Acute phase proteins in serum and cerebrospinal fluid in healthy cattle: possible use for assessment of neurological diseases


Use of acute-phase proteins (APPs) for assessment of health and disease in animals has increased greatly within the last decade. The objective was to determine the normal concentration of APPs in the serum and cerebrospinal fluid (CSF) of healthy cattle by polyacrylamide gel electrophoresis. Fifty crossbred animals (350±70kg of BW and 18±1.2 months of age), 25 heifers and 25 steers were used. CSF samples were collected from atlanto-occipital (AO) site and blood samples were obtained from the jugular vein. CSF and serum protein electrophoresis were performed by means of sodium dodecyl sulphate-polyacrylamide gel electrophoresis. Thirty-seven proteins with molecular weights ranging from 7 and 37kDa were identified in CSF of all animals. These eight were nominally identified with immunoglobulin A and G, ceruloplasmina, transferrina, albumina, α1-antitripsina, acidic glycoprotein and haptoglobin. All protein fractions in CSF did not differ between heifers and steers. In sera, 34 proteins with molecular weights between 7 and 244kDa were identified in heifers and steers. Similar proteins were nominally identified in the sera, but only the CSF presented α1-antitripsina. The serum values of acidic glycoprotein and immunoglobulin G were significantly higher in steers compared with heifers. In conclusion, measurement of CSF acute phase protein concentrations can be useful in diagnosing and monitoring the progression of bovine neurological diseases, perhaps even to guide therapeutic procedures. The CSF electrophoretic profile of healthy cattle does not change depending on gender.

INDEX TERMS: Acute phase response, protein, serum, cerebrospinal fluid, cattle, neurological diseases, polyacrylamide gel electrophoresis, cerebrospinal fluid biomarker, neuroinflammation.
INTRODUCTION

The physiological response to infections, trauma or surgery, stress, neoplasia, immunological disorders and injuries involves local inflammation and the start of events leading to a systemic response, also called acute phase reaction (APR) (Murata et al. 2004, Petersen et al. 2004, Cerón et al. 2005, Ceciliani et al. 2012). This multiplicity of changes is distant from the injury site, and includes fever, leukocytosis and quantitative and qualitative modification of a group of non-structurally related proteins present in blood and other biological fluids, collectively named acute phase proteins (APPs) (Ceciliani et al. 2012).

The APPs consist of ‘negative’ and ‘positive’ proteins that show a decrease and an increase in levels, respectively, in response to challenge. The negative APPs include albumin and transferrin. The positive ones include haptoglobin, C-reactive protein, serum amyloid A, caeruloplasmin, fibrinogen and alpha 1-acid glycoprotein (Murata et al. 2004). Acute phase proteins are primarily produced in the liver (Murata et al. 2004, Petersen et al. 2004, Ceron et al. 2005). However, extrahepatic production of APPs is also possible in most mammalian species (Jacobsen et al. 2006, Di Filippo et al. 2011, 2014a). Some APPs opsonize microorganisms and activate complements, while others scavenge cellular remnants and free radicals or neutralize proteolytic enzymes (Gruys et al. 2014a). Some APPs complement the defensive action of other APPs, while others scavenge cellular remnants and free radicals or neutralize proteolytic enzymes (Gruys et al. 2014a). Some APPs are also involved in non-structural processes such as coagulation, fibrinolysis, platelet aggregation, and complement activation (Petersen et al. 2004, Cerón et al. 2005). However, extrahepatic production of APPs is also possible in most mammalian species (Jacobsen et al. 2006, Di Filippo et al. 2011, 2014a). Some APPs complement the defensive action of other APPs, while others scavenge cellular remnants and free radicals or neutralize proteolytic enzymes (Gruys et al. 2014a).

The circulating concentrations of APPs are related to the severity of the disorder and the extent of tissue damage in the affected animal. Therefore, quantification of their concentration can provide diagnostic and prognostic information if proper timing of sampling is assured (Murata et al. 2004). The results of one study found that APPs were eight times more sensitive than leukocyte count (Cerón et al. 2005).

Recent studies have demonstrated an increase in the concentration of APPs in cerebrospinal fluid (CSF) in humans and dogs with neurological diseases and traumatic brain injury. A significant association between high levels of proinflammatory markers in CSF and severity of fatigue, depression, anxiety and cognitive impairment in individuals with Parkinson’s disease has been demonstrated (Lindqvist et al. 2013). Another study concluded that CSF APPs estimation is a useful marker to differentiate pyogenic from non-pyogenic meningitis, but it cannot differentiate between tuberculosis and fungal or viral meningitis (Anil Kumar et al. 2010). In turn, it has been demonstrated that the level of APPs is significantly greater in the CSF of dogs with acute spinal cord injury compared with healthy control dogs (Anderson et al. 2015). CSF APPs levels were positively associated with the severity of spinal cord damage and can serve as a prognostic indicator in dogs with intervertebral disc herniation (Roerig et al. 2013).

Therefore, analysis of APPs has become an important tool in the diagnosis, treatment and prognosis of neurological inflammatory diseases in humans as well as domestic animals. In view of the great importance of cattle not only in production of milk and meat, but also in transmission of disease to humans, it is advisable to determine APP levels in the CSF of healthy animals. The aim of this study was to determine the concentration of APPs in the CSF of healthy heifers and steers by polyacrylamide gel electrophoresis.
RESULTS

Thirty-seven proteins with molecular weights between 7 and 37kDa were identified in cerebrospinal fluid (CSF) of heifers and steers. CSF levels of proteins of 169kDa (immunoglobulin A), 104kDa (celuloplasmin), 85kDa (immunoglobulin G), 77kDa (transferrin), 64kDa (albumin), 58kDa (α1-antitripsin), 39kDa (acidic glycoprotein), and 43kDa (haptoglobin) were nominally identified (Table 1). All protein fractions in CSF did not differ between heifers and steers.

In sera, 34 proteins with molecular weights between 7 and 244kDa were identified in all animals. Serum levels of proteins of 105kDa (celuloplasmin), 171kDa (immunoglobulin A), 85kDa (immunoglobulin G), 77kDa (transferrin), 62kDa (albumin), 41kDa (haptoglobin), and 39kDa (acidic glycoprotein) were nominally identified (Table 2). The serum values of acidic glycoprotein and immunoglobulin G were significantly higher in steers compared with the values in heifers.

Table 1. Cerebrospinal fluid protein concentrations (mean ± SEM), determined by sodium dodecyl sulphate-polyacrylamide gel electrophoresis, in crossbred heifers and steers

<table>
<thead>
<tr>
<th>Protein</th>
<th>Cattle</th>
<th>Steers</th>
<th>Value of P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total serum protein</td>
<td>12.2±1.04</td>
<td>14.7±1.02</td>
<td>0.09</td>
</tr>
<tr>
<td>Albumin</td>
<td>5.29±0.45</td>
<td>7.08±0.74</td>
<td>0.04</td>
</tr>
<tr>
<td>Celuloplasmin</td>
<td>0.03±0.00</td>
<td>0.07±0.00</td>
<td>0.79</td>
</tr>
<tr>
<td>Transferrin</td>
<td>0.54±0.07</td>
<td>0.53±0.07</td>
<td>0.91</td>
</tr>
<tr>
<td>Haptoglobin</td>
<td>0.23±0.08</td>
<td>0.21±0.08</td>
<td>0.85</td>
</tr>
<tr>
<td>Acidic glycoprotein</td>
<td>0.21±0.04</td>
<td>0.17±0.03</td>
<td>0.47</td>
</tr>
<tr>
<td>Immunoglobulin A</td>
<td>0.07±0.02</td>
<td>0.11±0.02</td>
<td>0.19</td>
</tr>
<tr>
<td>Immunoglobulin G</td>
<td>3.05±0.31</td>
<td>3.76±0.30</td>
<td>0.11</td>
</tr>
<tr>
<td>α1-antitripsin</td>
<td>0.27±0.05</td>
<td>0.29±0.06</td>
<td>0.75</td>
</tr>
</tbody>
</table>

Different letters (A and B) in the same line indicate differences between groups (P<0.01).

Table 2. Serum protein concentrations (mean ± SEM), determined by sodium dodecyl sulphate-polyacrylamide gel electrophoresis, in crossbred heifers and steers

<table>
<thead>
<tr>
<th>Protein</th>
<th>Cattle</th>
<th>Steers</th>
<th>Value of P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total serum protein</td>
<td>8982.5±419.1</td>
<td>8488.7±166.3</td>
<td>0.28</td>
</tr>
<tr>
<td>Albumin</td>
<td>5142.1±157.4A</td>
<td>5021.7±98.8A</td>
<td>0.52</td>
</tr>
<tr>
<td>Celuloplasmin</td>
<td>11.00±0.81A</td>
<td>139.6±1.46A</td>
<td>0.08</td>
</tr>
<tr>
<td>Transferrin</td>
<td>335.60±22.01A</td>
<td>267.54±22.01A</td>
<td>0.03</td>
</tr>
<tr>
<td>Haptoglobin</td>
<td>9.44±1.44A</td>
<td>7.65±0.92A</td>
<td>0.303</td>
</tr>
<tr>
<td>Acidic glycoprotein</td>
<td>13.42±0.73A</td>
<td>7.98±0.73B</td>
<td>0.00</td>
</tr>
<tr>
<td>Immunoglobulin A</td>
<td>174.90±17.81A</td>
<td>138.60±17.81A</td>
<td>0.15</td>
</tr>
<tr>
<td>Immunoglobulin G</td>
<td>2799.36±95.162A</td>
<td>2070.74±95.162B</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Different letters (A and B) in the same line indicate differences between groups (P<0.01).

DISCUSSION

The serum electrophoretic profile values obtained were similar to those described in the literature for healthy cattle of the same age range (Fagliari et al. 2007, Simplício et al. 2013, Sampaio et al. 2015). The CSF profile has not yet been determined for bovines, so no comparative data are available.

The proteinogram of the cerebrospinal fluid (CSF) of the steers and heifers was similar to the serum subfractionation, except for α1-antitripsin, which was only present in the CSF. Different body fluids can have similar electrophoretic profiles (Kaneko et al. 2008). However, in cattle, the serum levels of α1-antitripsin can be so low that their systemic detection is difficult. Similar findings were described by Fagliari et al. (2007) and Sampaio et al. (2015).

The identification and quantification of α1-antitripsin in the CSF of the animals can be related to the local synthesis of this APP by the glial cells or other tissues of the central nervous system (CNS) (Johanson et al. 2008). Approximately 20% of the proteins present in the CSF are produced locally, with the other 80% coming from the blood. The plasma proteins selectively cross the blood-brain barrier before reaching the CSF (Johanson et al. 2008, Kaneko et al. 2008).

The APPs are often called blood proteins because they are primarily synthesized by the hepatocytes, and their production is part of the acute phase response (Ceciliani et al. 2012). However, it has been demonstrated that APPs can be produced by many other tissues and cells (Jacobsen et al. 2006, Di Filippo et al. 2014a, 2014b). Under normal physiological conditions, the production of amyloid A (McDonald et al. 2001) and α1 acid glycoprotein (Ceciliani et al. 2005) by the epithelial cells of the mammary gland of healthy bovines has been reported. Increases in the local expression of Hp were observed after intramammary infusion of LPS (Hiss et al. 2004) and infection by Escherichia coli (Buitenhuis et al. 2011). Hp was also detected in the ovaries and oviducts of milk cows in luteal and non-luteal phases of the estrus cycle (Lavery et al. 2003).

The local extrahepatic production of APPs appears to occur in disseminated form, mobilizing different tissues simultaneously with the liver (Gruys et al. 2005). The mechanism probably plays the role of counteracting the proinflammatory effects inside the injured tissue (Ceciliani et al. 2012). Measurement of the local levels of APPs, by providing information on the inflammatory/infectious status of an organ of interest, increases the diagnostic precision (Jacobson et al. 2006). In one study, an increase was noted in the concentration of transferrin (Tf) in the CSF of people suffering from restless leg syndrome (RLS). However, there was no change in the serum values of Tf (Mizuno et al. 2005). Similar results have been observed in people with multiple sclerosis (Khalil et al. 2014). Tf is one of the main carriers of iron in CNS, in humans acting as an important marker of neurodegenerative diseases (Murata et al. 2004).

Haptoglobin (Hp) is one of the most plentiful APPs in bovines (Murata et al. 2004). Hp binds to free hemoglobin, which is toxic and proinflammatory, and in the plasma, it reduces the oxidative damages associated with hemolysis (Yang et al. 2003). It has an inhibitory effect on the chemotaxis of granulocytes and on phagocytosis, as well as bacterial activity. It can also inhibit the proliferation of mast cells (El-Ghmati et al. 2002), impede the spontaneous maturation of...
Langerhans cells (Xie et al. 2000) or suppress the proliferation of T cells (Arredouani et al. 2003).

Increases in blood levels of Hp have been reported in cattle with mastitis, hepatic abscesses, pyometra, traumatic reticulitis and abomasal displacement (Eckersall & Conner 1988, Ceciliani et al. 2012). In the same study, dogs with severe spinal cord injury showed significantly higher concentration of Hp in the CSF than animals with slight to moderate injuries. However, no correlation was found between the Hp concentration in the CSF and the motor, posture or sensory results. Hp was considered the most important APP of the CSF after injury associated with IVDH in the dogs studied (Anderson et al. 2015). In humans, the concentration of Hp in the CSF was also found to be significantly higher after traumatic brain injury. Hp is an α2-glicoprotein produced by the liver and oligodendroglia in response to IL-6 and other proinflammatory cytokines (Yang et al. 2013). Therefore, in the presence of traumatic brain injuries, intracranial hemorrhages and autoimmune encephalitis, it is believed that Hp exerts neuroprotective effects by binding with free hemoglobin (Zhao et al. 2009).

Ceruloplasmin is primarily synthesized in the liver, but studies have demonstrated that it can also be synthesized by neurons and that lipopolysaccharides (LPS) can induce its extra-hepatic synthesis (Murata et al. 2004, Anil Kumar et al. 2010). Increases and significant concentrations of ceruloplasmin were found in the CSF of people suffering from pyogenic meningitis. However, no alterations were observed in the ceruloplasmin concentration in the CSF of people with non-pyogenic meningitis (Anil Kumar et al. 2010). The measurement of ceruloplasmin in the CSF also was found to enable early identification of neurodegenerative diseases such as Parkinson’s disease (Costa et al. 2011). A massive increase of apolipoprotein E (Apo-E), a biomarker used to diagnose Alzheimer’s disease, was found in the CSF (Wang et al. 2013). Another study demonstrated a significant increase in the ceruloplasmin concentration in the CSF of dogs with steroid-responsive meningitis-arteritis (SRMA) compared to healthy animals (Bathen-Nothen et al. 2008). According to the authors, the high concentration of ceruloplasmin in the CSF might have originated from a subarachnoid hemorrhage or the intrathecal production of ceruloplasmin by the leukocytes. Dogs with meningioma and hemangiosarcoma also presented significant increase of ceruloplasmin in the CSF due to changes in the blood-brain barrier and hemorrhages (Gabor & Vanderstichel 2006).

Ceruloplasmin is an iron oxide containing copper, able to oxidize toxic ferrous iron, transforming it into nontoxic ferric iron. It protects the issues from lesions triggered by iron-mediated free radicals and is involved in many antioxidant and cytoprotective activities (Gruys et al. 2005). It can also act as an anti-inflammatory agent by reducing the number of neutrophils adhered to the endothelium and by removing peroxides (Ceciliani et al. 2012). In cattle, the increase in serum ceruloplasmin is related to inflammatory processes with tissue damages (photosensitization). The reduction of its concentration, however, is associated with metabolic factors like malnutrition and hepatic and renal diseases that affect its production and excretion, respectively (Fagliari et al. 2007).

In cattle α1-acid glycoprotein (α1-GPA) is a clinically important APP and has been used to monitor affections such as pericarditis, arthritis, mastitis, pneumonia and babesiosis (Murata et al. 2004). Its concentration generally rises more slowly, but remains high for a longer period. Therefore, its measurement can be useful to establish the acute or chronic evolution of different ailments (Petersen et al. 2004, Cerón et al. 2005).

In this study, the steers presented higher serum levels of α1-GPA than the heifers. These differences can be associated with stress (Murata et al. 2004), ingestion or not of colostrum (Orro et al. 2008) and birth from multiparous or primiparous cows. Calves from primiparous cows generally present lower serum concentration of α1-GPA than those from multiparous cows (Rocha et al. 2013). There are no reports of α1-GPA levels in the CSF of cattle, but α1-GPA is considered a sensitive marker for bacterial meningitis in pigs and rabbits (Itoh et al. 1993). It is believed that α1-GPA levels in the CSF rise for the purpose of inhibiting TNF-α, the main cytokine involved in this type of infection (Itoh et al. 1993). Increases in the levels of α1-GPA in the CSF were also described in people suffering from neurodegenerative diseases like Alzheimer’s disease (Rohrer et al. 2009).

Among the immunoglobulins, IgG and IgA have been detected in electrophoretic band shifts. In livestock, the function of IgA in the immune response has not been totally elucidated. Nevertheless, IgA appears to be related to an inflammatory stimulation, acting as a second line of defense in the elimination of pathogens that have penetrated through the surface mucous barrier (Snoeck et al. 2006). IgA can also act as a secretory antibody within the intestinal tract and lungs, able to neutralize viruses and prevent adherence of bacterial pathogens to target tissues (Di Filippo et al. 2011).

We observed a difference in the serum levels of IgG in the steers and heifers. These findings can be related to the animal’s weight at birth, calving age of the cow, milking management, udder characteristics and stress (Fagliari et al. 2007). In another study, increased concentration of IgG was
observed in the CSF of humans with tuberculosis meningitis. The results were associated with the increased permeability of the blood-brain barrier or intrathecal production, triggered by an oligoclonal reaction (Sardella et al. 2010).

CONCLUSIONS

We believe that the measurement of cerebrospinal fluid (CSF) acute phase protein concentrations can be useful in diagnosing and monitoring the progression of bovine neurological diseases or traumas, perhaps even to guide therapeutic procedures.

The CSF electrophoretic profile of young cattle does not change based on sex.

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REFERENCES


Paula A. Di Filippo et al.