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Cimetidine effects on the immunosuppression induced by burn injury

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ABSTRACT

Although many studies on the immune response following burn injuries have been reported, more attention has been given to the immunosuppression mechanism and mediators that shape the process of immune suppression. Specifically, information is not available concerning the immunomodulatory effects of the drugs which are involved in the immune response restoration. In this study, we investigated the effects of Cimetidine on the modulation of immune response in patients with burn injury of 20–60%. Two groups of patients were involved in this study; the patients in one group were treated with 15 mg/kg per day of Cimetidine while the patients in the other group were treated with placebo. Peripheral blood mononuclear cell (PBMC) expressing CD3, CD4, CD8, CD19 and CD3/HLA-DR was analyzed by flow cytometry. Cell proliferation assay using H3 thymidine was performed on PBMC samples. The proliferation assay showed a significant suppression of cell proliferation rate in post-burn patients ($p = 0.001$). We observed a significant reduction in the lymphocyte count ($p = 0.001$) and frequency of CD3 ($p = 0.007$) and CD4 ($p = 0.001$) T cells in post-burn patients. Also, the frequency of CD 19+ and HLA DR+ cells was increased compare to normal donors following burn injury. Treatment with Cimetidine increased the frequency of CD8+ T cells in the patient's peripheral blood. The PBMC proliferation rate was restored following the treatment with Cimetidine ($p = 0.02$). Our data indicates that Cimetidine may have beneficial effects on cell mediated immunity following burn injury.

1. Introduction

Infection complication represents a major cause of death in patients with extensive burn injury [1,2]. Extensive burn injury causes profound alterations in normal host immune response. Thus, the main aim of treatment after resuscitation is to improve patient's immune response [3,4]. Reports have shown that the main contributors are impaired leukocyte and cytokine imbalance [5,6]. Bystrova, NA et al. described the failure of T cells to respond to T-dependent antigens following burn injury [7].

Several investigators have observed immunosuppression effects of low molecular weight peptides found in the serum of burn and trauma patients [7]. The impairment of T helper cell function due to polarization toward T helper 2-type response has been suggested as a major cause for post-traumatic immunodeficiency [8]. Improvement of the immune competence in post burn patients through different immunotherapeutic strategies has been reported; IFN gamma has been recognized as an effective antimicrobial prophylactic cytokine in trauma [9]. Effects of fat intake [10] and Ornithine Alpha-Ketoglutarate preteen have been investigated in modulation of immune response [11].

Cimetidine was administrated in this study because the drug is well documented with recognized immune potentiating properties [12].

Cimetidine is a histamine receptor antagonist which blocks H_2-histamine receptor and usually is used to reduce gastric acid secretion and gastric ulcer treatment [13]. Cimetidine also blocks the activation of regulatory T cells and facilitates cell-mediated immunity (CMI) by CD8+ T cells [14]. On the other hand, histamine induces the production of inflammatory cytokines such as IL-6 and IL-1β. Furthermore, H_4 and H_2 receptors of histamine control histamine-induced interleukin-16 release from human CD8+ T cells [15]. Thus, Cimetidine may modulate the immunomodulatory effects of histamine [16]. In thermal injuries Cimetidine modulates edema formation by blocking of burn induced inflammation [17,18]. T cell activation and regulation, which have very important roles in prevention of infection and wound healing, are impaired in thermal injuries [19]. However, CMI boosting could be useful in the treatment of burn wounds and Cimetidine may be a good candidate for this strategy [20]. In this study, we investigated the effects of Cimetidine on T cell proliferation, activation and other features of immune system in post-burn patients.

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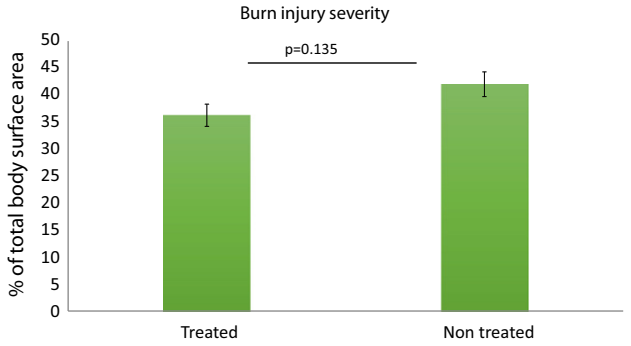

Fig. 1. Burn injury severity measured by total body surface area that defined by Wallace Rule of Nines, treated patients have received Cimetidine. Treated and nonretreated groups are match in terms of burn severity.

2. Material and methods

2.1. Samples

Peripheral blood samples were collected from twenty two healthy subjects and thirty-seven patients exposed to burn injuries that had severe acute phase burn injury with 20–60% of total body surface area. 18 of the patients were treated with Cimetidine and 19 untreated.

2.2. Drug

Cimetidine was purchased from Chemidarou Co. (Tehran, Iran). 15 mg/kg was chosen according to the standard procedures [21]. The drug was administered per 24 h for four days.

2.3. Immunophenotyping

For cell surface phenotyping, anti CD4, CD8, CD4/CD8, CD19, and HLA-DR (DAKO, Denmark) were used. We established the reference immunophenotypic pattern using standard procedures. In this study 100 μ of blood sample was treated as follows; each sample was immunostained with 10 μ mAbs directly conjugated with Fluorescein Isothiocyanate (FITC) or R-Phycoerythrin (RPE) in Q-Prep apparatus afterwards, in which three immunopreps were added automatically, 0.7 ml immunoprep A (Formic Acid 1.2 ml/L), 0.32 ml immunoprep B (Sodium Carbonate 6.0 g/L, Sodium Chloride 14.5 g/L, Sodium Sulfate 31.3 g/L) and 0.14 ml immunoprep C (Paraformaldehyde 10.0 g/L, Phosphate Buffer) 9Coulter. Each sample was then kept in 2–8 °C and dark for about 24 h [22].

2.4. Flow cytometry analysis

Cell samples were analyzed on a Coulter flow cytometer with serial filter configuration. The analysis was focused on the lymphoid areas of the forward and side scatters. Double stained cells were analyzed using Coulter software.

2.5. Proliferation assay

Five ml of peripheral heparinized bloods from Cimetidine treated and non-treated patients were collected and the lymphocyte was isolated by Ficoll hypaque. PBMC proliferation was measured by incorporation of [^{3}H]-thymidine. 2×10^5 PBMC from patients and healthy controls were stimulated with 5 μg/ml phytohemagglutinin (PHA) in RPMI 1640 medium supplemented with 2 mM/l glutamine, 10% heat inactivated AB+, 100 IU/ml penicillin and 100 mg/ml streptomycin in 96-well U-bottomed tissue culture plates and incubated in humidified CO_2 5% at 37 °C. After 3 days, 1 μCi [^{3}H]-thymidine was added to each well followed by further incubation for 18 h. The plates were harvested using Skatron A.S automatic harvester Flow Labs, Rockville MD and uptake of [^{3}H]-thymidine was determined in liquid Scintillation counter (Bekman). Proliferation responses were calculated by

$$\text{Stimulation Index} = \text{the increase in cpm in test/the increase in cpm of base lineCpm} = \text{count per minute.}$$

2.6. Statistics

In this study one-way analysis of variation (ANOVA) and Kruskal–Wallis nonparametric test and *T*-test analysis were employed using SPSS software.

3. Results

The initial stage of this work consisted an efforts aimed at obtaining an effective and acceptable stage of burn injuries which clearly displayed clinical signs of burn injury. The stage of burn used in this study was divided in two group so that the groups were matched for the extend of burn injury (Fig. 1). The results showed a significant suppression of immune system as indicated in Fig. 2. Injured patients display a significant reduction in the lymphocyte number comparing to control group as well as a significant change in surface markers of the immune cells comparing to the control group (Table 1).

3.1. Effect of Cimetidine on burn injury induced B cell immunosuppression

In order to assess the effect of Cimetidine on B cell status following burn injury, the protocol shown in Table 1 was employed for 37 patients

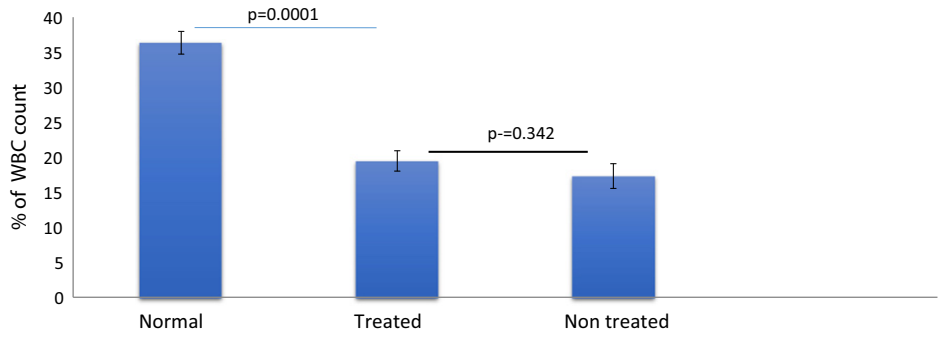

Fig. 2. WBC percent in two groups of patients and normal controls. Patients in treated and nontreated group showed significant WBC reduction.