STIMULATING EFFECT OF MYCOPLASMA BOVIS INFECTION ON PROINFLAMMATORY RESPONSE IN INFECTED CATTLE

KATARZYNA DUDEK, DARIUSZ BEDNAREK, AND EWELINA SZACAWA

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

Received: October 28, 2011   Accepted: December 6, 2011

Abstract

The aim of the study was to evaluate the alternations in the concentration of proinflammatory arachidonic acid metabolites in cattle infected with Mycoplasma bovis. The study was performed on two groups of cattle, differing in aged, i.e. calves (n=56) and adult animals (n=19). Additionally, two subgroups were selected among them: seropositive and seronegative for Mycoplasma bovis infection. The presence of serum specific antibodies against Mycoplasma bovis and the concentration of selected inflammatory parameters, such as prostaglandins (PGE$_2$, PGF$_2$α), leukotrien B$_4$, and tromboxan B$_2$ were determined. An increase in the concentration of the greater part of arachidonic acid cascade metabolites in cattle seropositive for Mycoplasma bovis, in both age groups was observed in comparison with the seronegative animals, except leukotrien B$_4$ in adult animals. The obtained results indicate the stimulating effect of Mycoplasma infection on proinflammatory response in infected cattle.

Key words: cattle, Mycoplasma bovis, arachidonic acid metabolites.

Mycoplasma bovis is considered to be one of the most important infectious agent of bovine respiratory disease (BRD), which is a major health and economic problem in the world’s cattle industry (1, 12, 15), including Poland (4). The disease is a result of interactions of both infectious and environmental factors, which effectively impair health status of animals, and in consequence lead to pneumonia. The most often isolated bacterial agents from BRD cases, except mycoplasmal factors, are Mannheimia haemolytica, Pasteurella multocida, Arcanobacterium pyogenes, and Histophilus somni, whereas microorganisms of viral origin, such as bovine respiratory syncytial virus (BRSV), parainfluenza-3 virus (PI3V), or bovine herpesvirus-1 (BHV1) are considered to be important in the specific role attributed to bovine viral diarrhoea virus (BVDV), especially in the context of coinfection with M. bovis (1, 11, 12).

During different viral or bacterial infections in livestock, proinflammatory response is usually reported, which is characterised by an activation of arachidonic acid cascade resulting in a significant increase in the production of suitable inflammatory mediators, such as prostanoids (prostaglandins, tromboxanes) and leukotriens (e.g. slow-reacting substance of anaphylaxis, leukotrien B$_4$). Leukotriens arise through lipoxygenase pathway as a result of arachidonic acid metabolism initiated by phospholipase A$_2$ (PLA$_2$). Leukotrien B$_4$ (LTB$_4$) is one of the most important inflammatory agents due to its strong chemotactic effect on some mononuclear leukocytes, i.e. neutrophils and eosinophils. In Mannheimia haemolytica infections in cattle, LTB$_4$ plays an important role as a strong pro-inflammatory agent (6), whose production is stimulated by the bacterial leukotoxin (LKT) in vitro (7). The stimulation is intracellular Ca$^{2+}$ dependent and related to the source of arachidonic acid (7, 8). Additionally, it was shown that the metabolites of arachidonic acid mediated cytolysis of bovine leukocytes caused by Mannheimia haemolytica LKT (13). An increase in LTB$_4$ concentration in the exhaled condensate of calves was associated with respiratory dysfunction following experimental infections with Pasteurella multocida or BRSV (18).

Prostaglandins and tromboxanes originate from cyclooxygenase pathway parallel to lipoxygenation process (20). The up-regulation of PGE$_2$ together with other inflammatory factors, such as TNF-α, IL-1, and IL-6 was observed following the stimulation of bovine Kupffer cells by bacterial lipopolysaccharide in vitro (21).

Tromboxanes are responsible for thrombocyte adhesion to the walls of blood vessels, which facilitate formation of intravascular thrombosis. The serum concentration of TXB$_2$ indicates actual thrombocyte aggregation in vivo (16). An activation of factors contracting vessels is often a result of tromboxan production (5). The inoculation with Mannheimia haemolytica in calves caused a significant rise in production of TXB$_2$ (6), which may prove the participation of this eicosanoid in the pathogenesis of fibrinous pneumonia.
The aim of the study was to evaluate the changes in the arachidonic acid cascade response in the seropositive cattle naturally infected with *Mycoplasma bovis*.

**Material and Methods**

**Animals.** The study was performed on Black and White Lowland breed cattle, which were divided into two age groups: 56 calves at the age from a few weeks to four months and 19 adult animals. Among both groups of animals, two subgroups were additionally distinguished on the basis of the presence or absence of serum *Mycoplasma bovis* antibodies, i.e. seropositive and seronegative (control) for *M. bovis*. The animals originated from different farms and regions of Poland and were kept in individual pens holding maximum 20 animals. The average herd size in which the laboratory samples were collected had about 100 animals.

**Sample collection and assays.** The blood samples were collected from the *vena jugularis externa* and then centrifuged to obtain the sera to further analysis. Before the analysis, the serum samples were stored under freeze conditions (-20°C) with no preservative substances. The duration of sample storage lasted 4-6 weeks. The presence of serum *M. bovis* antibodies and concentrations of selected inflammatory parameters, i.e. PGE$_2$, PGF$_{2\alpha}$, LTB$_4$, and TXB$_2$ were evaluated using ELISA commercial kit (Bio-X Diagnostics) for *M. bovis* antibodies and separate tests for the analysis of the individual eicosanoid concentration (Assay Designs, Inc.; USA).

**Data analysis.** Results were presented as arithmetic means with standard deviation (means ±SD). Statistical significance of differences between the values recorded in individual subgroups and their controls was compared using Student’s *t*-test at *P*<0.05, *P*<0.01, and *P*<0.001.

**Results**

The presence of *M. bovis* antibodies in 42 calves was observed from + to ++ of positiveness degree, whereas in 15 adult cattle it ranged between + and ++++. The other animals showed the lack of contact with the pathogen (negative results of serological examinations).

In calves seropositive for *M. bovis* an increase in serum concentration of the selected inflammatory parameters was observed in comparison with the seronegative animals. Statistically significant differences between both examined subgroups of animals were recorded for PGE$_2$, TXB$_2$ at *P*<0.01 and *P*<0.001, respectively (Table 1).

The serum concentrations of PGE$_2$, PGF$_{2\alpha}$, and TXB$_2$ were higher in adult cattle seropositive for *M. bovis* as compared with seronegative animals. A statistically significant increase in the eicosanoid concentration between the two subgroups of adult animals came to *P*<0.05 (PGE$_2$) and *P*<0.001 for TXB$_2$. In contrast, a decrease in LTB$_4$ serum concentration was observed in adult cattle seropositive for *M. bovis* in comparison with the seronegative animals. However, the differences between both subgroups were not statistically significant (Table 1).

**Discussion**

The clinical signs of pneumonia, i.e. coughing, hyperpnoea, dyspnoea, nasal discharge, and other nonspecific symptoms such as fever and depression were observed earlier in cattle in which the presence of specific *M. bovis* antibodies was diagnosed. On the other hand, in some animals seronegative for *M. bovis* a lack, or only mild clinical signs were noted, and basic clinical parameters, such as body temperature, breaths, and pulse did not exceed the reference values. The obtained results indicate a significant increase in TXB$_2$ serum concentration in both age groups of cattle seropositive for *M. bovis*. The rise was more than 7-fold in calves and 4-fold in adult animals. The results were consistent with our previous study, where the stimulation in production of all examined eicosanoids, i.e. TXB$_2$, LTB$_4$, PGE$_2$, and PGF$_2$ was observed following the experimental inoculation of calves with *M. bovis* (10). The tromboxans are considered to be strong activators of thrombocyte aggregation and factors responsible for contraction of blood vessels (5) and their production leads to thrombocyte adhesion to blood vessel walls. As a consequence of tromboxan stimulation, venous thrombosis in target tissues and organs may appear.

**Table 1**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Seropositive</th>
<th>Seronegative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>calves (n = 42)</td>
<td>adult animals (n = 15)</td>
</tr>
<tr>
<td>PGE$_2$ (pg/mL)</td>
<td>554.97 ± 807.17</td>
<td>489.05 ± 234.85*</td>
</tr>
<tr>
<td>PGF$_{2\alpha}$ (pg/mL)</td>
<td>44,564 ± 38,674b</td>
<td>34,932 ± 17,423</td>
</tr>
<tr>
<td>LTB$_4$ (pg/mL)</td>
<td>153.55 ± 156.53</td>
<td>103.57 ± 45.52</td>
</tr>
<tr>
<td>TXB$_2$ (pg/mL)</td>
<td>33,339 ± 39,007c</td>
<td>31,858 ± 21,587c</td>
</tr>
</tbody>
</table>

* *P*<0.05, b *P*<0.01, c *P*<0.001.
The study demonstrated a marked stimulation of PGE$_2$ and PGF$_2\alpha$ production in all examined animals, indicating their contact with *M. bovis*. An almost two-fold increase in their PGF$_2\alpha$ was observed in calves. The elevation of the serum concentrations of PGE$_2$ and PGF$_2\alpha$ in cattle with *M. bovis* antibodies resulted probably from prostaglandin synthesis and release by neutrophils, eosinophils, and macrophages (20). The stimulation of the prostaglandin synthesis could lead to an increased spread of the bacteria in infected organism following vasodilatation and rise of blood vessel permeability (14).

A slight increase in serum concentration of LTB$_4$ in seropositive calves probably initiated the activation of neutrophil and eosinophil migration to infected tissues (20), considering its strong chemotactic effect on these cells or the migration, which could be evidenced by a decreased LTB$_4$ in adult animals. The role of LTB$_4$ as a „potent neutrophil chemoattractant” in BHV1 infection in cattle was shown in the *in vitro* study with bovine bronchial epithelial cells (17).

The significance of eicosanoid role in the pathogenesis of *Mannheimia haemolytica* infection was demonstrated in a distinct elevation of concentrations of LTB$_4$, TXB$_2$, 6-keto-prostaglandin F$_1\alpha$, and PGE$_2$, as a result of calf inoculation with that pathogen. Chemotactic effect of these inflammatory mediators, especially LTB$_4$, was proved after treatment of calves with representatives of steroidal inflammatory drugs, which effectively inhibited the eicosanoid synthesis (6). *In vitro* studies indicated that LTB$_4$, TXB$_2$, and PGE$_2$ constitute the important mediators of inflammatory response in bacterial respiratory diseases of cattle (19).

The obtained results confirmed our previous studies evaluating the changes in selected immunological parameters under the same conditions. In *M. bovis* seropositive cattle, a significant increase in concentration of total protein, γ-globulins, haptoglobin, and serum amyloid A was observed (9). The confirmation of stimulation of production of the two latest parameters was obtained following calf challenge with *M. bovis* (10).

The arachidonic acid metabolites play an important role in both bacterial and viral respiratory infections of cattle. Our results confirmed the hypothesis that the eicosanoids constitute the important inflammatory mediators in calves suffering from pneumonia caused by *M. bovis*, because a significant increase in prosttanoid concentrations, *i.e.* extremely proinflammatory agents, was noted in the affected animals. Similar changes regarding leukotrien content were observed as a result of lipoxygenase activation, although to a lesser degree. The evaluation of selected metabolites of arachidonic acid has diagnostic importance and indicates therapeutic possibility of the use of cyclooxygenase inhibitors as supporting agents in the therapy of the cattle respiratory diseases, including *M. bovis* infections, especially considering the lack of effective vaccines and increasing resistance of the pathogen to antibiotics (2, 3).

**References**


