THE EFFECT OF ACUTE HYPERVITAMINOSIS A ON SUBMANDIBULAR GLANDS FUNCTION

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Abstract — Pure submandibular saliva was collected from anaesthetized vitamin - A - treated and control rats with using of pilocarpine as secretagogue, and then the serum was collected by cardiac puncutre. Intraperitoneal injection of a large single dose (100,000 IU/kg) of vitamin A changed total protein, calcium and sodium concentrations of saliva so that sodium level of submandibular saliva was reduced but total protein and calcium were found to be elevated significantly in rats. The levels of sodium and phosphate in serum of vitamin-A-treated animals were more higher than control values. In conclusion, if animal experiments can confirm in human, one can identify hypervitaminosis A by measuring protein and electrolytes of saliva.

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INTRODUCTION

Vitamin A exerts several important functions in relation to normal morphology and cell biology of epithelial tissues, e.g. protein synthesis and membrane metabolism (1,2). Since vitamin A can serve as a carrier of carbohydrate moiety to proteins, thus it affects the biosynthesis of glycoproteins (2,3) and it is previously reported that secretion of retinal and protein by the rabbit lacrimal gland appear to be closely related (4). Also there is evidence that, the short term vitamin A deficiency redue tissue protein and glycogen contents of rat(5). In addition Shilina (3) suggested that, in rats with vitamin A deficiency, the level of alpha 1 and alpha 2 glycoprotein were decreased simultaneously in blood plasma as a result of possible impairment of protein glycosylation in liver tissue.It has also been shown that the inhibition and activation of the synthesis of 286 and 101 proteins respectively, two hours after oral refeeding retinyl acetate as the source of retinol to retinol deficient rats (6). In relation to mineral metabolism, vitamin A toxicity causes alteration of bone and mineral metabolism (7,8).It has been reported that retinol can stimulate bone and cartilage resorption (7,9,10,11). Another study indicated that in animals with vitamin A toxicity, the alterations of serum magnesium level was unmarkable, but the serum concentration of phosphate was significantly higher than control values (12) and in lacrimal gland, the ionic composition of tears and lacrimal gland fluid of vitamin A-deficient rabbits showed that calcium levels in tears and lacrimal gland fluid were decreased (13). Hypervitaminosis A induces decrease of sodium excretion in rat kidney.(14). Vitamin A exist in salivary and lacrimal glands (4). Submandibular salivary gland and plasma vitamin A levels in rat are increased by feeding high levels of retinyl palmitate (15). With respect to these finding, we got interested to examine the effects of acute hypervitaminosis A on the secretion of fluids, electrolytes and total protein in rat submandibular saliva and serum.

MATERIALS AND METHODS

Chemicals

Dry vitamin A acetate was obtained from Roche. Vitamin A was prepared in 0.9% sodium chloride immediately before use. Phosphate kit was prepared from Zist Shimi Company (Iran). All other chemicals were purchased from Sigma (England).

Animals

Adult male albino rats weighing 200-250g were used. The rats had free access to a stock laboratory diet and water adlibitum. Following several days of acclimatization the rats were randomly distributed into experimental and control groups. They were caged in an environment at 21-24°C with a light/dark cycle of 14-10-hr starting at 06 AM. The experimental group were injected intraperitoneal (IP) with 100,000 IU/kg of vitamin A (retinol acetate) and the control animals injected with a volume of 0.9% sodium chloride solution equal to that used for drug administration. The recommended intake of vitamin A in rats is 100 IU/day and acute hypervitaminosis A may occure after ingestion over 100 times the recommended daily allowance (16).

Methods

The rats were treated by vitamin A or saline, 2 hours (6) before saliva collection. The animals were anaesthetized by i.p injection of sodium secobarbital (50mg/kg), secured in a supine position with tape and trachaeotomized to facillate respiration during experiment. Both submandibular ducts were cannulated

intra orally with polyethylene tubes (PE 10) by the method of Yoshida (17). All dissections were performed with the aid of dissecting microscope. As secretory stimuli pilocarpine nitrate (8 mg/kg) was dissolved in deionized water and administered i.p. with the exception of the initial two drops which were discarded, saliva was collected for 30 minute into per weighed stoppered microtubes kept in ice and stored at - 20°c for subsequent determination of electrolytes. After saliva collection, blood was collected by cardiac puncture and was then centrifuged after clotting to obtain serum. the right and left submandibular glands were dissected free of connective tissue and were weight.

Assays

Flow rates were expressed as milliliter of saliva per minute (ml/min) and microliter of saliva per minute per gram wet weight of gland and microliter of saliva per minute per kilogram of body weight. The volume of saliva secreted were estimated by weight, assuming the specific gravity of 1.0 (18). Total protein and phosphate were analyzed immediately after collection saliva and serum. The concentration of protein was determined by the lowry procedure (19). Method for phsophorus determination were based on, molybdate react with phosphate to form various heteropoly compounds and photometric measurement of the molybdenum blue formed by reduction of phosphomolybdate (20). That were done with kits manufactured by Zist Chimi Company (Iran). Calcium, potassium, sodium and magnesium concentration were determined by atomic absorption spectrophotometry (21).

Statistical Analysis

The data were statistically evaluated by using student's t-test. Differences were considered significant when ($P<0.05$). All values are mean ± SE for the pooled 30 minute collection.

RESULTS

Electrolytes levels, total protein and flow rate of submandibular saliva are shown in Table 1. Hypervitaminosis A increased calcium ($P<0.02$), total protein concentration ($P<0.05$) and reduced sodium ($P<0.01$) of submandibular saliva. The concentrations of total protein and electrolytes of serum are shown in Tabel 2. In serum, hyperviatminosis A caused a significant increase in sodium ($P<0.05$) and phosphate concentrations ($P<0.05$) when compared with control.

Table 1. Effects of acute hypervitaminosis A on submandibular saliva composition

	control	n	vitamin A 100,000 Iu/kg	n
Total protein (mg/100ml)	124.62 ± 25	6	208.44 ± 21.37*	8
Calcium (mg/100ml)	4.758 ± 0.332	7	6.746 ± 0.576**	8
Sodium (mg/100ml)	32.068 ± 4.142	7	16.499 ± 2.455***	8
Potassium (mg/100ml)	187.721 ± 16.653	6	200.664 ± 18.371	9
Magnesium (mg/100ml)	1.4142 ± 0.2071	7	1.8387 ± 0.1451	9
Phosphate (mg/100ml)	0.837 ± 0.142	7	0.811 ± 0.085	10
Flow rate (ml/min)	0.019 ± 0.00476	7	0.024 ± 0.00583	11
Flow rate (µlit/min./kg of body weight)	81.557 ± 20.627	7	81.015 ± 9.315	10
Flow rate (µlit/min./kg of gland weight)	51.49 ± 11.901	7	54.475 ± 6.992	10

n = the number of observation

* P < 0.05 ; ** P < 0.02 ; *** P < 0.01

Table 2. Effects of acute hypervitaminosis A on serum composition

	control	n	vitamin A 100,000 Iu/kg	n
Total protein (g/100ml)	7.76 ± 0.82	6	9.258 ± 0.94	6
Calcium (mg/100ml)	9.922 ± 0.602	9	0.304 ± 0.528	9
Sodium (mg/100ml)	281.292 ± 37.171	5	438.266 ± 49. 884*	6
Potassium (mg/100ml)	16.180 ± 1.021	7	21.229 ± 2.632	10
Magnesium (mg/100ml)	1.803 ± 0.140	8	2.1409 ± 0.284	10
Phosphate (mg/100ml)	6.82 ± 0.238	7	7.698 ± 0.284*	8

n = the number of observation ; * P < 0.05

DISCUSSION

Vitamin A toxicity is a well known cause of hypercalcemia (8,22). However hypercalcemia is not always observed in hypervitaminosis A in experimental animals (12). In the present study, hypervitaminosis A did not cause hypercalcemia. Since ionized calcium and albumin levels were not measured, therefore, the possibility that an increase in total calcium has been masked by a concomitant decrease in albumin resulting from vitamin A - induced liver toxicity can not be ruled out (12). In this study, the salivary calcium concentration was significantly elevated. Also previous findings showed that, in vitamin A - deficient rabbits, calcium levels in tears and lacrimal gland fluid was decreased (13) and in hypervitaminosis A, we observed increased calcium in saliva.

In relation to protein it has been shown that two hours after refeeding retinyl acetate, synthesis of proteins was activated (6). Short term vitamin A deficiency reduce tissue protein and glycogen contents (5). There is also evidence that in rats with vitamin A deficiency, the level of some glycoproteins was decreased simultaneously in blood plasma (3). Our results showed that total protein of submandibular saliva is elevated in treated group. Since the most secretory proteins in saliva are glycoproteins (23), that are synthesized by striated duct cell in salivary gland (23), the elevation of total protein of submandibular saliva may be related to increase of the biosynthesis of glycoproteins induced by vitamin A. Vitamin A can serve as a carrier of the carbohydrate moiety to proteins (2,24).

In our experiments salivary sodium level decreased and serum sodium elevated. It is previously reported that in hypervitaminosis A, sodium excretion is decreased in urine (14) and the structure of the striated duct cells in salivary glands is typical of tissue involved in water and electrolyte transport such as the kidney tubules (23). So it is possible that the hypervitaminosis A cause increase reabsorption of sodium in submandibular glands and kidney tubules.

Our findings show that, serum phosphate concentration was elevated. Hyperphosphatemia occure in hypervitaminosis A(12,22). and this may be related to bone resorption.

In conclusion, if animal experiments can confirm in human studies, one can identify hypervitaminosis A by measuring protein and electrolytes of saliva.

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