

Effects of Dexamethasone on Rats Infected with *Pasteurella multocida*

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Abstract: Two groups of Sprague-Dawley adult male rats each consist of 8 animals were used throughout the experiment. All rats were subcutaneous inoculated once with 10^8 cfu/ml of *Pasteurella multocida* strain PMB 202. In addition, Group 1 animals also received intra-muscular injection of dexamethasone (1.6 mg/animal/day) once daily for 9 days. Blood was collected from all animals before and after inoculation once daily for 9 days, for total and differential leucocytes count. Rats were scarified at day 9 for swabs culture and histological sections of liver, spleen and lung. In dexamethasone-treated animals (Group 1), total leucocytes count decreased after 24 hours (day 1) post-infection and then leucocytosis at day 4 (96 h), then leucopenia at day 8 and 9. Differential leucocytes count revealed gradual neutrophilia and lymphopenia at 120 h post infection (maximum), then neutropenia and lymphocytosis at day 7 and 9. Swab cultured from liver, spleen, and lung revealed positive for *P. multocida* and *Staphylococcus aureus*. Histological sections of such organs showed abscess formation surrounded by neutrophils. In *Pasteurella multocida* inoculated animals (Group 2), total leucocytes count started to increase gradually at day 1 and 2, then decreased to normal level at day 3 and leucocytosis at day 4 (maximum) and then gradual decreased to reach the normal level at day 9. Differential leucocytes count revealed, neutropenia and lymphocytosis at 24 hour post infection, and remain with the same level reaching maximum at day 9 with atypical lymphocytes. Swab cultured from liver, spleen and lungs showed negative for bacterial isolation. Histological sections of such organs revealed erythrocyte infiltration and mild infiltration of inflammatory cells.

Key words: *Pasteurella multocida*, rats, dexamethasone, histology, hematology

Introduction

Pasteurella multocida are not effectively controlled by immunization are important pathogens of most animals including humans (Rhoades and Rimler, 1989; Lukban and Baker, 1995; Machiels *et al.*, 1995 and Poumarat *et al.*, 2001). Thus *P. multocida* produce hemorrhagic septicemia in cattle as well as infections in buffalo, sheep, swine, cats, dogs, chickens and most laboratory animals (Chanter and Rutter, 1989; Christensen *et al.*, 1998; Donachie, 2000; Muhairwa *et al.*, 2001 and Rubies *et al.*, 2002).

Histologically, infection with *P. multocida* reveal, acute hepatitis with necrotic foci in the parenchyma (Glavits and Magyar, 1990) ovarian abscess (Johnson and Wolf, 1993); abscess complicating a femoral vein thrombosis (Petermann *et al.*, 1993); brain abscess (Li *et al.*, 1994) chronic lung abscess (Machiels *et al.*, 1995); tuboovarian abscess (Lukban and Baker, 1995); splenic abscess (Talbot *et al.*, 1995).

A variety of studies have shown that corticosteroid administration to sensitive species like, mouse, rat, hamster and rabbit can severely decrease the number of viable nucleated cells in thymus, spleen, and lymph nodes, the most severely affected being the thymus (Calman, 1972 and Dracott and Smith, 1979). Corticosteroids have at least three effects on cells of immune system: destruction, inhibition of function, and redistribution (Claman, 1972; Bach and Stroma, 1985). The susceptibility of these effects depends on the animal species and cell type, and its location, physiological maturity and state of activation (Haynes and Fauci, 1978 and Batch and Strom, 1985). A single and multiple injections of mice with the synthetic corticosteroid dexamethasone sodium phosphate have contrasting effects upon the antibody production (Benner *et al.*, 1978; Benner and Van Oudenaren, 1979) and background immunoglobulin production (Sabbele *et al.*, 1983) in spleen and bone marrow. Dexamethasone has also been used to mimic stress resulting in more severe lesions than in the absence of dexamethasone treatment (Chiang *et al.*, 1990).

The present study was undertaken to determine, the effect of dexamethasone on the hematological and histological parameters of rats experimentally infected with *P. multocida* and the rate of infection.

Materials and Methods

Experimental Animals: Pathogen free adult male *Sprague Dawley* rats aged 10 week-old weighing 180-200 grams were used throughout the experiment. Animals were separated into 2 groups of 12 rats each caged individually and were housed in isolation room provided by Faculty of Medicine, University Malaya. Water and sterilized food were supplied ad libitum.

Dexamethasone Injection: A dose of 1.5mg/rat/day (0.5 ml) dexamethasone (dexamethasone sodium phosphate, Intervet International B.V. Boxmeer-Holland) was injected intramuscularly to Group 1 animal once daily for 9 days. Group 2 animals received appropriate quantity of PBS (0.5 ml) once daily for 9 days.

Bacterial Culture and Rat Inoculation Procedure: *Pasteurella multocida* strain PMB 202 serotype was isolated by the Department of Medical Microbiology, Faculty of Medicine, University Malaya. *P. multocida* was grown in Brain Heart Infusion (BHI) broth for 24 hours at 37°C in a shaker bath. *P. multocida* was washed twice with PBS and re-suspended in PBS to give estimated concentration of bacteria between 10⁸-10⁹ live organisms/ml using Hemocytometer chamber. Each rat from both groups was inoculated with 1 ml of bacterial suspension subcutaneously once.

Hematological Parameters: Blood was collected (rat's tail) in EDTA tubes from all animals prior (0 hour) and after bacterial inoculation once daily for 9 days.

Total Leucocytes Count: Total leucocytes count were performed from the EDTA blood by deliver 0.95 ml of whit cell diluting fluid (Turk's solution) into a test tube, add 0.05 ml blood into the tested tube. Leucocytes count were made using hemocytometer and viability assessed by trypan blue exclusion..

Differential Leucocytes Count: Thin smear were prepared from EDTA blood and stained with Wright's stain and the differential whit blood cells counts were determined by a standard method described by Dacie and Lewis (1975).

Microbiological Parameter: All rats were sacrificed at day 9, and swabs cultured were taken aseptically from liver, spleen, and lungs for any bacterial isolation.

Histological Parameters

Histology and Preparation of Tissue Section for Analysis: Tissues such as liver, spleen and lungs of sacrificed rats were fixed immediately in 10% buffer formalin embedded in paraffin, section at 5 µm and stained with Hematoxylin and Eosin stain.

Results

Clinical Signs: Dexamethasone-treated rats were alert at 24 and 48 hours post-infection, but at day 3 to day 9 animals showed a roughened coat, variable degree of depression and 3 rats out of 12 were dead throughout the experiment. Reduction in the consumption of water and food was also noted. These signs increased in severity with the duration of experiment. However, *P. multocida* inoculated animals does not show such signs.

Blood Parameters: In dexamethasone-treated animals, total leucocytes count showed leucopenia after 24 hours post-infection and then leucocytosis gradually to reach maximum at day 4 (96 h), then decreased gradually to reach normal count at day 7 and then started leucopenia at day 8 and 9 (Fig. 1). Differential leucocytes count revealed gradual neutrophilia and lymphopenia to reach maximum at day 5 (120 h), then neutrophil gradually decreased and lymphocyte increased to reach maximum at day 7. Neutrophil and lymphocytes returned to normal at day 8, then neutrophil decreased and lymphocytes increased at day 9 (Fig. 2). Swab cultured from liver, spleen, and lungs revealed positive for *Pasteurella multocida* and *Staphylococcus aureus*. Histological sections of such organs showed abscess formation surrounded by neutrophils.

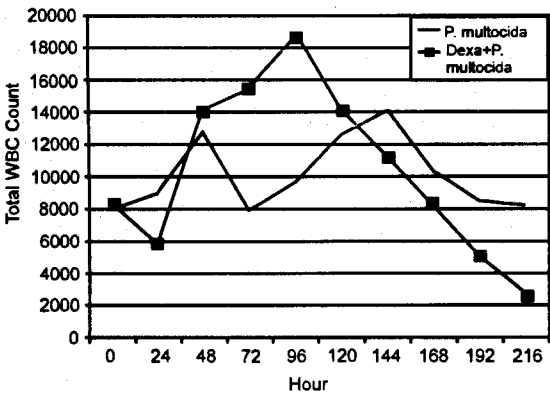


Fig.1: Effects of *P. multocida* alone or in combination with Dexa on Total WBC count

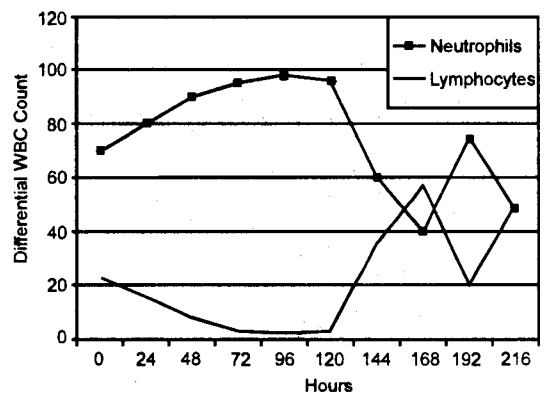


Fig.2: Effects of *P. multocida* in combination with Dexa on Differential WBC count

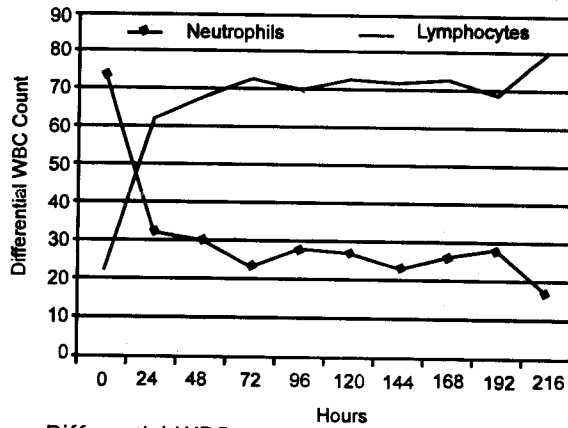


Fig.3: Effects of *P. multocida* on Differential WBC count

cida inoculated animals (Group 2), total leucocytes count started to increase gradually at day 1 and 2, then decreased to normal level at day 3 and leucocytosis at day 4 (maximum) and then gradual decreased to reach the normal level at day 9 (Fig. 1). Differential leucocytes count revealed, neutropenia and lymphocytosis at 24 hours post infection, then neutrophils started slightly to decrease, and lymphocyte began slightly to increase with atypical lymphocytes, to reach maximum at day 9 post infection (Fig. 3). Swab cultured from liver, spleen and lungs showed negative for bacterial isolation. Histological sections of such organs revealed erythrocyte infiltration and mild infiltration of inflammatory cells.

Microbiological Parameter: The microorganisms isolated from the liver, spleen and lungs in dexamethasone-treated group were, *P. multocida*, and *S. aureus*. However, no bacteria were isolated from swab cultured in rats inoculated with *P. multocida* only.

Gross Lesions: Dark red patches were seen only in the lung, spleen and liver of dexamethasone-treated animals, and the affected parts appear firm. The effect of *P. multocida* infection in rats indicated that severe lesions were produced in immunosuppressed-rats compared to animals inoculated with *P. multocida* only. Also in dexamethasone-treated group 3 rats were dead throughout of the experiment. No macroscopic lesions were found in *P. multocida* inoculated animals

Histological Parameter: *P. multocida* inoculated immunosuppressed-rats was found to produce severe lesions. The histological lesions of liver, spleen and lung of sacrificed rats in dexamethasone-treated animals consist of focal necrosis (abscess) of the epithelium surrounded by accumulation of inflammatory cells mainly neutrophils and the capillaries were congested (Plate 1). Tissue sections from *P. multocida* inoculated animal revealed mild to moderate inflammation with infiltration of erythrocytes and mild inflammatory cells (Plate 2). There were moderate to strong correlations between the clinical sign scores and the severity of lesions between both groups.

Discussion

The results of the present study showed that *P. multocida* were isolated from abscesses in liver, spleen and lungs in dexamethasone-treated group. Similarly, *P. multocida* were isolated from, the pus of a brain abscess following chronic purulent otitis, chronic lung abscess; splenic abscess; tuboovarian abscess and from *P. multocida* abscess complicating a femoral vein thrombosis in human (Petermann *et al.*, 1993; Li *et al.*, 1994; Lukban and Baker, 1995; Machiels *et al.*, 1995 and Talbodec *et al.*, 1995); and from ovarian abscesses, and necrotic foci in the parenchyma of liver in rabbits (Glavits and Magyar, 1990; Johnson and Wolf, 1993).

This study demonstrate that infection of adult rats with *P. multocida* renders them more susceptible to secondary infection following immunosuppression with dexamethasone as determinant by isolation of *Staphylococcus aureus*. Enhanced susceptibility to secondary infections in dexamethasone-treated rats revealed dexamethasone-mediated effects on both cell-mediated and humoral immunity.

In dexamethasone-treated animals, a relationship between the recovery rate of *P. multocida*, *S. aureus* and severity of necrotic lesion of internal organs was noted in the present investigation.

A variety of studies have shown that corticosteroid administration to sensitive species like mouse, rat, hamster and rabbit can severely decrease the number of viable nucleated cells in the thymus, spleen, and lymph nodes, the most severely affected being the thymus (Claman, 1972; Dracott and Smith, 1979). This is with the consistence of the

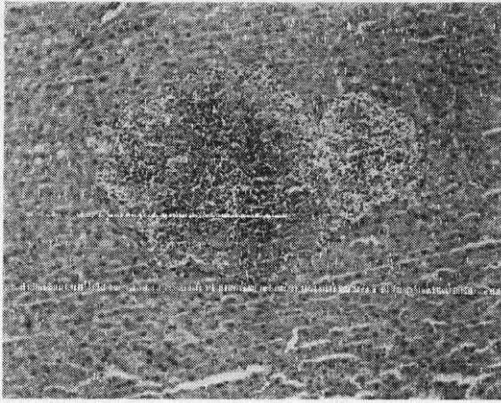


Plate 1A
Histological section of liver in dexamethasone-treated group and stained with H and E revealed abscess formation surrounded by neutrophils

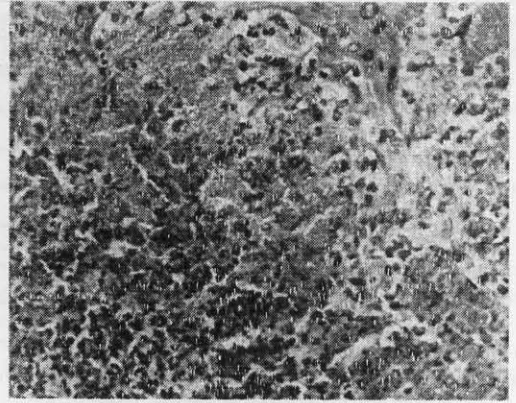


Plate 1B
High magnification of abscess area showing inflammatory cells

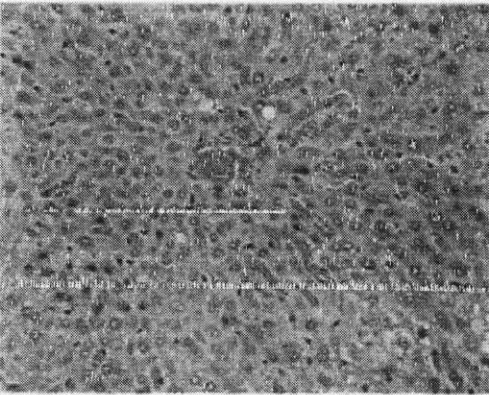


Plate 1A
Histological section of liver in *P. multocida* inoculated rats and stained with H and E. area



Plate 1B
High magnification of erythrocytes infiltrating

result of the present study.

Glucocorticoids cause alteration of various leukocyte cell subset kinetics in blood that may affect their accumulation at inflammatory sites (Issekutz and Issekutz, 1991). This alteration includes increased lymphocyte concentration in bone marrow and their depletion in blood (Fauci, 1975). On the other hand, steroids elevate blood neutrophil counts (Burton *et al.*, 1995) but cause monocytopenia (Steer *et al.*, 1997).

The lymphocytopenic effects observed here are in agreement with previous findings with prednisolone (Milad *et al.*, 1994 and Chakraborty *et al.*, 1999). Glucocorticoids affect both circulating and total number of monocytes and macrophages (Boumpas *et al.*, 1991). Glucocorticoids decrease lymphocyte numbers primarily through redistribution to bone marrow, spleen, lymph nodes, and thoracic duct. Glucocorticoids also activate a process known as programmed cell death, or apoptosis, especially in thymocytes and immature lymphocytes (Cohen, 1992). Glucocorticoids inhibit T-lymphocyte proliferative responses to both soluble and cellular antigens (Gillis *et al.*, 1979).

Our result showed that in dexamethasone-treated animals there were neutrophilia. Burton *et al.* (1995) reported pronounced neutrophilia after dexamethasone treatment, which may be the result of down-regulation of adhesion receptors, L-selection, and CD18 expression on circulation neutrophils. Glucocorticoids administration leads to an increase in the circulating pool of neutrophils due to premature release from bone marrow, demargination from vascular endothelium, and increased circulating half-life (Schleimer, 1990 and Lomas *et al.*, 1991)

Glucocorticoids also inhibit mononuclear cell production of chemotactic stimulants, including cytokines, prostaglandins, and leukotrienes, further reducing neutrophil migration. Neutrophil activation may be reduced as

well, possibly through effects on mononuclear cell production of IL-8 (Seitz *et al.*, 1991).

The results of the present study showed that 3 rats died out of 12 in dexamethasone-treated animals and infected with *P. multocida*, the histological section of sacrificed rats showed abscess formation in liver, spleen and lungs indicated that *P. multocida* could establish themselves and replicate freely and spread through the blood and ultimately damage tissues that could cause the death. These findings correlate with those reported by Collins and Woolcock (1976) and Smith *et al.*, (1981), who suggested that death of mice from *P. multocida* resulted from an overwhelming septicemia and extensive tissue damage.

From the present study, it is concluded that dexamethasone-treated rats produced more severe necrotic lesions and higher morbidity and greater severity of lung, liver, and lesions than *P. multocida* alone

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