MEDICAL SCIENCE MONITOR

LAB/IN VITRO RESEARCH

e-ISSN 1643-3750
© Med Sci Monit, 2019; 25: 9159-9166
DOI: 10.12659/MSM.917904

Received: 2019.06.03
Accepted: 2019.08.06
Published: 2019.12.02

Vitamin D3 Is Transformed into 1,25(OH)$_2$D$_3$ by Triggering CYP3A11(CYP3A4) Activity and Hydrolyzing Midazolam

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

BCDEF Hanfei Zhu
BCDF Ruihan Wu
BCF Zijun Gu
ADEG Minghui Ji
ABCD Qin Xu

School of Nursing, Nanjing Medical University, Nanjing, Jiangsu, P.R. China

Corresponding Authors: Minghui Ji, e-mail: jiminghui77@sina.com, Qin Xu, e-mail: qinxu@njmu.edu.cn
Source of support: This work was supported by the Natural Science Foundation of Jiangsu province (Grant No. BK20180678) and the Natural Science Foundation of Jiangsu University (Grant No. 17KJB330004)

Background: Vitamin D3 (VD3) is a commonly used supplement in clinical practice. Cytochrome P450 3A11 (CYP3A11) is the most important monomeric enzyme involved in metabolism of drugs. This study aimed to investigate effects of vitamin D3 (VD3) on CYP3A11 activity.

Material/Methods: Forty male Sprague-Dawley (SD) rats were randomly divided a Control group (peanut oil 0.1 ml/kg/d), a Low-VD3 group (100 IU/kg/d), a Medium-VD3 group (400 IU/kg/d), and a High-VD3 (1600 IU/kg/d) group. Blood samples were collected from the jugular vein after midazolam (MDZ) administration. CYP3A11 expressions in liver and colon were detected by Western blotting and immunohistochemistry (IHC) assay. The concentration of serum 25(OH)D$_3$ and serum 1,25(OH)$_2$D$_3$ were evaluated using ELISA. Effects of different dosages of vitamin D3 on metabolism of MDZ were evaluated using high-performance liquid chromatography (HPLC).

Results: Vitamin D3 significantly enhanced serum 25(OH)D$_3$ and 1,25(OH)$_2$D$_3$ levels in rats compared to Control rats (p<0.05). Expressions of hepatic CYP3A11 were more than 10-fold higher in rats treated with vitamin D3 compared to Control rats (p<0.05). Expressions of colon CYP3A11 were 5-fold higher than in Control rats (p<0.05). CYP3A11 expressions in vitamin D3-treated groups were significantly higher compared to the Control group (p<0.05). MDZ levels were significantly higher in vitamin D3-treated rats compared to that in Control rats (p<0.05). Concentrations of serum MDZ at every sampling point were remarkably lower in the vitamin D3-treated rats than in Control rats (p<0.05).

Conclusions: Vitamin D3 was transformed into 1,25(OH)$_2$D$_3$ by triggering CYP3A11 and CYP3A11 activity and by hydrolyzing MDZ.

MeSH Keywords: 24,25-Dihydroxyvitamin D3 • Basal Metabolism • Midazolam

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/917904

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Background

Vitamin D3 (VD3) is a commonly used supplement in clinical practice [1]. It not only participates in the metabolism of calcium and phosphorus in the human body, but also promotes its absorption and plays a key role in bone formation [2]. In recent years, more and more studies reported that the role of vitamin D3 far exceeds the traditionally accepted range, and has been proven to be related to endocrine diseases [3], cardiovascular diseases [4,5], tumors [6], and immunity [7]. Vitamin D3 is usually hydroxylated to the activated form of $25(OH)D_3$ in the livers of humans and animals undergoing 25-hydroxylase activation, and this process is a classical circulating cycle for vitamin D3. Its half-life is 2–3 weeks, so it is used to evaluate serum vitamin D3 levels. In the kidneys and the other extrarenal sites, $25(OH)D_3$ can also be hydroxylated by the CYP27B1 to generate the $1,25(OH)_2D_3$, which is a bioactive form with a half-life of only 4–6 h [8].

There are many subtypes in the cytochrome P450 3A (CYP3A) protein family, including CYP3A4 and CYP3A5 in adults. CYP3A4 has been proven to be the most critical monomeric enzyme involved in drugs metabolism. It is mainly found in the liver and small intestinal mucosal epithelial cells. More than 60% of drugs on the market today are metabolized by CYP3A4, which is related to the drug and drug interaction [9].

In rats, CYP3A11, a homologous cytochrome P450 (CYP) enzyme to CYP3A4, has potent interactions with vitamin D3 [10]. Effects of CYP3A enzymes have been proven to play important roles in colon and liver tissues and associated cells of rats and humans [11].

Midazolam (MDZ), which is a short-acting benzodiazepine, is used clinically for conscious sedation by CPY3A4 and CYP3A5 in humans and by CYP3A1 and CYP3A2 in rats. MDZ is regarded by the FDA as a promising probe for evaluating the activity of CYP3A11 (CYP3A4) based on *in vitro* and *in vivo* investigations. MDZ is also extensively applied for assessing the expression of CYP3A in rats and humans.

The present study was conducted to assess the induction effects of vitamin D3 on CYP3A11 (CYP3A4) in rats and CYP3A11 (CYP3A4)-mediated MDZ pharmacokinetic changes induced by vitamin D3.

Material and Methods

Chemicals and reagents

All reagents used were of the highest grade commercially available. MDZ, vitamin D3, and $1,25(OH)_2D_3$ were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol and acetonitrile of HPLC-MS grade were purchased from Merck (Darmstadt, Germany). Enzyme-linked immunosorbent assay (ELISA) kits for detecting serum $25(OH)D_3$ and $1,25(OH)_2D_3$ concentrations were purchased from Gene Beauty Biotechnology (Wuhan, China). All the other reagents were of commercial or analytical grade and required no further purification. All other solvents were of analytical grade or high-performance liquid chromatography (HPLC) grade. Ultra-pure water was prepared by using the Milli Q-plus system (Billerica, MA).

Animals

We obtained 40 male Sprague-Dawley (SD) rats (6 to 8 weeks old, weighting 200 to 220 g) from the Animal Center of Nanjing Medical University (Nanjing, China) and acclimatized them for at least 7 days before being assigned to their experimental groups. Rats were fed a standard laboratory diet and housed in an air-conditioned room maintained at 22°C to 24°C with a relative humidity of 55–60% and day/night cycle of 12 h/12 h. The rats were provided semi-purified rat chow (Charles River, Japan) and water *ad libitum*. The 40 male rats were randomly divided into 4 groups (using randomized blocks design method), including a Control group (n=10), a Low group (n=10), a Medium group (n=10), and a High group (n=10). Vitamin D3 was intragastrically administered once daily for 7 consecutive days at the final concentrations of 100, 400, and 1600 IU/kg for the Low, Medium, and High groups, respectively. Control rats were administered vehicle (with 0.1 ml/kg/d peanut oil) alone. Rats were fasted for 8 h after the last intragastric administration. After fasting for 16 h, MDZ (1 mg/ml) was injected into the tail vein at a dosage of 10 mg/kg. At different time points (0, 5, 10, 15, 30, 45, 60, 90, 120, 180, 240, and 480 min), a total of 0.2 ml blood was collected from the jugular vein of rats. Rats were killed on day 7 and the liver and intestinal tissues were collected for the following analyses. Blood was collected into citrated tubes to harvest the plasma by centrifuging at 600 g for 10 min and then at 18 000 g for 2 min (2-step approach). Then, the obtained plasma samples were stored at –80°C until use. Immediately prior to analysis, the serum was isolated from the plasma by centrifuging at 1000 g for 15 min. For the collected liver and intestinal tissues, half of the tissues were frozen at –80°C until use, and the half of tissues were embedded in paraffin and stored at –80°C. All samples were stored at –80°C until analysis. The whole study process is illustrated in Figure 1.

All animal experiments and tests were approved by the Experimental Animal Ethics Committee of Nanjing Medical University (Nanjing, China).

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

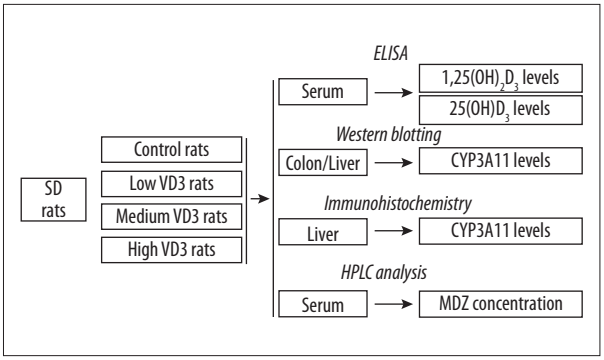

Figure 1. The study design graph for experiments.

Measurement of concentrations of serum 25(OH)D_3 and serum 1,25(OH)$_2D_3$

The concentrations of serum 25(OH)D_3 and serum 1,25(OH)$_2D_3$ were measured with the 25(OH)D_3 ELISA Detection kit (Cat. No. AE90635Ra, LianShuo Biol., Shanghai, China) and the 1,25(OH)$_2D_3$ ELISA kit (Cat. No. AE92083Ra, LianShuo Biol.), respectively. ELISA was conducted strictly according to the manufacturers' instructions and regular ELISA protocols. Stored plasma samples from every group were centrifuged at 1000×g at 4°C for 15 min, followed by ELISA. The absorbance (optimal density, (OD) value) of each well was measured sequentially with a micro-plate reader at a wave length of 450 nm. The standard curve regression equation was calculated, and the corresponding sample concentration was calculated based on the regression equation according to the OD value of samples. The final concentration was the actual measured concentration multiplied by 5.

Preparation of standard midazolam solution

Midazolam (5 mg/ml, 200 µl) was added into 800 µl of normal saline and diluted to a concentration of 1 mg/ml of midazolam solution, and then serially diluted to give a final concentration of 0.2 mg/ml, 0.02 mg/ml, 0.01 mg/ml, and 0.005 mg/ml. A standard stock solution of diazepam (IS) at 5 mg/ml was also diluted to obtain a working solution at 1 mg/ml. The stock solutions were stored at –80°C, and the working solutions were stored at –20°C and were warmed to room temperature before use. Calibrated standard samples were prepared by spiking blank rat plasma with standard working solutions.

HPLC analysis

Blood samples were collected and then immediately placed into heparin-prepared centrifuging-tubes, then centrifuged for 10 min at 3000×g. The obtained supernatants were placed into 1.5-ml centrifuge tubes. For some of these samples, we added 10 µl internal standard diazepam solution (1 mg/ml) into the plasma sample (40 µl). A total of 200 µl extracts (isopropyl alcohol: ethyl acetate=2: 8) were added into the mixture. Portions of other blood samples were used to conduct HPLC analysis. For both internal standard and samples, after the tube was vortex-mixed for 1.0 min using a vortex mixer, the sample was centrifuged at 10 000 rpm for 5 min at 4°C. With the supernatant removed, the sample was evaporated to dryness by a nitrogen bath at 37°C and reconstituted with 100 µl methanol, then the supernatant (5 µl) was injected directly into the HPLC system for analysis. All of the above blood or plasma samples were fresh, without cryopreservation.

Measurement of midazolam concentration

Analysis of midazolam concentration was performed with a 1260 Series liquid chromatograph (Agilent Technologies, Waldbronn, Germany) equipped with a quaternary pump for solvent, a degasser, and an auto-sampler for sample delivery. We used a Welchrom-C18 analytical column (with the volume of 4.6 mm×150 mm, thickness of 5 µm) purchased from Welch Materials (Austin, TX, USA) and maintained at 25°C. The mobile phase was composed of methanol and water with the proportion of 1: 1 from 0 to 5 min. The flow rate was 1.0 ml/min. The injection volume was 5 µl. The quantification was performed by the peak area method.

Protein extraction and Western blot detection

A total of 100 mg intestinal tissue samples and 100 mg liver tissue samples were isolated from rats and rinsed with phosphate-buffered saline (pH 7.4). Then, the tissues were smashed and lysed using radioimmunoprecipitation assay (RIPA) buffer containing protease inhibitor mixture. After homogenizing with a glass homogenizer for 1 min, tissues were homogenized using a professional ultrasonic cell disrupter. Then, the lysates were centrifuged at 12 000 rpm at 4°C for 4 min, and the supernatants were harvested. The protein contents of samples were measured using a bicinchoninic acid (BCA)-protein assay kit (Beyotime Biotechnology, Beijing, China). After quantification, a total of 20 µl protein sample was loaded into a 12.5% separation gel with 5% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride (PVDF) membranes using a 220 mA steady current for 110 min. PVDF membranes were then blocked using 5% skim milk dissolved in 0.1% Tris-buffered saline (TBS) at room temperature for 1 h. Then, the PVDF membranes were incubated with rabbit anti-rat CYP3A11 polyclonal antibody (Cat. No. ab195627, 1: 2000, Abcam Biotech., Cambridge, MA, USA) and rabbit anti-rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Cat. No. ab9485, 1: 2000, Abcam Biotech) at 4°C overnight. The PVDF membranes were washed 3 times with TBST (1×) for 10 min and incubated with horseradish peroxidase (HRP)-labeled goat IgG (Cat. No. ab6721, 1: 1000, Abcam Biotech) for 1 h at 37°C. PVDF membranes were then washed

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

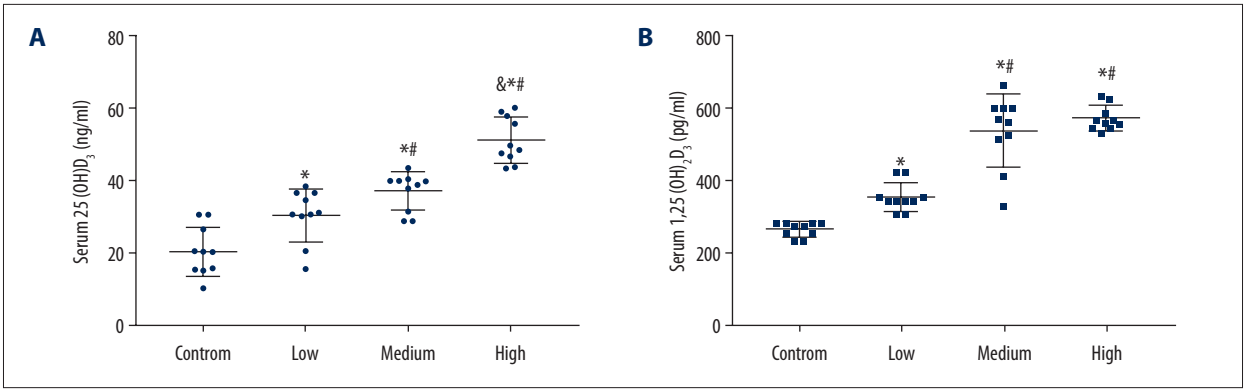

Figure 2. Effects of vitamin D3 on serum levels of serum 25(OH)D_3 and 1,25$(OH)_2D_3$ in rats. (**A**) Comparison of concentration of 25(OH)D_3 levels between vitamin D3-treated groups and Control group. (**B**) Comparison of serum 1,25$(OH)_2D_3$ concentrations between the vitamin D3-treated groups and the Control group. The dosages in the Low group, Medium group, and High group were 100 IU/kg, 400 IU/kg, and 1600 IU/kg, respectively. * $p<0.05$ *vs.* Control group. # $p<0.05$ *vs.* Low group. & $p<0.05$ *vs.* Medium group.

with TBST (1×) 3 times for 10 min each time. Subsequently, the membrane-bound antibodies were visualized with enhanced chemiluminescence (ECL) detection reagent (Thermo Scientific Pierce, Rockford, IL, USA). Analysis and quantification of optical density (OD) values of each color band were performed using Image J software (version: 1.45s, National Institutes of Health, Bethesda, MD, USA), with GAPDH as an internal control.

Immunohistochemistry detection

The rat liver tissues were cut into 3-μm-thick sections, placed on slides, and dried at 70°C for 2 h before immunohistochemical staining. Tissue sections were de-paraffinized in xylene before rehydration in graded alcohols, and rinsed 3 times with phosphate-buffered saline (PBS, Beyotime Biotech. Shanghai, China) for 5 min. Endogenous peroxidase activity of tissue slides was blocked by treatment with 3% H_2O_2 (Beyotime Biotech, Shanghai, China) for 20 min, and washed with PBS 3 times. Antigen unmasking was performed, followed by incubation in 10 mmol/l trisodium citrate (pH 6.1) at 95°C for 20 min. After the slides cooled, they were washed in PBS 3 times at room temperature. Then, the slides were blocked with 5% skim milk for 20 min at room temperature. Subsequently, slides were treated with rabbit anti-rat CYP3A11 (CYP3A4) polyclonal rabbit antibody (Abcam Biotech, Cambridge, MA, USA) at room temperature for 60 min. The sections were incubated with horseradish peroxidase (HRP)-labeled goat anti-rabbit IgG (Santa Cruz Biotech, Santa Cruz, CA, USA) for 20 min at room temperature. The sections were then rinsed with PBS and colored with 3,3-diaminobenzidin (DAB, Beyotime Biotech, Shanghai, China) at room temperature for 8 min. The colored sections were finished with distilled water and then stained with hematoxylin for 20 s. Finally, the slides were dehydrated using gradient alcohol methodology, mounted under cover slips, and analyzed by optical microscopy (Mode: BX51, Olympus, Tokyo, Japan).

Statistical analysis

Data are described as mean±standard deviation (SD) and statistical analysis was performed using SPSS software (version 22.0, SPSS, Inc., Chicago, IL, USA). The differences among the means of 4 groups were compared using Tukey's post hoc test validated by one-way ANOVA. Differences with a p value of 0.05 or less were considered significant.

Results

Vitamin D3 increased concentration of serum of 25(OH)D_3 and 1,25$(OH)_2D_3$ in rats

Our findings demonstrated that concentrations of serum 25(OH)D_3 in vitamin D3 administration groups were much higher than in the Control group (Figure 2A, $p<0.05$), and the concentration of serum 1,25$(OH)_2D_3$ in rats treated with vitamin D3 was remarkably higher than in the Control group (Figure 2B, $p<0.05$). Furthermore, concentrations of serum 1,25$(OH)_2D_3$ and serum 25(OH) D_3 in the Control, Low VD3, Medium VD3, and High VD3 groups gradually increased. Therefore, vitamin D3 administration enhanced the concentration of serum 1,25$(OH)_2D_3$ and serum 25(OH) D_3 in rats.

Vitamin D3 increased expression of CYP3A11 in colon tissues

We found that CYP3A11 expression in all 3 groups (Low, Medium, and High VD3 groups) were remarkably higher compared to the Control group (Figure 3A, $p<0.05$). Moreover, CYP3A11 expression was higher significantly in the Medium VD3 group compared to that in the Low VD3 group ($p<0.05$) in both colon tissues (Figure 3A) and liver tissues (Figure 3B),

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

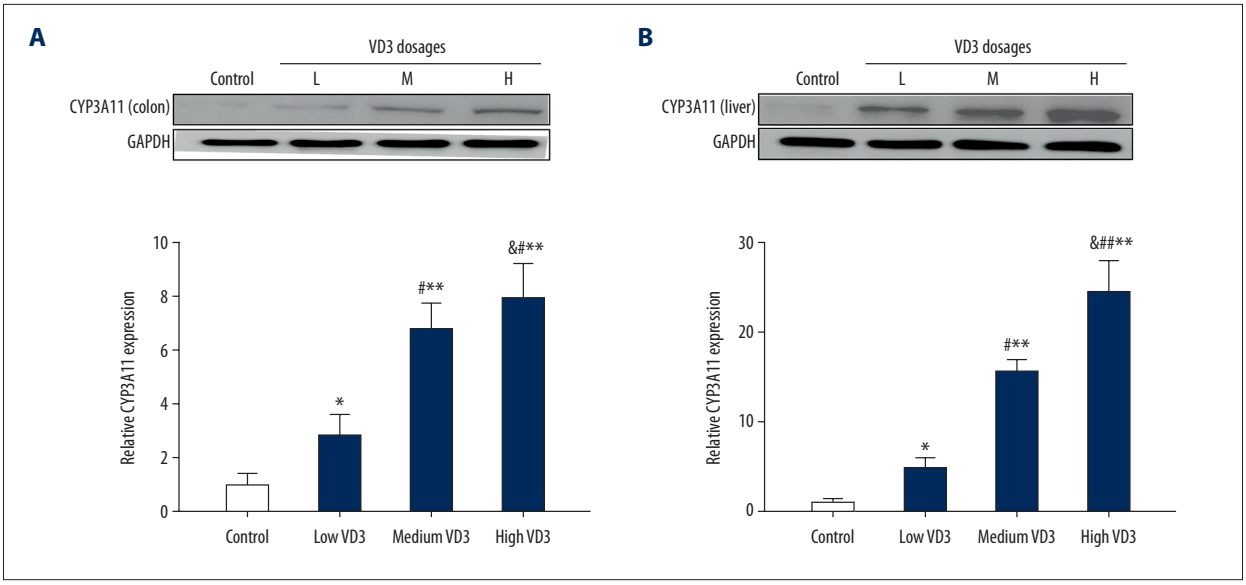

Figure 3. Determination of CYP3A11 expression by Western blotting assay. (**A**) Western blotting bands and statistical analysis of CYP3A11 expressions in colon tissues. (**B**) Western blotting bands and statistical analysis for CYP3A11 expressions in liver tissues. The dosages in the Low group, Medium group, and High group were 100 IU/kg, 400 IU/kg, and 1600 IU/kg, respectively. * $p<0.05$, ** $p<0.01$ *vs.* Control group. # $p<0.05$, ## $p<0.01$ *vs.* Low group. & $p<0.05$ *vs.* Medium group.

and CYP3A11 expression was also significantly higher in the High VD3 group compared to that in the Medium VD3 group in colon and liver tissues (Figure 3A, 3B, $p<0.05$).

Vitamin D3 increased CYP3A11 expression in liver tissues of rats

Western blot analysis showed that the Low VD3, Medium VD3, and High VD3 groups had significantly higher CYP3A11 levels compared to the Control group (Figure 3B, $p<0.05$). Moreover, CYP3A11 expression was significantly upregulated by increased concentrations of vitamin D3 in the Low VD3, Medium VD3, and High VD3 groups (Figure 3B, $p<0.05$).

Vitamin D3 enhanced CYP3A11 expression in liver tissues of rats

CYP3A11 expression in liver tissues was assessed by immunohistochemistry assay (Figure 4A). The results showed that the CYP3A11 expressions were significantly higher in the Low VD3, Medium VD3, and High VD3 groups compared to the Control group (Figure 4B, $p<0.05$), and the vitamin D intervention groups showed different degrees of elevation.

Vitamin D3 reduced the levels of MDZ in rats

The effects of different dosages of vitamin D3 on metabolism of MDZ were evaluated by HPLC method using a standard curve (Figure 5A) and sample curve (Figure 5B). We found that concentrations of serum MDZ at different sampling points were remarkably lower in rats treated with vitamin D3 than in the Control group (Figure 5C, $p<0.05$). Meanwhile, the inductive effects of vitamin D3 on MDZ metabolism were significantly increased by increasing concentrations of vitamin D3 (Figure 5C, $p<0.05$).

Discussion

A growing body of evidence shows there are strong interactions between CYP3A11 (CYPA4) and vitamin D3. Most of these studies focused on the metabolism mechanism of 1,25-DihydroxyVitamin D3 *in vitro*, but our study explored the induction effect of vitamin D3 in rats [12]. The effects of CYP3A enzymes on rats and human CYP3A enzymes in the colon and liver have been studied *in vitro* in both primary cultured hepatocytes and enterocytes, and in immortalized human cell lines such as HepG2 and Caco-2 cells. There have been clear demonstrations that CYP3A11 is expressed at substantially higher levels in the normal colon and colon cancers than in the liver [13]. CYP3A11 is associated with the anti-tumor activities of vitamin D3 against various types of cancers, including colon cancer [14]. Sun et al. [15] proved that microRNA-627 (miR-627) targeted CYP3A4 and suppressed CYP3A4 expression and reported that vitamin D3 decreased CYP3A4 levels in colon cancer cells. A previous study [16] clearly showed that $1,25(OH)2D_3$ induces the expression of CYP3A11 (CYP3A4) in human liver tissue. $1,25(OH)_2 D_3$ is the strongest inducer for the expression of CYP3A1, and is a slightly weaker inducer for the expression of CYP3A2 in the ileum [16]. Thus, we assessed $25(OH)_2D_3$ and

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

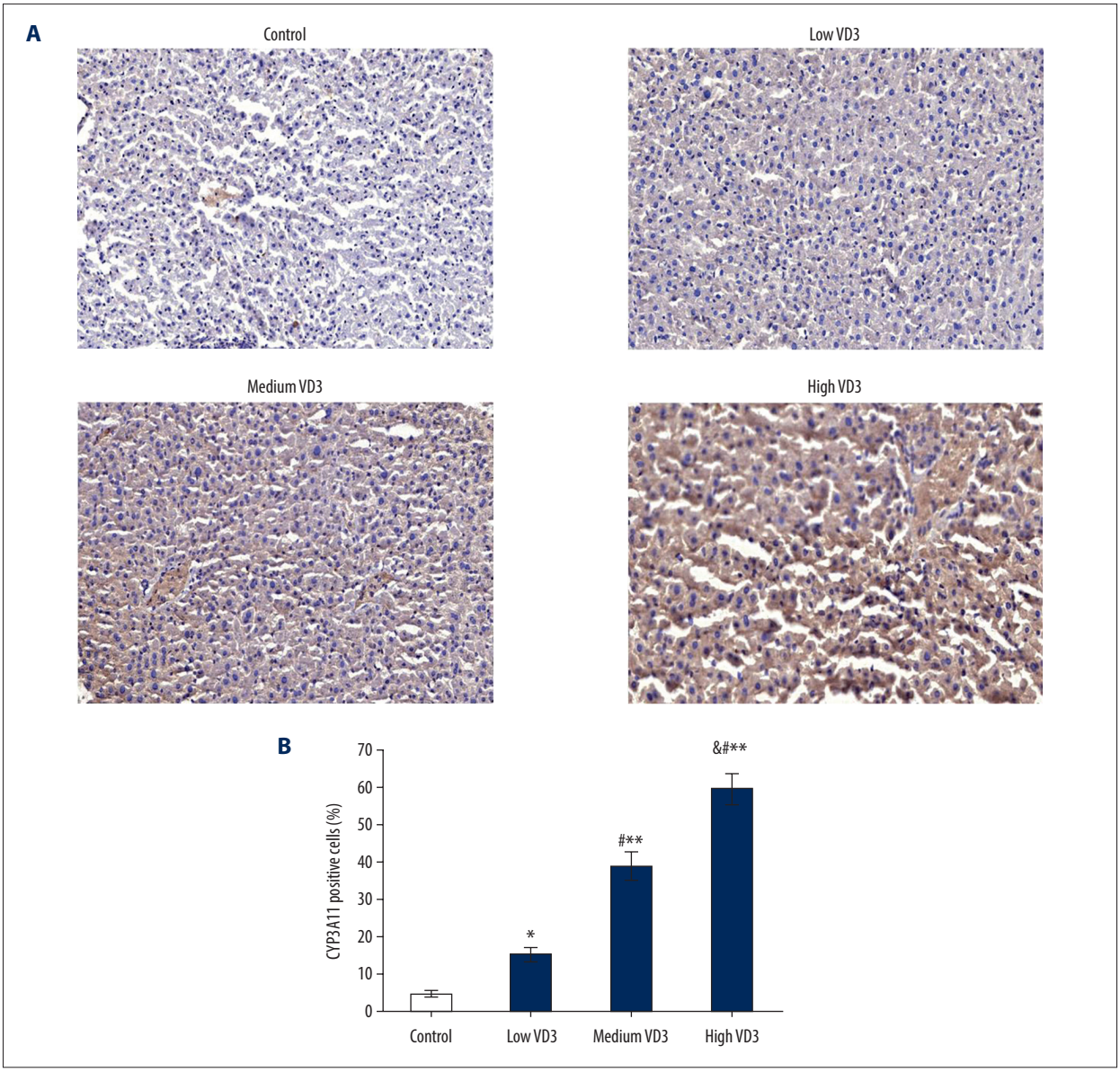

Figure 4. Evaluation of CYP3A4 expression using immunohistochemistry assay. (**A**) Immunohistochemistry assay images in different groups. (**B**) Statistical analysis of CYP3A11 expression. The dosages in the Low group, Medium group, and High group were 100 IU/kg, 400 IU/kg, and 1600 IU/kg, respectively. * $p<0.05$, ** $p<0.01$ *vs.* Control group. # $p<0.05$ *vs.* Low group. & $p<0.05$ *vs.* Medium group.

$1,25(OH)_2D_3$ levels in rats treated with vitamin D3, showing that vitamin D3 remarkably increased concentration of serum $25(OH)D_3$ and serum $1,25(OH)_2D_3$ in rats, similar to the results of a previous study [17]. Another study [18] also reported that vitamin D3-activated vitamin D receptor (VDR) could induce CYP3A11 (CYP3A4) expression in liver and colon tissues, which agrees with the present results. Therefore, CYP3A11 plays critical functions in $1,25(OH)_2D_3$ hydroxylating processes.

CYP3A11 (CYP3A4) is an important targeting enzyme for the pregnane X receptor (PXR) and the VDR in liver tissues and colon tissues. By binding to VDR, vitamin D3 activates a feed-forward catabolic pathway that increases CYP3A expression. According to a previous study [19], $1,25(OH)_2D_3$ can be used to increase CYP3A11 expression in some, but not all, human cell lines derived from tissues known to express CYP3A enzymes. Due to the roles of CYP3A11 (CYP3A4) in vitamin D3 administration and metabolism, we also assessed the expression of CYP3A11 in liver tissues and colon tissues. Our findings demonstrated that CYP3A11 was overexpressed in liver and colon tissues of rats treated with vitamin D3. A previous study [20] also found that vitamin D3 regulates the levels of

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

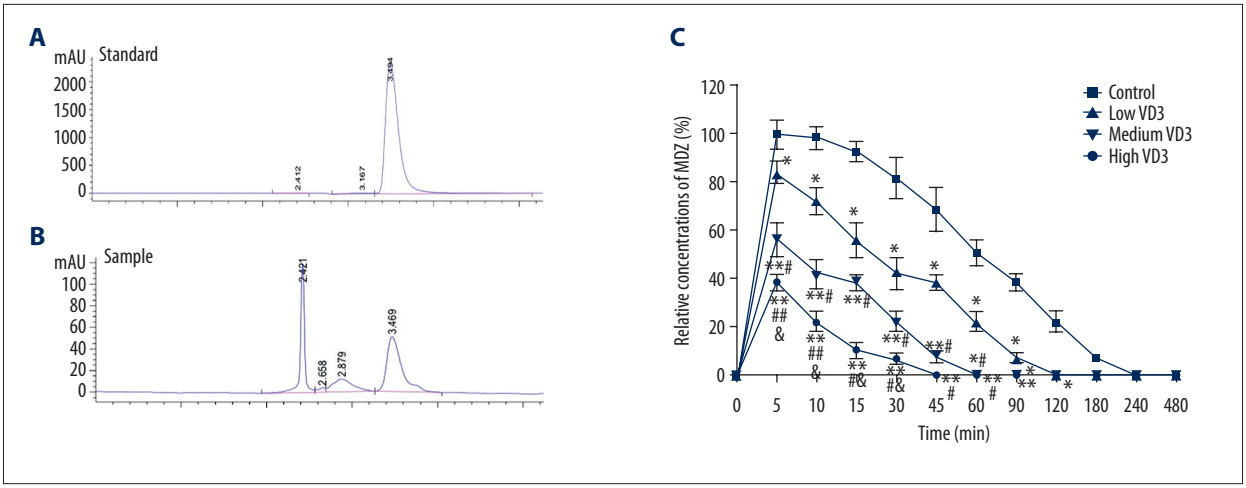

Figure 5. Serum concentration-time profiles of MDZ at 5, 15, 30, 45, 60, 90, 120, or 240 min after treatment with different dosages of vitamin D3 in rats. (**A**) Standard curve for the HPLC analysis. (**B**) Sample curve for the HPLC analysis. (**C**) Statistical analysis for the concentrations of MDZ in the serum of rats. The dosages in the Low group, Medium group, and High group were 100 IU/kg, 400 IU/kg, and 1600 IU/kg, respectively. * $p<0.05$, ** $p<0.01$ *vs.* Control group. # $p<0.05$ *vs.* Low group. & $p<0.05$ *vs.* Medium group.

CYP3A11 and CYP3A4 in liver and intestinal cells. However, in our study, the enhanced expressions of CYP3A11 were discovered in the liver and colon tissues of rats treated with vitamin D3, which further confirmed the previous conclusion [20].

MDZ is usually used as a specific biomarker for CYP3A11 (CYP3A4)-mediated metabolism in several cell lines [21]. MDZ is always metabolized by the CYP3A enzymes to 4-hydroxymidazolam and 1-hydroxymidazolam, both of which participate in calcium metabolism [22]. In our study, concentrations of serum MDZ at all sampling points were remarkably lower in the rats treated with vitamin D3 than in Control rats, suggesting that vitamin D3 regulates levels of CYP3A11 (CYP3A4) and calcium metabolism by hydrolyzing MDZ to 4-hydroxymidazolam and 1-hydroxymidazolam. To the best of our knowledge, this is the first study to explore the roles of hydrolyzing MDZ in the relationship between vitamin D3 and CYP3A11.

VDR also mediates vitamin D3-associated bone tissue growth, bone metabolism, cardiovascular functions, cell differentiation, and regulation of bile acid [23]. $1,25(OH)_2D_3$ also acts as an endogenous regulator of VDR-mediated CYP3A4 expression and thus may have a role in its own homeostasis and could potentially affect biotransformation of CYP3A substrates [24,25]. The above results suggest that vitamin D3 also enhances the $1,25(OH)_2D_3$ levels by directly and indirectly modulating bone metabolism of hydroxy midazolam.

Although our study produced some interesting results, it also has a few limitations. First, the transcriptions and protein expressions of VDR and PXR were not clarified in this study, which might help improve the effects of VD3 on calcium metabolism. Second, the drug-metabolizing genes that might explain the modified pharmacokinetics of MDZ, such as multi-drug resistance 1 (MDR1) or P-glycoprotein (Pgp), were not evaluated. In future research, we plan to assess the DMZ-associated MDR1 or Pgp gene expression.

Conclusions

Vitamin D3 administration increased serum $25(OH)D_3$ and $1,25(OH)_2D_3$ levels and enhanced CYP3A11 expression in rat livers and tissues. Vitamin D3 also induced hydrolysis of MDZ and reduced serum MDZ concentrations. In summary, we found that vitamin D3 is transformed into $1,25(OH)_2D_3$ by triggering CYP3A11 and CYP3A4 activity and by hydrolyzing MDZ.

Conflict of interest

None.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

References:

1. Chen L, Yuan X, Zou L et al: Effects of 1,25-Dihydroxyvitamin D3 on the prevention of chronic obstructive pulmonary disease (COPD) in rats exposed to air pollutant particles less than 2.5 micrometers in diameter (PM2.5). Med Sci Monit, 2018; 24: 356–62
2. Javed F, Malmstrom H, Kellesarian SV et al: Efficacy of vitamin D3 supplementation on osseointegration of implants. Implant Dent, 2016; 25: 281–87
3. Pittas AG, Dawson-Hughes B, Li T et al: Vitamin D and calcium intake in relation in type 2 diabetes in women. Diabetes Care, 2006; 29: 650–56
4. Wang TJ: Vitamin D and cardiovascular disease. Annu Rev Med, 2016; 67: 261–72
5. Garipardic M, Dogan M, Bala KA et al: Association of attention deficit hyperactivity disorder and autism spectrum disorders with mean platelet volume and vitamin D. Med Sci Monit, 2017; 23: 1378–84
6. Groschel C, Tennakoon S, Kallay E: Cytochrome P450 vitamin D hydroxylases in inflammation and cancer. Adv Pharmacol, 2015; 74: 413–58
7. AlSaleem A, AlE'ed A, AlSaghier A et al: Vitamin D status in children with systemic lupus erythematosus and its association with clinical and laboratory parameters. Clin Rheumatol, 2015; 34: 81–84
8. Feldman D, Krishnan AV, Swami S et al: The role of vitamin D in reducing cancer risk and progression. Nat Rev Cancer, 2014; 14: 342–57
9. Xu RA, Wen J, Tang P et al: Functional characterization of 22 CYP3A4 protein variants to metabolize lbrutinib *in vitro*. Basic Clin Pharmacol Toxicol, 2018; 122: 383–87
10. Roizen JD, Casella A, Lai M et al: Decreased serum 25-Hydroxyvitamin D in aging male mice is associated with reduced hepatic Cyp2r1 abundance. Endocrinology, 2018; 159: 3083–89
11. Novotna A, Krasulova K, Bartonkova I et al: Dual effects of ketoconazole cis-enantiomers on CYP3A4 in human hepatocytes and HepG2 cells. PLoS One, 2014; 9: e111286
12. Durk DR, Fan J, Sun H et al: Vitamin D receptor activation induces P-glycoprotein and increases brain efflux of quinidine: An intracerebral microdialysis study in conscious rats. Pharm Res, 2015; 32: 1128–40
13. Canaparo R, Nordmark A, Finnstrom N et al: Expression of cytochromes P450 3A and P-glycoprotein in human large intestine in paired tumour and normal samples. Basic Clin Pharmacol Toxicol, 2007; 100: 240–48
14. Deeb KK, Trump DL, Johnson CS: Vitamin D signalling pathways in cancer: Potential for anticancer therapeutics. Nat Rev Cancer, 2007; 7: 684–700
15. Sun M, Zhang Q, Yang X et al: Vitamin D enhances the efficacy of irinotecan through miR-627-mediated inhibition of intratsumoral drug metabolism. Mol Cancer Ther, 2016; 15: 2086–95
16. Khan AA, Chow EC, van Loenen-Weemaes AM et al: Comparison of effects of VDR versus PXR, FXR and GR ligands on the regulation of CYP3A isozymes in ratsand human intestine and liver. Eur J Pharm Sci, 2009; 37: 115–25
17. Biancuzzo RM, Clarke N, Reitz RE et al: Serum concentration of 1,25-dihydroxyvitamin D2 and 1,25-dihydroxyvitamin D3 in response to vitamin D2 and vitamin D3 supplementation. J Clin Endocrinol Metab, 2013; 98: 973–79
18. Makishima M, Lu TT, Xie W et al: Vitamin D receptor as an intestinal bile acid sensor. Science, 2002; 296: 1313–16
19. Deb S, Chin MY, Adomat H et al: Abiraterone inhibits 1-alpha,25-dihydroxyvitamin D3 metabolism by CYP3A4 in human liver and intestine *in vitro*. J Steroid Biochem Mol Biol, 2014; 144 Pt A: 50–58
20. Wang K, Shen S, Xie W et al: Retinoids induce cytochrome P450 3A4 through RXR/VDR-mediated pathway. Biochem Pharmacol, 2008; 75: 2204–13
21. Fisher JM, Wrighton SA, Watkins PB et al: First-pass midazolam metabolism catalyzed by 1 alpha, 25-dihydroxy vitamin D3 modified Caco-2 cell monolayers. J Pharmacol Exp Ther, 1999; 289: 1134–42
22. Moorthy GS, Jogiraju H, Vedar C et al: Development and validation of a sensitive assay for analysis of midazolam, free and conjugated 1-hydroxymidazolam and 4-hydroxymidazolam in pediatric plasma: application to pediatric pharamcokinetic study. J Chromatogr B Analyt Technol Biomed Life Sci, 2017; 1067: 1–9
23. Cheng J, Fang ZZ, Kim JH et al: Intestinal CYP3A4 protects against lithocholic acid-induced hepatotoxicity in intestine-specific VDR-deficient rats. J Lipid Res, 2014; 55: 455–65
24. Deb S, Pandey M, Adomat H et al: Cytochrome P450 3A-mediated microsomal biotransformation of 1alpha,25-dihydroxyvitamin D3 in mouse and human liver: Drug-related induction and inhibition of catabolism. Drug Metab Dispos, 2012; 40: 907–18
25. He R, Li Y, Ruan J: Serum alanine transaminase total bilirubin concentrations predict CYP3A activity as measured by midazolam and 1'-hydroxylation. Med Sci Monit, 2015; 21: 396–402

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]