Vitamin D3 reduced helicobacter pylori infection of gastric cells by strengthening liver lipid metabolism, blocking gastric JAK-STAT3 and NF-κB signalling pathways and inhibiting gastric mucosal inflammation

Wenjiao Xu

the Affiliated Hospital of Southwest Medical University

Min Kang

Southern Medical University Nanfang Hospital

Xiaolin Zhong

the Affiliated Hospital of Southwest Medical University

Muhan Lü

the Affiliated Hospital of Southwest Medical University

Lei Shi

the Affiliated Hospital of Southwest Medical University

Ping Chang Li (✉ 506854209@qq.com)

<https://orcid.org/0000-0002-8594-8321>

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Abstract

Background: *Helicobacter pylori* (Hp) is a unipolar, multi-flagellate, spiral, Gram-negative bacterium that is the only stomach bacterium found in humans. Hp is the leading cause of digestive tract disease and can cause stomach diseases, such as chronic gastritis, peptic ulcer, gastric cancer and gastric mucosa-associated lymphoma. Clinical studies have shown that Hp infection rates are significantly higher in patients with hyperlipidaemia than they are in non-hyperlipidaemic patients. In addition, many studies have shown that the serum vitamin D3 levels are reduced in patients with obesity and hyperlipidaemia. Lower serum vitamin D3 levels diminish the role of fat cells in storing fat; as a result, fat is released into the bloodstream, leading to elevated blood lipid levels. However, a specific mechanism has not been proposed to explain how VD3 can reduce HP infection in patients with hyperlipidaemia by lowering blood lipids. Methods real-time PCR was used to detect the expression of gastrin in VDR, insign-2 and gastric tissues of mice in each group. The expression of jak, stat 3, cox2 and lkba protein in gastric tissue of mice was detected by western blotting. We 6 and IL-8 in the gastric tissue of mice by Elisa method. The liver tissues of each group were stained with HE to observe the balloon-like changes of liver tissue, and the gastric tissues of mice in each group were stained by HE. The degree of gastritis in each group of mice. Conclusions In this article, we conducted animal experiments using VD3 as an intervention in hp-infected mice and then examined molecular changes in the VDR/Insig-2/SREBP-2 pathways in the mouse liver, the serum cholesterol levels, and the JAK-STAT3 and NF- κ B pathways. Vitamin D3 further reduced the gastric mucosa cholesterol level, destroyed lipid rafts, and reduced assembly of IL-6 receptor subunits by lowering the circulating cholesterol levels, thereby blocking the JAK-STAT3 signalling pathway. Infection of the gastric mucosa by CagA-positive *Helicobacter pylori* reduced gastrin secretion and inhibited COX2 expression. Moreover, NF- κ B activation and inflammatory cytokine expression were inhibited by blocking the JAK-STAT3 signalling pathway. Furthermore, the degree of infection of the gastric mucosal tissue was reduced.

Background

Lipid rafts are rigid microdomains in the cytoplasmic membrane. The major components of lipid rafts are phospholipids, sphingolipids, and cholesterol, which together form tight interactions [1]. IFNGR, IL-6R, and IL-22R are all assembled on the lipid raft structures of gastric cell membranes. Infection of the gastric mucosa by CagA+ hp under IL-6 stimulation activates the IL6R complex,

phosphorylates the JAK1 and JAK2 kinases, and destroys the lipid rafts by

consuming cholesterol, thereby preventing IL-6 from binding to IL6R and further

blocking the JAK/STAT3 signalling pathway [2] [3]. LDL has been reported to

NF- κ B, [4] which is a stimulatory transcriptional regulator. Hirata, et al recently reported [5] that a high-fat diet induced gastric mucosal damage in rats. That study showed that the production of superoxide anions increased gastric mucosal lipid peroxidation by 5.7-fold, whereas the high-fat diet increased this measure by 2.6-fold. Additionally, serum lipid metabolism had been demonstrated to be influenced by *H. pylori* infection [6] [7]. Jak/Stat3 activate NF- κ B and IL-8 expression in AGS cells, which mediates *H. pylori* infection. Inhibition of Jak/Stat3 inhibits NF- κ B activation and induces inflammatory cytokine IL-8 expression, thereby inhibiting *Helicobacter pylori*-induced gastritis. The IL-6/gp130/STAT3 pathway has been shown to play a role in the development of gastric cancer [8] [9]. Consumption of cholesterol by the gastric mucosa during lipid valve structure and results in failure to assemble the IL-6 receptor subunit, thereby blocking the JAK-STAT3 signalling pathway. IL-6 exerts its biological activity through the gp130 receptor subunit [10]. To elucidate whether *Helicobacter pylori*-associated proteins can induce gastrin expression, Zhou et al. [11] constructed the eukaryotic expression vector pcDNA3.1/cagA and the luciferase reporter vec

co-transfected them into gastric cancer cells, and found that CagA activated the

gastrin promoter and upregulated gastrin expression in SGC-7901 cells. Blocking

the JAK-STAT3 pathway with a chemical blocker under action of the CagA protein

has been reported to be effective in reducing gastrin mRNA expression. COX

is a key enzyme that catalyzes the formation of arachidonic acid (PGs) and other eicosanoids. COX can be divided into two types: constitutively expressed COX 1 and mitogen-induced COX-2 [12] [13]. *Helicobacter pylori* infection causes COX-2 inflammation, which participates in the inflammatory response [14]. COX-2 expression leads to the induction of pro-inflammatory prostaglandin PGE [15] [16]. PGs play an important role in the growth and stimulation of inflammation. Gastrin has been shown to mediate COX-2 induction in gastrointestinal cells, suggesting a direct association between gastrin and inflammation.

Vitamin D3 regulates calcium metabolism and promotes bone health by enhancing intestinal calcium absorption [17] [18]. Vitamin D3 and synthetic vitamin D were evaluated based on the relevant potency of the vitamin D receptor (VDR)

as a cholesterol lowering agent in mice. Some studies have shown that patients

with 25(OH)D deficiency have higher serum total cholesterol (TC) and low-density

lipoprotein cholesterol (LDL-C) levels than those with normal 25(OH)D levels [19] [20]. The increase in the cholesterol concentration caused by vitamin D3 deficiency

is mainly due to enhanced cholesterol biosynthesis. In the absence of vitamin D, the transcriptional activity of the vitamin D receptor (VDR) is reduced, resulting in

downregulation of Insig-2 expression, which inhibits its effect on binding of sterol regulatory elements. As a result, 3-hydroxy-3-methylglutaryl coenzyme A reductase expression is increased. Vitamin D3 causes elevated cholesterol because it increases VDR activity, thereby maintaining the activities of

INSIG-2 and TRAN [21].

Fig1

With improvement of living standards, the prevalence of hyperlipidaemia has gradually increasing. In the general public, the hp infection rate is 50%. Many clinical studies have also proven that the hp infection rate of patients

with hyperlipidaemia is increased, which means that more people have both hp infections and hyperlipidaemia. Therefore, we hypothesized that there is a substance that can reduce hp infection while decreasing fat. This study aimed

to prove this hypothesis

Methods

Animal model selection

C57BL/6mice(purchased from Chongqing Tengxin Experimental Animal Sales Co., Ltd., address: 11-2, Building B, Baike Building, No. 160 Luzhou Road, Jiulongpo District,Chongqing,website: www.cqtx123.com.cn)were selected as our animal model.

Preparation of the experimental animal diet

The AIN93G standard diet (catalogue No: LAD 3001G) and a high-cholesterol diet (catalogue No: TP06105) were manufactured by Trophic Animal Feed High-Tech Co., Ltd., China (address: Hainan Chengnan Development Zone, Jiangsu Province National High-tech Development Zone,website: www.trophic.cn).The high-cholesterol diet was prepared by adding 1.25% cholesterol and 0.5% sodium cholate to AIN-93G according to the manufacturer.

Bacterial culture

The standard HP strain NCTC11637 (a gift from the Third Military Medical University)was removed from the -80 degree freezer (Southwest Medical University Central Laboratory) and resuspended in liquid medium. The liquid medium containing the hp strain was placed in a carbon dioxide incubator (5% O₂, 10% CO₂, 85% air) (Manufacturer: Shenzhen Hongwumei Diagnostic Technology Co., Ltd., model: HM-310, website: www.hongmed-infagen.com). An N2 incubator was used for recovery and growth of the bacteria. After 2 days, a small amount of the bacterial suspension was placed in a rapid urease reagent to identify the cultured bacteria as HP (fast red colour development in the presence of urease), and then it was subcultured. After 2 passages, the identified HP bacteria were added to BHI containing 10% FBS for culture.The solution was adjusted to an OD= 0.1 in liquid using a visible spectrophotometer (manufacturer: Shanghai Aucy Scientific Instrument Co., Ltd., device number: 7221772003, website: www.aucybest.cn) and shaken for 20 hours in a carbon dioxide incubator.

Preparation of the VD3 stock solution:

A total of 2.4 mg of vitamin D3 (manufacturer: Macklin, item No.: C804669, website: www.macklin.cn) was added to 4 mL of olive oil (manufacturer:

Macklin, item No: 0815210, website: www.macklin.cn) to obtain a 600 µg/mL vitamin D3 stock solution. The solution was diluted to a concentration of 2.4 µg/ml (250:1) in olive oil.

Animal experiment

Animal studies were approved by the Animal Ethics Committee of the

Southwest Medical University (20181212-1), and animal experiments were conducted in accordance with the guidelines of Southwest Medical University. Taking into

account the welfare factors of all animals, we provide a comfortable, clean and quiet

animal living environment, namely the Experimental Animal Center of Southwest Medical University.

The animal center maintains a room temperature of 21±2.0°C and a humidity of 50±5%. In order to avoid animal stimulation, the environment is

kept quiet and there is a fixed opening time tomorrow. In order to keep the environment clean, the animal center has a special cleaning staff.

The feed used is provided by the professional feed processing company Nantong Trophy Animal Feed Production Co., Ltd. The quality of the feed is guaranteed. Rate, animal drinking water is sterilized distilled water, and the experimenter regularly changes water and feed to the animal center every day to ensure adequacy. In addition,

every day, the experimenter also needs to regularly observe the living state of the animal at the animal center, mainly to monitor the health status of the experimental animals by observing whether the mice are dead, the activity of the mice, the eating state, and whether there is a fight between the animals.

Establishment of the high-fat diet and hp infection models

C57BL/6 mice were housed in individual cages in the Southwestern Medical

University Animal Housing Facility under a controlled 12:12 hour light:dark

cycle. The room temperature was maintained at 21 ± 2.0 °C with 50 ± 5%

humidity. The mice were fed a purified AIN-93G diet with free access to the

diet and water. After one week of adaptation, the mice were randomly divided into four groups. Group A (blank control group) was given the purified AIN-93G diet at a dose of 6 g per mouse and intragastrically administered saline at

a dose of 5 ml/kg. Group B (HP-infected group) was given the purified

standard diet and intragastrically treated with a HP bacterial solution (OD=1) at a dose of 5 ml/kg. The mice were fasted for 12 hours before each gavage treatment. Each mouse was given 2.6 mg of metronidazole (manufacturer:

Hunan Hansen Pharmaceutical Co., Ltd., website: www.hansenzy.com), 5 mg of amoxicillin (manufacturer: North China Pharmaceutical Co., Ltd., website: www.ncpc.com.cn) and 1.2 mg of Lizhu Dele (manufacturer: Livzon Pharmaceutical

Co., Ltd., website: www.Livzon.com) 8 hours before the gavage, which

removed bacteria from the stomach. One hour before the gavage, 2% sodium

bicarbonate (manufacturer: Huiyin Bizhong Jiangxi East Asia Pharmaceutical

Co., Ltd., product batch number: 2018071824, website: www.mhuiyinbi.cn) was administered orally. Group C (HP infection group + high cholesterol group)

was fed the naturally increased high-cholesterol TP06105 diet at a dose of 6 g per mouse. The same method was used to inject the HP bacterial solution. In

the high cholesterol group (D), the naturally increased high-cholesterol TP06105 diet was provided at a dose of 6 g/kg per mouse, and the liquid medium

was administered in a volume of 0.2 ml per mouse.

Evaluation of successful test model construction

After 4 weeks, 2 mice from groups A, B, C, and D were fasted for 24

hours. Then we use a 1% sodium pentobarbital solution (manufacturer: Shanghai Biaozhuo Biotechnology Co., Ltd., website: www.app17.com/c68009, 30 mg/kg body weight, intraperitoneal injection) to inject the target mouse intraperitoneally at a dose of 150 mg/kg. After the treatment is completed, it is necessary

whether the heart of the mouse is beating, ensuring that the mouse is quiet and

without fear or anxiety. Die without pain. Blood samples collected from the four groups of mice were centrifuged (2000 x g for 5 min) to obtain sera, which was evaluated using an antibody against cholesterol. The results were compared among the 4 groups. The results obtained from the mouse sera determined the successful establishment of a high-cholesterol model. The stomach tissue was removed using a sterile surgical instrument. The stomach residue was rinsed with phosphate buffered saline (PBS) (manufacturer: Solarbio, item No.: P1020, Website: www.solarbio.com). Then, the stomach gland was cut, and one piece was placed in the rapid urease reagent. The colour changes from yellow to red.

Fig2

Vitamin D3 intervention

Group A (blank control group) did not receive any treatment. The remaining mice in each group were randomly divided into two groups to create groups B1, B2, C1, C2, D1, and D2, and groups B1, C1, and D1, which were sacrificed for analysis. The vitamin D3 stock solution in olive oil was administered orally by mouth. The amount of gastric juice was 5 ml/kg once daily for 4 weeks. Groups B2, C2 and D2 were orally administered olive oil alone. The amount of gastric juice was 5 ml/kg once daily by gavage for 4 weeks.

Preparation of sera and tissue

After 4 weeks of vitamin D3 administration, all mice were fasted for 24 hours. Then we use a 1% sodium pentobarbital solution (manufacturer: Shanghai Biaozhuo Biotechnology Co., Ltd., website: www.app17.com/c68009, 30 mg/kg body weight, intraperitoneal injection) to inject the target mouse intraperitoneally at a dose of 150 mg/kg. After the treatment is completed, it is necessary for the mouse to be beating, ensuring that the mouse is quiet and without fear or anxiety. Die without pain. Blood was collected from the mice by heart puncture and then

centrifuged at 2000 x g for 10 min with a high-speed centrifuge (manufacturer: Shanghai Anting Scientific Instrument Factory, website: www.sh-anting17.com).

The sera were stored in a -80 degree freezer for the cholesterol and IL-8 measurements. Figure 2A shows the liver of non-high cholesterol mice, figure 2B is the liver of high cholesterol mice. The stomach tissue was removed using a sterile surgical instrument, and the gastric residue was washed with PBS. The gastric gland was cut and divided. The colour of the rapid urease reagent changed from yellow to red (figure 2C). Additionally, one part was fixed with paraformaldehyde and paraffin sectioned for HE staining, and the degree of gastritis was observed under an inverted microscope. An additional piece was collected in a freezer tube and stored in a -80 °C freezer for western blotting to examine the STAT3, JAK, IKBa and cox 2 contents in the gastric mucosa. A final

piece was collected in a freezer tube and stored in a -80 °C freezer to examine gastrin gene expression by RT-PCR. The liver tissue from the mice was collected and stored in a -80 °C freezer. Insig-2 and VDR gene expression was examined by RT-PCR

Experimental detection methods

Real-time PCR analysis

PCR was performed using the Life Technology StepOne™ Real-Time PCR instrument as described above. Each sample was comprised of 3 replicate wells. The liver tissue and blood samples were tested using the QuFast

SYBR Green PCR Master Mix kit (ELK Biotechnology, EQ001) to evaluate

VDR, insig-2, and gastrin gene expression. The following nucleotide primers

were used: gastrin NM_010257.4 (Forward ACCAATGAGGACCTGGAACAG, Reverse AAGTCCATCCATCCGTAGGC), mouse liver tissue insig-2 NM_001271531.1 (Forward CAGTTTTCCCTCACACTGGCT, Reverse GGCAACCAAGAACGGACATAG), mouse liver tissue VDR NM_009504.4 (Forward CAGGACCGCCTATCCAACAC, Reverse GCCGAACACCTCTAGCACAAG), and GAPDH

NM_008084.3 (Forward TGAAGGGTGGAGCCAAAAG, Reverse AGTCTTCTGGGTGGCAGTGAT). The samples were pre-denatured at 95 °C for 1 minute and cycled 40 times for 10 seconds at 95 °C and 1 minute at 60 °C.

Western blotting analysis

Protein extraction: Total protein extraction from gastric tissue: Tissue block 2 was rinsed with pre-cooled PBS (brand: ASPEN, product number: AS1025, website: www.aspenpharma.com). The homogenate was transferred to a centrifuge tube and shaken. After incubating in an ice bath for 30 minutes and centrifuging at 12,000 rpm for 5 minutes at 4 °C, the

supernatant was collected as a total protein solution. 2) Quantification of the

protein concentration: The protein concentration of the samples was determined using a BCA protein concentration assay kit (brand: ASPEN, product number: AS1086, website: www.aspenpharma.com). 3) SDS-PAGE electrophoresis: The total of 40 µg of total protein was loaded for each sample, and an appropriate amount of protein loading buffer was added (30.3 g of Tris, 18.77 g of glycine, and 1.0 g of SDS) (brand: ASPEN, product code: AS1011, website: www.aspenpharma.com). Double-distilled water was added to obtain a volume of 1000 µl, and the mixture was incubated in a 95-100 °C boiling water bath for 5 minutes. To prepare the separating gel (see Table 1), the gel mixtures were shaken

immediately after adding TEMED (tetramethylethylenediamine). After approximately 45 minutes, the water covering the upper layer was poured out, and the remaining water was blotted with absorbent paper. The concentrating gel was

prepared (see Table 2) by filling and replenishing the remaining space in the

gel and inserting the comb into the concentrating gel. The comb was removed, and the electrophoresis rack was placed in the electrophoresis tank (manufacturer: Beijing Liuyi Instrument Factory, model: DYCZ-24DN, website: www.liuyi17.com). Then, the tank was filled with electrophoresis buffer, and samples were added to the sample wells. Constant pressure electrophoresis was applied at 80V for the concentrating gel and 120 V for the separating gel until bromophenol blue reached the lower edge of the rubber sheet. 4) Transfer to film: The

membrane filter paper and PVDF film were prepared (brand: Millipore, product number: IPVH00010, website: www.sns17.com). From the positive to the negative electrode, the transfer film sponge, three-layer filter paper, PVDF film, gel,

three-layer filter paper, and transfer film sponge were sequentially stacked.

Bubbles were removed from between the layers. The membrane transfer time was adjusted depending on the molecular weight of the target protein. 5)

Incubation with antibodies: The transferred membrane was added to blocking

solution and blocked at room temperature for 1 hour. The blocking solution

was removed, and the diluted primary antibody (brand: ASPEN, product number: AS1061, website: www.aspenpharma.com) was added, and the membrane was incubated at 4 °C overnight. The diluted primary antibody was recovered, and the membrane was washed with TBST. The secondary antibody diluted with

the secondary antibody diluent (brand: ASPEN, product number: AS1058, www.aspenpharma.com) was added, and the membrane was incubated for 30 minutes at room temperature and then washed four times with TBST on a shaker at

room temperature (manufacturer: Beijing Liuyi, instrument factory model:

WD-9405A, website: www.liuyi17.com) for 5 minutes per wash. 6) Chemiluminescence detection: Newly prepared ECL solution (A: B = 1:1) (solution A: 200 ml (2.422 g of Tris and 150 ml of H₂O) was adjusted to pH 8.6, and the

volume was adjusted to 200 ml. The solution was mixed (0.08858 g of luminol and 0.0131328 g of pcAcid) overnight in the dark. The protein side of the membrane was exposed in the darkroom. The exposure conditions were adjusted

according to the different light intensities, and the films were developed and

fixed.

ELISA detection method

The standards were diluted, and the blank sample and sample wells were prepared for a total of 7 wells. The liquid was removed from each well, but the wells were not washed. Then, 100 μ L of IL-6 antibody (brand: ELK Biotechnology, item No: ELK1157, website: www.elkbioch.com) or IL-8 antibody (brand: ELK Biotechnology, item No: ELK8323, website: www.elkbioch.com) was added to each well. The plate was covered with a plate sealant, incubated at 37 °C for 1 hour, and then washed with a solution. Next, 350 μ L of 1 $\times$ solution was added to each well, and the plate was allowed to stand for 1-2 minutes. The plate was washed completely 3 times. During the last wash, any remaining wash buffer was removed by aspiration or decantation or by adsorbing it on absorbent paper. Then, 100 μ L of streptavidin-HRP working solution was added to each well, and the plate was covered with a plate sealant and incubated for 30 minutes at 37 °C. The aspiration/washing process was repeated a total of 5 times as described in step 4. Next, 90 μ L of LTMB substrate solution was added to each well. The plate was covered with a new plate sealant and incubated at 37 °C for 10-20 minutes. The liquid turned blue after the addition of a TMB substrate solution. Finally, 50 μ L of stop reagent was added to each well. After the addition, the liquid turned yellow, and the reaction was stopped by mixing the liquid via tapping the side of the plate. Any water droplets and fingerprints were removed from the bottom of the plate, and the liquid surface was checked for air bubbles. The plate was read on a microplate reader immediately at 450 nm.

HE staining

Dewaxing of the paraffin sections in water: The sections were incubated successively in xylene I for 20 min, xylene II for 20 min, anhydrous ethanol I for 10 min, anhydrous ethanol II for 10 min, 95% alcohol for 5 min, 90% alcohol for 5 min, 80% alcohol for 5 min, and 70% ethanol for 5 min. Then, the sections were washed with distilled water. The sections were incubated in Harris haematoxylin staining solution (manufacturer: ASPEN, item, NOAS1055A, website: www.aspenpharma.com) for 3-8 minutes, rinsed with tap water, separated with 1% hydrochloric acid alcohol for a few seconds, rinsed with tap water, returned to 0.6% ammonia and rinsed with tap water. The sections were stained with Yihong dye solution (manufacturer: ASPEN, item number: AS1094, website: www.aspenpharma.com) for 1-3 minutes. Then, the sections were placed in 95% ethanol for 5 minutes, 95% ethanol for 5 minutes, absolute ethanol I for 1 minute, absolute ethanol II for 5 minutes, xylene I for 5 minutes and xylene II for dehydration and to make them transparent for 5 minutes; the sections were finally sealed with xylene from neutral chewing gum (manufacturer: Shanghai Yiheng Scientific Instrument Co., Ltd., model: DHG-9123A, website: www.yihenyiqi.com)

Statistical analysis

The statistical analysis was performed using the SPSS 19.0 software (SPSS Inc., Chicago, IL, USA, <http://www.presidion.com>), and $p < 0.05$ was considered statistically significant. Data from the animal studies were analysed using a t-test or one-way ANOVA, followed by Tukey's test. The results are expressed as the mean $\pm$ SD.

Results

VD3 in liver tissue enhances VDR activity, increases insig-2 expression, enhances cholesterol in circulation, and reduces liver cholesterol.

Fig3(A) shows that VDR expression does not significantly differ among the blank control, hp infection and hp+VD3 groups ($p > 0.05$). The differences among the blank control, cholesterol and cholesterol + VD3 groups were statistically significant ($p < 0.05$), whereas the differences among the blank control, cholesterol + hp, and cholesterol + hp + VD3 groups were not statistically significant ($p > 0.05$). No significant differences were found between the hp and hp+VD3 groups ($p < 0.05$), whereas the differences among

the cholesterol, cholesterol + VD3, cholesterol + hp, and cholesterol + hp + VD3 groups were statistically significant ($p < 0.05$). Therefore, VD3 increased VDR expression in the high-cholesterol and high-cholesterol +

hp groups but not in the hp-infected group. (B) shows that insig-2 expression was not significantly different among the blank control, hp infection and hp+VD3 groups ($p > 0.05$). Conversely, significant differences were found among the

blank control, cholesterol and cholesterol + VD3 groups. The differences

among the blank control, cholesterol + hp, and cholesterol + hp + VD3 groups were statistically significant ($p < 0.05$). No significant difference was found between the hp and hp+VD3 groups ($p > 0.05$), whereas the differences among

the cholesterol, cholesterol + VD3, cholesterol + hp, and cholesterol + hp + VD3 groups were statistically significant ($p < 0.05$). Fig4 shows that obvious fat balloon-like changes in (C) and (E), whereas the balloon-like changes in (D) and (F) were significantly reduced. But there were no differences in (A) and

(B).

Fig3 Fig4

Cholesterol circulation in the liver was weakened, serum cholesterol content was decreased, gastric mucosal cell fat was destroyed, and JAK and STAT3 protein expression was decreased.

Fig5(A) shows the jak and stat3 western blotting bands, and (C) and (D) show

the corresponding jak and stat3 protein content maps. (B) showed that the serum IL-6 levels were high in the seven groups. The IL-6 content was highest in the hp+cholesterol group, followed by the hp group. No significant difference in the IL-6

content was found between the hp+ high cholesterol group and the hp group supplemented with VD3. (C) and (D) showed that jak and stat3 protein expression was

significantly increased in the hp-infected and hp+VD3 groups compared with that of the blank control group ($p < 0.05$), indicating that hp infection induced the jak-stat3

signalling pathway. No significant differences in jak and stat3 expression were

observed between the high cholesterol and the high cholesterol + VD3 groups and

the blank control group ($p > 0.05$). The high cholesterol + hp and high cholesterol +

hp + VD3 groups were compared with the blank control group. The differences in

the jak and stat3 protein contents was statistically significant ($p < 0.05$), but no

significant difference was found in the jak and stat3 contents among

the hp infection, hp+VD3, high cholesterol and high cholesterol + VD3 groups. A statistically significant difference was

found in jak and stat3 expression between the high cholesterol hp and high cholesterol + hp + VD3 groups ($p < 0.05$).

Fig5

Blocking the JAK1/STAT3 signalling pathway inhibits NF- κ B activation and inflammatory cytokine expression.

Fig6(A) and (C) showed that the I κ B α protein content was significantly

increased in the hp-infected and hp+VD3 groups compared with that of the blank

control group ($p < 0.05$). No significant difference in the I κ B α protein content was noted between the high cholesterol and high cholesterol + VD3 groups ($p > 0.05$).

The I κ B α protein content in the high cholesterol+hp and high cholesterol+hp+VD3

groups was significantly lower than that of the blank control group ($p < 0.05$). Conversely, no significant difference in the I κ B α protein content was observed among the

hp-infected and hp+VD3 groups and among the high cholesterol, high cholesterol+VD3, high cholesterol + hp, and high cholesterol + hp groups. The difference in

the I κ B α protein content in the +VD3 groups was statistically significant ($p < 0.05$).

The NF- κ B pathway was blocked by blocking the jak-stat3 pathway to further

attenuate IL-8 expression, as shown in (D). At the same time, cox2 and gastrin secretion was inhibited, as shown in (B) and (E).

Fig6

Changes in inflammation of the gastric mucosa[22].

Fig 7 shows HE staining of the gastric mucosa from the seven groups of mice, and Table 3 provides a classification of the HE inflammation levels according to the degree of gastric mucosal inflammation. The pathology due to chronic gastritis (microscopic) observations was graded based on the following5 histological changes: 1) Hp infection, 2) chronic inflammatory response (mononuclear cell infiltration), 3) activity (neutrophil infiltration), 4) atrophy (intrinsic c body reduction, and 5) intestinal metaplasia. The 4 ratings were as follows: 0, + mild, ++ moderate, and +++ heavy

Table3Fig7

Discussion

Helicobacter pylori is one of the most infectious bacteria worldwide. The average infection rate of natural populations worldwide exceeds 50%[23].Significant differences exist in the infection rates in different regions.In developed countries, the infection rate is up to 90%[24]. With improvement of living standards and changes in dietary structure, the prevalence of hyperlipidaemia has also increased annually. Scholars have found a prevalence of hyperlipidaemia of 18% in Beijing, 24.2% in Guizhou[25], and 42.8% in Gansu[26]. As early as 1996, researchers in Finland found that TC and TGs were higher in hp-infected patients than in non-infected patients. Since then, many Linchuan studies have shown that the TC, TG and LDL concentrations are elevated in hp-infected patients. Therefore, the number of hyperlipidaemic patients infected with hp is also increasing. Hyperlipidaemia can be treated with lipid-lowering therapy, and hp-infected patients can take standard quadruple anti-hp therapy. However, many studies have shown that vitamin D3 can also reduce blood lipids through liver lipid circulation, and blood lipids are closely associated with hp infection. Therefore, we speculate that vitamin D3 can reduce blood lipids to reduce the risk of hp infection.

VDR proteins are present in adipose tissue (AT) and may contribute to the role of VD3 and its analogues in adipocytes [27][28]. Under vitamin D3 supplementation conditions, VDR activity is enhanced, and the SREBP cleavage-activating protein (SCAP) acts as an inducible protein and sterol sensor by forming a complex with SREBP-2 in the ER. The SCAP-SREBP complex in the ER is mediated by sterol-induced SCAP via binding to Insigs located on the ER membrane. Following binding to SCAP, the SCAP escorting the SREBP complex is transferred to the Golgi apparatus [29] [27].The vitamin Ddeficiency-induced increase in the cholesterol concentration is mainly due to enhanced cholesterol biosynthesis rather than reduced catabolism.Under vitamin D deficiency, the transcriptional activity of the vitamin D receptor (VDR) is decreased,leading to downregulation of insulin-induced gene 2(Insig-2)expression and thus its inhibitory role on sterol regulatory element-binding protein 2

activation; accordingly, 3-hydroxy-3-methylglutaryl-coenzyme A reductase

expression is increased. Vitamin D3 was protective against the vitamin D

deficiency-induced increase in cholesterol by maintaining the transcriptional

activity of VDR and Insig-2 [29]. Therefore, we used vitamin D3 as an intervention and evaluated VDR and Insig-2 expression in the seven groups of mice by PCR. We also examined the TC levels and stained the liver tissue with HE (figure 4).

From these data, we can conclude that vitamin D3 supplementation enhances

VDR activity, induces insulin-induced gene 2 (Insig-2) expression thus

enhancing its binding to sterol regulatory elements, and reduces cholesterol

synthesis by the liver.

The cholesterol in the circulating blood is reduced. Because the cholesterol

in the gastric mucosa is mainly derived from the blood, the cholesterol in the

gastric mucosa is further lowered. The main lipid raft component of the gastric

mucosa is cholesterol. Does the reduction of the cholesterol destroy lipid rafts,

leading to destruction of hp-infected gastric cell lipid rafts, and does the IL-6 secreted in response to hp infection fail

to assemble with its receptors and block the JAK-STAT3 signalling pathway? To answer these questions, we assessed IL-6 in the circulating blood and JAK and STAT3 protein expression in gastric mucosal tissue (figure 5). We concluded that lowering cholesterol levels in the liver and as a result in

the blood could reduce the gastric mucosal cholesterol content, destroy lipid

rafts, and affect IL-6 secretion in CagA-positive hp-infected gastric cells due to

lack of assembly of the IL-6 receptor subunit, thereby blocking the JAK-STAT3 signalling pathway.

1. *pylori* infection causes inflammation, and COX-2 is involved in the

inflammatory responses [30]. Interleukin-8 (IL-8) is an activator of neutrophils

that migrates to the infected gastric mucosa. [31] Upregulation of IL-8 was

found in gastric mucosal tissues isolated from patients infected with *H. pylori*. IL-8 expression is mediated by activation of the transcription factor NF- κ B in gastric epithelial cells. [32][33]. A decreased vitD3 status is associated with obesity, and recent in vitro studies have described the anti-inflammatory action exerted by 1,25(OH)₂D on adipocytes [34], [35]. In humans, a negative association was found between the serum 25(OH)-D concentration and the plasma interleukin (IL)-6 and TNF- α levels in normal-weight subjects. Nevertheless, vitD had no effect on inflammatory biomarkers in the sera or AT in obese subjects [36]. VitD exerts anti-inflammatory effects that are mediated by inhibition of the NF- κ B and

mitogen-activated protein kinase signalling pathways [37]. In the case of

JAK-STAT3 pathway blockade, we measured IL-8 and COX2 expression (Fig 5). We concluded that blocking the JAK-STAT3 signalling pathway inhibited

NF- κ B activation and inflammatory cytokine IL-8 and COX2 expression and reduced inflammation of the gastric mucosa.

Because the cholesterol in the circulating blood is reduced and the cholesterol in the gastric mucosa is mainly derived from the blood, the cholesterol in the gastric mucosa is further lowered. The main lipid raft component of the gastric mucosa is cholesterol. A decrease in cholesterol destroys lipid rafts, prevents

IL-6 from assembling with its receptor and blocks the JAK-STAT3 signalling

pathway. Blocking the JAK-STAT3 and inflammatory NF- κ B pathways reduces

secretion of inflammatory factor IL-8 and the pro-inflammatory substance

gastrin. Finally, the gastric tissue was stained with HE (Fig 6). We concluded that vitamin D3 supplementation conferred strong VDR activity, induced insulin-induced gene 2 (Insig-2) expression thus enhancing its binding to sterol regulatory elements, and reduced cholesterol synthesis in the liver and the level circulating in the bloodstream. The reduced cholesterol levels further reduced the gastric mucosal cholesterol, destroyed lipid rafts, and resulted in failure to assemble IL-6 receptor subunits,

thereby blocking the JAK-STAT3 signalling pathway. Infection with CagA-positive *H. pylori* can reduce the gastrin secretion levels and inhibit COX2 expression,

whereas blocking the JAK-STAT3 signalling pathway can inhibit NF- κ B activation and expression of the inflammatory cytokine IL-8. As a result, the degree of

infection of the gastric mucosal tissue is reduced. As shown in Fig3, VD3 can lower cholesterol in non-Helicobacter pylori-infected mice with high cholesterol but not in H. pylori-infected mice with low cholesterol. Fig4 shows no difference in gastrin expression between the high-cholesterol group not infected with Helicobacter pylori and the lower cholesterol group infected with H. pylori. Therefore, we can conclude that the cholesterol-lowering effect of VD3 is only effective in cases with high cholesterol and that VD3 does not alleviate the degree of gastritis in mice that are only infected with H. pylori. However, previous studies have shown that phosphatidylethanolamine (PE) carries myristic acid molecules in Helicobacter pylori lipid compositions and has a high binding affinity for non-esterified steroids, such as those that are free of charges. The carbon-3 positions of the cholesterol, pregnenolone and progesterone backbones play important roles in the interaction between the bacterial cells and these steroid compounds. PE (DMPE) has also been found to be one of the most prevalent PE molecular species in Helicobacter pylori cell membrane lipid compositions. Steroidal compounds, progesterone, induce lysis of H. pylori cells by binding to H. pylori molecules. Compounds with high binding affinity for Helicobacter pylori PE can confer selective antibacterial action. Vitamin D3 can attenuate the ability of H. pylori to infect the gastric mucosa by altering the bacterial cell membrane lipids. However, this study shows that vitamin D3 can alleviate gastritis by destroying the lipid rafts of the gastric mucosa and blocking the jak-stat3 pathway to further inhibit the inflammatory factor NF- κ B pathway and the expression of inflammatory factors. However, this study did not detect changes in Helicobacter pylori cell membrane lipids after VD3 intervention, and thus it did not accurately indicate that the reduction of gastritis was due only to the destruction of gastric cell lipid rafts, which needs further investigation.

Competing interests There is no interest between all the authors in this article, ranking disputes, all authors agree to publish papers in the magazine, no copyright disputes.

Conclusions

From this study, we can conclude that Vitamin D3 supplementation enhanced VDR activity, induced insulin-induced gene 2 (Insig-2) expression thus enhancing its binding to sterol regulatory elements, and reduced cholesterol synthesis in the liver. Reduction of cholesterol levels in the liver and blood following infection of gastric cells by CagA-positive hp reduced the gastric mucosal cholesterol content, destroyed lipid rafts, and enhanced IL-6 secretion due to failure of IL-6 receptor subunit assembly, which blocked the JAK-STAT3 signalling pathway. Blocking the JAK-STAT3 signalling pathway could inhibit NF- κ B activation and inflammatory cytokine IL-8 and COX2 expression and could reduce gastric mucosal inflammation.

Abbreviations

Hp Helicobacter pylori

VD3 Vitamin D3

COX Cyclooxygenase

VDR Vitamin D receptor

NF-κB Increase expression of nuclear factor-κB

TC Total cholesterol

LDL-C Lipoprotein cholesterol

Insig-2 Insulin-induced gene 2

PBS Phosphate buffered saline

TEMED Tetramethylethylenediamine

AT Adipose tissue

RT-PCR Real-time PCR

SCAP SREBP cleavage-activating protein

Declarations

Ethics approval and consent to participate

The animal research study was approved by the Animal Ethics Committee of Southwestern Medical University(20181212-1),and the animal experiments were conducted in accordance with the Southwest Medical University guidelines.

Consent for publication

Not applicable.

Aavailability of data and material

All data generated or analyzed during this study is included in this article.

Competing interests

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Authors' contributions

WX to participate in the whole process of the experiment and wrote t

his manuscript. CL guided the design of the experiment and the revision of

the manuscript. MK participated in the analysis of the experiment and experime

ntal data. X Z participated in the animal model of the study. The constructi

on of LS participated in the method detection of this experiment.ML

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Figures

Figure 1

Vitamin D3 can further reduce gastric mucosal cholesterol, destroy lipid rafts, block JAK and STAT3 signaling pathways. When CagA-positive *Helicobacter pylori* infected the gastric mucosa, it reduced the secretion of gastrin and inhibited the expression of COX2. On the other hand, blocking the JAK1 / STAT3 signaling pathway, inhibiting the activation of NF-κB and the expression of inflammatory cytokines are inhibited

Figure 2

The result of model construction. (A) shows the liver of non-high cholesterol mice, (B) is the liver of high cholesterol mice, and (C) is the result of rapid urease test of gastric antrum tissue in mice of hp (B) group 2 weeks after hp infection.

Figure 3

The effect of VD3 on hepatic cholesterol metabolism. VD3 was used to intervene in hp group, cholesterol group and cholesterol + hp group to detect the mRNA expression of VDR(A) by real-time PCR, insig-2(C) by real-time PCR. Determination of cholesterol content by cholesterol antibody(B).

Figure 4

HE staining of liver tissue showed in seven groups (C) representatives the cholesterol group,(E) representatives the cholesterol + hp groups,(D) representatives the cholesterol + VD3 group,(F)representatives cholesterol + hp + VD3 groups,(G) representatives the control group,(A) representatives the hp infection group, (B) representatives the hp infection + VD3 group).

Figure 5

The liver cholesterol cycle is weakened, serum cholesterol levels are reduced, gastric mucosal cell fat is destroyed, and JAK and STAT3 protein expression is weakened. The Elisa method was used to detect the serum IL6 levels in 7 groups (B). The expression of jak and stat3 protein in 7 groups was detected by western blot. (A) is the band diagram of jak and stat 3 measured by western blot method.(C) and (D) respectively represent jak and stat3 proteins content respectively.

Figure 6

VD3 disrupts gastric cell lipid rafts, blocks the jak-stat3 pathway, further blocks the NF- κ B pathway, and attenuates IL8 and cox2 expression. Western blot was used to detect the expression of I κ B α protein in 7 groups (A) and the expression of cox2(B) was detected by PCR. (C) is the comparison of I κ B α protein content in (A) of the corresponding group. (D) was used to detect the expression of IL8 in serum in Elisa. (E) is the gastrin RNA content detected by PCR.

Figure 7

Represents HE staining images of seven groups of gastric mucosa, respectively. The index of gastritis is shown in table 1.(C) representatives the cholesterol group,(E)representatives the cholesterol + hp groups,(D) representatives the cholesterol + VD3 group,(F) representatives cholesterol + hp + VD3 groups,(G) representatives the control group,(A) representatives the hp infection group,(B) representatives the hp infection + VD3 group.

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