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COMPARATIVE EFFICACY OF HYPERTONIC SALINE AND NORMAL SALINE SOLUTIONS IN EXPERIMENTALLY INDUCED ENDOTOXIC SHOCK IN DOGS

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ABSTRACT

This study was contemplated to determine the comparative beneficial effects of hypertonic saline solution and sterile saline solution in induced endotoxic shock in dogs. For this purpose, 12 healthy Mongrel dogs were randomly divided into two equal groups (A and B). All the animals were induced endotoxaemia by slow intravenous administration of *Escherichia coli* endotoxins 0111:B4. Group A was treated with normal saline solution @ 90 ml/kg BW, while group B was given hypertonic saline solution @ 4 ml/kg BW, followed by normal saline solution @ 10 ml/kg BW. Different parameters were observed for evaluation of these fluids including clinical and haematological parameters, serum electrolytes, mean arterial pressure, and blood gases at different time intervals up to 24 hours post treatments. After infusion of respective fluids, all parameters returned to baseline values in both the groups but group B showed better results than group A except bicarbonates, which better recovered in group A. Thus, it was concluded that a small-volume of hypertonic saline solution could be effectively used in reversing the endotoxaemia. Moreover, it provides a rapid and inexpensive resuscitation from endotoxic shock.

Key words: Endotoxins, *Escherichia coli*, induction, fluid therapy, resuscitation.

INTRODUCTION

Endotoxaemia, associated with gram-negative bacteria, is still one of the most common causes of death in humans and animals. Endotoxaemia is caused by endotoxins or lipopolysaccharide (LPS), a toxic structural component of gram-negative bacteria (Luderitz *et al.*, 1982). These endotoxins, released from bacteria either by body's defense systems or by antibiotics, are recognized by a variety of cell types in the body, including monocytes/macrophages (Evans, 1996). When these cells sense the presence of LPS, they respond by producing numerous inflammatory mediators including tumor necrosis factor alpha (TNF-α), interleukin-1 (IL-1) and interleukin-6 (IL-6) in response to LPS (Dinarello, 1991; Dinarello, 1996). These substances have profound effects on the circulatory system including myocardial depression, pronounced vasodilatation and hypovolaemia. Reduced cardiac output and oxygen delivery to tissues result in impaired regional blood flow and disturb the end-organ function, leading to multiple organ failure (MOF) which culminates in death (Gow *et al.*, 1998; Somell *et al.*, 2007).

Early restitution of fluid is the basic tenet for the management of endotoxic shock. The goal of fluid administration is to restore intravascular volume for maintaining an adequate blood pressure, thereby increasing cardiac output and oxygen delivery to the tissues (Somell *et al.*, 2007). There are certain crystalloids already in vogue, however, administration of these fluids are difficult and expensive to accomplish in the field because of requiring large volume, more than one catheterization, labour and proper restraining. A practical and effective method for intravenous (IV) fluid administration would, therefore, be extremely advantageous (Constable, 1999).

With small volume resuscitation using hypertonic saline (HSS), the main effect is a rapid plasma volume expansion induced by mobilization of fluids from the intracellular compartment through the extravascular fluid spaces. The volume expansion is two to four times the infused volume (Velasco *et al.*, 1989; Tollofsrud *et al.*, 2001; Kramer, 2003). Osmotically drawing volume expansion of intracellular and interstitial water into the vascular space is approximately 3 ml for every 1 ml of hypertonic saline infused (Constable, 1999). Plasma volume expansion is, therefore, achieved earlier with less free water administration than with isotonic solutions. So, HSS can be useful to veterinary practitioners because it provides a rapid, inexpensive and practical technique for the initial resuscitation of endotoxin shock in animals.

This study, thus, was planned to evaluate the comparative beneficial effects of hypertonic saline and normal saline solutions in induced endotoxic shock in dogs.

MATERIALS AND METHODS

Experimental animals

Twelve adult, healthy Mongrel dogs of either sex were selected for experiment and acclimatized for one

week. All the animals were fed on the same feed, with fresh water available a*d libitum* and were subjected to complete blood count, faecal and urine examination to ascertain their health status.

Instrumentation

Each dog was sedated only for intravenous cannulations with a combination of ketamine HCl and acepromazine @ 10 and 0.2 mg/kg, respectively, intramuscularly. For infusion of respective solutions, IV catheters (Vasocan® Brannula) of 18-gauge were introduced percutaneously into the left cephalic veins in all the animals. Medial side of the right limb was shaved and cannulated with 22-gauge IV catheter in the femoral artery to measure the mean arterial blood pressure (MAP) of each animal using saline sphygmomanometer during whole of the experiment.

Induction of endotoxic shock

Endotoxic shock was induced by slow (over 5 min) intravenous administration of *Escherichia coli* endotoxins (lipopolysaccharides *E. coli,* 0111:B4-Sigma Chemical Co, St. Louis, MO, USA) at 1 mg/kg BW. The main criterion of endotoxaemia was mean arterial pressure (MAP) ≤60 mmHg (Cohen *et al.*, 1996; Batmaz *et al.*, 2003).

Treatment protocol

At the treatment stage, animals were randomly divided into two groups i.e. A and B, each comprised of six dogs. Animals in group A were infused with normal saline solution (0.9% NaCl) @ 90 ml/kg BW, while animals in group B were infused with hypertonic saline solution (7.5% NaCl) @ 4 ml/kg BW, followed by sterile saline solution @ 10 ml/kg BW.

Evaluation parameters

The parameters observed for evaluation of therapeutic efficacy of normal saline (NS) and hypertonic saline solution (HSS) were respiration rate, pulse rate, rectal temperature, mean arterial pressure (MAP), haemoglobin (Hb) concentration, haematocrit, sodium (Na^+), chloride (Cl^-), potassium (K^+), bicarbonates (HCO_3^-) and blood pH values. These parameters were observed at baseline (before induction of shock), during endotoxic shock, and 30, 60 minutes, 3, 8, 12, and 24 hours after infusion of respective fluids.

Venous blood samples were collected with and without anticoagulant. The blood samples with anticoagulant were used to determine Hb concentration and haematocrit values, while the samples without anticoagulant were used to harvest serum for biochemical profiles and stored at -20^{0}C until assayed. The haemoglobin concentration was determined by cyannmethemoglobin method and haematocrit values were determined with microhematocrit method, as described by Benjamin (1978). Serum Na^+, Cl^- and K^+ were determined with the help of EasyLyte Analyzers (Medica Corporation, Bedford UK) and HCO_3^- with the help of Microlab-200 (S. No. 8-0939, Vital Scientific, Merck, Netherlands).

Statistical analysis

Statistical analyses were performed using student t-test. All analysis was performed using the Statistical Software Package (SPSS Version 11.5). Statistical significance was assigned at $p<0.05$.

RESULTS

Baseline values for the systemic parameters of the both groups are shown in Tables 1 and 2 and are not significantly different. After endotoxic shock, there was significant change in the values of all the parameters from the baseline but difference was again non-significant within groups.

Clinical parameters

Body temperature

There was an increase in body temperature during endotoxaemia in all the animals of both the groups. After instituting the respective treatments, rapid fall in body temperature was noted in both the groups. But this decrease in body temperature was more rapid in group B than in animals of group A. Within 6 hours, body temperature was near to normal in group B and was normal within 12 hours (Fig. 1). But in group A, it did not attain its normal value even after 24 hours.

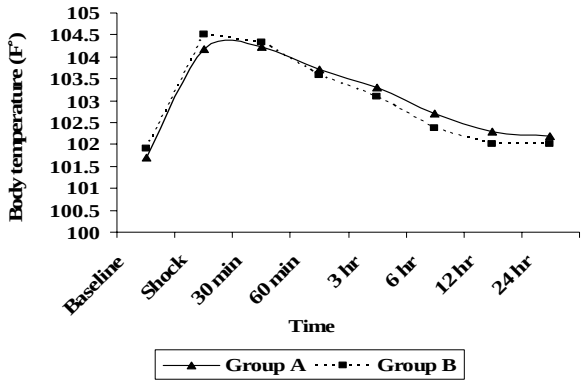

Fig. 1: Body temperature in dogs of two groups at different time intervals after treatment

Pulse rate

A sharp increase in pulse rate was noted in endotoxic shock. After treatment, it decreased more rapidly in group B as compared to group A. In group A, first its trend was increasing after treatment but then it decreased and became near to normal within 24 hours. On the other hand, group B showed good recovery and values became normal within study time (Fig. 2).

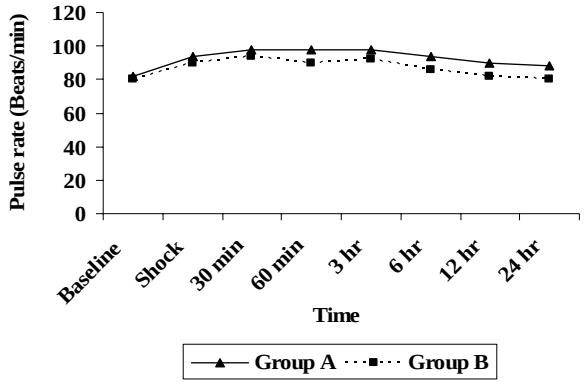

Fig. 2: Pulse rate in dogs of two groups at different time intervals after treatment

Respiration rate

There was a marked increase in respiration rate of animals in both the groups during endotoxic shock. After administration of fluids, group B showed falling trend towards normal and respiration rate in this group became normal within 12 hours (Fig. 3). Group A showed falling trend but slower than its counterpart. After 24 hours, respiration rate was near to normal in group A but did not become normal.

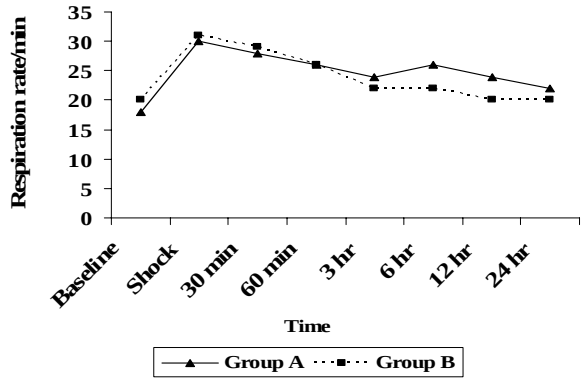

Fig. 3: Respiration rate in dogs of two groups at different time intervals after treatment

Haematological parameters

Haemoglobin concentration

Concentration of haemoglobin increased in endotoxic shock. After infusion of respective treatments, group B showed significant difference compared to group A ($P<0.05$) within 60 minutes in reversing the Hb levels towards normal (Table 1). The values were normal in animals of group B within 24 hours but animals of group A could not achieve normal values within observing time and Hb concentration was still high than the normal.

Haematocrit

Values of haematocrit also increased than its normal values during endotoxic shock. In group B, the haematocrit values started to return towards normal after infusion of allotted fluid and it showed significant difference ($P<0.05$) over group A within first 60 minutes. Group A showed recovery towards normal but slower than group B and did not return to normal within 24 hours. On the other hand, animals of group B showed rapid recovery and the value was near to normal within observing time (Table 1).

Blood gases

Among blood gases, only HCO_3^- and blood pH were measured.

Bicarbonates

Administration of endotoxins induced significant decrease in bicarbonate ions concentration. After fluid resuscitation, bicarbonate (HCO_3^-) values started to recover in group A and it became significantly higher compared with group B ($P<0.05$) within first hour and these values remained higher than the group A up to 6 hours (Table 1). But after 6 hours, group B showed rapid recovery and reached baseline within 24 hours as for group A.

Blood pH

After development of endotoxic shock, blood pH decreased in all the animals. After administration of respective fluids to the animals, pH rapidly recovered and became normal within 24 hours after administration of HSS to the animals of group B. While in group A, values of blood pH recovered but slowly and did not return to normal within observing time (Table 1).

Serum electrolytes

Sodium

There was an increase in sodium ions (Na^+) concentration from the baseline during shock state in all the animals. After instituting the treatments, group A showed good recovery towards normal and showed significant differences ($P<0.05$) throughout the study period over group B (Table 2). While in group B, there was an increasing trend in the Na^+ ions concentration for first 3 hours but after that there was rapid fall in the values and it was near to baseline within 24 hours.

Chloride

Same observations were observed in the chloride ions (Cl^-) concentration as for sodium (Na^+) ions. After administration of respective fluids, group A showed good recovery towards normal and significant differences ($P<0.05$) were observed over group B from 60 minutes to 6 hours. After that, group B showed rapid recovery towards baseline and it was near to baseline within observing time (Table 2).

Table 1: The results of haematological examination, bicarbonates and blood pH before and after treatment in both groups

Variables	Groups	Baseline	Shock	Time after treatment					
				30 min	60 min	3 hrs	6 hrs	12 hrs	24 hrs
Haematocrit (%)	A	35 ± 1.05	43 ± 2.28	44 ± 2.04	49 ± 1.60	48 ± 1.55	46± 1.21	42 ± 1.97	40 ± 1.47
	B	35 ± 1.41	43 ± 1.05	42 ± 1.75	43 ± 2.40*	42 ± 2.07*	41 ± 2.25*	39 ± 1.87	36 ± 1.87*
Hb conc. (g/dl)	A	12 ± 1.05	14 ± 0.82	16 ± 0.75	17 ± 0.82	17 ± 1.10	16 ± 1.17	15 ± 1.17	15 ± 0.75
	B	13 ± 1.38	16 ± 0.89	16 ± 1.26	15 ± 0.84*	14 ± 0.82*	15 ± 0.75	14 ± 0.75	13 ± 0.89*
HCO_3^- (mEq/l)	A	19 ± 1.52	14 ± 0.98	14 ± 1.26	17 ± 1.51*	20 ± 1.26*	21 ± 1.03*	19 ± 1.17	19 ± 0.89
	B	20 ± 1.79	14 ± 1.51	13 ± 1.51	14 ± 0.98	17 ± 1.03	17 ± 1.63	19 ± 1.03	20 ± 1.33
Blood pH	A	7.27 ± 0.1	6.87 ± 0.2	6.95 ± 0.1	6.95 ± 0.6	7.11 ± 0.4	7.10 ± 0.6	7.15 ± 0.2	7.14 ± 0.8
	B	7.32 ± 0.4	6.85 ± 0.1	6.85 ± 0.2	6.97 ± 0.5	7.21 ± 0.1*	7.25 ± 0.1*	7.28 ± 0.8*	7.31 ± 0.7*

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

Table 2: The results of serum electrolytes and mean arterial pressure before and after treatment in both the groups

Variables	Groups	Baseline	Shock	Time after treatment					
				30 min	60 min	3 hrs	6 hrs	12 hrs	24 hrs
Sodium (mEq/l)	A	139 ± 2.4	146 ± 3.2	144 ± 3.4	142 ± 2.9*	140 ± 2.7*	142 ± 2.8*	143 ± 3.1*	138 ± 2.7*
	B	138 ± 2.6	144 ± 2.6	146 ± 2.2	152 ± 2.9	154 ± 1.5	150 ± 0.9	146 ± 2.7	143 ± 3.3
Chloride (mEq/l)	A	106 ± 2.1	110 ± 1.6	112 ± 2.3	105 ± 1.8*	109 ± 1.6*	109 ± 1.5*	111 ± 1.6	107 ± 2.3
	B	108 ± 2.8	112 ± 2.5	118 ± 1.6	120 ± 2.7	119 ± 1.3	119 ± 2.1	117 ± 2.1	110 ± 3.1
Potassium (mEq/l)	A	3.8 ± 1.47	3.0 ± 0.63	2.8 ± 0.75	3.1 ± 0.50	3.3 ± 0.39	3.6 ± 0.23	3.8 ± 0.27	3.8 ± 0.10
	B	4.1 ± 0.52	3.1 ± 0.16	3.1 ± 0.23	3.0 ± 0.21	3.3 ± 0.24	3.5 ± 0.28	3.8 ± 0.34	4.0 ± 0.51
MAP (mm Hg)	A	118 ± 8.16	59 ± 8.1	67 ± 4.7	71 ± 2.4	79 ± 4.9	89 ± 6.3	96 ± 5.1	105 ± 2.7
	B	117 ± 7.58	54 ± 3.7	76 ± 3.8	84 ± 3.2*	92 ± 3.7*	99 ± 4.4*	109 ± 6.6*	116 ± 6.6*

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

Potassium

There was decreasing trend in the potassium ions (K^+) concentration during endotoxic shock. After administration of allotted fluids to the animals of both the groups, no significant differences were observed at each observational time in recovering the values towards baseline (Table 2). And potassium ions became normal within 24 hours in both the groups.

Haemodynamic parameters

The MAP was ≤60 mm Hg in all groups, as it was cut-off point for induction of endotoxic shock. After initiation of treatment, MAP rose quickly in both the groups but group B showed rapid increase throughout the study period and showed significant differences from group A (P<0.05) at each observational time from 60 minutes onward. Within 24 hours, MAP values became normal in group B but it was not so in group A (Table 2).

DISCUSSION

In the present study, increased body temperature, heart and respiration rates, haematocrit and haemoglobin concentrations and decreased mean arterial pressure indicated endotoxaemia in all the dogs and these findings are in agreement with other studies (Kreimeier *et al.*, 1991; Batmaz *et al.,* 2003). Mean arterial pressure of ≤60 mm Hg was accepted as typical hypotension after endotoxin administration (Cohen *et al.*, 1996).

The cornerstone of management of endotoxic shock is rapid fluid transfusion to maintain the circulating volume, to rapidly increase plasma volume and to restore cardiovascular function, thereby increasing cardiac output, oxygen delivery and tissue perfusion (Kreimeier *et al.*, 1991). Most regimens emphasize the importance of administering crystalloids and/or colloids as early shock therapy which results in increased vascular volume, mean arterial pressure and cardiac output (Luypaert *et al.*, 1986).

In the present study, increased values of haematocrit and Hb concentration from baseline values clearly indicated the presence of dehydration in the endotoxic shock which is supported by the previous studies (Cohen *et al.*, 1996; Batmaz *et al.*, 2003). After administration of the respective fluids, there was significant decrease in both the haematological values towards the baseline. Group B showed rapid fall in the values towards normal than group A which showed that plasma volume increased rapidly after administration of HSS, as reported previously (Velasco *et al.*, 1989; Constable *et al.*, 1991). This showed the efficacy of hypertonic saline solution to reverse the hypovolaemia developed in endotoxic shock.

The key feature for successful resuscitation of hypovolaemia or endotoxaemia in animals is the total amount of sodium (Constable, 1999). In endotoxaemia,

the sodium ions concentration increased. After initiation of the treatment, the increased sodium level in shocked condition started to decrease and reached baseline values within 24 hours. In group B, the sodium ions concentration increased maximally up to 154 mEq/l for a short interval and this value was not beyond the limits of hypernatraemia that is 160 mEq/l (Tyler *et al.*, 1994). So, we could suggest that infusion of HSS is safer and it does not cause hypernatraemia in endotoxic shock, although some animals might be at risk, as reported by other investigators (Ajito *et al.*, 1999). So, HSS showed good results to resuscitate the animals from endotoxic shock due to its small volume.

Mean arterial pressure is of great importance for the evaluation of circulatory function during hypovolaemia and endotoxaemia. Principally, rise in serum osmolality is thought to cause the major effect by extraction of water from the interstitium and expansion of the intravascular volume (Constable *et al.*, 1996). Fall in MAP during endotoxic shock clearly indicates decreased intravascular volume which ultimately decreased cardiac output (Kreimeier *et al.*, 1991; Constable *et al.*, 1996; Batmaz *et al.*, 2003). In the present study, administration of HSS in animals of group B increased MAP more rapidly than in animals of group A. This impulsive increase in MAP showed that hypertonic saline could efficiently increase intravascular volume more rapidly with a small volume when comparing to normal saline infusion. Similar observations were made by Velasco *et al.* (1989). However, Ajito *et al.* (1999) reported that MAP decreased immediately after HSS infusion.

Blood pH is an indicator of acidosis. In this study, decreased blood pH during endotoxic shock clearly indicated occurrence of acidosis to the animals. After infusion of respective fluids, blood pH increased but results were very satisfactory in animals of group B. In this group, blood pH increased rapidly to baseline within observing time but it was not seen in group A. These observations are in accordance to the previous studies (Kreimeier *et al.*, 1991; Constable *et al.*, 1996; Batmaz *et al.*, 2003). So, HSS had shown well affectivity against acidosis.

Body temperature either rises in endotoxaemia or it may fall. In the present study, there was increased body temperature in all the animals administered with *E. coli* endotoxins. This increase was sufficient to declare that animals were in the phase of endotoxic shock. After infusion of respective treatments to the animals, there was decrease in their body temperature but this trend was better in group B, showing the efficiency of hypertonic saline solution. Similarly, pulse and respiration rates also increased during endotoxic shock which returned to their baseline values within 24 hours in both the groups but group B showed better results.

Conclusion

Depicted from the results of this study, hypertonic saline (HSS) is a small-volume resuscitative solution and seems to be handy and practical method for the initial management of endotoxic shock compare to normal saline solution.

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