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Desialylation Modulates Alkaline Phosphatase Activity in Zebu Cattle Experimentally Infected with *Clostridium chauvoei*: A Novel Report

Nicodemus M. Useh[1,2*], Deborah I. Arimie[1], Emmanuel O. Balogun[3,4], Najume D. G. Ibrahim[1], Andrew J. Nok[3] and King A. N. Esievo[1]

[1]*Department of Veterinary Pathology and Microbiology, Ahmadu Bello University, Zaria, Nigeria;*

[2] *Laboratory of Molecular Biology of Infectious Diseases, Department of Population Medicine & Diagnostic Sciences, College of Veterinary Medicine, Cornell University,Ithaca, 14853 New York, USA;*

[3]*Department of Biochemistry, Ahmadu Bello University, Zaria, Nigeria;*

[4]*Laboratory of Biomedical Chemistry, School of Medicine,The University of Tokyo, Tokyo, Japan*

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Abstract

Blackleg, which was known for many decades as an animal disease has been confirmed to be fatal to human beings. The present study was conducted to investigate the role of sialidase (neuraminidase) and toxins produced by the bacteria in the surge in plasma alkaline phosphatase activity that is usually associated with *C. chauvoei* infection. Fourteen Zebu cattle were allocated into four experimental groups. They were administered *C. chauvoei* (n=4), toxins (n=3), neuraminidase (n=4) and a control group (n=3) respectively. Results obtained indicate that mean alkaline phosphatase level was highest in the bacteria-infected group, followed by the neuraminidase and toxin-administered groups. The mean alkaline phosphatase activities of the four groups were significantly different ((P<0.05) and this suggest liver and intestinal mucosal cell damage. The possible role of neuraminidase and toxins produced by the bacteria in the pathogenesis of blackleg is discussed. This is the first study to demonstrate that neuraminidase and toxins produced by *C chauvoei* either cleaved sialic acids or caused necrosis to liver hepatocytes and intestinal mucosal cells, leading to increased alkaline phosphatase activity in these organs. The current study also provides baseline data on the pattern of variation of alkaline phosphatase activity in Zebu cattle experimentally infected with *C chauvoei*.

Keywords: Alkaline phosphatase activity; neuraminidase; toxins; *Clostridium chauvoei*; Zebu cattle; desialylation

1. Introduction

Blackleg is a fatal disease of cattle and sheep caused by *C. chauvoei* and was first reported in 1870 (Armstrong and MacNamee, 1950). In Nigeria, the disease was first reported in 1929 (Osiyemi, 1975) and has remained a major problem of cattle in the country (Useh *et al*., 2010a). The prevalence of blackleg is known to be very high during years of high average annual rainfall (Uzal *et al*., 2003; Useh *et al*., 2006a). 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 annually (Useh *et al*., 2006a). 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 (Abdu *et al*., 2000). 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. chauvoei which is the known cause of blackleg has been reported to produce neuraminidase (Useh *et al*., 2004). Neuraminidases (sialidases, EC 3.2.1.18) are involved in the pathogenesis of some infectious diseases, whose aetiologic agents produce the enzyme (Nok and Balogun, 2003). The enzyme is of great importance in medicine and pharmaceutical industry for the analysis of oligosaccharides and the development of neuraminidase inhibitors (Traving and Schauer, 1998). There is no consensus on the pathogenesis of blackleg, but toxins and neuraminidase produced by the bacteria are believed to play significant contributory roles in the mechanisms of the disease (Useh *et al*., 2003). Recent studies on the haematology and some biochemical changes in Zebu cattle

* Corresponding author. e-mail: nicodemus.useh@fulbrightmail.org.

infected with *C. chauvoei* revealed novel findings (Useh *et al*., 2008). In this report, we present for the first time, the role of desialylation of the liver and intestinal mucosal cells on the surge in alkaline phosphatase activity in Zebu cattle experimentally infected with *C. chauvoei*, its toxins and neuraminidase.

2. Materials and Methods

2.1. Animal acquisition, acclimatization and grouping

Fourteen (14) Zebu bull-calves were purchased, acclimatized, grazed, aged and grouped into 4 groups. Groups A (n=4), B (=3) and C (n=4) were administered *C. chauvoei* (Jakari strain), toxins and neuraminidase from the bacteria respectively, while group D (n=3) served as control. 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 (Wosu, 2002) 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 to ensure that there was no significant difference (P> 0.05) between the mean ages or weights of all the experimental groups investigated in the study (Chatfield, 1983).

2.2. 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 (Princewill, 1965). The preparation of the bacteria and infection of Zebu bull-calves was carried out using the method described by Useh *et al*. (2008) and the experiment lasted for 21 days.

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

Lyophilized *C. chauvoei* (Jakari strain) was cultivated and neuraminidase was isolated as described previously (Useh *et al*., 2004). The extracellular sialidase released in the medium was partially purified as described earlier (Useh *et al*., 2006 b).

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

The method of Jayaraman *et al.* (1962) was used to cultivate the bacteria and produce the toxins which were administered to experimental group B. Exactly 40 IU of toxins produced by the bacteria was administered intramuscularly to animals in group B (n=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 (Bankowski, 1985). At the end of the experiment, the surviving animals were treated with penicillin (20,000 IU/kg) (Tennyson, China) and they all recovered.

2.5. Determination of alkaline phosphatase activity in plasma

Blood samples were 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) for determination of alkaline phosphatase activity. Enzyme activity was determined by hydrolysis of phosphate by the enzyme to release inorganic phosphate and alcohol. The substrate paranitrophenyl phosphate (20 mg) was dissolved in 100 mL of phosphate buffer pH 7. An aliquot (0.1 mL) of the paranitrophenyl phosphate solution was added to 5 mL of the sample. The set up was incubated at 37 ^{o}C for 1 h in a water bath and absorbance was measured spectrophotometrically at 420 nm.

2.6. Determination of sialic acid concentration in liver

One (1) g each of liver and intestinal mucosal cells was collected from animals that died during the experiment (one animal per each experimental group) and thoroughly homogenized. The resultant slurries were scooped into 2 mL containers and 1 mL of distilled water added. Sialic acid in the homogenized liver was determined (n=3) as described earlier (Aminoff, 1961).

2.7. Determination of plasma and intestinal mucosal cell neuraminidase activity

Neuraminidase activity in the blood (plasma) and homogenized intestinal mucosal cells was determined using the method described by Webster and Campbell (1972).

2.8. 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 (Chatfield, 1983).

3. Results

Alkaline phosphatase activity was highest in the bacteria-infected group, followed by neuraminidase and toxin-administered groups (Fig. 1). There were 3 peaks of mean alkaline phosphatase activity in the bacteria-infected group, with the highest peak noticed on day 10 (230 h), followed by day 8 (189 h), and the lowest peak on day 3 (81 h). Four peaks of mean alkaline phosphatase activity were observed in the neuraminidase-administered group with the highest peak noticed on day 8 (189 h), followed by day 10 (230 h), day 4 (97 h) and the lowest observed on day 2 (46 h) respectively. In the toxin-administered group, 3 peaks of mean enzyme activity were observed with the highest peak on day 7 (65 h), followed by day 10 (230 h) and the lowest on day 1 (24 h). In the control group mean enzyme activity peaked only on day 3 (81 h) of the experiment and did not vary significantly (P>0.05) throughout the experiment. There was a statistically significant difference (P<0.05) between mean alkaline phosphatase activity of control, bacteria-infected, neuraminidase, and toxin-administered groups respectively

(Fig. 1). Mean sialic acid complement of bacteria-infected animals was lower than the controls (P<0.05).

Results of sialic acid assays from the liver and neuraminidase activity in the liver and intestinal mucosal cells are presented in Table 1.

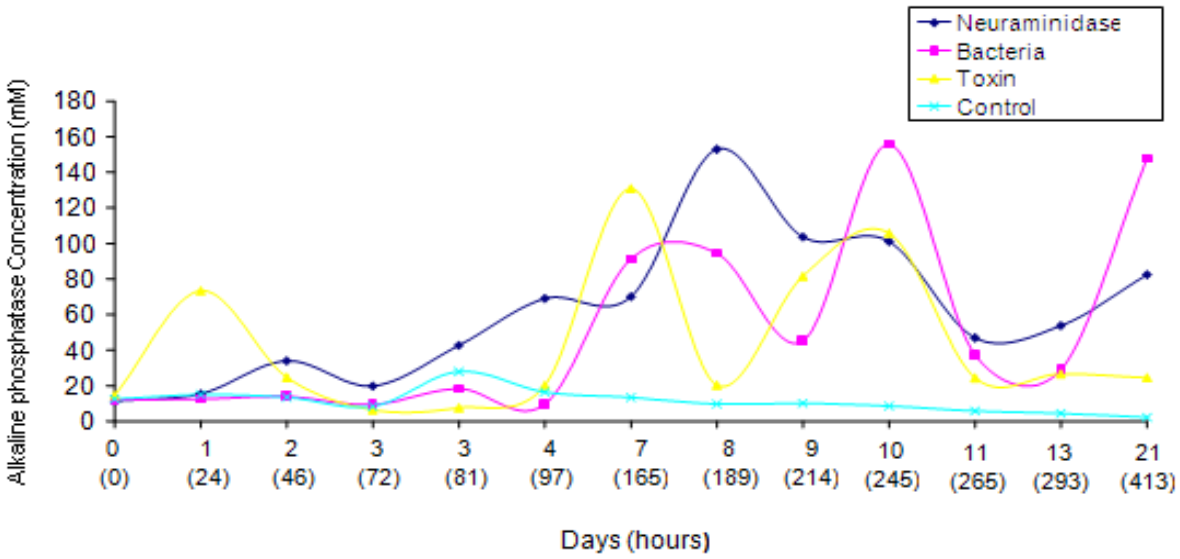

Figure 1: Variation in the mean alkaline phosphatase concentration in Zebu cattle administered *Clostridium chauvoei* (Jakari strain), it's toxins and neuraminidase.

Table1. Mean sialic acid concentration and neuraminidase activity in the organs of Zebu cattle experimentally infected with C. *chauvoei*, its toxins and neuraminidase

	C. chauvoei-infected group	Toxin-administered group	Neuraminidase-administered group	Control group
Sialic acid µM (Liver)	3.43±0.16	5.20±0.18	2.73±0.20	5.17±0.20
Neuraminidase activity in liver (µMmin^{-1})	3.74±0.11	1.30±0.36	2.85±0.15	1.30±0.27
Neuraminidase activity in intestinal mucosal cells (µMmin^{-1})	6.65±2.00	1.70±0.60	6.45±2.41	1.70±0.70

4. Discussion

C. chauvoei infection has a long history of veterinary importance (Radostits *et al*., 2000). During the period, molecular diagnostic techniques were not available, hence the difficulty in distinguishing *C chauvoei* from *C septicum* which are phylogenetically closely related species.

Alkaline phosphatase is produced in the liver, kidney, bone, intestinal mucosal cells and placenta for the metabolic processes in these organs (Friedman *et al*., 1996). In *C. chauvoei* infection, the neuraminidase and toxins produced by the bacteria *in vivo* possibly caused pathology in the above organs through desialylation or cell death (necrosis). Damage of these organs has been reported in cattle with blackleg (Singh *et al*., 1993), although the exact mechanism by which this occurs has yet to be known. An earlier report suggested possible leucophagocytosis by Kupfer cell lectins in the liver of Zebu cattle experimentally infected with *C. chauvoei* (Useh *et al*., 2010b).

Mean alkaline phosphatase activity in the plasma of the bacteria-infected, neuraminidase and toxin-administered experimental groups increased significantly (P<0.05), compared to the control and the data coincided with simultaneous increase in plasma neuraminidase activity and decreased sialic acid concentration in the liver of the bacteria-infected and neuraminidase-administered experimental groups (Table 1). Damage to the liver in the toxin-administered group, leading to increased alkaline phosphatase activity may be as a result of possible necrosis to the tissues by the toxins. Results from the current study further supports earlier reports that neuraminidase and toxins produced by *C. chauvoei* work in tandem with each other in causing pathology in blackleg (Useh *et al*., 2007, 2008). It is safe to report, based on these findings, that the surge in alkaline phosphatase activity in *C. chauvoei* infected cattle is predicated both on sialic acid cleavage and necrosis caused by neuraminidase and toxins

(respectively) produced by the bacteria *in vivo* to cause hepatic and intestinal mucosal cell damage. The high alkaline phosphate activity is in agreement with another study in which liver, kidney, spleen and intestinal cell damages were reported (Pemberton *et al*., 1974). It is concluded that future research should target both neuraminidase and toxins produced by the bacteria in chemotherapy and novel molecular vaccination strategies.

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