The Veterinary Journal 184 (2010) 212–218

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Characterisation of acid–base abnormalities in pigs experimentally infected with *Chlamydia suis*

Petra Reinhold [a,*], Helmut Hartmann [b], Peter D. Constable [c]

[a] Institute of Molecular Pathogenesis in the Friedrich-Loeffler-Institut (Federal Research Institute for Animal Health), Naumburger Str. 96a, 07743 Jena, Germany
[b] Institute of Veterinary Physiology, Veterinary Faculty, Free University of Berlin, Oertzenweg 19b, 14163 Berlin, Germany
[c] Department of Veterinary Clinical Sciences, School of Veterinary Medicine, Purdue University, 625 Harrison St, West Lafayette, IN 47907, USA

ARTICLE INFO

Article history:
Accepted 3 February 2009

Keywords:
Strong ion difference
Strong ion gap
Anion gap
Metabolic acidosis
Pigs
Chlamydia suis

ABSTRACT

This study characterises the acid–base abnormalities in pigs experimentally infected with *Chlamydia suis* (Henderson–Hasselbalch equation and Constable's simplified strong ion equation). Eight pigs were challenged with the respiratory pathogen *C. suis* and four pigs served as non-infected controls. Pigs were monitored from 7 days before challenge to 8 days post-inoculation. Clinical examination was performed twice daily and venous blood samples were collected every two days. Blood-gas analysis, haemoxymetry, serum biochemical analysis and electrophoresis were performed in order to characterise the acid–base derangement. Aerosol challenge with *C. suis* resulted in severe acid–base disturbance characterised by acute respiratory acidosis and strong ion (metabolic) acidosis secondary to anaerobic metabolism and hyper L-lactataemia. Maximal changes were seen at day 3 post-inoculation when severe clinical signs of respiratory dysfunction were evident. The results of the study provide new information regarding the pathophysiology of respiratory infection caused by *C. suis* and the applicability and diagnostic utility of different approaches for assessing acid–base status in pigs.

Introduction

The pathogenic role of chlamydial infections in respiratory system infection remains controversial in domestic animals and humans. It is well known from human medicine that the clinical outcome of respiratory infection with either *Chlamydophila pneumoniae* or *Chlamydophila psittaci* ranges from clinically inapparent infections to life threatening respiratory disease (Kikawada et al., 2004; Haas et al., 2006; Pandeli and Ernest, 2006; Droemann et al., 2007; Wolf and Daley, 2007).

Chlamydia suis infections in pigs are associated with a spectrum of respiratory disease ranging from clinically inapparent to severe pulmonary dysfunction (Reinhold et al., 2005). Results from a previous experimental study (Reinhold et al., 2008) indicated that respiratory challenge with *C. suis* can produce a clinical outcome in pigs comparable to rare reports of fulminant cases of chlamydial infection in humans with multi-organ failure that requires mechanical ventilation for successful treatment. Respiratory dysfunction in our pig model of aerogenous chlamydial challenge was characterised by increased peripheral airway resistance and decreased diffusion capacity of the lung, resulting in abnormal gas exchange and profound respiratory distress (Reinhold et al., 2005, 2008). Characterisation of the acid–base imbalance associated with respiratory *C. suis* inoculation in pigs has not been undertaken but would be helpful in increasing our understanding of the pathophysiology of chlamydial infection and would assist in the development of effective treatment protocols. To do so, different approaches for assessing acid–base status need to be applied and compared for their diagnostic utility in the pig.

Clinical assessment of acid–base status has traditionally been based on blood-gas analysis and application of the Henderson–Hasselbalch equation in order to describe the relationship between pH, partial pressure of CO_2 (pCO_2) and the concentration of bicarbonate ($cHCO_3^-$) in plasma. The bicarbonatecentric Henderson–Hasselbalch equation characterises the metabolic component of an acid–base derangement by calculating the plasma bicarbonate concentration or base excess and the anion gap (AG) (Constable, 2000). Two alternative quantitative physicochemical approaches have been developed to describe acid–base balance (Kurtz et al., 2008), namely, Stewart's strong ion equation (Stewart, 1983) and Constable's simplified strong ion equation (Constable, 1997). The strong ion approach categorises the metabolic component of an acid–base derangement into two components, (1) changes in the strong ion difference (SID), which is the difference in charge between the sum of strong cations (such as sodium and potassium) and the sum of strong anions (such as chloride and L-lactate) and (2) changes in the total plasma concentration of non-volatile weak acids (A_{tot}) such as albumin, globulin, and phosphate. According to

* Corresponding author. Tel.: +49 3641 804269; fax: +49 3641 804228.
E-mail address: petra.reinhold@fli.bund.de (P. Reinhold).

1090-0233/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tvjl.2009.02.005

the strong ion difference theory, a change in pH or bicarbonate concentration is dependent on a change in either pCO_2, SID or A_{tot} (Stewart, 1983; Constable, 1997).

The SID theory is increasingly used to characterise complex acid–base derangements in domestic animals, but almost all of these studies have either focused on determining the mechanism of an acid–base derangement that is primarily metabolic in origin (Constable et al., 2005), or have been applied to anaesthetised animals under experimental conditions (Frischmeyer and Moon, 1994; Kaplan et al., 2006). The present authors are not aware of any studies that have applied the SID theory to acid–base disturbances in conscious pigs, or pigs undergoing experimental challenge with a respiratory pathogen such as *C. suis*. Consequently, the aim of this study was to characterise the *C. suis* challenge model with respect to acid–base balance using the traditional bicarbonatecentric approach (Henderson–Hasselbalch equation) and the SID theory.

Material and methods

Animals

Twelve female German Landrace pigs were obtained from Charles River Germany. The pigs had been fed colostrum and conventionally raised in a closed swine rearing facility. Pigs were transported to the institute at 3–4 weeks of age and healthy pigs were enrolled in the study after a quarantine period of at least 10 days. The animals were housed in conformity with the guidelines for animal welfare and fed twice a day with a commercially available swine grower diet that did not contain antibiotics. Water was supplied ad libitum. Pigs were housed in groups of four animals, with pens of four pigs being separated from each other.

Experimental design

The study had the ethical approval of the Commission for the Protection of Animals of the State of Thuringia and was performed in a containment of biosafety level 2.

Eight pigs were exposed to *C. suis* at 39–44 days of age and the remaining four pigs served as uninfected controls. The challenge model has been described previously (Sachse et al., 2004). Briefly, each pig inhaled 35–40 L of aerosol produced from 1.0 mL of either chlamydia cell culture (strain DC6) containing 10^9 inclusion forming units (ifu) or a non-infected culture (controls). A jet nebuliser (Pari I, Medanz) was used for aerosol production, with the aerosol being administered via a tightly fitting facemask to each pig over a period of 10–15 min.

Clinical observations were recorded twice daily and included feed intake, rectal temperature, respiratory rate, and the presence or absence of clinical signs of diarrhoea or respiratory disease, such as cough or nasal discharge. Preprandial jugular venous blood samples were collected in the morning by restraining the pig in dorsal recumbency for less than 5 min before sampling. Blood samples were obtained at following time points: 2 days (−2) and 1 day (−1) before challenge in treated and control pigs; 3 and 5 days after aerosol administration in animals exposed to *C. suis*; 4 and 6 days after aerosol administration in non-infected controls. Blood was collected anaerobically into heparinised 2 mL plastic syringes (PICO 50, Radiometer) for acid–base analysis and into 7.5 mL plastic syringes (S-Monovette, Sarstedt) for serum biochemical analysis.

Pigs were euthanised for necropsy at different times after aerosol administration: one *C. suis* exposed pig was euthanised each at 3, 4, 5, 7, 10, and 17 days post-inoculation, and the remaining two *C. suis* exposed pigs were euthanised on 24 days post-inoculation; one control pig was euthanised at 6, 8, 11, and 25 days after challenge. Studies documenting the histological findings and changes in respiratory function testing of challenged and control pigs have been published elsewhere (Reinhold et al., 2005, 2008).

Analysis of jugular venous blood

Venous blood samples were transported to the laboratory at room temperature and analysed within 10 min of collection using a combined blood-gas- and electrolyte-analyser (ABL system 605, Radiometer) and haemoxymeter (OSM3, Radiometer). The following factors were measured in the venous blood: pH(v), pCO_2(v), the concentration of total haemoglobin (ctHb), the fraction of oxyhaemoglobin in total haemoglobin (FO_2Hb(v)), the fraction of deoxyhaemoglobin in total haemoglobin (FHHb(v)), and the plasma concentration of sodium (cNa^+), potassium (cK^+), calcium (cCa^{2+}) and chloride (cCl^-) by ion-selective potentiometry. Plasma concentrations of glucose (cGlucose) and L-lactate (cL-lactate) were measured in the same equipment using enzymatic electrodes.

Serum biochemical analysis

Serum was harvested by centrifugation and stored at −20 °C until analysed. Serum concentrations of total protein (Biuret method) and inorganic phosphate (ammonium-molybdate) were measured spectrophotometrically (Cobas 6000, Roche/Hitachi). Capillary electrophoresis was performed to determine concentrations of albumin and globulin, as well as the globulin spectra (Capillarys2, Sebia). Protein concentration data was not available after day 5 post-challenge because of sample availability.

Calculated acid–base variables

The following variables were then calculated using proprietary equations included in the software of the blood-gas- and electrolyte-analyser: blood pH and pCO_2(v) corrected for the actual body temperature (BT) of the animal as measured rectally via digital thermometer before each blood collection (pH_{BT}, $pCO_2(v)_{BT}$), haematocrit (Hct), and traditional variables of acid–base balance, i.e. standard bicarbonate ($cHCO_3^-$(st)), actual base excess (cBase), and standard base excess (cBase (Ecf)).

Henderson–Hasselbalch equation and anion gap

$cHCO_3^-$ was calculated using the Henderson–Hasselbalch equation, temperature corrected values for pH_{BT} and $pCO_2(v)_{BT}$, and assumed values for S and pK1′ at 38 °C of 0.0301 (Austin et al., 1963) and 6.120 (Putnam and Roos, 1991), respectively. The Henderson–Hasselbalch approach quantifies the unmeasured anion concentration by calculating the AG as follows (Constable, 2000):

$$AG = (cNa^+ + cK^+) - (cCl^- + cHCO_3^-) \quad (1)$$

SID and strong ion gap (SIG)

Two quantitative physicochemical approaches to acid–base balance have been developed (Kurtz et al., 2008), namely, Stewart's strong ion model (Stewart, 1983) and Constable's simplified strong ion model (Constable, 1997). Both models provide a novel insight into the pathophysiology of mixed acid–base disorders and are based on the assumption that plasma pH is a dependent variable and as such its value is determined by three independent factors: pCO_2, the SID, and A_{tot}. Strong ions are those that dissociate completely at physiological pH, existing either as strong cations (Na^+, K^+, Ca^{2+}, Mg^{2+}) or strong anions (principally Cl^- and L-lactate). This charge difference generates a positive value for SID, which is counterbalanced by the negative charges residing on bicarbonate, albumin, globulin, and phosphate (Constable, 1997).

The simplified strong ion approach quantifies the unmeasured strong ion equation by calculating the SIG from measured values for plasma Na^+, K^+, Cl^-, and total protein concentrations, and calculated values for A_{tot} and pKa (Constable et al., 1998a; Constable, 2000). Because the buffer capacity of pig plasma appeared similar to that of human plasma over the pH range 7.29–7.62 (Weiskopf et al., 1983), values for A_{tot} and Ka (the effective equilibrium constant of non-volatile buffers in plasma) of pig plasma were assumed to be similar to that of human plasma. Accordingly, the SIG was calculated from the total protein concentration (cProtein total) in g/L and temperature corrected blood pH using the SIG equation for human plasma as follows (Staempfli and Constable, 2003):

$$SIG = A_{tot}/(1 + 10^{(pKa - pH)}) - AG \quad (2)$$

$$= [cProtein\ total] \times [0.224/(1 + 10^{(7.10 - pH)})] - AG \quad (3)$$

Measured SID ($SIDm_4$) was calculated as follows based on the number of strong ions measured [m] in plasma:

$$SIDm_4 = (cNa^+ + cK^+) - (cCl^- + cL\text{-lactate}) \quad (4)$$

Statistical analysis

Baseline values before challenge were calculated using the average between the two pre-challenge values measured on days −2 and −1 for each animal. Within groups, significant differences compared to baseline data were evaluated by multifactorial analysis of variance (ANOVA) using multiple range test based on least significant difference (LSD). The Mann–Whitney–Wilcoxon-test (*W*-test; comparison of medians) was explored to compare groups at one time point. Numeric data are presented as median, minimum and maximum. Since the given *P* values are $\leqslant 0.05$, there is a statistically significant difference at the 95% confidence level in both tests. AG and SIG were regressed against plasma L-lactate concentration and the resultant linear regression equation compared to the line of identity (slope = −1, intercept = 0) for SIG and for equivalence (slope = 1) for AG.