

EFFECT OF SHORT TERM USE OF SEDATING AND NON-SEDATING ANTIHISTAMINES ON WOUND HEALING AND IMMUNE RESPONSE IN RATS

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ABSTRACT

Objective: The present study was designed to evaluate the suspected role of short-term use of sedating and non-sedating antihistamines on wound healing and immune response in animal model of experimentally-induced infected wound.

Method: Experimentally non-infected and infected wound were induced in rats which were allocated into 3 groups treated with either vehicle and served as control or with 0.88 mg/kg loratidine (LOR) or 2.25 mg/kg diphenhydramine (DPH); healing time, total WBC count, and immunoglobulin-G levels were measured after 15 days of treatment.

Results: Treatment with DPH prolongs healing time and decrease IgG levels only in infected wound model; meanwhile, both LOR and DPH decreased IgG levels with predominant effect for DPH in this respect.

Conclusion: DPH prolongs the time required for complete wound healing only in the presence of bacterial infection, which may be attributed to interference with cellular and humoral immune response, while LOR has no such effect.

Keywords: Loratidine, Diphenhydramine, Wound healing, Infected wound.

INTRODUCTION

Histamine is one of the monoamines {2-(imidazol-4-yl) ethylamine} with the broad spectrum of activities in various physiological and pathological situations. It is involved in aminergic neurotransmission and numerous brain functions (sleep/wakefulness, emotion, learning, memory, locomotor activity, nociception, food intake and aggressive behavior), secretion of pituitary hormones, regulation of gastrointestinal and circulatory functions, as well as inflammatory reactions and modulation of the immune response [1]. Undoubtedly, the fundamental pleiotropic regulatory character of histamine in cellular events is attributed to its binding to four subtypes of G-protein-coupled receptors (GPCR), designated H1, H2, H3 and H4 [2]. The ability of histamine to induce the production of cytokines such as interleukin-6 (IL-6) and IL-8 by endothelial cells [3,4] suggests that this mediator can act as an important inflammatory signal in addition to its well-recognized function as a vasoactive substance [5]. Histamine plays an important role in immune reactions, the coagulation cascade, and in various related inflammatory reactions, and activation of H2 receptors is known to accelerate cell proliferation, thereby affecting both lymphocyte and immune system response [6,7]. Many antihistamines (both H1 and H2 receptors blockers) are available in many countries without a prescription. However, many reports very well documented through direct evidence the association of antihistamines intake and enhanced susceptibility to bacterial infection [8]. The involvement of histaminergic signaling after tissue injury and wound healing is well documented, and the inability to resolve the extent of innate/acquired response at the site of injury due to the interference with histamine signaling can lead to poor wound healing, immune suppression and recurrent infections episodes [9]. The present study was designed to evaluate the suspected role of short-term use of sedating and non-sedating antihistamines on wound healing and immune response in animal model of experimentally-induced infected wound.

MATERIALS AND METHODS

Preparation of *Staphylococcus aureus* Suspension

Pure strain of pathogenic *Staphylococcus aureus* was isolated from pus discharge of patients in Baghdad Teaching Hospital, Medical City. From the culture of this pathogen, single isolated colony was selected and inoculated into 10 ml brain-heart infusion broth (OXOID, England) and incubated over night at 37°C. The resultant suspension was adopted to

contain 10⁹ cells/ml, and used to induce infected wound in rats by 0.5 ml of this suspension [10].

Animal model for non-infected and infected wound

Thirty-six rats of both sexes, 200-300 g body weight are used to induce skin lesions. They were housed in the animal house of the College of Pharmacy, University of Baghdad, under conditions of controlled temperature, with free access to food and water *ad libitum*. At the day of the experiment they were shaved at the abdomen area with razor blade about 4 cm in diameter; a sharp wound was made in the bases of three skin incisions of 2 cm long using a scalpel blade. Then the incised area was inoculated with 0.5 ml of *Staphylococcus aureus* suspension; the inoculum spread well on the injured area to induce infected wound. After 24 hrs, signs of infection were monitored, reported and verified by microbiological techniques; also direct signs of the inflammatory signs (edema, redness, swelling and pus formation) were documented [10].

Drug treatment and monitoring the response

The animals were allocated into two groups: group 1: Non-infected wound model, include 18 rats used to evaluate the difference in the effects of sedating and non-sedating antihistamines on wound healing and immune response in experimentally induced non-infected wounds as follow: **A:** Six animals treated with normal saline orally at the day of inducing wound and continued for 15 days and served as control (CTRL); **B:** Six animals treated orally with a single daily dose of 2.25 mg/kg Diphenhydramine (DPH) (SDI, Iraq), at the day of inducing wound and continued for 15 days; **C:** Six animals treated orally with a single daily dose of 0.88 mg/kg Loratidine (LOR) (Julphar, UAE) at the day of inducing the wound and continued for 15 days. group 2: Infected wound model, includes 18 rats used to evaluate the difference in the effect of sedating and non-sedating antihistamines on wound healing and immune response in experimentally induced infected wounds as follow: **A:** Six rats with infected wound treated with normal saline and 89.28 mg/kg Ceftriaxone (Mepha, Switzerland) given I.M once daily (*Staphylococcus aureus* sensitive to Ceftriaxone) at the day of inducing the infected wound; the antibiotic treatment continued for 7 days [10], while administration of normal saline continued for 15 days; **B:** Six animals with infected wound treated at the day of inducing the infected wound with a single daily dose of 2.25 mg/kg DPH for 15 days, and 89.28 mg/kg Ceftriaxone once daily for 7 days; **C:** Six animals with infected wound treated at the day of inducing the infected wound with

a single daily dose of 0.88 mg/kg LOR for 15 days and 89.28 mg/kg Ceftriaxone once daily for 7 days. Evaluation of response includes reporting the time required for complete healing within the specified group, in addition to the signs of inflammation including swelling, edema, redness and pus formation. Moreover, after animal scarification, blood samples were collected from all animals in all groups and divided into two parts, the first aliquot (1 ml) collected in EDTA tube for total and differential white blood cells count according to standard laboratory methods [11]; the second aliquot (5 ml) collected in plane tube for serum preparation to be used for the estimation of immunoglobulin-G (IgG) by radial immune-diffusion technique using ready-made kits (LTA s.r.l, Milano, Italy) for this purpose [12].

Statistical Analysis

Statistical evaluation of data was performed utilizing SPSS 19 software for Windows. Paired *t-test* was performed to compare between means. Differences were considered significant at *p* value <0.05.

RESULTS

Despite of the reported differences regarding the time required for complete wound healing in group 1 (Figure 1), they remain non-significantly different; although both types of treatment (LOR and DPH) slightly prolong the time required for complete healing, they demonstrated no significant differences in this respect.

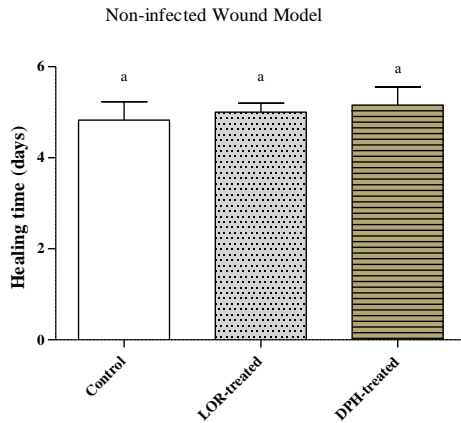


Fig. 1: Healing time of experimentally induced non-infected wound in rats; values with non-identical letters are significantly different (*P*<0.05).

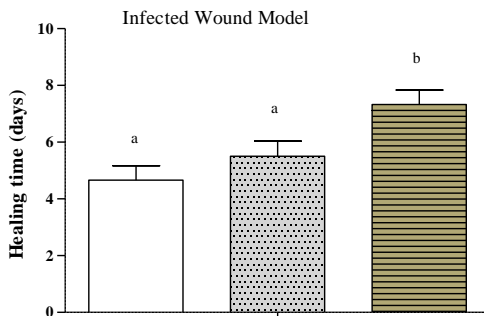


Fig. 2: Healing time of experimentally induced infected wound in rats; values with non-identical letters (a,b) are significantly different (*P*<0.05).

Figure 3 showed that treatment with LOR did not significantly affecting T-WBC count, while DPH significantly lowers T-WBC count (*P*<0.05) compared to both control and LOR-treated groups (35.5% and 19.5%, respectively). In the infected wound model, LOR demonstrates non-significant changes in T-WBC count compared to control, while DPH also decreased the value of WBC count significantly compared to

control and LOR-treated group (30.1% and 33.8%, respectively) (Figure 4).

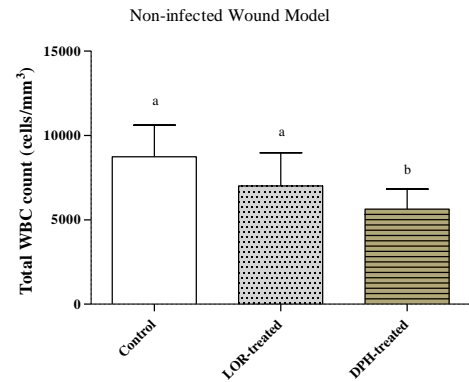


Fig. 3: Total WBC count in rats with experimentally induced non-infected wound; values with non-identical letters (a,b) are significantly different (*P*<0.05).

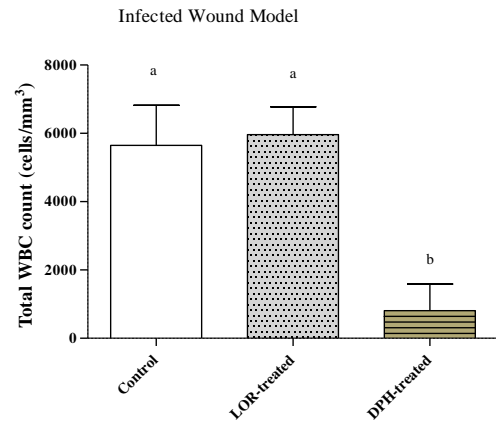


Fig. 4: Total WBC count in rats with experimentally induced infected wound; values with non-identical letters (a,b) are significantly different (*P*<0.05).

In figure 5, both LOR and DPH did not produce significantly different changes in the serum levels of IgG in the group of non-infected wound (group1); however, in infected wound model (group 2) both drugs produced significant decrease in serum IgG levels (33.8% for LOR and 57.8% for DPH) compared with control group. Moreover, DPH produced greater effect in this respect, and found to be significantly different compared to LOR (Figure 6).

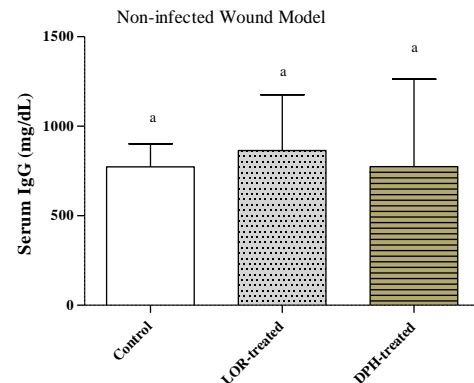


Fig. 5: Serum IgG levels in rats with experimentally induced non-infected wound; values with non-identical letters are significantly different (*P*<0.05).

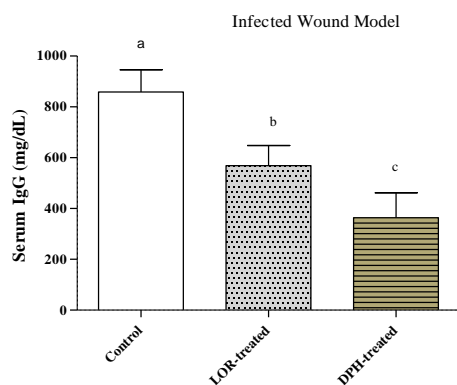


Fig. 6: Serum IgG levels in rats with experimentally induced infected wound; values with non-identical letters (a,b,c) are significantly different ($P < 0.05$).

DISCUSSION

Antihistamines are widely incorporated and used in a variety of cold preparations, many of them considered over the counter medications; so they are used extensively without medical supervision or control. There is substantial evidence that a defect in innate effector functions of phagocytes (neutrophils, monocytes and macrophages) plays an important role in the weakened resistance to pathogenic bacteria [12]. Improvement of the function of the immune system or not depressing it can be considered as an important factor in combating infections, through the use of antimicrobial drugs for both improving efficacy and not prolonging the time period needed for complete recovery from bacterial infection. Talreja *et al.* (2004) showed that histamine amplifies the immune-stimulatory effects of Gram-positive and Gram-negative bacterial cell wall components [13]. Several studies demonstrated the importance of the recruitment of inflammatory cells into wounds; in addition, they have been shown to act directly or indirectly on resident cells, there by regulating re-epithelialization, angiogenesis and myofibroblast differentiation [2]. The effect of sedating antihistamine in prolonging the time required for wound healing in the presence of pathogenic bacteria may be due to blocking the effect of histamine in amplifying the stimulatory effects of Gram-positive and Gram-negative bacterial cell wall components, or the antihistamine depresses the host's innate immunity [13]. The results of the present study demonstrate that the use of antihistamines whether sedating or non-sedating did not affect the time required for wound healing in non-infected wound model, while only the sedating antihistamines prolonged the time required for complete wound healing in infected wound model. Within few hours after tissue injury, inflammatory cells invade the wound area. They produce a wide variety of proteases as a defense against contaminating microorganisms and they are involved in the phagocytosis of cell debris [14]. Additionally, inflammatory cells are also an important source of growth factors and cytokines, which initiate the proliferation phase of wound repair [15]. Histamine is known to modulate the functions of many cell types, including monocytes/macrophages [16,17], eosinophils [18,19], T-cells [20], neutrophils [21] and endothelial cells [22]. The profound negative effect of short-term use of sedating antihistamine on WBC count in the absence or presence of infection in rats may be attributed to the effect of histamine on the chemotactic immune-modulatory response. The results showed that sedating antihistamine (DPH) decreased T-WBC count in both non-infected and infected wound model, and this effect is undesirable in the presence of bacteria or in immune-compromised patients, while the non-sedating antihistamine (LOR) did not show such effect. It has been previously reported that treatment with oral doses of the sedating antihistamine DPH, the H2R blocker cimetidine and H3/4R blocker thioperamide impairs optimal innate immune responses in severe murine bacterial sepsis. However, these adverse effects are not mediated by H1R, as mice deficient for H1R show similar rates of morbidity and mortality after cecal ligation and puncture as their wild-type controls. Similarly, the non-sedating antihistamine desloratadine affects neither morbidity nor mortality after cecal ligation and puncture [23]. Immunoglobulin-G (IgG), a major effector molecule of

the humoral immune response, accounts for about 75% of the total immunoglobulins in plasma of healthy individuals [24]. Antibodies of the IgG class express their predominant activity during a secondary antibody response. Thus, the appearance of specific IgG antibodies generally corresponds with the maturation of the antibody response, which is switched on upon repeated contact with an antigen. In comparison to antibodies of the IgM class, IgG antibodies have a relatively high affinity and persist in the circulation for longer time [25]. IgG can bind to many kinds of pathogens, for example viruses, bacteria, and fungi, and protects the body against them by agglutination and immobilization, complement activation (classical pathway), opsonization for phagocytosis, and neutralization of their toxins [26]. The present study demonstrated that treatment with LOR and DPH has no effect on plasma IgG levels in non-infected wound model, while in the infected wound model both LOR and DPH decreased the plasma IgG levels significantly compared to control, and the effect of DPH was significantly greater than that produced by LOR.

CONCLUSIONS

DPH prolongs the time required for complete wound healing only in the presence of bacterial infection, which may be attributed to interference with cellular and humoral immune response, while LOR has no such effect.

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