International Immunopharmacology 52 (2017) 176–184

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Concurrent administration effect of antibiotic and anti-inflammatory drugs on the immunotoxicity of bacterial endotoxins

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ARTICLE INFO

Keywords:
Anti-inflammatory
Bacterial endotoxins
Immunoglobulin
Immunotoxicity
P. aeruginosa
Dexamethasone

ABSTRACT

Pseudomonas aeruginosa (*P. aeruginosa*) is a gram-negative bacterium that causes a variety of diseases in compromised hosts. Bacterial endotoxins such as lipopolysaccharide (LPS) are the major outer surface membrane components that are present in almost all gram-negative bacteria and act as extremely strong stimulators of innate immunity and inflammation of the airway. This study was undertaken to determine the effect of combined administration of Gentamicin (GENT) as an antibiotic and Dexamethasone (DEXA) as an anti-inflammatory drug on some immunological and histological parameters. After determination of LD_{50} of *P. aeruginosa*, mice groups were injected with DEXA, GENT and lipopolysaccharide alone or in combination. Lipopolysaccharide single injection caused a significant increase of total leukocyte count, lymphocytes, neutrophils and levels of IgM and IgG. DEXA induced an increase of neutrophilia and lymphopenia. Immunological examination demonstrated that combined treatment has a significant effect of decreasing lymphocytes and IgG levels than single treatment does. Histological examination demonstrated that the inflammation of thymus, spleen, lymph node and liver decreases in mice that received combined treatment than those that received individual treatment. Concurrent administration of DEXA and GENT has a great effect on protecting organs against damage in case of endotoxemia.

1. Introduction

P. aeruginosa is an opportunistic pathogen that causes a wide range of acute and chronic infections [1,2]. Endotoxin or lipopolysaccharide (LPS) is a component of the outer membrane of gram-negative bacteria and it has been implicated as an important inducer of the local and systemic responses to such a bacterial infection [3]. It is excessively released during antibiotic therapy, and activates the immunological and inflammatory reaction [4]. However, in conditions where the body is exposed to bacterial endotoxin excessively (during severe infection and sepsis with gram-negative bacteria) or systemically (when endotoxin enters the blood stream “endotoxemia”), a systemic inflammatory reaction can occur, leading to tissue injury, metabolic and neuroendocrine changes, multiple organ damage and/or dysfunction, circulatory shock, and a potential death [5].

Glucocorticoids (DEXA) are important modulators of immune reactions. They are capable of antagonizing several effects of the bacterial endotoxin by inhibiting endotoxin-induced leukocyte activation, and producing cytokines as inflammatory mediators [6] Dexamethasone (DEXA) is a synthetic glucocorticoid used in both humans and animals [7].

Gentamicin (GENT) is an aminoglycoside antibiotic, which has a wide utility in many bacterial infections. It has a broad spectrum of activities against some common pathogens, both gram-positive and gram-negative. It has a strong activity against *P. aeruginosa* [8].

This study aims at evaluating the effect of administration of GENT and DEXA singly or concurrently on mice affected by LPS through assessment of various immunological and histological parameters.

2. Materials and methods

2.1. Animals

Adult male Swiss albino mice, (20–25 g) were obtained from the breeding colony at the animal house of the National Organization for Drug Control and Research [NODCAR], Giza, Egypt and were housed under controlled temperature [23 ± 2 °C], humidity [60 ± 10%], with light/dark (12/12 h) cycle. The animals were kept on standard diet laboratory chow and water *ad libitum*. Animal handling was in accordance with the guidelines and ethical procedures and policies approved by the Ethical Research Committee of Faculty of Science, Cairo University, Cairo, Egypt, which comply with the Guide for the

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http://dx.doi.org/10.1016/j.intimp.2017.09.006
Received 23 June 2017; Received in revised form 22 August 2017; Accepted 8 September 2017
Available online 18 September 2017

Table 1
Effect of administration of DEXA, GENT and LPS on Leukocytes profile in mice.

Animals groups	Total leukocyte count	% of change	Lymphocytes	% of change	Segmented neutrophils	% of change
A: Control	4537.5 ± 141.38		57.17 ± 1.83		34.66 ± 1.69	
B: DEXA	6133.3 ± 236.88**	35.17	51.67 ± 2.33	− 9.62	40.00 ± 2.00	15.40
C: GENT	3766.7 ± 162.62** □□ γγ	− 16.99	54.00 ± 2.92γγ	− 5.54	45.33 ± 2.72**	30.78
D: LPS	8266.7 ± 138.84** □□	82.19	67.67 ± 0.76** □□	18.36	49.00 ± 1.92**	41.37
E: DEXA + LPS	5375.0 ± 280.10 ** ΔΔ γγ ♠♠	18.46	50.17 ± 3.35γγ	− 12.24	61.00 ± 3.09** □□ γ ΔΔ	76.00
F: GENT + LPS	4075.0 ± 84.41** □□ γγ	− 10.19	41.33 ± 2.12** Δ γγ	− 27.70	53.50 ± 3.09** □□	54.36
G: DEXA + GENT	6025.0 ± 416.28** ΔΔ γγ ♠♠	32.78	39.17 ± 1.97** □ ΔΔ γγ Φ	− 31.49	58.50 ± 1.50** □□ Δ	68.78
H: DEXA + GENT + LPS	5883.3 ± 247.21** ΔΔ γγ ♠♠	29.65	48.00 ± 2.58 ** γγ	− 16.04	56.00 ± 3.18** □□	61.57

All data are represented as mean ± SE of 6 animals. % of change from control group. □ Significant difference ($P < 0.05$) between gr B and the next groups. Δ Significant difference ($P < 0.05$) between gr C and the next groups. γ Significant difference ($P < 0.05$) between gr D and the next groups. Φ Significant difference ($P < 0.05$) between gr E and the next groups. ** Highly significant difference ($P < 0.01$) between gr A and the next groups. □□ Highly significant difference ($P < 0.01$) between gr B and the next groups. ΔΔ Highly significant difference ($P < 0.01$) between gr C and the next groups. γγ Highly significant difference ($P < 0.01$) between gr D and the next groups. ♠♠ Highly significant difference ($P < 0.01$) between gr F and the next groups.

Care and Use of Laboratory Animals [9].

2.2. Bacterial challenge test

Lipopolysaccharides (LPS) act as endotoxins which are released in the circulation during infection. Endotoxins prepared in the laboratory according to the method of Kwapinski and El-Mosallamy et al. [10,11]. LD_{50} of *P. aeruginosa* LPS was determined using 56 adult male Swiss albino mice which were divided into 7 groups (8 mice per cage). Mice groups were injected intraperitoneally (i.p.) with different doses of LPS (100, 250, 350, 400, 500, 700 and 1000 μg). The number of surviving animals was recorded 48 h after the bacterial infection. The survival index was calculated according to Howard's et al. method [12] using the following formula. The curve was plotted and the dose at which LD_{50} occurred was calculated using the standard curve. The chosen dose is 300 μl/mice.

$$\text{Survival index} = \frac{\text{Number of survival animals}}{\text{Total number of animals}} \times 100$$

2.3. Drugs

- Dexamethasone (DEXA) sodium phosphate (Amriya Pharma Industries Egypt, 8 mg/2 ml ampoule). DEXA was used as an anti-inflammatory [13] and antiallergic drug [14]. It was used as a single intramuscular (i.m) injection in a dosage of 100 μl (contains 20 μg of DEXA).
- Garamycin sulfate (GENT) (Memphis Co. for Pharmaceutical and Chemical Industries, Egypt. 40 mg/ml ampoule). Each mice received an intramuscular (i.m.) dose of 100 μl (contains 100 μg of GENT).

The dose of each drug is equivalent to the human therapeutic dose as extrapolated relative to the body surface area tables according to the surface area ratio between man and mice [15].

2.4. Experimental design

Adult Swiss albino mice (48 male) were divided into 8 groups. (A) Normal control group. (B) Mice that received a dose of 100 μl DEXA. (C) Mice that received a dose of 100 μl GENT. (D) Mice that received a dose of 300 μl LPS. (E) Mice that received a dose of 100 μl DEXA and 300 μl LPS. (F) Mice that received a dose of 100 μl GENT and 300 μl LPS. (G) Mice that received a dose of 100 μl DEXA and 100 μl GENT. (H) Mice that received a dose of 100 μl DEXA, 100 μl GENT and 300 μl LPS.

At 48 h post treatment, blood samples were collected individually and stored at − 20 °C until they are used for the biochemical and immunological parameters estimation.

2.5. Immunological investigation

Erythrocyte (RBCs), total Leukocyte (WBCs) counts and different types of WBCs were determined by the method of Hayahoe and Flemans [16] using the Leishman staining technique. Measurement of serum Immunoglobulin level (IgG) was carried out by Bindarid and Nanorid Laboratory reagent kit using the immunodiffusion technique [17].

2.6. Histological examination

Different organs (spleen, liver, thymus and lymph node) were isolated and 3 to 4 μm paraffin sections were stained by Hematoxylin and Eosin according to Banchroft et al. method [18].

2.7. Statistical analysis

Statistical analyses were done for all data using one-way ANOVA. The student *t*-test was used to detect the differences between the control group and the other groups of animals. All values were reported as (mean ± standard error). Statistical significance differences were $P \leq 0.05$ and $P \leq 0.01$ [19].

3. Results

3.1. Determination of LD_{50} of P. aeruginosa

The survival index was calculated after 48 h and the 300 μg dose at which 50% lethality (LD_{50}) was determined.

3.2. Effect of administration of DEXA, GENT and LPS on leukocytes profile in mice

The LPS injection (gr D) induced a significant ($P < 0.01$) elevation in the total leukocyte count due to significant ($P < 0.01$) increase in lymphocytes and segmented neutrophils with % of change (PC) = 82.19, 18.36 and 41.37%, respectively, in comparison to normal levels of healthy mice (gr A) (Table 1 and Fig. 1). DEXA administration on healthy (gr B) or infected mice (gr E) caused a significant increase ($P < 0.01$) in total leukocyte count with PC = 35.17% and 18.46%, respectively. This may be due to the recorded increase in segmented neutrophils with PC = 15.40% and 76.00%, respectively. Meanwhile, the number of lymphocytes was not affected significantly by DEXA treatment in normal or infected mice with PC = − 9.62% and − 12.24%, respectively. On the other hand, administration of combination of DEXA and GENT (gr G) for normal mice induced a significant ($P < 0.01$) increase in segmented neutrophils which may lead to an increase in total leukocyte count with PC = 32.78% and 68.78%, respectively. Meanwhile, a concurrent treatment of DEXA and GENT to LPS injected mice (gr H) recorded a significant ($P < 0.01$) increase in