ASPIRIN INHIBITION OF HYPERCALCEMIC EFFECT OF 1 α -HYDROXYVITAMIN D₃ IN RATS

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Abstract—Interaction of calcium regulating hormone, active form of vitamin D₃, and aspirin (ASA) was studied in rats. One alpha-hydroxyvitamin D₃ (1 α -OH-D₃) produced a hypercalcemia in parathyroidectomized, thyroparathyroidectomized and thyroparathyroidectomized nephrectomized rats. The effect of 1 α -OH-D₃ was inhibited by treatment with 200, 50 and 20 mg/kg p.o. of ASA. ASA did not stimulate the gut absorption of calcium in vitamin D deficient rats. These data suggest that ASA acts by inhibiting 1 α -OH-D₃ induced bone resorption. This is the first report demonstrating the inhibitory effect of ASA on bone resorption as induced by 1 α -OH-D₃, although partial inhibition of bone resorption *in vitro* has been reported.

Recently, special interest has focussed on the role of prostaglandins (PG) in the process of bone resorption. PG E₁ or PG E₂ stimulates bone resorption both *in vivo* and *in vitro* (1), and aspirin (ASA) has been demonstrated to be an inhibitor of cyclooxygenase in PG biosynthesis, as stimulated by parathyroid hormone and PG E₁ *in vitro* (2). However, no information is available on the effect of ASA *in vivo*. The purpose of the present work was to demonstrate the hypocalcemic effect of ASA *in vivo* and to clarify the related mechanism.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing 180-250 g, fed a low calcium and/or vitamin D deficient diet, were subjected to parathyroidectomy (PTx), thyroparathyroidectomy (TPTx), or TPTx plus nephrectomy (Nx) one day before the experiment. Rats in which the plasma calcium levels were lower than 6 mg/dl after an overnight fast were used. Each experiment was done on a different day. ASA was given p.o. to rats 30 min before 1 α -hydroxyvitamin D₃ (1 α -OH-D₃) i.p. Drugs used were ASA (Yoshida Seiyaku K.K.) and 1 α -OH-D₃ (Teijin Ltd.). Blood samples were taken by orbital puncture at appropriate time intervals according to the procedure reported by Kawashima et al. (3) and the plasma calcium level was measured

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by the method of Connerty and Briggs (4). Statistical analyses were performed by using Student's *t*-test.

RESULTS

1) *Effect of ASA in PTx rats*: Table 1 shows the inhibitory effect of ASA on the elevation of plasma calcium level as induced by 1α -OH- D_3 in PTx rats. Administration of 200 mg/kg p.o. of ASA completely inhibited the elevation of plasma calcium levels 20 hr after the administration of 1α -OH- D_3 (2.5 μ g/kg i.p.).

2) *Effect of ASA in TPTx and TPTx plus Nx rats*: Tables 2 and 3 show the effect of ASA on the 1α -OH- D_3 induced hypercalcemia in TPTx rats and in TPTx plus Nx rats, respectively. ASA (200 mg/kg p.o.) inhibited the effect of 1α -OH- D_3 (2.5 μ g/kg p.o.) in TPTx rats 20 hr after the administration and in TPTx plus Nx rats (ASA 20 and 50 mg/kg p.o.) 8 hr after the administration of 1α -OH- D_3 .

3) *Effect of ASA on the intestinal absorption of calcium in vitamin D deficient rats*: Twenty rats were separated into 4 groups of 5. Group I: controls without any treatment,

TABLE 1. Effect of aspirin on elevation of plasma calcium level induced by 1α -OH- D_3 in parathyroidectomized rats

1α -OH- D_3 μ g/kg i.p.	Drug		Change in plasma Ca level Δ Ca (mg/100 ml) mean $\pm$ s.e.m.
		Aspirin mg/kg p.o.	
0		0	-0.33 ± 0.39 (8)
2.5		0	$1.68 \pm 0.18^*$ (17)
0		200	-0.69 ± 0.35 (10)
2.5		200	-0.52 ± 0.38 (17)

Numbers of rats in each group are shown in parentheses. Rats parathyroidectomized under ether anesthesia and fasted overnight, and in which the plasma calcium levels were lower than 6 mg/dl, were used. One alpha-hydroxyvitamin D_3 was given i.p. 24 hr after surgery and plasma calcium levels were determined 20 hr after the drug administration. Aspirin was administered p.o. 30 min prior to administration of 1α -OH- D_3 .

*; different from control, $p < 0.001$

TABLE 2. Effect of aspirin on elevation of plasma calcium level induced by 1α -OH- D_3 in thyroparathyroidectomized rats

1α -OH- D_3 μ g/kg i.p.	Drug		Change in plasma Ca level Δ Ca (mg/100 ml) mean $\pm$ s.e.m.
		Aspirin mg/kg p.o.	
2.5		0	$1.45 \pm 0.23^*$ (7)
2.5		200	-1.21 ± 0.42 (6)

Numbers of rats in each group are shown in parentheses. Rats thyroparathyroidectomized under ether anesthesia and fasted overnight, and in which the plasma calcium levels were lower than 6 mg/dl, were used. One alpha-hydroxyvitamin D_3 was given i.p. 24 hr after surgery and plasma calcium levels were determined 20 hr after the administration. Aspirin was administered p.o. 30 min prior to administration of 1α -OH- D_3 .

*; different from aspirin treated group, $p < 0.001$

TABLE 3. Effect of aspirin on elevation of plasma calcium level induced by 1α -OH- D_3 in thyroparathyroidectomized nephrectomized rats

Drug		Change in plasma Ca level
1α -OH- D_3 μ g/kg i.p.	Aspirin mg/kg p.o.	Δ Ca (mg/100 ml) mean $\pm$ s.e.m.
2.5	0	0.39 $\pm$ 0.29 (7)
2.5	20	-0.48 $\pm$ 0.14* (5)
2.5	50	-0.79 $\pm$ 0.43* (4)

Numbers of rats in each group are shown in parentheses. Rats bilaterally nephrectomized immediately after thyroparathyroidectomy and fasted overnight, and in which the plasma calcium levels lower than 6 mg/dl, were used. Twenty-four hours after the surgery, rats were treated with 2.5 μ g/kg i.p. of 1α -OH- D_3 and plasma calcium levels were measured 8 hr after the administration. Aspirin was given p.o. 30 min prior to the administration of 1α -OH- D_3 . *: different from the group without aspirin, $p < 0.01$

TABLE 4. Effect of aspirin on intestinal absorption of calcium induced by 1α -OH- D_3 in vitamin D deficient rats

Drug		Intestinal Ca transport	Plasma Ca level
1α -OH- D_3 μ g/kg i.p.	Aspirin mg/kg p.o.	$^{45}\text{Ca}(\text{S})/^{45}\text{Ca}(\text{M})$ mean $\pm$ s.e.m.	Ca (mg/100 ml) mean $\pm$ s.e.m.
0	0	2.20 $\pm$ 0.31	4.48 $\pm$ 0.09
0.25	0	4.59 $\pm$ 0.25*	5.74 $\pm$ 0.17*
0	200	2.19 $\pm$ 0.27	4.14 $\pm$ 0.04
0.25	200	4.60 $\pm$ 0.19*	4.09 $\pm$ 0.07

Rats, fed a vitamin D deficient diet, were given 0.25 μ g/kg i.p. of 1α -OH- D_3 and/or 200 mg/kg p.o. of aspirin, and intestinal absorption of calcium and plasma calcium levels were measured 20 hr after the administration. Aspirin was given 30 min prior to the administration of 1α -OH- D_3 (5 rats in each group). *: different from control, $p < 0.01$

Group II: 0.25 μ g/kg of 1α -OH- D_3 was given i.p. 20 hr before the experiment, Group III: 200 mg/kg of ASA was given p.o. 20 hr before the experiment, Group IV: 0.25 μ g/kg of 1α -OH- D_3 was given 20 hr before the experiment and 200 mg/kg of ASA was given 30 min prior to the administration of 1α -OH- D_3 . Rats were sacrificed 20 hr after the administration of 1α -OH- D_3 and the small intestine was excised for determination of calcium, everted sac method, the plasma calcium levels were also determined.

The ingestion of 1α -OH- D_3 produced a significant increase in both gut absorption of calcium and in the plasma calcium levels. Administration of ASA did not inhibit the 1α -OH- D_3 induced gut absorption of calcium, however, the hypercalcemic effect of 1α -OH- D_3 was inhibited by ASA (Table 4).

DISCUSSION

The present study clearly demonstrated that ASA inhibited 1α -OH- D_3 induced hypercalcemia in PTx, TPTx and TPTx plus Nx rats.

Since the hypocalcemic effect of ASA was evident in TPTx rats and in TPTx plus Nx rats, it is possible that neither the thyroid nor the kidney is an essential component of the

mechanism responsible for the action of ASA. It was shown that ASA did not stimulate the gut absorption of calcium in vitamin D deficient rats. Therefore it is highly possible that ASA acts by inhibiting the 1α -OH- D_3 induced bone resorption. According to preliminary data, successive administration of ASA increases the bone mass in vitamin D deficient rats. Although the inhibitory effect *in vitro* of ASA on bone resorption has been demonstrated by Powles et al. (2), this effect has never before been demonstrated *in vivo*.

In view of our present data, ASA probably also inhibits bone resorption *in vivo*.

Since ASA is a potent inhibitor of cyclooxygenase in PG biosynthesis, it is tempting to speculate that PG is involved in the process of 1α -OH- D_3 induced bone resorption. Further studies are under way to clarify the possible involvement of PGs.

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