MEDWELL PUBLISHING
Journal of Animal and Veterinary Advances 8 (4): 755-759, 2009
ISSN: 1680-5593

Avian Cationic Antimicrobial Peptides in Health and Disease: A Mini Review

[1]Omar Bennoune, [1]Mohamed Melizi, [2]Kamel Khazal and [3]Romyla Bourouba
[1]Laboratory of Health, Animal Production and Environment (ESPA), University of Batna, Algeria
[2]School of Veterinary Medicine, Tuskegee University, Tuskegee Alabama, AK 36088, USA
[3]Department of Biology, University of Batna, Algeria

Abstract: The cationic antimicrobial peptides, including avian β-defensins called gallinacins in chickens, are endogenous natural antibiotics and constitute bioactive molecules of innate immune system. Marked by their broad spectrum antimicrobial activity and lack of antibioresistance, these antimicrobial peptides act promptly and form the first line of host defense against bacteria, fungi and enveloped viruses. They have been isolated from different species and constitute the most active molecules of the non-oxidative killing mechanism in chicken heterophils and expressed in the chicken reproductive system, tongue and bursa of fabricius and they constitute the backbone for new class of antibiotics and alternatives of feed additives. Their expression shows variability in health and disease and their low level is incriminated in some genetic disorders.

Key words: Cationic peptides, avian, antimicrobial activity, heterophils, avian β-defensins

INTRODUCTION

Widely distributed, the cationic antimicrobial peptides exist in all living species and >24 different antimicrobial peptides have been found in a single animal (Hancock and Diamond, 2000), In contrast, to the immune response characterized by its high specificity and relative slowness and discrimination between self and non-self, the cationic antimicrobial peptides have rapid response with broad spectrum activity and low host cytotoxicity (Hancock and Scott, 2000), the rapid response is crucial in determining the outcome of infection (Namavar *et al*., 1984). They exist in all forms of life and they were isolated from Human Immunodeficiency Virus type 1 (HIV-1), Simian Immunodeficiency Virus (SIV) and from Equine Infectious Anemia Virus (EIAV) (Tencza *et al*., 1997), they were also isolated from plants, these latter combat infections by the production of specific cationic peptides, thionins (Hancock *et al*., 1995).

An antimicrobial peptide with 51 residues and 3 intramolecular disulfide linkages, named royalisin was found in royal jelly of the honeybee *Apis mellifera* (Fujiwara *et al*., 1990). The cationic peptides tend to be mainly found in the most exposed parts of the body to environmental infection (Hancock and Scott, 2000). The 4th isolated and characterized defensins were from rabbit lung macrophage and named HCP-1 and MCP-2 (Selsted *et al*., 1983).

The cationic antimicrobial peptides in avian blood cells: The chicken heterophils lack myeloperoxidase, an essential enzyme of the respiratory burst (Brune *et al*., 1972; Brune and Spitznagel, 1973; Penniall and Spitznagel, 1975; Styrt, 1989; Harmon, 1998). The avian heterophils produced few oxygen radicals (Conlon *et al*., 1991) and their antimicrobial activity depends mainly on non-oxidative killing mechanism like cationic antimicrobial peptides and lysosyme (Harmon *et al*., 1992). Many researchers in the field of natural cationic antimicrobial peptides have been attracted by the avian heterophils due to their antimicrobial activity which depend primarily on oxygen-independent mechanisms (Harmon, 1998).

Three cationic antimicrobial peptides or β-defensins named gallinacins (Gal-1α, Gal-1, Gal-2) were isolated from chicken leukocytes Gal-2, these peptides contained 36-39 amino acid residues and they were relatively cationic due to their lysine and their arginine residues, gallinacins showed sequence homology to the β-defensin subfamily. The gallinacins showed 9 invariant residues (6 cysteines, 2 glycines and 1 proline) common to bovine β-defensins (Harwig *et al*., 1994) and 2 other cationic antimicrobial peptides named chicken heterophil peptides (CHP1 and CHP2) were purified from the granule extract of chicken heterophils. Three Turkey antimicrobial peptides were isolated from turkey heterophils and named turkey heterophil peptides (THP1, THP2 and THP3) (Evans *et al*., 1994). Recently a new nomenclature of avian β-defensins have been proposed (Lynn *et al*., 2007).

Corresponding Author: Omar Bennoune, Laboratory of Health, Animal Production and Environment (ESPA), University of Batna, Algeria

The expression of cationic peptides in normal and pathologic states: The gallinacin-3 expression was found to be strongly prominent in the chicken tongue, bursa of Fabricius and trachea and was also moderately expressed in the skin, esophagus and air sacs; weaker expression of GaL-3 was seen in the large intestine, kidney and ovary but only its tracheal expression was increased in response to *Haemophilus paragallinarum* experimental infection. In contrast to GaL-3, the expression of GaL-1 (and-1α) and GaL-2 was limited to bone marrow cells (Zhao *et al*., 2001).

Another β-defensin named gallinacin-6 (Gal-6) with potent antimicrobial activity against food borne pathogens was found in the proximal digestive tract (van Dijk *et al*., 2007). Twelve avian β-defensins genes (AvβD-1, AvβD-2... and AvβD-12) were identified in the reproductive tract; the expression of some of them was increased following lipopolysaccharide injection (Abdel Mageed *et al*., 2008).

The chicken Liver-Expressed Antimicrobial Peptide 2 (cLEAP-2), with its 4 conserved cysteine amino acids and antibacterial activity, was expressed in the small intestine, liver, lung and kidney. Its expression was upregulated following oral challenge with *salmonella* (Townes *et al*., 2004).

Biosynthesis of avian β-defensins: They are ribosomally synthesized peptides (Hancock and Chapple, 1999). In chicken, it has been shown that the peptide precursors of the four avian β-defensins (Gal-1/CHP-1, Gal-2, THP-1 and THP-2) were composed by a signal sequence with about 19 amino acid residues and it is hydrophobic, a propiece with >40 amino acid residues and it is highly acidic and the cationic mature peptide (Brockus *et al*., 1998). The propiece has a role in the inactivation of the mature peptide and has been demonstrated *in vitro* (Valore *et al*., 1996).

Antimicrobial activity: The conserved killing activity of the polymorphonuclear leukocytes against certain bacteria and fungi even in anaerobic conditions where their ability to generate reactive oxygen intermediates is completely abolished explain the existence of non-oxidative mechanism (Vel *et al*., 1984). The cationic antimicrobial peptides are natural antibiotic and their antimicrobial activity has been extensively studied. They are active against enveloped viruses such as HIV, *Herpesvirus* and *Vesicular stomatitis* virus (Lehrer *et al*., 1985) and Gram-negative and/or Gram-positive bacteria, fungi, parasites such as trypanosomas and plasmodia and even cancer cells (Hancock and Diamond, 2000). They were also active against three clinical isolates of *Mycobacterium tuberculosis* (Miyakawa *et al*., 1996). Bacterial killing occurs in minutes and in most cases requires bacterial cell growth (Huttner and Bevins, 1999).

Quantitative assays revealed that CHP-1 kill 80% of the *E. coli* at 2.7 μg mL^{-1}, while 80% of the *S. aureus* was killed at only 0.7 μg mL^{-1}. The CHP-2 tested by using radial diffusion method and was bactericidal against both *S. aureus* and *E. coli*. The THP-1 is less potent than chicken heterophil peptides and kills 80% of the *S. aureus* at 2.6 μg mL^{-1} while killing of the *E. coli* require higher concentrations and 80% of the *E. coli* was killed at 5.3 μg mL^{-1}. The other 2 Turkey Heterophil Peptides (THP-2 and THP-3) fail to kill *E. coli in vitro* at 21 μg mL^{-1} and killed 80% of the *S. aureus* at 10.5 μg mL^{-1} (Evans *et al*., 1994).

Other chicken and turkey heterophil peptides (CHP-1, CHP-2, THP-1 and THP-3) were tested against a variety of microorganisms including avian pathogens and human pathogens which are harbored by birds. The three first peptides were effective against all the bacteria tested at a concentration of 16 μg mL^{-1}, except for *Pasteurella multocida* serotype A:3, strain 1059 where none of the peptides including a known microbicidal peptide: protamine sulfate (that used as a positive control) were active against this serotype at the maximum concentration of 16 μg mL^{-1} also the THP-3 has no effect on *Bordetella avium*, *E. coli* and *Salmonella typhimurium* at the maximum concentration of 16 μg mL^{-1}. The THP-1 is the most potent of the tested avian heterophils peptides. It has been shown that bacterial survival reduction is concentration dependent and the four peptides were unable to neutralize Infectious Bronchitis Virus (IBV) in the experimental conditions (Evans *et al*., 1995).

By using an ultrasensitive radial diffusion method, GAL-1 (CHP-1) and Gal-1α were powerful against *E. coli* and they were 10 fold more potent than HNP-1, Gal-2 (CHP-2) expressed less efficacy against *E. coli* but far exceeded that of HNP-1. All 3 gallinacins have almost equal antibacterial activity against *L. monocytogenes* Gal-1 and Gal-1α killed *C. albicans* whereas Gal-2 was ineffective even in a concentration of 400 μg mL^{-1} (Harwig *et al*., 1994).

Cationic peptides and cells: The cationic antimicrobial peptides have cytotoxic effect on the host cells especially blood cells. The biological activity of the antimicrobial peptides depends on the environment in which it is found. The cytotoxic peptides found in antimicrobial secretions function primarily by providing a 1st line of defense against invading microorganisms whereas, some cytotoxic peptides present in the venom function as toxins and are used by organisms for defense (Kourie and Shorthouse, 2000).

The outer leaflet of the cell membrane is less negatively charged in eukaryotic cells compared to bacteria and this may partially explains the different effects of the peptides on prokaryotic cells compared to eukaryotic cells (Gudmundsson and Agerberth, 1999). Their action on the cell membranes depends on their lipid composition (Hristova *et al*., 1997).

Other activities of cationic antimicrobial peptides: Unlike most of antibiotics, the cationic antimicrobial peptides have antiendotoxinic activity through preventing septicemia which usually caused by release of lipopolysaccharides from the outer membrane of Gram-negative bacteria; the toxicity of this endotoxin (LPS) is contained within its lipid a portion. Gram-positive septicemia is also presumed to be caused by the released of bacterial cell wall components (Hancock and Scott, 2000).

These antimicrobial peptides have the ability to block the activation of macrophages by LPS, possibly by blocking the binding of LPS to LPS binding protein and prevent the initial step of LPS signaling that consist on the transfer of LPS to CD14 by LPS binding protein and this reduce or prevent the LPS-induced inflammatory responses (Scott *et al*., 2000).

Some cationic antimicrobial peptides have histamine releasing activity and degranulate rat peritoneal mast cells (Befus *et al*., 1999). The defensins increase epithelial wound repair *in vitro* and mucin production (Aarbiou *et al*., 2004). These peptides are regulators of inflammation and immunity (Bals and Hiemstra, 2004).

DISCUSSION

The cationic antimicrobial peptides include avian β-defensins exist in blood heterophils where their antimicrobial activity against microorganisms depends on the non-oxidative killing mechanism and not on the respiratory burst and these peptides are the principal component of the 1st one. Works on cationic antimicrobial peptides open a new window on the best comprehension of genetic disorders associated with decreased cationic antimicrobial peptides and disruption of barriers against microorganisms (Chanock and Foster, 1999; Wehkamp *et al*., 2005).

Recent advances on cationic antimicrobial peptides may help in the development of new feed additives and exploited to replace the formerly used antibiotics.

CONCLUSION

The relative paucity of information on avian cationic antimicrobial peptides, compared to defensins in humans, leads to more confusion and ambiguities in studying the innate immune system and particularly the avian non-specific part of the innate host defense mechanisms. Little is known about the effect of major avian diseases on these molecules that play a key role in protection against microorganisms especially in this species where the antimicrobial activity of heterophils depends mainly on cationic antimicrobial peptides.

REFERENCES

Aarbiou, J., R.M. Verhoosel, S. van Wetering, W.I. de Boer, J.H.J.M. van Krieken, S.V. Litvinov, K.F. Rabe and P.S. Hiemstra, 2004. Neutrophil defensins enhance lung epithelial wound closure and mucin gene expression *in vitro*. Am. J. Respir. Cell. Mol. Biol., 30: 193-201. DOI: 10.1165/rcmb.2002-0267OC. PMID: 12871849. http://ajrcmb.atsjournals.org/cgi/content/short/30/2/193.

Abdel Mageed, A.M., Isobe, N. and Y. Yoshimura, 2008. Expression of avian β-defensins in the oviduct and effects of lipopolysaccharide on their expression in the vagina of hens. Poult. Sci., 87: 979-984. DOI: 10.3382/ps.2007-00283. PMID: 18420991. http://ps.fass.org/cgi/content/abstract/87/5/979.

Bals, R. and P.S. Hiemstra, 2004. Innate immunity in the lung: how epithelial cells fight against respiratory pathogens. Eur. Respir. J., 23: 327-333. DOI: 10.1183/09031936.03.00098803. PMID: 14979512. http://erj.ersjournals.com/cgi/reprint/23/2/327.pdf.

Befus, A.D., C. Mowat, M., Hu, J. Gilchrist, S. Solomon and A. Bateman, 1999. Neutrophil defensins induce histamine secretion from mast cells: Mechanisms of action. J. Immunol., 163: 947-953. PMID: 10395691. http://www.jimmunol.org/cgi/reprint/163/2/947.pdf.

Brockus, C.W., M.W. Jackwood and B.G. Harmon, 1998. Characterization of β-defensin prepropeptide mRNA chicken and turkey bone marrow. Anim. Genet., 29: 283-289. PMID: 9745666.

Brune, K. and J.K. Spitznagel, 1973. Peroxidaseless chicken leukocytes: Isolation and characterization of Antibacterial Granules. J. Infect. Dis., 127 (1): 84-94. PMID: 4566622.

Brune, K., M.S. Leffell and J.K. Spitznagel, 1972. Microbicidal activity of peroxidaseless chicken heterophile leukocytes. Infect. Immun., 5: 283-287. PMID: 4564557. http://iai.asm.org/cgi/reprint/5/3/283.

Chanock, S.J. and C.B. Foster, 1999. SNPing away at innate immunity J. Clin. Invest., 104 (4): 369-370. DOI: 10.1172/JCI7986. PMID: 10449427. http://www.jci.org/articles/view/7986.

Conlon, P., S. Dale and T. Gowlett, 1991. Oxygen Radical Production by Avian Leukocytes. Can. J. Vet. Res., 55: 193-195. PMID: 1884301. http://ukpmc.ac.uk/picrender.cgi?artid=8799&blobtype=pdf.

Evans, W.E., F.G. Beach, K.M. Moore, M.W. Jackwood, J.R. Glisson and B.G. Harmon, 1995. Antimicrobial activity of chicken and Turkey heterophil peptides CHP1, CHP2, THP1 and THP3. Vet. Microbiol., 47: 295-303. DOI: 10.1016/0378-1135(95)00126-3. PMID: 8748545.

Evans, E.W., G.G. Beach, J. Wunderlich and G.B. Harmon, 1994. Isolation of antimicrobial from avian heterophils. J. Leuko. Biol., 56: 661-665. PMID: 7964174.

Fujiwara, S., J. Imai, M. Fujiwara, T. Yaeshima, T. Kawashima and K. Kobayashi, 1990. A potent antibacterial protein in royal jelly purification and determination of the primary structure of royalisin. J. Biol. Chem., 265 (19): 11333-11337. PMID: 2358464. http://www.jbc.org/cgi/reprint/265/19/11333.pdf.

Gudmundsson, G.H. and B. Agerberth, 1999. Neutrophil Antibacterial Peptides, Multifunctional Effector Molecules in the Mammalian Immune System. J. Immunol. Methods, 232: 45-54. DOI: 10.1016/S0022-1759(99)00152-0. PMID: 10618508.

Hancock, R.E.W. and D.S. Chapple, 1999. Peptide antibiotics. Antimicrob. Agents Chemother., 43 (6): 1317-1323. PMID: 10348745. http://www.pubmedcentral.nih.gov/picrender.fcgi?tool=pmcentrez&artid=89271&blobtype=pdf.

Hancock, R.E.W. and G. Diamond, 2000. The role of cationic antimicrobial peptides in innate host defences. Trends Microbiol., 8 (9): 402-410. DOI: 10.1016/S0966-842X(00)01823-0. PMID: 10989307.

Hancock, R.E.W. and M.G. Scott, 2000. The role of antimicrobial peptides in animal denfenses. Proc. Natl. Acad. Sci. USA., 97 (16): 8856-8861. DOI: 10.1073/pnas.97.16.8856. PMID: 10922046. http://www.pnas.org/content/97/16/8856.full.pdf+html.

Hancock, R.E.W., T. Falla and M. Brown, 1995. Cationic bactericidal peptides. Adv. Microb. Physiol., 37: 135-175. DOI: 10.1016/S0065-2911(08)60145-9. PMID: 8540420.

Harmon, B.G., 1998. Avian heterophils in inflammation and disease resistance. Poult. Sci., 77: 972-977. PMID: 9657606. http://ps.fass.org/cgi/reprint/77/7/972.

Harmon, B.G., J.R. Glisson and J.C. Nunally, 1992. Turkey macrophage and heterophil bactericidal activity against pasteurella multocida. Avian Dis., 36: 986-991. DOI: 10.2307/1591559. PMID: 1485882.

Harwig, S.S.L., K.M. Swiderek, V.N. Kokryakov, L. Tan, T.D. Lee, E.A. Panyuticha, G.M. Aleshina, O.V. Shamova and R.I. Lehrer, 1994. Gallinacins: Cysteine-rich antimicrobial peptides of chicken leukocytes. Federation of Eur. Biochem. Societies Lett., 342 (3): 281-285. DOI: 10.1016/0014-5793(94)80517-2. PMID: 8150085.

Hristova, K., M.E. Selsted and S.H. White, 1997. Critical role of lipid composition in membrane permeabilization by rabbit neutrophil defensins. J. Biol. Chem., 272 (39): 24224-24233. DOI: 10.1074/jbc.272.39.24224. PMID: 9305875. http://www.jbc.org/cgi/reprint/272/39/24224.pdf.

Huttner, K.M. and C.L. Bevins, 1999. Antimicrobial peptides as mediators of epithelial host defense. Pediatr. Res., 45 (6): 785-794. DOI: 10.1203/00006450-199906000-00001. PMID: 10367766.

Kourie, J.I. and A.A. Shorthouse, 2000. Properties of cytotoxic peptide-formed ion channels. Am. J. Physiol. Cell Physiol., 278: C1063-C1087. PMID: 10837335. http://ajpcell.physiology.org/cgi/reprint/278/6/C1063.pdf.

Lehrer, R.I., K. Daher, T. Ganz and M.E. Selsted, 1985. Direct inactivation of viruses by MCP-1 and MCP-2, natural peptide antibiotics from rabbit leukocytes. J. Virol., 54 (2): 467-472. PMID: 2985808. http://jvi.asm.org/cgi/reprint/54/2/467.pdf.

Lynn, D.J., R. Higgs, A.T. Lloyd, C. O'Farrelly, V. Herve-Grepinet, Y. Nys, F.S.L. Brinkman, P.L. Yu, A. Soulier, P. Kaiser, G. Zhang and R.I. Lehrer, 2007. Avian β-defensin nomenclature: A community proposed update. Immunol. Lett., 110: 86-89. DOI: 10.1016/j.imlet.2007.03.007.PMID:17467809.http://www.massey.ac.nz/~pyu/Lynn_Immunology_Letters.pdf.

Miyakawa, Y., P. Ratnakar, A.G. Rao, M.L. Costello, O. Matieu-Costello, R.I. Lehrer and A. Catanzaro, 1996. *In vitro* activity of the antimicrobial peptides human and rabbit defensins and porcine leukocyte protegrin against mycobacterium tuberculosis. Infect. Immun., 64 (3): 926-932. PMID: 8641802. http://iai.asm.org/cgi/reprint/64/3/926.pdf.

Namavar, F., A.M.J.J. Verweij-Van Vught, W.A.C. Vel, M. Bal and MacLaren, 1984. Polymorphonuclear leukocyte chemotaxis by mixed anaerobic and aerobic bacteria. J. Med. Microbiol., 18: 167-172. PMID: 6387126.

Penniall, R. and J.K. Spitznagel, 1975. Chicken neutrophils: Oxidative metabolism in phagocytic cells devoid of myeloperoxidase. Proc. Natl. Acad. Sci. USA, 72: 5012-5015. DOI: 10.1073/pnas.72.12.5012. PMID: 174090. http://www.pubmedcentral.nih.gov/picrender.fcgi?artid=388865&blobtype=pdf.

Scott, M., A.C.E. Vreugdenhil, W.A. Buurman, R.E.W. Hancock and M.R. Gold, 2000. Cutting edge: Cationic antimicrobial peptides block the binding of Lipopolysaccharide (LPS) to LPS binding protein. J. Immunol., 164: 549-553. PMID: 10623792. http://www.jimmunol.org/cgi/reprint/164/2/549.pdf.

Selsted, M.E., D.M. Brown, R.J. DeLange and R.I. Lehrer, 1983. Primary structures of MCP-1 and MCP-2, natural peptide antibiotics of rabbit lung macrophages. J. Biol. Chem., 258 (23): 14485-14489. PMID: 6643497. http://www.jbc.org/cgi/reprint/258/23/14485.pdf.

Styrt, B., 1989. Species variation in neutrophil biochemistry and function. J. Leuko. Biol., 46: 63-74. PMID: 2543725. http://www.jleukbio.org/cgi/reprint/46/1/63.pdf.

Tencza, S.B., J.P. Douglass, D.J. Creighton, Jr., R.C. Montelaro and T.A. Mietzner, 1997. Novel antimicrobial peptides derived from human immunodeficiency virus type 1 and other lentivirus transmembrane proteins. Antimicrob. Agents Chemother., 41: 2394-2398. PMID: 9371339. http://aac.asm.org/cgi/reprint/41/11/2394.pdf.

Townes, C.L., G. Michailidis, C.J. Nile and J. Hall, 2004. Induction of cationic chicken liver-expressed antimicrobial peptide 2 in response to *Salmonella enterica* infection. Infect. Immun., 72: 6987-6993. DOI: 10.1128/IAI.72.12.6987-6993.2004. PMID: 15557621. http://iai.asm.org/cgi/reprint/72/12/6987.pdf.

Valore, E.V., E. Martin, S.S.L. Harwig and T. Ganz, 1996. Intramolecular inhibition of human defensin HNP-1 by its propiece. J. Clin. Invest., 97 (7): 1624-1629. DOI: 10.1172/JCI118588. PMID: 8601627. http://www.jci.org/articles/view/118588/pdf.

Van Dijk, A., E.J.A. Veldhuizen, S.I.C. Kalkhove, J.L.M. Tjeerdsma-van Bokhoven, R.A. Romijn and H. P. Haagsman, 2007. The β-defensin gallinacin-6 is expressed in the chicken digestive tract and has antimicrobial activity against food-borne pathogens. Antimicrob. Agents Chemother., 51 (3): 912-922. DOI: 10.1128/AAC.00568-06. PMID: 17194828. http://www.pubmedcentral.nih.gov/picrender.fcgi?artid=1803155&blobtype=pdf.

Vel, W.A.C., F. Namavar, A.M.J. Werweij, A.N.B. Pubben and D.M. MacLaren, 1984. Killing capacity of human polymorphonuclear leukocytes in aerobic and anaerobic conditions. J. Med. Microbiol., 18: 173-180. PMID: 6436491.

Wehkamp, J., M. Schmid, K. Fellermann and E.F. Stange, 2005. Defensin deficiency, intestinal microbes and the clinical phenotypes of Crohn's disease. J. Leukoc. Biol., 77: 460-465. DOI: 10.1189/jlb.0904543. PMID: 15618294. http://www.jleukbio.org/cgi/rapidpdf/ jlb.0904543v1.pdf.

Zhao, C., T. Nguyen, L. Liu, R.E. Sacco, K.A. Brogden and R.I. Lehrer, 2001. Gallinacin-3, an inducible epithelial β-defensin in the chicken. Infect. Immun., 69 (4): 2684-2691. DOI: 10.1128/IAI.69.4.2684-2691.2001. PMID: 11254635. http://www.pubmedcentral.nih.gov/picrender.fcgi?artid=98207&blobtype=pdf.