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Pathological Changes in Mice Experimentally Injected *Clostridium chauvoei* Toxins

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Abstract: Blackleg is an economically important disease of cattle, sheep and other ruminants which is endemic in both developed and developing countries of the world. Toxins and neuraminidase produced by *Clostridium chauvoei* have been reported to play significant complimentary roles in the pathogenesis of the disease. In this study, the pathological changes caused by exogenous toxins produced following the culture of *C. chauvoei* at 24 and 48 h respectively were investigated and it was observed that the 24 h toxin produced more severe pathological changes, compared to the 48 h toxin. Necrosis was observed in the tissues examined, both grossly and histopathologically and was attributed probably to impaired cellular (mitochondrial) respiration. It was concluded that, although toxins produced by *C. chauvoei* play an important role in the mechanisms of blackleg, the role of leukotrienes (C4, D4 and E4), cytokines (interleukin-1, IL-1; tumour necrosis factor-, TNF-), platelet-activating factor, interferon, complement fragments (anaphylatoxins C5a and C3a), prostaglandins and neuraminidase in the pathogenesis of *C. chauvoei* infection in mice need to be thoroughly investigated.

Key words: Pathological changes, mice, *Clostridium chauvoei*, toxins

INTRODUCTION

Blackleg is a disease of cattle, sheep and other ruminants caused by *C. chauvoei* (Radostist *et al*., 1994; Useh *et al*., 2003). It is an economically important disease in both developed and developing countries of the world, where it is known to be endemic (Useh *et al*., 2006a). In Nigeria, the disease was first reported in 1929 (Osiyemi, 1975) and has remained a major problem of cattle in the country. The economic losses of cattle to blackleg in Nigeria have been estimated at US dollar 4.3 million annually (Useh *et al*., 2006b).

There is no consensus on the pathogenesis of blackleg in cattle (Jubb *et al*., 1993; Useh, 2002), although toxins (Singh *et al*., Useh, 2006) and neuraminidase (Useh *et al*., 2004; 2006; 2006a) produced by *C. chauvoei* have been reported to play significant complimentary roles in the mechanisms of the disease. The pathological changes caused by toxins produced by *C. chauvoei* in blackleg have been documented in cattle (Singh *et al*., 1993; Useh, 2006) and guinea pigs (Singh *et al*., 1993). In the present study, we report here for the first time, the pathological changes observed in Balb C albino mice, experimentally injected exogenous toxins produced by *C. chauvoei*, to further support earlier reports that toxins produced by *C. chauvoei* are partly responsible for the pathological changes that occur in blackleg.

MATERIALS AND METHODS

Bacteria strain: A highly pathogenic strain of *C. chauvoei* (Jakari strain) donated by the *Clostridium chauvoei* group of Ahmadu Bello University, Zaria, Nigeria was used for the experiment. The bacterium was first isolated from blackleg-infected Zebu cattle and its pathogenicity indices have been fully determined (Princewill, 1965).

Toxin production: The bacterium was cultured for 24 and 48 h, respectively and its toxins were obtained at these times as described by Jayaraman *et al*. (1962).

Experimental animals and injection of toxins: Twenty Specific Pathogen Free (SPF) Balb C albino mice, each weighing between 18-19 g were obtained at Ahmadu Bello University, Zaria, Nigeria and divided into 3 groups of 6,

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Table 1: Clinical presentation of mice experimentally injected toxin obtained from 24 h culture of C. chauvoei (Jakari strain)

Mice No.	Volume of Inoculums (mL)	Time of inoculation of toxin (pm)	Onset of clinical signs post-inoculation (Min)		
			Respiratory Distress	Convulsion	Death
1	0.1	6	28	30	2880(48 h)
2	0.2	6.05	24	15	2880 (48 h)
3	0.3	6.1	10	None	1440 (24 h)
4	0.4	6.15	2	20	360 (6 h)
5	0.2	6.2	2	20	720 (12h)
6	0.4	6.25	27	None	30
7	0.2	6.3	15	30	33

Table 2: Clinical presentation of mice experimentally injected toxin obtained from 48 h culture of C. chauvoei (Jakari strain)

Mice No.	Volume of Inoculums (mL)	Time of inoculation of toxin (pm)	Onset of clinical signs post-inoculation (Min)		
			Respiratory Distress	Convulsion	Death
8	0.1	7.30	25	40	2880 (48 h)
9	0.2	7.35	10	15	2440 (24 h)
10	0.3	8.00	None	None	2880 (48 h)
11	0.4	8.10	10	None	None
12	0.5	8.20	10	None	2880 (48h)
13	0.1	8.30	none	None	None
14	0.2	None	15	None	4320 (72 h)

7 and 7 mice respectively and identified as groups A, B and C respectively. Group A (n = 6) served as control, while group B (n = 7) and group C (n = 7) were injected toxins produced at 24 and 48 h of culture respectively intravenously (iv) through the tail vein (Table 1 and 2).

Gross and microscopic examination of carcasses: These were carried out using the procedure described by Igbokwe 1989. Tissue sections for histopathology were obtained from various organs. They were preserved and fixed in 10% buffered neutral formalin for at least 48 h. The tissues were sectioned at 5-6 μm and stained with haematoxylin and eosin (H and E).

RESULTS

Clinical presentation of mice: The clinical picture of mice experimentally injected *C. chauvoei* toxins produced after 24 and 48 h of cultivation are presented in Table 1 and 2, respectively.

Postmortem findings: Necrosis and haemorrhages were obtained in the liver, pancreas and kidney on gross examination of the afore-mentioned organs injected both 24 and 48 h toxins (Plates not shown). Similar histopathological changes were observed in mice injected 24 and 48 h toxins, although the former were more severe than the later. The histopathological changes observed following the injection of 24 h toxins are presented in Plates 1-3. There was congestion of the blood vessels and focal areas of necrosis in the liver 33 min post-injection of the 24 h toxin, while in the pancreas, there was diffuse necrosis of the pancreatic cells in the mice that died 1440 min post-injection of the 24 h toxin. In the kidney, Atrophied Glomeruli (AG), Glomerular (G) and Renal (R) tubular necrosis, with Mononuclear (M) cellular infiltration were observed following the injection of the toxin obtained after 24 h culture.

DISCUSSION

The pathological changes in Balb C albino mice caused by exogenous toxins produced *in vitro* by *C. chauvoei* (Jakari strain) at 24 and 48 h of culture respectively and injected intravenously through the tail vein were investigated in the present study and all the seven (7) mice (100%) injected the 24 h toxin showed marked respiratory distress, while 5 (71%) convulsed and 7 (100%) died respectively. In the group injected the 48 h toxin, 5 (71%) showed respiratory distress, while 2 (29%) convulsed and 6 (86) died at the various times reported in Table 1 and 2.

The gross lesions observed in the liver, pancreas and kidney of mice injected 24 and 48 h toxins were essentially similar and these include necrosis and haemorrhages. Histopathological changes were more severe in the former than the later and hence we have presented the histopathology of the organs injected the 24 h toxin in Plates 1-3. The necrosis observed in this study tally with the reports of Singh *et al.* (1993; 1992) and Useh (2006). It is tempting to assume that since some toxic chemicals (for instance, azaanthraquinone) are known to cause cell

death in long slender forms of trypanosomes through the inhibition of mitochondrial respiration (Nok, 2002), the same mechanism may be applicable with toxins produced by *C. chauvoei* in clinical blackleg. This assumption is

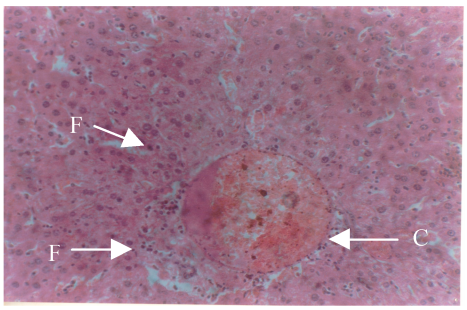

Plate 1: A Microscopic section of liver showing Congested blood vessels (C) and focal areas of necrosis (F) in mice that died 33 minutis post-injection of *C. Chauvoei* toxins (H and E stain×400)

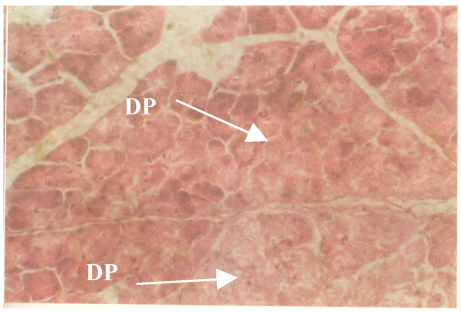

Plate 2: A Microscopic section of pancreas showing diffuse areas of necrosis (DP) of the pancreatic cells in mice that died 1440 minutis post-injection of *C. Chauvoei* toxins (H and E stain×400)

strongly supported by previous reports that the oxygen tension in the tissues of blackleg patients is usually low (Useh, 2006; Jones *et al*., 1997), thus impairing cellular (mitochondrial) respiration in the affected tissues and hence necrosis. In the present study, exogenous toxin produced after 24 h cultivation of *C. chauvoei* were more potent and produced more severe pathological changes in the tissues of the injected mice, compared to the 48 h toxin. This finding agrees with Jayaraman *et al.* (1962) who reported that the potency of *C. chauvoei* toxin decreased with increased culture duration.

It is concluded that, although toxins produced by *C. chauvoei* play an important role in the mechanisms of blackleg, the role of leukotrienes (C4, D4 and E4), cytokines (interleukin-1, IL-1; tumour necrosis factor-, TNF-), platelet-activating factor, interferon, complement

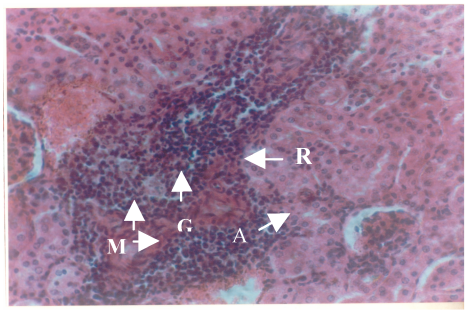

Plate 3: A Microscopic section of the kindly showing Atrophied Glomerulus (AG) Glomerular (G) and Renal (R) tubular necrosis and Monouclear (M) cells infiltration in mice that died 2880 minutis post- injection of *C. Chauvoei* toxins (H and E stain×400)

fragments (anaphylatoxins C5a and C3a), prostaglandins and neuraminidase in the pathogenesis of *C. chauvoei* infection in mice should be thoroughly investigated.

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REFERENCES

Igbokwe, I.O., 1989. Postmortem examination of carcasses. Zariya Vet., 4: 145-152.

Jayaraman, M. S., L. Roshan and M. R. Dhana, 1962. Toxin production by *Clostridium chauvoei.* Indian Vet. J., 39: 481-484.

Jubb, K.V.F., P.C. Kennedy and N. Palmer, 1993. Pathology of domestic animals, Academic press, London, San Diego, (4th Edn.), pp: 183-264.

Jones, C. J., R. C. Hunt and N. W. King, 1997. Blackleg. Veterinary Pathology, Williams and Wilkins, Baltimore, MD,(6th Edn.), pp: 417-418.

Nok, A.J., 2002. Azaanthraquinone inhibits respiration and *in vitro* growth of long slender blood stream forms of *Trypanosoma congolense*. Cell Biochem. Funct., 20: 205-212.

Osiyemi, T.I.O., 1975. The aetiology and data on seasonal incidence of clinical blackleg in Nigerian cattle. Bull. Anim. Hlth. Prod. Af., 23: 367-370.

Princewill, T.J.T., 1965. Effect of calcium chloride on germination and pathogenicity of spores of *Clostridium chauvoei*. J. Comp. Pathol., 75: 343 -351.

Radostits, O.M., D.C. Blood and C.C. Gay, 1994. Veterinary Medicine. A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses, ELBS, London, (8th Edn.), pp: 684-686.

Singh, K.P., N.S. Parihar and B.N. Tripathi, 1993. Pathology of experimental *Clostridium chauvoei* infection in hill bulls. Indian Vet. J., 70: 511-514.

Singh, K.P., N.S. Parihar and B.N. Tripathi, 1992. Pathology and pathogenesis of *Clostridium chauvoei* infection in guinea pigs. Indian J. Anim. Sci., 62: 611-615.

Useh, N.M., A.J. Nok and K.A.N. Esievo, 2003. Pathogenesis and pathology of blackleg in ruminants: The role of toxins and neuraminidase. Vet. Quart., 25: 155-159.

Useh, N.M., A.J. Nok and K.A. N. Esievo, 2006a. Blackleg in ruminants. Perspectives Agric., Vet. Sci., Nutr. Nat. Res., 1: 1-8.

Useh, N.M., N.D.G. Ibrahim, A.J. Nok and K.A.N. Esievo, 2006b. The relationship between annual rainfall and outbreaks of blackleg of cattle in Zaria, Nigeria. Vet. Rec., 158: 100-101.

Useh, N.M., 2002. The production and characterization of neuraminidase (sialidase) from *Clostridium chauvoei* (Jakari strain). M.Sc. Thesis, Ahmadu Bello University, Zaria, Nigeria, pp: 224.

Useh, N.M., 2006. The possible role of clostridial neuraminidase (sialidase) in the pathogenesis of blackleg in Zebu cattle. Doctoral (Ph.D.) Dissertation, Ahmadu Bello University, Zaria, Nigeria, pp: 172.

Useh, N.M., 2004. The production and characterization of neuraminidase (sialidase) from *Clostridium chauvoei* (Jakari strain). Masters Abst. Int., 42: 05M.

Useh, N.M., A.J. Nok, O.J. Ajanusi, E.O. Balogun, S.B. Oladele and K.A.N. Esievo, 2004. *In vitro* production of neuraminidase (sialidase) by *Clostridium chauvoei* (Jakari strain). Vet. Arhiv, 74: 289-298.