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Effect of Slow Release Pentoxifylline and Captopril on Delayed Pulmonary Complications of Mustard Gas in Animal Models

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ABSTRACT

Background: *Considering the effect of pentoxifylline on the immune system and reducing oxidative stress and also the anti-oxidative properties of captopril, these drugs are indicated for prevention and treatment of delayed pulmonary complications due to exposure to sulfur mustard (SM). Therefore, we decided to study the effect of slow release pentoxifylline and captopril on SM-induced delayed pulmonary complications in animal models.*

Materials and Methods: *Pentoxifylline and captopril were administered for two weeks to mice exposed to sulfur mustard. Biochemical and pathological analyses included: hydroxyproline assay, alveolar space percentage and severity of inflammatory cell infiltration. The results were compared between groups using ANOVA statistical test.*

Results: *Hydroxyproline content of the lungs was significantly lower in the negative control group in comparison to positive control, captopril intervention and pentoxifylline intervention groups. There was no significant difference between groups in image analysis figures. However, there was a significant difference in extent of fibrosis, inflammation, and lymphocyte and PMN percentage between different groups.*

Conclusion: *Pentoxifylline only resulted in decreased pulmonary inflammation without any effects on other indices. On the other hand, increase in hydroxyproline content of the lung in the captopril group compared to controls showed that captopril had accelerated the process of fibrosis. Hence, more research is recommended to study the effect of captopril on pulmonary fibrosis.* ***(Tanaffos 2009; 8(1): 41-49)***

Key words: *Pentoxifylline, Captopril, Lung, Slow release pump, Sulfur mustard*

INTRODUCTION

Mustard gas or sulfur mustard (SM) was first synthesized by Despretz in 1822 and used in many combats. The last application was reported in the Iraq/Iran war between the years 1983-1988 (1). SM is an electrophilic compound. Its alkyl group can be transferred to live cell proteins, nucleic acids and components of complement factors producing cross links between target molecules (2,3) and eventually causing cellular death.(4).

One of the organs undergoing acute and chronic injury due to this gas is the respiratory system. Damage to the respiratory epithelium gradually develops within few days and subsequently, a mild to

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severe inflammatory reaction occurs which is accompanied by epithelial necrosis and eventually, acute inflammation of the upper and lower respiratory tracts (5). Even following resolution of acute complications, a persistent respiratory distress can occur in the exposed victims. In follow up of 200 Iranian patients with severe exposure to SM, near one-third of the individuals showed chronic respiratory symptoms two years later. Reported complications included: bronchitis, asthma, laryngitis, tracheobronchitis, recurrent pneumonia, and bronchiectasis (6-8).

In acute SM exposure, oxidative stress is responsible for at least partial toxicity to internal organs and local tissues. In cell culture environments, contact of this agent with live cells leads to loss of anti-oxidant protection by the cells.(9) On the other hand, role of oxidative stress in the pathogenesis of tissue inflammation has already been established (10) and there is a possibility that oxidative stress, active oxygen radicals and lack of anti-oxidant protection are involved in delayed complications due to SM considering the chronic inflammation of the lower respiratory tract and its progressive nature (11, 12). As a result, medications which can decrease oxidative stress may prevent or decrease damage or treat late side effects in those exposed to SM.

Pentoxifylline is a methylxanthine derivative that participates in immune modulation by preventing lymphocyte (13) and neutrophil cytotoxicity (14, 15). This drug decreases inflammatory cytokine release such as TNF-alpha (16, 17); on the other hand, it decreases oxidative stress (18) and lipid peroxidation (19). As a result, it is believed to decrease SM toxicity at the time of exposure and also due to chronic destructive mechanism of this agent, it may influence the outcome of chronic SM-induced lung injury.

Another important consideration is the influence of hypoxia on the formation of pulmonary fibrosis (20-22) and the effect of fibrosis on inducing hypoxia (23) which leads to a destructive cycle and progression of the condition. Also, pentoxifylline can decrease hypoxia and prevent progression of disease through its vasodilatory effect and decreasing blood viscosity (24).

Captopril decreases lipid peroxidation, potentiates the intrinsic anti-oxidant system (25) and prevents alveolar epithelial cell apoptosis (26). Therefore, it can have therapeutic effects.

Additionally, several studies on lung fibrosis secondary to paraquat, amiodarone and radiation have shown beneficial effects of this drug (25-27).

The important point about these two medications is their short half life. The half life of captopril is less than 3 hours and if the original form of the medication is used, its half life would be less than 2 hours (28). The half life of pentoxifylline is 0.4 to 0.8 hour. This time is 1-1.6 hours for its metabolites (24). Therefore, a protocol that can maintain an effective constant concentration of these drugs in tissues without their continuous administration can increase their effectiveness.

Considering the current opinions about the effectiveness of these two medications in prevention and treatment of SM-related late pulmonary complications, this study was planned to evaluate the effectiveness of slow release forms of these medications in animal models.

MATERIALS AND METHODS

During an experimental study, 30 female mice with the mean weight of 23±2g were selected. The mice did not have clinical signs of respiratory or gastrointestinal infection. They were provided by the Pasteur Institute of Iran and were kept in similar standard nutritional and environmental conditions and a 12-hour light/12 hour dark cycle. The mice were kept for two weeks and when we got reassured

about their health, we randomly divided them into 5 groups each containing 6 mice.

Group 1: Negative control (in this group no intervention was done and it was selected for possible environmental confounders influencing the experimental results)

Group 2: An intervention group receiving pentoxifylline

Group 3: An intervention group receiving captopril

Group 4: Positive control for captopril (included mice exposed to sulfur mustard and no medical intervention)

Group 5: Positive control for pentoxifylline (included mice exposed to sulfur mustard and no medical intervention)

(Groups 2 and 3 included mice exposed to sulfur mustard that underwent medical intervention.)

Initially, ALZET pumps (DURECT corporation/CA) were loaded with the medication under sterile conditions. These pumps have a daily release of medication equal to 6 microliters. Therefore, to prepare ALZET pumps for group 2 (pentoxifylline), 200 mg pentoxifylline (1/2 Tab) was dissolved in 2.85 ml of distilled water and 200 microliters of this solution was administered into the pumps. To prepare these pumps in group 3 (captopril), 250mg captopril (5 tablets, each 50 mg) was dissolved in 1.2 ml distilled water and 200 microliter of this solution was administered into the pumps. In group 2, pentoxifylline 20mg/kg/day was administered via the ALZET pump starting 48 hours before the administration of sulfur mustard and was continued for 20 days. Group 3 also received captopril via the described method at a dose of 50 mg/Kg/day via ALZET pump starting 48 hours before the administration of mustard gas and continued for 14 days. The pumps were implanted into the mice as follows:

Initially, the mice were anesthetized with intraperitoneal injection of ketamine and xylocaine (with a 2 to 1 ratio). Then, the hair behind their neck was shaved and the skin was disinfected with antiseptics. Under sterile conditions, a 1.5 cm sagittal incision was made at the mentioned site and a space was created under the mouse's skin where ALZET pumps were placed. The incision site was sutured with chromic 2/0 surgical suture. Groups 4 and 5 (positive controls) also had plastic pumps in the dimensions of the ALZET pumps inserted subcutaneously as placebo. Forty-eight hours after implantation of the pumps, 24 of the 23±2 gram female mice (Groups 2,3,4, and 5) were placed in a closed container of sulfur mustard gas with concentration of 600 mg/m^3. At the same time, 6 healthy mice without gas exposure (group 1) were selected as the negative controls for the study.

After completion of the experiment, the mice were euthanized and their lungs were removed. The right lungs were placed in 10% formalin for microscopic slide preparation and pathologic evaluation. The left lungs were placed in special containers and dried in incubator for hydroxyproline assay.

For the hydroxyproline assay, the left lungs were weighed after being dried in the oven for 12 hours at 90°C, and then the Woessner method was applied to assess their total hydroxyproline content (see below) (36). Additionally, by using MATLAB software the percentage of alveolar space was estimated and compared among the groups.

Pathologic Evaluation:

After tissue preparation, the right lungs of the mice were cut for slides and stained with H&E to determine the severity of fibrosis and inflammation by a pathologist. Indices for fibrosis and inflammation were graded from 1 to 4 using 400x magnification. Percentage of mononuclear and multinuclear cells in the samples was also determined. All slides were evaluated at the same

time and the pathologist was unaware of their classification.

Hydroxyproline Assay:

To measure the quantity of hydroxyproline by the Woessner method (29), the lungs were weighed after drying in an oven for 12 hours at 90°C. Then, the tissue sample was hydrolyzed in 6 molar hydrochloride acid at 110°C for 12 hours. Subsequently, 50 microliter citrate acetate buffer (containing 5% citric acid, 1.2% glacial acetic acid, 7.24% sodium acetate, and 3.4% sodium hydroxide) and one ml of chloramine T solution (0.564 chloramine T, 4 ml propanol, 32 ml citrate acetate buffer) were added to the samples and then the samples were placed at room temperature for 20 minutes. Afterwards, one ml of Ehrlich solution (4.5 mg dimethyl-amino-benzaldehyde in 18.6 ml propanol and 7.8 ml perchloric acid) was added and incubated at 65°C for 15 minutes. Finally, light absorption of samples was measured at 550nm using a spectrophotometer and was compared to pure hydroxyproline standardized measuring curve. All measurements were performed at the same time and the biochemistry technician was unaware of the grouping of the samples.

Computerized Estimation of the Index of Fibrosis:

A method for assessing the severity of fibrosis and bronchitis is estimation of the total lung capacity (TLC). To measure this index in pathologic slides, the ratio of alveolar space to parenchyma in a microscopic field was assessed. By using the MATLAB 6 program, 5 slide fields with 100x magnification were selected and percentage of alveolar space was measured as an index of TLC and a mean was calculated for each slide. Since the alveolar space is white on microscopic examination, special software was used to estimate the percentage of white color compared to other colors (lung parenchyma) and this characteristic was used as an index for fibrosis (30).

Analysis of Results:

The results were analyzed using SPSS software version 13 and expressed as mean ± SD. Initially, normal distribution of the variable was evaluated using the Kolmogorov-Smirnov statistical test. Quantitative measures were analyzed using ANOVA and Tukey Post Hoc analyses and qualitative variables using the Chi-square test. P-value less than 0.05 was considered significant. Additionally, for a more comprehensive comparison between the groups, the mean values for the two positive control groups were determined and presented as one single group.

RESULTS

In the present study, all mice survived and had no infection or other complications during the study and follow-up period. Numerical results from pathological and biochemical analyses are presented in Table 1.

Table 1. Mean and standard deviation of indices among the study groups

Groups / Indices	Negative Control	Positive Control	Captopril	Pentoxifylline	P-value
Percentage of lymphocytes	97.2±3.01	78.5±12	85±10	91.42±3.77	P<0.05
Percentage of polymorphonuclear	3.3±3.7	28±13.9	20±14.4	11.49±6.9	P<0.05
Hydroxyproline content (µg/lung)	156.79± 36.7	235.13± 28.35	257±13.91	232.92±25.64	P<0.05
Percentage of alveolar space	54.76±1.22	61.8±4.73	61.03±3.51	62.25±5	P>0.05
Degree of fibrosis	0.58±0.66	2.9±0.56	1.7±0.5	2.42±1.51	P<0.05
Degree of inflammation	1.08±0.9	3±0.66	2±0.81	2±0.81	P<0.05

Based on the results of hydroxyproline measurements, a significant difference was observed in the hydroxyproline content of the lung among different groups and the lung hydroxyproline level in the negative control group was significantly lower than that in the positive control groups ($p<0.05$), the captopril study group ($p<0.05$) and the pentoxifylline study group ($p<0.05$). No significant difference was found among the other groups.

Additionally, results of pictorial analysis of other groups did not show significant differences between them.

In pathologic evaluation, significant difference was found in the percentage of lymphocytes in the lung tissue among the various groups. The lymphocyte percentage in the negative control group was significantly higher than the positive control group ($p<0.05$) and the captopril intervention group ($p<0.05$). Additionally, the lymphocyte percentage in the pentoxifylline intervention group was significantly higher than the positive control group ($p<0.05$). No significant difference was found in lymphocyte percentage among the other groups.

Results of multinucleated cell percentage in the lung tissues showed significant difference between the groups. Accordingly, the percentage of multinucleated cells in the negative control group was significantly less than the positive control group ($p<0.001$) and the captopril intervention group ($p<0.05$). Even though, the percentage of lymphocytes in the negative control group was less than the pentoxifylline intervention group, this difference was not statistically significant. Furthermore, a significant difference in the percentage of polymorphonuclear cells between the pentoxifylline and positive control groups was observed ($p<0.05$).

Evaluation of the degree of tissue fibrosis showed significant difference between the groups ($P<0.05$). Accordingly, the extent of tissue fibrosis in the negative control group was significantly less than the positive control group ($p<0.05$) and the pentoxifylline intervention group ($p<0.05$). However, there was no significant difference in the degree of fibrosis between the captopril intervention group and negative controls ($p<0.05$). Additionally, no significant difference in the degree of fibrosis was found among pentoxifylline group, captopril group and the positive control group. Also, no significant difference was found between the captopril and pentoxifylline intervention groups.

Results of tissue inflammation evaluation showed a significant difference in degree of tissue inflammation between the positive control group compared to the negative control group ($p<0.05$). No significant difference was observed between other groups.

DISCUSSION

Based on the present study, mustard gas led to pulmonary fibrosis in exposed mice and administration of slow release forms of captopril and pentoxifylline did not decrease lung fibrosis indices in them. The two positive and negative control groups had a significant difference in fibrosis index which supports the study method for induction of fibrosis and the role of mustard gas in causing this complication.

Mustard gas is an electrophilic alkylating agent which has been used as a vesicant chemical warfare agent in combats especially in the Iraq/Iran war causing toxicity in many Iranian soldiers. At present, about 34,000 SM victims are inflicted with late complications of this agent (31). To date, a diverse range of pulmonary ailments have been reported among war veterans, but lung fibrosis is considered as one of the most important and deleterious chronic SM complications and has been the cause of death in many patients (32).

Years after exposure to SM, patients continue to

have complications of toxicity with this poison. In a study by Khateri and colleagues on 34,000 Iranian chemical warfare patients 13 to 20 years post exposure, the most common delayed complications involved lungs (42.5%) followed by the eyes (39%) and skin (24.5%) (31).

Pulmonary disease is the most important cause of long-term disability among mustard gas victims (1). Based on a study on 197 Iranian soldiers 10 years after extensive exposure to mustard gas, pulmonary complications such as chronic bronchitis (58%), pulmonary fibrosis (12%), asthma (10%), narrowing of large airways (9%) and bronchiectasis (8%) were observed (33).

The pathogenesis of SM toxicity includes inflammatory cells infiltration (34), aggregation of inflammatory mediators such as TNF-alpha (35), acute inflammation followed by chronic inflammation and alveolar epithelial cell apoptosis (1) and eventually, activation of fibroblasts and resultant pulmonary fibrosis (11).

According to a study performed by Emad and Rezaian, neutrophilic accumulation in alveolar inflammation (neutrophilic alveolitis) due to release of free radicals and proteases from the neutrophils is the probable cause of initiation of fibrosis (33).

Considering the role of hypoxia, oxidative stress and inflammation in the process of fibrosis, it is suggested that captopril and pentoxifylline may influence at least one of these mechanisms, leading to treatment or preventing progression of fibrosis. In this study, lung fibrosis was induced in animal models using mustard gas and the effect of slow release captopril and pentoxifylline (which are usually used for treatment of other forms of fibrosis) was evaluated.

The Effect of Pentoxifylline on Pulmonary Fibrosis:

In previous studies, role of hypoxia in inducing pulmonary fibrosis has been supported (20-22). Additionally, tissue inflammation in acute or chronic phases has been proposed as a mechanism of fibrosis formation (33). Consequently, it seems that pentoxifylline as a vasodilating agent with significant anti-inflammatory properties (13-17) might treat or prevent progression of fibrotic lung disease.

In some studies use of this medication has decreased tissue fibrosis (36,37).

However, in this study despite the probable anti-inflammatory effect of pentoxifylline on the mice lungs, it could not treat pulmonary fibrosis. The results of this study are similar to those of Keremer and colleagues in which pentoxifyline did not improve lung fibrosis induced by bleomycin (38).

The reason may be the insufficient vasodilatory effect of pentoxifylline on the pulmonary vessels. Therefore hypoxia cannot be revered effectively to prevent the process of fibrosis. But, considering the anti-inflammatory effect of this drug on the lungs and the accepted role of inflammation in the process of formation and progression of fibrosis (33), this medication can be used as a supplementary treatment for lung fibrosis. However, further studies are required to support this theory. It is recommended that future studies focus on the effect of more specific pulmonary vasodilators such as sildenafil on the progress of SM-induced lung fibrosis.

The Effect of Captopril on Pulmonary Fibrosis:

Captopril is an angiotensin converting enzyme inhibitor (ACEI) currently used as the primary treatment for hypertension. This drug reinforces the intracellular antioxidant system (25) and inhibits respiratory epithelial cell apoptosis (26) and pulmonary fibroblasts' proliferation (39). It has also been used for treatment of some other forms of lung fibrosis with promising results (25-27). Therefore, in this study we evaluated the effect of this drug on SM-induced pulmonary fibrosis.

Based on the results of this study, even though captopril decreased inflammation and fibrosis in pathologic samples, we did not notice any difference in quantitative and biochemical indices. Since the quantitative content of hydroxyproline is the most accurate index of fibrosis, the above mentioned difference may be justified by knowing that on microscopic examination, decreased severity of inflammation is associated with decreased density of the pulmonary interstitial tissue which may provide us with an incorrect impression of decreased interstitial connective tissue .To avoid this error, specific collagen and elastin staining should be used for detection of fibrosis in microscopic examination. Additionally, decrease in the extent of inflammation with no reduction in the percentage of mono and polymorphonuclear inflammatory cells may be due to the decrease in the exact number of inflammatory cells without affecting the relative percentage of these cells. However, considering the results of hydroxyproline assay in the captopril intervention group and the positive control group and higher levels of hydroxyproline in the lungs of those receiving captopril, it seems that more precise studies are required to evaluate the effect of this drug on the progression of pulmonary fibrosis.

REFERENCES

1. Balali-Mood M, Hefazi M. The pharmacology, toxicology, and medical treatment of sulphur mustard poisoning. ***Fundam Clin Pharmacol*** 2005; 19 (3): 297- 315.
2. Papirmeister B; Feister AJ; Rabinson SI; et al. Medical defense against mustard gas: toxic mechanism and pharmacological implications. Florida: CRC Press; 1991; p180-99.
3. Meier HL,Millard CB. Alterations in human lymphocyte DNA caused by sulfur mustard can be mitigated by selective inhibitors of poly(ADP-ribose) polymerase. ***Biochim Biophys Acta*** 1998; 1404 (3) :367- 76.
4. Moser J, Meier HL. Comparison of cell size in sulfur mustard-induced death of keratinocytes and lymphocytes. ***J Appl Toxicol*** 2000; 20 Suppl 1: S23- 30.
5. Papirmeister B; Feister AJ; Ford RD. Medical Defense against mustard gas: Toxic mechanisms and pharmacological implications. Florida: CRC Press; 1991; p 6-23.
6. Willems JL. Clinical management of mustard gas casualties. ***Ann Med Milit*** 1989; 3: 1-61.
7. Balali M. Clinical and laboratory findings in Iranian fighters with chemical gas poisoning. ***Arch Belg*** 1984; Suppl: 254- 9.
8. Balali Mood M. First report of delayed toxic effects of yperite poisoning in Iranian fighters. In new compounds in biological and chemical warfare, Second World Congress. International Association of Forensic Toxicologists, 23rd European International Meeting. Ghent, 24-27 August.
9. Han S, Espinoza LA, Liao H, Boulares AH, Smulson ME. Protection by antioxidants against toxicity and apoptosis induced by the sulphur mustard analog 2-chloroethylethyl sulphide (CEES) in Jurkat T cells and normal human lymphocytes. ***Br J Pharmacol*** 2004; 141 (5): 795- 802. Erratum in: ***Br J Pharmacol*** 2004; 142 (1): 229.
10. Mitchell RN; Cotran RC. Cell injury, adaptation and death. In: Kumar V; Cotran R; Robbins SL. Robbins Basic Pathology 7th ed. Philadelphia: WB Saunders company 2003; p9-11.
11. Emad A, Rezaian GR. Immunoglobulins and cellular constituents of the BAL fluid of patients with sulfur mustard gas-induced pulmonary fibrosis. ***Chest*** 1999; 115 (5): 1346- 51.
12. Aghanouri R, Ghanei M, Aslani J, Keivani-Amine H, Rastegar F, Karkhane A. Fibrogenic cytokine levels in bronchoalveolar lavage aspirates 15 years after exposure to sulfur mustard. ***Am J Physiol Lung Cell Mol Physiol*** 2004; 287 (6): L1160- 4.
13. Lazarczyk M, Grzela T, Niderla J, Golab J, Korczak-Kowalska G, Oldak M, et al. Pentoxifylline inhibits leukocyte infiltration and splenocyte cytotoxicity against

murine colon adenocarcinoma. ***Oncol Rep*** 2003; 10 (4): 839- 43.

14. Currie MS, Rao KM, Padmanabhan J, Jones A, Crawford J, Cohen HJ. Stimulus-specific effects of pentoxifylline on neutrophil CR3 expression, degranulation, and superoxide production. ***J Leukoc Biol*** 1990; 47 (3): 244- 50.
15. Sourbier P; Perianin A; Hakim J. In vitro effect of pentoxifylline on human neutrophil function. In: Mandell GL; Novick WJ. Pentoxifylline and leucocyte function. Hoechst-Roussel Pharm Inc., FL, USA 1987; p56-67
16. Takehana H, Inomata T, Niwano H, Nishii M, Matsuda C, Kohno K, et al. Immunomodulatory effect of pentoxifylline in suppressing experimental autoimmune myocarditis. ***Circ J*** 2002; 66 (5): 499- 504.
17. Marcinkiewicz J, Grabowska A, Lauterbach R, Bobek M. Differential effects of pentoxifylline, a non-specific phosphodiesterase inhibitor, on the production of IL-10, IL-12 p40 and p35 subunits by murine peritoneal macrophages. ***Immunopharmacology*** 2000; 49 (3): 335- 43.
18. Shukla V, Gude RP. Amelioration of B16F10 melanoma cells induced oxidative stress in DBA/2 mice by pentoxifylline. ***J Exp Clin Cancer Res*** 2003; 22 (3): 407- 10.
19. Radfar M, Larijani B, Hadjibabaie M, Rajabipour B, Mojtahedi A, Abdollahi M. Effects of pentoxifylline on oxidative stress and levels of EGF and NO in blood of diabetic type-2 patients; a randomized, double-blind placebo-controlled clinical trial. ***Biomed Pharmacother*** 2005; 59 (6): 302- 6.
20. Helfman T, Falanga V. Gene expression in low oxygen tension. ***Am J Med Sci*** 1993; 306 (1): 37- 41.
21. Falanga V, Martin TA, Takagi H, Kirsner RS, Helfman T, Pardes J, et al. Low oxygen tension increases mRNA levels of alpha 1 (I) procollagen in human dermal fibroblasts. ***J Cell Physiol*** 1993; 157 (2): 408-12.
22. Vujaskovic Z, Anscher MS, Feng QF, Rabbani ZN, Amin K, Samulski TS, et al. Radiation-induced hypoxia may perpetuate late normal tissue injury. ***Int J Radiat Oncol Biol Phys*** 2001; 50 (4): 851- 5.
23. King TE; Schwarz MI. Idiopathic interstitial pneumonias. In: Murray JF; Nadel JA. Textbook of respiratory medicine 3rd ed. Philadelphia: WB Saunders Company 2000; p1675- 6.
24. Sweetman SC. MARTINDALE the complete drug reference 34th ed. London: pharmaceutical press 2005; p 980.
25. Candan F, Alagözlü H. Captopril inhibits the pulmonary toxicity of paraquat in rats. ***Hum Exp Toxicol*** 2001; 20 (12): 637- 41.
26. Uhal BD, Wang R, Laukka J, Zhuang J, Soledad-Conrad V, Filippatos G. Inhibition of amiodarone-induced lung fibrosis but not alveolitis by angiotensin system antagonists. ***Pharmacol Toxicol*** 2003; 92 (2): 81- 7.
27. Molteni A, Moulder JE, Cohen EF, Ward WF, Fish BL, Taylor JM, et al. Control of radiation-induced pneumopathy and lung fibrosis by angiotensin-converting enzyme inhibitors and an angiotensin II type 1 receptor blocker. ***Int J Radiat Biol*** 2000; 76 (4): 523- 32.
28. Sweetman SC. MARTINDALE the complete drug reference 34th ed. London: pharmaceutical press 2005; p 879.
29. WOESSNER JF Jr. The determination of hydroxyproline in tissue and protein samples containing small proportions of this amino acid. ***Arch Biochem Biophys*** 1961; 93: 440- 7.
30. Kremer S, Breuer R, Lossos IS, Berkman N, Christensen TG, Connor MW, Goldstein RH, Or R. Effect of immunomodulators on bleomycin-induced lung injury. ***Respiration*** 1999; 66 (5): 455- 62.
31. Khateri S, Ghanei M, Keshavarz S, Soroush M, Haines D. Incidence of lung, eye, and skin lesions as late complications in 34,000 Iranians with wartime exposure to mustard agent. ***J Occup Environ Med*** 2003; 45 (11): 1136- 43.
32. Emad A, Rezaian GR. Immunoglobulins and cellular constituents of the BAL fluid of patients with sulfur mustard gas induced pulmonary fibrosis. ***Chest*** 1999; 115: 1346-51.

33. Emad A, Rezaian GR. The diversity of the effects of sulfur mustard gas inhalation on respiratory system 10 years after a single, heavy exposure: analysis of 197 cases. ***Chest*** 1997; 112 (3): 734- 8.
34. Papirmeister B; Feister AJ; Ford RD. Medical defense against mustard gas: toxic mechanisms and pharmacological implications. Florida: CRC Press 1991; p 66-70.
35. Arroyo CM, Burman DL, Kahler DW, Nelson MR, Corun CM, Guzman JJ, et al. TNF-alpha expression patterns as potential molecular biomarker for human skin cells exposed to vesicant chemical warfare agents: sulfur mustard (HD) and Lewisite (L). ***Cell Biol Toxicol*** 2004; 20 (6): 345- 59.
36. Entzian P, Zähringer U, Schlaak M, Gerlach C, Galle J, Zabel P. Comparative study on effects of pentoxifylline, prednisolone and colchicine in experimental alveolitis. ***Int J Immunopharmacol*** 1998; 20 (12): 723- 35.
37. Entzian P, Gerlach C, Gerdes J, Schlaak M, Zabel P. Pentoxifylline inhibits experimental bleomycin-induced fibrosing alveolitis. ***Pneumologie*** 1997; 51 (4): 375- 80.
38. Kremer S, Breuer R, Lossos IS, Berkman N, Christensen TG, Connor MW, et al. Effect of immunomodulators on bleomycin-induced lung injury. ***Respiration*** 1999; 66 (5): 455- 62.