Technical Note: Vaginal Absorption of 1,25(OH)₂D₃ in Cattle

N. Okura,^{1,4} N. Yamagishi,² Y. Naito,³ K. Kanno,⁴ and M. Koiwa¹

¹Large Animal Clinical Center, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069-8501, Japan

²Research Center for Animal Hygiene and Food Safety, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan

³Department of Veterinary Clinical Medicine, Faculty of Agriculture, Iwate University, Morioka, Iwate 020-8550, Japan

⁴Central Veterinary Clinical Center, Kamikawa Chuo Agricultural Mutual Aid Association, Asahikawa, Hokkaido 078-8208, Japan

ABSTRACT

Although exogenous 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃] administered via intravenous, intramuscular, and oral routes has been tested for efficacy in preventing parturient hypocalcemia in dairy cows, there are few reports concerning intravaginal administration. For this study, 1,25(OH)₂D₃ was administered via the bovine vaginal lumen, and subsequent changes in blood chemistry, including levels of 1,25(OH)₂D₃ and several minerals, were measured to confirm vaginal absorption. Each of 5 heifers received a single intravaginal dose of 1 µg of 1,25(OH)₂D₃/kg body weight; a single control heifer received the ethanol carrier alone. In heifers receiving 1,25(OH)₂D₃, the plasma 1,25(OH)₂D₃ levels increased markedly from baseline (88.3 ± 20.3 pg/mL) within 2 h and reached a peak at 6 h after treatment (1967.4 ± 1139.6 pg/mL). Plasma Ca levels increased from baseline (10.4 ± 0.4 mg/dL) to a peak of 11.96 ± 0.7 mg/dL at 24 h. The levels of inorganic phosphate in plasma increased over time from 7.3 ± 0.5 to 8.1 ± 0.8 mg/dL by 6 h and were maintained at a plateau level (9.1 ± 0.7 to 8.6 ± 0.6 mg/dL) from 24 to 96 h after treatment. Plasma magnesium decreased from a baseline level of 2.1 ± 0.1 mg/dL to a plateau level of 1.8 ± 0.1 mg/dL, which was sustained from 24 to 48 h after treatment. The present study provides evidence of the absorption of exogenous 1,25(OH)₂D₃ from the bovine vaginal wall, as shown by the marked elevation of plasma 1,25(OH)₂D₃ levels by 2 h after administration, and indicates the possible utility of intravaginal administration of 1,25(OH)₂D₃ for prophylaxis of hypocalcemia.

(**Key words:** heifer, intravaginal administration, 1,25-dihydroxyvitamin D₃)

Abbreviation key: 1,25(OH)₂D₃ = 1,25-dihydroxyvitamin D₃, iP = inorganic P, VD₃ = vitamin D₃.

Vitamin D₃ (VD₃) is commonly used for prophylaxis of parturient hypocalcemia in dairy cows (Julien et al., 1977). Vitamin D₃ is metabolized in the liver to 25-hydroxyvitamin D₃ and subsequently in the kidney to 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃] (Horst and Reinhardt, 1983). Because 1,25(OH)₂D₃ is the most physiologically active form of VD₃ in the regulation of Ca metabolism, several investigators have tested the effects of administration of this form of VD₃ to dairy cows for the prevention of hypocalcemia (Gast et al., 1979; Hoffsis et al., 1979).

The vagina has been recognized as a route of drug administration since ancient Egyptian times (Benziger and Edelson, 1983). Currently, the intravaginal delivery of progesterone is widely applied to control the estrous cycle in cattle (Tjondronegoro et al., 1987). To the best of our knowledge, the vaginal absorption of 1,25(OH)₂D₃ has not been studied. We describe here the changes in blood levels of 1,25(OH)₂D₃ and several minerals after intravaginal administration of 1,25(OH)₂D₃ to heifers. The aim of the present study was to confirm the vaginal absorption of exogenous 1,25(OH)₂D₃ administered via the bovine vaginal lumen.

Six clinically healthy Holstein heifers (3 to 6 mo of age, 97 to 118 kg of BW) were penned and habituated to handling for at least 1 wk. They were fed 1.56 kg of grass hay, 0.55 kg of grain, and 1.44 kg of alfalfa hay cube daily, measured on a DM basis, and were given water ad libitum. The daily intake of minerals (21 g of Ca, 13 g of P, and 4 g of Mg) was well above NRC recommendations. The protocol and experimen-

Received January 11, 2004.

Accepted April 5, 2004.

Corresponding author: N. Okura; e-mail: n-ohkura@mvg.biglobe.ne.jp.

tal design were approved by the Rakuno Gakuen University Laboratory Animal Care and Use Committee.

Five heifers each received an intravaginal dose of 1 µg of 1,25(OH)₂D₃/kg of BW. The 1,25(OH)₂D₃ used in this study was in the form of crystal powder (a gift of the Mercian Corporation), which was dissolved in 99% ethanol at 1 mg/mL and frozen at -20°C until use. The preparation was administered into the intravaginal lumen using a 64-mm, 14-ga catheter sheath (Surflo, Terumo Co., Ltd., Tokyo) and a plastic syringe. A control heifer received 3.0 mL of 99% ethanol intravaginally.

Heparinized blood samples were taken from the jugular vein just before the administration of 1,25(OH)₂D₃ (0 h) and at 2, 6, 12, 24, 48, 72, and 96 h after treatment. The blood samples were centrifuged immediately to separate the plasma, which was frozen at -20°C until analyzed. The plasma concentration of 1,25(OH)₂D₃ was determined by radioimmunoassay (1,25(OH)₂D RIA kit; Immunodiagnostic Systems Limited, UK). The plasma Ca concentration was determined by the orthocresolphthalein complexone method (Connerty and Briggs, 1966), inorganic P (iP) by the Mo method (Drewes, 1972), and Mg by the xylyl blue method (Chromy et al., 1973).

The blood chemistry values were expressed as means ± standard deviations. Repeated measures one-way ANOVA was used to determine the significance of variation in the 1,25(OH)₂D₃-treated group. Dunnett's multiple comparison test was used to determine the significance of values in comparison with the baseline value (0 h). The threshold for significance was $P < 0.05$.

There were significant changes in the plasma concentrations of 1,25(OH)₂D₃, Ca, iP, and Mg in the heifers that received an intravaginal dose of 1,25(OH)₂D₃, whereas the plasma concentrations of those variables, with the exception of iP, were not affected by the administration of ethanol (Figures 1 and 2). The plasma 1,25(OH)₂D₃ levels increased significantly from 88.3 ± 20.3 pg/mL at 0 h to 1967.4 ± 1139.6 pg/mL ($P < 0.01$) at 6 h after the administration of exogenous 1,25(OH)₂D₃ and fell thereafter. The plasma Ca concentration was significantly higher at 12 to 72 h ($P < 0.01$) after the administration of 1,25(OH)₂D₃ compared with the level at 0 h (10.4 ± 0.4 mg/dL); it peaked at 24 h (11.96 ± 0.7 mg/dL). The changes in the plasma iP concentration observed in the treatment group were similar to those in the control heifer, which received ethanol only. The plasma iP levels were significantly higher at 6 h ($P < 0.05$) and from 24 to 96 h ($P < 0.01$) after treatment compared with baseline levels. The plasma Mg levels in the treatment group were significantly lower at 24 and 48 h (1.8 ± 0.1 and 1.8 ± 0.1 mg/dL,

Figure 1. Changes in plasma concentrations of 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃] in heifers receiving intravaginal 1,25(OH)₂D₃ (n = 5; ●) or ethanol (n = 1; ■). Significant difference from the value before administration of 1,25(OH)₂D₃ (0 h): ** $P < 0.01$.

respectively) compared with the level at 0 h (2.1 ± 0.1 mg/dL; $P < 0.01$).

The results of this study present evidence of measurable absorption of exogenous 1,25(OH)₂D₃ from the bovine vaginal wall, as indicated by a marked elevation in the plasma 1,25(OH)₂D₃ concentration at only 2 h after treatment. The major biological actions of 1,25(OH)₂D₃ are to increase the plasma Ca and iP concentrations by increasing intestinal absorption (Garabedian et al., 1974). However, in our study, a diphasic change in the plasma iP level was observed in the control heifer that received ethanol only; the pattern was similar in the 1,25(OH)₂D₃-treated group. We suggest that the changes in plasma iP concentration described here resulted not only from the administration of exogenous 1,25(OH)₂D₃, but also from ethanol absorbed through the vaginal wall, consistent with a previous report (Matsubara et al., 1982). There have been several reports regarding hypomagnesemia after the intramuscular administration of 1,25(OH)₂D₃ in adult cows (Hoffsis et al., 1979; Hove et al., 1983; Moate et al., 1987). The present study also demonstrated a decrease in plasma Mg concentration at 24 and 48 h after the intravaginal administration of 1,25(OH)₂D₃. The cause of this hypomagnesemia is unclear, but it has been suggested that 1,25(OH)₂D₃

Figure 2. Changes in plasma concentrations of Ca, inorganic P, and Mg in heifers receiving intravaginal 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃] (n = 5; ●) or ethanol (n = 1; ■). Significant difference from the value before administration of 1,25(OH)₂D₃ (0 h): **P* < 0.05; ***P* < 0.01.

causes an increase in renal excretion of Mg directly (Levine et al., 1980) or indirectly (Littledike and Goff, 1987).

Goff and Horst (1990) indicated 3 problems that impede the widespread use of the intramuscularly injection of 1,25(OH)₂D₃ or its analogues for the prevention

of parturient hypocalcemia. The principal problem is the difficulty in timing the treatment appropriately; the potent toxicity of these drugs and the occurrence of delayed parturient paresis also pose difficulties. We suggest that the intravaginal administration of 1,25(OH)₂D₃ could become a useful means of overcoming these problems because of the advantages offered by this route of administration as enumerated by Woolfson et al. (2000): its accessibility without medical equipment, the efficient absorption of substances from the vagina without first-pass metabolism by the liver, and the possibility of multiple forms for drug delivery (e.g., gels, tablets, pessaries, microspheres, rings). Therefore, we conclude that the intravaginal administration of 1,25(OH)₂D₃ offers a possible alternative treatment of prophylaxis of parturient hypocalcemia, although further studies are needed to confirm the absorption of 1,25(OH)₂D₃ from the vagina of adult cows.

ACKNOWLEDGMENTS

The authors thank A. Watanabe, Pharmaceuticals & Chemicals Division, Mercian Corporation, for supplying 1,25(OH)₂D₃.

REFERENCES

- Benziger, D. P., and J. Edelson. 1983. Absorption from the vagina. *Drug Metab. Rev.* 14:137–168.
- Chromy, V., V. Svoboda, and I. Stepanova. 1973. Spectrophotometric determination of magnesium in biological fluids with xylidyl blue II. *Biochem. Med.* 7:208–217.
- Connerty, H. V., and A. R. Briggs. 1966. Determination of serum calcium by means of orthocresolphthalein complexone. *Am. J. Clin. Pathol.* 45:290–296.
- Drewes, P. A. 1972. Direct colorimetric determination of phosphorus in serum and urine. *Clin. Chim. Acta* 39:81–88.
- Garabedian, M., Y. Tanaka, M. F. Holick, and H. F. Deluca. 1974. Response of intestinal calcium transport and bone calcium mobilization to 1,25-dihydroxyvitamin D₃ in thyroparathyroidectomized rats. *Endocrinology* 94:1022–1027.
- Gast, D. R., R. L. Horst, N. A. Jorgensen, and H. F. Deluca. 1979. Potential use of 1,25-dihydroxycholecalciferol for prevention of parturient paresis. *J. Dairy Sci.* 62:1009–1013.
- Goff, J. P., and R. L. Horst. 1990. Effect of subcutaneously released 24F-1,25-dihydroxyvitamin D₃ on incidence of parturient paresis in dairy cows. *J. Dairy Sci.* 73:406–412.
- Hoffsis, G. F., C. C. Capen, M. E. Placke, and A. W. Norman. 1979. The use of 1,25-dihydroxycholecalciferol in the prevention of parturient hypocalcemia in dairy cows. *Bovine Pract.* 13:88–95.
- Horst, R. L., and T. A. Reinhardt. 1983. Vitamin D metabolism in ruminants and its relevance to the periparturient cow. *J. Dairy Sci.* 66:661–678.
- Hove, K., R. L. Horst, and E. T. Littledike. 1983. Effects of 1 α -hydroxyvitamin D₃, 1,25-dihydroxyvitamin D₃, 1,24,25-trihydroxyvitamin D₃, and 1,25,26-trihydroxyvitamin D₃ on mineral metabolism and 1,25-dihydroxyvitamin D concentrations in dairy cows. *J. Dairy Sci.* 66:59–66.
- Julien, W. E., H. R. Conrad, J. W. Hibbs, and W. L. Crist. 1977. Milk fever in dairy cows. VIII. Effect of injected vitamin D₃ and calcium and phosphorus intake on incidence. *J. Dairy Sci.* 60:431–436.

- Levine, B. S., N. Brautbar, M. W. Walling, D. B. N. Lee, and J. W. Coburn. 1980. Effects of vitamin D and diet magnesium on magnesium metabolism. *Am. J. Physiol.* 239:E515–E523.
- Littledike, E. T., and J. Goff. 1987. Interactions of calcium, phosphorus, magnesium and vitamin D that influence their status in domestic meat animals. *J. Anim. Sci.* 65:1727–1743.
- Matsubara, K., M. Nakahara, S. Takahashi, and Y. Fukui. 1982. Acute effects of ethanol and acetaldehyde on plasma phosphate level. *J. Pharm. Pharmacol.* 34:373–376.
- Moate, P. J., K. M. Schneider, D. D. Leaver, and D. C. Morris. 1987. Effect of 1,25-dihydroxyvitamin D₃ on the calcium and magnesium metabolism of lactating cows. *Aust. Vet. J.* 64:73–75.
- Tjondronegoro, S., P. Williamson, G. J. Sawyer, and S. Atkinson. 1987. Effect of progesterone intravaginal devices on synchronization of estrus in postpartum dairy cows. *J. Dairy Sci.* 70:2162–2167.
- Woolfson, A. D., R. K. Malcolm, and R. Gallagher. 2000. Drug delivery by the intravaginal route. *Crit. Rev. Ther. Drug Carrier Syst.* 17:509–555.