ACUTE PHASE RESPONSE IN CALVES AS A RESULT OF EXPERIMENTAL CHALLENGE WITH MYCOPLASMA BOVIS

KATARZYNA DUDEK, DARIUSZ BEDNAREK, AND MONIKA SZYMAŃSKA-CZERWIŃSKA

Department of Cattle and Sheep Diseases, National Veterinary Research Institute, 24-100 Pulawy, Poland
katarzyna.dudek@piwet.pulawy.pl

Received for publication September 03, 2010

Abstract

The aim of the study was to investigate the influence of Mycoplasma bovis challenge in calves on the alteration of acute-phase response (APR). The study was performed on twelve calves aged 4-8 weeks. The animals were divided into two equal groups: experimental and control. Calves in the experimental group were intratracheally challenged with pathogenic strain of Mycoplasma bovis, whereas controls received sterile physiological saline as placebo. The blood samples were collected before (1st d) and after the mycoplasma challenge (3, 5, 7 and 9th d). The following parameters were assayed in serum: acute phase proteins (APPs), i.e. haptoglobin (Hp) and amyloid A (SAA), and eicosanoids such as prostaglandin E2 (PGE2), prostaglandin F2α (PGF2α), leukotrien B4 (LTB4), and tromboxan B2 (TXB2). In calves of experimental group, a significant increase in concentrations of Hp and SAA was observed when compared with the controls. A decrease in both APPs to the initial values, i.e. before the challenge, was noted on the 9th d of experiment. On the other hand, the inoculation of Mycoplasma bovis caused a significant increase of the examined eicosanoids, which maintained elevated during the whole study. The stimulation of synthesis of APPs and eicosanoids in response to the challenge with Mycoplasma bovis probably indicates the effective activation of APR under these conditions.

Key words: calves, Mycoplasma bovis, acute phase proteins, eicosanoids.

Acute phase response (APR) is a complex of organism non-specific reactions, i.e. immunological, behavioural, metabolic, and others in response to infection or trauma (16). Acute phase proteins (APPs) are one of the most essential components of APR. In cattle, haptoglobin (Hp) and amyloid A (SAA), α1-acid glycoprotein (27), fibrinogen (10, 13), or lipopolysaccharide binding protein (32) are also important APPs (25). However, Hp and SAA have the most diagnostic significance in cattle (23, 27). The changes of Hp concentrations were observed in different bacterial, and viral infections, and other diseases, i.e. Mannheimia haemolytica infection (25), Pasteurella multocida infection (7), bovine respiratory syncytial virus (BRSV) infection (18), bovine viral diarrhea (BVD), respiratory disease, mastitis, metritis, hepatic lipidosis, and foot and mouth disease (27), and in such states as parturition, starvation, or transport stress (9, 37). An increase in SAA concentrations were observed in different bacterial, and viral infections, and other diseases, i.e. Mannheimia haemolytica infection (25), Pasteurella multocida infection (7), bovine respiratory syncytial virus (BRSV) infection (18), bovine viral diarrhea (BVD), respiratory disease, mastitis, metritis, hepatic lipidosis, and foot and mouth disease (27), and in such states as parturition, starvation, or transport stress (9, 37). An increase in SAA concentration was also observed in Pasteurella multocida infection (7), Mannheimia haemolytica infection (25), BRSV infection (18), mastitis, and BVD (27).

Eicosanoids are the metabolites of arachidonic acid cascade and they establish important markers of inflammation in animals. Prostaglandins E2 (PGE2) and F2α (PGF2α), leukotrien B4 (LTB4), and tromboxan B2 (TXB2) are the most useful in the diagnosis or recognition of pathogenesis of many cattle diseases. For example, the stimulation of synthesis of LTB4 occurred in response to leukotoxin (6, 31) or lipopolysaccharide (LPS) of Mannheimia haemolytica (31), Pasteurella multocida, or BRSV infection (29) and in mastitis following Klebsiella pneumoniae infection (30). Chemotactic function of the eicosanoid after inoculation of bovine herpes virus-1 (28) or Mannheimia haemolytica (3) was also showed. Similarly, an evaluation of prostaglandin occurrence in a course of disease or infection has an important diagnostic significance. The rise of PGE2 release was observed after stimulation of LTB4 occurred in response to leukotoxin (6, 31) or lipopolysaccharide (LPS) of Mannheimia haemolytica (31), Pasteurella multocida, or BRSV infection (29) and in mastitis following Klebsiella pneumoniae infection (30). Chemotactic function of the eicosanoid after inoculation of bovine herpes virus-1 (28) or Mannheimia haemolytica (3) was also showed. Similarly, an evaluation of prostaglandin occurrence in a course of disease or infection has an important diagnostic significance. The rise of PGE2 release was observed after stimulation of LTB4 occurred in response to leukotoxin (6, 31) or lipopolysaccharide (LPS) of Mannheimia haemolytica (31), Pasteurella multocida, or BRSV infection (29) and in mastitis following Klebsiella pneumoniae infection (30). Chemotactic function of the eicosanoid after inoculation of bovine herpes virus-1 (28) or Mannheimia haemolytica (3) was also showed. Similarly, an evaluation of prostaglandin occurrence in a course of disease or infection has an important diagnostic significance. The rise of PGE2 release was observed after stimulation of LTB4 occurred in response to leukotoxin (6, 31) or lipopolysaccharide (LPS) of Mannheimia haemolytica (31), Pasteurella multocida, or BRSV infection (29) and in mastitis following Klebsiella pneumoniae infection (30). Chemotactic function of the eicosanoid after inoculation of bovine herpes virus-1 (28) or Mannheimia haemolytica (3) was also showed. Similarly, an evaluation of prostaglandin occurrence in a course of disease or infection has an important diagnostic significance. The rise of PGE2 release was observed after stimulation of LTB4 occurred in response to leukotoxin (6, 31) or lipopolysaccharide (LPS) of Mannheimia haemolytica (31), Pasteurella multocida, or BRSV infection (29) and in mastitis following Klebsiella pneumoniae infection (30). Chemotactic function of the eicosanoid after inoculation of bovine herpes virus-1 (28) or Mannheimia haemolytica (3) was also showed. Similarly, an evaluation of prostaglandin occurrence in a course of disease or infection has an important diagnostic significance. The rise of PGE2 release was observed after stimulation of LTB4 occurred in response to leukotoxin (6, 31) or lipopolysaccharide (LPS) of Mannheimia haemolytica (31), Pasteurella multocida, or BRSV infection (29) and in mastitis following Klebsiella pneumoniae infection (30). Chemotactic function of the eicosanoid after inoculation of bovine herpes virus-1 (28) or Mannheimia haemolytica (3) was also showed. Similarly, an evaluation of prostaglandin occurrence in a course of disease or infection has an important diagnostic significance. The rise of PGE2 release was observed after stimulation of LTB4 occurred in response to leukotoxin (6, 31) or lipopolysaccharide (LPS) of Mannheimia haemolytica (31), Pasteurella multocida, or BRSV infection (29) and in mastitis following Klebsiella pneumoniae infection (30). Chemotactic function of the eicosanoid after inoculation of bovine herpes virus-1 (28) or Mannheimia haemolytica (3) was also showed. Similarly, an evaluation of prostaglandin occurrence in a course of disease or infection has an important diagnostic significance. The rise of PGE2 release was observed after stimulation of LTB4 occurred in response to leukotoxin (6, 31) or lipopolysaccharide (LPS) of Mannheimia haemolytica (31), Pasteurella multocida, or BRSV infection (29) and in mastitis following Klebsiella pneumoniae infection (30). Chemotactic function of the eicosanoid after inoculation of bovine herpes virus-1 (28) or Mannheimia haemolytica (3) was also showed. Similarly, an evaluation of prostaglandin occurrence in a course of disease or infection has an important diagnostic significance. The rise of PGE2 release was observed after stimulation of LTB4 occurred in response to leukotoxin (6, 31) or lipopolysaccharide (LPS) of Mannheimia haemolytica (31), Pasteurella multocida, or BRSV infection (29) and in mastitis following Klebsiella pneumoniae infection (30).
Bovine respiratory disease (BRD) is a multifactor syndrome caused by infectious and environmental agents (4, 5). Actually, Mycoplasma bovis is one of the most important infectious factors of BRD. The significance of M. bovis participation in BRD was increased in the last years in Europe and other continents (1, 2, 12, 21, 24). For some time now, M. bovis is regarded as the primary or start factor of the syndrome, rather than the secondary factor (1). In Poland (2007-2010), the seroprevalence of M. bovis was 76.67% with reference to positive samples of respiratory diseases in cattle. Strongly positive results were demonstrated in 12.59% of the animals (2).

The aim of the study was to investigate the stimulation of selected APR parameters in response to intratracheal inoculation of Mycoplasma bovis in calves. Not sufficient information concerning the changes of APPs or eicosanoids during infection of Mycoplasma bovis in cattle requires a completion of knowledge in this scope.

Material and Methods

Animals. Twelve clinically-healthy, Black and White Lowland breed calves, aged 4-8 weeks, were divided into two equal groups. Experimental procedures and animal management protocols were undertaken in accordance with the requirements of the Local Ethic Committee on Animal Experimentation of the University of Life Sciences in Lublin, Poland.

Study protocol and sample collection. Calves in the experimental group (group I) were intratracheally challenged with a broth culture of pathogenic strain of Mycoplasma bovis propagated in Eaton’s medium with the final titre of 3 x 10^9 (CFU)/mL. The strain was isolated from a calf with symptoms of bronchopneumonia and arthritis. The control animals (group II) received by the same route a sterile physiological saline as placebo. The blood samples were collected into tubes containing K3EDTA as the anticoagulant (0.07 mol/mL of blood) before the day of inoculation (zero test; the first day of experiment) and then with 48 h intervals of blood before the day of inoculation (zero test; the first day of experiment) and then with 48 h intervals of observation. Statistically significant (P<0.05) differences between examined groups were noted in a period of 3-9 d of the experiment with reference to PGF2α and LTB4, and also on days 3, 5, and 7 for TXB2.

Blood serum analysis. The following parameters were assayed in the serum: the concentration of selected acute phase proteins (APPs), i.e. haptoglobin (Hp) and serum amyloid A (SAA), and the content of eicosanoids: prostaglandin E2 (PGF2α), prostaglandin F2α (PGF2α), leukotrien B4 (LTB4), and tromboxan B2 (TXB2). The analysis of all examined parameters was done using commercial ELISA kits; Hp, SAA - Tridelta Development Limited (Ireland), eicosanoids – Assay Designs, Inc., (USA).

Statistical analysis. Statistical significance of differences between the mean values recorded in the experimental group and controls was compared using Student’s t-test at P<0.05.

Results

The concentration of Hp in experimental calves was statistically significantly (P<0.05) higher on days 3, 5, and 7 of observation in comparison with the controls (Table 1). The values were similar in both experimental and control groups on day 9 of the experiment. In response to inoculation of Mycoplasma bovis, the increase in SAA was observed when compared with the controls (Table 1). This increase was noted during the whole experiment, starting from day 3 of the observation. Statistically significant (P<0.01) differences between both examined groups of calves were observed on days 3, 5, and 7 of the study (Table 1).

In response to inoculation of Mycoplasma bovis, an increase in eicosanoid concentrations in comparison with controls was observed, starting from day 3 of the experiment (Table 1). These elevated concentrations were maintained until the 9th d of observation. Statistically significant (P<0.05) differences between examined groups were noted in a period of 3-9 d of the experiment with reference to PGF2α and LTB4, and also on days 3, 5, and 7 for TXB2.

In case of PGF2α, the differences were significant (P<0.01) between 5th and 9th d of observation (Table 1).

Discussion

APPs are synthesised in the liver in response to infection (positive APPs), or their concentration decrease after contact with infectious or inflammatory factor (negative APPs) (34). SAA and Hp belong to the first group of APPs (33). Intratracheal inoculation of Mycoplasma bovis caused an increase in serum concentration of Hp and SAA in calves. This finding is consistent with the data of previous study, in which an increase in total protein and γ-globulin concentration was observed under the same conditions (8). Hp plays an important role in monitoring of the acute inflammatory state in cattle. It is a very useful parameter of low or undetectable content in serum of healthy cattle (33). It is worth noting that during APR a multiple increase in Hp concentration, of even 50-100 times, is observed in blood plasma in response to inflammatory impulse (4, 5, 15, 17, 33). However, Hp is a slow reactive protein on this impulse. It means that its multiple rise is not noted until 12-24 h after initiation of inflammatory process, whereas a peak of haptoglobin rise is present approximately between 72 and 96 h. The fall of Hp concentration to reference values was noted between 8th and 14th d from the onset of inflammatory impulse (33). In response to inoculation of Mycoplasma bovis, the concentration of Hp was similar to the control at the 9 d of observation. Hp is characteristic for its high specificity, so used alone it seems to be more available in estimation of the health status of calves. Its sensitivity will be higher, however, when used in combination with APPs. For example, the Hp and fibrinogen concentrations may be used as diagnostic parameters for determination of treatment validity in feedlot calves with bronchopneumonia (20).

SAA belongs to the fastest reactive APPs in cattle. Its increase is observed after approximately 6-8 h, whereas maximum concentration of SAA usually appears on 24-48 h from initiation of the inflammatory process.
Gradual fall of SAA concentration to reference values takes place during the few following days (33). Inoculation of Mycoplasma bovis in calves caused an increase in SAA content as early as on 3 d of observation when compared with the controls. That rise maintained during the whole study. The SAA concentration in blood serum of challenged animals decreased to the values before inoculation at the 9 d of observation.

In response to Mycoplasma bovis challenge, the stimulation of production and release of selected chemotactic factor for neutrophils and eosinophils. Then LTB4 is responsible for intensification of phagocytic ability of neutrophils. This process resulted from the ability of this eicosanoid to increase intensification synthesis of LTB4 is present on the route of lipooxygenation (36). Whereas, the rise of concentration of TXB2 in response to lipooxygenation (36). The evaluation of selected APPs in bovine sera during M. bovis infection could have a practical significance and new diagnostic applications especially concerning diagnostic problems in the bacteria identification in acute phase of disease, when specific antibodies are not detectable. The significance increases in subclinical cases of the infection emphasises the need of estimation of both fast (SAA) and slow reactive (Hp) proteins in the affected animals. The distinct changes of concentration in blood serum of challenged animals decreased to the values before inoculation at the 9 d of observation.

Table 1
Mean concentrations of Hp, SAA and selected eicosanoids (PGE2, PGF2α, TXB2, LTB4) in the serum of calves challenged with Mycoplasma bovis (group I) and controls (group II)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I</th>
<th>Group II</th>
<th>Group I</th>
<th>Group II</th>
<th>Group I</th>
<th>Group II</th>
<th>Group I</th>
<th>Group II</th>
<th>Group I</th>
<th>Group II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hp (mg/mL)</td>
<td>0.047 ± 0.003</td>
<td>0.048 ± 0.004</td>
<td>0.075 ± 0.013b</td>
<td>0.045 ± 0.002</td>
<td>0.059 ± 0.006a</td>
<td>0.051 ± 0.004</td>
<td>0.108 ± 0.023c</td>
<td>0.053 ± 0.011</td>
<td>0.05 ± 0.003</td>
<td>0.048 ± 0.004</td>
</tr>
<tr>
<td>SAA (µg/mL)</td>
<td>17.60 ± 0.39</td>
<td>17.30 ± 1.25</td>
<td>22.83 ± 4.48b</td>
<td>15.11 ± 1.09</td>
<td>24.70 ± 1.46c</td>
<td>17.75 ± 0.53</td>
<td>22.03 ± 1.66c</td>
<td>14.04 ± 0.07</td>
<td>11.6 ± 0.07</td>
<td>6.90 ± 0.16</td>
</tr>
<tr>
<td>PGE2 (pg/mL)</td>
<td>1,565 ± 178</td>
<td>1,388 ± 363</td>
<td>2,150 ± 356</td>
<td>1,275 ± 204</td>
<td>2,550 ± 362b</td>
<td>1,455 ± 234</td>
<td>2,333 ± 175b</td>
<td>1,281 ± 114</td>
<td>2,358 ± 317b</td>
<td>1,228 ± 121</td>
</tr>
<tr>
<td>PGF2α (pg/mL)</td>
<td>670 ± 210</td>
<td>570 ± 280</td>
<td>6,100 ± 2210b</td>
<td>540 ± 180</td>
<td>6,680 ± 180</td>
<td>540 ± 180</td>
<td>3,160 ± 234</td>
<td>220 ± 175b</td>
<td>880 ± 114</td>
<td>160 ± 120b</td>
</tr>
<tr>
<td>TXB2 (pg/mL)</td>
<td>28,000± 9,040</td>
<td>29,600± 10,680</td>
<td>79,000± 38,800d</td>
<td>30,600± 12,000</td>
<td>82,000± 17,700d</td>
<td>48,270± 21,830</td>
<td>58,530± 7,940d</td>
<td>15,470± 7,400</td>
<td>28,200± 1,980</td>
<td>21,460± 9,604</td>
</tr>
<tr>
<td>LTB4 (pg/mL)</td>
<td>83 ± 5.65</td>
<td>89.16 ± 12.81</td>
<td>242 ± 40.67b</td>
<td>120 ± 24.28</td>
<td>312 ± 53.07b</td>
<td>128 ± 34.88</td>
<td>367 ± 70.88</td>
<td>145 ± 25.88</td>
<td>516 ± 70.88</td>
<td>113 ± 18.90</td>
</tr>
</tbody>
</table>

α – P<0.05; b – P<0.01; c – P<0.001; ± - SD.


