Effects of experimental hyper- and hypocalcemia on bile flow and composition in sheep

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Summary

Experiments were carried out on sixteen Iranian cross-bred sheep aged between 2-2.5 years old, with body weight between 35-45 kg. All operative procedures were performed under anaesthesia by intra-jugular infusion of sodium thiopental. One cannula into the proximal duodenum and the second into gallbladder were inserted. Experiments commenced approximately 10 days after surgical preparations. Bile and blood samples were obtained simultaneously at one-hour intervals for six hours. Bile flow and concentrations of sodium, potassium, chloride, calcium, magnesium, cholesterol, bilirubins, total solids of bile and calcium of serum were measured. Bile flow and composition as well as calcium of serum in this stage were considered as control group. Hypercalcemia was induced by intra-jugular administration of calcium borogluconate. Blood and bile samples were obtained using the same procedure as control group. After 10 days, hypocalcemia was induced by intra-jugular administration of Na2EDTA. Blood and bile samples were obtained using the same procedure as control group. The results indicated that, bile flow decreased significantly in hypercalcemic group (P<0.05), but did not change significantly in hypocalcemic group. The concentrations of biliary calcium, total bilirubin, indirect bilirubin and total solids increased significantly in hypercalcemic group (P<0.05). During hypocalcemia the concentration of biliary potassium decreased significantly (P<0.05). Bile flow and biliary calcium decreased respectively and increased significantly more in male than in female sheep (P<0.05). In conclusion hypercalcemia decreases bile flow and increases biliary calcium, but hypocalcemia did not change bile flow and biliary calcium.

Keywords: Sheep, Bile flow, Bile composition, Hypocalcemia, Hypercalcemia

Introduction

Calcium is one of the abundant mineral elements in the animal body and plays a vital role in many of life’s processes. Since serum calcium concentration is normally maintained within a relatively narrow range (Smith, 2002), therefore changes in serum calcium concentration can cause various disorders. Some of these disorders include reduction of the motility of the ruminant stomach (Huber et al., 1981), hypotension, hyperhaemoglobinemina, increasing of the serum cortisole concentration in calves (Desmecht et al., 1996), reduction of blood flow to ovaries in sheep (Jonsson and Daniel, 1997), polyuria, polydipsia (Kaneko, 1989), decreased atrial activity and increased ventricular activity (Littlledike et al., 1976). It is thought that bile formation and bile flow are dependent processes (Blitzer and Boyer, 1982). Evidence also suggests that hypercalcemia decreases bile flow and increases biliary calcium in carnivores (Layer et al., 1986; Lillemoe et al., 1988; Magnuson et al., 1989; Ahrendt et al., 1995). Moreover, calcium plays a critical role in the formation of most, if not all, gallstones (Moore, 1984). There are many reports about prevalence of gallstones in carnivores and ruminants (Petruzzi et al., 1988; Cavallini et al., 1991). Despite the significance of the calcium ion in gallstones pathogenesis, the complex interrelationships between biliary, serum calcium and bile flow remain incompletely defined in ruminants. In the present study we
examined the effects of hyper- and hypocalcemia on bile flow and composition in sheep.

**Materials and Methods**

Sixteen (8 males and 8 females) Iranian crossbred sheep aged between 2-2.5 years old, with body weights between 35-45 kg were used. They were all fed a ration, based on hay and barley. Water was offered ad libitum. They were in good condition and clinically normal. Prior to the experiment, they were dewormed using albendazole (7mg/kg, orally).

**Surgical techniques**

After an overnight fast, allowing water ad libitum, animals were anaesthetized by 16 mg/kg intravenous sodium thiopental, with additional quantities given to maintain adequate surgical anaesthesia. Bile duct was tightly ligated distal to the junction of cystic and hepatic duct but proximal to the entrance of the pancreatic duct and cholecystotomy was performed. One cannula in the gallbladder and a second in the proximal of duodenum were inserted. Details of the cannula used and technique for insertion have already been described (Mostaghni and Howard, 1975; Mostaghni, 1985). To establish enterohepatic circulation, bile was returned to the animal via duodenum slowly. Animals were kept under standard conditions and stabilized over one week. During this period, clinical and paraclinical factors were considered to confirm the general health status of the animals.

**Experimental procedures**

Experiments commenced approximately 10 days after surgical preparation. Serum samples were obtained hourly for Six hours through the jugular vein. Bile was also collected hourly for six hours. After measuring the volume of secreted bile, sufficient quantity was taken for analysis and the remaining bile was pooled and kept separately, which was returned to the animal via duodenum slowly at the end of experimental period. The samples of bile were quickly frozen at -20°C until analyzed. Bile flow and composition as well as calcium of serum in this stage were considered as control group. Hypercalcemia was induced by intravenous infusion of 40% calcium borogluconate at dose rate of 0.005 mmole/kg/min (Littledit *et al.*, 1976; Ahrendt *et al.*, 1995). During hypercalcemia, serum and bile samples were collected using the same procedure as control group. After 10 days, hypocalcemia was induced by the infusion of 4.7% Na₂EDTA via a catheterized jugular vein at dose rate of 0.2 mmole/kg/min. For IV injection, Na₂EDTA solution was adjusted to PH 7.0 with 6N NaOH (Huber *et al.*, 1981; Desmecht *et al.*, 1995; Desmecht *et al.*, 1996; Jonsson and Daniel, 1997). During hypocalcemia, serum and bile samples were obtained using the same procedure as above.

**Analytical methods**

Bile samples were analyzed for calcium and magnesium by using atomic absorption spectrophotometry (Shimadzoo AA-670, Shimadzoo Corporation, Kyoto, Japan), sodium and potassium by the flame photometric method (Flame photometer FLM2, Bach-Simpson. Ltd, Ontario, Canada), cholesterol by Lieberman-Burchard method, chloride by the colorimetric thiocyanate method, bilirubins by the colorimetric method (modified Vandenbergs), total solids by evaporation of 1 ml portions of bile at 105°C. Serum calcium by atomic absorption spectrophotometer (Burtis and Ashwood, 1999).

**Statistical analysis**

Results are expressed as mean±SE. Statistical analysis of bile flow and composition data between groups were assessed by paired t-test. Differences of bile flow and composition data between male and female sheep in different groups were assessed by independent t-test. Significance was accepted at p<0.05 level.

**Results**

The effects of hyper- and hypocalcemia on bile flow, concentrations of biliary and serum calcium in male and female sheep and bile composition are presented in Table 1 and 2. Average rate of bile secretion during normocalcemia was 13.01±0.45 ml/hour, with an average total solids of 54.99±3.11 mg/ml. During hypercalcemia, clinical signs
including polyuria, frequent defecation, bradycardia and increased heart contractility were seen. Hypercalcemia caused a significant (P<0.05) decrease in bile flow. Concentrations of the biliary calcium, total bilirubin, Indirect bilirubin and total solids increased significantly (P<0.05). Concentrations of biliary sodium, potassium, chloride, cholesterol, magnesium and direct bilirubin remained unchanged. During hypocalcemia, clinical signs including tachycardia, decreased cardiac contractility, tremor and reduction of ruminal contraction were observed. Bile flow did not change significantly. Concentration of biliary potassium decreased significantly (P<0.05), but the concentrations of biliary sodium, chloride, calcium, magnesium and bilirubins did not show significant changes. Bile flow and composition between male and female sheep in normo and hypocalcemic animals did not show significant differences. In hypercalcemic animals, bile flow decreased significantly (P<0.05) more in male than in female sheep, but biliary calcium increased significantly (P<0.05) more in male than in female sheep. Other compositions of bile did not show any significant differences between male and female sheep.

Discussion

Effects of hypercalcemia

The results of the present study showed that bile flow decreased significantly in hypercalcemic animals which is in agreement with the observations of Ahrendt et al., (1995) and layer et al., (1986) in carnivores. Increased biliary calcium in hypercalcemic animals were also similar to those described by Lillemoe et al., (1988) and Ahrendt et al., (1995). It has been claimed that increased biliary calcium may probably be due to passive entering of calcium from blood to bile across paracellular channels (Cummings and Hofman, 1984; Knym et al., 1989; Rege et al., 1990). Decreased bile flow in Hypercalcemic animals may be due to calcium dependent processes of bile formation and Bile flow (Blitzer and Boyer, 1982). Bile flow is also dependent on osmotic pressure of bile composition (Moore et al., 1986). Moreover the osmotic pressure of bile is strongly dependent on biliary calcium (Roda et al., 1983; Van der meer et al., 1988). High affinity binding of biliary calcium to other compositions of bile reduce osmotic pressure which decreases bile flow (Moore and Sanyal, 1989; Ahrendt et al., 1995). In addition Chenderovitch et al., (1963) and Guzelia and Boyer, (1974) reported that acutely increased plasma osmolarity by infusing 40% calcium borogluconate (hypertonic Solution) inhibits bile secretion in man. Observation of Polyuria in hypercalcemic animals in present study also could be explained by inhibiting antidiuretic hormone and hypertention (Kaneko, 1989). Although the hypercalcemia seems to increase blood pressure and vagal efferenct activity that may lead to increasing bile flow (Littledike et al., 1976; Mostaghi, 1985), according to our results it appears that the causes of reduction of bile flow are more effective than causes of increasing of bile flow. The results of this study also showed that the concentrations of the total and indirect bilirubin and total solids of bile increased significantly in hypercalcemic animals. To our knowledge there is no report on the effects of hypercalcemia on bile composition in relation to bile flow. Biliary calcium is of great importance in the pathogenesis of gallstones (Rege et al., 1990). Cavallini et al., (1991) believed that sheep with high total bilirubin concentration in bile, have high frequency of gallstones. Moreover study from patients with gallstones have shown an excess concentration of indirect bilirubin in most cases (Moore et al., 1982). Therefore it can be suggested that increased indirect bilirubin and biliary calcium in hypercalcemic animals of our study may increase likelihood of the formation of gallstones.

Effects of hypocalcemia

The results of the present study showed that bile flow as well as biliary calcium did not change significantly in hypocalcemic animals. To our knowledge there is no report on the effects of hypocalcemia on bile flow and composition in animals. During hypocalcemia clinical signs including tachycardia and cool extremities were observed, which may be due to reduction of cardiac output and hypotention (Jonsson and Daniel, 1997). Reduction of cardiac output
and hypotension in hypocalcemic animals may prevent bile flow to increase. Moreover non-significant reduction of biliary calcium in hypocalcemic animals, causes bile secretion to remain unchanged. The results of this study indicated that the concentration of biliary potassium decreased in hypocalcemic animals. With regard to passive entering of potassium from plasma to bile (Rege et al., 1990), decreased biliary potassium may result from reduction of potassium in plasma. This reduction may be attributed to alkalosis, which is induced by stasis of digestive tract in hypocalcemic animals (Allen and Roussel, 1999).

Comparison of bile flow and composition between male and female sheep

The findings of the present study in hypercalcemic animals showed that, bile flow decreased more in male than in female sheep, probably due to more increase biliary calcium concentration in males. Bile flow and biliary calcium did not show any differences between male and female sheep in normo and hypocalcemic animals. Petruzzi et al., (1988) demonstrated that the prevalences of gallstones is greater in male than in female sheep. It seems that, male sheep are more sensitive to increased biliary calcium and decreased bile flow in hypercalcemic state. In conclusion hypercalcemia decreases bile flow and increases biliary calcium concentration, but hypocalcemia did not change bile flow and biliary calcium.

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References

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