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Acute Effects of Sulphur Mustard Gas on the Number of Lymphocytes in the Rat's Spleen

Efectos Agudos del Gas Mostaza sobre el Número de Linfocitos en el Bazo de Rata

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SUMMARY: Sulphur mustard (SM), commonly known as mustard gas is an alkylating agent that causes serious blisters upon contact with human skin. SM is frequently used as a chemical warfare agent. There is some evidence for sulfur mustard-induced lymph system effects in humans. Between 2000-2001, 42 male albino Wistar rats were used. After accommodation with environment, we divided rats to control, sham and experimental groups (2.5 mg/kg, 5 mg/kg, 10 mg/kg, 20 mg/kg and 40 mg/kg). Then we injected sulphur mustard oil in rat's intraperitoneal space. Then their spleens were removed for histological verification. Our results showed that significant difference in lymphocytes number in experimental groups after 24 hours. The number of lymphocytes in 5, 10, 20 and 40 mg/kg groups was increased and this increase in 40 mg/kg group was more than the other groups. We concluded that the number of lymphocytes increased due to exposure of mustard gas and there is a relationship between the increase of lymphocytes and dose of exposure.

KEY WORDS: Mustard gas; Lymphocytes; Spleen.

INTRODUCTION

Sulphur mustard (SM), commonly known as mustard gas and chemically, bis [2-chloroethyl] sulphide is an alkylating agent that causes serious blisters upon contact with human skin. SM is frequently used as a chemical warfare agent (Wormser, 1991; Eisenmenger *et al.,* 1991; Momeni *et al.*, 1992). Due to the simple method of preparation, SM being used clandestinely during war or by terrorist groups still remains a threat, inspite of the successful implication of the Chemical Weapons Convention (Krutzsch & Trapp, 1994). SM forms sulphonium ion in the body and alkylates DNA leading to DNA strand breaks and cell death (Papirmeister *et al.*, 1991; Rao *et al.*, 1999). Due to the high electrophilic property of the sulphonium ion, SM binds to a variety of cellular macromolecules (Somani & Babu, 1989). Eyes, skin and the respiratory tract are the principal target organs of SM toxicity (Papirmeister *et al*.; Balali, 1984; Vijayaraghavan, 1997).

Several animal studies indicate effects of sulfur mustard on the hemopoietic system following intravenous or subcutaneous administration of sulfur mustard (Kindred, 1947).

There is some evidence for sulfur mustard-induced lymph system effects in humans. Lymph node discoloration and spleen pathology were found in autopsies of sulfur mustard victims (Alexander, 1947). Additional animal studies also indicated sulfur mustard-induced damage to the lymph system (Cameron *et al*., 1946; Coutelier *et al.*, 1991; Venkateswaran *et al.*, 1994).

Sulfur mustard tissue distribution data from an Iranian soldier who died 7 days after inhalation and/or dermal exposure to sulfur mustard indicated distribution: brain > kidney > liver > spleen > lung (Drasch *et al*., 1987), whereas radiolabel concentration data in rats 4 days after an intravenous injection of radiolabeled sulfur mustard indicate a different distribution pattern to these organs: kidney > lung > liver > spleen > brain (Maisonneuve *et al.*, 1994).

Since there are rare documents about the number of lymphocytes in spleen after exposure of mustard gas, therefore the aim of this study was evaluation of the lymphocytes number in the rat's spleen after 24 hours of exposure of mustard gas.

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MATERIAL AND METHOD

Between 2000-2001 years, 42 male albino Wistar rats (250 - 300 g) obtained from Pasteur institute of Iran were used. Rats were housed in large plastic cage, food and water were available. Animals were maintained under standard conditions and 12 h / 12 h light / dark cycle with lights on at 7.00 a.m. After accommodation with environment, we divided rats to control, sham and experimental groups (2.5 mg/kg, 5 mg/kg, 10 mg/kg, 20 mg/kg and 40 mg/kg) according of Coutelier report in 1991 (Coutelier *et al*.); in each group we had 6 rats.

We mixed the sulphur mustard oil with the Tyrods buffer and then we injected this solution in intraperitoneal space according the rats weight. For sham group we injected the buffer without the mustard.

After examinations, animals were dissected after ether anesthesia and their spleen were removed for histological verification, at first the spleens fixed in Buoin solution and two weeks later impregnated with paraffin wax. After histological processing, slices of 5 µm (serial section of anterior to posterior of spleens) were produced with Leitz rotary microtome (Microm, Germany, HM, 325) (One of 10 sections were selected for staining and morphometeric measurements). For staining, haematoxylin-eosine (H&E) staining was used.

Morphometric measurement were carried out using on Olympus DP 12 digital camera and BX 51 microscope (Olympus Optical Co. LTD, Tokyo, Japan). We selected a field (100000 μm^2) within the spleens. Randomly selected, non-overlapping photographs using a x40 objective lens were taken from the designated areas. Images were saved by the Bioreporter program and further processed using the Adobe Photoshop 6.0 program (Adobe System Inc., San Jose, CA, USA).

For cell counts, photographs at a magnification of x40 (objective lens) were taken throughout the longitudinal axis of the spleen and further processed as described above. All of the lymphocytes shown on thisfield were counted and then the mean and SD of lymphocytes number were measured.

Statistical analysis. Data was expressed as mean SD differences among areas were statistically evaluated using the one-way analysis of variance (ANOVA). Probabilities of $P < 0.01$ were considered significant.

RESULTS

Immediately after the injection of mustard solution, the rats secluded to one corner of cage. The mean and SD of the number of lymphocytes (per 105 μm^2) in control, sham and experimental groups are depicted on Table I.

There were not significant differences in lymphocytes number between control and sham groups, also between sham and 2.5 mg/kg differences were not significant, but the differences between sham and 5 mg/kg groups, between sham and 10 mg/kg groups, between sham and 20 mg/kg groups and also between sham and 40 mg/kg groups were significant.

The number of lymphocytes in 40 mg/kg group was more than the other groups and in 2.5 mg/kg group it was less than the other groups.

Table 1. The mean of lymphocytes number in control, Sham and experimental groups P<0.01 is significant.

P- value	Std. Deviation	Area	Mean	Group
NS	24.1	μm^2 100000	1440	Control
NS	12.1	μm^2 100000	1410	Sham
NS	8.9	μm^2 100000	1394	2.5 mg/kg
P<0.01	26	μm^2 100000	1596	5 mg/kg
P<0.01	14.2	μm^2 100000	1631	10 mg/kg
P<0.01	62.6	μm^2 100000	1568	20 mg/kg
P<0.01	5.6	μm^2 100000	1632	40 mg/kg

DISCUSSION

The short time effects of mustard oil indicate that the number of lymphocytes increased due to the interance of pathogenic matter, and the lymphocytes reaction against it via the proliferation or migration. We found there is a relationship between dose of exposure and increase of lymphocytes number. The related documents about the effects of mustard gas on the spleen are as follow:

Venkateswaran *et al.* reported that sulfur mustard was topically applied a single time at doses of 3.88, 7.75, or 15.5 mg/kg to the shaved backs of Balb/c mice (16/group/

dose). Sulfur mustard produced a significant dose-related decrease in the weight of the spleen (12–59%), and peripheral (12-44%) and mesenteric lymph nodes (significant only at high dose, 18%). Incidence and severity of histological changes in the thymus and spleen were also dose-related. Spleen histopathology included hypocellularity, atrophy of the lymphoid follicles, degeneration of germinal centers, and red pulp infiltrated with macrophages.

A significant dose-related decrease in the cellularity of the spleen (24-45%) was measured. A dose-related decrease in the cellularity of the thymus was also found, significant at the mid and high doses (36-42%) (Venkateswaran *et al*.).

Coutelier *et al*. reported that: A significant dose-related reduction in spleen cell number was measured in mice 7 days after intraperitoneal injection of sulfur mustard (23% at 5 mg/kg and 49% at 10 mg/kg). A 26% increase in spleen T-lymphocytes and a 44% decrease in B-lymphocytes were measured 7 days following injection with 10 mg/kg of sulfur mustard (Coutelier *et al*.).

Also Cameron *et al.,* Coutelier *et al*. and Venkateswaran *et al.* showed that sulfur mustard-induced damage to the lymph system and lymph node discoloration and spleen pathology were found in autopsies of sulfur mustard victims.

Consistent with observations of the human spleen, Pant & Vijayaraghavan (1999) measured a significant 38% reduction in spleen-to-body weight ratio in mice exposed to 84.7 mg/m^3 for 1 hour. In the majority of cases, the spleen was described as shrunken in size with pale color. Microscopically only 2 of 32 spleens examined showed degeneration or necrosis; pyknosis and karyorrhexis of lymphocytes in some corpuscles was observed in one and slight necrosis of the malpighian follicle in the other (Pant & Vijayaraghavan).

Kindred in 1947 reported that single intravenous injection of 0.5 mg/kg of sulfur mustard dissolved in thiodiglycol in young male rats caused degenerative damage to the spleen, thymus, and bone marrow.

As the mention studies were in long period of exposure, therefore they showed decrease of lymphocytes, number in spleen, but in our study the number of lymphocytes counted after 24 hours and in this time due to immunological responses the number of lymphocytes was increased.

We concluded that there is a dose-related increase in number of lymphocytes due to exposure of mustard gas.

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RESUMEN: El sulfuro de mostaza (SM), comúnmente conocido como gas mostaza, es un agente alquilante que causa graves ampollas en contacto con la piel humana. SM se utiliza con frecuencia como un agente de guerra química. Hay algunas evidencias que indican que el SM induce efectos en el sistema linfático en seres humanos. Entre los años 2000-2001, fueron utilizadas 42 ratas albinas Wistar macho. Después de la acomodación con el medio ambiente, las ratas se dividieron en grupos control, impostor y experimental (2,5 mg / kg, 5 mg / kg, 10 mg / kg, 20 mg / kg y 40 mg / kg). Luego se inyectó aceite de SM en el espacio intraperitoneal de las ratas. A continuación, sus bazos fueron removidos para la verificación histológica. Los resultados mostraron una diferencia significativa en el número de linfocitos en el grupo experimental después de 24 horas. El número de linfocitos en los grupos de 5, 10, 20 y 40 mg / kg fue mayor siendo este incremento en el grupo de 40 mg / kg más alto que en los otros grupos. Concluimos que el número de linfocitos aumenta debido a la exposición de gas mostaza existiendo una relación entre el aumento de linfocitos y la dosis de exposición.

PALABRAS CLAVE: Gas mostaza; Linfocitos; Bazo.

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