Antibacterial Activity of Heterophils:  
A Protective Shield Against Infections in Broilers

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Abstract: Heterophils in broilers are the equivalent of mammalian neutrophils. They constitute the angular stone of the innate immune system against invading microorganisms with highly potential antimicrobial equipment. The non-oxidative mechanism is well developed and depends mainly on cationic antimicrobial peptides localized within cytoplasmic granules and characterized by broad spectrum antimicrobial activity and lack of antibiotic resistance. This study was carried out on twenty five, one day old chicken heterophils to show their antibacterial activity. Heterophils were collected at 35 days old during the exudative phase of the inflammatory response induced by intraperitoneal injection of 3% starch soluble then purified and homogenized. The cytoplasmic granules obtained by centrifugation gradient were extracted and their contents were tested against standard strains of Staphylococcus aureus and Escherichia coli by using an ultrasonic radial diffusion method. This study confirms the existence of very efficient non-oxidative mechanism and molecules with potent antibacterial activity. One-dimensional acid-urea polyacrylamide gel electrophoresis (AU-PAGE) of granule extract showed remarkable cathodal mobility and this confirm the cationic nature of these molecules and their remarkable antibacterial activity.

Key words: Broilers • Heterophils • Cytoplasmic granules • Antibacterial activity

INTRODUCTION

Chicken heterophils, called also pseudoeosinophils, are the counterpart of neutrophils in mammals, with rod shaped or spherical cytoplasmic granules; they are the most numerous blood leucocytes and constitute the first line of defense against invading microorganisms, where the initial response of the body to microbial invasion and the events in the first few hours are crucial in determining the outcome of infection [1]. The heterophils lack myeloperoxidase, an essential enzyme of the respiratory burst [2-6] and their antimicrobial activity depends mainly on non-oxidative mechanism. The cationic antimicrobial peptides located in the cytoplasmic granules are the most active molecules of the non-oxidative killing activity of heterophils. Three cationic antimicrobial peptides called "gallinaeins" have been isolated from chicken leucocytes namely Gal-1, Gal-la and Gal-2, [7]. The cationic antimicrobial peptides have a wide killing activity; they are active against enveloped viruses, Gram-negative and /or Gram-positive bacteria, fungi, parasites and even cancer cells [8]. Bacterial killing occurs in minutes and in most cases requires bacterial cell growth [9]. Recently, a new nomenclature for the avian cationic antimicrobial peptides has been proposed [10].

The acute exudative phase of the inflammatory response, induced either by artificial irritants or infectious agents or their fractions such lipopolysaccharids (LPS), in avian tissues is characterized by the predominance of the heterophils [6].

Many researchers in the field of non-oxidative killing activity have been attracted by the avian heterophils due to their antimicrobial activity which depend primarily on oxygen-independent mechanisms [6] and were very useful in studying oxygen independent killing activity.

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MATERIALS AND METHODS

Animals: Twenty five, one day old unsexed broilers "TSA" were used in this study. The birds were housed in wire cages throughout the experiment, water and antibiotic free were offered ad libitum. The trial lasted 35 days.

Cell Isolation: Intraperitoneal injection (i.p.) of irritant solutions is one of the best methods to get high proportion of heterophils and it is the most used. The interval between the i.p. injection of irritant solutions and lavage of the peritoneal cavity with heparinized or non-heparinized buffer solution vary from 6 hours to 24 hours [2, 7, 11, 12]. For best yield of heterophils, they were collected at 35 days old during the exudative phase of inflammatory process induced by i.p. injection of 60cc of sterile 3% starch soluble. The intraperitoneal injection was done according to the technique described by Sabet et al., [13]. The animals were sacrificed and the peritoneal cavity was washed with PBS: pH=7.2 and the heterophils were collected and washed twice with PBS.

Granule Isolation and Extraction: For best release of cytoplasmic granules, the heterophils were sonicated in an ice bath to prevent molecules denaturation, with an ultrasonic homogenizer (BANDELIN sonopuls HD 200) with a power of KE76/D; the granules were pelleted at high speed centrifugation (27000 x g) for 30 min in a cooled centrifuge. The granule extraction was carried out overnight in 5% acetic acid for 16 hours with a magnetic stirrer at 4°C. Followed by high speed centrifugation 27000 x g and the pelleted material was reextracted.

Antibacterial Activity: After extraction the supernatant was dialysed overnight against 1 % glacial acetic acid using dialysis tubing with a MW cutoff of 1000 Da and magnetic stirrer at 4°C. The granule extract was concentrated by lyophilisation. The lyophilized material was resuspended in 0.01 % glacial acetic acid and protein content was determined using the Lowry method [14].

The antibacterial activity was tested against standard strains of bacteria E. coli ATCC 25922 and staphylococcus aureus ATCC 25923 by using an ultrasonic sensitive radial diffusion method described by Lehrer et al., [15].

AU-PAGE: AU-PAGE of granule extract was carried using disc gel electrophoresis with reverse current (cathode on bottom).

RESULTS AND DISCUSSION

The crude extract of heterophils' cytoplasmic granules tested against standard strains by ultrasonic method where the wells, with a diameter of 3 mm, loaded with 5μl of the solution to be tested. The antibacterial activity against E. coli ATCC 25922 with adjusted protein concentration showed a marked antibacterial activity (Table 1) and the diameter of the inhibition zone is quietly visible even the small amount of the tested material (Figure 1).

Fig. 1: Antibacterial activity against E. coli (1: acetic acid0.001%, 2: granule extract)

Fig. 2: AU-PAGE with reverse current of chicken heterophil's granule extract stained with coomassie brilliant blue (R-250) with different protein concentrations.
Table 1: Results of the radial diffusion assay against E. coli 25922

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<th>Concentration (mg/ml)</th>
<th>Diameter of the IZ (cm)</th>
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<td>0.08</td>
<td>0.7 - 1.1</td>
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* : Inhibition zone

In another experiment, the total protein was adjusted to 20 μg/ml and the wells were loaded with 5 μl of the solution to be tested against E. coli ATCC 25922 and S. aureus ATCC 25923. Even with the very small amount of loaded material and its very low protein concentration its antibacterial activity is conserved and an inhibition zone of 0.6 cm was observed against E. coli ATCC 25922 and an inhibition zone of 0.68 cm against Staphylococcus aureus.

The heterophils crude granule extract showed striking antibacterial activity even at very low concentration either against gram positive or gram negative bacteria (20 μg/ml) and the AU-PAGE showed great cathodal mobility and this confirm the presence of non-oxidative mechanisms and potent antibacterial molecules like cationic antimicrobial peptides [7, 11].

CONCLUSION

Chicken heterophils are major blood leukocytes with remarkable antibacterial activity of their cytoplasmic granules this study confirm the antibacterial effect of their cytoplasmic granules on either Gram negative or Gram positive bacteria. This antibacterial killing activity of heterophils is an oxygen independent mechanism where AU-PAGE of their granule extract showed their cationic nature. The cytoplasmic granules are equipped with powerful molecules against bacteria and responsible for the main activity of heterophils. Chicken heterophils, by their potent antibacterial activity, play a key role in the innate immune system and constitute very efficient and protective shield against invading microorganisms.

REFERENCES