Effects of experimental proximal duodenal obstruction on clinical, hematological, serum and urine biochemical changes in sheep

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Summary

The objectives of this experiment were to determine clinical and clinicopathological changes after proximal duodenal obstruction in sheep. Experiments were carried out on seven Iranian crossbred male sheep aged between 2-2.5 years old and weighing 45-50 kg. All operative procedures were performed under general anesthesia by intravenous infusion of sodium thiopental. A polyethylene tube reinforced with braided cotton tape was surgically placed around the duodenum in 5 to 6 cm caudal to the pylorus. Experimental observations commenced two days before tightening the ligatures and inducing obstruction. Measurements at this stage were considered as the control values. Clinical (appetite, urination, defecation, ruminal contractions, temperature, respiratory and heart rates) and clinicopathological indices (hematological parameters, concentrations of sodium, chloride, potassium, calcium, magnesium, inorganic phosphorous, urea nitrogen, creatinine and acid-base status in serum and urine) were determined simultaneously at 12 hrs intervals for 72 hrs. At the end of the experiment the animals became lethargic, anorexic, weak and dehydrated. Finally, six sheep died and one sheep was slaughtered. There were significant differences ($P<0.05$) between temperature, respiratory and heart rates of the control and experimental values. Moreover, significant increases ($P<0.05$) in hematocrit, RBC and WBC counts, hypokalemia ($P<0.05$), hypochloremia ($P<0.05$) and metabolic alkalosis were seen in experimental sheep. Significant increases ($P<0.05$) were detectable in serum concentrations of inorganic phosphorous, urea nitrogen and creatinine. There were significant declines ($P<0.05$) in urinary concentrations of sodium, potassium, chloride, magnesium, inorganic phosphorous, urea nitrogen and creatinine as well as a significant decrease ($P<0.05$) in urinary pH. In conclusion, proximal duodenal obstruction in sheep leads to clinical and clinicopathological changes which are important for diagnosis.

Key words: Sheep, Duodenal obstruction, Hematological and biochemical changes

Introduction

Obstruction of the lumen of the small intestine with subsequent impairment to the flow of luminal content is a relatively common occurrence in animals (Radostits *et al*, 2000). Obstruction leads to dilatation of the bowel proximally and disrupts peristalsis (Burkitt *et al*., 1996). The manner of presentation depends on the level, completeness of obstruction and involvement in the lumen of the bowel, vascular compromise of the bowel wall, or both (Anderson, 1992).

A great number of information are available on the effects of intestinal obstruction in horses (Green and Tong, 1988; White, 1988; Moore and Morris, 1992; Yvorchuk *et al*., 1993). In ruminants, foreign bodies, enteroliths, partial displacement of the abomasum, (Whitlock, 1976; Escamilla *et al.*, 1995), intestinal adenocarcinoma (Cordes and Shortidge, 1971) and hair ball (Escamilla *et al.*, 1995) account for the majority of intestinal obstructions. There are a few reports on intestinal obstruction in sheep (Gingerich and Murdick, 1975; Purohit *et al*, 1984).

The present work aimed to induce experimental proximal duodenal obstruction in sheep to clarify the clinical, hematological and biochemical changes.

Materials and Methods

Seven Iranian crossbred rams aged 2-2.5

years old and weighing between 45-50 kg were used. They were all fed a ration, based on hay and barley. Water was offered *ad libitum*. The experimental animals were in good condition and clinically normal. Prior to the experiment, they were dewormed using albendazole (7.5mg/kg, orally).

Surgical techniques

After an overnight fasting, allowing water *ad libitum*, animals were anesthetized by intravenous injection of sodium thiopental (16mg/kg), with additional quantities given to maintain adequate surgical anesthesia. A polyethylene tube reinforced with braided cotton tape was surgically placed around the duodenum about 5 to 6 cm caudal to the pylorus. The ends of tubing were brought through the skin in such a manner that, at a later time, the ligature could be tightened and the duodenum could be occluded against the body wall (Gingerich and Murdick, 1975). The animals were kept under standard conditions and stabilized over one week. During this period, clinical and clinicopathological factors were considered to confirm the general health status of the animals. When it become apparent that the condition of each sheep had stabilized, as evidenced by the resumption of normal appetite, rumination, passage of feces, output of urine and stability of the blood gas reading, the duodenal ligatures were tightened to occlude the lumen.

Experimental procedures

Experimental observations commenced two days before tightening the ligatures. Measurements determined within the 48 hours preceding the occlusion of the duodenum were designated as control period measurements. The animals were kept under 12 hrs intervals observation to notice any clinical changes (appetite, urination, defecation, ruminal contraction). The temperature, respiratory and heart rates were measured. Blood and urine samples were obtained at 12 hrs intervals for 72 hrs after intestinal ligation. Samples for acid-base determinations were anerobically collected by jugular venipuncture into heparinized plastic syringes and analyzed within 10 minutes of collection. Blood samples for erythrocytes and leukocytes counts, PCV, as well as hemoglobin concentration were collected by jugular venipuncture into glass vials with Na_2EDTA anticoagulant. Blood samples for electrolytes (sodium, chloride, potassium, calcium, magnesium and inorganic phosphorous), urea nitrogen, creatinine and serum total protein concentrations were taken by jugular venipuncture into glass vials without anticoagulant and then blood samples were kept at room temperature for complete clotting and centrifuged for the separation of serum and stored at -20˚C. Simultaneously with blood sampling, urine samples were also obtained by manipulation of the penis. Urinary pH was initially measured with reagent strips (Whatman-BDH, England) after sampling and then urinary samples were frozen at -20˚C and stored for later analysis.

Analytical analysis

Serum and urine samples were analyzed for sodium, potassium and magnesium using atomic absorption spectrophotometry (Shimadzu AA-670, Shimadzu Corporation, Kyoto, Japan). The concentrations of serum and urine calcium, chloride, inorganic phosphorous, urea nitrogen and creatinine were analyzed, using colorimetric method (reaction with methyl-tymol blue), colorimetric thiocyanate method, ammonium molybdate method, colorimetric diacetyl monoxime method and colorimetric Jaffe's method respectively. Serum total protein concentration was analyzed by biuret method. Blood gas analysis as well as hemoglobin concentrations were determined by ABL^{TM}5 blood gas system (Radiometer Copenhagen, Denmark) (Burtis and Ashwood, 1999).

Statistical analysis

Results are expressed as Mean ± SE. The statistical significance of the measured data was determined by performing one-way analysis of variance from 48 hrs before obstruction to 72 hrs after obstruction.Whe n significant values were found, the Duncan test was preformed to determine where the differences occurred. Significance was

accepted at $p<0.05$ level.

Results

Clinical observations

During intestinal obstruction, the animals were characterized by lethargy, anorexia, generalized weakness, kicking at the belly, semi-crouching and frequent changes from standing to a recumbency position. In early stages of obstruction feces were voided, often more frequently than normal in small quantities of changed consistency. The feces then became increasingly scanty and hard. Finally, they passed no feces. The pattern of ruminal contractions and the ruminal contents after the onset of obstruction were extremely variable. Contractions were usually suppressed and were totally abolished. Urine output was decreased. The effects of duodenal obstruction on temperature, respiratory and heart rates are presented in Table 1. The heart rate and temperature increased significantly ($P<0.05$) by 12 and 24 hrs after obstruction respectively and continued to increase throughout the post-obstruction period. There was a significant difference ($P<0.05$) between the control and post-obstruction respiratory rates at 24 hrs after obstruction and continued to decrease throughout the post-obstruction period. In the end of the experiment (72 hrs after obstruction), six sheep died and one was slaughtered.

Clinicopathological changes

A significant increase in the mean values of hematological parameters (erythrocytes, leukocytes, neutrophils and lymphocytes counts), PCV and serum total protein were observed during obstruction (Table 2). Hemoglobin concentration did not change significantly, but there was a significant inverted neutrophil to lymphocyte ratio ($P<0.05$) after obstruction.

Metabolic alkalosis occurred abruptly in all of the animals after obstruction. The blood pH, bicarbonate concentration and the partial pressure of CO_2 increased significantly ($P<0.05$) by 24 hrs and reached peak values by 72 hrs after obstruction (Table 3). Serum chloride, potassium and calcium concentrations decreased significantly ($P<0.05$) by 24, 12 and 36 hrs after obstruction respectively and continued to decrease throughout the post-obstruction period. Concentrations of serum sodium and magnesium did not show significant changes. The serum concentrations of inorganic phosphorous, urea nitrogen and creatinine increased significantly ($P<0.05$) by 60, 48 and 12 hrs after obstruction respectively with the peak of concentrations, at 72 hrs after obstruction (Table 4).

Urinary pH decreased to 9 by 24 hrs after obstruction, but the changes were not statistically significant. Significant decline to less than 7 occurred as the experiment progressed. The urinary chloride concentrations also decreased significantly ($P<0.05$) by 12 hrs after obstruction and the decreased in each of the animals to near zero at 72 hrs after obstruction. A significant decline ($P<0.05$) in urinary concentrations of sodium, magnesium, inorganic phosphorous, urea nitrogen and creatinine occurred by 60, 60, 72, 60 and 48 hrs after obstruction, respectively ($P<0.05$). Urine calcium concentration was also measured, but significant changes were not found (Table 5).

Discussion

Clinical observations

The present study indicated that duodenal obstruction in sheep caused anorexia, rumen stasis, abdominal pain, decreased urine volume, tachycardia, fever and death. The main symptoms of duodenal obstruction were dehydration, absence of ruminal contractions and loss of defecation. The clinical symptoms might be due to bowel distension that results from intraluminal accumulation of gas, ingesta and fluids. This finding is in agreement with observations of Alexander and Hickson (1969) and King and Gerring (1989). Bowel distension and increases in intraluminal pressure have inhibitory effect on normal gastrointestinal function, causing a feedback loop in which the pain inhibits normal gut motility and absorption (Radostits *et al.*, 2000). The presence of dehydration, tachycardia and fever in this study were also

similar to those described by Reeves *et al*., (1991). It has been claimed that dehydration occurs as a result of movement of water from the intracellular to the extracellular fluid space and then from the extracellular fluid space to the lumen or wall of the obstructed intestine (Singer and Smith, 2002).

Clinicopathological changes

The clinicopathological changes indicated significant increases in the erythrocytes and leukocytes counts, hematocrit, serum total protein, urea nitrogen and creatinine concentrations. These conditions can be explained on the basis of reduction of the circulating blood volume and prerenal azotemia due to hypovolemia (Pearson and Pinsent, 1977; Radostits *et al.*, 2000).

In this study, significant increases in the blood pH, bicarbonate concentration and the PCO_2 were found to occur abruptly after obstruction. These observations could be explained by findings of Gingerich and Murdick (1975) and Kaneko (1989), who noticed that metabolic alkalosis results primarily from loss of HCl sequestered in the abomasum and secondarily from the effects of dehydration. Under normal condition, HCl moves out of the abomasum and into the intestinal tract to be buffered and reabsorbed (Hammond *et al*, 1964).

The reduction of serum potassium concentration from 12 hrs after obstruction up to the end of experiment was similar to those described by Whitlock *et al.,* (1977) in cattle and Gingerich and Murdick (1975) in sheep. Hypokalemia in proximal duodenal obstruction can result from sequestration of the cation in the bowel lumen, or continued urinary loss in the face of no potassium intake (Swenson, 1977). According to our results, serum concentrations of inorganic phosphorous, urea nitrogen and creatinine increased which may be due to dehydration and renal hypoperfusion (Radostitis *et al.*, 2000).

Results of the present study also showed that, in the face of systemic alkalosis, urinary pH decreased significantly in the animals. The observed paradoxical aciduria has been explained on the basis of electrolyte imbalances (Gingerich and Murdick, 1975). Depletion of the blood volume is a strong stimulus for renal conservation of Na, reabsorption of Na allows reabsorption of water (Anderson, 1992). To maintain electroneutrality, Na reabsorption must be accompanied by either reabsorption of an anion, usually Cl, or by the secretion of cation, usually H or to a lesser extent K (Cunningham, 2002). Under conditions of hypochloremia, hypokalemia and metabolic alkalosis, similar to what occurs in proximal duodenal obstruction, Na resorption will be increasingly accompanied by renal secretion of acid (Radostits *et al.*, 2000).

The data obtained from the present study showed that, urinary sodium concentration decreased 60 hours, after obstruction, whereas adequate serum sodium concentration was maintained. This indicates that increased renal resorption of sodium occurred at the same time that urinary pH decreased (Anderson, 1992).

The decrease in the urinary concentrations of sodium, magnesium, urea nitrogen and creatinine beyond the 6o hours after obstruction and potassium and inorganic phosphorous at the 72 hrs after obstruction may, in fact, represent the loss of ability of the kidney to eliminate them as a result of dehydration (Pearson and Pinsent, 1977; Singer and Smith, 2002).

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Table 1: Changes in vital signs before and after induction of surgical duodenal obstruction in sheep (Mean ± SE)

Time (hours)	-48	-36	-24	-12	0	12	24	36	48	60	72
Heart Rate (beat/min)	82 (±1.2)	81 (±1.1)	80 (±1.3)	80 (±2.0)	84 (±1.7)	95* (±1.4)	100* (±1.3)	106* (±2.1)	111* (±2.2)	111* (±2.9)	113* (±5.2)
Respiratory Rate (/min)	18 (±0.4)	17 (±0.6)	17 (±1.0)	17 (±1.7)	18 (±0.5)	17 (±0.2)	15* (±0.4)	13* (±0.7)	11* (±0.5)	10* (±0.4)	9* (±0.4)
Temperature(˚C)	39.11 (±0.05)	39.15 (±0.06)	39.10 (±0.04)	39.05 (±0.07)	39.11 (±0.03)	39.32 (±0.04)	39.58* (±0.03)	39.81* (±0.05)	40.01* (±0.11)	40.28* (±0.18)	40.20* (±0.20)

*Significantly different at (P<0.05)

Table 2: Changes in hematological parameters before and after induction of surgical duodenal obstruction in sheep (Mean ± SE)

Time (hours)	-48	-36	-24	-12	0	12	24	36	48	60	72
PCV (L/L)	0.34	0.34	0.34	0.33	0.35	0.37	0.45*	0.46*	0.45*	0.44*	0.45*
Total protein (g/L)	65.4 (±2.9)	65.2 (±2.6)	65.4 (±2.7)	65.0 (±2.9)	65.4 (±2.7)	70.2 (±2.7)	77.7* (±1.9)	82.1* (±1.8)	86.1* (±1.8)	89.2* (±2.0)	92.1 (±2.1)
Hb (g/L)	108.5 (±4.6)	108.5 (±5.0)	108.5 (±4.7)	107.7 (±4.5)	108.2 (±4.7)	108.7 (±4.5)	110 (±4.8)	110 (±0.45)	110.2 (±4.3)	110.4 (±4.5)	110.4 (±4.5)
RBC ($\times 10^{12}$/L)	10.44 (±0.53)	10.36 (±0.50)	10.28 (±0.53)	10.27 (±0.50)	10.35 (±0.48)	10.71 (±0.42)	11.04 (±0.40)	11.38 (± 0.41)	11.99 (±0.56)	12.58* (±0.70)	12.80* (±0.69)
WBC ($\times 10^{9}$/L)	9.25 (±0.63)	9.20 (±0.55)	9.27 (±0.58)	9.10 (±0.56)	9.01 (±0.64)	9.38 (±0.72)	10.47 (±0.64)	12.17* (±0.58)	13.38* (±0.57)	12.57* (±0.83)	8.31* (±1.09)
Lymphocytes ($\times 10^{9}$/L)	6.42 (±0.42)	6.44 (±0.34)	6.45 (±0.38)	6.48 (±0.35)	6.08 (±0.43)	5.12 (±0.34)	4.92 (±0.63)	3.87* (±0.29)	3.64* (±0.35)	3.17* (±0.31)	2.12* (±0.28)
Neutrophils ($\times 10^{9}$/L)	2.78 (±0.30)	2.75 (±0.24)	2.81 (±0.27)	2.60 (±0.26)	2.92 (±0.31)	4.22 (±0.45)	5.50* (±0.17)	8.27* (±0.61)	9.67* (±0.66)	9.39* (±0.70)	6.18* (±0.83)

*Significantly different at (P<0.05)

Table 3: Changes in acid-base status before and after induction of surgical duodenal obstruction in sheep (Mean ± SE)

Time (hours)	-48	-36	-24	-12	0	12	24	36	48	60	72
PH (venous)	7.39 (±0.005)	7.39 (±0.004)	7.39 (±0.004)	7.39 (±0.003)	7.39 (±0.004)	7.41* (±0.004)	7.45* (±0.005)	7.50* (±0.01)	7.52* (±0.01)	7.54* (±0.006)	7.55* (±0.002)
PCO_2 (kPa)	5.14 (±0.05)	5.16 (±0.03)	5.16 (±0.01)	5.12 (±0.02)	5.2 (±0.03)	5.43* (±0.04)	5.62* (±0.04)	5.81* (±0.03)	6.06* (±0.05)	6.21* (±0.03)	6.55* (±0.03)
Bicarbonate (mmol/L)	20.57 (±0.52)	20.14 (±0.45)	20.57 (±0.36)	20.28 (±0.47)	20.85 (±0.59)	23.57* (±0.89)	27.28* (±0.91)	31.14* (±0.82)	34.85* (±0.91)	37.42* (±0.64)	39.42* (±0.48)

*Significantly different at (P<0.05)

Table 4: Changes in biochemical parameters before and after induction of surgical duodenal obstruction in sheep (Mean ± SE)

Time (hours)	-48	-36	-24	-12	0	12	24	36	48	60	72
Chloride (mmol/L)	100.21 (±1.13)	100.32 (±1.35)	100.01 (±1.09)	99.08 (±1.33)	98.84 (±0.57)	96.67 (±0.96)	92.84* (±1.04)	88.64* (±0.76)	85.87* (±1.11)	81.92* (±1.58)	79.10* (±0.51)
Sodium (mmol/L)	149.70 (±1.50)	149.07 (±1.84)	148.80 (±1.40)	148.21 (±1.17)	148.80 (±1.52)	146.24 (±1.39)	145.18 (±1.55)	145.20 (±1.83)	146.30 (±1.56)	146.95 (±1.53)	147.35 (±1.59)
Potassium (mmol/L)	5.21 (±0.12)	4.95 (±0.12)	5.04 (±0.18)	4.87 (±0.21)	4.85 (±0.06)	4.38* (±0.05)	4.28* (±0.07)	4.15* (±0.12)	3.90* (±0.11)	3.68* (±0.05)	3.58* (±0.04)
Calcium (mmol/L)	3.06 (±0.20)	3.07 (±0.19)	3.04 (±0.25)	3.01 (±0.28)	3.05 (±0.19)	2.95 (±0.30)	2.86 (±0.30)	2.80* (±0.28)	2.76* (±0.26)	2.73* (±0.27)	2.68* (±0.23)
Magnesium (mmol/L)	1.08 (±0.06)	1.05 (±0.06)	1.08 (±0.09)	1.07 (±0.07)	1.03 (±0.08)	0.94 (±0.07)	0.90 (±0.07)	1.00 (±0.07)	1.06 (±0.07)	1.13 (±0.06)	1.16 (±0.08)
Phosphorus (mmol/L)	1.92 (±0.24)	1.89 (±0.2)	1.93 (±0.32)	1.90 (±0.27)	1.94 (±0.23)	1.85 (±0.23)	1.82 (±0.28)	1.97 (±0.24)	2.08 (±0.25)	2.15 (±0.26)	2.24* (±0.20)
Urea nitrogen (mmol/L)	1.39 (±0.07)	1.40 (±0.06)	1.37 (±0.07)	1.37 (±0.11)	1.41 (±0.06)	1.43 (±0.06)	1.54 (±0.22)	1.72 (±0.24)	3.61* (±1.04)	7.25* (±0.42)	7.68* (±0.43)
Creatinine (µmol/L)	94.58 (±3.53)	99.00 (±3.52)	98.12 (±4.42)	97.24 (±5.03)	94.58 (±3.53)	114.92* (±4.42)	136.13* (±4.42)	151.16* (±4.42)	159.12* (±3.53)	172.38* (±3.53)	175.03* (±2.65)

*Significantly different at (P<0.05)

Table 5: Changes in urine analysis before and after surgical induction of duodenal obstruction in sheep (Mean ± SE)

Time (hours)	-48	-36	-24	-12	0	12	24	36	48	60	72
PH	9.42 (±0.20)	9.57 (±0.20)	9.71 (±0.18)	9.71 (±0.18)	9.57 (±0.20)	9.57 (±0.20)	9.00 (±0.30)	8.42* (±0.20)	8.14* (±0.26)	7.57* (±0.29)	6.95* (±0.18)
Chloride (mmol/L)	27.75 (±3.19)	29.44 (±2.47)	30.32 (±2.80)	30.58 (±3.21)	28.70 (±2.32)	18.70* (±2.41)	7.34* (±1.20)	2.97* (±0.61)	0.48* (±0.33)	0.21* (±0.21)	0.00*
Sodium (mmol/L)	33.14 (±1.9)	32.61 (±1.75)	32.55 (±1.64)	31.81 (±2.12)	32.72 (±1.70)	34.02 (±1.62)	31.61 (±1.85)	30.70 (±1.33)	28.18 (±1.17)	22.18* (±1.39)	16.47* (±1.08)
Potassium (mmol/L)	129.18 (±2.59)	129.14 (±2.42)	129.14 (±1.79)	129.05 (±1.57)	129.71 (±1.89)	129.14 (±3.26)	129.95 (±2.19)	129.02 (±1.42)	126.68 (±1.69)	122.32 (±1.84)	118.64* (±1.94)
Calcium (mmol/L)	4.77 (±0.14)	4.4 (±0.12)	4.50 (±0.16)	4.36 (±0.16)	4.55 (±0.15)	4.63 (±0.09)	4.49 (±0.06)	4.47 (±0.08)	4.38 (±0.08)	4.37 (±0.13)	4.33 (±0.19)
Magnesium (mmol/L)	11.22 (±0.24)	11.31 (±0.23)	11.13 (±0.23)	11.14 (±0.21)	11.22 (±0.25)	10.75 (±0.33)	11.30 (±0.30)	11.27 (±0.26)	10.78 (±0.27)	10.03* (±0.28)	9.38* (±0.28)
Phosphorus (mmol/L)	6.20 (±0.14)	6.50 (±0.26)	6.46 (±0.28)	6.22 (±0.11)	6.41 (±0.12)	6.27 (±0.15)	6.52 (±0.18)	6.37 (±0.28)	6.02 (±0.10)	5.74 (±0.12)	5.50* (±0.11)
Urea nitrogen (mmol/L)	69.68 (±0.11)	69.78 (±0.18)	69.90 (±0.27)	69.58 (±0.09)	69.61 (±0.09)	69.97 (±0.22)	70.27 (±0.24)	70.35 (±0.32)	69.72 (±0.56)	61.32* (±0.24)	63.47* (±0.17)
Creatinine (µmol/L)	2834.98 (±15.02)	2880.07 (±28.28)	2650.23 (±24.44)	2870.34 (±20.33)	2908.36 (±23.86)	2758.08 (±57.46)	2662.60 (±48.62)	2579.51 (±56.57)	2484* (±42.43)	2408.9* (±32.70)	2362.04* (±22.98)

*Significantly different at ($P<0.05$)