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A Preliminary Study of Evaluation of the Role of Interleukin-1β (IL-1β) and Effects on Post-Treatment in Goats Experimentally Induced for Mannheimiosis

Fathin FAAHIMAAH ABDUL HAMID[1], Mohd FARHAN HANIF REDUAN[1], Jasni SABRI[1], Faez FIRDAUS JESSE ABDULLAH[2], Mohammed NAJI ODHAH[1], Nur ATHIRAH BINTI ABDUL MANAF[1], Mohd JEFRI NORSIDIN[2], Siti NOR CHE YAHYA[1], Intan NOOR AINA KAMARUZAMAN[1], Abd RAHMAN AZIZ[1], Nur ZUL IZZATI MOHD RAJDI[1]

[1]Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, Pengkalan Chepa, Kelantan, Malaysia

[2]Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine, Universiti Putra Malaysia, Selangor, Malaysia

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ORCID IDs of the authors: F.F.A.H. 0000-0002-7098-6608, M.F.H.R. 0000-0003-0815-8761, J.S. 0000-0002-9552-4776, F.F.J.A. 0000-0001-8119-3331, M.N.O. 0000-0001-9650-7145, N.A.B.A.M. 0000-0001-5966-4141, M.J.N. 0000-0002-2524-2669, S.N.C.Y. 0000-0003-4088-9206, I.N.A.K. 0000-0002-4702-5323, A.R.A. 0000-0001-8042-0862, N.Z.I.M.R. 0000-0002-1182-1969.

Abstract

Pro-inflammatory cytokines are necessary for an inflammatory response to infection or tissue injury. Interleukin-1β (IL-1β) is a pro-inflammatory cytokine involved in the pathophysiology of acute lung damage and respiratory distress syndrome. The study aims to evaluate the effect of treatments on the levels of IL-1β in goats induced with Mannheimiosis. Twenty male goats were divided into five groups ($n=4$). All groups except group 1 (negative control) were inoculated intranasally with *Mannheimia haemolytica* (10^7 CFU/mL). Goats in group 2 served as positive control. Goats in groups 3 and 4 were treated with an antimicrobial (oxytetracycline, SID) on days 6 and 9 of post-infection and an anti-inflammatory drug (Flunixin meglumine, BID) for 5 days of post-infection, respectively. Goats in group 5 received both treatments. Blood samples were collected via jugular vein at 24 hours, days 5, 9, and 11 of post bacteria inoculation for interleukin-1β analysis. The levels of IL-1β within 24 hours and day 5 of inoculations were significantly increased ($p < .05$) in groups (2, 3, 4, 5) compared to the negative control. On days 9 and 11, the levels of IL-1β in groups 2 and 3 remained significantly higher ($p < .05$) than in other groups. These findings indicated a synergistic effect of antibiotic and anti-inflammatory drugs combination in reducing the levels of IL-1β in the blood in goats induced with *Mannheimia haemolytica*.

Keywords: Biomarkers, goats, mannhemiosis, pneumonia, pro-inflammatory cytokines

Introduction

Cytokines are tiny secreted proteins released from cells that specifically influence cell connections and communications (Zhang & An, 2007). Pro-inflammatory cytokines are necessary to initiate an inflammatory response when responding to infection or tissue injury. These cytokines trigger each other into a signaling cascade, hence potentiating pathogenic effects synergistically. Measurements of serum cytokines provide clues to the underlying inflammatory damage processes that have taken place. In addition, they may serve as diagnostic, prognostic, and therapeutic biomarkers for both health and disease situations (Gulati et al., 2016; Mack, 2007).

The interleukin-1 (IL-1) cytokine is a heat-labile protein that causes fever. They are involved in the activity of leukocyte chemotaxis, which attracts more inflammatory cells to the site of injury. Interleukin-1β has a role in the pathophysiological changes in acute lung damage and acute respiratory distress syndrome. That is a part network of cytokines that cause, promote, and prolong lung injury (Stylianou et al., 2003). Interleukin-1β often acts on the nearby cells to initiate and amplify inflammation. It leads to the vascular endothelial cells becoming adhesives for neutrophils in recruiting them to the site of infection. During the peak of infection, it circulates in the bloodstream together with tumor necrosis factor-alpha (TNF-α) and is responsible for the manifestation of sickness in animals. It also acts on the brain and thus producing fever and lethargy in the animals. It also initiates other interleukins to act upon the liver cells to induce the production of acute-phase proteins against the infection (Tizard, 2013).

Mannheimiosis is one of the common diseases of the respiratory system in small ruminants (Jesse et al., 2019). Sheep and goats are most susceptible and cause a high mortality rate in farm animals (Kumar et al., 2000). The bacteria often induced pneumonia characterized by acute cranioventral pleuropneumonia (Emikpe et al., 2010). The diagnosis of this disease is based on history, clinical signs, post-mortem examination, histopathological findings, and molecular techniques of identification and isolation using polymerase chain

Corresponding Author: Mohd FARHAN HANIF REDUAN • **E-mail:** farhan.h@umk.edu.my

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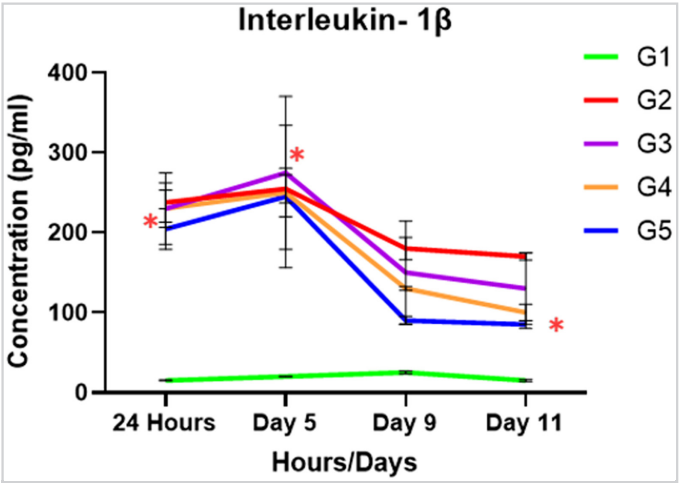

Figure 1.
Mean ± standard deviation of the interleukin-1β levels at 24 hours, day 5, day 9, and day 11 in 5 different groups of goats. Group 1: Negative control; Group 2: positive control (no treatment); Group 3: administration of oxytetracycline (20 mg/kg, SID); Group 4: administration of flunixin meglumine (1.1 mg/kg, BID); Group 5: administration of oxytetracycline (20 mg/kg, SID) and flunixin meglumine (1.1 mg/kg, BID). Asterisk (*) indicates value with significant differences ($p < .05$).

reaction (Taunde et al., 2019). However, there is still limited information on the role and levels of interleukin-1β (IL- β) in experimentally induced mannheimiosis in goats. This study aimed to measure the interleukin-1β levels, their alteration in the induced goats, and the post-treatment effect in goats.

Methods

Twenty male goats were divided into five groups (Groups 1, 2, 3, 4, 5). Goats in group 1 were kept as negative control and goats in groups 2, 3, 4, and 5 were inoculated with 10^7 CFU/mL of *Mannheimia haemolytica* intranasally. Goats in the Group 2 did not receive any treatment. Meanwhile, goats in group 3 were treated with antimicrobial (oxytetracycline, 20 mg/kg SID) on days 6 and 9 post-infection. Group 4 was treated only with an anti-inflammatory drug (flunixin meglumine, 1.1 mg/kg BID) for 5 days post-infection. Group 5 received both antimicrobial (oxytetracycline, 20 mg/kg SID) on days 6 and 9 with anti-inflammatory drug (flunixin meglumine, 1.1 mg/kg BID) treatment for 5 days post-infection**.**

For interleukin-1β (IL-1β) analysis, blood samples were collected via jugular vein before inoculation and at 24 hours, days 5, 9, and 11 days post bacteria inoculation. The levels of inflammatory cytokines, IL-1β, were quantified from serum samples using commercially available goat ELISA Kits (Fine Test, Wuhan Fine Biotech Co., Ltd., China). Analysis of variance tests were done to compare the data differences between and within groups. Post hoc analysis using the Duncan test was used to determine the level of statistical significance which was set at $p < .05$.

Results

The levels of IL-1β within 24 hours and day 5 of inoculations were significantly increased ($p < .05$) in groups (2, 3, 4, and 5) compared to the negative control. On day 11, the levels of IL-1β in groups 4 and 5 were significantly lower ($p < .05$) than in other groups. Interleukin-1β increased upon onset of infection to initiate, stimulate, and attract more leucocytes to the injury site. After day 5 of post-inoculation, the goats received treatment of anti-inflammatory (G4) shows that levels of IL-1β are generally declined. Group 5 (G5) was administered a combination of the antibiotic and anti-inflammatory drug and had a more significant reduction in the IL-1β levels than the positive control and single antibiotic treatment group (G3) (Figure 1).

Discussion

In innate immunity, ILs are the first-line defense and are essential to fight against infection; however, they may intensify the damage to the organs during acute tissue injuries and chronic inflammation. Therefore, inhibition of these cytokines can reduce inflammatory reactions (Dinarello, 2005). Using non-steroidal anti-inflammatory drugs (NSAIDs) such as flunixin meglumine for treatment may interfere with the transcriptional factor and production of cytokines, and thus inhibit these inflammatory cytokines (Lisboa et al., 2017). Besides that, it can also be said that inhibition of cyclooxygenase enzymes by NSAIDs can promote leukocyte phagocytic uptake and reactive oxygen intermediates in killing bacteria (Stables et al., 2010). The tetracycline antibiotics also have non-antimicrobial and anti-inflammatory activities that could help reduce cytokine levels (Sun et al., 2015). One of their actions is by inhibiting matrix metalloproteinases. Besides, their other actions include the regulation of cytokines, antioxidant effects, and modulation of leukocyte chemotaxis and activation (Monk et al., 2011).

The present findings indicated that treatments could reduce the levels of interleukin-1β in the treated goats compared to the goats that have not received any or receive only a single treatment of antibiotics. This agrees with the findings of (Rizk et al., 2017), who demonstrated that the calves affected with bronchopneumonia treated with anti-inflammatory and steroidal drugs as a single treatment or combination of drugs reduce the pro-inflammatory responses of interleukin-1β. Therefore, using a combination of treatment regimes shows that the drugs can synergistically lessen the level of cytokines during the infection. These could assist in the healing progression, eliminating the pathogens and its factor to be released. Thus, it alleviates the severity of tissue damage caused by infection.

Conclusion and Recommendation

Interleukin-1β (IL-1β) release is a part of the first-line defense mechanism for combating infection. Treatments with anti-inflammatory drugs and antibiotics reduced the levels of IL-1β during infection, and this is indicated as a potential reliable biomarker in diagnosing early infection of Mannheimiois in goats.

Ethics Committee Approval: The experimental procedure was conducted under the approval of the Animal Care and Use Ethics Committee (ACUC), Universiti Malaysia Kelantan (Date: 2021; Approval No: UMK/FPV/ACUC/PG/3/2021).

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Declaration of Interests: The authors declare that they have no competing interest.

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