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Biochemical profiles during endotoxic shock and after hypertonic saline solution, Dexamethasone and Flunixin meglumine administration in buffalo calves

G.S. Ghuman, and D.V. Singh[1*]

Department of Veterinary Physiology
Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141 004 (India)

Abstract

Endotoxic shock was produced in five apparently healthy male buffalo calves aged between 4 months to 1 year by I/v infusion of *E.coli* endotoxin @ 5 μg/Kg BW/ hour for 3 hours. The animals were further observed for a period of 7 days. A general hypoproteinemia was observed during endotoxin infusion along with significant ($P < 0.01$) hypoalbuminemia, hypoglycemia, decrease in plasma globulin, creatinine, BUN and chloride while plasma sodium, potassium, showed non-significant alterations during endotoxin infusion for 3 hours. All the endotoxemic buffalo calves were infused I/v hypertonic saline solution (7.2% NaCl acq.) @ 4ml/Kg BW in 6.5 minutes followed by dexamethasone @ 4mg/Kg BW and flunixin meglumine @ 1.1 mg/Kg BW as one time infusion and plasma proteins showed a significant decrease at 4, 5 and 6 hour and on day 2 and 3while albumin showed a declining trend throughout the observation period with a significant fall at 3, 4, 5 hour and on day 6 of start of endotoxin infusion. Plasma globulin and other proteins also decreased. Plasma fibrin increased significantly at 6th hour to day 7 i.e., end of the observation period. Hypoglycemia continued from 2nd to 6th hour followed by day 1 -5 accompanied by a significant fall in BUN on day1-3.The plasma creatinine along with sodium and potassium did not alter significantly.

Key Words: Biochemistry, Endotoxemia, Hypertonic saline, Dexamethasone, Flunixin meglumine, Buffalo calves

Introduction

Septic or endotoxic shock results from rapid liberation of endotoxin into circulation that results into cardiovascular collapse accompanied by severe vasodilation, pallor of mucosa, cool skin and extremities, diarrhoea, decreased systemic blood pressure and muscle weakness (Radostits *et al.*, 2000). Endotoxemia is a state of high level of endotoxin circulation in plasma or blood. During shock, there is a substantial morbidity and mortality in cattle especially neonates (Gerros *et al.*, 1993). Therefore the consequences of endotoxemia are either a considerable morbidity or mortality of the animals leading to severe economic losses to the dairy farmers due to decrease in production or death of productive animals. The present investigation was undertaken with the objective of studying the biochemical changes induced during endotoxemia and after administration of hypertonic saline solution, dexamethasone and flunixin meglumine in buffalo calves.

***Corresponding author:**
E-mail: Digvijay231@rediffmail.com

Materials and Methods

Normal healthy buffalo calves (5), aged between 4 months to 1 year with body weight range 70-110 kg procured from local market were dewormed a week before the experiment with fenbendazole @ 5 mg/kg BW. The *E. coli* endotoxin (lyophilized, phenol extracted 0111:B4 lipopolysacchride, SIGMA chemicals, USA) was reconstituted by dissolving it in 0.9% normal saline to make a stock solution of 1 mg/ml. Endotoxin concentration of 5 μg/ml was prepared by dissolving 1 ml of stock solution in 199 ml of normal saline. Endotoxin was infused in the animals @ 5 μg/kg BW/hr for 3 hr followed immediately with infusion of hypertonic saline solution (HSS) @ 4 ml/kg BW, dexamethasone @ 4 mg/kg BW and flunixin meglumine @ 1.1 mg/kg BW.

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The blood samples from normal buffalo calves were collected immediately before and after 1, 2, 3, 4, 5, 6, 7 hr of start of endotoxin infusion from the carotid artery followed by samples at an interval of 12 and 24 hr of last sample of day 1 till day 7. The biochemical constituents were monitored from all these samples by blood chemistry analyzer RA-50 (Ames Technician-RA-50-Bayer diagnostics-Miles India Ltd-Baroda, Gujarat, India) using AUTOPAK analytical kits. Total plasma protein was estimated by Biuret method and the color was read at 540 nm. Plasma albumin concentration was estimated by Bromocresol Green method and the colour was measured at 628 nm. Glucose was estimated by oxidase/peroxidase method. The intensity of red colour was measured at 505 nm. Blood urea nitrogen was calculated by determining the absorbance decrease per minute relative to the urea nitrogen at 340 nm. Picrate method was employed to estimate blood creatinine. The rate of color development is proportional to the concentration of creatinine and is measured at 540 nm. Plasma chloride was measured colorimetrically by using mercuric thionate. Fibrinogen was estimated using a portable refractrometer by comparing the protein in non-heated plasma and that in the sample heated to 56-58° C for 3 min (Thomas 2000). Plasma Na and K in plasma were analysed using flame photometer (Systronics-128, Systronics India Ltd, Ahmedabad-Gujarat, India).

The data were pooled and analyzed using completely Randomized Design ANOVA and t-test (Snedecor and Cochran 1976).

Results and Discussion

The normal mean plasma protein was 6.32 ± 0.15 g/dL which is close to 6.82 ± 0.24 g/dL (Sobti *et al*., 1981), 6.40 ± 0.19 g/dL (Singh *et al*., 2004) but lower than 7.72 ± 0.25 g/dL (Kumar, 1989). A significant hypoproteinemia was observed at 2nd to 6th hour and on 2nd and 3rd day (Table 1). Similar decrease in proteins after endotoxin infusion has been reported in buffalo calves. The hypoproteinemia was perhaps due to the increased protein breakdown and ability of carbon skeleton of amino acids to enter Krebs cycle. Additionally the decreased ability of anoxic liver to metabolize amino acids may partially contribute to hypoproteinemia (Singh *et al*., 2004).

The mean normal plasma albumin was observed to be 3.20 ± 0.19 g/dL which is similar to 3.29 g/dl reported by Kaneko *et al*. (1997). A significant ($P < 0.01$) hypoalbuminemia persisted at 2nd to 5th hour and even at 6th day of start of endotoxin infusion. Singh *et al*. (2004) also observed hypoalbuminemia in buffalo calves after E. coli endotoxin administration. The sensitivity of albumin synthesis to protein and nitrogen loss that occurs in any form of diarrhoea and consequent loss of albumin impairs albumin synthesis and further compounds hypoalbuminemia. Due to this sensitivity, decreased albumin developed generalized hypoproteinemia.

The normal mean plasma globulin concentration was 2.76 ± 0.13 g/dL which is lower than 3.90 ± 0.39 g/dL (Singh, 2000). A significant hypoglobulinemia was observed at 3rd, 4th, 6th hour and day 2nd and 4th of the observation. This may be due to increased protein breakdown and hypo proteinemia .

The fibrinogen concentration was significantly ($P < 0.01$) higher than normal pre infusion levels from 6th hour till day7 of endotoxin infusion which may be due to the fact that endotoxin accelerates fibrinogen synthesis rate. Deldar *et al*. (1984) observed that disseminative intravascular coagulopathy (DIC) because endotoxemia is characterized by two phases with an initial decline in blood platelets count and plasma fibrinogen which was indicative of consumptive coagulopathy and followed by an increase in plasma fibrinogen concentration and blood platelet count during reparative phase of disseminative intravascular coagulopathy in endotoxemic calves.

The normal glucose concentration was 75.2 ± 6.25 mg/dL which is close to 67.50 ± 1.74 mg/dL (Singh, 2000). A significant hypoglycemia was observed from 2nd till 6th hour and during 1st, 2nd, 4th,5th and 6thday. The prolonged hypoglycemia may be due to increased utilization of glucose by muscle tissues, inhibition of glucose synthesis from non-carbohydrate sources, decreased hepatic glucose

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Table 1: Mean concentrations (mean ± SE) of biochemical components in blood plasma at different stages of endotoxic shock and after treatment with hypertonic saline solution, flunixin meglumine and dexamethasone in buffalo calves.

	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr	24 hr	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Protein (g/dL)	6.32 ±0.15	5.68 ±0.19	5.36* ±0.22	4.74* ±0.38	4.30* ±0.14	5.14* ±0.83	5.16* ±0.10	6.40 ±0.38	6.28 ±0.11	5.44* ±0.31	5.32* ±0.96	5.68 ±0.23	6.08 ±0.43	6.50 ±0.10	6.10 ±0.00
Albumin (g/dL)	3.20 ±0.19	3.18 ±0.30	2.34* ±0.33	2.64* ±0.07	2.44* ±0.15	2.56* ±0.11	3.02 ±0.11	2.86 ±0.06	2.70 ±0.20	2.76 ±0.14	2.62 ±0.11	3.32 ±0.06	2.82 ±0.15	1.93* ±0.21	2.92 ±0.00
Globulin (g/dL)	2.76 ±0.13	2.14 ±0.33	2.72 ±0.28	1.78* ±0.35	1.52* ±0.19	2.14 ±0.86	1.54* ±0.18	2.60 ±0.15	2.84 ±0.43	1.98* ±0.44	2.08 ±0.16	1.84* ±0.33	2.74 ±0.45	4.05 ±0.28	2.72 ±0.00
Fibrinogen (g/dL)	0.36 ±0.02	0.36 ±0.02	0.30 ±0.00	0.32 ±0.04	0.34 ±0.05	0.44 ±0.09	0.60* ±0.09	0.68* ±0.05	0.74* ±0.04	0.70* ±0.03	0.62* ±0.02	0.52* ±0.08	0.52* ±0.08	0.52* ±0.08	0.46* ±0.04
Glucose (g/dL)	75.2 ±6.25	80.8 ±7.54	55.6* ±11.4	57.4* ±8.24	44.2* ±4.21	45.6* ±2.25	45.4* ±4.32	60.0 ±6.08	52.2* ±3.34	58.2* ±2.30	60.6 ±6.08	46.0* ±8.08	52.8* ±5.50	53.0* ±4.25	66.0 ±0.00
BUN (g/dL)	18.2 ±1.93	18.1 ±1.80	16.1 ±1.41	13.1* ±1.00	13.3* ±0.71	14.4* ±0.78	13.2* ±0.40	12.1* ±0.26	14.1* ±1.17	15.1* ±0.35	15.4* ±0.35	17.5 ±0.92	17.60 ±0.83	18.1 ±0.67	20.1 ±0.00
Creatinine (g/dL)	1.46 ±0.01	1.43 ±0.15	1.30 ±0.15	1.32 ±0.16	1.01 ±0.60	1.05 ±0.70	1.94 ±0.61	1.25 ±0.16	1.34* ±0.18	1.25 ±0.93	1.15 ±0.93	1.30 ±0.73	1.16 ±0.06	1.20 ±0.06	1.55 ±0.00

* Significant at 5% level

Table 2: Mean concentrations (mean ± SE) of sodium, potassium and chloride in blood plasma at different stages of endotoxic shock and after treatment with hypertonic saline solution, flunixin meglumine and dexamethasone in buffalo calves

	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr	24 hr	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Chloride (mEq/L)	98.8 ±7.35	99.1 ±4.03	81.9* ±6.75	79.5* ±4.45	115.8* ±3.12	95.9 ±4.45	94.0 ±8.92	94.7 ±8.12	89.7 ±6.45	91.3 ±2.20	90.0 ±3.01	93.3 ±2.25	90.9 ±1.06	90.5 ±0.50	92.4 ±0.00
Sodium (mEq/L)	136.6 ±5.51	131.1 ±1.07	122.6 ±3.40	123.7 ±2.21	127.8 ±0.98	135.9 ±2.08	127.4 ±3.07	127.9 ±12.1	128.0 ±2.08	133.7 ±2.25	134.4 ±6.22	129.3 ±3.38	137.4 ±1.23	133.2 ±5.01	129.5 ±0.00
Potassium (mEq/L)	2.94 ±1.05	2.27 ±0.42	2.27 ±0.50	2.59 ±0.18	2.24 ±0.73	2.42 ±0.78	2.26 ±0.11	1.82 ±0.43	2.47 ±0.38	2.23 ±0.38	2.13 ±0.17	1.95 ±0.14	2.49 ±0.48	2.59 ±0.42	2.41 ±0.00

* Significant at 5% level

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synthesis, depletion of glycogen storage pools and macrophage–like activity, limited amount of available carbon for glucose synthesis and macrophage derived glucocorticoid-antagonizing factors.

Normal mean blood urea nitrogen was 18.2 ± 1.93mg/dL which is a little less than 20-30 mg/dL (Kaneko *et al*, 1997) but higher than 12.18 ± 4.32 mg/dL (Singh, 2000). A significant decrease in BUN was observed from 3rd hour to 3rd day. Van *et al*. (1988) observed decrease in BUN concentration in goats injected with *E. coli* endotoxin. HSS treatment may be responsible for decrease in BUN because it draws water from intracellular spaces (Constable *et al*., 1991) causing hemodilution.

Normal mean plasma creatinine was observed to be 1.46 ± 0.01mg/dL (Table 1) which is within the physiological range of 1-2 mg/dL. Apart from general declining trend, plasma creatinine decreased significantly at 24 hours of start of endotoxin infusion. The mean sodium, potassium and chloride concentrations were 136.6 ± 5.51, 2.94 ± 1.05 and 98.8 ± 7.35 mEq/L, respectively. Non-significant variations were observed in sodium and potassium concentrations but chloride concentration decreased significantly at 2nd and 3rd hour followed by a significant increase at 4th hour which could be due to infusion of hypertonic saline solution (Table 2).

On the basis of the observations of the present investigation, it can be concluded that administration of hypertonic saline solution, dexamethasone and flunixin meglumine do not bring about any characteristic beneficial change in biochemical profiles of endotoxemic buffalo calves.

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