhesion molecules, which facilitate the neutrophil influx at the site of infection [30–33]. Increased neutrophil infiltration in the lungs observed in this study point toward such a mechanism contributing toward influx due to altered cytokine levels.

The early increase in IL-10 production in flaxseed oil fed mice points toward its role in early resolution of infection in this animal group. Earlier results indicate that IL-10 regulates cytokine and chemokine production but does not play an essential role in antibacterial host defense during murine pneumococcal meningitis [34]. These results taken together to prove that the dietary fish oil has immunomodulatory role mediated in part by its effect on pro and anti-inflammatory cytokines. Recently, a new group of ω-3 fatty acid derivatives, called the resolvins, was introduced by Serhan et al. [28]. This group is anti-inflammatory and possesses immunoregulatory action. The presence of this compound in flaxseed oil might explain the positive effects of the n-3 PUFA supplementation on the course of *Streptococcus pneumoniae* infection in our study.

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References

1. Rudan I, Boschi-Pinto C, Biloglav Z, Mulhollandd K, Campbelle H (2008) Epidemiology and etiology of childhood pneumonia. Bull World Health Organ 86:408–416
2. Blue CE, Paterson GK, Kerr AR, Berge M et al (2003) ZmpB, a novel virulence factor of *Streptococcus pneumoniae* that induces tumor necrosis factor alpha production in the respiratory tract. Infect Immun 71(9):4925–4935
3. Kadioglu A, Andrew PW (2004) The innate immune response to pneumococcal lung infection: the untold story. Trends Immunol 25:143–149
4. Kudsk KA (2006) Immunonutrition in surgery and critical care. Annu Rev Nutr 26:463–479
5. Gebauer SK, Psota TL, Harris WS, Kris-Etherton PM (2006) n-3 fatty acid dietary recommendations and food sources to achieve essentiality and cardiovascular benefits. Am J Clin Nutr 83:1526S–1535S
6. Harper CR, Edwards MJ, DeFilipis AP, Jacobson TA (2006) Flaxseed oil increases the plasma concentrations of cardioprotective (n-3) fatty acids in humans. J Nutr 136:83–87
7. Ander BP, Weber AR, Rampersad PP, Gilchrist JS et al (2004) Dietary flaxseed protects against ventricular fibrillation induced by ischemia–reperfusion in normal and hypercholesterolemic rabbits. J Nutr 134:3250–3256
8. Prasad K (2000) Flaxseed: a source of hypocholesterolemic and antiatherogenic agents. Drug News Perspect 13:99–104

9. Prasad K (2005) Hypocholesterolemic and antiatherosclerotic effect of flax lignan complex isolated from flaxseed. Atherosclerosis 179:269–275
10. Dupasquier CM, Weber AM, Ander BP, Rampersad P et al (2006) Effects of dietary flaxseed on vascular contractile function and atherosclerosis during prolonged hypercholesterolemia in rabbits. Am J Physiol Heart Circ Physiol 291:H2987–H2996
11. Zeng X, Moore TA, Newstead MW, Deng JC et al (2005) Interferon-inducible protein 10, but not monokine induced by gamma interferon, promotes protective type 1 immunity in murine *Klebsiella pneumoniae* pneumonia. Infect Immun 73(12):8226–8236
12. Greenberger MJ, Steriter RM, Kunkel SL, Danforth JM et al (1996) Neutralization of MIP-2 attenuates neutrophil recruitment and bacterial clearance in murine *Klebsiella pneumoniae*. J Infect Dis 173:159
13. Ohkawa H, Ohishi N, Yagi K (1979) Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95:351–358
14. Tsai WC, Strieter RM, Zisman DA, Wilkowski JM et al (1997) Nitric Oxide is required for effective innate immunity against *Klebsiella pneumoniae*. Infect Immun 65:1870–1875
15. Bergeron Y, Ouellet AM, Deslauriers M, Simard M et al (1998) Cytokine kinetics and other host factors in response to pneumococcal pulmonary infection in mice. Infect Immun 66:912–922
16. Thors VRSN, Ttir APR, Ttir HE, Einarsson II et al (2004) The effect of dietary fish oil on survival after infection with *Klebsiella pneumoniae* or *Streptococcus pneumoniae*. Scotl J Infect Dis 36:102–105
17. Rubin RH, Wilkinson RA, Xu L, Robinson DR (1989) Dietary marine lipid does not alter susceptibility of mice to pathogenic microorganisms. Prostaglandins 38:251–262
18. Barton RG, Wells CL, Carlson A, Singh R et al (1991) Dietary omega-3 fatty acids decrease mortality and Kupffer cell prostaglandin E2 production in a rat model of chronic sepsis. J Trauma 31:768–773
19. Rayon JI, Carver JD, Wyble LE, Wiener D et al (1997) The fatty acid composition of maternal diet affects lung prostaglandin E2 levels and survival from group B Streptococcal sepsis in neonatal rat pups. J Nutr 127:1989–1992
20. Chang HR, Arsenijevic D, Pechere JC, Piguet PF et al (1992) Dietary supplementation with fish oil enhances in vivo synthesis of tumor necrosis factor. Immunol Letts 54:13–18
21. Fritsche KL, Shahbazian LM, Feng C, Berg JN (1997) Dietary fish oil reduces survival and impairs bacterial clearance in C3H/Hen mice challenged with *Listeria monocytogenes*. Clin Sci (Lond) 92:95–101
22. de Pablo MA, Puertollano MA, Galves A, Ortega E et al (2000) Determination of natural resistance of mice fed dietary lipids to experimental infection by *Listeria monocytogenes*. FEMS Immunol Med Microbiol 27:127–133
23. Anderson M, Fritsche KL (2002) (n-3) Fatty acids and infectious disease resistance. J Nutr 132:3566–3576
24. Moreland JG, Bailey G (2006) Neutrophil transendothelial migration in vitro to *Streptococcus pneumoniae* is pneumolysin dependent. Am J Physiol Lung Cell Mol Physiol 290:L833–L840
25. Malley R, Henneke P, Morse SC, Cieslewicz MJ et al (2003) Recognition of pneumolysin by Toll-like receptor 4 confers resistance to pneumococcal infections. Proc Natl Acad Sci USA 100:1966–1971
26. Bedirli A, Sozuer EM, Saraymen R, Ersoz N, Kilic E (2002) Protective effect of nitric oxide in lung injury associated with ceruleininduced acute pancreatitis. Turk J Med Sci 32:463–468
27. Braun JS, Novak R, Gao G, Murray PJ, Shenep JL (1999) Pneumolysin, a protein toxin of Streptococcus pneumoniae, induces nitric oxide production from macrophages. Infect Immunol 67(8):3750–3756
28. Serhan CN, Savill J (2005) Resolution of inflammation: the beginning programs the end. Nat Immun 6:1191–1197
29. Lokesh BR, Sayers TJ, Kinsella JE (1990) IL-1 and TNF synthesis by mouse peritoneal macrophages is enhanced by dietary n-3 polyunsaturated fatty acids. Immunol Lett 23:281–286
30. Petursdottir DH, Olafsdottir I, Hardardottir I (2002) Dietary fish oil increases tumor necrosis factor secretion but decreases interleukin-10 secretion by murine peritoneal macrophages. J Nut 132:3740–3743
31. Mackay F, Loetscher H, Stueber D, Gehr G, Lesslauer W (1993) Tumor necrosis factor α (TNF-α)-induced cell adhesion to human endothe-lial cells is under dominant control of one TNF receptor type, TNF-R55. J Exp Med 177:1277–1286
32. Neumann B, Machleidt T, Lifka A, Pfeffer K et al (1996) Crucial role of 55-kilodalton TNF receptor in TNF-induced adhesion molecule expression and leukocyte organ infiltration. J Immunol 156:1587–1593
33. Zwijnenburg PJG, van der Poll T, Florquin S, Roord JJ, Marceline van Furth A (2003) Interleukin-10 negatively regulates local cytokine and chemokine production but does not influence antibacterial host defense during murine pneumococcal meningitis. Infect Immun 71(4):2276–2279