

**Effect of Treatment With SB 242784 on Soft Tissue Calcification in Rats Treated With Vitamin D**

Tissue, $\mu\text{mol}$	Vitamin D Only	Vitamin D+10 mg SB 242784	Vitamin D+40 mg SB 242784	Age-Matched Control
Abdominal aorta	$3.1 \pm 1.3$	$1.4 \pm 0.5^*$	$0.3 \pm 0.03^\dagger$	$0.3 \pm 0.02$
Kidney	$39.6 \pm 32.5$	$20.7 \pm 11.9$	$2.6 \pm 0.2^\ddagger$	$1.1 \pm 0.2$
Lungs	$95.5 \pm 24.3$	$26.7 \pm 33.6$	$3.8 \pm 0.3\$$	$3.4 \pm 0.5$
Trachea	$2.0 \pm 0.2$	$1.9 \pm 0.3$	$0.9 \pm 0.7  $	$0.6 \pm 0.2$

Seven-week-old male rats received subcutaneous injections of 500,000 IU of vitamin D/kg body wt at  $t=0$ , 24, and 48 hours. Subsets of these animals were also injected with either 10 or 40 mg SB 242784/kg body wt per day beginning at 2 days before the first injection of vitamin D. Animals were killed 96 hours after the first vitamin D injection, and the abdominal aorta, one kidney, the lungs, and an anatomically uniform 1 cm of trachea were ashed (see Materials and Methods for further details). The ashed tissues were extracted with acid to dissolve minerals, and each acid extract was analyzed for calcium as described in Materials and Methods. Results are given as  $\mu\text{mol}$  calcium in the indicated tissue. Vitamin D treatment does not result in any significant increase in the size or wet weight of the tissues analyzed. Therefore, the increase in calcium content seen in the tissues from the vitamin D treatment group cannot be due to changes in the size or mass of the tissues (authors' unpublished observations, 2002).

Data are mean  $\pm$  SD,  $n=4$ , for all groups.

\* $P=0.06$  compared with vitamin D only.

$^\dagger P=0.005$  compared with vitamin D only.

$^\ddagger P=0.063$  compared with vitamin D only.

$\$P=0.0003$  compared with vitamin D only.

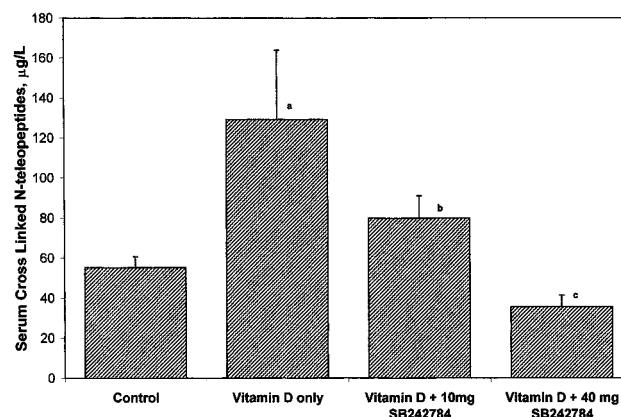
$||P=0.023$  compared with vitamin D only.

control animals (Table). Calcium levels measured on serum obtained 96 hours after the first vitamin D injection were as follows:  $13.4 \pm 1.2$  mg/dL for rats treated with vitamin D only ( $n=4$ ),  $14.0 \pm 0.9$  mg/dL for rats treated with vitamin D plus the 10-mg dose of SB 242784 ( $n=4$ ) ( $P>0.5$  versus vitamin D alone),  $10.8 \pm 0.5$  mg/dL for rats treated with vitamin D plus the 40-mg dose of SB 242784 ( $n=4$ ) ( $P<0.01$  versus vitamin D alone), and  $10.9 \pm 0.3$  mg/dL for untreated control rats ( $n=4$ ) ( $P<0.001$  versus vitamin D alone).

To confirm that SB 242784 did in fact inhibit bone resorption at the doses used in the above experiments, serum samples obtained at death were analyzed to determine the level of cross-linked N-telopeptides, a specific marker for bone resorption activity that is released during the osteoclast-mediated breakdown of bone matrix collagen. As seen in Figure 4, the level of cross-linked N-telopeptides was elevated by 140% in rats treated with vitamin D alone and was reduced to below control levels in rats treated with vitamin D plus the higher dose of SB 242784. This result

supports the hypothesis that vitamin D treatment induces arterial calcification by accelerating bone resorption and shows that SB 242784 treatment dose-dependently inhibits bone resorption activity in the vitamin D-treated rat.

The effect of SB 242784 on arterial calcification was also examined in rats treated with warfarin, a treatment that inactivates the calcification-inhibitory activity of matrix Gla protein and thereby induces rapid calcification of arteries and cartilage. In the first experiment, weanling rats were treated for 1 week with warfarin alone or with warfarin plus 10 mg SB 242784/kg per day. This experiment showed that the 10-mg dose of SB 242784 did not significantly inhibit arterial calcification as assessed by alizarin red staining. Subsequent analysis of bone resorption activity using the cross-linked N-telopeptide assay showed that the 10-mg dose of SB 242784 also did not significantly reduce bone resorption activity in the warfarin-treated rats. When this experiment was repeated at the higher dose (40 mg/kg per day) of SB 242784, we observed that the drug strongly inhibited arterial



**Figure 4.** Effect of SB 242784 on bone resorption activity as measured by serum cross-linked N-telopeptides. Serum was obtained from each animal 96 hours after the first vitamin D injection in the experiment described in the Figure 1 legend, and serum was also obtained from 4 age-matched control rats. Each serum sample was analyzed to determine the level of cross-linked N-telopeptides (OSTEOMARK NTx, Ostex), a specific marker for bone resorption activity (see Materials and Methods). Results are presented as mean  $\pm$  SD. a,  $P<0.01$  compared with control; b,  $P<0.05$  compared with vitamin D only; and c,  $P<0.001$  compared with vitamin D only.