

Effect of Immunomodulator Pyrimethamine and Cimetidine on Immunosuppression Induced by Burn Blister Fluid

Behnaz Gharegozloo¹, Zuhair M. Hassan², Sussan K. Ardestani³, and Nasser Tavassoli⁴

¹ Department of Immunology, School of Paramedical Sciences, Iran University of Medical Sciences, Tehran, Iran

² Department of Immunology, School of Medical Sciences, University of Tarbiat Modarres, Tehran, Iran

³ Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran

⁴ Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

ABSTRACT

Despite recent advances in burn wound management, sepsis remains the main cause of death in patients resuscitated after major thermal injury. Increased susceptibility to infections has been related to severe suppression of the immune system.

The aim of this study was to induce immune suppression with blister fluid injection, and to modulate immune response by use of cimetidine and pyrimethamine in animal model.

Male Balb/c mice were injected with blister fluid intraperitoneally (ip). Fluids were collected from parital-thickness burn blisters and then the delayed type hypersensitivity (DTH) to sheep red blood cell (SRBC) and the effects of different doses of immunomodulators (Cimetidine and Pyrimethamine) on this response were quantitated.

A marked suppression of DTH was observed in mice injected with blister fluid. Pyrimethamine and Cimetidine at all three doses caused a significant enhancement of DTH response to SRBC compared with blister fluid injected in control group.

This finding represents evidence of a host defense defect within the burn wound and also indicates the blister fluid exhibit immunosuppressor factor that can modulate with immunomodulatory drugs like cimetidine and pyrimethamine.

Keywords: Blister, Cimetidine, Immunomodulators, Pyrimethamine

INTRODUCTION

Susceptibility to infection and septicemia due to depression of immune system can lead to increased morbidity and mortality. Deteriorated immune system remains the major cause of death following exposure to chemical and physical burn.¹⁻³ Extensive burn injury causes profound alterations in various essential elements of normal host immune response and the main aim of treatment after resuscitation is to maintain or even improve host resistance.⁴

Reports have indicated a severe suppression in the immune response due to impaired leukocyte and cytokine balance and the failure of T cell response to T-dependent antigen.⁵⁻⁷

The factors incriminated also include neutrophil dysfunction, abnormality in opsonic activity, macrophage dysfunction, production of soluble immunosuppressive substances, stress associated hormones, and an increase in the PGE2 level.¹

Several investigators have observed immunosuppression effects of low molecular weight peptides found in serum of burn and trauma patients.⁸

Impairment of T helper cell function and polarization toward T helper 2- type cytokine synthesis has been postulated to represent a major cause for postburn immunodeficiency.^{7,9}

Corresponding Author: Dr. Zuhair M Hassan, Department of Immunology, School of Medical Sciences, University of Tarbiat Modarres, Tehran, Iran. Tel: (+98 21) 801 1001, Fax: (+9821) 800 6544, E-mail: hasan_zm@modares.ac.ir

Improving immune competence through immunotherapy was also studied. The effects of fat intake¹⁰ and ornithine α -ketoglutarate¹¹ in immunomodulation of burn patients have been reported.

Improving of the immune competence through immunotherapy is an important issue for survival of burn patients. Therefore, we set up two experimental studies. Firstly, we studied the induction of immunosuppression by burn blister fluid. In the second step, we investigated the effect of two drugs with immunomodulating properties; pyrimethamine, an anti malaria agent,¹² and cimetidine, an anti peptic ulcer drug.¹³

Cimetidine was chosen because it is well-documented drug with recognized immunopotential properties. The drugs were chosen on the basis of report that sulfur mustard induced immunosuppression in mice was improved by administration of Pyrimethamine and Cimetidine.¹⁴ In our study the effect of these two immunomodulators on immune parameters was evaluated in Balb/c mice suppressed by receiving burn blister fluid.

MATERIALS AND METHODS

Animals

Eight to ten weeks male Balb/c mice were purchased from Pasteur Institute, Tehran, Iran. They were given sterilized water and autoclaved standard mouse chow ad Libitum throughout the study.

Blister Fluid

Blister Fluid was collected 1-40 hours after grade two thermal injury from 26 patients. Blisters were cleaned by povidone iodide before taking the fluid. 0.2 ml of unprocessed blister fluid was injected intraperitoneally (i.p.) to each mouse.

Drug

a) Pyrimethamine (Daraprim) was obtained from Wellcome Company, England. Three doses of 2.5, 5, and 10 mg/kg were selected on the basis of the drug's effectiveness and toxicity. One single injection on day 0 was sufficient to produce the desired effects. The drugs were injected 3-4 h after blister fluid i.p. injection to prevent the cross-interaction of the drug and blister fluid.

b) Cimetidine (Tagamet) was purchased from Chemidarou co., Iran. Doses of 5, 10 and 15 mg/kg of

this drug were administered by daily i.p. injection from day 0 up to day 5.

All drugs were administered in saline in a total volume of (0.1-0.5 ml).

Antigen

Sheep red blood cells were obtained from the Pasteur Institute, Tehran, Iran and preserved in sterile Alsevers' solution. After 3 times washing, mice were primed with 1×10^9 washed SRBC injected intraperitoneally for humoral study and 1×10^8 washed SRBC injected subcutaneously in the back for cellular study on day 0.

Antibody response

The mice were bled by intracardiac puncture on day 5 and the antibody responses were assessed. Two fold dilutions of the sera were made in phosphate buffered saline with a total volume of 0.2 ml. Then 0.2 ml volumes of 3% v/v washed SRBC were added to 96 well Nunc microtitre plate. Plates were then incubated at 37°C for one hour and observed for hemagglutination under an inverted microscope. Results were expressed as Log₂ titer.¹⁴

Delayed Type Hypersensitivity (DTH) Test

The sensitized animals were challenged with 1×10^9 SRBC injected subcutaneously on the left hind footpad on day 5. Foot thickness was measured 24 hours later with a Mause dial caliper and the results expressed as percent increase.

Histological Preparation of the Spleen

Spleen (removed upon autopsy on day 5) were preserved in 10% formalin and processed for histological work. Tissues were dehydrated and embedded in paraffin. Sections of tissues were cut at 3 μ m and stained with hematoxyline and eosin.

Spleen weight and organ index: Since the spleen is one of the most important organs indicative of immune function, the animals' body weight and the weight of their spleens were recorded on day 5 and the weight index was calculated according to the following formula:

$$\text{Spleen weight index} = \frac{\text{spleen weight}}{\text{animal weight}} \times 100$$

Statistical Methods

The results of hemagglutination titers expressed as log₂ titer. To determine statistical significance for these

Table 1. Effect of different time harvested blister fluid on immunoparameter after SRBC injection.

Group	BF hrs	Total Protein (mg/ml) of BF	Hemagglutination Mean Titer (Log2)	Spleen Index	Spleen Histology	% Footpad Increased (DTH)
1	1-10	26.1	5.96	5.8	Low grade hyperplasia, enlarged follicles	6.16*
2	10-20	23.1	5.52	5.84	Low grade hyperplasia, enlarged follicles	7.2*
3	20-30	27.2	5.46	5.5	Low grade hyperplasia, enlarged follicles	7.35*
4	30-40	29.5	6.1	6.2	Low grade hyperplasia, enlarged follicles	6.2*
5	Control (+) ¹	0	6.12	6.8	Hyperplasia, enlarged follicles	21.15*
	Saline					
	Control (-) ²	0	0	4.5	Normal histology	0
	Saline					

*P<0.05 indicates a significant difference in comparison with control group as shown by Kruskal-Wallis test.

1. SRBC injection only

2. Without BF and SRBC injections

data the t-test for independent means was used. In all analyses statistical significance was claimed at the 5% level ($p<0.05$).

RESULTS

Effect of blister fluid on Immunoparameters (Table 1). At first we wanted to know whether blister fluid had any effect on immune responses. For this reason, mice were injected (i.p.) with blister fluid and sensitized with SRBC. Then spleen histology and spleen weight index were assessed and humoral immune response and DTH were measured.

Blister fluids did not induce any significant effect on spleen weight index and humoral immunity but these fluids significantly ($p<0.05$) decreased DTH and caused low hyperplasia and enlarged follicles in the spleen. The immunomodulatory effect of pyrimethamine and cimetidine were studied.

Mice were injected (i.p.) with blister fluid and DTH was measured. Delayed type hypersensitivity test was conducted in a group of mice with constant dose of 27mg/ml blister fluid and the results showed a mean 30% suppression of footpad thickness as compared to a mean control injected with SRBC only.

Effect of Pyrimethamine on blister fluid induced immunosuppression (Table 2).

In order to assess the effect of pyrimethamine on the DTH in mice injected with blister fluid at the dose of 27mg/ml, 50 mice were divided into 5 groups. Group 1-3 took 2.5, 5 and 10 mg/kg pyrimethamine. Group 4 and 5 took blister fluid and PBS respectively. Results indicate that all three pyrimethamine doses caused a significant ($p<0.05$) increase in DTH responses compared to blister fluid injected in the control group. 5 mg/kg of pyrimethamine had the best effect on DTH of immunosuppressed mice.

Table 2. Effect of pyrimethamine on immune parameters following blister fluid and SRBC injection.

Group	Pyrimethamine (mg/kg)	Spleen Index	Spleen Histology	% Footpad Increased (DTH)
1	5	0.64	Hyperplasia, enlarged follicles	10.6*
2	10	0.61	Hyperplasia, enlarged follicles	14.6*
3	15	0.75	Hyperplasia, enlarged follicles	13.0*
4	Control (+), Blister fluid	0.66	Low grade hyperplasia, enlarged follicles	4.8
5	Control (-), Saline	0.77	Normal histology	16.3*

*P<0.05 indicates a significant difference in comparison with group 4 as shown by Kruskal-Wallis test.

Table 3. Effect of cimetidine on immune parameters following blister fluid and SRBC injection.

Group	Cimetidine (mg/kg)	Spleen Index	Spleen Histology	% Footpad Increased (DTH)
1	2.5	0.66	Hyperplasia, enlarged follicles	12.6*
2	5	0.71	Hyperplasia, enlarged follicles	14.6*
3	10	0.82	Hyperplasia, enlarged follicles	12.2*
4	Control (-), Blister fluid	0.76	Low grade hyperplasia, enlarged follicles	4.8
5	Control (+), Saline	0.77	Normal histology	16.3*

* P<0.05 indicates a significant difference in comparison with group 4.

The mean Log2 titer of antibody has shown no significant changes comparing to control group and we measured DTH only because blister fluid did not show any significant effect on humoral immunity.

Effect of Cimetidine on blister fluid induced immunosuppression. In order to evaluate the effect of cimetidine on DTH response, following blister fluid injected in mice, another 50 mice were divided into 5 groups. Results indicated that all the doses of cimetidine caused a significant ($p<0.01$) enhancement of the DTH response to SRBC compared with blister fluid injected in the control group (Table 3). Also, 10 mg/kg cimetidine had the best effect on DTH.

DISCUSSION

The initial stage of this work consisted of efforts aimed at obtaining an effective model of blister fluid, which displayed signs of immunosuppression. Mice were injected blister fluid with defined dose of 27mg/ml which was similar to that present in the human blister fluid and the immunosuppression was evaluated by DTH response.

The second stage of this work is the immunopharmacologic studies on the ability of pyrimethamine and cimetidine to reverse immunosuppression induced by blister fluid. Pyrimethamine is a 2,4-diaminopyrimidine developed almost fifty years ago for the treatment of malaria¹⁵ its major mechanism of action appears to be the inhibition of the dihydrofolate reductase enzyme, which is essential for the biosynthesis of purines and pyrimidines.¹⁶ Cimetidine is a histamine (H₂) antagonist widely used for the treatment of duodenal ulcers and other gastric hypersecretory conditions. It has been shown that cimetidine can reverse histamine induced suppression of the immune response.¹⁷ Cimetidine has also been implicated in augmentation of cell-mediated cytotoxicity and in the abrogation of suppressor T-cell function.^{13,18} Cimetidine, at the dose similar to that employed in our work, has been proved to abrogate the

immunosuppression caused by burn in mice.^{19,20} Cimetidine has been effective in countering the burn wound itch,²¹ tumor immunotherapy, as well as certain degrees of protection against infection in experimental animals.¹⁷

In our study, pyrimethamine (administered only once) at doses 2.5, 5, and 10mg/kg could significantly increase DTH responses to SRBC in comparison with untreated. Thong et al¹² had shown that pyrimethamine administered to C57/bl mice could stimulate both humoral and cell mediated immunity against SRBC.

Cimetidine administered daily could augment DTH response. Since cimetidine is thought to act by the inhibition of T-suppressor cell function, it is reasonable to postulate that increasing doses inhibit more suppressor cell clones to the extent that a great deal of suppressor cell activity is abolished and the immune response is augmented.

Up to now several hundred immunomodulator agents have proved to be promising in the cancer and AIDS therapy and they may also be effective in restoring burn injury immunosuppression and preventing the grave consequences. Further experimental and clinical studies may elucidate a possible role for immunomodulators such as Leflunomide²² in this regard.

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