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Review

Is the metabolism of 25-hydroxyvitamin D_3 age-dependent in dairy cows?

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ABSTRACT

It has recently been demonstrated that prepartum administered 25-hydroxyvitamin D_3 (25-OHD_3) is a promising candidate to assist the maintenance of peripartal calcium homeostasis in dairy cows. Since the incidence of peripartal hypocalcemia and the reported beneficial effects of the treatment are both associated with the lactation number, we investigated pharmacokinetic aspects of 25-OHD_3 related to the age of dairy cows. The daily oral administration of 3 mg 25-OHD_3 in rapeseed oil as well as a treatment with 4 and 6 mg included in the feed during the last eight to ten days of gestation resulted in linear dosage- and age-dependent increases in plasma 25-OHD_3. After parturition the administration was stopped and blood samples were taken to calculate the plasma half-life. Irrespective of the supplemented dosage, cows starting the 2nd lactation showed a significantly longer plasma half-life of 25-OHD_3 than cows starting the 3rd or higher lactation. Age-dependent differences in the increase of plasma 25-OHD_3 could already be found before parturition when calcium homeostasis was not yet significantly challenged. Additionally, no correlations between plasma half-life of 25-OHD_3 and 1,25-dihydroxyvitamin D_3, PTH or the bone resorption marker CrossLaps were observed after parturition. Thus we conclude that the influence of the lactation number on the pharmacokinetics of 25-OHD_3 is related directly to the age of the cows.

This article is part of a Special Issue entitled 'Vitamin D Workshop'.

Contents

1. Introduction

It has recently been demonstrated that prepartum administered 25-hydroxyvitamin D_3 (25-OHD_3) is a promising candidate to assist the maintenance of peripartal calcium (Ca) homeostasis in dairy cows [1]. Since the incidence of peripartal hypocalcemia [2] and the reported beneficial effects of the treatment are both associated with the lactation number [1], we investigated pharmacokinetic aspects of 25-OHD_3 related to the age of dairy cows.

2. Materials and methods

2.1. Animals, treatments and sampling

Handling, treatment and sampling of the animals were approved and the conduct of the experiment was supervised according to the German Animal Welfare law.

In the 1st series, a total of 14 multiparous dairy cows were individually supplemented orally with 3 mg 25-OHD_3 (Rovimix Hy-D®,

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DSM Nutritional Products, Basel, Switzerland) dissolved in rapeseed oil once daily from the 270th day of gestation until parturition. Blood samples were taken every other day from the beginning of the experiment to evaluate the increase in plasma 25-OHD_3.

In the 2nd series 90 cows were divided into three groups. Two experimental groups were provided from the calculated day −10 until parturition (day 0) with either 4 or 6 mg 25-OHD_3 provided in the mineral feed additive, while the remaining 30 cows served as the untreated control group. Blood samples were taken every other day from the beginning of the experiment until parturition and on day 1, day 2, day 4, day 8, day 16 and day 32 postpartum.

2.2. Analytical methods

The concentrations of 25-OHD_3 and 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$) were determined by HPLC by the Analytical Research Center of DSM Nutritional Products (Kaiseraugst, Switzerland). Ionized calcium (Ca^{2+}) was determined in whole blood using a blood gas analyzer (Chiron Diagnostics, RapidLab 348, Bayer, Fenwald, Germany). Analyses for parathyroid hormone (PTH) and C-terminal telopeptide of type I collagen (CrossLaps, CL), a marker for bone resorption, were done using commercial kits (Bovine Intact PTH ELISA Kit, Immutopics Inc., San Clemente, CA; Serum CrossLaps ELISA, IDS GmbH, Frankfurt, Germany).

2.3. Calculations and statistical analyses

For the calculation of plasma half-life plasma concentrations of 25-OHD_3 in untreated animals were used as basal values to correct those of supplemented animals. The plasma concentrations versus time curves obtained for each individual animal in each treatment group (starting on day 1 after parturition when no additional 25-OHD_3 was supplemented until day 32 after calving) were analyzed with the PK Solutions 2.0 (Ashland, OH) computer software. Pharmacokinetic parameters were determined using the method of a non-compartmental model. The terminal (elimination) half-life ($t_{1/2}$el) was calculated as $\ln 2/(z$ where (z is the elimination rate.

Statistical analyses were performed using SPSS 19.0 (SPSS, Chicago, IL). Values are expressed as mean ± standard error of the mean. The numbers of animals investigated are given in brackets in the figure legends. Linear regression was used to analyze the prepartum increases in plasma 25-OHD_3 during the 1st series. In order to reveal effects of age on the peripartal time courses of plasma parameters we used repeated measurements ANOVA (lactation number and dosage) separately for untreated and HyD-supplemented cows. The Fisher Least Significant Difference (LSD) test was applied for comparison of estimated marginal means at each time point. Calculation of the Pearson product-moment coefficient was used to estimate the dependence between two variables (e.g. 25-OHD_3 half-life and PTH plasma concentration). Plasma half-lives of 25-OHD_3 were compared by two-way ANOVA (lactation number, dosage, interaction) followed by the Bonferroni post-test.

In all cases, P values < 0.05 were considered significant.

3. Results and discussion

3.1. Increase of plasma 25-OHD_3 during daily oral treatment before parturition

Fig. 1 shows the linear correlation between the prepartum plasma 25-OHD_3 concentrations and the duration of daily oral treatment with individually administered 25-OHD_3 (3 mg in rapeseed oil). The calculated slopes of the two different lactation number groups differ significantly (2nd lactation: 16.07 ± 0.87, ≥3rd lactation: 12.92 ± 0.69; P < 0.01).

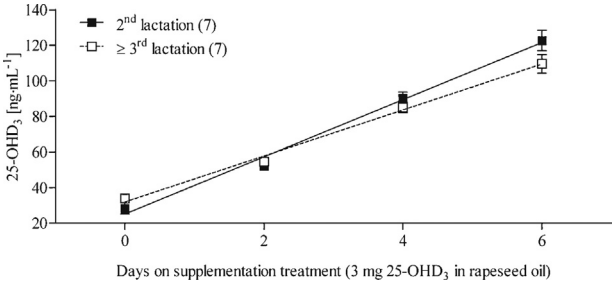

Fig. 1. Increase of plasma 25-OHD_3 during daily oral treatment with 3 mg 25-OHD_3 (1st series). Significant linear correlations can be found in supplemented animals. The slopes for the different lactation number groups differ significantly (P < 0.01).

From these results it can be concluded that either the absorption of 25-OHD_3 is more efficient in younger animals or the rate of elimination is faster in older cows.

3.2. Peripartal parameters of Ca homeostasis and plasma half-life of 25-OHD_3 postpartum

Regardless of whether cows received 25-OHD_3 supplementation or not, the decrease in whole blood Ca^{2+} concentrations at parturition was more pronounced in older cows (2nd lactation: 1.16 ± 0.08 mmol L^{-1}; ≥3rd lactation: 1.07 ± 0.13 mmol L^{-1}; Fisher LDS test: P < 0.01). This was accompanied by higher PTH levels on day 1 after parturition (2nd lactation: 105 ± 19 pg mL^{-1}; ≥3rd lactation: 202 ± 46 pg mL^{-1}; Fisher LSD test: P < 0.05) and a sharper increase in 1,25-$(OH)_2D_3$ concentrations in animals at the 3rd or higher lactations compared to younger cows (data not shown; ANOVA for repeated measurements: P < 0.05). In supplemented cows, plasma 25-OHD_3 concentrations of younger cows were significantly higher during the entire observation period (Figs. 1 and 2; ANOVA for repeated measurements: P < 0.05). An effect of age on CL concentrations could only be found in supplemented cows before parturition (data not shown).

The observation that peripartal hypocalcemia and the respective hormonal responses are more pronounced in older cows is in line with former studies [2]. Immediately after parturition, the higher

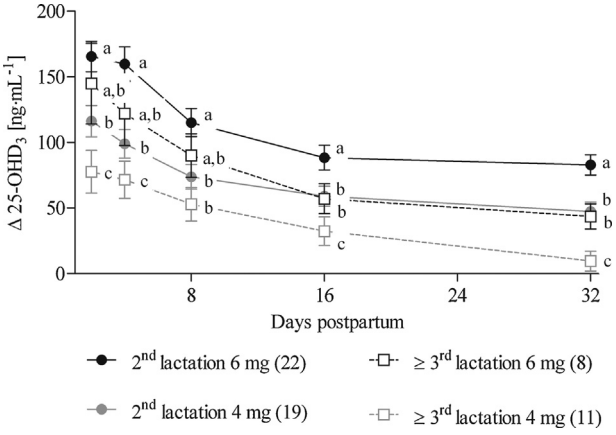

Fig. 2. Time courses of plasma 25-OHD_3 after parturition (2nd series), concentrations of supplemented animals corrected with respective values of untreated control animals. Effects of time (P < 0.001), dosage (P < 0.001), lactation number (P < 0.01) and an interaction of dosage and lactation number (P < 0.01) were revealed in supplemented animals using repeated measurements ANOVA. Results of Fisher LSD test for comparison of estimated marginal means are given within the figure.

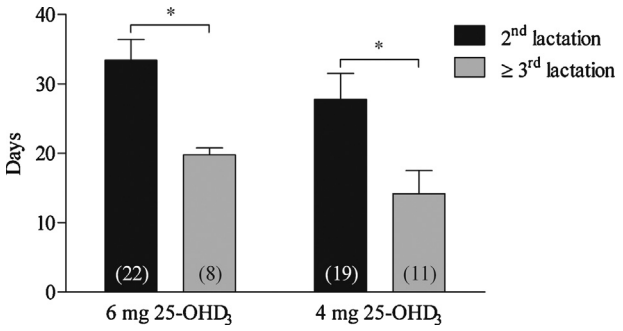

Fig. 3. Plasma half-lives of 25-OHD_3 in supplemented cows. Two-way ANOVA (dosage and lactation number) revealed an effect of lactation number ($P < 0.001$). Asterisks indicate results of Bonferroni post-test: $^*P < 0.05$.

1,25-$(OH)_2D_3$ concentrations might induce different pathways of vitamin D catabolism [3] and thus, a faster elimination of 25-OHD_3.

However, no correlations between plasma half-life of 25-OHD_3 and 1,25-$(OH)_2D_3$, peak concentrations of 25-OHD_3, PTH or the bone resorption marker CrossLaps could be found. Plasma half-life of 25-OHD_3 was only positively correlated with basal Ca^{2+} concentrations (0.342; $P < 0.01$), basal 25-OHD_3 (0.330; $P < 0.05$), 25-OHD_3 on day 8 postpartum (0.292; $P < 0.05$), on day 16 (0.409; $P < 0.01$) and on day 32 (0.579; $P < 0.01$).

When data of supplemented animals were compared with respect to age it was found that cows starting the 2nd lactation treated with 4 mg daily showed a significantly longer plasma half-life of 25-OHD_3 than cows starting the 3rd or higher lactation ($P < 0.01$). The same significant difference in plasma half-life could be observed in animals treated with 6 mg 25-OHD_3 daily ($P < 0.01$). Although plasma half-life of animals dosed with 6 mg seemed to be longer compared to the respective 4 mg group, an effect of dosage could not be verified statistically (Fig. 3).

As no impact of the more pronounced hypocalcemia in older cows and the corresponding stronger hormonal response to this challenge could be found, the longer plasma half-life of 25-OHD_3 in younger animals is most probably caused by an age-dependent slower rate of degradation of 25-OHD_3. This could involve a lower expression and/or activity of the 24-hydroxylase of younger animals in comparison to older individuals which has already been demonstrated in the rat [4]. Although this assumption is supported by the positive correlation between the calculated plasma-half life postpartum and the basal concentrations of 25-OHD_3 prepartum when 1,25-$(OH)_2D_3$ levels were low, further studies are needed to prove this hypothesis.

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