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The Impact of Concurrency of Colchicine and Simvastatin on Variation of Serum Immunoglobulins IgG, IgE, IgM and IgA in Mustard Gas-Wounded Patients

Ali Emad* and Ali Koushki

Department of Internal Medicine, Section of Pulmonary Diseases,
Shiraz University of Medical Sciences, Shiraz, Iran.

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Mustard gas as a chemical weapon causes devastating effects on different tissues of the body and there is no perfect antidote against it. This gas is a very strong and non-specific alkaline agent that is capable of causing inflammation as well as increase of immunoglobulins in the patients. This gas causes irreparable and sever damage to all the body tissues; so finding of effective medicines to treat the mustard gas-exposed patients is of great importance and is the main purpose of this study. This study was conducted as double-blind intervention during 2 months. The population society was consisted of 40 mustard gas-wounded patients. Their illness had previously been approved by Foundation of Oppressed and the Crippled and other valid government agencies. These patients were referred as OPD for treatment to pulmonary clinic related to Shiraz University of Medical Sciences (Motahari, Imam Reza and Shahid Faghihi Clinics). The studied patients in this study were divided into two groups: 1- The placebo group that included 20 patients who did not receive colchicine and simvastatin. 2- The medication group included 20 patients who received simultaneously colchicine and simvastatin with daily dosage of 1 mg/d and 10 mg/d, respectively. Then blood samples were obtained from the under studied patients and the concentration of serum immunoglobulins IgG, IgE, IgM and IgA was measured by PFT method and using Prestige 24i automate analyzer device in before and after the beginning of the treatment. The results showed that there is no significant relationship between the concentrations of IgG, IgE, IgM and IgA before and after receiving the medicines and placebo (p value: 1). The obtained results from this study indicates that both colchicine and simvastatin are not effective in patients with mustard gas toxicity and thereby it will be possible to cut the treatment with both medicines in these patients and to reduce by this way the costs related to the treatment of these patients and to ignore the continues of an useless treatment.

Key words: Colchicine, Simvastatin, Immunoglobulin, Mustard Gas.

Chemical warfare modern weaponries were built and used for the first time especially by Germany during the World War I. These weaponries began by throwing acid and simple diffusion of lethal gases such as chlorine. They hope to be able latter to launch these materials to far distances in order to kill more enemy soldiers. Germany has been always the leader of this industry. Use of more than 124 thousand tons of chemicals during the First World War injured one million people and killed one hundred of soldiers from both side. After the end of the First World War and by ratification of 1925 Geneva Protocol by most countries of the world, the use of these weapons were banned[1]. One widely used chemical weapon is mustard gas. Mustard gas or sulfur mustard by chemical formula of 1,5-dichloro-3-thiapentane is a chemical compound containing of

* To whom all correspondence should be addressed.
Tel :+989177190079;
E-mail: lungdep@yahoo.com

chlorine and sulfur and has been synthesized for the first time by Despretz in 1822 [2]. Its density is 1.27 grams per liter, its melting point and boiling point is respectively 14.4°C and 217 °C[2, 3]. It was stated in studies that mustard gas was used for the first time in 1917 as a chemical weapon in the First World War. After this war, the broadest use of chemical weapons had been occurred in Iran-Iraq war[2, 3]. It has also been stated that this gas has long-term complications including blindness, infertility as well as different kinds of cancer and defects of organs before birth[1]. Exposure to very high doses of mustard gas, causes convulsion and in extreme cases leads to death in less than an hour[4]. Partly mustard gas poising was also reported in workers who worked in an ammunition factory during Iran-Iraq war, which has led to incidence of injury in their respiratory tracts[5]. Evidence indicates that mustard gas can lead to cellular and humoral immune dysfunctions[6]. Diyhimi *et al*. were studied 100 victims of chemical warfare during one year after the initial contact; although a premature rise and a slow decline to initial amount was observed in IgG and IgA levels, any classes of Immunoglobulin did not show variation outside the normal range[7]. Studies have also shown that often Immunoglobulin levels especially IgG and IgM are higher than normal range during the first week to six weeks after exposure to mustard gas[8]. It also indicted that suppression of cellular immunity in Iranian victims was recorded one year or two and three years after contact to the gas[9].

Colchicine medicine with chemical formula $C_{22}H_{25}NO_6$ is an anti-gout drug that pharmacologically classified as alkaloid from colchicum autumnale [10]. Studies indicated that colchicine has anti-inflammatory effects and this property makes possible its usage for improvement of diseases leading to infection [10]. It was stated in previous studies about evaluation of the therapeutic effects of colchicine on delayed lung injury caused by mustard gas in animal model that this medicine due to reduction of inflammation and oxidative stress induced by mustard gas can be effective limitedly in reduction of delayed lung complications caused by it[11].

Simvastatin by chemical formula of $C_{25}H_{38}O_5$ is a component of cardiovascular medicines and is pharmacologically a component of HMG-CoA reductase inhibitor[10]. Simvastatin is consumed as an adjunct drug along with diet in treatment of high blood cholesterol caused by LDL cholesterol in patients at risk of coronary artery disease and the patients that do not respond to diet therapy[10]. It was also stated that simvastatin has anti-inflammatory properties and does not have significant effect on improvement of pulmonary bronchiectasis caused by mustard gas[12].

According to the above, this study is aimed to evaluate the effect of colchicine and simvastatin on serum Immunoglobulin changes in mustard gas-exposed patients.

Methods

This study was conducted as double-blind intervention during 2 months. Before the beginning of the research, all rules and ethical issues approved by Islamic Countries, Iran Ministry of Health and Shiraz University of Medical Sciences were taken into consideration and after face to face explanation and completion of moral questionnaire; patients with complete knowledge and understanding were enrolled to the study. The population society was consisted of 40 mustard gas-wounded patients and their illness had previously been approved by Foundation of Oppressed and the Crippled and other valid government agencies and they have had chemical wounded patients' card. These patients were referred as OPD for treatment to pulmonary clinic related to Shiraz University of Medical Sciences (Motahari, Imam Reza and Shahid Faghihi Clinics).

The studied patients in this research were divided into two groups as follows:

1- The placebo group that included 20 patients who did not receive colchicine and simvastatin.

2- The medication group included 20 patients who received simultaneously colchicine and simvastatin with daily dosage of 1 mg/d and 10 mg/d, respectively.

It should be noted that patients and researchers were kept unaware of the name of the class and the type of taken medicine during the study period. The patients were prevented from taking new drugs and they were evaluated in terms of incidence of side effects of taking medicine during the verbal interviews and the medication was discontinued when it was required.

Blood sampling was taken twice (before

and after consumption of the medicines) from the patients' vein, so that 5 ml of the blood samples were prepared from each patient. The samples were then transferred into a centrifuge tube and after completion of coagulation in 4°C, centrifugation was carried out at 2500 × g for 10 min. Blood serum was separated and then the concentrations of immunoglobulins IgG, IgE, IgM and IgA were measured by PFT method and using Prestige 24i automate analyzer device in before and after the beginning of the treatment. The obtained data were analyzed using SPSS 16, and Mc Nemar test was used to investigate the relationship between Immunoglobulin before and after the intervention.

RESULTS

Twelve patients in the medication group had normal IgG before the intervention and no change was observed after the intervention and the same 12 patients had normal IgG. In the case of IgM, 16 patients were normal before the intervention and 14 patients were normal after the intervention. In the case of IgA, 15 and 16 patients were normal before and after the intervention, respectively. In the case of IgE, 14 and 14 patients were normal before and after the intervention (Table 1).

In the placebo group, 13 and 17 patients were respectively normal in the term of IgG before and after the intervention. In the case of IgM, 19 and 17 patients were respectively normal before and after the intervention. In the case of IgA, 14 and 15 patients were normal before and after the intervention, respectively. In the case of IgE, 15 and 17 patients were respectively normal before and after the intervention (Table 2).

No significant relationship was observed in the relationship between IgG before and after taking the medicine (p value: 1). This means that no significant change was observed in normal individuals in the terms of IgG and the medicines had no effect in this case.

As expected, no significant relationship was observed between IgG before and after taking the placebo (p value: 0.12).

No significant relationship was observed in the relationship between IgM before and after taking the medicine (p value: 0.62). This means that after taking the medicines no significant change was observed in normal individuals in the terms of IgM and the medicines had no effect in this case, too.

As expected, no significant relationship was observed between IgM before and after taking the placebo (p value: 0.5).

No significant relationship was observed in the relationship between IgA before and after taking the medicine (p value: 1). This means that after taking the medicines no significant change was observed in normal individuals in the terms of IgA and the medicines had also no effect in this case.

As expected, no significant relationship was observed between IgA before and after taking the placebo (p value: 1).

No significant relationship was observed in the relationship between IgE before and after taking the medicines (p value: 1). This means that after taking the medicines no significant change was observed in normal individuals in the terms of IgE and the medicines had also no effect in this case.

As expected, no significant relationship was observed between IgE before and after taking the placebo (p value: 0.5).

DISCUSSION

The results of serum immunoglobulins IgG, IgE, IgM and IgA in the placebo and the medication groups does not have significant change in this study, which shows the medicines were not effective in treating and reducing of inflammation.

It was stated in studies that mustard gas is an alkylating agent that is highly toxic for lymphatic system and bone marrow cells. It was also indicated that leukocytosis occurs in the first few days after exposure to mustard gas. Evaluation of bone marrow in 15 patients showed that the amount of cellularity and kariorrhexis reduces severely in progenitors of white and red blood cells, which are dependently effective on inflammatory responses and factors. It was also reported that severe leucopenia is a sign of incidence of secondary infections and inflammation and eventually death in patients[13].

Studies found that mustard gas poisoning causes disruption of cellular and humoral immune

Table 1. The frequency of individuals in terms of Immunoglobulins normality and abnormality before and after taking the medicines

Ig Levels	IgG before taking the medicines	IgG after taking the medicines	IgM before taking the medicines	IgM after taking the medicines	IgA after taking the medicines	IgA before taking the medicines	IgE before taking the medicines	IgE after taking the medicines
NL	12 (60%)	12 (60%)	16 (80%)	14 (70%)	15 (75%)	16 (80%)	14 (70%)	14 (70%)
abNL	8 (40%)	8 (40%)	4 (20%)	6 (30%)	5 (25%)	4 (20%)	6 (30%)	6 (30%)

Table 2. The frequency of individuals in terms of Immunoglobulins normality and abnormality before and after taking the placebo

Ig Levels	IgG before taking the medicines	IgG after taking the medicines	IgM before taking the medicines	IgM after taking the medicines	IgA after taking the medicines	IgA before taking the medicines	IgE before taking the medicines	IgE after taking the medicines
NL	13 (65%)	17 (85%)	19 (95%)	17 (85%)	14 (70%)	15 (75%)	15 (75%)	17 (85%)
abNL	7 (35%)	3 (15%)	1 (5%)	3 (15%)	6 (30%)	5 (25%)	5 (25%)	3 (15%)

system and causes incidence of various clinical signs in individuals, including increased level of Immunoglobulins IgG and IgM in the first weak to the sixth month, which is involved in enhancement of inflammation in respiratory tract[13]. It was also stated that even eight years after contact to the chemical, a significant percentage of patients have significantly increased levels of immunoglobulins IgG, IgE, IgM and IgA compared to the sham group[8]. Studies on 40 Iranian mustard gas-wounded patients with severe late toxicity with sulfur mustard showed that the level of IgM is still significantly higher than the sham group even in 16 to 20 years after contact. Cellular immune suppression in Iranian victims was reported one year to three years after contact[4].

So in people who are suffering from mustard gas, there is a relatively higher percentage of Immunoglobulins in their blood that indicates the occurrence of inflammatory reactions in these patients. This agrees with the result of the present study so that among studied patients some of them had abnormal Immunoglobulin but the majority of patients had normal Immunoglobulin before intervention of the medicines. This indicates that over time and after long period of exposure to sulfur mustard (30 years) it is possible that the concentration of Immunoglobulins returns to normal depending on the severity of the disease. This agrees with the results of this study, as expressed that a substantial increase occurs in the level of Immunoglobulins in the first week to 6th month of contact and eventually after 20 years of contact and then depending on the activity of the immune system of the patients may be reduced to normal. So, duration of exposure to mustard gas is very important in related changes to Immunoglobulins and it depends to overactive and underactive immune system in various people. Given to the results of this study, the number of patients who have abnormal IgG is proportionally more in the medication group (by intervention) and the placebo group. This can be considered as an assumption that the concentration of IgG in those patients changes more in inflammatory reactions. Further researches are recommended for this assumption.

Studies suggested that pulmonary complications in mustard gas-exposed patients is dependant to respiratory ducts inflammation and has immune source. These responses can be seen in chronic bronchitis, obstructive bronchitis, bronchiectasis, tracheoomalacia and tracheal obstruction. Any agent that could reduce inflammation and inflammatory Immunoglobulins in these patients can be used in recovery and treatment of the related complications to poisoning caused by mustard gas[14-16]. Other studies on the role of oxidative stress and inflammation caused by mustard gas in these patients stated that due to the proven role of oxidative stress in pathogenesis of inflammatory tissue[17], the possibility of involvement of oxidative stress and reactive oxygen species and deficiency of defensive antioxidants of the body against them are also raised in the case of the late injury caused by mustard gas due to stability of inflammatory process in lower respiratory tracts [18] and progressive nature of the disease. Therefore, medicines that can reduce the severity of oxidative stress and as a result reduces the intensity of inflammation can be effective to prevent progression of injury and/or to treat late complications in pulmonary injured patients.

Hothersall et al expressed that consumption of Statins causes a significant reduction in the number of inflammatory cells and factors in patients with respiratory diseases taking corticosteroid medicines. However, consumption of Statins does not improve the disease in short time, and long term use of these drugs is recommended[20]. Studies showed that Statins have anti-inflammatory effects by inhibiting G protein Isoprenylation small component and preventing peroxidation and oxidative stress. Also found that Statins are used due to inflammatory and immune modulating properties in asthma models and toxicity by mustard gas. However, Statins cannot completely improve the patients and long term of use of Statins is recommended[21-24]. Studies also expressed that colchicines is an anti-inflammatory drug that acts through binding to intra cellular protein called tubulin and as a result prevention from its polymerization with microtubules and prevention from migration of leukocytes and phagocytes [25]. Aside from that, colchicine is a treatment protocol component of idiopathic pulmonary fibrosis and is an inflammation reducer [26], as in performed studies on investigation of colchicines in improvement of inflammatory factors

and reduction of its concentration found that this medicine can reduce infiltration of lymphocytes and inflammatory factors[11], and in this study, it is also expected that inflammatory immunoglobulins can be reduced in patients taking this drug. But in this study, no significant change in the Immunoglobulins levels was observed compared to the placebo group after taking the medicines. The related results to pulmonary tests as well as the function and activity of the patients are a confirmation of ineffectiveness of the medicines in effective treatment, which is related to inflammatory changes. Emad et al in their studies on evaluation of 52 patients with bronchiectasis caused by mustard gas and investigation on anti-inflammatory effect of simvastatin in these patients concluded that no significant difference was observed between control and experimental groups after two months of treatment, and only the number of white blood cells and serum levels of Immunoglobulin IgM in the experimental group showed a significant reduction and in the control group, only Immunoglobulin IgM and IgG showed a significant reduction. It seems that the use of simvastatin in bronchiectasis caused by mustard gas does not have an important impact on the lung function tests and blood inflammatory markers[12]. This is consistent with the results of this study.

As the obtained results from this research showed, during a 2 months period no significant changes in the medication group was observed in inflammatory Immunoglobulins before and after the intervention compared to the placebo group. This shows that the medicines did not have any effect on variation of serum levels of immunoglobulins IgG, IgE, IgM and IgA. This is probably dependant to features of subjects, different stages of the disease as well as many other factors. According to the mentioned results, prescription of these two medicines is not recommended for improvement of inflammatory factors in mustard gas-exposed patients.

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