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

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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\textsubscript{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
Lipopolysaccharides (LPS) act as endotoxins which are released in the circulation during infection. Endotoxins prepared in the laboratory 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 LD50 occurred was calculated using the standard curve. The chosen dose is 300 μL/mice.

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

### Table 1

<table>
<thead>
<tr>
<th>Animals groups</th>
<th>Total leukocyte count</th>
<th>% of change</th>
<th>Lymphocytes</th>
<th>% of change</th>
<th>Segmented neutrophils</th>
<th>% of change</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: Control</td>
<td>4537.5 ± 141.38 μl</td>
<td>57.17 ± 1.83</td>
<td>34.66 ± 1.69</td>
<td>6133.3 ± 236.88 μl</td>
<td>51.67 ± 2.33</td>
<td>40.00 ± 2.00</td>
</tr>
<tr>
<td>B: GENT</td>
<td>3766.7 ± 162.62 μl</td>
<td>16.99</td>
<td>18.50</td>
<td>280.10 μl</td>
<td>39.15 ± 1.20</td>
<td>61.57 ± 3.09</td>
</tr>
<tr>
<td>C: LPS</td>
<td>8266.7 ± 138.84 μl</td>
<td>18.29</td>
<td>18.36</td>
<td>100 μl</td>
<td>12.24</td>
<td>76.00</td>
</tr>
<tr>
<td>D: DEXA + LPS</td>
<td>5375.0 ± 280.10 μl</td>
<td>18.46</td>
<td>18.36</td>
<td>100 μl</td>
<td>12.24</td>
<td>76.00</td>
</tr>
<tr>
<td>E: DEXA + GENT</td>
<td>4075.0 ± 84.41 μl</td>
<td>10.19</td>
<td>12.79</td>
<td>100 μl</td>
<td>12.24</td>
<td>76.00</td>
</tr>
<tr>
<td>F: GENT + LPS</td>
<td>6025.0 ± 416.28 μl</td>
<td>32.78</td>
<td>31.49</td>
<td>100 μl</td>
<td>12.24</td>
<td>76.00</td>
</tr>
<tr>
<td>G: DEXA + GENT</td>
<td>5863.3 ± 247.21 μl</td>
<td>29.65</td>
<td>27.70</td>
<td>100 μl</td>
<td>12.24</td>
<td>76.00</td>
</tr>
<tr>
<td>H: DEXA + GENT</td>
<td>5863.3 ± 247.21 μl</td>
<td>29.65</td>
<td>27.70</td>
<td>100 μl</td>
<td>12.24</td>
<td>76.00</td>
</tr>
</tbody>
</table>

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. γγ 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. γγγ 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.

#### 2.2. Bacterial challenge test

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.

#### 2.3. Drugs

- Dexamethasone (DEXA) sodium phosphate (Amriya Pharma Chemical 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

#### 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 immunofluorescence 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 ≤ 0.05 and P ≤ 0.01 [19].

#### 3. Results

#### 3.1. Determination of LD50 of P. aeruginosa

The survival index was calculated after 48 h and the 300 μg dose at which 50% lethality (LD50) 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 = 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...
total leukocyte count and segmented neutrophils with PC = 29.65% and 61.57%, respectively.

### 3.3. Effect of administration of DEXA, GENT and LPS on the levels of different immunoglobulins in serum

The data mentioned in Table 2 improved the mild significant

\[(P < 0.05)\] effect of DEXA (gr B) and GENT (gr C) on the immune response by increasing IgM level in comparison to its recorded normal level with PC = 63.58% and 54.06%, respectively. Moreover, combination of both DEXA and GENT immunization alone (gr G) triggered a good response as immunostimulants recording as they caused a highly significant \((P < 0.01)\) increase with PC = 89.51% referring to control group (gr A) and individual injection with DEXA (gr B) or GENT (gr C). Again, LPS-treatment (gr D) clarified that the infection mode stimulates a highly significant \((P < 0.01)\) increase in IgM secretion or with DEXA (gr E) which recorded no effect on infection with a very high PC = 196.01% and 207.69%, respectively. Meanwhile, GENT (gr F) has a great effect on the infection in inflammation and it decreases the IgM level with PC = 88.95% in comparison to LPS-treated group alone. On the other hand, combination of both DEXA and GENT treatment to LPS-treated mice (gr H) illustrates a mild decrease in IgM level during infection with PC = 176.55%. IgG profile was slightly affected by a mild significant increase after treatment singly with DEXA (gr B) recording with PC = −0.05% (Fig. 2).

![Fig. 1. Percentage of the change of the total leukocyte count, the lymphocytes and the segmented neutrophils in mice groups treated with DEXA, GENT and LPS singly or concurrently.](image)

![Fig. 2. Percentage of the change of serum IgM and IgG in mice groups treated with DEXA, GENT and LPS singly or concurrently.](image)

### Table 2

Effect of administration of DEXA, GENT and LPS on the levels of different immunoglobulins in serum.

<table>
<thead>
<tr>
<th>Animals groups</th>
<th>IgM % of change</th>
<th>% of change</th>
<th>IgG % of change</th>
<th>% of change</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: Control</td>
<td>119.87 ± 17.82</td>
<td>63.58</td>
<td>15,625 ± 1709.0</td>
<td>12.53</td>
</tr>
<tr>
<td>B: DEXA</td>
<td>196.08 ± 23.59*</td>
<td>54.06</td>
<td>15,617 ± 929.67</td>
<td>38.08</td>
</tr>
<tr>
<td>C: GENT</td>
<td>184.67 ± 11.94*</td>
<td>196.01</td>
<td>21,575 ± 1082.30**</td>
<td>5.92</td>
</tr>
<tr>
<td>D: LPS</td>
<td>354.83 ± 68.19**</td>
<td>88.95</td>
<td>12,413 ± 939.9**</td>
<td>38.08</td>
</tr>
<tr>
<td>E: DEXA + LPS</td>
<td>507.80 ± 39.41**</td>
<td>92.49</td>
<td>19,823 ± 1209.0**</td>
<td>28.04</td>
</tr>
<tr>
<td>F: GENT + LPS</td>
<td>226.50 ± 22.52**</td>
<td>89.51</td>
<td>18,887 ± 261.62**</td>
<td>5.92</td>
</tr>
<tr>
<td>G: DEXA + GENT</td>
<td>227.17 ± 19.28**</td>
<td>112.43</td>
<td>18,887 ± 261.62**</td>
<td>28.04</td>
</tr>
<tr>
<td>H: DEXA + GENT + LPS</td>
<td>331.50 ± 26.70**</td>
<td>176.55</td>
<td>16,550 ± 745.1**</td>
<td>28.04</td>
</tr>
</tbody>
</table>

All data are represented as mean ± SE of 6 animals. % of change from control group. * Significant difference \((P < 0.05)\) between gr A and the next groups. □ Significant difference \((P < 0.05)\) between gr B and the next groups. △ Significant difference \((P < 0.05)\) between gr C and the next groups. ◆ Significant difference \((P < 0.05)\) between gr E and the next groups. ■ Significant difference \((P < 0.05)\) between gr F and the next groups. ** Highly significant difference \((P < 0.01)\) between gr A and the next groups. ΔΔ Highly significant difference \((P < 0.01)\) between gr B and the next groups. Δγ 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. ΦΦ Highly significant difference \((P < 0.01)\) between gr E and the next groups. ♠♠ Highly significant difference \((P < 0.01)\) between gr F and the next groups. ♦♦ Highly significant difference \((P < 0.01)\) between gr G and the next groups.
3.4. Histological microscopic examination

The thymus revealed a significant decrease in the cortex/medulla ratio in the animals treated with DEXA (Fig. 3-2). Mild morphological changes were detected in animals treated with GENT (Fig. 3-3). LPS (Fig. 3-4) caused remarkable thickening in the cortex associated with heavy cellularity both in the cortex and medulla with congested blood vessels. Administration of DEXA in animals treated with LPS (Fig. 3-5) markedly reduced the inflammatory response and similar observations were detected in animals treated with LPS-GENT (Fig. 3-6). The concomitant administration of DEXA and GENT in animals treated with LPS-GENT (Fig. 3-8) markedly ameliorated the inflammatory response to the thymus tissue of animals treated with LPS.

In mice treated with DEXA (Fig. 4-2), the periarтерiolar lymphoid sheath PALS region appears typically “moth-eaten” due to a moderate degree of scattered lymphocyte apoptosis that appears as picnotic lymphocytic cells; also the presence of apoptotic cells within the periphery of the marginal zone could be recognized. The proportion of white pulp underwent a significant decrease and the size of splenic lymphoid follicles diminished. GENT (Fig. 4-3) caused mild alteration in the morphometry of the spleen. LPS treated animals (Fig. 4-4) have got severe inflammatory responses in the spleen represented as increased cellularity both in the white and red pulps, megakaryoblasts and congested blood vessels. When treated with DEXA or GENT, these LPS treated groups were immunomodulated (Fig. 4-5 and -6, respectively). Concomitant administration of DEXA and GENT in LPS treated animals (Fig. 4-8) showed mild improvement of inflammatory responses.

Histological analysis of control animals’ lymph nodes (Fig. 5-1) showed normal follicular lymphocytic areas adjacent to a compact paracortex with no evidence of necrotic lesions or lymphoid hyperplasia. The effect of DEXA on lymph nodes (Fig. 5-2) was demonstrated...
as lymphocytic depletion. The effect of GENT was less pronounced in lymph nodes (Fig. 5-3). LPS injected group (Fig. 5-4) showed increased immature germinal centers in the nodes, hyperplasia, and multiple necrotic foci located at the edge of the lymph nodes and close to the germinal centers or in the paracortex areas of necrosis containing neutrophils, eosinophilic amorphous material, and many apoptotic nuclei. DEXA markedly protected the lymph nodes from inflammatory reaction of LPS (Fig. 5-5). Similar effect was produced by GENT (Fig. 5-6). Concomitant administration of DEXA and GENT in LPS treated animals restored the normal structure of the lymph nodes (Fig. 5-8).

Liver of animals in the control group (Fig. 6-1) showed normal hepatic architecture, where the hepatocytes are arranged around the central vein and alternate with blood sinusoids. Each hepatic cell possesses a limiting membrane, centrally placed large nucleus and prominent nucleoli. DEXA administration (Fig. 6-2) resulted in severe microvesicular steatosis selectively localized to the periportal areas. Microscopic examination of animal livers treated with GENT (Fig. 6-3) depicted marked hepatocellular changes, dilated and congested sinusoids with hemorrhage. GENT (Fig. 6-3) induced injuries in the liver with a severe level of leukocytes infiltration and hydropic degeneration of hepatocytes. LPS (Fig. 6-4) caused focal necrosis with neutrophil infiltration in the liver, areas of hepatocyte apoptosis and steatohepatitis. DEXA administration markedly prevented LPS-induced liver injury (Fig. 6-5). GENT did not show a comparable effect (Fig. 6-6). Co-administration of DEXA and GENT in LPS treated animals ameliorated the inflammatory outcome of LPS (Fig. 6-8).

3.5. Discussion

In the current study, LPS injected group, morphological changes were associated with liver injury such as confusion of hepatic lobule structure with inflammatory cell infiltration, serious vacuolation of
hepatic cells and necrosis. In contrast, these pathological changes were significantly attenuated in DEXA treated infected group. These results are agreed with the study of Wei et al. who reported that dexamethasone attenuates LPS-induced liver injury by affecting GITRL in Kupffer cells [20]. In the current study, the administration of DEXA and GENT concurrently to LPS treated infected group showed the same attenuation effect.

LPS has been reported to induce protein synthesis in B lymphocytes [21], and has enhanced T lymphocyte proliferation [22] by an unknown molecular mechanism. Similar observations were detected in the current study where massive number of leukocytes and megakaryoblasts were shown in the congested red pulps of spleen of mice injected with LPS. The current study proved that when mice were injected with DEXA, they showed a non-significant change in lymphocytes and a non-significant increase in neutrophils. Jeklova et al. reported that after a one-day administration of DEXA [23], a marked lymphopenia was found in percentage and absolute number, a significant decrease was clear in the percentage and absolute number of monocytes, eosinophils and basophils and significant neutrophilia.

The current study showed leukocytosis associated with significant increase in neutrophils percentage in LPS treated animals. These results agree with Chaves et al. who mentioned that acute bacterial infection was associated with positive blood cultures, higher WBC counts, and higher percentages of neutrophils [24]. In the current study, the absolute numbers of neutrophils, lymphocytes and total leukocyte count were markedly decreased after DEXA treatment. Similar observations were recorded by Garcia-Porrua et al. [25] and Detilleux [26] who illustrated that once an inflammatory response of bacterial infection has started in response to a pathogen, one observes a massive influx of blood polymorphonuclear neutrophils (PMN). Kohno et al. recognized leukocytosis and neutrophilia in bacterial infections such as *P. aeruginosa* [27]. In the current study, the absolute numbers of neutrophils,
lymphocytes and total leukocyte count were markedly decreased after DEXA treatment compared to LPS alone. It seems that DEXA inhibits acute inflammation. Again, these findings agree with the work results of Yi et al. who reported that dexamethasone inhibits the LPS-initiated vascular leak of plasma proteins and inhibit LPS-initiated emigration of neutrophils and lymphocytes [28]. Fauci and Dale reported that the anti-inflammatory effect of DEXA in LPS-inhibited acute inflammation appears to be mediated by the down regulation on a broad spectrum of proinflammatory molecules [29].

This study was associated with depletion in white pulps of the spleen, showing lymphoid depletion in the medullary portion of the spleen and lymphoid depletion in the cortical follicles of the lymph node after DEXA, GENT administration singly or concurrently with each other. Chung et al. reported that in mice, DEXA administration caused an increase in the percentage of radiolabeled lymphocytes in bone marrow [30]. This was associated with the decrease of lymphocytes in the mesenteric and regional lymph nodes and the spleen. This finding agreed with our results in which marked degeneration and reduced cellularity in the thymus, the spleen and the lymph node sections in mice group injected with DEXA.

Corticosteroids have at least three effects on the immune system cells: destruction, inhibition of function and redistribution [31]. DEXA, is a pharmacologic glucocorticoid, strongly inhibits polymorphonuclear emigration [28]. Issekutz et al. reported that glucocorticoids cause alteration of various leukocyte cells subset kinetics in blood that may affect their accumulation at the inflammatory area [32]. Moreover, DEXA treatment could greatly reduce the number of B cells in the spleen, but the bone marrow B-cell compartment was quite resistant to DEXA [33]. Roth et al. reported an increased release of neutrophils from bone marrow following the administration of corticosteroids [34]. Jain explained that glucocorticoids cause neutrophilia primarily by inducing the increased release of neutrophils from the bone marrow.

Fig. 6. T.S of the liver of control and different treated groups of mice (H & E ×40). 1: (gr A) Normal structure of the central vein (cv) and hepatocytes (h). 2: (gr B) Massive no. of inflammatory cells infiltration with dilatation in the portal vein (pv). 3: (gr C) Severe dilatation in the central vein (cv). 4: (gr D) Focal necrosis in the hepatic parenchyma (n) and congestion in the central veins (cv). 5: (gr E) Dilatation in the central (cv), portal vein (pv) and bile duct (bd). It shows karyocytomegaly and multiple double nuclei in the hepatocytes. 6: (gr F) Dilatation of portal (pv) and central vein (cv) and degeneration in the hepatocytes (d). 7: 8: (gr G & H) Diffused kupffer cells proliferation in between the hepatocytes (arrow).
reserve through the circulation [35]. He added that DEXA synthetic glucocorticoid-induced lymphopenia is attributed to lympholysis in blood, DNA damage, and increased shift of lymphocytes from the blood to other body compartments. Therefore, lymphocytes in circulation were decreased. Thanasak et al. reported that these changes in the number of circulating lymphocytes and lymphocyte subsets are thought to be the effects of glucocorticosteroid on the expression of lymphocyte adhesion molecules that mediate cell-to-cell interactions and leukocyte extravasations [36]. Such a decrease in expression could impair lymphocyte adhesion to lymphatic vessels in the tissues with a consequent decreased re-entry into the circulation.

The in vitro LPS-induced IgM response of the residual B cells from both the spleen and the bone marrow and their capacity to switch from IgM to IgG and IgA secretion were not affected. These data indicate that DEXA can decrease the total number of B cells but not the functional capacity of the residual LPS-reactive B cells [33]. Previous studies revealed that antibody formation to the T-dependent antigen sheep red blood cells and the background IgM, IgG and IgA synthesis in the bone marrow were quite resistant to massive doses of DEXA, whereas the response of the spleen was greatly reduced [37]. In agreement with our results Shannon et al. reported that DEXA enhanced IgG level [38].

Patients with chronic P. aeruginosa infection have higher concentration of antibodies directed against multiple P. aeruginosa protein antigens, including cell surface and secreted exoproducts, which indicates that these are made during infection [39]. IgGs are produced in a very high titer against specific P. aeruginosa antigens during a chronic infection and can be used as diagnostic indicators of the progression of the infection [40]. IgM antibodies are the primary antibody based immune reaction upon stimulation by bacterial antigens such as LPS [41,42]. These results agreed with the present study as IgM and IgG levels were found to have increased significantly higher after LPS injection which marked a highly significant increase of serum globulin after LPS injection.

Antibiotics, nonsteroidal anti-inflammatory drugs and glucocorticoids are used in the treatment of endotoxemia. The increased level of nitric oxide (NO) was inhibited by low and high dose of DEXA. Renal damage was inhibited and hepatic damage was partially inhibited after the treatment with low and high dose of DEXA [43]. These results agreed with our results as sections in the liver of mice injected with DEXA showed massive number of inflammatory cells infiltration in the portal area with dilatation in the portal vein and no significant change of IgG after DEXA administration.

In the current study, concurrent administration of GENT and DEXA to mice injected with LPS caused a highly significant increase in IgM and a non-significant increase of IgG instead of a highly significant increase of IgG after individual DEXA injection. This was indicated by lymphoid hyperplasia in the thymus, the spleen and the lymph node.

Tsui et al. reported that 24 h after LPS injection, large foci necrosis as well as inflammatory cell infiltration was observed in livers from wild-type mice [44] which were compatible with the present results as focal necrosis in the hepatic parenchyma and the congestion in the central veins that were observed in the liver 48 h after LPS.

A variety of studies have shown that corticosteroid administration on sensitive species like mice, rats, hamsters and rabbits severely decrease the number of viable nucleated cells in the thymus, the spleen, and the lymph nodes; and the most severely affected being thymus [45,46,47]. They have presented evidence that only a minor part of all peripheral leukocytes are redistributed to the bone marrow after corticosteroid treatment. Since all other tissues show severe depletion, the latter must be mainly due to destruction. Drobbin reported that DEXA does not impair the antibacterial efficacy of GENT for P. aeruginosa. This supports the role of DEXA as an otoprotectant with aminoglycoside therapy [48].

3.6. Conclusion

In conclusion, concurrent administration of DEXA and GENT in LPS treated animals ameliorated the inflammatory outcome of LPS as it has an effect in protecting organs against damage in case of endotoxemia. This protection occurred through decreasing liver damage and serum total protein elevation. As single administration of GENT to LPS infected mice caused liver injury. Although concurrent administration of DEXA and GENT showed protective properties in case of endotoxemia, it could be recommended that further investigations are required to determine those effects on physiological, immunological and histological parameters.

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References
