

EFFECT OF OMEGA3+VITAMIN E ON EXPERIMENTAL INFECTION OF LABORATORY MICE WITH *PSEUDOMONAS AERUGINOSA* AND *KLEBSIELLA PNEUMONIAE*

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ABSTRACT

In present study, 40 adult male mice were used and divided into 5 equal groups of 8 mice each. The first group was the control group, in which the mice were fed with standard ration along the period of experiment; the second and third groups were fed on fats free ration for 14 days while the fourth and fifth groups were fed on Omega3+Vitamin E supplemented ration for 14 days too. The test organisms were suspended in phosphate buffer solution (PBS) and 10-folds serial dilutions were done for (CFU) count. Then, the animals of the second and fourth groups were injected intramuscularly with 22×10^5 (CFU) of *Pseudomonas aeruginosa* bacteria, while the animals of the third and fifth groups were injected intramuscularly with 18×10^6 (CFU) of *Klebsiella pneumoniae* bacteria. After 24 hours all mice of the third group and 3 mice of the fifth group died; their thigh muscles, livers, lungs, spleens and stomachs were taken, weighed and homogenized with phosphate buffer solution (PBS) to count the (CFU) in these organs. 2 days later, the remainder 5 mice of the fifth group were euthanized and the previous processes were done. For the second and fourth groups, after 48 hours of the bacterial injection all mice of the second group and 4 mice of the fourth group died, the previous processes were done for the bacterial count. 2 days later the remainder 4 mice of the fourth group were euthanized for the bacterial count purpose. The results revealed that the use of Omega3+Vitamin E as a supplements to ration increased the survival of mice and the organs bacterial count of the Omega3+Vitamin E fed mice decreased significantly as compared to those of the fats free fed animals at ($P \leq 0.05$).

INTRODUCTION

Pseudomonas aeruginosa represents important problems as an opportunistic pathogen in medicine and veterinary medicine (1). *Klebsiella pneumoniae* is one of the pathogens cultured from samples from infected hospitalized patients and is a serious complication in patients with malignancies (2). Regarding host resistance against *P. aeruginosa* in mice, genetic- and age-related resistance have been reported (3, 4). Most adult mice exhibit a certain degree of resistance to *P. aeruginosa*. Accordingly, a high challenge dose must be used in infection experiments unless virulence-enhancing substances are used (5). Omega-3 is reported to have anti-inflammatory and immunomodulatory activities (6, 7, and 8). Most of the biological activity of these fatty acids is thought to arise from their ability to antagonize the conversion of arachidonic acid to a family of lipid mediators, known as eicosanoids, and to diminish the production of proinflammatory cytokines (9 and 10). Vitamin E are important in host antioxidant defense and immune function. Vitamin E is a potent peroxyl radical scavenger that prevents lipid peroxidation (11) and is found in high concentrations in immune cells (12). Deficiency in vitamin E is associated with increased oxidative stress (13) and impaired immune function, including both humoral and cell-mediated immunity, phagocyte function, and lymphocyte proliferation (14). Age-related declines in immune function can be restored by vitamin E supplementation (15).

MATERIALS AND METHODS

Experimental animals and diets

Animals and diets: 40 male BALB/c mice (10 weeks old) were purchased from the animal house of the college of veterinary medicine, University of Baghdad. Animals received a fat-free diet -without fat addition- which was purchased from the college of agriculture, university of Baghdad for 1 week, and then they were divided into five groups of 8 mice each as follows:

1. Control group, received standard ration with 3.5% of soy oil (normal fat content).
2. Second group, received standard ration with 3.5% of soy oil (normal fat content).
3. Third group received standard ration with 3.5% of soy oil (normal fat content).

4. Fourth group, received standard ration supplemented by omega-3+ vitamin E.

5. Fifth group, received standard ration supplemented by omega-3+ vitamin E.

Each group received one of the following diets: (i) 3.5% of soy oil (normal fat content: control, second and third group), and (ii) (omega-3, content 1.30%, + vitamin E (50 mg/kg of D- α -tocopherol acetate)). Food pellets were changed daily to preserve lipid content and properties.

Challenge experiments

After 5 weeks on the fat-specific diets, mice of the second and fourth groups were gently injected intramuscularly with 22×10^5 (CFU) of *Pseudomonas aeruginosa* bacteria (Provided by Al-Muthanna General Hospital). Thigh muscle, lung, kidney, liver and spleen were removed, weighed and homogenized in sterile PBS. The number of viable bacteria present in the organs was determined by plating on Cetrimide agar plates. The results were expressed as number of CFU/g of tissue.

The mice of the third and fifth groups were gently injected with 18×10^6 (CFU) of *Klebsiella pneumoniae* (Provided by Al-Muthanna General Hospital) . Thigh muscle, lung, kidney, liver and spleen were removed, weighed and homogenized in sterile PBS. The number of viable bacteria present in the organs was determined by plating on agar (BHI). The results were expressed as number of CFU/g of tissue. Plates were incubated at 37 C° for 24 hours and CFU were scored.

Cetrimide Agar Preparation

The medium containing 0.03 per cent. cetrimide was prepared in the manner described by Brown and Lowbury (1965). The basal medium consisted of Proteose peptone, 20 g; New Zealand agar, 15 g; glycerol, 10 g; distilled water, 1000 ml. It was adjusted to pH 7.2 and autoclaved for 15 min. at 121°C. The following ingredients were added to 100 ml of the melted base: 1 ml. of a 15 per cent solution of K₂HPO₄ (anhydrous) and 1 ml of a 15 per cent. Solution of MgSO₄; these solutions were prepared with distilled water and Seitz-filtered. A 2 per cent. Seitz-filtered solution of cetrimide was added to the basal medium to give a final concentration of 0.03 per cent.

Statistical analysis

The results of this study were statistically analyzed to find the significant differences among the treated groups by the use of (t) test at probability of ($P \leq 0.05$), using SPSS analysis program version 19.

RESULTS AND DISCUSSION

The results showed that the use of Omega-3+vitamin E as a supplementations to the ration of mice has increased the number and ratio of survived mice after they were injected intramuscularly with 22×10^5 (CFU) of *Pseudomonas aeruginosa* bacteria (fourth group) as compared to those fed with Omega-3+vitamin E free ration (second group) where all of those mice died as it's shown in table (1). The results also showed that the use of Omega-3+vitamin E as a supplementations to the ration of mice has increased the number and ratio of survived mice after they were injected intramuscularly with 18×10^6 (CFU) of *Klebsiella pneumoniae* bacteria (fifth group) as compared to those fed with Omega-3+vitamin E free ration (third group) where all of those mice died as it's shown in table (2).

The results also revealed that the numbers of the cultured bacteria which were isolated from different organs of the animals were greater significantly in the groups that did not receive Omega-3+vitamin E than those which received Omega-3+vitamin E as a supplementations in their ration in both cases of either *Pseudomonas aeruginosa* or *Klebsiella pneumonia* injections as it is clear from tables (3) and (4) respectively.

The role of Omega-3 + in decreasing the numbers of bacteria in the vital organs as it was shown in tables and hence to increase the numbers of survived animals can be explained as the role of this poly unsaturated fatty acid in immunity. Macrophages have classically been recognized as scavenger cells possessing phagocytic and intracellular digestive properties. The secretory function of macrophages however, maybe as important as their phagocytic capacity (16). For example, depending on the nature of the stimulus and the local pulmonary environment, the alveolar macrophage can be triggered to selectively release arachidonic acid AA (20:4 6) (17). Free AA thus becomes the substrate for the COX and 5-LO enzyme cascades that convert 20:4 6 to a complex array of prostanoids and

leukotrienes. These eicosanoids may contribute to the immunoregulatory (17). Eicosanoids are responsible for many of the effects found in acute inflammation and host defense functions exercised by the alveolar macrophage(18). Some studies demonstrated that early exposure to Omega-3 during the fetal and neonatal period has a prolonged impact on immune responses and T-cell cytokine profiles (19). Omega-3 fatty acids mainly act as eicosapentaenoic acids (EPA) and docosahexaenoic acids (DHA), both had anti-inflammatory effects. EPA and DHA reduce the release of arachidonic acid-derived pro-inflammatory eicosanoids, and generate a group of lipid mediators called resolvins (E- and D-series) and protectins with potent anti-inflammatory and inflammation resolution properties (20). It is also mentioned that Omega-3 causes increase in macrophage infiltration and consistently higher serum levels of mRNA expression of anti-inflammatory cytokines and this is might increase the survival of mice after being challenged with bacteria (21).

The role of vitamin E in improving the immunity of the host animals against infections was widely established. Studies mentioned that vitamin E helps elevate the titer of immunoglobulins especially IgM and IgG in chicken, sheep and mice (22 and 23). Vitamin E showed a significant impact on gene expression profiles of T cells in few different categories of genes, including those involved in cell cycle and Th1/Th2 balance. Vitamin E increased expression of cyclin B2, Cdc2, and Cdc6 in old T cells, which might contribute to the increased ability of T cells from the old mice to progress through cell division and proliferate, Vitamin E also, had a significant impact on expression of genes involved with a Th1/Th2 balance. Vitamin E increased gene expressions of IL-2 and IL-1 receptor antagonist, and decreased the expression of IL-4, a major Th2 cytokine and stimulator of Th2 response. IL-1 receptor antagonist was reported to play a role in up-regulation of Th1 response (24). In conclusion, studies have demonstrated an improvement in immune response in animals and humans supplemented with more than the recommended level of vitamin E. This enhancement of immune response is associated with increased resistance to infectious diseases in animal models. This immunoregulatory effect of vitamin E is mediated indirectly by a reduced production of suppressive factors such as PGE2 by macrophages and directly by an increase in cell division capacity and IL-2 production by naïve T cells and by the changes in expression of genes related to cell cycle and Th1/Th2 balance of T cells (25).

Table (1). Represents the effect of omega-3+vitaminE on mice survival after being challenged with *Pseudomonas aeruginosa*.

Groups	Total mice number	Number of died mice	Number of survived mice	Ratio of survived mice %
Control	8	0	8	100
Second	8	8	0	0
Fourth	8	4	4	50

Table (2). Represents the effect of omega-3+vitaminE on mice survival after being challenged with *Klebsiella pneumonia*.

Groups	Total mice number	Number of died mice	Number of survived mice	Ratio of survived mice %
Control	8	0	8	100
Third	8	8	0	0
Fifth	8	3	5	62.5

Table (3). The effect of omega-3+vitamin E on the numbers of cultured *Klebsiella pneumonia* bacteria isolated from different organs. The numbers represent the means. The letters on the numbers refer to the significant difference among groups at ($P \leq 0.05$) (vertical comparison).

Groups	Organs					
	Muscle	Lung	Kidney	Liver	Spleen	Stomach
Control	0 ^C	0 ^C	0 ^C	0 ^C	0 ^C	0 ^C
Klebs.(2 nd group)	35×10 ⁷ ^A	20×10 ⁷ ^A	30×10 ⁷ ^A	8×10 ⁷ ^A	19×10 ⁷ ^A	17×10 ⁷ ^A
Klebs+Ome g (4 th group)	57×10 ⁶ ^B	12×10 ⁶ ^B	17×10 ⁶ ^B	32×10 ⁶ ^B	41×10 ⁶ ^B	36×10 ⁶ ^B
LSD	2.18	547.03	670.46	327.72	516.45	488.84

Table (4). The effect of omega-3+vitamin E on the numbers of cultured *Pseudomonas aeruginosa* bacteria isolated from different organs. The numbers represent the means. The letters on the numbers refer to the significant difference among groups at ($P \leq 0.05$) (vertical comparison).

Groups	Organs					
	Muscle	Lung	kidney	Liver	Spleen	Stomach
Control	C 0	C 0	C 0	C 0	C 0	C 0
Pseudo.(3 rd group)	A 27×10^7	A 22×10^7	A 20×10^7	A 30×10^7	A 33×10^7	A 29×10^7
Pseud+Omega (5 th group)	B 31×10^6	B 14×10^6	B 12×10^6	B 29×10^6	B 33×10^6	B 30×10^6
L.S.D.	1854.32	1693.06	1615.66	1962.24	6.46	1926.51

تأثير الأوميغا - 3 وفيتامين هـ على أصابة الفئران المختبرية التجريبية بجراثيم

Klebsiella pneumoniae و *Pseudomonas aeruginosa*

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الخلاصة

أستخدم في هذه الدراسة 40 ذكرا بالغا من الفئران المختبرية وقسمت إلى خمس مجاميع متساوية بواقع ثمانية فئران لكل مجموعة. مثلت المجموعة الأولى مجموعة السيطرة والتي غذيت فئرانها على عليقة قياسية طويلة فترة التجربة، المجموعتين الثانية والثالثة غذيت فئرانها على عليقة خالية من الدهون لمدة 14 يوما بينما المجموعتين الرابعة والخامسة غذيت فئرانها على عليقة مدعمة بفيتامين هـ وأوميغا-3 لمدة 14 يوما ايضا.

تم تعليق جراثيم الاختبار في محلول فوسفات بفر وتم عمل 10 تخافيف متسلسلة لعد الوحدات المكونة للمستعمرات (CFU). ثم تم حقن فئران المجموعتين الثانية والرابعة داخل عظمة الفخذ بـ 22×10^5 (CFU) من جراثيم *Pseudomonas aeruginosa* ، بينما فئران المجموعتين الثالثة والخامسة تم حقنها داخل عظمة الفخذ بـ 18×10^6 (CFU) من جراثيم *Klebsiella pneumoniae* . بعد 24 ساعة نفقت جميع فئران المجموعة الثالثة و 3 فئران من المجموعة الخامسة، وتم أخذ عظامات أفخاذها، الأكباد، الرئى، الطحال والمعدة وتم مزجها مع محلول فوسفات بفر لعد الخلايا الجرثومية في هذه الاعضاء. بعد يومين، الفئران الخمس المتبقية

من المجموعة الخامسة تم قتلها وطبقت عليها العملية السابقة. بالنسبة للمجموعتين الرابعة والثانية فبعد 48 ساعة من الحقن الجرثومي نفقت جميع فئران المجموعة الثانية وأربعة من فئران المجموعة الرابعة، وتم إجراء العمليات سابقة الذكر لغرض العد الجرثومي. بعد يومين، الفئران الأربع المتبقية من المجموعة الرابعة تم قتلها لغرض العد الجرثومي في الاعضاء.

أظهرت نتائج هذه الدراسة أن استخدام فيتامين هـ و أوميغا-3 كمكملات لعليقة الفئران قد سبب زيادة في عدد الفئران التي بقت على قيد الحياة وسبب أيضا انخفاض معنوي في العد الجرثومي في أعضاء الفئران التي غذيت على فيتامين هـ وأوميغا-3 مقارنة مع تلك التي غذيت على عليقة خالية من فيتامين هـ وأوميغا-3 عند مستوى احتمال ($P \leq 0.05$).

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