ACTION OF SOLANUM MALACOXYLON ON CALCIUM METABOLISM IN THE RAT[+]

by

A. Uribe, M.F. Holick, N.A. Jorgensen and H.F. DeLuca*

Departments of Biochemistry and Dairy Science, College of Agricultural and Life Sciences, University of Wisconsin-Madison, Madison, Wisconsin 53706

Received March 18,1974

The administration of an aqueous extract of the leaves from Solanum malacoxylon to vitamin D-deficient rats fed a normal calcium, normal phosphorus diet markedly increased serum calcium concentration within 48 hours. The Solanum malacoxylon extract also stimulated intestinal calcium transport in the vitamin D-deficient rat but was without effect on the mobilization of calcium from bone. The extract from 100 mg of dry Solanum malacoxylon leaves was more effective than 25 units of vitamin D given daily to vitamin D-deficient rats in stimulating intestinal calcium transport but its effect was not additive to that of the vitamin D. The results demonstrate that the action of Solanum malacoxylon is independent of vitamin D and, although it can substitute for vitamin D in the stimulation of intestinal calcium transport activity, it cannot substitute for vitamin D in the mobilization of calcium from bone.

A disease of cattle known as "Enteque seco" in Argentina and as "Espichamento' in Brazil, presenting gross symptoms of severe wasting, stiffness and massive calcification of heart, lungs and large blood vessels, is caused by animals grazing leaves of the shrub, Solanum malacoxylon (1). Oral administration of the dried leaves or administration of an aqueous extract of the leaves is capable of reproducing the disease in bovines (2) (3). The aqueous extract produces hypercalcemia and hyperphosphatemia and soft tissue calcification in ovines (4), guinea pigs (5), and rabbits (6). In cattle the Solanum malacoxylon extract reversed a negative calcium balance within a few days as a result of greater absorption of calcium from the intestine (7). Similar findings in sheep and guinea pigs have been reported (4) (5). Because of this action, it

[+]This work was supported by PHS grant No. AM-14881 and an A.E.C. contract No. AT-(11-1)-1668.

*To whom to address correspondence.

Table 1. Intestinal calcium transport and serum calcium responses of vitamin D-deficient rats to vitamin D and Solanum malacoxylon (S.M.)

Supplement	^{45}Ca serosal/^{45}Ca mucosal (mean ± SEM)	Serum calcium (mg/100 ml) (mean ± SEM)
Vitamin D-deficient	1.52 ± .34	6.03 ± .18
Vitamin D_3, 25 IU daily	2.67 ± .59	8.23 ± .48
Vitamin D_3, 25 IU plus 200 mg S.M. daily	3.93 ± .54[a]	9.18 ± .29[a]
S.M., 200 mg daily	3.83 ± .28[a]	9.03 ± .14[a]

Weanling rats were fed a vitamin D-deficient diet containing 0.47% calcium and 0.3% phosphorus for 5 weeks. They were then given the indicated dietary supplements orally. Fifteen days later the rats were sacrificed for measurement of intestinal calcium transport and serum calcium determination. There were 4 rats in each group.

[a] Significantly different from control $p < 0.005$.

has been suggested that the leaves of Solanum malacoxylon contain one or more active principles which stimulate calcium absorption in a manner similar to vitamin D; however, its action is more rapid in onset and of shorter duration than that of vitamin D (6). Also, the active material(s) is water soluble. Recently, it has been demonstrated that Solanum malacoxylon stimulates intestinal calcium absorption in vitamin D-deficient rats suggesting that it may possess vitamin D activity (8).

In this report we demonstrate that Solanum malacoxylon acts like vitamin D in intestine, but unlike vitamin D it does not cause a mobilization of calcium from bone.

Weanling male albino rats were obtained from the Holtzman Co., Madison, Wisconsin. They were housed individually in overhanging wire cages and were

given food and distilled water ad libitum. The diet was essentially that described by Suda et al.(9). Two levels of dietary calcium were used. Primarily the diet containing .47% calcium and .3% phosphorus was employed. Where indicated the very same diet was fed except that calcium was omitted giving a diet containing .02% calcium. At the termination of the experiments or during the experiments, serum calcium concentration was determined in the presence of 0.1% lanthanum chloride by atomic absorption spectrometry using a Perkin-Elmer Model 403 instrument. The blood was collected from the tail vein or from animals killed by decapitation. Serum was obtained from clotted blood by centrifugation. Intestinal calcium transport activity was determined by the everted sac method as described by Martin and DeLuca (10).

Vitamin D-deficient rats fed a diet adequate in calcium and phosphorus but without vitamin D for a period of 14 days became severely hypocalcemic (Table 1). Some of these animals were then given a single subcutaneous injection of the Solanum malacoxylon extract (2), such that each rat received the active product from 100 mg of leaves dissolved in 0.1 ml of distilled H_2O. Control animals received the distilled H_2O alone. The Solanum malacoxylon extract brings about a marked elevation of serum calcium concentration from 6.0 mg/100 ml to 8.0 mg/100 ml within 24 hours in the vitamin D-deficient rat on this diet. This elevation is still obvious 48 hours following the administration of the active extract. The response is dose dependent and thus constitutes a usable bioassay animal for the active component in the Solanum malacoxylon extract. However, the elevation of serum calcium can be due either to a stimulation of intestinal calcium transport or to a stimulation of bone calcium mobilization or both. The results shown in Table 1 reveal that, like vitamin D, Solanum malacoxylon stimulates intestinal calcium transport as measured in vitro by the everted sac method. It is of great interest that the Solanum malacoxylon extract equivalent to 200 mg of dried leaves was more effective in stimulating intestinal calcium transport than 25 units of vitamin D_3 (Philips-Roxane, Inc., N.Y.C.) given orally each day in 0.1 ml cottonseed oil. Additionally, there

Table 2. Failure of Solanum malacoxylon (S.M.) to stimulate bone calcium mobilization in vitamin D-deficient rats.

Group	Treatment	Serum Ca, mg/100 ml (mean ± SEM) hours after administration 0	48	Δ
1	Control	5.2	5.3	.1 ± 0.08
2	325 pmole 1,25-$(OH)_2D_3$[a] orally	5.5	7.5	2.0 ± 0.14
3	200 mg S.M. orally	5.5	5.3	-.2 ± 0.18
4	100 mg S.M. orally (normal calcium diet)	5.6	7.6	+2.0 ± 0.16

Weanling rats (groups 1, 2 and 3) were fed a calcium and vitamin D-deficient diet for 2 weeks while group 4 received the vitamin D-deficient diet containing 0.47% calcium. Serum calcium was determined in all groups and then each rat was given the indicated compounds orally. Forty-eight hours later serum calcium was determined.

[a] Synthesized chemically by the method of Semmler et al. (11). It was given in 0.1 ml propylene glycol.

was a correlation between the increased intestinal calcium transport and the elevation of serum calcium concentration. The Solanum malacoxylon when given together with the vitamin D brought about the same elevation of intestinal calcium transport and serum calcium concentration as it did when it was given in the absence of vitamin D_3. Thus it might appear that the Solanum malacoxylon can replace vitamin D in the intestinal calcium transport system, which would suggest that the Solanum malacoxylon extract might contain an active vitamin D derivative. Because of the aqueous solubility of the Solanum malacoxylon material, one might expect it to be a derivative of vitamin D which has been rendered water-soluble either by multiple hydroxylations or by conjugation with an aqueous-soluble material. The results in Table 2, however, show

that the Solanum malacoxylon differs markedly from 1,25-dihydroxyvitamin D_3[1](11). the known active form of vitamin D in the intestine, in that it does not elevate serum calcium of rats maintained on a vitamin D-deficient, calcium-deficient diet. It is important to note in Table 2 that 100 mg of Solanum malacoxylon given orally to animals on a normal calcium diet will, like the subcutaneously administered extract, elevate the serum calcium as already shown in Table 1.

The active principle of the Solanum malacoxylon leaf extract has been demonstrated previously to have profound effects on calcium metabolism, especially on intestinal calcium absorption (4) (5). It has been suggested that the active principle in this plant acts very much like vitamin D. In agreement with this conclusion a recent report has demonstrated that Solanum malacoxylon will stimulate intestinal calcium transport in vitamin D-deficient rats (8). The present study provides additional evidence that Solanum malacoxylon active principle will markedly increase serum calcium concentration and intestinal calcium transport of vitamin D-deficient rats. This demonstrates that Solanum malacoxylon can act quite independently of vitamin D and does not require the presence of vitamin D for it to elicit its marked effects on calcium metabolism. The present results also suggest that the active material in Solanum malacoxylon might well be a vitamin D-like compound since it can substitute for vitamin D in elevating serum calcium and in stimulating intestinal calcium transport of vitamin D-deficient rats. However, unlike vitamin D or its active metabolites, 25-hydroxyvitamin D_3[2] and 1,25-$(OH)_2D_3$, Solanum malacoxylon cannot elevate serum calcium of vitamin D-deficient and calcium-deficient rats. Thus if the active material is a vitamin D analog it must differ from these metabolites.

It is of interest that the Solanum malacoxylon material is just as effective as 25 international units of vitamin D_3 given daily in the elevation of serum calcium and in the stimulation of intestinal calcium transport of animals which are vitamin D-deficient but which have adequate amounts of calcium in

[1] 1,25-$(OH)_2D_3$ [2] 25-OH-D_3

their diet. Surprisingly when the 25 units of vitamin D was given together with the Solanum malacoxylon extract each day, their effects were not additive. This would suggest that the Solanum malacoxylon activity on intestinal calcium transport may be at the same site of the active metabolite of vitamin D, 1,25-$(OH)_2D_3$.

From these and previous results it can be concluded that: one, the active principle from Solanum malacoxylon can function independently of vitamin D and that vitamin D is not required for its function; two, that unlike vitamin D and its active metabolites it is unable to stimulate the mobilization of calcium from bone; and three, it may function at the same site as 1,25-$(OH)_2D_3$ in the stimulation of intestinal calcium transport.

This substance, therefore, is of considerable interest as a possible tool in the elucidation of mechanisms of calcium metabolism and may even be of some practical value in disease states.

References

1. Worker, N.A. and Carrillo, B.J. (1967) Nature 215, 72-74.
2. Carrillo, B.J., Gaggiano, P., Ruksan, B., Lando, E., Deshpande, P.D., Tilley, J.M.A., and Worker, N.A. (1966) VII Pan American Congress of Pharmacy and Biochemistry, Buenos Aires, Argentina.
3. Carrillo, B.J., and Worker, N.A. (1967) Rev. Invest. Agrop. INTA, Buenos Aires, Argentina, Series 4, Pat. Ani. 6, 9
4. Camberos, H.R., Djafar, M.I., Davis, G.K. (1969) Florida Academy of Science, Gainesville, Florida.
5. Camberos, H.R., Davis, G.K., Djafar, M.I., Simpson, C.F. (1970) Am. J. Vet. Res. 31, 685-696.
6. Mautalen, C.A. (1972) Endocrinology 90, 563-567.
7. Sansom, B.F., Vagg, M.J., Döbereiner, J. (1971) Res. Vet. Sci. 12, 604-605.
8. O'Donnell, J.M. and Smith, M.W. (1973) Nature 244, 357-359.
9. Suda, T., DeLuca, H.F., and Tanaka, Y. (1970) J. Nutr. 100, 1049-1052.
10. Martin, D.L. and DeLuca, H.F.(1969) Am. J. Physiol. 216, 1351-1359.
11. Semmler, E.J., Holick, M.F., Schnoes, H.K. and DeLuca, H.F. (1972) Tetrahedron Letters 40, 4147-4150.