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Role of Hyperlipidemia and Sensitization in Airway Inflammation in the Guinea-Pig

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Abstract: The prevalence of asthma has increased in many countries, several hypotheses have been raised; among them are changes in the dietary pattern, particularly the increased consumption of unsaturated fatty acids. Epidemiological studies have suggested that the dietary pattern may be associated with asthma. On evidence, this study was done examining the effect of changes in blood lipoprotein profile on manifestation of asthma and pulmonary inflammation in an animal model. 32 guinea pigs were randomly categorized into four groups: control, allergic, hyperlipidemic, hyperlipidemic-allergic groups. Hyperlipidemia was induced with a regimen enriched with 15% corn oil and 1.6% cholesterol, Sensitization was done by intraperitoneal (i.p.) injection of ovalbumin, 100 μg per animal and aluminium hydroxide 100 mg on day 1, with the addition of a booster injection of the same solution on day 5. 17 days after 1st injection, aerosol inhalation was done and blood samples were taken then animals were euthanized with CO_2 gas and BAL was performed. Fatty diet in the present study increased plasma levels of cholesterol, LDL and VLDL but not that of HDL. Although not reaching the level of statistical significance, allergy *per se* was shown to increase HDL levels tremendously. Interestingly, a significant synergy between allergy and the fatty diet, led to a significant elevation of the HDL levels, a sign reported in asthmatic subjects as well. OVA sensitization increases the number of eosinophils and neutrophils, recovered from the BALF. Hyperlipidemia does not lead to cell infiltration in the respiratory system of either healthy or allergic guinea-pigs.

Key words: Asthma · Hyperlipidemia · Inflammation · Guinea pig

INTRODUCTION

The prevalence of asthma has increased in many developed and developing countries in the past decades. This phenomenon is most likely the result of complex interactions between multiple environmental and genetic factors [1, 2]. Several hypotheses have been raised; among them are the changes in the dietary pattern, particularly the increased consumption of unsaturated fatty acids [3, 4].

Since 1968 several studies were done to determine the relationship between asthma and serum lipoproteins levels in patients with asthma [5-8].

Epidemiological studies have suggested that the dietary pattern may be associated with the prevalence of asthma. Yeh and colleagues reported that an increased intake of foods of animal origin was associated with the occurrence of allergic rhinitis and asthma in adolescents. They also demonstrated that supplementing cholesterol in the diet aggravated antigen-induced pulmonary inflammation in murine model of asthma [9]. In a cross-sectional survey in Taiwan, the prevalence of allergic rhinitis and asthma in adolescents was associated with the intake frequency of several food items, including liver, deep-fried foods and meat [10]. The association of hypercholesterolemia and obesity with airway hyperresponsiveness has drawn increasing attention to the potential role of cholesterol and lipid homeostasis in lung physiology and in chronic pulmonary diseases such as asthma [11].

Dysregulation of cholesterol homeostasis may be important in the pathogenesis of diseases such as asthma. Regarding presence of evidences about relation between plasma levels of lipoproteins and asthma, this study aimed

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at examining the effect of changes in blood lipoprotein profile on manifestation of asthma, pulmonary inflammation in the animal model.

MATERIAL AND METHODS

Animals: Thirty two male Dunkin-Hartley guinea pigs (250- 350 g) were purchased from Pasteur institute of Iran (Iran, Tehran) and acclimatized in the animal house (12-hour light/dark periods, temperature of 22-25°C) for 5 days and received a regular chow diet. Animal welfare and experimental procedures were undertaken in accordance with the Animal Scientific Procedures Act 1986 under Home Office personal and project licenses. After acclimation period, guinea pigs were randomly categorized into four groups of eight animals each as follows: (1) control, (2) allergic, (3) hyperlipidemic, (4) hyperlipidemic-allergic groups.

Induction of Hyperlipidemia and Ovalbumine Sensitization: Hyperlipidemia was induced with a regimen enriched with 15% corn oil and 1.6% cholesterol which was fed to guinea pigs of hyprlipidemic and hyperlipidemic-allergic groups for 1 week. Occurrence of hyperlipidemia after one week was confirmed by measuring blood lipoproteins level. Sensitization was done in allergic and hyperlipidemic-allergic groups by intraperitoneal (i.p.) injection of ovalbumin (Merck, Germany), 100 μg per animal and aluminium hydroxide (Loba Chemie, India) 100 mg, in 3 ml of normal saline, in divided doses bilaterally. This was administered as an injection on day 1, with the addition of a booster injection of the same solution on day 5. 17 days after the first sensitization injection, sensitized animals were placed in clear plastic chamber and exposed to a nebulized solution of OA (10 μg in saline), generated by a nebulizer(Shinmed, Taiwan) supplied with air at a pressure of 20 psi at 0.3 ml min-1 for 1 hour. Respiratory inflammation was measured post challenge. Control groups of animals received injections by the same protocols but without ovalbumin and, were exposed to aerosolized saline.

Bronchoalveolar Lavage: After aerosol inhalation, 17 days post first sensitization injection, blood samples were taken from the hearts of guinea pigs and then animals were euthanized with CO_2 gas. BAL was performed with 10 ml normal saline solution. The lavage was centrifuged at 300 *g* for 10 min. Supernatants of centrifuged lavages were removed for protein measurement and sediments were kept for total count and differential count of BAL cells. Protein measurement was done by a biophotometer (Eppendorf, Germany). Viability of BAL cells was done by 0.4% trypan blue dye and observation with a light microscope.

Blood Lipoprotein Measurement: Total serum cholesterol, LDL, HDL, VLDL and triglyceride concentrations in plasma were measured by colorimetric procedure with an autoanalizer (BSM, China).

Chemicals and Drugs: The followings were the main materials used in this study: ovalbumine (Merck, Germany), aluminium hydroxide (Loba Chemie, India), cholesterol (Loba Chemie, India), trypan blue (Merck, Darmstadt, Germany).

Statistical Analysis: Data were expressed as mean ± SEM. Means of 4 experimental groups were compared by 1 way analysis of variance (ANOVA) and then group by group by the *post hoc* test of *Bonferroni's t test*, where permitted. $P<0.05$ was considered to be statistically significant.

RESULTS

Blood Lipoproteins Profile: Feeding diet enriched with 15% corn oil and 1.6% cholesterol induced hyperlipidemia in guinea pigs as shown by the increased levels of cholesterol, triglyceride, LDL and VLDL compared to control animals (Figure 1). Antigen sensitization did not affect blood lipoproteins levels. Level of HDL in hyperlipidemic-allergic group was statistically significant in comparison with groups of control and hyperlipidemic (Figure 1).

BALF Cells-Eosinophils: Difference between number of eosinophils in control ($124.2\pm33.6\times10^3$/ml) and hyperlipidemic ($126.6\pm30.2\times10^3$/ml) groups was not statistically significant whereas number of eosinophils in allergic group ($464.2\pm62.4\times10^3$/ml) reaches approximately 273.7% higher and hyperlipidemic-allergic group ($509.2\pm30.2\times10^3$/ml) increases 309.9%, these changes in comparison to control group were statistically significant ($P<0.001$) (Figure 2A).

BALF Cells-Macrophages: Number of macrophages in allergic ($264.4\pm38.1\times10^3$/ml) and hyperlipidemic-allergic ($289.2\pm23.4\times10^3$/ml) groups was higher in comparison to control ($172.8\pm30.8\times10^3$/ml) and hyperlipidemic ($174.4\pm17.9\times10^3$/ml)groups, ($P<0.05$) but difference between groups was not statistically significant (Figure 2B).

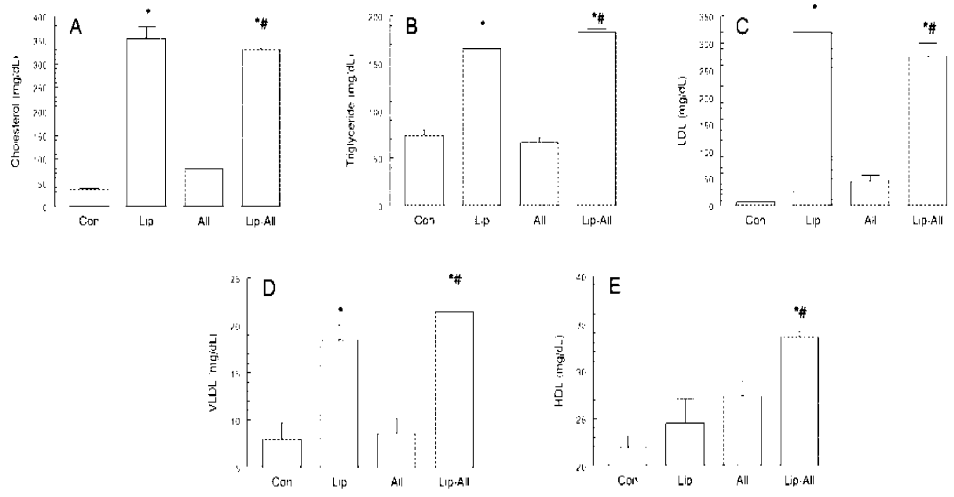

Fig. 1: Blood lipid concentrations (mg/dL) of control (Con), hyperlipidemic (Lip), allergic (All) and hyperlipidemic plus allergic (Lip-All) guinea-pigs. Figures show the levels of cholesterol (A), triglyceride (B), LDL (C), VLDL (D) and HDL (E). * P<0.05 compared to the control animals; # P<0.05 compared to the allergic animals.

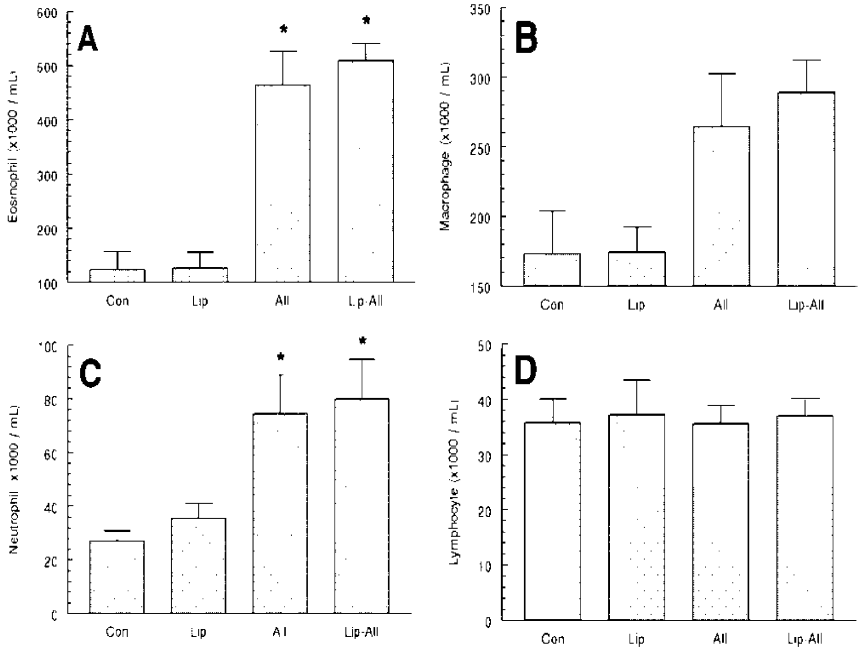

Fig. 2: Cells recovered from the BALF (×1000/mL) of control (Con), hyperlipidemic (Lip), allergic (All) and hyperlipidemic plus allergic (Lip-All) guinea-pigs. Figures show the numbers of eosinophils (A), macrophages (B), neutrophils (C) and lymphocytes (D). * P<0.05 compared to the control animals; # P<0.05 compared to the allergic animals.

BALF Cells-Neutrophils: Number of neutrophils in allergic (74.4±14.5×10^3/ml) and hyperlipidemic-allergic (80±14.7×10^3/ml) groups in comparison to control (27±4×10^3/ml) group was higher and statistically significant (P<0.001). Number of neutrophils in hyperlipidemic group was (35.6±5.3×10^3/ml) (Figure 2C).

BALF Cells-Lymphocyte: Number of lymphocytes in different groups did not show significant changes (P=0.99) (Figure 2D).

BALF Protein: Protein measurement showed significant increase in hyperlipidemic, allergic and hyperlipidemic-allergic groups in comparison to control animals.

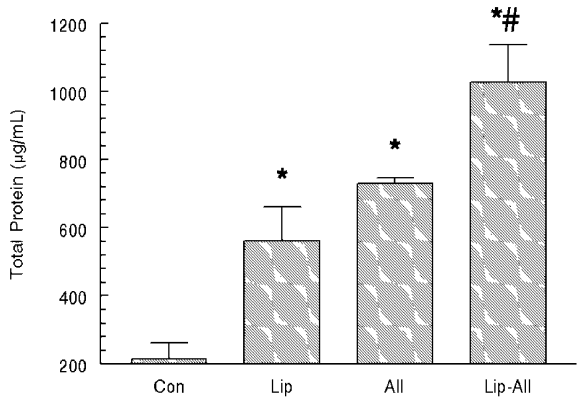

Fig. 3: Total protein in the BALF (µg/mL) of control (Con), hyperlipidemic (Lip), allergic (All) and hyperlipidemic plus allergic (Lip-All) guinea-pigs. * P<0.05 compared to the control animals; # P<0.05 compared to the allergic animals.

Difference between hyperlipidemic-allergic animals and hyperlipidemic animals was statistically significant ($p<0.001$) (Figure 3).

Viability of Cells: There was no significant difference in viability of cells in different groups of guinea pigs.

DISCUSSION

Present study showed that adding 1.6% cholesterol and 15% corn oil to guinea pigs regular diet caused significant increase in cholesterol, LDL, VLDL and triglyceride levels in plasma. It was in line with findings of Chapman and Mills (1997), which showed that such a diet caused hyperlipidemia in guinea pigs [12]. In this model, OVA sensitization had no significant effects on plasma lipoproteins except an increased HDL levels. Yeh and Huang reported that in murine model, OVA sensitization and challenge did not affect serum cholesterol, a finding in agreement with present study [9]. In coordination with our findings, Shenoi *et al* (1992) showed that serum concentrations of HDL were significantly higher in children with respiratory allergy and those of total cholesterol were lower in children with respiratory allergy when compared to controls [8]. Although not reaching the level of statistical significance, allergy *per se* was shown to increase HDL levels tremendously. Interestingly, a significant synergy between allergy and the fatty diet, led to a significant elevation of the HDL levels, a sign reported in asthmatic subjects as well.

There are evidence that an association exists between serum cholesterol and asthma, but little is known about underlying mechanism. Nagel and colleagues (2009) found that high apolipoprotein A-I is associated with the manifestations of asthma and atopy. Apolipoprotein A-I is the primary protein constituent of HDL-cholesterol [13]. Chilton and coworkers (2008) suggested that the dramatic increase in the ingestion of saturated and n-6 fatty acids and concomitant decrease in n-3 fatty acids are thought to be major drivers of the increase in the incidence of inflammatory diseases such as asthma, allergy and atherosclerosis [14]. Moreover, Al-Shawwa and co-workers (2006) found that hypercholesterolemia is associated with increased frequency of asthma even when other potential confounding variables such as age, gender and obesity were controlled [15].

OVA sensitization caused significant increase in the number of eosinophils and neutrophils and a slight increase in the number of macrophages, obtained from the guinea-pig BALF. In common with these findings, Underwood *et al.* (1995) demonstrated that number of eosinophils macrophages and neutrophils, but not lymphocytes, increased significantly after antigen challenge [16]. Vries *et al.* (2006) reported that the percentage of eosinophils increased in OVA-sensitized animals as compared with non-sensitized animals [17]. Westerhof *et al.* (2001) reported that neutrophil and eosinophil numbers in sensitized guinea pigs increased in different airway compartments after ovalbumin inhalation [18]. Also, Verbout *et al.* (2007) described that antigen challenge in sensitized guinea pigs enhanced the number of eosinophils in lavage fluid significantly [19]. In agreement with our results, several studies have demonstrated increased neutrophil numbers in BAL fluid from asthmatics and from guinea pigs after allergen challenge [20-22]. Moreover, there are increasing indications that neutrophils may play a role in the pathogenesis of acute severe asthma. In common with other workers [23-25], we observed no significant change in the number of lymphocytes recovered from the BALF of guinea-pig lungs after antigen challenge.

Hyperlipidemia does not lead to cell infiltration in the respiratory system of either healthy or allergic guinea-pigs, however, Yeh and Huang (2001) showed that in mice fed a cholesterol diet numbers of eosinophils, neutrophils and lymphocytes were all significantly higher than in OVA-challenged mice fed a control diet, number of eosinophils in BALF were 8 and 4 fold higher in mice fed cholesterol [9]. Species differences as well as the protocol specifications could account for different findings of these two studies. In present study, hyperlipidemia did not cause a significant change in inflammatory

manifestation of asthma. Mechanism by which hyperlipidemia can aggravate asthma may be other than just pulmonary inflammation.

As expected, allergy caused a protein leakag from blood into the alveolar spaces as measured by the level of total protein in the BALF. Interestingly, the fatty diet had a similar effect in the animal model of our study. Combination of allergy and the fatty diet caused an additive effect, i.e. the protein leakage was more profound in the allergic-hyperlipidemic group than the healthy, allergic and hyperlipidemic guinea-pigs. Microvascular leakage and due to it, increase in protein content of BALF, may be another factor which causes increase in incidence of asthma in hyperlipidemic individuals. Determining the exact relation between asthma and hyperlipidemia and the underlying mechanism needs more investigations.

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