The influence of chronic verapamil treatment on calcium absorption and homeostasis in the geriatric rat

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It has been demonstrated that verapamil produces a significant reduction in calcium transport in rat everted gut sacs in vitro. The purpose of the present study was to determine the effect of oral verapamil treatment on calcium absorption and homeostasis in vivo in the geriatric rat. Verapamil was administered (either oral or parenteral) to groups of 12-month-old female rats at a moderate (5 mg/kg) to large (15 mg/kg) dose over a period of 8 weeks. At the end of the 8-week treatment period, calcium transport was examined in duodenal segments and femoral bone was removed to measure bone density and mechanical strength. Blood levels of verapamil as measured by high pressure liquid chromatography were consistent with the administered dose. The results of this study indicate that chronic verapamil treatment at a dose of 15 mg/kg caused an increase in calcium transport, a reduction in calcium uptake into duodenal tissue, and an increase in serum and urinary calcium. Therefore, these results support the concept that chronic oral and/or parenteral use of calcium channel blocking agents may alter calcium homeostasis in the geriatric patient. (J. Nutr. Biochem. 5:547–550, 1994.)

Keywords: verapamil; Ca homeostasis; Ca retention; intestinal transport; bone

Introduction

Calcium (Ca) channel blocking agents are used therapeutically in the treatment of cardiac disorders such as myocardial ischemia,[1] cardiac arrhythmias,[2,3] and hypertension,[4,5] which occur most often in the geriatric segment of the population. These agents act primarily by binding to specific receptor sites within the membrane Ca channel, resulting in an inhibition of the slow inward Ca current in cardiac and smooth muscle and the cardiac conductile system,[6–8] and possibly by inhibition of a slow sodium current.[9]

In addition to their action on cardiovascular and contractile tissues, Ca channel blocking agents have been reported to reduce Ca translocation in a variety of other biologic tissues,[10,11] including the intestine.[12,13] In a previous study we examined the influence of verapamil on Ca transport in isolated segments of rat intestine and Ca uptake into isolated enterocytes.[13,14] Our results indicate that verapamil (1 mM) produced a significant reduction in Ca transport and Ca uptake in duodenal and jejunal segments[13] and reduced Ca uptake in isolated enterocytes at a concentration of 0.3 mM.[14] Similar in vitro results with verapamil have been reported by others in rat duodenum,[12,15] rat cecum,[16] duodenal loops,[17] organ cultured chick duodenum,[18] and isolated brush border vesicles.[19]

Chronic oral administration of verapamil in young rats at a dose within the therapeutic dose range causes an increase in parathyroid hormone (PTH) secretion, a decreased level of 1,25-$[OH]_2$ vitamin D_3, and an unexpected increase in duodenal Ca absorption.[20,21] Because the rat has been reported to be a good model for predicting changes in Ca homeostasis in the human,[22] the results of these in vivo and in vitro studies suggest that chronic use of these drugs may alter Ca homeostasis. Accordingly, a clinical study has reported that a Ca channel blocking drug decreased Ca balance in hypertensive patients,[23] while others have not observed this effect.[24] Taken together, these results suggest that chronic use of these drugs in the geriatric patient population may place this group at greater risk of developing Ca metabolic disorders. Therefore, the purpose of the present study was to evaluate the influence of chronic (8-week) oral and paren-

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teral verapamil administration on Ca absorption and homeostasis in the geriatric female rat.

Methods and materials

Animals

These studies were conducted over an 8-week verapamil-treatment period in which 12-month-old female Sprague-Dawley rats were used. The 12-month-old rat was used in this study to examine the influence of chronic verapamil treatment on Ca homeostasis in a geriatric animal model.

Following delivery, the animals were given a 5-day acclimation period and randomly distributed into experimental groups containing six rats each. The rats were housed individually in stainless-steel metabolic cages in a controlled environment with a 12 hr light-dark cycle. An oral dose of vitamin D (200 units) was administered to all animals 2 days before each experiment to optimize Ca transport activity under the experimental conditions used in this study as previously reported.[25] All animal weights were recorded at the beginning of each experiment and at weekly intervals thereafter. The rats were allowed to eat food (Purina Lab Chow; Ralston Purina, St. Louis, MO USA) and water ad libitum, which was removed 18 hr before each transport study.

Experimental design

Verapamil was administered orally in a phosphate buffer containing 140 mM NaCl, 4.75 mM KCl, 1.5 mM $NaH_2PO_4 \cdot H_2O$, and 2.5 mM Na_2HPO_4. This solution was heated to 40° C to maintain drug solubility. Verapamil administered parenterally (s.c.) was solubilized in a solution of 60% sesame oil, 20% ethyl alcohol, and 20% DMSO. In each experiment the animals were divided into three experimental groups (group I, control; group II, verapamil 5 mg/kg; group III, verapamil 15 mg/kg). The verapamil doses in groups II and III represent a normal and large therapeutic dose, respectively, for the treatment of cardiovascular disorders in humans.[26]

Each 8-week balance study was divided into 7-day periods during which animals were weighed weekly. During the last 7-day period the urine was collected daily and pooled for each individual rat. The urine was acidified with HCl and refrigerated. The 7-day pooled urine samples were prepared for Ca determination. At the conclusion of the 8-week balance study, the rats were fasted overnight, bled by cardiac puncture under light ether anesthesia for serum Ca determination, and then euthanized. The right femurs were removed for mineral density and mechanical strength determination and the duodenum removed and used immediately for in vitro Ca transport and Ca uptake study.

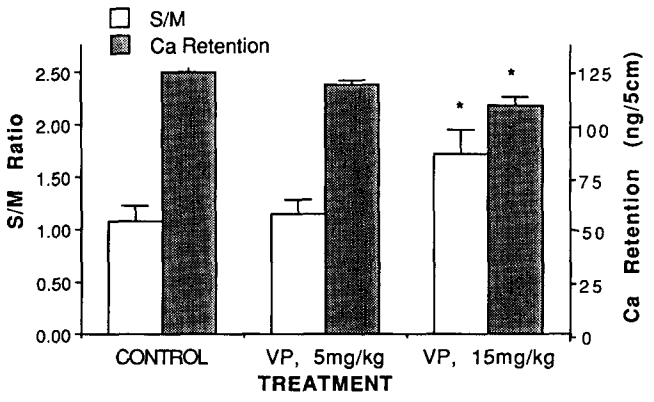

Figure 1 Influence of oral verapamil treatment on Ca uptake and transport. Each column represents the mean ± SEM from five rats. *Significantly different from the control group ($P < 0.05$).

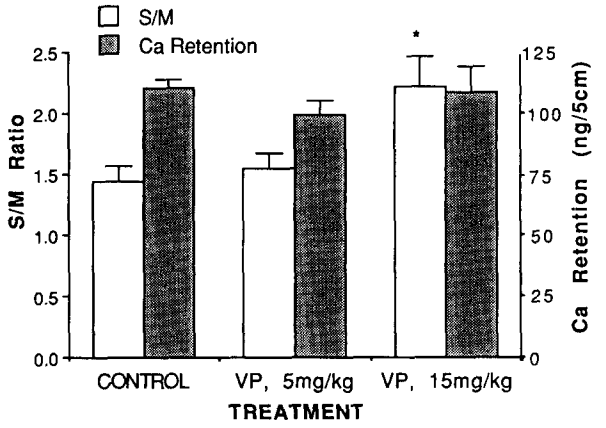

Figure 2 Influence of parenteral verapamil treatment on Ca uptake and transport. Each column represents the mean ± SEM from five rats. *Significantly different from the control group ($P < 0.05$).

Experimental methods

Active Ca transport was measured in proximal duodenal segments of rat intestine using a modification of the everted gut-sac technique of Wiseman,[27] which has been described in detail.[25]

The femurs were removed by blunt dissection, freed of all soft tissue, dried in a vacuum oven at 70° C for 24 hrs, cooled, and weighed. The bone mineral density was measured using a Hologic bone densitometer. The mechanical properties of the femurs were measured using an Instron Universal Testing machine (Instron Corp., Canton, MA USA) as previously described.[28]

Serum and urine Ca levels were determined by atomic absorption spectrophotometry (Varian model 1200, Melbourne, Australia). Serum verapamil levels were determined by high pressure liquid chromatography (HPLC) using methodology previously described.[29]

Statistical analysis

Multiple group means were compared with the analysis of variance using a CLR ANOVA program (Clear Lake Research Inc., Houston, TX, USA). Duncan's New Multiple Range test was used for multiple comparisons between group means. Statistical comparisons resulting in P values of less than 0.05 were considered significant.

Results

Animal weights remained relatively constant between control and treatment groups (in both oral and parenteral treatment studies) during the 8-week period (data not shown). Plasma verapamil was not detectable in the control groups. The 5 mg/kg and 15 mg/kg oral verapamil treatment groups had plasma levels of 36.8 ± 16.4 and 219.1 ± 64.2, respectively, and the parenteral verapamil treatment groups had plasma levels of 469.4 ± 18.2 and 1541.3 ± 77, respectively.

Ca transport and uptake in duodenal segments in the oral administration study are presented in *Figure 1*. Oral verapamil at a dose of 15 mg/kg caused a significant increase in Ca transport (S/M) and a significant decrease in tissue-associated Ca ($P < 0.05$). Similar results were observed with

Table 1 Influence of oral (and parenteral) verapamil treatment on the density and mechanical properties of bone

Treatment	Bone density*	Mechanical properties†			
		Stiffness‡	Ductility§	Toughness‖	Strength¶
Control	0.34 ± 0.005#	0.97 ± 0.18	0.039 ± 0.007	1.85 ± 0.40	47.25 ± 3.4
	(0.32 ± 0.009)	(0.99 ± 0.11)	(0.049 ± 0.004)	(1.74 ± 0.16)	(44.40 ± 1.8)
Verap	0.33 ± 0.008	0.97 ± 0.08	0.039 ± 0.003	1.63 ± 0.16	42.60 ± 3.5
(5 mg/kg)	(0.32 ± 0.009)	(0.87 ± 0.06)	(0.035 ± 0.002)	(1.63 ± 0.09)	(46.60 ± 1.9)
Verap	0.33 ± 0.005	0.73 ± 0.039	0.029 ± 0.002	1.37 ± 0.12	47.00 ± 2.3
(15 mg/kg)	(0.32 ± 0.006)	(0.79 ± 0.16)	(0.032 ± 0.006)	(1.34 ± 0.29)	(38.00 ± 3.7)

*Density (gms/cm^2), measured using the Hologic scanner.
†Measured using the Instrom Testing Machine.
‡lbs/inch.
§inches.
‖inches × lbs.
¶lbs.
#Values are means ± SEM. (parenteral data).

verapamil administered by the parenteral route for calcium transport (S/M) ($P < 0.05$); however, Ca retention was not significantly altered *(Figure 2)*. Verapamil administered by the oral route appears to have a greater effect on Ca uptake and transport in the intestine than s.c. administration, although the verapamil blood level was found to be much greater with parenteral administration. A direct action of verapamil on the intestine during oral absorption may explain this apparent discrepancy.

The influence of oral and parenteral verapamil on the density and mechanical strength of femurs is summarized in *Table 1*. Chronic verapamil treatment by both routes of administration produced a small but consistent decrease in bone mechanical properties; however, these decreases were not statistically significant.

The effects of oral verapamil treatment on serum and urine Ca are presented in *Table 2*. In this study, verapamil caused a significant ($P < 0.05$) increase in both plasma and urine calcium.

Table 2 Influence of oral verapamil treatment on serum and urine Ca concentrations

Treatment	Serum Ca (mg%)*	Urine Ca (mg%)†
Control	9.5 ± 0.31‡	26.91 ± 5.51
Verap (5 mg/kg)	10.26 ± 0.32	35.19 ± 6.10§
Verap (15 mg/kg)	10.74 ± 0.15§	36.20 ± 4.59§

*Serum collected at the end of the 8-week treatment period.
†Urine collected during the last week of the treatment period.
‡Values are means ± SEM.
§Significantly different from the control group ($P < 0.05$).

Discussion

The results of the present study indicated that an 8-week course of oral verapamil treatment at 15 mg/kg produced a significant decrease in Ca uptake into isolated segments of intestinal tissue. Accordingly, this observation supports the results of our previous findings in which verapamil was applied directly to intestinal duodenal segments or isolated enterocytes in vitro.[13,14] In contrast, oral and parenteral administration of verapamil increased Ca transport in this study. This finding is similar to that reported by Fox and Della-Santina in their study with young male rats.[21] Because many investigators have found that acute verapamil treatment in vitro decreased Ca transport,[12,15,16,18,19] this apparent discrepancy suggests that chronic treatment in vivo with this drug alters the homeostatic regulation of the process of Ca transport in the intestine. Accordingly, it has been reported that verapamil treatment is associated with an increase in parathyroid hormone and a decrease in $1,25(OH)_2D_3$.[20,21] In addition, the verapamil-induced increases in plasma and urinary Ca levels observed in the present study also indicate a significant alteration in homeostatic regulation. Further support for the concept of a verapamil-induced alteration of Ca homeostasis is the small but consistent decrease in the mechanical strength of bone that was observed in this study.

The increase in serum and urinary Ca found in the high dose group of verapamil (15 mg/kg) may be from an overall effect of Ca mobilization. In intestinal segments verapamil could be mobilizing Ca by increasing transport and decreasing tissue retention. In addition, verapamil may be mobilizing Ca indirectly or directly from other sources such as kidney and bone. Thus, it is possible that the slight decrease in bone strength may have resulted from a similar overall Ca mobilizing effect of verapamil on bone. Although we do not know the biochemical mechanism responsible for the alterations in Ca homeostasis observed in this study, it is clear that these changes were not due to general verapamil toxicity or a decrease in food consumption because the body weights were not different among the treatment and control groups during the 8-week study period.

Taken altogether the results of this study indicate that chronic verapamil treatment may alter Ca absorption and homeostasis in the geriatric rat. Further these results suggest that chronic treatment with verapamil and possibly other Ca blocking agents in elderly patients may increase the risk of osteoporosis and/or other Ca metabolic disorders. Accordingly, it has been reported that nifedipine treatment

for 2 weeks in patients with essential hypertension reduced Ca balance by increasing urinary Ca excretion (as observed in the present study with verapamil) and by decreasing Ca transport in the intestine.[23] However, it should be noted that another clinical study in men and women failed to detect any significant effect of diltiazem on the intestinal absorption of Ca.[24] It may be found that Ca antagonist drugs vary considerably in their effects on Ca transport. Thus, additional studies will be necessary to determine drug-specific effects on Ca absorption and homeostasis, especially in the geriatric patient.

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