Efficacy of 1α hydroxyvitamin D_3 in the Prevention of Bovine Parturient Paresis

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One hundred and seventeen Israeli-Friesian cows from herds with a milk fever incidence of more than 15 percent were injected intramuscularly with either 350 μg 1α hydroxyvitamin D_3 (1αOHD_3) in propylene glycol or with the vehicle alone, close to calving. If parturition had not occurred within 72 hours a second injection was administered; parturition was induced two days after the second injection if necessary. There were 10 cases of milk fever among 57 control cows as opposed to two cases among the 60 animals treated with 1αOHD_3. In an attempt to prolong the effect of the drug, Israeli-Friesian cows were injected intramuscularly with 350 μg 1αOHD_3 in either 10 ml propylene glycol or arachis oil. 1αOHD_3 in arachis oil did not prolong the effect of the drug. 1αOHD_3 in propylene glycol increased plasma calcium concentrations more rapidly than when the drug was administered in oil. Additional cows of the same breed and age were injected intramuscularly with 350 μg 1αOHD_3 in propylene glycol. Five of the animals received a second dose four days, and five received a second dose five days after the first injection. Five animals served as uninjected controls. The plasma calcium levels of the injected cows were significantly higher ($P<0.01$) than those of the controls from the second until the 14th day after the first injection. Based on these results 451 Israeli-Friesian cows from herds with a milk fever incidence of more than 15 percent were injected intramuscularly with 1αOHD_3 close to calving. If parturtion had not occurred up to 100 hours later, a second injection was administered; parturition was induced two days after the second injection if necessary. There were 27 cases (6 percent) of milk fever among the 451 injected cows. The incidence in 68 animals injected less than one day before calving was 23.5 percent (16 cows). However, a significantly lower incidence (2.9 percent, $P<0.01$) occurred in the 383 cows which were injected more than one day before calving, with less than four days between the two injections of 1αOHD_3, and when parturition occurred not more than four days after the last injection of the drug either with or without induction of parturition.

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PARTURIENT paresis (milk fever) is a metabolic disorder of periparturient adult cows which is characterized by hypocalcaemia leading to ataxia, paresis, coma and death if it is not treated in time (Hibbs 1950, Ramberg and others 1970, Jorgensen 1974, Littledike and others 1981, Horst and Reinhardt 1983). The 1-hydroxylated metabolites of vitamin D or their derivatives have been studied to ascertain their effectiveness in preventing the hypocalcaemia associated with this disorder. These derivatives have been found to increase the plasma concentrations of the active hormonal form of vitamin D, 1,25 dihydroxyvitamin D (1,25$(OH)_2$D) calcium, and inorganic phosphate (Marquardt and others 1974, Sansom and others 1976, Barlet 1977, Sachs and others 1977, Hoffsis and others 1978, Gast and others 1979, Bar and others 1980, Manston and others 1981, Hove and others 1983). Parenteral administration of 1αhydroxyvitamin D_3 (1αOHD_3) to dairy cows increases the plasma 1,25 $(OH)_2D_3$ concentration for longer than injecting the equivalent amount of 1,25 $(OH)_2D_3$ (Hove and others 1983). An intramuscular injection of 350 μg of 1αOHD_3at least 24 hours before parturition protects cows from milk fever for three to four days (Sachs and others 1977). The efficacy of 1αOHD_3 depends on the accurate prediction of the calving time. In several cases, in order to ensure six days of protection, two injections of 1αOHD_3 were required followed if necessary by the induction of parturition (Bar and others 1980).

The purpose of the present study was first to investigate the efficacy of 1αOHD_3 in raising and maintaining plasma calcium levels in dry cows by changing the vehicle from propylene glycol to arachis oil or by increasing the time interval between the two injections and secondly to evaluate the efficacy of 1αOHD_3 injected at different intervals and either with or without the induction of parturition, in the prevention of milk fever under field conditions.

Materials and Methods

Trial 1

The purpose of this trial was to evaluate the efficacy of 350 μg 1αOHD_3 (Vetalpha; Teva) in 10 ml propylene glycol, in

preventing milk fever in comparison with animals injected with propylene glycol alone.

The drug and the placebo were supplied unlabelled to veterinarians for use in a 'double blind' study on the prevention of milk fever in Israeli-Friesian cows in herds with an incidence higher than 15 percent. The suggested treatment regimen was as follows.

(a) The first intramuscular injection of $1\alpha OHD_3$ or placebo was to be administered 72 to 24 hours before the estimated time of calving.

(b) A second injection was to be administered if calving had not occurred 72 hours after the first injection.

(c) If calving had not occurred 48 hours after the second injection parturition was to be induced by using 2.5 mg flumethasone (Fluvet; Teva).

Milk fever cases were to be treated with an intravenous injection of 600 ml of a 24 percent solution of calcium borogluconate containing 10.6 calcium.

Trial 2

The purpose of this trial was to compare the changes in plasma calcium concentrations in dry cows injected with 350 μg $1\alpha OHD_3$ in propylene glycol or arachis oil.

Twelve dry Israeli-Friesian cows which had calved at least three times were fed a commercial diet containing approximately 80 g of calcium and 50 g of phosphorus per day. They were divided into two groups of six animals and injected intramuscularly with either 350μg $1\alpha OHD_3$ in 10 ml propylene glycol or 350μg $1\alpha OHD_3$in 10 ml arachis oil. The cows were bled daily for 21 days after injection.

Trial 3

The purpose of this trial was to compare the changes in plasma calcium concentrations in dry cows injected twice with 350 μg $1\alpha OHD_3$ in propylene glycol at intervals of either four or five days.

Fifteen dry cows of the same breed and fed as above were divided into three groups of five. Cows in the first group were injected twice intramuscularly with 350 μg $1\alpha OHD_3$ in 10 ml propylene glycol at an interval of four days; cows in the second group were injected at an interval of five days; cows in the third group served as uninjected controls. Blood samples were taken from one day before the first injection until 20 days after the second injection.

Trial 4

The purpose of this trial was to evaluate the efficacy of 350 μg $1\alpha OHD_3$ in 10 ml propylene glycol, injected at different intervals, in preventing milk fever under field conditions.

The drug was supplied to veterinarians for use in herds of Israeli-Friesian cows with an incidence of milk fever higher than 15 percent. The suggested treatment regimen was as follows.

(a) The first intramuscular injection was to be administered between four days and one day before the estimated time of calving.

(b) A second injection was to be administered, if calving had not occurred, three to four days after the first injection.

(c) If calving had not occurred two days after the second injection parturition was to be induced.

Milk fever cases were to be treated as in trial 1.

Analytical procedures

Blood samples were taken into heparinised tubes and analysed for calcium as described previously (Sachs and others 1977).

Statistical methods

Means, standard deviations and standard errors, Student's t test, analysis of variance and χ^2 tests were computed according to standard procedures (Snedecor and Cochran 1968).

Results

Trial 1

Sixty cows were injected with the drug and 57 with the vehicle alone. The results are given in Table 1. Milk fever occurred in 10 of the 57 placebo treated cows (17.5 percent) and in two of the 60 cows treated with the drug (3.3 percent; $P<0.001$). Among the cows treated with $1\alpha OHD_3$ or the placebo the incidence of milk fever was not significantly ($P>0.05$) affected by the injection procedures. The two treated cows which developed milk fever had been injected with the drug less than 24 hours before parturition. There were no local or systemic clinically detectable signs of toxicity in any of the injected animals.

Trial 2

The changes in plasma concentration following the injection of 350 μg $1\alpha OHD_3$ in either propylene glycol or arachis oil are shown in Fig. 1. The maximal increases in plasma calcium were observed seven to eight days after the injection (1.51 ± 0.46 mg/dl and 1.00 ± 0.13 mg/dl for propylene glycol and arachis oil respectively). Forty-eight hours after injection plasma calcium concentrations were significantly higher ($P<0.05$) in cows injected with propylene glycol than in cows injected with arachis oil and they continued to be higher, although not significantly ($P>0.05$) for a further six days. Plasma calcium concentrations were still increased in

TABLE 1: Effect of injecting $1\alpha OHD_3$ pre-partum on the occurrence of parturient paresis (PP) (trial 1)

Number of injections	Induction after 2nd injection	Control Number of cows	Control Number of PP cases	Control PP incidence (%)	$1\alpha OHD_3$-treated Number of cows	$1\alpha OHD_3$-treated Number of PP cases	$1\alpha OHD_3$-treated PP incidence (%)
1	–	21	4	19·0	32	2	6·2
2	–	14	3	21·4	11	0	0
2	+	22	3	13·6	17	0	0
Total		57	10	17·5	60	2	3·3

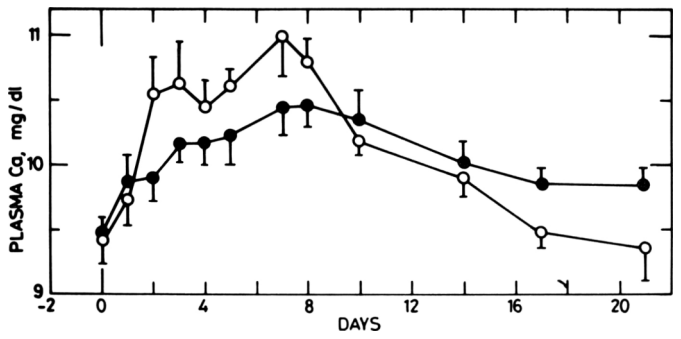

FIG 1: Changes in plasma calcium concentrations (mean±se) of six cows injected with 350 μg 1αOHD$_3$ in either propylene glycol (o) or arachis oil (●) during 21 days after the administration of the drug

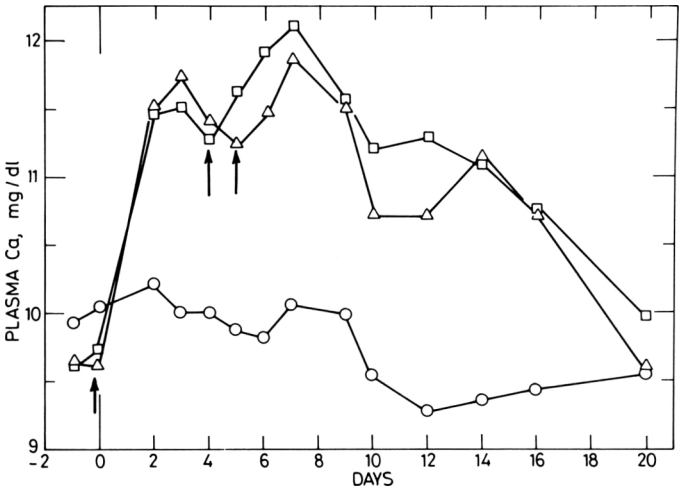

FIG 2: Changes in plasma calcium concentrations (mean±se) of five cows injected twice with 350 μg 1αOHD$_3$ in propylene glycol at either four (□) or five (△) day intervals, and of untreated cows (o) during 20 days after the administration of the drug

both groups 14 days after the injection.

Trial 3

The changes in plasma calcium concentrations following the injection of 350 μg 1αOHD$_3$ in propylene glycol at intervals of four and five days are shown in Fig. 2. Plasma calcium was significantly ($P<0.01$) increased by nearly 2 mg/dl in both groups of cows 48 hours after the first injection. In the cows injected at four day intervals a further rise of about 0.8 mg/dl was observed three days after the second injection, whereas in cows injected at five day intervals a rise of about 0.7 mg/dl was observed two days after the second injection.

The plasma calcium concentrations of the injected animals were significantly higher ($P<0.01$) than those of the controls from the second until the 14th day after the first injection. On the 16th day after the first injection plasma calcium was still higher ($P<0.05$) than in the uninjected cows.

Trial 4

Altogether 568 cows were injected with the drug. The data from 399 animals were reported in hours (Sachs and others 1983) and from the remaining cows in days. Three hundred and eighty-three cows were treated according to the protocol and the results, reported either in hours or days, are presented in Table 2. Thirty of the cows had calved three times and the rest four or more times. Three hundred and six animals were injected once and 43 and 102 cows were injected twice at intervals of three and four days, respectively. One hundred and eighty-five cows were not treated according to the protocol; 68 of them were injected only once and less than 24 hours before calving (Table 2).

The incidence of milk fever in those cows injected less than 24 hours before calving was 23.5 percent (16 of 68 cows), whereas in those injected between four days and one day before calving the incidence was only 2.5 percent (6 of 238 cows). This difference was significant ($P<0.001$).

The incidence of milk fever in the 24 cows injected twice at an interval of three days without induction of parturition was 4.2 percent and in the 19 cows in which parturition was induced it was 5.3 percent. Parturition was induced two days after the second injection and calving occurred not more than two days later.

None of the 58 cows injected at an interval of four days suffered from milk fever, whereas there were three cases in the 44 animals in which parturition was induced and calving occurred as above, an incidence of 6.8 percent. The overall incidence of the condition in the 383 cows injected according to the protocol was 2.9 percent (11 cases).

Induction of parturition was accomplished in most cases by an intramuscular injection of 2.5 mg flumethasone (Fluvet; Teva). The remaining animals were induced with either 20 mg dexamethasome phosphate (Dexivit; Vitamed), 0.5 mg cloprostenol (Estrumate; ICI) or 25 mg dinoprost tromethamine (Lutylase; Upjohn).

The clinical signs of milk fever were ataxia, paresis, coma and death. One of the 27 clinical cases died and one was slaughtered; severe fatty degeneration of the liver was found in the slaughtered cow. There were no clinically detectable signs of toxicity in any of the injected animals and neither the cow which died nor the slaughtered animal showed any signs of soft tissue calcification.

TABLE 2: Effect of injecting 1αOHD$_3$ pre-partum on the occurrence of parturient paresis (PP) (Trial 4)

Number of injections	Interval between injections (days)	Induction after 2nd injection	Calving after last injection (days)	Number of cows	Number of PP cases	PP incidence (%)
1	–		<1	68	16	23·5
1	–	–	1–4	238	6	2·5
2	3	–	0–2	24	1	4·2
2	3	+	<4	19	1	5·3
2	4	–	0–2	58	0	0
2	4	+	<4	44	3	6·8
Total				451	27	6·0
Total (without cows which calved <24 hours after injection)				383	11	2·9

Discussion

The results obtained in the first trial confirmed earlier observations (Sachs and others 1977, Bar and others 1980) that when $1\alpha OHD_3$ is injected 72 to 24 hours before parturition good protection against milk fever is achieved. Prolonging the effect of $1\alpha OHD_3$ appears to be essential for the most efficient use of the drug in preventing parturient paresis. Two methods were used to extend the effect of a single injection of $1\alpha OHD_3$. The results (Fig 1) indicate clearly that $1\alpha OHD_3$ in propylene glycol was more efficient in rapidly raising and maintaining plasma calcium levels than $1\alpha OHD_3$ in arachis oil. It is also clear (Fig 2) that plasma calcium concentrations remained above pre-injection levels for up to 16 days after dry cows were injected twice with $1\alpha OHD_3$ at an interval of four or five days.

On the basis of these results a field trial was designed in which propylene glycol was used as the vehicle for $1\alpha OHD_3$ and two injections were given up to four days apart instead of the two to three days suggested previously (Sachs and others 1977, Bar and others 1980). The results of this field trial confirmed previous observations on the efficacy of $1\alpha OHD_3$ in preventing milk fever. The overall incidence of milk fever in the 451 treated cows was 6.0 percent (27 cases), but there was a significant difference ($P<0.01$, χ^2 test) between those animals injected less than 24 hours before calving (23.5 percent) and those injected according to the protocol (2.9 percent). The lack of efficacy of a single dose of $1\alpha OHD_3$ given less than 24 hours before calving has been reported previously (Sachs and others 1977, Davies and others 1978, Bar and others 1980, Vagg and others 1981).

The authors' previous observations showed that two injections of $1\alpha OHD_3$ administered at an interval of two to three days ensured a high degree of protection (Bar and others 1980). The present results (Table 2) indicate that good protection was also achieved when the interval between the two injections was increased to four days, because none of the 58 cows contracted milk fever.

The reason for the failure of $1\alpha OHD_3$ to protect 11 of the 383 animals (Table 2) injected as recommended is not clear. It may have been due to variations in the rate of release of the drug from the site of injection or in its rate of hydroxylation to 1,25 $(OH)_2D_3$, or to the inefficient absorption of calcium from the small intestine of these animals.

Owing to practical considerations it was not possible to introduce a control group among the commercial herds. The incidence of milk fever in cows given different treatments (Table 2) was therefore compared either with the incidence among those injected less than 24 hours before calving or with the incidence reported previously in similar herds (Sachs and others 1977, Bar and others 1980). In either case there was a significantly ($P<0.05$ to $P<0.001$) lower incidence of milk fever in those cows injected with $1\alpha OHD_3$ as recommended.

The results suggest that 350 μg $1\alpha OHD_3$ in propylene glycol injected 96 to 24 hours before calving markedly reduced the incidence of milk fever. The reduced incidence was maintained for an additional 100 hours as a result of administering a second injection of the drug 96 hours after the first.

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