Further Observations on the Use of lα-Hydroxycholecalciferol in the Prevention of Bovine Parturient Paresis

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Abstract

Varying doses of lαhydroxycholecalciferol (lα-HCC) (50, 150, 250 and 350 micrograms) in propylene glycol were injected intramuscularly into 30 dry adult Israeli Friesian cows. Four of these animals received a second dose (250 or 350 Hg) 48 hours later or ten (350 Hg) 72 hours after the first dose.

Plasma calcium rose after 24 hours at all dose levels except 50 Hg. A dose-dependent peak in plasma calcium was reached after 3-4 days, followed by a return to baseline 5 days (150 Hg) and 8 days (250 and 350 Hg) post-injection, respectively. Repeating the injection 48 or 72 hours later increased the time span by 3 or 4 days, respectively.

The effect on plasma inorganic phosphate was double that on plasma calcium. Plasma magnesium declined slightly 3 days post-injection.

High calcium feeding in conjunction with one or two injections of 350 Hg lαHCC did not modify the response plasma calcium.

An injection of 350 ug of lα-HCC was given once to 40 parturient-prone cows of the same breed, and twice — at 72-hour intervals — to 37 such cows. Six of these animals received 5 mg of flumethasone together with the second injection, and 13 received it 48 hours later, in order to induce parturition, which occurred 24-48 hours post-injection. None of the cows injected earlier than 24 hours prepartum developed parturient paresis in comparison with 22 out of 60 control animals which developed the condition.

There were no local or systemic clinically detectable signs of toxicity in any of the injected animals.

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The results suggest that lα-HCC is a valuable agent in the prevention of bovine parturient paresis.

Procedures against bovine parturient paresis under field conditions have met with only partial success. Dietary calcium restricted for a limited period before calving, although sometimes effective, is not always practical.

Another approach has been the administration of vitamin D and its metabolite, 25-hydrosycholecalciferol (Hibbs and Pounden, 1955; Manston and Payne, 1969; Olson *et al.*, 1973). The use of both met with limited success due to toxic complications in some cases and to a delay in response. This delay has been shown to be shorter when potent, faster acting vitamin D derivatives have been used (Barlet, 1975; Sansom *et al.*, 1976; Sachs *et al.*, 1977; Hoffsis *et al.*, 1978).

The active hormonal form of vitamin D is 1,25-dihydroxycholecalciferol (1,25-DHCC); this metabolite has been shown to be the most potent in stimulating the absorption of calcium by the gut and the mobilization of calcium from bone (Haussler, 1974; Norman and Henry, 1974; Fraser, 1975; DeLuca, 1979). 1,25-DHCC is formed from vitamin D in two hydroxylation steps: (i) via a hydroxylase system in the liver, producing 25-hydroxycholecalciferol, and (ii) in the kidney, yielding 1,25-DHCC. The second hydroxylation step is regulated by the calcium and/or parathyroid hormone concentration in the plasma.

The form lα-hydroxycholecalciferol (lα-HCC) is an active synthetic analogue of 1,25-DHCC. Its biological action is independent of kidney hydroxylation and it becomes active after hydroxylation in the liver (Holick *et al.*, 1973). In the chicken and rat, lα-HCC has an antirachitic action 3-6 times greater on a weight basis and acts three times faster in inducing calcium absorption in the small intestine than does vitamin D itself (Haussler *et al.*, 1973; Holick *et al.*, 1973; Corck *et al.*, 1974). Barlet (1975), Sansom *et al.*, (1976) and Sachs *et al.* (1977) demonstrated the efficacy of lα-OHCC in raising calcium and phosphorus levels in the bovine and Braithwaite (1978) in the ovine. Hoffsis *et al.* (1978) produced the same effect using 1,25-DHCC. Both products required 24 hours to achieve the desired effect.

The short-term response, together with its high clearance rate from the body (Holick *et al.*, 1976), could be considered

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desirable qualities in the use of this compound for the prevention of parturient paresis, without long-term risks of sustained hypercalcaemia and soft tissue calcification. Sachs *et al.* (1977), using a dose of 350 ug given no longer than 24 hours prepartum, were able to prevent completely parturient paresis and to reduce the degree of postpartum hypocalcaemia. Sansom *et al.* (1976) and Barlet (1977) injected the material at the time of parturition. On the assumption that post-parturient paresis is the result of bypocalcaemic disturbances which start ante partum, Sachs et al. (1977) and Wittwer and Ford (1978) used the material before parturition took place, and Davies et al. (1978) up to 6 hours post-partum. Sachs et al. (1977) used a dose of 350 Hg, the other workers used 500 Hg.

Results varied among the different workers and seemed to be governed by the time of injection in relation to parturition, posology, and the feeding regime in the treated herds.

The effective use of this derivative in practice depends, therefore, on the accurate prediction of calving. Furthermore, the minimal effective dose has not been determined. In the present study, dose response curves to lα-HCC were obtained, and parturition induction by flumethasone has been tried in an effort to overcome the difficult problem of timing.

Materials and Methods

Trial 1

The purpose of this trial was to determine changes in plasma calcium following injection of lα-HCC (synthesized by Y. Mazur, The Weizmann Institute, Israel) once, at doses of 0, 50, 150, 250 and 350 Hg; and twice, at 48-hour intervals, at doses of 250 and 350 Hg.

Twelve dry Israeli Friesian cows, 4th calving and upwards, fed a commercial diet containing approximately 90 g of calcium per day and 60 g of phosphorus per day, were divided into five groups and injected intramuscularly with 0 (2 cows), 50 (3 cows), 150 (3 cows), 250 (2 cows) and 350 (2 cows) ug of lα-HCC in 5 ml of propylene glycol. Four additional cows were injected twice with 250 (2 animals) and 350 (2 animals) ug of the same material, at 48-hour intervals. Blood samples were taken from one day pre-injection until 9-16 days post-injection.

Trials 2 and 3

The purpose of this trial was to determine the effect of high-calcium feeding in conjunction with the single or double injection of lα-HCC (Teva, Israel) at 72-h intervals, on plasma calcium, inorganic phosphorus and magnesium.

Nine dry cows of the same breed and fed as in Trial 1, were divided equally into three groups. Cows in the first group were injected once with 350 ug of the material, those in the second group received the same injection but were fed an additional 100 g of calcium per day in the form of ground limestone, the third group served as a control.

The experiment was repeated on an additional 15 cows, in groups of five cows each. This time a second injection of 350 ug of lα-HCC was given 72 hours after the first. The calcium supplementation was started 4 days prior to injection.

Blood samples were taken at 24-h intervals ranging from 2 days pre-until 12 days post-injection.

Trial 4

A field trial was carried out to test the prophylactic efficiency of lα-HCC when given as (1) a single injection of 350 ug (not shown in Fig. 5); (2) two injections at 72-h intervals instead of 48, as reported by us previously (Sachs et al., 1977); and (3) adding 5 mg of flumethasone (Fluvet, Teva, Israel) to the second injection of lα-HCC (Teva, Israel), or giving it 48 hours after the second injection of the material.

Flumethasone was used in order to induce parturition while the cow was still under the prophylactic effect of lα-HCC. It obviated the need for a third injection of the material, because of (a) the additional cost and (b) the danger of a delayed hypocalcaemia as a result of a prolonged interference with normal calcium homeostasis (unpublished results).

For this purpose 77 milk-fever-prone cows of the same breed and kept under similar conditions of management and feeding as in the above trial, were injected with 350 ug of lα-HCC in 5 ml of propylene glycol, 72-14 hours prepartum. Thirty seven animals, which had not calved 72 hours after the first injection, and of which six were bled, were injected again with the same material 122-89 hours prepartum.

Six out of the thirty seven cows were given 5 mg flumethasone intra-muscularly together with the second dose of lα-HCC and 13 animals, of which eight were bled, received 5 mg of flumethasone 48 h after the second injection. Sixty milk-fever-prone cows served as controls. Five of these which did not develop the condition were bled and results are shown in Fig. 5.

Blood samples were taken up to 280 h post-partum.

Analytical procedures

Blood samples were taken in heparinized tubes (vacucontainers) from the jugular vein. Each blood sample was centrifuged at 3000 rmp for 45 minutes. The plasma was separated and used for calcium, inorganic phosphate and magnesium determinations. Calcium was determined by EGTA direct titration using the Automatic Calcium Titrator (Precision Systems, Sudbury, Me., U.S.A.). For the inorganic phosphate assay the plasma was treated with a 10% trichloracetic acid solution, centrifuged, and phosphate was then determined in the filtrate fraction by a modification of the Gomori method (1942) using an Autoanalyser (Technicon, Tarrytown, N.Y., U.S.A.). Magnesium was determined by the colorimetric method as described by Merck (1970).

Results

Trial 1

The time response function of plasma calcium following lα-HCC injection is presented in Fig. 1, and the dose

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response curve in Fig. 2.

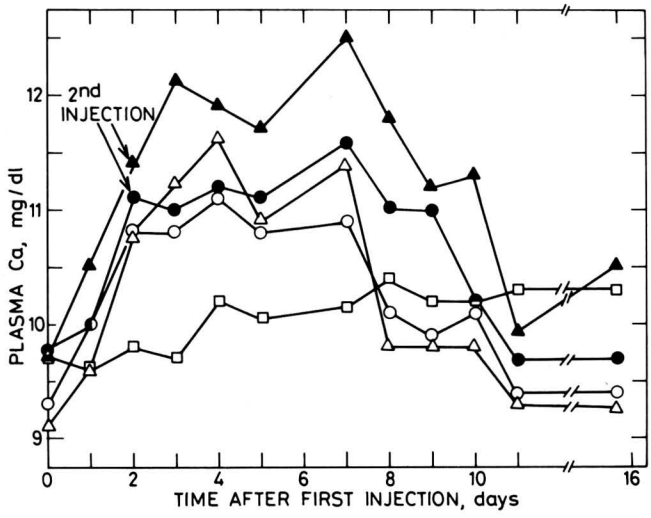
A first marked increase in plasma calcium was observed 24 hours after injection at all dose levels except 50 ug (not shown in Fig. 1). A dose-dependent peak was reached at 3-4 days with a return to baseline at 5 days (150 ug, not shown in Fig. 1) and 8 days (250-350 ug) post-injection.

When plasma calcium peaked, a linear dose response was observed (Fig. 2). The second injection 48 hours later increased the time span by a further 3 days (Fig. 1).

Trials 2 and 3

The additional intake of calcium did not affect significantly the response of cows to a single dose of lα-HCC (Fig. 3), although a slight tendency for plasma calcium levels to remain higher for a longer period existed in the calcium-supplemented cows.

The second injection increased the time response by a further 4 days with no significant effect of calcium supplementation (Fig. 4). Plasma inorganic phosphate levels responded more rapidly to lα-HCC. When the material was given twice, two peaks appeared, the first 3 days after injection one, and the second 3 days after the second injection. Plasma magnesium declined (0.3 ± 0.06 mg/dl) concurrently with the calcium plasma peaks on day 3 post-injection. No effect of dietary calcium supplementation was noted on either plasma phosphate or magnesium.



Legends to Figures

Fig. 1. Changes in plasma calcium concentration of dry cows as a result of a single or double injection of varying doses of lα-HCC.
□, control; 0, 250 ug injected once; ●, 250 ug injected twice at an interval of 48 hours; △, 350 ug injected once; ▲, 350 ug injected twice at an interval of 48 hours.

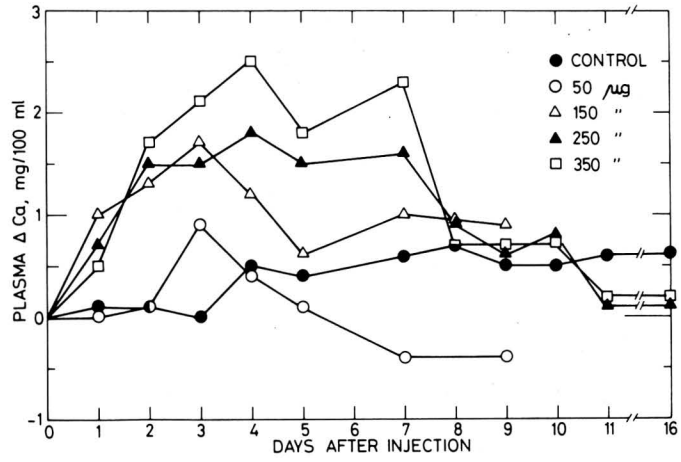


Fig. 2. The increase noted in plasma calcium cncentration of dry cows as a result of administering varying doses of lα-HCC.

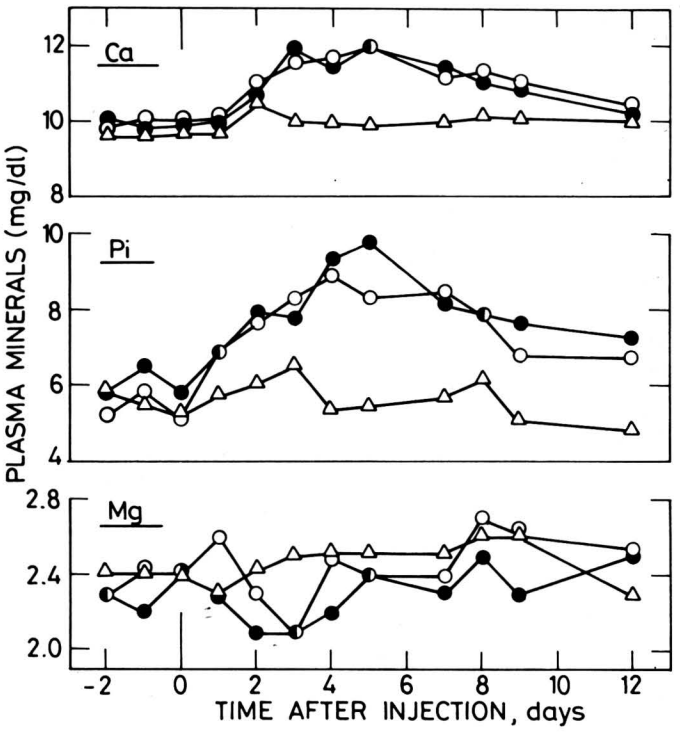


Fig. 3. The effect on plasma calcium, inorganic phosphorus and magnesium levels of dry cows as a result of the injection of a single dose of 350 ug lα-HCC in conjunction with feeding calcium-rich fodder.
△, normal feed without lα-HCC; 0, one injection of 350 Hg lα-HCC; feed plus 100 g calcium per day; ●, one injection of 350 ug lα-HCC; normal feed.

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Trial 4

About one-third of the control group developed parturient paresis. Only two out of 77 of the lα-HCC-injected cows developed the condition. In both cases the interval between the last injection and parturition was less than 24 hours. Flumethesone did not alter the response to lα-HCC in the prevention of parturient paresis. The results are presented in Table 1 and Fig. 5.

The non-injected animals, which did not develop milk fever, showed the typical physiological hypocalcaemia at the time of calving (Fig. 5).

The changes in blood calcium in those cows injected twice at 72-h intervals prepartum resembled those where the second injection was given after 48 hours, as described by Sachs *et al.* (1977). Plasma calcium concentrations rose slightly prepartum (11.09 ± 0.46 mg/dl) which was significantly ($P<0.01$) higher than that of the control cows (6.7 ± 0.5 mg/dl) and remained so for 94 hours.

Flumethasone, either given with the second injection of the material or 48 h later, did not alter the hypercalcaemic response of lα-HCC. Parturition occurred 24-48 h (avg. 30 h) post-injection. The incidence of retained placenta, however, was 64%, but all the affected animals were free of any uterine infection 4-6 weeks postpartum.

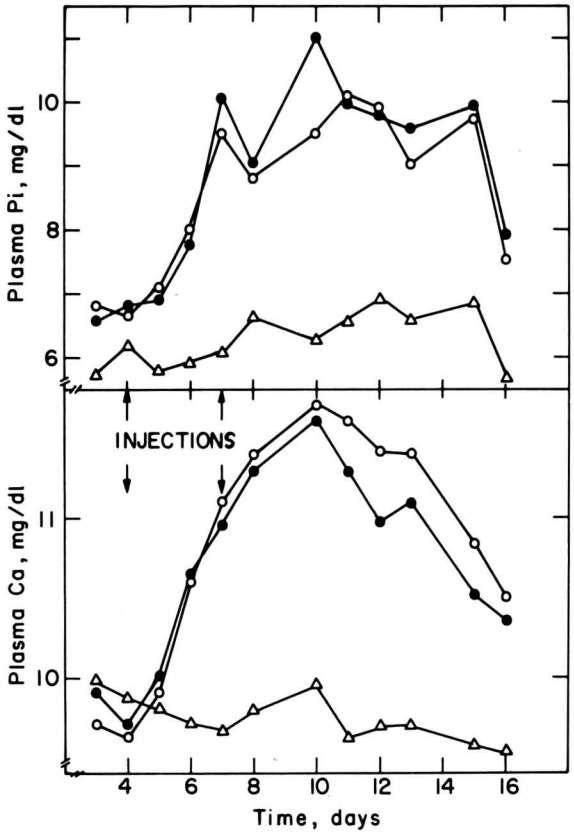

Fig. 4. The effect on plasma calcium and inorganic phosphate concentrations of dry cows as a result of two injections of 350 ug lα-HCC at an interval of 72 hours, in conjunction with feeding calcium-rich fodder.
△, normal feed without lα-HCC; 0, two injections of 350 ug lα-HCC; feed plus 100 g calcium per day; ●, two injections of 350 ug lα-HCC; normal feed.

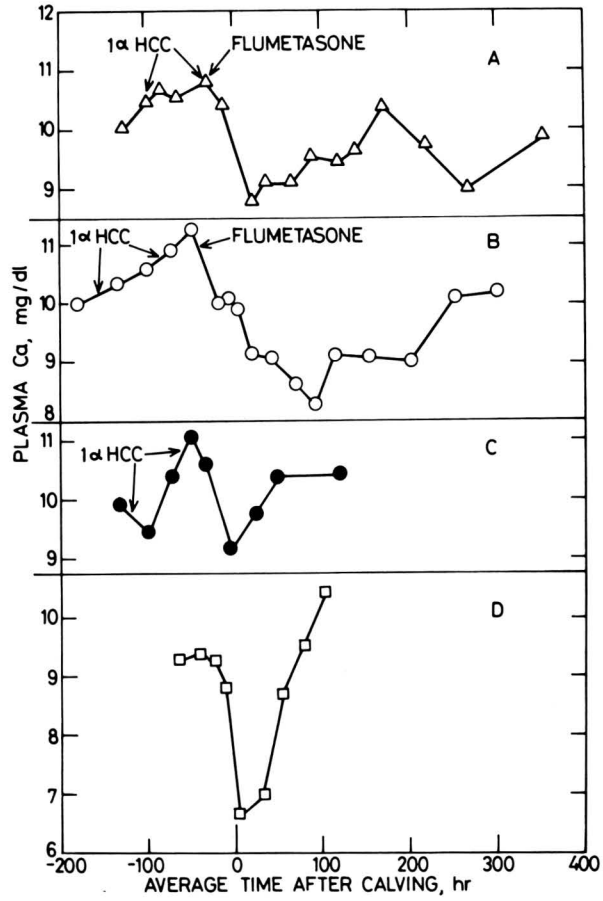

Fig. 5. lα-HCC in the prevention of parturient paresis:
A. 350 ug lα-HCC injected twice prepartum at an interval of 72 hours; 5 mg flumethasone administered together with the second injection (average of 6 cows).
B. The same as in A but flumethasone injected 48 hours after the second injection of lα-HCC (average of 8 cows).
C. Two injections of 350 ug of lα-HCC prepartum at an interval of 72 hours (average of 6 cows).
D. Physiological hypocalcaemia without parturient paresis at the time of parturition (average of 5 cows).

Discussion

The present results indicate that lα-HCC has the ability to increase bovine plasma calcium levels at a dose as low as 50 ug and as soon as 24 hours after injection. At doses of 250 ug and 350 ug, the peak was reached 3-4 days post-injection, and remained elevated for 7 days. Repeating the dose 48 or 72 hours later increased the time span by 3-4 days,

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Table 1. Effect of lα-HCC injected prepartum on the occurrence of parturient paresis (trial 4)

No. of injections	No. of cows	No. contracting parturient paresis
0 (Control	60	22
1	40	1 (a)
2	18	1 (b)
2 + flumethasone with 2nd injection	6	0
2 + flumethasone 48 h after 2nd injection	13	0

(a) The material was injected less than 18 hours prepartum.
(b) The second injection was given less than 10 hours prepartum.

giving protection for 10-11 days. These levels were obtained in dry cows and served as a guide for successful prophylaxis of bovine parturient paresis.

High-calcium feeding, a common practice in countries using legumes for feed, such as alfalfa, in conjunction with one or two doses of the material, did not lead to toxic hypercalcaemia as observed in the chick by Hurwitz *et al.* (in preparation).

The use of parturition-inducing agents like flumethasone, whether given in conjunction with lα-HCC or 48 hours later, is a method of ensuring that calving occurs whilst the cow is still under cover of lα-HCC and avoids the excessive use of the latter material.

The effect on plasma inorganic phosphate levels was double that on plasma calcium ones. Plasma magnesium levels were affected only minimally and none of our cases or those reported by other workers developed hypomagnesaemia as a result of being injected with lα-HCC. It should be pointed out, however, that the total feeding schedule includes an adequate supply of magnesium. Some of the failures to respond to lα-HCC reported by Davies *et al.* (1978) were attributed to hypomagnesaemia being present in the herd.

The success of prophylactic treatment depends upon administering the drug at the correct time ante-partum. At least 24 hours are required before an effect is noted. The criteria for the anticipated parturition was the calving date, relaxation of the sacroiliac ligaments, appearance of the cervical mucous plug, and the state of the udder and teats. The 10-11 days of protection covered most of our cases, the addition of flumethasone covering the rest. Our observation on 77 treated and 60 control animals showed that lα-HCC is a valuable agent in the prevention of bovine parturient paresis.

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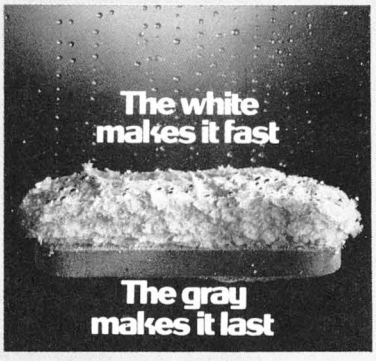

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