

THERAPEUTIC EVALUATION OF HYPERTONIC SALINE SOLUTION IN DIARRHEIC BUFFALOES

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ABSTRACT

This study was conducted to determine the effectiveness of rapid IV administration of hypertonic saline solution (HSS) for resuscitating buffaloes suffering from diarrhea. Twenty diarrheic buffaloes were selected from the field and randomly divided into two equal groups viz group A and B. Buffaloes of group A were treated with normal saline (NS; 0.9% NaCl) solution @ 32 ml/kg BW, while group B was infused with hypertonic saline solution (HSS; 7.5% NaCl) @ 4 ml/kg BW, followed by normal saline solution @ 10 ml/kg BW. Buffaloes were then monitored for 48 hours after initiation of fluid replacement. Diarrhea had profound effects on the partial pressure of O₂ and increased pressure of CO₂, mean arterial pressure (MAP), relative plasma volume (rPV) and blood pH. Infusion of HSS in buffaloes caused significant increase in MAP (74 ± 2 to 100 ± 5), plasma volume, systemic O₂ delivery and blood pH (7.12 ± 0.03 to 7.41 ± 0.05) in comparison to NS. Significant reduction in PaCO₂ (39 ± 2 to 28 ± 1 mm Hg) was also observed in group B. It was concluded from the study that hypertonic saline solution has beneficial effects in resuscitating the animals from hypovolemia and acidemia caused by diarrhea.

Key words: Hypertonic saline solution, therapeutic, diarrheic

INTRODUCTION

Diarrhea in buffaloes is a serious welfare problem and an important cause of economic loss because of high mortality, treatment costs and poor growth in the dairy industry. The etiology of diarrhea is multifactorial and involves a wide range of infectious (bacterial, viral, protozoan) and non-infectious (environmental, nutritional, etc.) factors (Radostits et al., 2007). Diarrhea induces fluid and electrolyte loss results in hypovolemia and metabolic acidosis (Hall et al., 1992; Barragry, 1994). Correction of hypovolemia and acidosis using oral or intravenous electrolyte solutions is a major goal of treatment (Radostits et al., 2007; Barragry, 1994).

Hypertonic saline solution (HSS; 7.5% NaCl @ 4 ml/kg of body weight, IV) has been used to resuscitate sheep (Nakayama et al., 1984; horses (Schmall et al.), dogs (Velasco et al., 1980) and human beings (Holcroft et al., 1987) with hypovolemic shock. Hypertonic saline solution increases cardiac output, mean systemic arterial pressure and oxygen delivery (Velasco et al., 1980; Constable et al., 1991; Suzuki et al., 1998; Kramer et al., 2003). Administration of HSS intravenously causes an initial rapid fluid influx into the vasculature due to the sudden hypertonic state of plasma in a relatively short time (Constable et al., 1991; Tyler et al., 1994). Plasma volume expansion is, therefore, achieved with less free water administration than with isotonic plasma expanders. Since administration of a hypertonic should lead to recruitment of extravascular fluids into the

vascular compartment, HSS seems likely to produce a more rapid response and marked hemodynamic effects than isotonic solutions (Zafar et al., 2004).

Many researchers have investigated the potential use of HSS in diarrheic calves (Constable et al., 1996; Constable et al., 1991a; Constable et al., 1991b; Senturk, 2003), however, to the author's knowledge; there are no controlled experimental studies that investigated the effects of hypertonic saline solution in adult diarrheic buffaloes. So, the objective of this study was to assess the effects of small volume intravenous HSS and to compare this novel therapy with large volume intravenous isotonic saline solution in the diarrheic buffaloes.

MATERIALS AND METHODS

Experimental Animals: The experimental protocol for this study was approved by the Animal Ethics Committee, University of Agriculture, Faisalabad-Pakistan. Twenty adult buffaloes suffering from diarrhea were selected for this study.

Experimental Design: On meeting the aforementioned criteria for entering the treatment phase of the study, all buffaloes were randomly divided into two groups, 10 buffaloes per group. Group A served as control and was treated with infusion of normal saline solution (NS; 0.9% NaCl) @ 32 ml/kg of body weight, IV along with Ceftiofur HCl @ 6 mg/kg BW, IM (Excenel RTU[®]; Pfizer Animal Health Division, Pakistan) and flunixin meglumine @ 2 mg/kg BW, IV (Ketoject[®]; Selmore

Pharmaceuticals, Lahore-Pakistan). Group B was infused with hypertonic saline (HSS; 7.5% NaCl; 2400 mmol NaCl/L) solution alone @ 4 ml/kg followed by normal saline solution @ 10 ml/kg BW. This solution provided 4.8 mmol of sodium/kg to each buffalo. Other than HSS, animals of group B were also treated with cefotiofur HCl and flunixin meglumine in the same manner as in group A.

Instrumentation: For infusion of solutions and collection of blood samples, catheters of 14-gauges were placed in jugular veins, respectively. Jugular catheter was flushed with 1 ml saline (0.9% NaCl) solution at the time of infusion solutions and sampling, containing 5 U of sodium heparin/ml. Mean arterial pressure (MAP) was measured with the help of saline sphygmomanometer.

Measurements and analysis of samples: Venous blood samples were collected at baseline, 3, 6, 12, 24 and 48 hours after treatment. The venous blood samples without anticoagulant were used to harvest serum after centrifugation and stored at -20 °C until assayed. Serum sodium (Na⁺), potassium (K⁺) and chloride (Cl⁻) were determined with the help of electrolyte's analyzer (Medica Corporation, Bedford). Changes in relative plasma volume (rPV) were calculated from hemoglobin concentration and hematocrit, using accepted formula (Greenleaf et al., 1979; Tyler et al., 1994).

Blood samples were obtained for determination of arterial and mixed venous blood gases at the same intervals as other parameters. Blood was collected anaerobically in 1-mL heparinized syringe and the tip was capped. The syringe was placed on ice and was processed within an hour of collection. Systemic arterial and mixed venous blood gas tensions, pH and bicarbonates (HCO₃⁻) were measured by automatic gas

analyzer (Medica Corporation, Bedford) at 37°C. Values were automatically corrected to rectal temperature.

RESULTS

Diarrhea affected profoundly on the oxygen delivery to the tissues (Table-1). It decreased partial pressure of oxygen and increased carbon dioxide (Table 1). The values of partial pressure of oxygen did not return to baseline after administration of NS plus ceftiofur and flunixin meglumine in group A. But infusion of HSS along with ceftiofur and flunixin meglumine in group B induced progressive and significant alteration in values toward normal.

Mixed venous pH and bicarbonates (HCO₃⁻) values decreased significantly during diarrhea (Table 1). The NS infusion in animals of group A induced metabolic acidosis by decreasing the values of arterial and mixed venous pH and HCO₃⁻ within 3 hours after infusion and then these values returned near to normal within 12 hours post-infusion, while buffaloes of groups B did not showed any metabolic acidosis after treatment and values decreased and returned near to baseline within 12 hours in group B (Table 1).

Diarrhea induced significant decrease in mean arterial pressure. Peak effects were observed in the animals having severe intensity of diarrhea. Rapid return toward normal followed by HSS administration in groups B (Table 2) was observed, while animals of group A showed less significant improvement in MAP. All the buffaloes were hypovolemic during diarrhea, as evidenced by decrease in plasma volume (Fig 1).

Table 1. Results of blood gases for samples obtained from diarrheic buffaloes before and after treatment in both the groups

Variables	Groups	Time after treatment					
		Baseline	3 hrs	6 hrs	12 hrs	24 hrs	48 hrs
PaO ₂ (mm of Hg)	Group A	38 ± 6	51 ± 4	61 ± 2	68 ± 1	72 ± 4	78 ± 3
	Group B	40 ± 4	58 ± 2	70 ± 4*	74 ± 2	82 ± 3*	90 ± 2
PvO ₂ (mm of Hg)	Group A	14 ± 1	30 ± 3	30 ± 4	33 ± 3	36 ± 2	35 ± 3
	Group B	13 ± 2	33 ± 2	38 ± 2*	42 ± 1*	39 ± 3	39 ± 4
PaCO ₂ (mm of Hg)	Group A	37 ± 4	40 ± 2	43 ± 2	38 ± 3	34 ± 1	34 ± 3
	Group B	39 ± 2	38 ± 3	40 ± 1	33 ± 4*	30 ± 2*	28 ± 1*
Blood pH	Group A	7.12 ± .03	7.15 ± .02	7.20 ± .02	7.26 ± .04	7.30 ± .01	7.30 ± .05
	Group B	7.12 ± .03	7.20 ± .01	7.28 ± .03	7.36 ± .02*	7.40 ± .02*	7.41 ± .05*
HCO ₃ ⁻ (mEq/l)	Group A	16 ± 1.1	14 ± 1.1	19 ± 1.3	17 ± 1.3	20 ± 1.5	23 ± 1.7
	Group B	17 ± 1.3	15 ± 1.6	18 ± 1.1	21 ± 2.2	23 ± 1.4	23 ± 1.1

*Significantly different from other group ($P < 0.05$)

Group A= Treated with Normal saline solution plus Ceftiofur HCl and flunixin meglumine; Group B= Hypertonic saline solution in combination with Ceftiofur HCl and flunixin meglumine.

Table 2. Results of mean arterial pressure and serum electrolytes for samples obtained from diarrheic buffaloes before and after treatment in both the groups

Variables	Groups	Time after treatment					
		Baseline	3 hrs	6 hrs	12 hrs	24 hrs	48 hrs
MAP (mm Hg)	Group A	70 ± 4	80 ± 6	78 ± 4	86 ± 3	90 ± 4	92 ± 6
	Group B	74 ± 2	88 ± 6*	94 ± 7*	100 ± 2*	96 ± 5	100 ± 5*
Sodium (mEq/L)	Group A	120 ± 3	124 ± 2	128 ± 3*	126 ± 3*	130 ± 2*	136 ± 2
	Group B	118 ± 4	150 ± 5	161 ± 5	152 ± 4	142 ± 4	134 ± 3
Chloride (mEq/L)	Group A	94 ± 2.4	100 ± 2.4*	99 ± 1.3*	104 ± 2.5	106 ± 3.4	104 ± 3.0
	Group B	90 ± 4.0	110 ± 3.1	114 ± 1.8	108 ± 2.8	104 ± 1.1	106 ± 2.4
Potassium (mEq/L)	Group A	3.2 ± 0.4	3.7 ± 0.7	3.5 ± 0.5	3.5 ± 0.8	3.8 ± 0.3	3.9 ± 0.5
	Group B	3.0 ± 0.6	3.2 ± 0.4	3.4 ± 0.3	3.6 ± 0.7	3.8 ± 0.6	4.0 ± 0.8

*Significantly different from other group ($P < 0.05$)

Group A= Treated with Normal saline solution plus Ceftiofur HCl and flunixin meglumine; Group B= Hypertonic saline solution in combination with Ceftiofur HCl and flunixin meglumine.

Significant increase was observed in animals of group B, while infusion of NS could not induce progressive and significant alteration in values of plasma volume toward normal in animals of group A (Fig 1).

Serum sodium and chloride concentrations decreased significantly while marked decrease in potassium concentration was noted during diarrhea due to hypovolemic condition. Hypertonic saline solution infusion produced significant increase in sodium and chloride ions concentration in the group B, compared with baseline values (Table 2) and then returned to normal values within monitoring time. While potassium ions concentration transiently decreased after HSS infusion in group B and then returned toward normal. Normal saline infusion in buffaloes of group A showed better recovery of sodium and chloride in attaining the normal values and potassium ions concentration significantly increased toward normal (Table 2).

DISCUSSION

This is the first study evaluating the potential benefits of hypertonic saline solution (7.5% NaCl; 4 ml/kg BW) to resuscitate the buffaloes from diarrhea. Our most conspicuous findings relate to the substantial improvement in systemic oxygenation, cardiac performance and reversing acidosis.

Blood gas analysis is frequently used to determine the imbalance of acid/base status and it also helps to develop effective treatment plan to resuscitate the patient from acidemia. Metabolic acidosis is the result of an accumulation of metabolic waste products and lack of buffer system in hypovolemia. In the present study, decreased pressure of oxygen was observed accompanied by increase in carbon dioxide (PaCO_2). Diarrhea may be linked to activation of host granulocytes and release of endogenous arachidonic acid metabolites, such as

thromboxane and prostaglandin $\text{F}_{2\alpha}$, which are important contributors to hypovolemia leading to endotoxemia. Other than these, decreased venous blood pH and decreased bicarbonates (HCO_3^-) during hypovolemia/endotoxemia were indicators of metabolic acidosis in buffaloes. As flunixin meglumine is a potent inhibitor of arachidonic acid metabolites (Plumb, 1999), so, its administration along with HSS recovered the animal from acidosis more effectively in less time than normal saline (Ajito et al., 1999; Constable, 1999; Kreimeier et al., 1991).

Hypertonic saline solution infusion induced significant increase in serum sodium and chloride concentration, which were accompanied by a transient decrease in serum potassium concentration. The key feature for successful resuscitation of hypovolemia and/or endotoxaemia in animals is the total amount of sodium (Constable, 1999). In group A, the sodium ions concentration increased beyond the limit of hypernatraemia that is 160 mEq/l (Tyler et al., 1994), but this increase was temporary and then these values became below this level within 6 hours. So, infusion of HSS is safer and it does not cause hypernatremia but not for a prolonged period (Constable et al., 1991; Tyler et al., 1994; Ajito et al., 1999). The mild decrease in potassium concentration after HSS infusion has been observed previously (Constable et al., 1991; Suzuki et al., 1998), but was not considered clinically important.

In the present study, hypotension occurred which was characterized by decreased MAP and decreased plasma volume during hypovolemia. Decreased MAP and plasma volume may be interpreted as an indicator of hypotension and hypovolemia (Cohen et al., 1996) reported that was accepted as typical hypotension during hypovolemia and/or endotoxemia (Constable et al., 1991b; Zafar et al., 2009). After administration of HSS plasma volume was restored and

ultimately MAP was increased to normal values more effectively than other group.

Conclusion: It was concluded from the study that hypertonic saline solution has beneficial effects in resuscitating the animals from hypovolemia and academia. It also improved cardiovascular performance and oxygenation.

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