Advances in Dairy Research
ISSN: 2329-888X

Ahmadzadeh, et al., J Adv Dairy Res. 2015, 3:3
DOI: 10.4172/2329-888X.1000142

Research Article Open Access

Effect of Prostaglandin $F_{2\alpha}$ on Growth of *Mycoplasma bovis* Associated with Bovine Mastitis

Ahmadzadeh A[1*], Fox LK[2], McGuire MA[1], and Carnahan KG[1]

[1]*Department of Animal and Veterinary Science, University of Idaho, 875 Perimeter Drive, MS 2330, Moscow, ID 83844, USA*

[2]*College of Veterinary Medicine, Washington State University Pullman, WA 99164-6610, USA*

[*]**Corresponding author:** Ahmadzadeh A, Department of Animal and Veterinary Science, University of Idaho, 875 Perimeter Drive, MS 2330, Moscow, ID 83844, USA, Tel: 208 885 7409; Fax: 208 885 6420; E-mail: amin@uidaho.edu

Rec date: May 14, 2015; **Acc date: July** 02, 2015; **Pub date: July** 10, 2015

Copyright: © 2015 Ahmadzadeh A, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abstract

Mycoplasma bovis (*M. bovis*) is a major mastitis pathogen that has been reported to be refractory to antibiotic treatment. Certain fatty acids have been shown to inhibit the growth of mastitis pathogens such as *Staphylococcus aureus* (S. *aureus*). *In vitro* experiments were conducted to determine the effects of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) on growth of *M. bovis*. Five strains of *M. bovis* of bovine origin were selected for the study. Two strains were reference strains (ATCC 25025 and 25523) and the other strains were isolated from diseased cattle. Isolates were cultured and suspended in saline to achieve an optical density of 0.2 at 520 nm, a suspension of approximately 1×10^7 to 2×10^8 CFU/ml. Subsequently, *M. bovis* suspensions were incubated in culture media containing $PGF_{2\alpha}$ (dinoprost tromethamine) at final concentrations of 0 (control), 2, 4, and 8 mg/ml, for 8 h at 37°C. A sample from each treatment group was obtained and cultured on agar plates for 10 d and bacterial growth assessed as CFU/ml. The entire experiment was repeated four times using duplicate tubes per $PGF_{2\alpha}$ concentrations for each strain. Data were analyzed by ANOVA and the model included the effect of treatment, strain, and their interaction. Treatment affected ($P<0.01$) *M. bovis* growth, and mean CFU/ml decreased with concentrations of $PGF_{2\alpha}$ at 4 and 8 mg/ml but not 2 mg/ml (43.6, 42.1, 24.3, 7.8 [±1.1] for 0, 2, 4, 8 mg/ml, respectively). However, an effect of treatment by strain interaction on mean CFU/ml was detected ($P<0.05$), indicating that the effect of $PGF_{2\alpha}$ on bacterial growth was not consistent across strains. Overall, the 2 mg/ml $PGF_{2\alpha}$ decreased CFU/ml in only one strain compared with control, whereas 4 and 8 mg/ml $PGF_{2\alpha}$ decreased CFU/ml in all strains compared with control. These *in vitro* results provide evidence, for the first time, that the fatty acid $PGF_{2\alpha}$, in the form of dinoprost tromethamine, has inhibitory effects on growth of *M. bovis*, and this bacteriostatic effect appears to be strain and dose dependent.

Keywords: *Mycoplasma*; Fatty acids; Prostaglandin $F_{2\alpha}$; Bacteriostatic

Introduction

Mastitis causes the greatest economic loss to the dairy industry. It has been estimated that costs associated with mastitis for the US dairy industry exceed $2 billion per year [1,2]. Antibiotic treatments and other mastitis management strategies have improved the control of contagious pathogens; nonetheless, mastitis caused by microorganisms is still a major problem, even in well-managed dairy farms [3,4].

Mycoplasmas are highly contagious organisms and can cause various diseases in humans and animals [5]. In dairy cows, *Mycoplasma bovis* (*M. bovis*) is known to be the most common and virulent bovine Mycoplasma species in the United States [6].The financial losses of *M. bovis* infections, as a result of loss of weight gain and carcass value and other associated diseases, are estimated to be approximately $108 million per year with infection rates of up to 70% in a herd [7]. *Mycoplasma bovis* is characteristically refractory to Beta lactam antibiotics because it does not possess a cell wall. Furthermore, evidence is accumulating that antibiotics, including tetracycline, tilmicosin and spectinomycin, are not effective for the treatment of *M. bovis* [8]. Thus, finding alternative yet effective treatments against *M. bovis* is vital.

Extensive research has shown that the various fatty acids have antimicrobial effects and may be used as an inhibitory agent against bacteria [9-11]. Kelsey et al. [12] demonstrated that linoleic acid inhibited growth of two different mastitis strains of *Staphylococcus aureus* (S. *aureus*). Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is synthesized via a metabolic pathway commencing with arachidonic acid which is previously derived from linoleic acid [13]. Results from a recent study in our laboratory [14,15] indicate that the fatty acid $PGF_{2\alpha}$, in the form of dinoprost tromethamine, inhibits the growth of *Streptococcus uberis* and *S. aureus*, in a dose dependent manner. These findings resemble the actions of fatty acids in a previously described study [12]. *Mycoplasma bovis* is phylogenetically related to gram positive bacteria [16] and therefore, $PGF_{2\alpha}$ may have similar inhibitory effects on growth as previously observed with S. *aureus*. To our knowledge there is no information on the effect of any prostaglandin on Mycoplasma species. It was hypothesized that $PGF_{2\alpha}$ has inhibitory effects on the growth of *M. bovis* associated with bovine mastitis. Thus, the objective was to determine the effect of $PGF_{2\alpha}$ on *M. bovis* growth *in vitro*.

Materials and Methods

Bacteria strains, experimental design, and growth culture

Five strains of *M. bovis* from bovine origin were selected for the study. Two strains were reference strains (ATCC 25025 and 25523). The other three strains were isolated from diseased cattle, two from

milk of cows with clinical mastitis (MKB and CS-UI) and one from a swabbing solution of a cow's nasal passage with clinical pneumonia (VP-UI). *Mycoplasma bovis* isolates were purified, cultured and suspended in sterile phosphate buffered saline to achieve an optical density of 0.2 at 520 nm, yielding approximately 1×10^7 to 2×10^8 CFU/ml as previously described [17]. Subsequently, *M. bovis* suspensions were incubated in culture media containing $PGF_{2\alpha}$ (dinoprost tromethamine) at final concentrations of 0 (control), 2, 4, and 8 mg/ml, for 8 h at 37°C in duplicate, where two sample tubes per treatment concentration per strain of Mycoplasma species were tested. After the 8 h incubation a sample from each tube was obtained, serially diluted, and cultured on three modified Hayflick's agar plates for 10 d and bacterial growth assessed as CFU/ml. The CFU/ml counts from each of the three agar plates were averaged for each corresponding tube. The entire experiment was repeated on 4 different days to account for variation associated with a day effect, categorized as run.

Statistical analysis

Data were analyzed by least squares analysis of variance using GLM procedures of SAS and the model included the effect of experimental run (4 runs), treatment (4 doses of $PGF_{2\alpha}$) , strain (5 strains), and their interaction.

Results

Based on CFU/ml, $PGF_{2\alpha}$, in the form of dinoprost tromethamine, had inhibitory effects on *M. bovis* growth; mean CFU/ml decreased ($P<0.01$) with increasing concentrations of $PGF_{2\alpha}$ and 8 mg/ml of $PGF_{2\alpha}$ being the most inhibitory (Figure 1). Mean CFU/ml (106/ml) was significantly decreased from 43.6 ± 1.1 in control (0 mg/ml $PGF_{2\alpha}$) to 24.3 and 7.8 ± 1.1, in 4 and 8 mg/ml $PGF_{2\alpha}$ treatment (Figure 1).

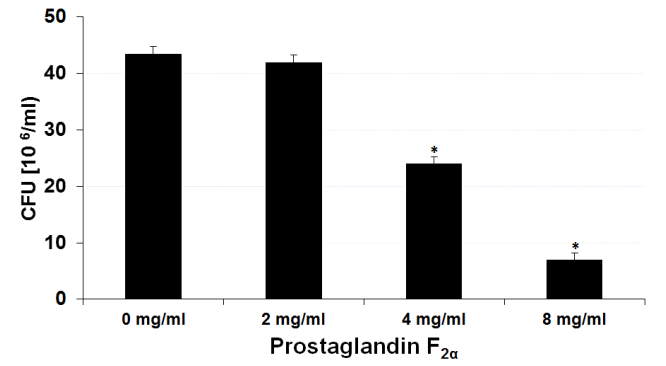

Figure 1: Effect of various doses of prostaglandin $F_{2\alpha}$ on *Mycoplasma bovis* growth across all five strains[1]. *There was an effect of treatment dose on mean CFU/ml of *M. bovis*. Across all strains, mean CFU/ml of *M. bovis* was less ($P<0.05$) for 4 and 8 mg/ml prostaglandin $F_{2\alpha}$ compared to 0 mg/ml. The error bars indicate the standard errors associated with the mean. [1]ATCC 25025 and 25523 were reference strains, MKB=isolated from milk of cows with clinical mastitis, CS-UI=isolated from milk of cows with clinical mastitis, VP-UI=swabbing solution of a cow's nasal passage with clinical pneumonia.

However, an effect of treatment by strain interaction on mean CFU/ml was detected ($P<0.05$) providing evidence that *M. bovis* growth across all $PGF_{2\alpha}$ doses were not similar among strains. Pre-planned contrasts were conducted to compare the mean log CFU/ml values between strains for each treatment. Overall, the 2 mg/ml $PGF_{2\alpha}$ decreased ($P<0.05$) CFU/ml only in one strain compared with control, whereas 4 and 8 mg/ml $PGF_{2\alpha}$ decreased ($P<0.01$) CFU/ml in all strains compared with control (Figure 2).

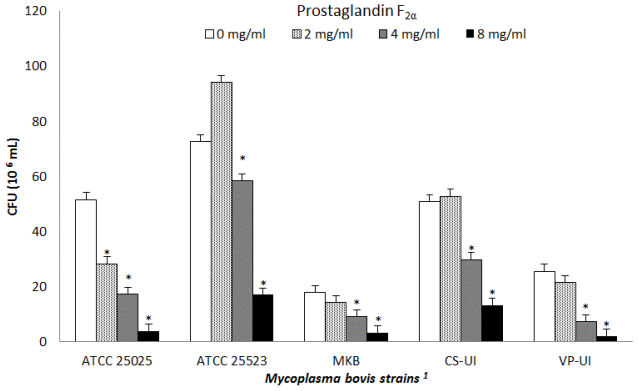

Figure 2: Effect of various doses of prostaglandin $F_{2\alpha}$ on each strain of *Mycoplasma bovis* growth[1]. There was an effect ($P<0.05$) of treatment by strain interaction on mean CFU/ml. [1]ATCC 25025 and 25523 were reference strain, MKB=isolated from milk of cows with clinical mastitis, CS-UI=isolated from milk of cows with clinical mastitis, VP-UI=swabbing solution of a cow's nasal passage with clinical pneumonia. *Different from 0 mg/ml treatment within strain ($P<0.05$). The error bars indicate the standard errors associated with the mean.

Discussion

Our results provide evidence, for the first time, that $PGF_{2\alpha}$, in the form of dinoprost tromethamine has inhibitory effects on growth of *M. bovis*. Moreover, the effect of $PGF_{2\alpha}$ on the bacterial growth was not consistent across strains. Nevertheless, if $PGF_{2\alpha}$ were to be considered as a potential treatment for *M. bovis*, then a dose for final concentration and administration routes remains to be determined, using *in vivo* experiments. The use of intra-mammary treatment of mycoplasma mastitis is questionable and needs further research [18].

The antibacterial effects of fatty acids on pathogens have been studied for years and reviewed [9-12]. Our previous results also showed that [19] $PGF_{2\alpha}$ has inhibitory effects on growth of *S. aureus* and *Streptococcus uberis* in vitro [14,15]. In addition, growth of *S. aureus* and Streptococcus agalactiae was inhibited by certain long-chain fatty acids *in vitro* [12].

The mechanism by which $PGF_{2\alpha}$ affected the growth of *M. bovis* cannot be determined from the current study. Based on current evidence in the scientific literature, we postulate that one potential mechanism of action centers on the ability of fatty acids to penetrate the plasma membrane of bacteria, increasing the negative charge of the bacterial membrane surface, and ultimately disrupting cell membrane integrity [20]. Another possibility involves the hindering of bacterial growth via an interaction of the fatty acids at the cell membrane, resulting in a change in membrane permeability [21] or the disruption of transduction cascades leading to cell lysis [22]. Additionally, since $PGF_{2\alpha}$ retains some of its hydrophobic properties from its precursor

arachidonic acid, it is possible that it is being incorporated into the plasma membrane and may be interfering with membrane fluidity and cellular signaling and transduction cascades similar to mechanisms previously suggested above [21,22].

Mycoplasma are unable to synthesize cholesterol needed to regulate membrane fluidity and must obtain sterols (cholesterol) from their environment to maintain proper fluidity [23,24]. When *M. laidlawii* were treated with palmitic and steric acids, cell permeability changed, cells appeared irregular, osmotic fragility increased, and cell growth was inhibited [25].

The $PGF_{2\alpha}$ concentrations used in the current *in vitro* study were relatively high, and therefore its feasibility as clinical therapeutic agent warrants further investigation. Previous research [12] has shown that linoleic acid and arachidonic acid, precursors of $PGF_{2\alpha}$, are potent bacteriostatic fatty acids and at low doses of these fatty acids can inhibit the growth of several gram positive bacteria. The results of the current research provide an opportunity to examine the effect of these and other fatty acids on *M. bovis*, which currently has no effective treatment.

Conclusions

These *in vitro* results provide evidence, for the first time, that $PGF_{2\alpha}$, in the form of dinoprost tromethamine, has inhibitory effects on the growth of *M. bovis*, and the bacteriostatic effect appears to be strain and dose dependent. The clinical application of $PGF_{2\alpha}$ and its efficacy for treatment of *M. bovis* requires further investigation. These findings provide further research opportunities to investigate the effect of fatty acids on *M. bovis*.

Acknowledgments

This study was made possible by the support of Zoetis Animal Health, Florham Park, NJ, in providing pure prostaglandin $F_{2\alpha}$ (dinoprost tromethamine), Idaho Dairymen's Association, NIH P20 RR15587, and by the Idaho Agricultural Experiment Station. The authors are thankful to Mr. Ben Enger for the careful review of the manuscript.

References

1. DeGraves FJ, Fetrow J (1993) Economics of mastitis and mastitis control. Vet Clin North Am. Food Anim. Pract 9: 421-434.
2. Philpot WN, Nickerson, SC (2000) Winning the fight against mastitis. Westfalia Surge, Inc. Naperville, IL.
3. Oliver SP (1988) Frequency of isolation of environmental mastitis-causing pathogens and incidence of new intramammary infection during the nonlactating period. Am J Vet Res 49: 1789-1793.
4. Todhunter DA, Smith KL, Hogan JS (1995) Environmental streptococcal intramammary infections of the bovine mammary gland. J Dairy Sci 8: 2366-2374.
5. Maunsell FP, Woolums AR, Francoz D, Rosenbusch RF, Step DL, et al. (2011) Mycoplasma bovis infections in cattle. J Vet Intern Med 25: 772-783.
6. Carter GR, Chengappa MM, Roberts AW, Claus GW, Rikihisa Y (1995) Essentials of Veterinary Microbiology. (5thedn) Williams & Wilkins, Philadelphia, PA.
7. Rosengarten R, Citti C (1999) The role of ruminant mycoplasmas in systemic infection. In: Stipkovits L, Rosengarten R, Frey J (eds) Mycoplasmas of Ruminants: Pathogenicity, Diagnostics, Epidemiology and Molecular Genetics, European Commission, Brussels, pp:14–17.
8. Nicholas RA, Ayling RD (2003) Mycoplasma bovis: disease, diagnosis, and control. Res Vet Sci 74: 105-112.
9. Hogan JS, Pankey JW, Duthie AH (1987) Growth-inhibition of mastitis pathogens by long-chain fatty-acids. J Dairy Sci 70: 927-934.
10. Khulusi S, Ahmed HA, Patel P, Mendall MA, Northfield TC (1995) The effects of unsaturated fatty-acids on Helicobacter-pylori in-vitro. J Med Microbiol 42: 276-282.
11. Knapp HR, Melly MA (1986) Bactericidal effects of polyunsaturated fatty acids. J Infect Dis 154: 84-94.
12. Kelsey JA, Bayles KW, Shafii B, McGuire MA (2006) Fatty acids and monoacylglycerols inhibit growth of Staphylococcus aureus. Lipids 41: 951-961.
13. Marcel YL, Christiansen, K, Holman, RT (1968) The preferred metabolic pathway from linoleic acid to arachidonic acid in vitro. Biochem Biophys Acta 164: 25-34.
14. Autran CA, Shafii B, Dalton JC, McGuire MA, Carnahan KG, Ahmadzadeh A (2013). Effect of Prostaglandin F2α on Growth of Staphylococcus aureus Associated with Bovine Mastitis. J Veterinar Sci Technol 4: 2-7.
15. Autran CA, Shafii B, Dalton JC, McGuire MA, Carnahan KG, Ahmadzadeh A (2011) Effect of prostaglandin F2α on growth of Escherichia coli and Streptococcus uberis associated with bovine mastitis. J Dairy Sci 94: 347.
16. Lysnyansky I, Sachse K, Rosenbusch R, Levisohn S, Yogev D (1999) The vsp locus of Mycoplasma bovis: gene organization and structural features. J Bacteriology 181: 5734-5741.
17. Punyapornwithaya V, Fox LK, Gay GM, Hancock DD, Alldredge JR (2009) The effect of centrifugation and resuspension on the recovery of Myscoplasma species from milk. J Dairy Sci 92: 4444-4447.
18. Kirk JH, Lauerman LH (1994) Mycoplasma mastitis in dairy cows. Compend Contin Educ Prac. Vet 16: 541-551.
19. Wang LL, Johnson EA (1992) Inhibition of Listeria monocytogenes by fatty acids and monoglycerides. Appl Environ Microbiol 58: 624-629.
20. Kondo E, Kanai K (1972) The lethal effect of long-chain fatty acids on mycobacteria. Jpn J Med Sci Biol 25: 1-13.
21. Neiman C (1954). Influences of trace amounts of fatty acids on growth of microorganisms. Bacteriol Rev 18: 147-161.
22. Chamberlain NR, Mehrtens BG, Xiong Z, Kapral FA, Boardman JL, et al. (1991) Correlation of carotenoid production, decreased membrane fluidity, and resistance to oleic-acid killing in staphylococcus-aureus 18z. Infec Immun 59: 4332-4337.
23. Boonyayatra S, Fox LK, Besser TE, Sawant A, Gay JM (2010) Effects of storage methods on the recovery of Mycoplasma species from milk samples. Vet Microbiol 144: 210-213.
24. McElhaney RN (1983) Section D4 manipulation of membrane lipid composition, In: Tully J.G., Razin S. (Eds.) Methods in mycoplasmology I. Academic Press, New York, pp. 235-239
25. Razin S, Cosenza BJ, Tourtellotte ME (1966) Variations in Mycoplasma morphology induced by long-chain fatty acids. J Gen Microbiol 42: 139-145.