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ORIGINAL ARTICLE

Selected Serum Biochemical and Electrolyte Findings of Splenectomised Dogs Transfused with Whole Blood, Isoplasma® and Haemaccel®

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SUMMARY

Blood transfusion therapy is necessitated due to high amount of blood and fluid loss. Of recent, Veterinary hemotherapists have resorted to the use of haemaccel® and/or isoplasma® as whole blood substitutes in times of hemotherapy due to unavailability of whole blood or its components. However, there is dearth of information on their serum biochemical and electrolyte effects as reports emanating from their effects are that of human studies. Twelve healthy splenectomised Nigerian dogs were randomly assigned to 4 groups of 3 dogs each. Group A was untreated and served as control, group B received whole blood, group C received hemacccel®, group D received isoplasma®. On post-surgical days (psd)- 14 and 21, the mean total plasma protein (TPP) of dogs in groups B, C and D were significantly ($p<0.05$) higher than that of the control while for the sodium, the reverse was the case. The mean albumin of dogs in groups B, C and D were significantly ($p<0.05$) higher than that of the control on day psd-21 while the mean globulin of group B value was significantly ($p<0.05$) higher than groups A, C and D on psd-10 and 21. On psd-1, the mean creatinine of dogs in groups B, C, D were significantly ($p<0.05$) higher than that of group A while on psd-7, the reverse was the case. For the blood urea (BU) group B values were significantly ($p<0.05$) higher than other groups on psd-14. The findings of this work showed that the blood substitutes used benefited the animal as much as the whole blood but showed a better outcome than the control.

Key words: anaemia, blood, splenectomy, isoplasma®, hemaccel®

INTRODUCTION

The metabolic effect observed in a surgical patient is mostly characterized by increased catabolism which mobilizes substrates to provide energy sources, and a mechanism to retain salt and water and maintain fluid volume and cardiovascular homeostasis (Desborough, 2000).These effects include alterations in protein, glucose, fat

metabolism and changes in water and electrolyte balance induced by accidental or surgical trauma, and an increase also occurs in the overall metabolic rate ((Davidson, 1989; Desborough 2000). To combat these observed effects, administration of fluids such as whole blood or colloids is indicated (Laurent, 2010).

Whole blood is transfused to maintain the vital functions of the body (Hohenhaus, 2012). Colloid expanders are generally non-protein substances such as dextran, hydroxyethyl starch (HES), or gelatin based (Ezio *et al*., 1990; Salmon and Mythen, 1993.). They do not carry oxygen themselves but are used to maintain sufficient blood volume to allow the heart to continue circulating whatever red blood cells exist to carry oxygen (Lamke and Liljedahl, 1976). In addition to sodium chloride, calcium chloride and potassium chloride, haemaccel® also contains polygeline which is a degraded gelatin polymer. It is a synthetic polymer (polypeptide) of molecular weight (MW) 30,000 that does not interfere with blood grouping, cross matching and is non-antigenic. It has the capacity of expanding the plasma volume for 12 hours (Davidson, 1989). On the other hand, Isoplasma® infusion contains polyvinyl pyrolidine, potassium chloride and sodium chloride as active ingredients. Polyvinylpyrrolidine (PVP) is a synthetic polymer of average MW 40,000 used as a 3.5% solution (Davidson, 1989; Ezio *et al*., 1990).

This study was designed to observe changes in selected serum biochemicals and electrolytes of splenectomiseddogs transfused with whole blood, isoplasma® and haemaccel® and thereafter observe if there is any side effects that might hinder the use of isoplasma® and haemaccel® in conditions of emergency that might call for their use.

MATERIALS AND METHOD

Animals

Fifteen (15) Nigerian indigenous adult dogs weighing 10.5±0.49 kg aged 2-3 years were used for this study. They were housed in Department of Veterinary Surgery dog kennel and acclimatized for 30 days before the commencement of the experiment. They were fed with commercial dog food (JO-JO® France) and water provided *ad libitum*. Within this period, they were confirmed free of blood and gastrointestinal parasites through blood smear and fecal floatation test respectively. The dog's vaccination history was up to date. Three of the dogs acted as blood donors. The remaining 12 dogs were randomly assigned into 3 group's designated A, B, C and D.

Experimental Design

Group A (n=3); splenectomised dogs (control)

Group B (n=3); splenectomised dogs transfused with whole blood.

Group C (n=3); splenectomised dogs transfused with haemaccel®.

Group D (n=3); splenectomised dogs transfused with isoplasma®.

Ethical Permit

Animal studies were performed in conformity with the guidelines of University of Nigeria ethical codes and regulations for animal use and that of National institute of health (NIH) revised guidelines for laboratory animals care and use (NIH, 1985).

Methodology

Baseline values (BV) were determined for serum biochemical and electrolyte changes prior to the surgery. Thereafter, the dogs were splenectomised and monitored post operatively. Serum biochemical and electrolyte parameters were re-assayed on psd 1, 3, 7, 10, 14 and 21. Whole blood, haemaccel® (Abbott, Piramel Healthcare UK limited) and isoplasma® (Juhel Pharmaceuticals, Nigeria) were administered to the appropriate groups at the rate of 15ml/kg body weight.

For the BV, 5 ml of venous blood was used to assay for the serum biochemical and electrolytes values. Subsequently, on days 1, 3, 7, 10, 14 and 21, 5ml of blood was harvested to re-assay the parameters assayed on the baseline. Blood was collected into plastic vacutainers containing ethylene diamine tetra acetic acid (EDTA) anticoagulant. Serum was gotten after proper

centrifugation using Denley BS400 centrifuge at 3,000 rpm for ten minutes and the supernatant was used for analysis of its biochemical and electrolyte concentration.

Total plasma protein (TPP) and creatinine were assayed as described by Lubran (1978) and Blass *et al.* (1974) respectively. Other parameters such as blood urea were assayed as described by Fawcett and Scott (1960), serum albumin and globulin as described by Doumas *et al*. (1971) and Doumas and Peters (1997). Serum electrolytes parameters such as calcium was assayed as described by Connerty and Briggs (1966), potassium as described by Hillman and Beyer (1967) sodium as described by Trinder (1951) and chloride as described by Skeggs and Hochstrasser (1964).

Data Analysis

Data obtained were summarized as mean (standard error of mean). To determine the effect of the treatments, mean data on serum biochemical and electrolyte assay were compared between groups using one-way analysis of variance followed by LSD test. Probability <0.05 were considered as significant.

RESULTS

Serum Biochemical Parameters

The mean creatinine of dogs in group A was significantly ($p<0.05$) lower than those of groups B, C and D on psd-1. Also, mean creatinine of group C was significantly ($p<0.05$) lower than those of groups B and D while there was no significant ($P>0.05$) difference between mean creatinine of groups B and D. On psd-3, the mean creatinine of dogs in groups A and C was significantly ($p<0.05$) lower than that of groups B and D. On psd-7, the mean creatinine of dogs in group A was significantly ($p<0.05$) higher than those of groups B, C and D while that of group B was significantly ($p<0.05$) higher than those of groups C and D. Mean creatinine of groups C and D did not vary significantly ($p>0.05$) with each other. On psd-10, the mean creatinine of dogs in groups A and B were not significantly ($p>0.05$) different but were significantly ($p<0.05$) higher than mean creatinine of groups C and D. On psd-14, there was no significant ($p>0.05$) difference in mean creatinine of groups A and C but they were significantly ($p<0.05$) higher than that of group D. However, mean creatinine of group B did not differ significantly ($p>0.05$) with that of the other groups. On psd-21, mean creatinine of groups A, C and D showed no significant ($p>0.05$) different among themselves, although mean creatinine of groups A and D was significantly ($p<0.05$) lower than that of group B (TABLE I).

The mean blood urea of dogs in group A did not vary significantly ($p>0.05$) with those of groups B, C and D on psd-1. On psd- 3, the mean blood urea of dogs in groups A and C did not vary significantly ($p>0.05$) with each other but were significantly ($p<0.05$) higher than those of groups B and D. Mean blood urea of groups A, B, and D did not vary significantly ($p>0.05$) among themselves on psd-7 but that of group C was significantly ($p<0.05$) lower than blood urea of others. On psd- 10 and 14, mean blood urea of dogs in groups B was significantly ($p<0.05$) higher than blood urea of group C. On psd-21, the mean blood urea of dogs in groups C and D were significantly lower than that of group B (Table I).

There was no significant difference in the mean TPP of dogs in groups A, B, C and D on psd-1, 3, and 7. On psd-10, the mean total plasma protein of dogs in group B was significantly ($p<0.05$) higher than that of group A but did not vary significantly ($p>0.05$) from that of groups C and D. The mean TPP of dogs in group A on psd-14 was significantly ($p<0.05$) lower than those of

Table I
Changes in the mean Creatinine and Blood Urea post-spleenectomy

Creatinine (mg/dl)	Days						
	1	3	7	10	14	21	
oup A	92.58±6.38[a]	60.83±15.21[a]	57.68±28.07[a]	110.96±5.32[a]	139.47±35.20[a]	112.93±8.20[a]	89.09±12.57[a]
B	*87.80±6.46[a]*	123.90±11.85[b]	108.99±31.10[b]	94.74±9.12[b]	104.39±3.74[ab]	101.98±11.37[ab]	103.94±2.46[b]
C	90.57±7.85[a]	88.60±5.60[c]	85.96±6.25[a]	75.00±0.66[c]	87.06±2.11[b]	105.7±15.60[a]	88.38±5.90[a]
D	91.81±4.11[a]	121.05±12.19[b]	99.56±2.11[b]	79.17±3.04[c]	88.38±13.84[b]	87.94±2.49[b]	92.30±3.16[ab]

Blood urea (mg/dl)	Days						
	0	1	3	7	10	14	21
oup A	8.90±3.14[a]	12.33±0.68[a]	11.44±2.52[a]	11.49±4.18[a]	8.47±1.81[a]	8.03±4.04[a]	4.75±1.80[a]
B	7.87±2.88[a]	9.08±1.085[a]	7.34±1.51[b]	7.43±3.28[ab]	9.06±3.32[b]	10.62±1.13[b]	8.58±3.01[ab]
C	5.64±0.83[a]	11.57±4.64[a]	10.65±2.00[a]	4.70±2.23[b]	4.41±2.10[c]	6.20±4.41[c]	3.90±2.50[ac]
D	5.48±1.99[a]	9.19±2.60[a]	5.69±2.07[b]	8.3±1.02[ab]	5.57±1.08[a]	3.89±2.84[a]	3.83±2.22[ac]

Table II: Changes in the mean TPP, Albumin and Globulin post-spleenectomy

TPP (g/dl)	Days						
	0	1	3	7	10	14	21
Group A	7.55±2.30[a]	5.29±1.84[a]	4.90±0.13[a]	6.89±2.21[a]	4.28±0.38[a]	3.06±0.09[a]	2.29±0.33[a]
B	7.01±0.54[a]	6.95±0.47[a]	6.09±0.29[a]	6.33±1.16[a]	5.92±0.78[b]	6.32±0.66[b]	6.95±0.15[b]
C	5.77±1.48[a]	5.15±0.88[a]	5.88±1.59[a]	5.17±0.91[a]	4.84±0.79[ab]	5.04±1.13[b]	5.92±0.74[b]
D	5.82±0.44[a]	3.33±1.79[a]	5.97±2.05[a]	4.66±2.09[a]	4.97±0.45[ab]	4.85±1.02[b]	4.09±1.31[c]

Albumin (g/dl)	Days						
	0	1	3	7	10	14	21
Group A	3.77±1.12[a]	2.98±1.32[a]	3.64±0.52[a]	3.96±1.39[a]	3.35±0.79a	2.57±0.48[a]	1.74±0.28[a]
B	3.76±0.79[a]	3.78±0.90[a]	3.95±1.13[a]	3.97±1.38[a]	3.56±0.42a	2.80±0.98[a]	2.44±0.03[b]
C	4.38±0.84[a]	3.50±0.87[a]	4.37±1.35[a]	4.04±0.36[a]	4.20±1.05a	3.76±0.60[a]	3.86±0.28[c]
D	4.64±0.73[a]	2.28±1.47[a]	3.32±0.50[a]	3.39±0.84[a]	3.93±0.47a	3.77±0.62[a]	3.03±0.46[d]

Gobulin (g/dl)	Days						
	0	1	3	7	10	14	21
Group A	3.78 ±2.09[a]	2.31±0.62[a]	1.26±0.39[a]	2.93±1.46[a]	0.94±0.57[a]	0.49±0.53[a]	0.55±0.51[a]
B	3.26±0.60[a]	321±1.31[ab]	2.14±1.32[a]	2.36±0.27[a]	2.37±0.51[b]	3.52±1.58[b]	4.52±0.17[b]
C	1.37±1.67[a]	1.65±0.53[a]	1.50±0.65[a]	1.12±0.77[a]	0.64±0.39[a]	1.28±0.86[ab]	2.06±0.93[c]
D	1.17±0.80[a]	1.12±0.81[ab]	2.64±2.13[a]	1.27±1.41[a]	1.04±0.91[a]	1.09±1.54[a]	1.07±0.86[ac]

Different superscript [a,b,c,d] in a column represents significance between means at p<0.05.

groups B, C and D. On psd-21, mean TPP of groups B and C did not vary significantly

The mean sodium of dogs in group B was significantly higher than those of groups A, C and D on psd 1. On psd- 3, 7, 10, 14 and 21, the

Table III: Changes in the mean Sodium and Chloride post-splenectomy

Sodium(mEq/L)	Days						
	0	1	3	7	10	14	21
Group A	124.78±17.12^{a}	90.83±17.54^{a}	100.65±1.37^{a}	162.08±8.91^{a}	163.27±6.25^{a}	164.49±6.15^{a}	190.88±9.37^{a}
B	137.49±13.36^{a}	133.21±2.73^{b}	131.47±8.36^{b}	138.36±2.64^{b}	128.04±3.48^{b}	128.39±8.34^{b}	125.35±0.12^{b}
C	127.31±22.98^{a}	84.32±11.18^{a}	137.50±4.68^{b}	151.79±3.11^{c}	155.31±5.18^{ac}	130.28±5.18^{b}	124.39±1.52^{b}
D	124.39±4.91^{a}	86.12±4.69^{a}	127.97±5.46^{b}	140.60±2.21^{b}	146.18±5.23^{c}	134.42±2.29^{b}	135.40±1.86^{c}

Chloride	Days						
(mg/dl)	0	1	3	7	10	14	21
Group A	110.38±17.88^{a}	137.71±5.65^{a}	135.48±5.18^{a}	134.93±3.48^{a}	128.27±2.25^{a}	125.61±4.88^{a}	123.88±2.24^{a}
B	110.86±5.00^{a}	110.04±1.86^{b}	109.81±5.22^{b}	113.69±3.61^{b}	121.53±2.64^{b}	111.75±3.62^{b}	106.52±4.84^{b}
C	101.00±2.39^{a}	129.32±7.17^{ac}	122.83±5.59^{c}	130.79±5.55^{a}	128.31±1.85^{a}	125.49±5.09^{a}	118.72±2.88^{ab}
D	107±5.89^{a}	121.05±2.72^{c}	125.97±4.56^{ac}	116.96±10.07^{b}	114.59±4.36^{c}	104.85±5.69^{b}	102.13±12.67^{b}

($p>0.05$) with each other but were significantly ($p<0.05$) higher than that of group D (TABLE II). The mean albumin level of dogs in groups A, B, C and D did not vary significantly ($p>0.05$) among themselves on psd-1, 3, 7, 10 and 14. On PSD- 21, the mean albumin level of dogs in group A was significantly ($p<0.05$) lower than albumin level of groups B, C and D (TABLE II).

The mean globulin level of dogs in groups A, B, C and D did not vary significantly ($p>0.05$) among themselves on psd-1, 3, and 7. On psd- 10 and 14 mean globulin level of dogs in group B was significantly ($p<0.05$) higher than that of dogs in groups A, C and D. There was no significant ($p>0.05$) difference in mean globulin level of dogs in groups A, C and D. The mean globulin level of dogs in group 4 on psd- 21 did not significantly ($p>0.05$) differ from that of group A and C but was significantly ($p<0.05$) lower than that of group B (TABLE II).

mean sodium of dogs in groups B, C, and D was significantly higher than that of group A (TABLE III).

On psd-1, the mean chloride level of dogs in groups A and C did not vary significantly ($P>0.05$) with each other although that of group B was significantly ($P<0.05$) lower than those of the other groups. The mean chloride level of dogs in group D did not significantly ($P>0.05$) vary from that of group C on psd-1. On psd- 3, the mean plasma chloride level of dogs in groups A and D did not differ significantly ($p>0.05$) with each other but were significantly ($p<0.05$) higher than those of groups B and C. On 7 and 14, the mean chloride level of dogs in groups A and C did not vary significantly ($p>0.05$) with each other but were significantly ($p<0.05$) higher those of groups B and D. On psd- 10, the mean chloride level of dogs in group D was significantly ($p<0.05$) lower than that of dogs in group C while chloride level of both groups were

Table IV: Changes in the mean potassium and calcium post-splenectomy

Potassium (mMol/L)	Days						
	0	1	3	7	10	14	21
Group A	3.82±0.86^{a}	4.60±1.44^{a}	3.30±0.55^{a}	4.38±0.67^{a}	4.18±0.81^{a}	4.76±0.68^{a}	4.73±0.40^{a}
B	4.49±0.63^{a}	4.38±0.33^{a}	3.95±0.74^{a}	2.52±1.52^{a}	2.38±2.40^{a}	1.89±0.41^{b}	2.28±0.34^{b}
C	4.14±1.79^{a}	3.14±0.93^{ab}	3.18±1.61^{a}	3.56±1.49^{a}	2.74±1.04a	3.41±1.64^{ab}	3.07±0.49^{bc}
D	3.43±0.20^{a}	1.52±0.73^{b}	2.93±0.69^{a}	3.88±0.41^{a}	2.31±0.50^{a}	3.04±0.44^{ab}	2.67±0.30^{b}
Calcium (mg/dl)	**Days**						
	0	1	3	7	10	14	21
Group A	5.27±0.85^{a}	5.10±1.65^{a}	7.60±1.17^{a}	10.52±0.52^{a}	10.48±0.62^{a}	9.09±0.55^{a}	6.13±1.31^{a}
B	4.24±1.84^{a}	3.98±1.75^{a}	3.73±0.02^{b}	3.15±0.10^{b}	5.47±1.49^{b}	5.04±0.08^{b}	5.09±0.09^{a}
C	4.75±2.37^{a}	5.89±1.04^{a}	6.18±0.48^{c}	5.05±0.88^{c}	5.44±0.35^{b}	5.43±1.48^{b}	2.40±0.47^{b}
D	4.79±2.21^{a}	5.15±2.71^{a}	7.72±0.60^{a}	11.38±0.15^{a}	11.52±0.43^{a}	7.77±3.03^{ab}	1.93±1.53^{b}

significantly ($p<0.05$) lower than those of groups A and B. The mean chloride level of dogs in groups B, C and D did not significantly ($P>0.05$) vary among themselves but that of groups B and D were significantly ($P<0.05$) lower than those of group A on psd- 21 (TABLE III).

On psd-1, the mean potassium level of dogs in group D was significantly ($p<0.05$) lower than those of dogs in groups A, B and C. On psd - 3, 7 and 10, there was no significant ($p>0.05$) difference in potassium level of dogs in groups A, B, C and D. The mean potassium level of dogs in group B was significantly ($p<0.05$) lower than those of groups A, C and D on psd- 14. On psd-21, group A potassium level was significantly ($p<0.05$) higher than those of groups B, C and D (TABLE IV).

The mean calcium level of dogs in groups A, B, C and D did not vary significantly ($p>0.05$) on psd- 1. On psd- 3 and 7, mean plasma calcium level of dogs in group B was significantly ($p<0.05$) lower than that of group C. On psd-10 and 14, the mean calcium of dogs in groups A and D did not significantly ($p>0.05$) vary with each other but was significantly ($p<0.05$) higher than those of groups B and C. On psd- 21, the mean calcium level of dogs in groups A and B was significantly ($p<0.05$) higher than those of groups C and D (TABLE IV).

DISCUSSION

Surgical stress leads to acute compensatory responses such as alteration in physiologic, neuroendocrine and metabolic status of animals. These changes occur in the body's attempt to maintain homeostasis as well as hasten the rate of catabolism (Udegbunam *et al.*, 2015).

Valeri and Fortier (1969) reported that elevated levels of red cell creatinine have been found parallel to increased concentrations of 2,3 DPG in patients suffering from red cell mass deficits or from cardiopulmonary insufficiency. Kopriva *et al.* (1972) demonstrated that red cell creatinine concentration rises with increased erythropoiesis and concluded that the level of creatinine in the circulating red cell is a sensitive indicator of the mean age of the red cell population. Increased red cell creatinine usually indicates a young mean red cell population, while decreased red cell creatinine usually indicates an old mean red cell population. The proposed reasons by Valeri and Fortier (1969) and Kopriva *et al.* (1972) might explain the lower creatinine level in the control group as against the higher levels in the treatment groups.

Post-operative catabolic phase is marked by low feed intakes and gradual lysis of skeletal muscle cells. This leads to negative nitrogen balance as amino acids are deaminated in turn leading to marked increase in creatinine (Udegbunam *et al.*, 2015). This could be the cause of the observed increase in creatinine level in the control group during the post splenectomy period.

Blood urea (BU) rises when their endogenous production from skeletal muscle catabolism outweighs the rate of urea excretion or if glomerular filtration rate decreases. So, we can conclude that the pattern of recorded changes in each experimental group corresponded with the degree of surgical stress and bodies attempt to maintain homoestasis as well as hasten the rate of catabolism. In dogs and cats, BU may be more influenced by pre-renal effects on tubular reabsorption and diffusion rate, and effects of diet and protein metabolism than is creatinine (Bessey and Lowe, 1993). However, the decrease in BU recorded across the groups on psd-21 might suggest that the glomerular filtration in these dogs increased post-surgery as a result of the effect of the fluid.

Hypernatraemia obtained from the control group as against the treated groups may imply that water moved from intracellular space into extracellular fluid (ECF) compartment resulting in probable cell shrinkage and intracellular fluid (ICF) deficit. As reported by Marilyn and Jesse (2000), possible causes of hypernatraemia include fever, heat stroke, extensive burn, watery diarrhoea, water deprivation or sodium gain from excessive intake, intravenous (IV) hypertonic saline and hyperaldosteronism (Cushing's). From our finding, sodium levels of the treated groups were within normal range while hypernatraemia was recorded in the control group, we suggest that recorded results might be as a result of the treatment meted out to the treated groups and the surgical stress encountered by the dogs in control group.

Chloride is the main extracellular fluid anion associated directly with sodium and inversely with bicarbonates. High levels of chloride are seen in cerebrospinal fluid, bile, gastric and pancreatic juices. It helps to maintain extracellular fluid osmolality and acid-base balance (Zaloga, 1991). Observed hyperchloraemia from the control group against the treated group might be as a result of the surgical stress.

According to the findings of Marilyn and Jesse (2000), a large amount of citrated blood or hemodilution usually leads to hypocalcemia. Hypocalcemia occurs in up to 70% of patients in intensive care units. It may be caused by either reduced ionized or protein-bound calcium. In critically ill patients, calcium loss due to increased tissue sequestration of calcium may be associated with pancreatitis, septicemia, burns or toxic shock (Zaloga, 1991). However, hypocalcemia is only important clinically when the physiologically active, ionized fraction (40% of total) is reduced. Ionized calcium is lowered by alkalosis, by high circulating free fatty acids, as in pancreatitis or sepsis, and by agents that chelate calcium, such as albumin infusions, bicarbonate administration and citrate loads administered with massive blood transfusion (Zaloga, 1991; Marilyn and Jesse, 2000). It could hence be assumed that haemaccel®

administered caused hemodilution thus resulting to the hypoglycaemia observed.

From this study we can conclude that the fluids administered (whole blood, haemaccel® and isoplasma®) at 10ml/kg/day did not alter the animals biochemical and electrolyte status of the treated groups. In other words, clinical conditions resulting to drop in blood volume can be managed with haemaccel® and isoplasma® especially in situations where whole blood is not handy. Also, we suggest that in cases of acute anemia, 10ml/kg/day can be the recommended dose as at this dose, no hemodilution was observed and no post transfusion challenges.

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