Application of a Reservoir-Type Calcitriol Transdermal Patch in Dairy Cattle

Norio YAMAGISHI1,2,*, Toru NAMIOKA1, Norimoto OKURA4, Shigeru SATO1,3, Danil KIM3, Kazuhisa FURUHAMA2,3 and Yoshitsa NAITO1

1)Departments of Veterinary Clinical Medicine and 2)Veterinary Basic Medicine, Faculty of Agriculture, Iwate University, Morioka, Iwate 020–8550, 3)United Graduate School of Veterinary Sciences, Gifu University, Gifu 501–1193 and 4)Biei Veterinary Clinical Center, Kamikawa Chuo Agricultural Mutual Aid Association, Biei, Hokkaido 071–0214, Japan

(Received 25 January 2009/Accepted 17 February 2009)

ABSTRACT. Our objective was to evaluate the effects of a reservoir-type calcitriol (5 mg/animal) transdermal patch on plasma calcitriol and calcium (Ca) concentrations in dairy cattle. A group of six heifers received three different types of patches: an encapsulated reservoir solution containing calcitriol alone (CAL), calcitriol with dodecylamine (CAL+D), or vehicle (CONT). The patches were applied to the skin of the tail for duration of 2 days at intervals of at least 3 weeks. Cattle that received CAL or CAL+D showed significant increases in plasma calcitriol and Ca concentrations on day 2 and days 2 and 3, respectively. The areas under the plasma concentration-time curve (AUC) values for plasma calcitriol and Ca in the CAL and CAL+D groups increased significantly compared to the CONT group. The data of this study describe evidence of measurable transdermal absorption of exogenous calcitriol from a patch and its sufficient biological action to elevate plasma Ca concentrations.

KEY WORDS: calcitriol, calcium, cattle, reservoir-type transdermal patch.

Vitamin D₃ (VD₃), which is metabolized in the liver to 25-hydroxyvitamin D₃ and subsequently to 1,25-dihydroxyvitamin D₃ (calcitriol) in the kidneys [6], is commonly used as a prophylaxis for parturient hypocalcemia in dairy cows [8]. Calcitriol is the most active form of VD₃ involved in the regulation of calcium (Ca) metabolism; however, it is difficult to accurately determine a feasible time course for the administration of calcitriol due to difficulties in predicting the actual calving date. Therefore, opinions vary as to whether the intramuscular or intravaginal administration of calcitriol in dairy cows prevents hypocalcemia [5, 16–18, 20].

Within the veterinary field, the topical application of a wide range of pharmaceutical agents has been reported to be a useful alternative to traditional delivery routes [12]. For example, compared to parenteral administration, drug delivery via the skin has many benefits. Specifically, topical application is a non-invasive procedure that supplies a constant level of the drug within a narrow therapeutic window (thus minimizing the risk of adverse effects or the reduction of efficiency) and avoids gastrointestinal disturbances and hepatic first-pass effects [3]. Previously, we reported that the topical delivery of a calcitriol liquid formulation onto the ventral root skin of the tail elicits incremental increases in plasma calcitriol and subsequent increases in plasma Ca in dairy cattle [13]. Unfortunately, this delivery method is unlikely to be suitable for clinical use; the liquid formulation was applied to cattle in a supine recumbent position under xylazine sedation.

A reservoir-type transdermal patch is convenient method for the systemic delivery of human medicines. With the patch, drug permeability can be controlled over a long period relative to topical delivery [19]. To our knowledge, however, little information is available on the use of transdermal calcitriol patches in cattle. Here, we examined the effects of experimentally fabricated reservoir-type transdermal patches containing calcitriol (5 mg/animal) on plasma calcitriol and Ca concentrations in dairy cattle. Additionally, the additive effects of dodecylamine, an enhancer used to increase drug permeability via the skin [1], were also investigated.

Six healthy, non-pregnant Jersey heifers (age, 12–23 months; body weight, 135–311 kg) were used in this investigation. Grass hay (0.4% Ca and 0.3% phosphate of dietary dry matter) and water were provided ad libitum throughout the study. Subjects were treated with one of three types of patches containing a 500 μl reservoir solution made of 10 μg/μl calcitriol in vehicle (99% ethanol; CAL), 10 μg/μl calcitriol with 10 μg/μl dodecylamine in vehicle (CAL+D), or vehicle alone as a control (CONT). Treatments were assigned according to a randomized block design. Treatment intervals were at least 3 weeks in length. During the experiments, each patch was applied once to the ventral root skin of the tail and bandaged with elastic adhesive tape (Sylkytex-E, Alcare Co., Ltd., Japan). The patch was removed 2 days later. The dosages and/or concentrations of calcitriol and dodecylamine were chosen based on the results of previous studies examining the topical administration of calcitriol [13] and the efficacy of testosterone patches [10], respectively. The study protocol and the experimental design were approved by the Iwate University Laboratory Animal Care and Use Committee.

Crystalline calcitriol (powder form) was provided by Mercian Corporation (Tokyo, Japan). Dodecylamine was
The reservoir solution were made of 10 µg/µl calcitriol in vehicle (99% ethanol; CAL), 10 µg/µl calcitriol with 10 µg/µl dodecylamine in vehicle (CAL+D), or vehicle alone as a control (CONT).

Heparinized blood samples were drawn from the jugular vein of each heifer immediately before application of the patches (day 0) and again at 0.25, 0.5, 1, 2, 3, and 5 days later. The day of application was designated as day 0. The blood was centrifuged immediately to separate plasma for the determination of calcitriol and Ca concentrations. Plasma specimens were frozen at –50°C until analyzed.

Plasma calcitriol concentrations were measured by radioimmunoassay (1,25(OH)2D RIA kit, Immunodiagnostics Systems Ltd., UK) and plasma Ca concentrations were measured using the orthocresolphthalein complexone method [2].

All numerical data are expressed as means ± standard deviations. One-way repeated measures analysis of variance (ANOVA), followed by Dunnett’s tests for multiple comparisons, was performed to analyze changes in plasma calcitriol and Ca concentrations. Specifically, each value was compared with the baseline value (day 0) for each patch. Differences in plasma calcitriol and Ca concentrations among the three patches were compared using one-way ANOVA and Tukey-Kramer multiple comparison tests. The areas under the plasma concentration-time curves (AUCs) for plasma calcitriol and Ca concentrations from 0 to 120 hr (days 0 to 5) were calculated using the linear trapezoidal rule. One-way ANOVA and Tukey-Kramer multiple comparison tests were performed to compare differences in the AUC0–120 hr among the three patches. The threshold for statistical significance was p<0.05.

Although erythema developed on the area of skin that contacted the patches in all groups (including the CONT group on day 2), this reaction disappeared within a few days.

Changes in plasma calcitriol and Ca concentrations are shown in Fig. 2A. Compared to the baseline value (day 0), no change in plasma calcitriol or Ca concentration was observed in the CONT group throughout the experimental period. In the CAL group, mean plasma calcitriol concentrations increased significantly from 41.3 pg/ml on day 0 to 169.6 pg/ml on days 1 and 2 (p<0.05), respectively. After day 2, calcitriol levels dropped. In the CAL+D group, mean plasma calcitriol concentrations increased from 69.3 pg/ml on day 0 to 236.6 pg/ml on day 2 (p<0.05), and fell thereafter. Compared to the CONT group, cattle exposed to either CAL or CAL+D exhibited significantly higher mean plasma calcitriol concentrations on day 2 (274.7 pg/ml in CAL and 236.6 pg/ml in CAL+D, p<0.05) and lower concentrations on day 5 (25.0 pg/ml in CAL and 22.1 pg/ml in CAL+D, p<0.05) . Likewise, mean plasma Ca concentrations increased significantly in both groups on days 2 and 3 (approximately 11 mg/dl, p<0.05) compared to the CONT group (10.0 to 10.3 mg/dl).

The AUC0–120 hr values for plasma calcitriol and Ca concentrations are shown in Fig. 2B. Both CAL and CAL+D groups displayed significantly higher AUC0–120 hr values for plasma calcitriol (p<0.01) and Ca (p<0.05) concentrations compared to the CONT group.
To the best of our knowledge, this study is the first to report evidence of measurable transdermal absorption of exogenous calcitriol from a patch. We observed elevated plasma calcitriol concentrations (approximately 250 pg/ml) on day 2 and an increased AUC_{0-120 hr} value for plasma calcitriol within the CAL and CAL+D groups. Calcitriol increases plasma Ca concentrations by facilitating intestinal absorption [4]. In cows, Hove et al. [10] reported that maximal increase in plasma Ca occurred when plasma calcitriol reached 250 pg/ml after calcitriol administration (intramuscular or oral administration of calcitriol or its analogues). In our study, we noted that cattle given CAL or CAL+D showed elevations in plasma Ca concentrations (approximately 11 mg/dl) on days 2 and 3. In addition, the AUC_{0-120 hr} values appear to be identical to findings in cattle treated with a topical liquid formulation of calcitriol [17]. These results demonstrated that calcitriol absorbed via the skin exerts sufficient biological action.

Meanwhile, plasma calcitriol concentrations decreased on day 5 in both the CAL and CAL+D groups. Previous investigations have also reported that the treatment of parturient cows with exogenous calcitriol causes prolonged decreases in plasma calcitriol concentrations approximately one week after intramuscular injection [20]. These findings are likely the result of endogenous calcitriol synthesis inhibition via 1α-hydroxylase due to exogenous calcitriol administration [11, 14].

Dodecylamine is a typical unsaturated fatty amine that has been shown to increase the skin permeability of various drugs dispensed through a patch (e.g., testosterone) [1, 10], ethanol, which was used as a vehicle in this study, is also reported to increase skin permeability in a wide range of in vivo and in vitro systems [9]. The mutual permeability-enhancing effect between dodecylamine and ethanol has been reported in a patch with testosterone [10]. However, in this study, no significant difference in AUC_{0-120 hr} for plasma calcitriol or Ca concentration was noted between the CAL and CAL+D groups. These results suggest that skin permeability of calcitriol was not improved by the addition of dodecylamine. It is also possible that the skin permeability of calcitriol in 99% ethanol has already reached its threshold, despite the addition of dodecylamine.

In conclusion, our results suggest that a patch procedure using calcitriol is easy, versatile, and reliable. Specifically, we found that plasma calcitriol concentrations increase to a physiologically acceptable level (approximately 250 pg/ml) [7] and that plasma Ca is maintained at a reasonable concentration (approximately 11 mg/dl) on days 2 and 3 post-application. For clinical use, however, further studies are necessary to improve skin permeability of calcitriol via changes in the device and the selection of appropriate enhancers and vehicles. Because percutaneous absorption may decrease with age [15], future studies are required to determine the rate of percutaneous absorption in adult cows.

ACKNOWLEDGMENTS. The authors thank Dr. A. Watanabe (Pharmaceuticals & Chemicals Division, Mercer Corporation) for supplying calcitriol and Mr. Y. Miyazaki (Drug Delivery System Project Department, 3M Health Care Limited) for the supplying 3M Scotchpack 9730 Backing and 3M CoTran 9728 Membranes. We also thank Mr. S. Akasaka, Mr. G. Abe, and Mr. H. Chida (University Research Farms, Iwate University) for their diligent care of the animals.

REFERENCES


