Slov Vet Res 2007; 44 (3): 91-6
UDC 636.2.09:579.62:579.844-074

Original Research Paper

PLASMA PYRUVIC ACID CHANGES IN ZEBU CATTLE EXPERIMENTALLY INFECTED WITH CLOSTRIDIUM CHAUVOEI

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Summary: A study was carried out to investigate the pyruvic acid concentration in Zebu cattle experimentally infected with *Clostridium chauvoei*. In the experimental design, 14 Zebu bull-calves were divided into 4 groups namely: groups A, B, C and D, and administered *C. chauvoei*, toxins, neuraminidase and control respectively. There was pyruvate accumulation in the plasma of all but the control group and the mean pyruvic acid concentrations in the *C. chauvoei*, toxin and neuraminidase groups had some peaks, with the highest peak value in the neuraminidase-administered group, followed by *C. chauvoei* and toxin-administered groups in that order. The control group had no peak pyruvic acid concentration and the values were almost similar throughout the experimental period ($P>0.05$). There was a statistically significant difference ($P<0.05$) between the plasma pyruvic acid levels of *C. chauvoei*, neuraminidase, toxin-administered and control groups, respectively. The significance of pyruvate accumulation in the pathogenesis of blackleg is discussed.

Key words: cattle disease; *Clostridium* infections–pathology–diagnosis; pyruvic acid–blood; neuroaminidase–blood; bacterial toxins–blood

Introduction

Blackleg is a fatal disease of cattle and sheep caused by *C. chauwoei* and was first reported in 1870 (1). In Nigeria, the disease was first reported in 1929 and has remained a major problem of cattle in the country (2,3). The prevalence of blackleg is known to be very high during years of high average annual rainfall (4,5). Vaccination against the disease has been carried out since 1930, but sporadic outbreaks are recorded annually. The economic losses of cattle to blackleg in Nigeria have been estimated at about 4.3 million United States dollars annually (3). The nomadic Fulani pastoralists of rural Nigeria, who own about 70-80% of livestock in the country, rear the Zebu breed of cattle that is highly susceptible to blackleg. They migrate from one place to another in search of pasture for their livestock and many of them request blackleg vaccination for their cattle, only if there are outbreaks of the disease in neighboring herds.

C. chauwoei which is the known cause of blackleg has been reported to produce neuraminidase (6,7). Neuraminidases (sialidases, EC 3.2.1.18) are involved in the pathogenesis of some infectious diseases, whose aetiologic agents produce the enzyme (8-12). The enzyme is of great importance in medicine and the pharmaceutical industry for the analysis of oligosaccharides and development of neuraminidase inhibitors (13-16). There is no consensus on the pathogenesis of blackleg, but toxins and neuraminidase produced by the bacteria are believed to play a significant role in the mechanisms of the disease (17-19). No studies have been carried out so far, on the biochemical changes in Zebu cattle infected with *C. chauvoei*. In this report, we present for the first time, the changes in plasma pyruvic acid concentration in Zebu cattle experimentally infected with *C. chauvoei* and the possible role of neuraminidase and toxins produced by the bacteria in the derangements observed.

Received: 31 July 2007
Accepted for publication: 23 August 2007

Materials and methods

Animal acquisition, acclimatization and grouping

Fourteen (14) Zebu bull-calves were purchased, acclimatized, grazed, aged and grouped into 4 groups as described by Useh (3). Groups A (n=4), B (=3) and C (n=4) were infected with *C. chauvoei* (Jakari strain), toxins and neuraminidase from the bacteria respectively, while group D (n=3) served as control. During the period of acclimatization, the animals were grazed on free range, because of the abundant pasture that characterize the rainy season in Zaria, Nigeria, but when the experiment commenced they were confined in the appropriate experimental pens and fed a combination of groundnut hay and hay prepared from *Andropogon gayanus, Hyprrhenia rufens, Pennisetum pedicellatum and Elionurus probeguinii* until the experiment was terminated. They were supplied feed commensurate with 4% of their individual body weights daily and water *ad libitum*. The weights of the animals were estimated using waist band and ranged between 80-140 kg. The animals were aged using dental eruption (20) and their ages ranged between 19-23 months. Analysis of variance (ANOVA, Duncan multiple range test) was used to compare means ± standard deviations (SD) of the ages cum weights of the experimental animals on day zero of the experiment and there was no statistically significant difference (P>0.05) between the mean age and the mean weight of all the animal groups on day zero of the experiment.

Cultivation of C. chauvoei for infection

Lyophilized *C. chauvoei* (Jakari strain) donated by the National Veterinary Research Institute (NVRI), Vom, Plateau state, Nigeria was used for the experiment. The organism was first isolated from Zebu cattle with blackleg and its pathogenicity indices have been fully determined (21). The preparation of the bacteria and infection of Zebu bull calves was carried out using the method described by Singh *et al.* (22) and the experiment lasted for 21 days.

Culture of C. chauvoei (Jakari strain) for neuraminidase production

Lyophilized *C. chauvoei* (Jakari strain) was obtained from the National Veterinary Research Institute (NVRI), Vom, Plateau state, Nigeria. It was cultivated and neuraminidase was isolated as described previously (12). The neuraminidase was partially purified as described earlier (23) and administered to one experimental group using conventional protocols (3).

Cultivation of C. chauvoei (Jakari strain) for toxin production

The method of Jayaraman et al. (24) was used to cultivate the bacteria and produce the toxins which were administered to one experimental group. The protocol including the amount of toxins administered is described elsewhere (3). Although there are no definite ethical guidelines of animal experimentation in Nigeria, the Zebu cattle were treated as humanely as possible during the experimental period in accordance with international provisions (25). At the end of the experiment, the surviving animals were treated with penicillin (20,000 IU/kg) (Tennyson, China) and they all recovered.

Determination of pyruvic acid concentration in plasma

Blood was collected on days 1 (24 h), 2 (48 h), 3 (72 & 81 h), 4 (105 h), 7 (165 h), 8 (189 h), 9 (214 h), 10 (245 h), 11 (265 h), 13 (293 h) and 21 (413 h) of bacteria, neuraminidase, and toxin-administration respectively and plasma was prepared from the whole blood (26). The same applied to the control group. Pyruvic acid levels were determined from the plasma using the dinitrosalicylic acid (DNS) method (27).

Statistical analysis

Data obtained from the study was computed as mean± standard deviation (SD), analyzed using analysis of variance (ANOVA, Duncan multiple range test) and values of P<0.05 were statistically significant (28).

Results

Pyruvic acid concentrations were higher in the bacteria, neuraminidase and toxin administered groups, compared to the control group. There was a statistically significant difference (P<0.05) between the mean pyruvic acid concentrations of the bacteria (*C. chauvoei*), neuraminidase, toxin-administered and control groups respectively (Fig. 1). From day 1-21 of the experiment, mean pyruvic acid concentration in the control group did not vary significantly (P>0.05). There were 4 peaks of mean pyruvic acid concentration in the bacteria (*C. chauvoei*) administered-group, with the highest peak of 1120.33 ± 186.29 g/L occurring on day 10 (245 h),

followed by 678.58 ± 205.84 g/L on day 4 (105 h), 413.52 ± 103.40 g/L on day 3 (72 h) and 330.68 ± 24.54 g/L on day 1 (24 h) respectively. In the neuraminidase administered-group, there were also 4 peaks of mean pyruvic acid concentration, with the highest peak of 1228.31 ± 198.66 g/L recorded on day 8 (189 h), followed by 649.31 ± 120.67 g/L on day 11 (265 h), 393.89 ± 97.24 g/L on day 3 (72 h) and 99.39 ± 68.72 g/L on day 1 (24 h) respectively. On the contrary, however, three peaks of mean pyruvic acid concentration were recorded in the toxin-administered group, the highest peak of 803.19 ± 94.72 g/L occurring on day 10 (245 h), followed by 452.79 ± 86.09 g/L on day 1 ((24 h) and 423.34 ± 100.60 g/L on day 3 (81 h) respectively. The highest peak of mean pyruvic acid concentration in the neuraminidase-administered group (1228.31 ± 198.66 g/L) was higher than the highest peak in the bacteria (1120.33 ± 186.29 g/L) and toxin-administered (803.19 ± 94.72 g/L) groups in that order. The control group had no peak of mean pyruvic acid levels and the concentrations were almost similar throughout the experimental period (P>0.05) (Fig. 1).

Discussion

This study is a continuation of a series of works to identify the role of neuraminidase in the pathogenesis of blackleg. It is believed that the changes in pyruvic acid levels of ruminants in blackleg, if established, will further assist in understanding the pathogenic roles of neuraminidase and toxins produced by *C. chauvoei* in the disease. Glucose which is a product of carbohydrate metabolism in diet is transformed either to pyruvate in plasma aerobically or lactate anaerobically in muscles (29). In the former, pyruvate is converted to acetyl Coenzyme A which enters the Kreps cycle and is oxidized to yield energy. In blackleg, anaerobiosis occurs following a chain of pathological events: neuraminidase produced by *C. chauvoei* cleaves sialic acid from the muscles and erythrocytes of infected ruminants (18), resulting in the exposure of galactose on the erythrocyte surfaces. Since galactose has a high affinity for lectins, this phenomenon is thought to subject the erythrocytes whose sialic acid is removed to erythrophagocytosis leading to reduced erythrocyte concentration and haemoglobin (3), similar to the erythrophagocytosis reported in trypanosomiasis (30,31) . Since haemoglobin is the oxygen carrying pigment in the body, this results in reduced oxygen tension in the muscles and red blood cells (RBC), leading to impaired cellular (mitochondrial) respiration and hence anaerobiosis. Similarly, toxins produced by *C. chauvoei* triggers necrosis through impaired cellular (mitochondrial) respiration, leading to anaerobiosis (32,33). It can therefore be explained that the anaerobic environment created by neuraminidase and toxins produced by *C. chauvoei* prevents pyruvate metabolism, leading to its accumulation in the plasma as observed in the present study.

Although haemoconcentration has been reported in blackleg (34, 35), recent studies suggest that the pathogenesis of the haemoconcentration is due to the masking effects of anaemia which occurs due to desialylation of erythrocytes (3). The haematological and biochemical changes in blackleg (*C. chauvoei* infection) have been exhaustively reported (34,35), but studies on the variation in plasma pyruvate concentration during the infection have been ignored. In the present study, it was observed that pyruvic acid catabolism was impaired by anaerobiosis in the bacteria, neuraminidase and toxin-administered groups, leading to a build up of high amounts of mean pyruvic acid in the plasma of these animals (Fig. 1), compared to the control animals which were healthy and whose pyruvate catabolism continued unabated. This study suggests that anaerobiosis created by neuraminidase and toxins produced by *C. chauvoei* is the major cause of impaired pyruvate metabolism in blackleg. It has further confirmed that neuraminidase and toxins produced by the bacteria work in tandem with each other in blackleg, and may be playing key roles in the mechanisms of the disease. Further studies should be conducted to investigate the beneficial effects of neuraminidase and toxin inhibitors, if used clinically to manage blackleg.

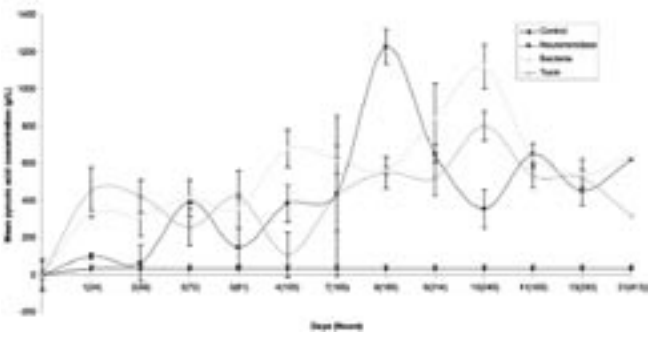

Fig. 1.: Variation in mean pyruvic acid concentration of Zebu cattle experimentally administered *Clostridium chauvoei*, its toxins and neuraminidase

Acknowledgements

Mrs. Chinwe Useh was very supportive when this research was designed and executed. Also, the arrival of Johnmark Kerter Useh provided the intellectual enthusiasm to write this paper.

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SPREMEMBE V NIVOJU PIRUVIČNE KISLINE V KRVNI PLAZMI PRI GOVEDU ZEBU, POSKUSNO OKUŽENIM S *CLOSTRIDIUM CHAUVOEI*

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Povzetek: V raziskavi smo ugotavljali nivo piruvične kisline v krvni plazmi pri govedu zebu, ki je bilo umetno okuženo z bakterijo *Clostridium chauvoei*. Štirinajst moških telet pasme zebu je bilo razdeljenih v 4 skupine. Skupine so bile tretirane z bakterijo *C. chauvoei*, toksinom te bakterije ali encimom nevraminidaza, četrta skupina pa je bila kontrolna in ni bila tretirana. Pri vseh treh poskusnih skupinah smo ugotovili povišanje nivoja piruvične kisline v primerjavi s kontrolno skupino, najvišje koncentracije so bile ugotovljene v skupini, ki je bila tretirana z nevraminidazo. V kontrolni skupini se koncentracija piruvične kisline ni spremenila ves čas poskusa, v ostalih skupinah pa je bila ves čas trajanja poskusa statistično zanesljivo višja ($p < 0,05$) kot v kontrolni skupini. V članek je vključena tudi razprava o morebitnem pomenu dviga piruvične kisline pri patogenezi bolezni črnih nog.

Ključne besede: govedo, bolezni; klostridij infekcije–patologija–diagnostika; piruvična kislina–kri; nevraminidaza–kri; bakterijski toksini–kri