Iranian J Publ Health, Vol. 36, No.4, 2007, pp.87-90

Original Article

The Short-Term Effects of Sulfur Mustard Gas on the Complement System

*AH Keyhani [1], *MR Nowroozi [2]*

[1]Dept. of Immunology, Faculty of Medicine, Medical Sciences/University of Tehran, Iran
[2]Dept. of Urology, Faculty of Medicine, Medical Sciences/University of Tehran, Iran

(Received 3 Apr 2007; accepted 30 Oct 2007)

Abstract

Background: The serum levels of C_3, C4, and the total hemolytic activity of complement system (CH50) of patients exposed to mustard gas (MG) in the battlefield were measured in this study.

Methods: C3 and C4 were measured by single radial immunodifusion and CH50 was evaluated according to total hemolytic activity of the serum.

Results: The serum levels of C_3 showed a significant increase on day 3 after exposure to MG. The serum levels of C_3 in the sera of patients collected during 4-18 and 19-31 days after exposure to MG were also found to have increased significantly comparing to those of the controls. The levels of C_4 in the sera of patients collected from the patients during 4-18 and 19-31 days after exposure were also found to be significantly higher than those of the controls. However the increase in the serum levels of C_4 were not in the same magnitude as those of the C_3. The serum CH50, which is a measure of the total hemolytic activity of classical complement system also showed a significant increase in patients' sera on day 3 and in the sera collected during 4-18 days after exposure. However, the CH50 in the sera of patients showed a decrease in the sera collected during 19-31 days after exposure to MG.

Conclusion: Since C3 is an acute phase protein as well as a complement component, these results are discussed in terms of a vigorous acute phase response and activation of the complement system.

Keywords: *C_3, C_4, CH50, Mustard gas, Sulfur mustard, Iran*

Introduction

Mustard gas (MG) is one of the well-known chemical substances which have been used as a chemical warfare agent. The principal toxic component of MG is sulfur mustard. As an alkylating agent, sulfur mustard has an affinity for nucleophilic parts of other molecules, reacting with arginine, cysteine and lysine of proteins. It also reacts with nucleic acids in particular with DNA, altering their normal functions (1, 2).

In recent times, exposure of soldiers and civilians to MG occurred during Iraq-Iran conflict in 1985, when hundreds of soldier and civilians were exposed to MG by the Iraqi army.

The ill-effects of the MG in various organs and systems of the victims have previously been reported (3- 5).

The cellular and molecules elements of the immune system in particular showed considerable changes in exposed victims (6). In an investigation on immunoglobulin levels in patients who had been exposed to MG in the battlefield, it was found that the serum levels of IgG had decreased significantly on day 3 after exposure. However serum levels of IgG showed a significant increase by 19-31 d after exposure to MG (7). Humoral immune system produces its effector molecules, the specific antibodies which are structurally immunoglobulins, with a delay of a week or longer after stimulation since B cells which are the essential cellular elements of this system must undergo differentiation to become antibody producing plasma cells. The molecules of the complement system, on the other hand, are available

* **Corresponding author:** Tel: + 98 21 88632333, E-mail: Nowrooziclinic@yahoo.com

in normal concentrations in serum and tissues to be activated by various stimuli. Thus, the effector molecules of this system which contributes to inflammation such as C3a, C4a, opsonization such as C3b and membrane attack complex C5b-C9 are produced in a shorter period of time.
The present study was undertaken to find out, the short-term effects of MG on the complement system in patients exposed to MG in the battlefield. The serum levels of C3 and C4 are determined and the total hemolytic activity of serum (CH50) is measured during one month after exposure to MG.

Materials and Methods

The patients were soldiers, all male and mostly in their 3rd decade of life which had been exposed to MG in the battlefield in Iraq-Iran war during 1985, these victims had been transferred initially to a military hospital and very soon to a hospital in Tehran for special treatment. Aliquots of the venous blood samples which had been collected from these patients for routine hospital investigations were also used for determination of serum complement components C3, C4 and CH50 activity.
The control sera were obtained from male university students who were in the same age range as the patients.
The plates of single radial immunodiffusion (SRID) (Behring, Germany) were used for quantitative determination of the serum variables. The patient's sera were tested along and concurrently with the sera from the control for measurement of C3 and C4 in the SRID plates according to manufacturer protocols and recommendation.
Total hemolytic activity of the classical complement system (CH50) was determined as stated earlier (8). Fresh sheep RBC and rabbit anti sheep antiserum where obtained from Razi Institute, Karaj, Iran. All buffers for this test were made in our own laboratory. Patients and controls sera which had been kept frozen at -70ºC were thawed and used for this test. The reciprocal of the dilution of sera that lysed 50% of the sensitized sheep RBC were determined in one milliliter of each serum.
The *t*-Student test was employed using SPSS PC and Stat graphics. The $P< 0.05$ was considered significant.

Results

Complement components-the serum levels of C3 in patients on day 3, during 2 wk after day 3 and last two weeks past exposure to MG were found to be substantially higher than those of the control. C3 showed a steady increase in patient's sera from days 3 onward (Table 1). Serum level of C4 in patients was also found to be higher than the serum C4 level of the control, in particular during the two weeks after day 3 and last two weeks past exposure to MG (Table 1).
The results of the total hemolytic activity of the complement system (CH50) are presented in Table 2. The CH50 in patient's sera collected on day 3 and during 4-18 d after exposure to MG were significantly higher than those of the controls. The CH50 in patients sera collected during 19-31 d after exposure showed a noticeable decrease, yet it did not decline to the same CH50 level as those of the controls sera.

Table 1: Serum concentrations of C3 and C4 levels in patients during one month after exposed to Mustard Gas

Time Day(s)	n		Complement component	Mean concentration mg/ml		SD		*P*
	Patient	Control		Patient	Control	Patient	Control	
3	18	14	C3	136.8	128.3	27.4	30.7	0.42
			C4	31.9	30.0	9.0	11.6	0.61
4-18	22	14	C3	184.4	128.3	29.9	30.7	< 0.0001
			C4	42.7	30.0	9.0	11.6	0.001
19-31	22	14	C3	188.3	128.3	40.4	30.7	< 0.0001
			C4	42.0	30.0	8.6	11.6	0.001

Table 2: Total hemolytic activity of complement system (CH50) in the sera of patients during one month after exposure to Mustard Gas with results of their normal controls

Time	n		CH50 Unit		SD		*P*
Day(s)	Patient	Control	Patient	Control	Patient	Control	
3	15	14	137.8	105.1	24.8	32.7	0.005
4-18	20	14	136.5	105.1	21.8	32.7	0.002
19-31	20	14	117.2	105.1	27.6	32.7	0.25

Discussion

The short outcome of sudden exposure to a highly toxic compound such as MG is a trauma characterized by tissue injury and inflammation. Such a disturbance of the integrity and well-being of an individual leads to a series of reactions including production of acute phase proteins and activation of complement system. The purposes of these diverse responses are to restore homeostasis and to remove the cause of the disturbance (9). The acute phase responses are initiated by cytokines in particular by IL-1 and IL-6 leading to activation of hepatic cells for synthesis of acute phase proteins and complement components (9).

Among various serum variables which were studied in patients exposed to MG, C_3 in particular showed noticeable alterations comparing with those of the normal controls.

C_3 and C_4 the two complement components which were measured in the sera of patients were found to be in higher levels than those of the normal controls (Table 1). These two components are produced by the liver and cells of the monocyte-macrophage linage in response to various stimuli (10) while the rise in the serum levels of C_4 in patients was almost moderate. Serum C_3 levels were found to have increased significantly on day 3 after exposure to MG and its levels showed significant increase in the patient's sera collected during 4-18 d and 19-31 d exposure. C3 is one of the acute phase proteins which often increase considerably after infection and tissue injuries. The significant increase in C3 in subjects exposed to MG indicates clearly the vigorous response of the hepatic tissue and the devastating effect of this chemical agent on organs and tissues of the victims. The increase in C_3 and probably other acute phase proteins in subjects exposed to MG are mainly due to the extensive tissue injuries rather than microbial invasion since others who investigated the status of the hospitalized patients exposed to MG did not find infection at this acute phase of the trauma to be a special problem of these victims (11).

The highest CH50 levels in the patient's sera were found on day 3 after exposure. Thereafter, CH50 levels showed a gradual decrease in the patients sera collected during 4-18 d and 19-31 d after exposure to MG (Table 2). Whereas the levels of C3 which is also an acute phase proteins remained significantly in high levels during the whole period of one month which this component was measured in patients sera and did not show any indication of a decrease even in the sera collected during 19-31 d after exposure to MG, CH50 levels showed a decrease in particular during 19-31 d after exposure. These results clearly indicate the significance of C3 as an acute phase proteins in this trauma.

In conclusion, the available data presented in this article indicate that following exposure to MG, rapid penetration and absorption of this chemical agent occur in various organs, resulting to tissue injuries, necrosis and release of inflammatory and vasoactive molecules. Moreover, the high production of acute phase proteins in particular the 3rd component of the complement system by the liver and macrophages in due to the rapid reaction of the innate immune system to maintain necessary support and protection of the victims in this trauma. The antibodies and other

elements of the adoptive immunity which are produced with a delay of several days would further assist the protection of these patients.

Acknowledgements

This study was supported by Gehad Daneshgahi of the Faculty of Medicine, Tehran university of Medical Sciences. The authors wish to express their sincere appreciation to Behzad Eftekhar MD, Mohammad Razavi MD and Abass Forootan MD, for invaluable assistance in this study.
The authors declare that they have no conflict of Interests.

References

1. Roberts JJ, Warwicke Gp (1963). Studies of the mode of action of alkylating agents: IV-the metabolism of the bis-2-chloroethyl-sulfide (mustard gas) and related compounds. *Biochem Pharmacol*, 12:1329-34.
2. Qujeq D, Taghikhani M (1991). High performance Liquid chromatographic analysis of sulfur mustard reaction with amino acids and protein. *Med J IR Iran*, 5:55-8.
3. Zuhair M, Hassan and Massoumeh Ebtekar (2001). Modeling for Immuno suppression by sulfur mustard. *International immunopharmacology*, 1:605-10.
4. Moradi A, Sodeifi M, Abdollahi A, Pakdaman A, vessal K (1980). Clinical presentation of chemical wasface liusies. *Iranian J Med Science*, 13:1-5.
5. Azizi F, Elyasi H, Sohrab pour H, Jalali N, Nafar abadi M (1984). Serum concentration of various Hormones following Exposure to chemical weapons containing sulfure mustard. *Medical J* Iranian Republic of Iran, 3:105-8.
6. Hassan ZM, Ebtekar M, Ghanai M, Taghikhani M, Noori Daloii MR, Ghazanfari T (2006). Immunobiological consequences of sulfur Mustard contaminations. *Iran J Allergy Asthma Immunol*, 5(3):101-8.
7. Keyhani AH, Eslami MB, Razavimanesh H (2007). The short term effects of mustard gas on the serum immunoglobulin Levels. *Iran J Allergy Asthma Immunol*, 6(1):15-9.
8. Henry JB (1984). *Clinical diagnosis and Management by Laboratory Methods*. W.B.Saunders.
9. Moshage H (1997). Cytokine and Hepatic Acute phase response. *J Pathology*, 181: 257-66.
10. Goldsby RA, Kindt TJ, Osborne BA, Kuby J (2006). *Immunology*. Freeman & Co (Publisher). P.312.
11. Motakallem MH (1988). Evaluation of 17 patients severely injured with sulfur Mustared. *Med J Islamic republic Iran*, 2:99-104.