Animal Nutrition 9 (2022) 269–279

Contents lists available at ScienceDirect

Animal Nutrition

journal homepage: http://www.keaipublishing.com/en/journals/aninu/

Original Research Article

Cytochrome P450 enzymes mediated by DNA methylation is involved in deoxynivalenol-induced hepatoxicity in piglets

Aimei Liu [a], Yaqin Yang [a], Jingchao Guo [a], Yan Gao [c], Qinghua Wu [d], Ling Zhao [b], Lv-hui Sun [b, *], Xu Wang [a, c, *]

[a] National Reference Laboratory of Veterinary Drug Residues and Key Laboratory for Detection of Veterinary Drug Residues, Huazhong Agricultural University, Ministry of Agriculture (MOA), Wuhan, 430070, China
[b] Hubei Hongshan Laboratory, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, 430070, China
[c] MOA Laboratory for Risk Assessment of Quality and Safety of Livestock and Poultry Products, Wuhan, 430070, China
[d] College of Life Science, Yangtze University, Jingzhou, 434025, China

ARTICLE INFO

Article history:
Received 1 July 2021
Received in revised form
4 November 2021
Accepted 9 November 2021
Available online 21 February 2022

Keywords:
Deoxynivalenol
Hepatoxicity
Growth inhibition
CYP450 enzyme
DNA methylation
Piglet

ABSTRACT

Deoxynivalenol (DON) is an inevitable contaminant in animal feed and can lead to liver damage, then decreasing appetite and causing growth retardation in piglets. Although many molecular mechanisms are related to hepatoxicity caused by DON, few studies have been done on cytochrome P450 (CYP450) enzymes and DNA methylation. To explore the role of CYP450 enzymes and DNA methylation in DON-induced liver injury, male piglets were fed a control diet, or diet containing 1.0 or 3.0 mg/kg DON for 4 weeks. DON significantly raised the activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and glutamyl transpeptidase (GGT) ($P < 0.01$), leading to liver injury. In vivo study found that DON exposure increased the expression of CYP450 enzymes (such as CYP1A1, CYP2E1, CYP3A29) ($P < 0.05$), and disturbed the expression of nicotinamide N-methyltransferase (NNMT), galanin-like peptide (GALP) and insulin-like growth factor 1 (IGF-1) ($P < 0.05$), in which DNA methylation affected the expression of these genes. In vitro study (human normal hepatocytes L02) further proved that DON elevated the expression of CYP1A1, CYP2E1 and CYP3A4 ($P < 0.05$), and inhibited cell growth in a dose-dependent manner, resulting in cell necrosis. More importantly, knockdown of CYP1A1 or CYP2E1 could alleviate DON-induced growth inhibition by promoting IGF-1 expression. Taken together, increased CYP450 enzymes expression was one of the mechanisms of hepatoxicity and growth inhibition induced by DON, suggesting that the decrease of CYP450 enzymes can antagonize the hepatoxicity in animals, which provides some value for animal feed safety.

1. Introduction

Deoxynivalenol (DON) is the most frequent mycotoxin produced by several pathogenic fungi, such as *Fusarium graminearum* (Zhou et al., 2020). DON is mainly present in wheat, barley, oats, corn and animal feeds (Wang et al., 2019), bread, biscuits (Suman et al., 2019), animal milk, eggs (Molina et al., 2019) and fruit (Pallares et al., 2019). DON contamination levels in grains and cereals range from 0.250 to 50.289 mg/kg (Streit et al., 2012). It is reported that the concentration of DON in the primary effluent samples of a Swiss waste water treatment plant is 32 to 118 ng/L (Wettstein and Bucheli, 2010). Among all organs, the liver is the most important target organ for DON-induced toxicity. Therefore, in view of the universality and severity of DON contamination, its hepatotoxicity should be paid great attention.

Cytochrome P450 (CYP450) enzymes in the liver do not directly metabolise DON (Peng et al., 2017), but these enzymes may be related to hepatic damage. Previous studies have found many

* Corresponding authors.
E-mail addresses: lvhuisun@mail.hzau.edu (L-h. Sun), wangxu@mail.hzau.edu.cn (X. Wang).

Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.

Production and Hosting by Elsevier on behalf of KeAi

https://doi.org/10.1016/j.aninu.2021.11.009

mechanisms of liver injury caused by DON, such as lipid peroxidation, oxidative stress (Yu et al., 2019), apoptosis (Zhou et al., 2020), inflammatory response (Reddy et al., 2018) and autophagy (Peng et al., 2019b). CYP450 enzymes are the most important enzyme for drug metabolism. However, it has been discovered in recent years that even though CYP450 does not metabolize drugs, it may also be an important target of drug-induced liver toxicity (Balakina et al., 2017; Bou-Fakhredin et al., 2021). Moreover, the literature showed that a diet containing 8 mg DON/kg feed fed to pigs for 4 weeks resulted in an increased expression of CYP1A26 in the liver (Reddy et al., 2018). Therefore, we speculate that CYP450 may also be closely related to liver damage caused by DON. However, it is unclear whether these enzymes mediated DON-induced hepatoxicity and growth inhibition.

In addition, DNA methylation is closely associated with toxin-induced liver damage and growth inhibition (Liu et al., 2019a). However, to the best of our knowledge, the potential role of DNA methylation in DON-induced CYP450 enzymes expression has been unclear. The expression of growth hormone, growth hormone receptor and insulin-like growth factor 1 (IGF-1) were influenced by DNA methylation (Ouni et al., 2015; Strobl et al., 1986). A study showed that 8 mg/kg of DON in the diet was responsible for significant decreases in the mRNA expression of nicotinamide N-methyltransferase (NNMT), galanin-like peptide (GALP) and IGF-1, but significantly promoted the expression of insulin-like growth factor-binding protein 2 (IGFBP2) in pig liver, resulting in growth stunting of pigs (Reddy et al., 2018). However, whether DNA methylation affects the expression of these genes in DON-exposed liver is unclear.

Thus, the present study was designed to investigate whether CYP450 enzymes and DNA methylation were involved in DON-induced hepatoxicity and growth inhibition, which used piglets as an in vivo model and human normal hepatocytes L02 as an in vitro model to study. The dose used in this study was selected on the basis of previous research reports on DON in piglets (Gerez et al., 2015; Wang et al., 2018; Van Le Thanh et al., 2016; Zhang et al., 2020b), and piglets were exposed to toxic levels of 1 and 3 mg/kg for 4 weeks of treatment, which resulted in weight gain and feed intake of piglets (Liu et al., 2020a, b; Zhang et al., 2020b). The effect of DON on the liver function was determined by analysing the changes of liver function indices in serum. The expression of CYP450 enzymes, growth- and DNA methylation-related genes was tested by quantitative real-time PCR (qPCR) and Western blot. Furthermore, the level of 5-methyl cytosine (5-mC) in the whole genome was detected by colorimetry. The methylation level of CYP450 enzyme gene promoter and growth-related gene promoter in the liver was detected by methylation-specific PCR (MSP) and bisulfite sequencing (BSP), respectively. The effect of DON on CYP450 enzymes expression, cell growth and pathological changes were further studied in vitro. In L02 cells, after siRNA interference of CYP1A1 and CYP2E1, the role of CYP450 enzymes in DON-induced growth inhibition was further verified.

2. Materials and methods

2.1. Experimental animals and design

The animal protocol for this study was approved by the Institutional Animal Care and Use Committee of Huazhong Agricultural University, China. In total, 21 male castrated crossbred ([Duroc × Landrace] × Large white) weanling piglets (3 weeks of age) were randomly allocated to 3 treatment groups; each group was assigned to 7 pens of 1 piglet/pen. Piglets were allowed free access to water and fed twice a day (08:00 and 17:00) with a corn-soybean based diet (control; Table S1) formulated to meet the NRC nutritional requirements (National Research Council (NRC), 2012), or the control diet spiked with 1.0 or 3.0 mg/kg DON. The experiment lasted 28 d, and during this period piglets were immunised with the OVA (0.5 mg + Quil A adjuvant) pseudorabies (PS) vaccine, swine fever (SF) vaccine and porcine circoviruses (PC) vaccine at 1 and 2 weeks into the feeding trial. The use dose of these vaccines (Huapai Bio-engineering Group Co., Ltd. Sichuan, China) was 1/2 recommended dose. On 28 d of experiment, all pigs were humanely sacrificed to harvest blood and liver samples. The blood was centrifuged to obtain serum and stored at −80 °C for subsequent biochemical detection. The tissues were washed with ice-cold saline, then divided into aliquots that were snap-frozen in liquid nitrogen and stored at −80 °C for further analysis. All animal work was in compliance with NIH Publication, The Development of Science Based Guidelines for Laboratory Animal Care.

2.2. Cell culture and cell viability assay

DON (CAS NO. 514810−8) was dissolved in dimethyl sulfoxide (DMSO) (Sigma−Aldrich, St. Louis, MO, USA) and then was diluted in RPMI-1640 (Gibco) medium. The L02 cell line was purchased from Suer Biotechnology Co., Ltd of Shanghai, China. L02 cells were cultured in RPMI-1640 medium containing 10% fetal bovine serum (FBS, Gibco), 1% glutamine, 1% penicillin and 1% streptomycin, at 37 °C in a 5% CO_2 incubator. The cells were sub-cultured 3 to 4 times and then used in subsequent experiments. To determine the optimal incubation concentration and time for the toxicity of DON to impact L02 cells, the cells (1×10^5) were seeded in 96-well plates and treated with different concentrations of DON for 12, 24 and 36 h, respectively. After incubation, the cells were treated with 10 μL/well with CCK-8 reagent for 2 h at 37 °C. The optical density (OD) was measured using a micro-quant plate reader (Bio-Tek Instruments) (TECAN A-5082, Magellan, Austria). Cells were cultured in cell plates and incubated with DON (2.5, 5, 10 μmol/L) for 24 h for follow-up experiments. Every experiment was carried out thrice on three separate occasions.

2.3. Measurement of hepatic biochemical indices

The 21 blood samples from piglets were analyzed biochemically. The activity of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and glutamyl transpeptidase (GGT) was determined by an autoanalyzer (Hitachi Model 7600 analyzer; Hitachi, Tokyo, Japan) using AST, ALT and GGT assay kits purchased from Mindray (Shenzhen, China) as described previously (Peng et al., 2019a; Santana et al., 2018; Wang et al., 2018a).

2.4. qPCR examination

Total RNA Extraction Reagent was used to obtain RNA from 21 piglets' liver and L02 cells. The quality of RNA was determined by micro ultraviolet spectrophotometer. Then the RNA was reverse-transcribed into cDNA using HiScript II 1st Strand cDNA Synthesis Kit (Vazyme, Nanjing, China) for qPCR. The qPCR primers are shown in Table S2 and Table S3. The qPCR reaction system was based on work previously described (Liu et al., 2019a). In this study, the housekeeping gene β-actin was used as an internal calibrator reference gene for expression profiling of genes. Data were analyzed and quantified using the $2^{-\Delta\Delta Ct}$ methods: $\Delta\Delta Ct = (Ct_{\text{experimental group target gene}} - Ct_{\text{experimental group β-actin}}) - (Ct_{\text{control target gene}} - Ct_{\text{control β-actin}})$.

2.5. Protein extraction and Western blot assay

The protein from 21 piglets' liver tissue and L02 cells were collected with RIPA Lysis Buffer (Servicebio, Wuhan, China) containing protease inhibitor cocktail and phosphatase inhibitors (Servicebio, Wuhan, China). The proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore, MA, USA). Blots were blocked with 5% bovine serum albumin (BSA) for 1 h and incubated with primary antibodies (species specificity: Human, Mouse, Rat) (ABclonal Technology, Wuhan, China) for NNMT Rabbit pAb (A18033) (1:500), CYP1A1 Rabbit pAb (A2159) (1:1,000), CYP2E1 Rabbit pAb (A2160) (1:1,000), CYP3A4 Rabbit pAb (1:1,000), IGF1 Rabbit pAb (A11985) (1:500), GALP Rabbit pAb (A17445) (1:1,000) and GAPDH Rabbit mAb (A19056) (1:2,000) dilution overnight at 4 °C. The PVDF membranes were subsequently incubated with secondary antibodies HRP Goat Anti-Rabbit IgG (H + L) (AS014) (ABclonal Technology, Wuhan, China) (1:5,000) at room temperature for 1 h before developing the membranes with a LAS-4000 luminescent image analyzer (Fujifilm, Tokyo, Japan). Finally, we applied Quantity One software to analyze the protein gray value.

2.6. Genome-wide DNA methylation and MSP assays

In order to detect the effect of DON on the methylation of genomic DNA, a colorimetric method was used to detect the level of 5-mC in all nucleotides in genomic DNA of the liver tissue. The specific steps of this method were based on work previously described (Liu et al., 2019a).

To determine the 5-mC level of gene promoter regions related to the metabolism and growth in liver tissue, the EZ DNA Methylation-Gold kit was used for bisulfite conversion of guanine-cytosine-rich DNA. Methylated and unmethylated primers for CYP450 enzymes and *NNMT*, *GALP*, *IGF-1* and *IGFBP2* genes are shown in Table S4. MSP analysis was conducted according to a previous study (Liu et al., 2019a).

2.7. BSP analysis

DNA of piglet liver was modified with bisulfite to detect cytosine-phosphate-guanine (CpG) dinucleotide methylation in the promoter regions of NNMT, GALP, IGF-1 and IGFBP2. These gene promoter regions were amplified from the bisulfite-modified sequence by BSP primers in Table S5. For the follow-up steps, please refer to previous studies (Liu et al., 2019a, 2019b). In short, the PCR products were recovered and purified, then connected with the vector, cloned and sequenced by selecting white single colonies.

2.8. Hematoxylin and eosin (H&E) and 5-ethynyl-2′-deoxyuridine (EdU) staining of L02 cells

L02 cells (1×10^4) were inoculated in 12-well plates covered with glass coverslips and grown to 70% to 80% confluency. DON (2.5, 5, 10 μmol/L) was added to L02 cells. Then, after 24 h, the cells were fixed and stained with H&E and EdU kit (Guangzhou RiboBio Co., Ltd.). Cell damage, including whether the cell edge was clear, the change of nuclear morphology, and whether the cell had necrosis was observed under an inverted microscope (Olympus BX 41, Japan).

2.9. CYP1A1 and CYP2E1 gene silencing by siRNA and cell proliferation assay

CYP1A1 and *CYP2E1* were transiently knocked out by siRNA transfection under the guidance of the manufacturer. Briefly, L02 cells were cultured to 60% to 70% confluence. The negative control (siNC), siCYP1A1 (sense: GUGAGCACUUCCAAAUGCAdTdT, anti-sense: UGCAUUUGGAAGUGCUCACdTdT) and siCYP2E1 (UGGUGGUGAUGCACGGCUA) were mixed with Lipofectamine 2,000 reagent (Invitrogen, USA) and added to the culture medium. After 6 h of transfection, the culture medium was changed to fresh medium for a further 18 h. The interference efficiency of *CYP1A1* and *CYP2E1* was tested by qPCR and Western blot assays.

L02 cells (1×10^5) were seeded in 24-well plates covered with glass coverslips and grown to 60% to 70% confluency. SiRNA was transfected into L02 cells, and then L02 cells were cultured for 24 h, followed by DON (5 μmol/L) incubating for 24 h. The cells were stained with EdU kit and the cell proliferation was analyzed by fluorescence microscope (Olympus, Tokyo, Japan).

2.10. Statistical analysis

Results were presented as mean ± standard error of the mean (SEM). Statistical analyses were adopted using SPSS 13.0 (Chicago, IL, USA). Data were analyzed by a one-way ANOVA with a significance level of $P < 0.05$. The Tukey–Kramer method was used for multiple mean comparisons. The BSP data were presented by the Mann–Whitney non-parametric test for multiple comparisons.

3. Results

3.1. DON affected serum liver biochemical indices in piglets

To observe the effect of DON on piglet liver function, serum chemical parameters including AST, ALT, and GGT were measured. According to the data presented in Fig. 1A, serum ALT and AST activity in the DON group significantly increased compared to 0 mg/kg group ($P < 0.01$). It should be noted that the ratio of AST to ALT (AST/ALT >1) in the DON-treated groups exhibited higher levels than that of the control group ($P < 0.01$), suggesting that the liver was damaged to a certain extent. Additionally, a sharp increase was observed in GGT activity following DON exposure when compared to the control group ($P < 0.01$).

3.2. DON exposure caused changes in CYP450 enzymes expression by DNA methylation in piglet liver

To check whether DON exposure caused abnormal expression of CYP450 enzymes, the mRNA level was examined. As shown in Fig. 1B, the mRNA expression levels of CYP450 enzymes, including CYP1A1, CYP1A2, CYP450 2B22 (CYP2B22), CYP450 2C33 (CYP2C33), CYP450 3A22 (CYP3A22), CYP450 2D25 (CYP2D25), CYP2E1 and CYP450 3A29 (CYP3A29), were significantly increased after DON administration at 3 mg/kg ($P < 0.05$). However, the expression of CYP2D25 and CYP2E1 in the DON group at 1 mg/kg exhibited a significant decrease compared to the control group ($P < 0.05$). The trend of protein expression and mRNA expression of CYP1A1, CYP2E1 and CYP3A29 was consistent in Fig. 1E. These results showed that DON exposure changed the expression of CYP450 enzymes.

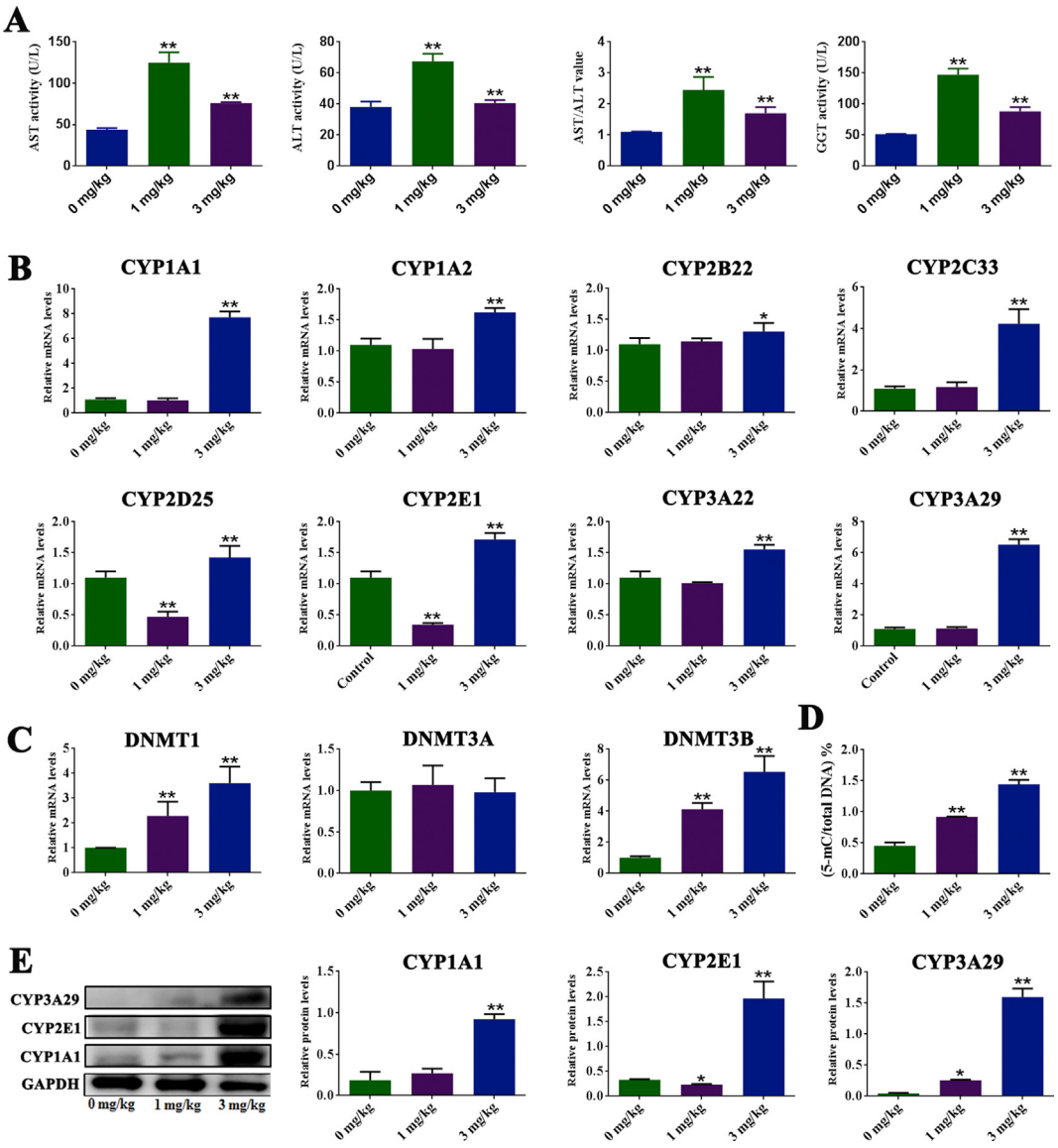

Fig. 1. Liver functional indicators, CYP450 enzyme, DNA methyltransferase expression and genome-wide 5-mC level in the liver of piglets fed diets containing DON-contaminated corn ($n = 7$). (A) Change of serum liver functional indicators AST, ALT and GGT. (B and C) The mRNA levels of CYP1A1, CYP1A2, CYP2B22, CYP2C33, CYP2D25, CYP2E1, CYP3A22, CYP3A29 and DNMT1, DNMT3A and DNMT3B were detected by qPCR. (D) The 5-mC level of genomic genes was assessed by the colorimetric. (E) The protein levels of CYP1A1, CYP2E1 and CYP3A29 were detected by Western blot. *$P < 0.05$, **$P < 0.01$ versus 0 mg/kg group. ALT = alanine aminotransferase; AST = aspartate aminotransferase; CYP = cytochrome P; DNMT = DNA methyltransferases; GGT = glutamyl transpeptidase; 5-mC = 5-methyl cytosine.

In DNA methylation analysis, we focused primarily on the level of 5-mC, which is regulated by DNA methyltransferases (DNMT1, DNMT3A, DNMT3B). As shown in Fig. 1C, the mRNA expression levels of DNMT1 and DNMT3B were significantly increased after DON exposure compared to the control group ($P < 0.01$). However, the DNMT3A expression of DON groups showed no significant difference compared to the control group. We tested the genomic 5-mC levels in pig liver, where our results uncovered that DON (1 or 3 mg/kg) significantly increased the total level of 5-mC compared with the control group ($P < 0.01$) (Fig. 1D).

The MSP was used to detect the methylation levels of CpG sites in the CYP450 enzymes gene promoter region. As shown in Fig. 2A and B, in one DON exposure group (3 mg/kg), the methylation levels of CYP1A1 ($P < 0.05$), CYP2D25 ($P < 0.05$), CYP2E1 ($P < 0.01$), and CYP3A29 ($P < 0.05$) promoters were significantly decreased compared to the control, thus promoting the expression of these genes. Additionally, the methylation level of CYP1A2 promoter in DON-exposure groups was lower than the control group ($P < 0.01$), which possibly promoted the transcription of the *CYP1A2* gene. Although the methylation level of CYP2B22 promoter was increased ($P < 0.05$), the expression of *CYP2B22* gene was raised.

3.3. DON exposure caused changes of growth-regulating gene expression by DNA methylation in piglet liver

To investigate the effect of DON exposure on piglet growth, we also examined the expression of growth-related genes in the liver (Fig. 3A, and B). The results demonstrated that the expression levels of *NNMT* were markedly decreased after DON administration (1 or 3 mg/kg; $P < 0.01$) compared to the control group. DON exposure at 3 mg/kg sharply elevated the expression of *GALP* ($P < 0.01$), *IGF-1* ($P < 0.05$) and *IGFBP2* ($P < 0.01$) when compared to the control group ($P < 0.05$), which might be the compensatory effect of DON-induced growth suppression toxicity.

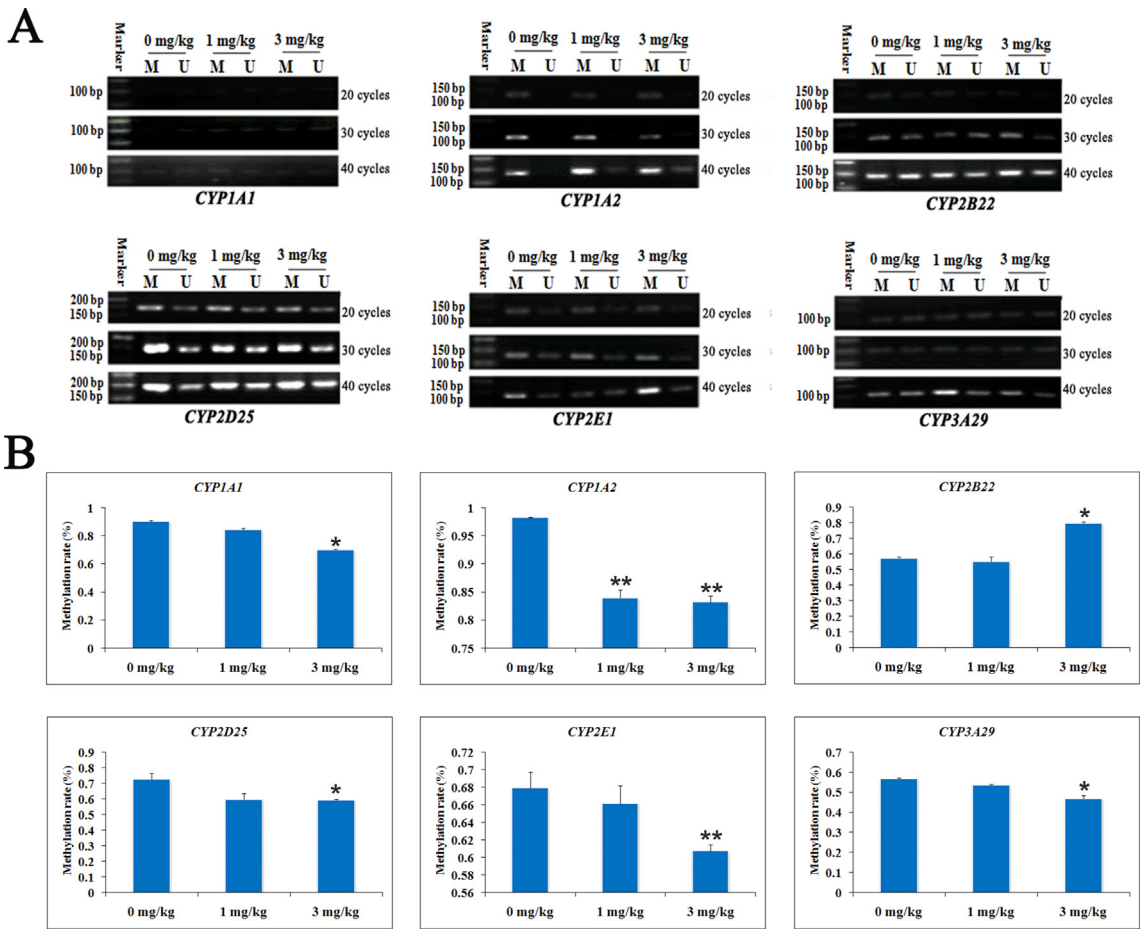

Fig. 2. The methylation levels of CYP450 enzyme genes in the liver of piglets fed diets containing DON-contaminated corn ($n = 7$). (A) The modified liver genomic DNA was amplified by methylation-specific PCR (MSP). In the figure, M stands for methylation, and U stands for unmethylation. (B) The quantitative analysis of the product brightness in Fig. 2A. *$P < 0.05$, **$P < 0.01$ versus 0 mg/kg group.

To further determine whether DNA methylation was involved in the expression of *NNMT*, *GALP*, *IGF-1* and *IGFBP2* after exposure to DON in pig liver, MSP and BSP were applied (Fig. 3C, and D). For these genes, the CpG sites of MSP assay were included in the sequence of BSP analysis. Although only methylated primers amplified the bands of all groups, the methylated bands of the DON treatment group were stronger, suggesting an increased methylation level of NNMT to some extent. BSP results of *NNMT* gene also revealed that some CpG sites in DON-exposed groups were completely methylated compared to the control group, thus promoting *NNMT* transcription. The methylation level of *GALP* in one DON group (3 mg/kg) was lower than the control group and induced *GALP* expression. DON treatment could decrease the promoter methylation level of *IGF-1*, promoting *IGF-1* expression. However, the *IGFBP2* methylation level in DON groups exhibited no significant difference compared to the control group.

3.4. DON exposure increased the expression of CYP450 enzymes and decreased the expression of growth-related genes in L02 cells

The results of **c**ell viability assay displayed a dose-and time-dependent decrease in cell viability induced by DON in L02 cells (Fig. S1). Considering the median inhibitory concentration (IC50) and the state of L02 cells, DON concentration (2.5, 5, 10 μmol/L) and 24 h of exposure time were selected for the subsequent studies. As shown in Fig. 4A, DON (5, 10 μmol/L) significantly ($P < 0.01$) increased the mRNA expression of *CYP1A1*, *CYP2E1* and *CYP3A4* genes, which is consistent with the results of our in vivo study. In addition, the expression of growth-related genes (*IGF-1*, *IGFBP2* and *NNMT*) was significantly ($P < 0.01$) downregulated after DON exposure. The protein level of CYP450 enzymes and growth-related genes was consistent with the mRNA expression trend (Fig. 4B, and C).

3.5. DON led to cell necrosis and inhibited cell growth in a dose-dependent manner

In the control group, the nucleus was oval and chromatin was clearly distributed in the nucleus. Compared with the control group, DON exposure resulted in chromatin marginalization, nuclear pyknosis and nuclear karyorrhexis, which is a typical feature of cell necrosis. Importantly, DON treatment inhibited cell growth in a dose-dependent manner (Fig. 4E).

3.6. Silencing CYP1A1 increased the expression level of IGF-1, promoting cell growth

To prove whether CYP1A1 mediates growth inhibition induced by DON, L02 cells were transfected with CYP1A1-specific siRNA. The knockdown efficiency was detected by qPCR and Western blot analysis. The results illustrated that the expression level of CYP1A1 was significantly reduced ($P < 0.01$) in CYP1A1-siRNA-transfected cells compared with the siNC group (Fig. 5A, and B). To detect the

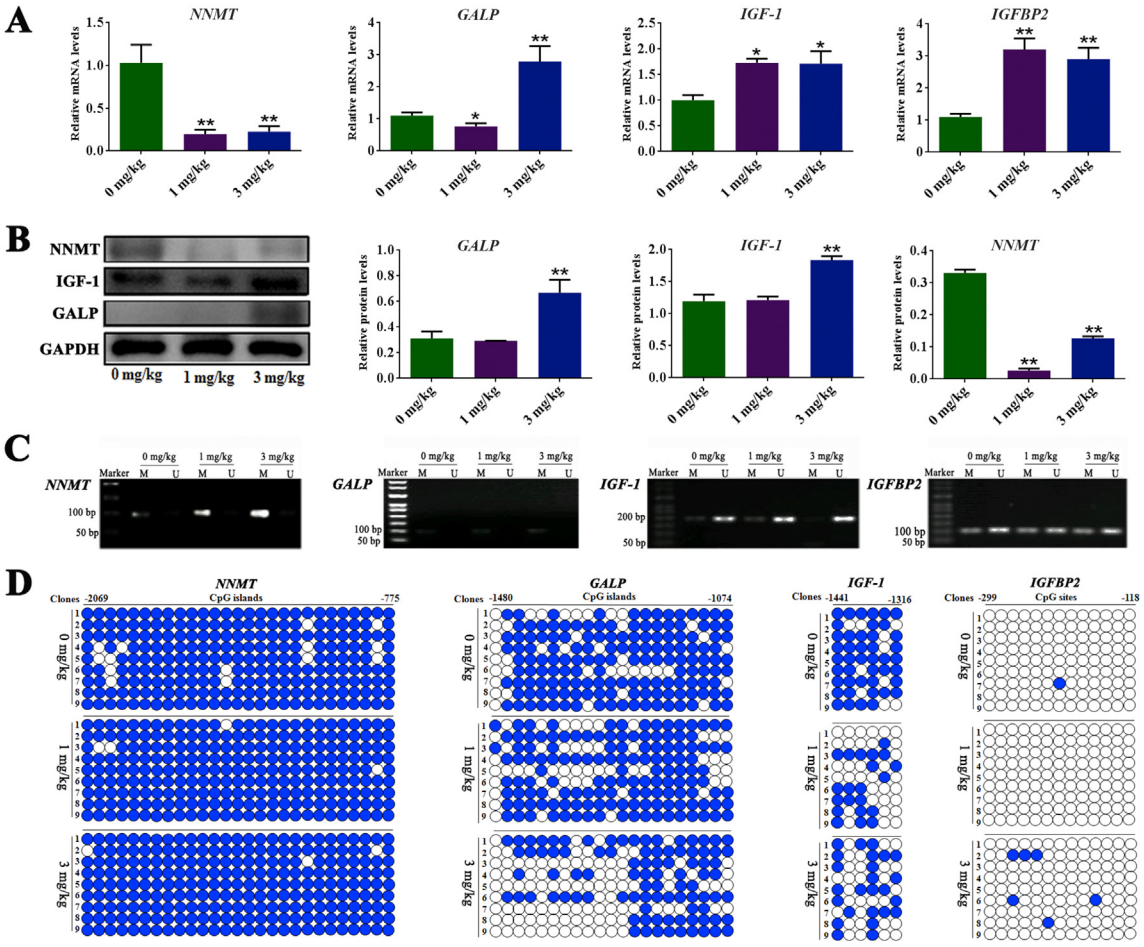

Fig. 3. The expression and methylation levels closely related to growth (NNMT, GALP, IGF-1, IGFBP2) in the liver of piglets fed diets containing DON-contaminated corn ($n = 7$). (A) The mRNA levels of NNMT, GALP, IGF-1 and IGFBP2 were detected by qPCR. (B) The protein expression of NNMT, GALP and IGF-1 was detected by Western blot. (C) The methylation status of CpG sites in gene promoter region was detected by methylation-specific PCR (MSP). M for methylation, U for unmethylation. (D) The methylation levels in gene promoter regions were detected by bisulfite sequencing (BSP). The blue dots for the CpG sites of methylation, and the white dots for the unmethylated CpG sites. $^{*}P < 0.05$, $^{**}P < 0.01$ versus 0 mg/kg group. CpG = cytosine-phosphate-guanine; GALP = galanin-like peptide; NNMT = nicotinamide N-methyltransferase; IGF-1 = insulin-like growth factor 1; IGFBP2 = insulin-like growth factor-binding protein 2.

effect of CYP1A1 inhibition on the expression of cell growth-related genes, the mRNA and protein expression of IGF-1 was analysed at 24 h after transfection. Knockout of *CYP1A1* gene significantly increased IGF-1 expression ($P < 0.01$) (Fig. 5A, and B). Correspondingly, compared with the siNC group, CYP1A1 knockout promoted cell growth ($P < 0.01$) (Fig. 5C, and D). Interestingly, decreased CYP1A1 alleviated growth inhibition induced by DON to some extent ($P < 0.01$) (Fig. 5C, and D).

3.7. Silencing CYP2E1 promoted the expression level of IGF-1, promoting cell growth

To prove whether CYP2E1 also mediates the growth inhibition induced by DON, L02 cells were transfected with CYP2E1-specific siRNA or siNC. The knockdown efficiency was detected by qPCR and Western Blot analysis. The results illustrated that the expression level of CYP2E1 was significantly decreased in CYP2E1-siRNA-transfected cells compared with the siNC group ($P < 0.01$) (Fig. 6A, and B). In addition, CYP2E1 silencing promoted IGF-1 expression ($P < 0.01$) (Fig. 6A, and B). Correspondingly, compared with the siNC group, knockdown of CYP2E1 induced cell growth ($P < 0.01$) (Fig. 6C). Noticeably, low expression of CYP1A1 also alleviated the growth inhibition induced by DON ($P < 0.01$) (Fig. 6C, and D).

4. Discussion

Due to high toxicity levels and widespread occurrence in animal feed and food, DON is considered an unavoidable contaminant in nature, which leads to hepatoxicity and even growth retardation of animals (Wang et al., 2012). The liver is an important organ for metabolism and detoxification, and its damage is closely related to stunting growth under DON exposure. Our previous study found that 1.0 or 3.0 mg/kg DON reduced the body weight of piglets (Liu et al., 2020a; Zhang et al., 2020b), however, the role of DON-induced liver injury and the mechanism in growth retardation is unclear. Additionally, although there are increasing studies on DON induced-hepatoxicity, the role of CYP450 enzymes and DNA methylation is still unclear. The present study showed DNA demethylation elevated the expression of CYP450 enzymes in DON-treated piglet livers, with an increase of AST and ALT. DON exposure reduced the expression of NNMT and GALP through DNA methylation, with the decrease in feed intake and weight of piglets (Liu et al., 2020a; Zhang et al., 2020b). In vitro studies found that different concentrations of DON inhibited cell growth and led to cell necrosis. In addition, it was found for the first time that CYP450 enzymes such as CYP1A1 and CYP2E1 could mediate DON-induced cell growth inhibition, although they do not metabolize DON.

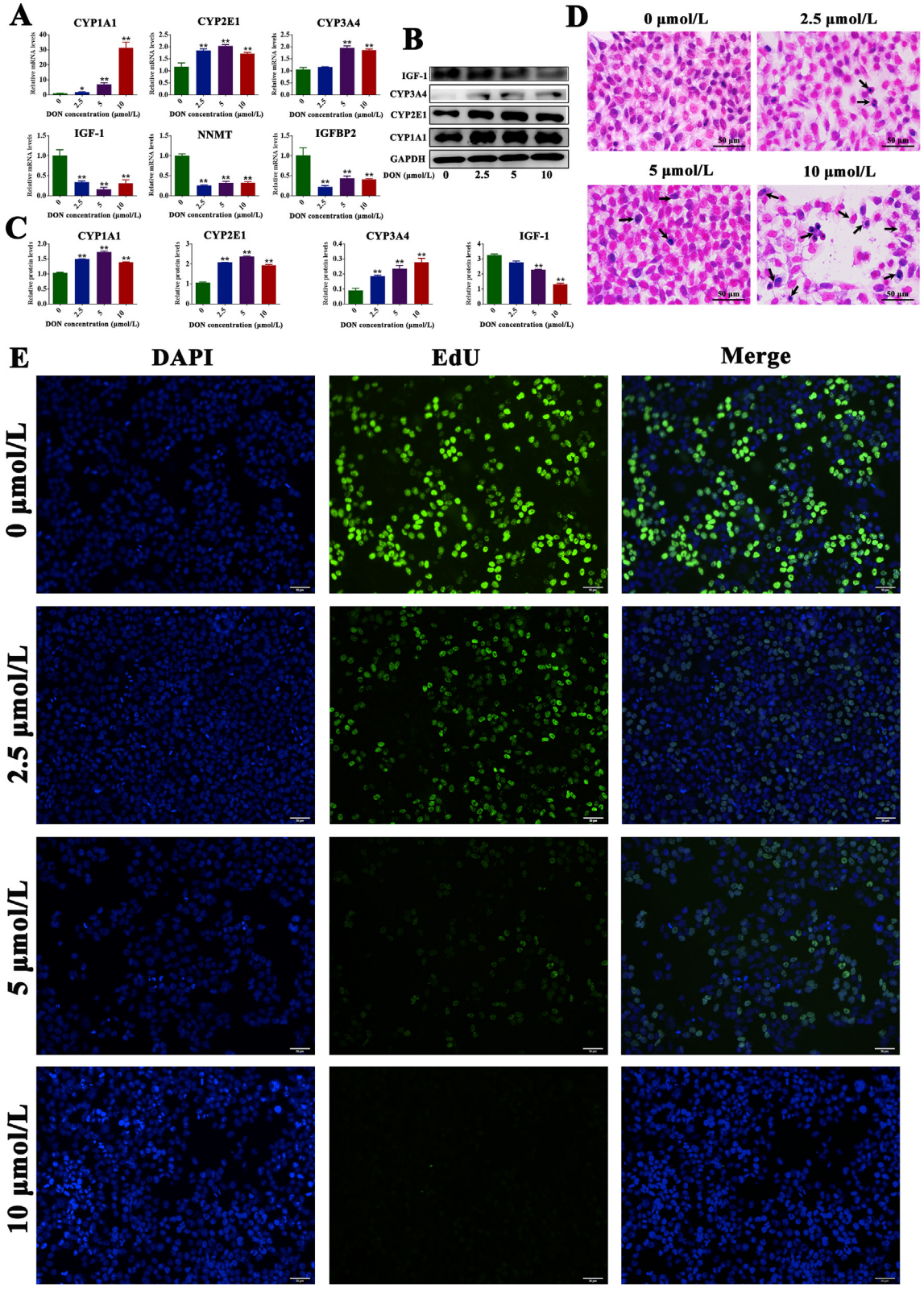

Fig. 4. The effects of different concentrations of DON on the expression of CYP450 enzymes and growth-related genes, on cell damage and cell growth were tested in human normal hepatocytes L02 cells. (A) The mRNA levels of CYP1A1, CYP2E1, CYP3A4, NNMT, IGF-1 and IGFBP2 were detected by qPCR. (B and C) The protein levels of CYP1A1, CYP2E1, CYP3A4, and IGF-1 were detected by Western blot. (D) The pathological changes of L02 cells under DON exposure were detected by H&E staining. (E) L02 cell proliferation after DON treatment was detected by the EdU reagent (100 × magnification, scale bar = 50 μm). $^{*}P < 0.05$, $^{**}P < 0.01$ versus the control group. EdU = 5-ethynyl-2′-deoxyuridine.

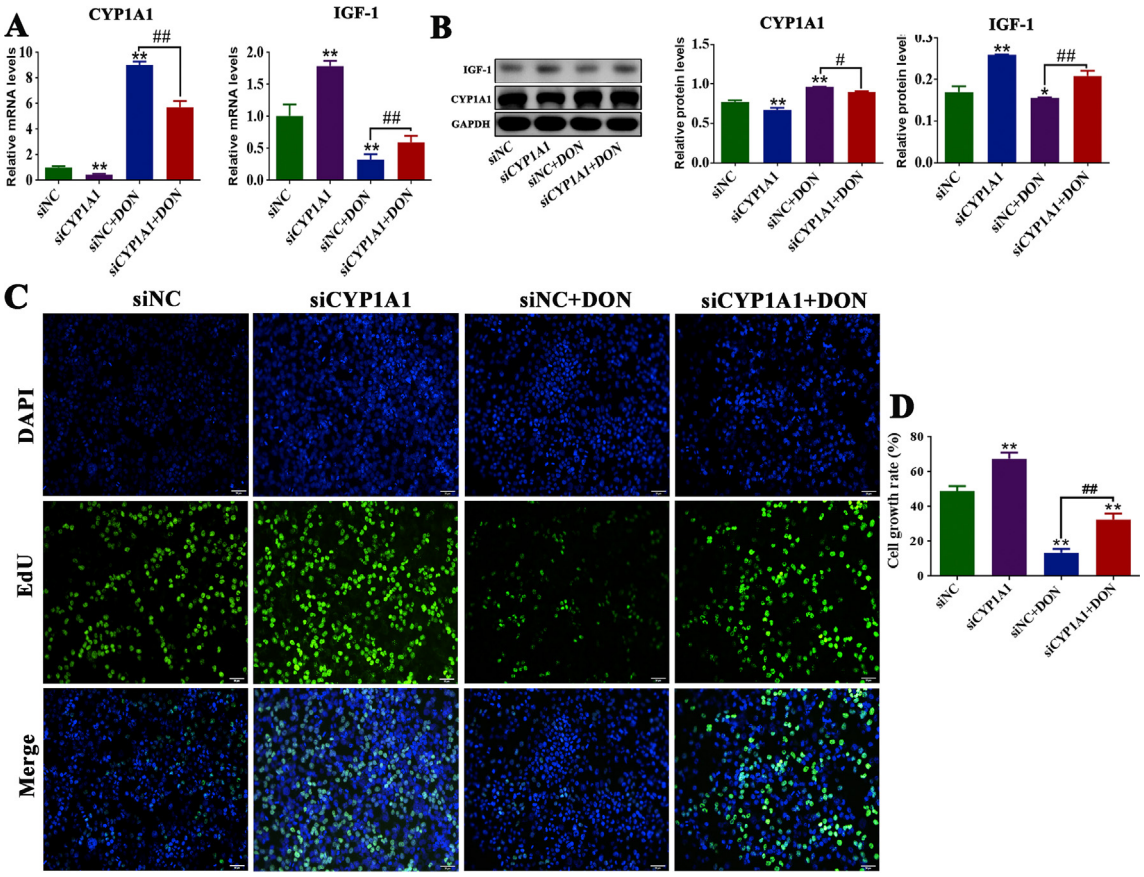

Fig. 5. Effects of CYP1A1 silencing on cell proliferation. After L02 cells were transfected with the siNC and siCYP1A1 for 24 h, and then added DON (5 μmol/L) for 24 h. (A and B) The expression levels of CYP1A1 and IGF-1 were detected by qPCR and Western blot. (C) L02 cell proliferation was detected by the EdU reagent (100 × magnification, scale bar = 50 μm). (D) The level of cell proliferation in Fig. 5C was quantified. *$P < 0.05$, **$P < 0.01$ versus the siNC group; #$P < 0.05$, ##$P < 0.01$ versus the siNC + DON group, and the data were expressed as SD ($n = 3$).

Feed levels of 1 and 3 mg/kg of DON are possible daily doses for piglets. In Portugal, by using high-pressure liquid chromatography (HPLC) to analyse 307 samples of plant crops, the highest level of DON concentration was found to be 17.9 mg/kg (Marques et al., 2008). It was reported that the average content of 23,980 samples contaminated by DON was as follows: wheat, 9.9 mg/kg; corn, 4.772 mg/kg; rice, 0.183 mg/kg; barley, 6.349 mg/kg; oats, 0.537 mg/kg; and rye, 0.190 mg/kg (Tardivel et al., 2015). DON was also detected in pasta with the highest level in the European Union of 3.2 mg/kg (Marin et al., 2013). Therefore, the dose used in this study was within the exposure dose range of animals to DON.

Although CYP450 enzymes do not participate in the direct metabolism of DON, abnormal upregulation in CYP450 enzymes were the mechanisms for DON-induced hepatoxicity and then growth inhibition. Recent literature suggested that CYP450 enzymes are involved in oxidative stress, apoptosis and inflammatory response (Cong et al., 2019; Xie et al., 2018). One study reported that DON (4.6 mg/kg feed) had no effect on the mRNA expression of CYP1A4 and CYP3A37 in broiler chickens' liver (Antonissen et al., 2017). However, the present study stated clearly that DON (3 mg/kg feed) could significantly increase the expression level of CYP1A1, CYP2E1 and CYP3A4 in piglet liver. The differences in the result may be related to the DON dose, and the sensitivity of piglets to DON.

IGF-1 is an endocrine hormone produced in the liver and a sensitive index reflecting the function of growth hormone. DON (3 mg/kg) increased the expression of IGF-1 and IGFBP2 in piglet liver, but their expression was reduced under DON exposure in vitro. This may be related to the different self-repair environments in which in vivo and in vitro cells are located. The adverse consequences of DON in the body of piglet growth retardation may stimulate the production of IGF-1 and IGFBP2 to protect the body. However, the cell environment in vitro is simple and the compensatory effect is poor (Paravidino et al., 2021). Therefore, when DON inhibited growth, the expression of IGF-1 decreased.

Additionally, in vitro studies have found that forced reduction of CYP1A1 and CYP2E1 could raise the expression of IGF-1 and alleviate DON-caused growth inhibition. However, in vivo studies have shown that DON promoted the expression of CYP450, and the expression of IGF-1 also increased. Danicke et al. (2005) found that when 300 μg pure DON/kg was fed to 34 female pigs for 2 weeks after weaning under standardized conditions, serum IGF-1 levels were increased, but were decreased in 1,200 μg pure DON/kg groups at 8 weeks compared to the control group (Danicke et al., 2005). Therefore, the effect of DON on the level of IGF-1 in pigs may be related to the dose of DON, animal sensitivity and growth environment. In addition, DON inhibits the synthesis of growth hormone and affects the growth hormone/IGF axis (Guo et al., 2021; Liu et al., 2020b), thereby stimulating the expression of IGF-1 to resist the adverse effects of DON. At the same time, the highly expressed IGF-1 may also activate the PI3 kinase (PI3K)/serine kinase (AKT) pathway, thereby reducing the degree of cell growth inhibition caused by DON (Li et al., 2020).

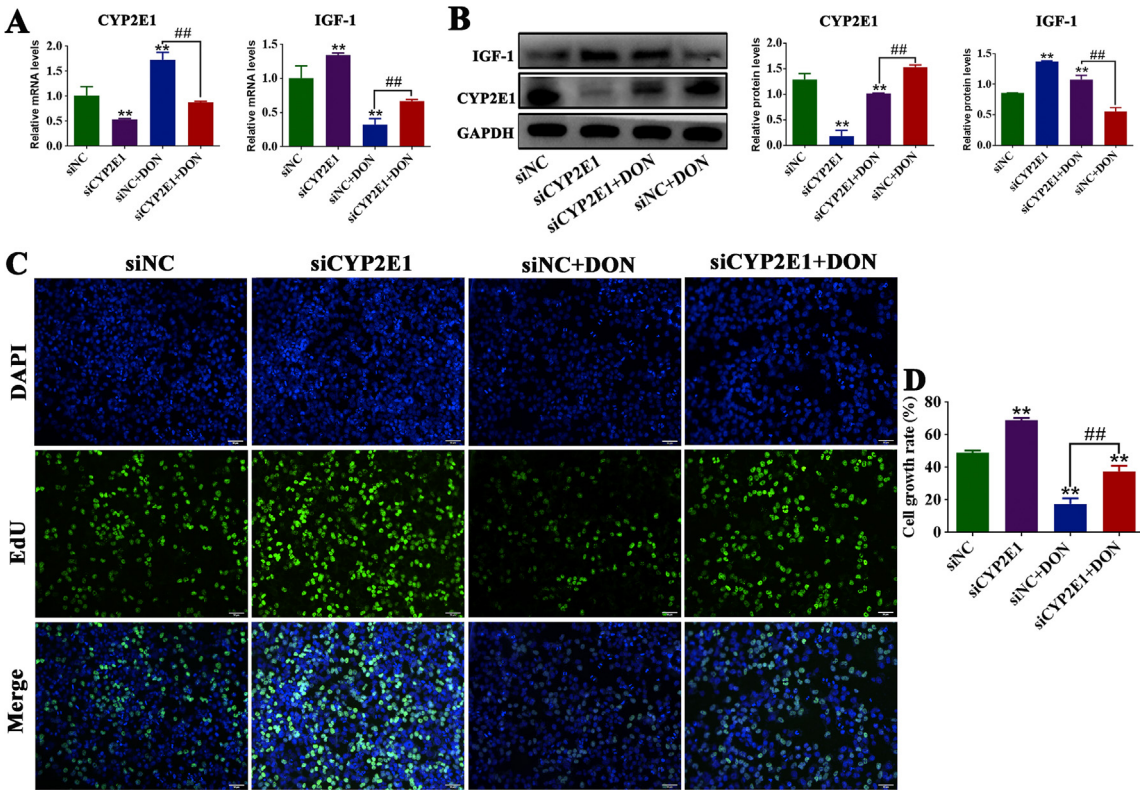

Fig. 6. Effects of CYP2E1 silencing on cell proliferation. After L02 cells were transfected with the siNC and siCYP2E1 for 24 h, and then added DON (5 μmol/L) for 24 h. (A and B) The expression levels of CYP2E1 and IGF-1 were analyzed by qPCR and Western blot methods. (C) L02 cell proliferation was detected by the EdU reagent (100 × magnification, scale bar = 50 μm). (D) The level of cell proliferation in Fig. 6C was quantified. *$P < 0.05$, **$P < 0.01$ versus the siNC group; #$P < 0.05$, ##$P < 0.01$ versus the siNC + DON group, and the data were expressed as SD ($n = 3$).

Piglets are one of the most sensitive species to DON contaminated feed (Amuzie and Pestka, 2010; Pestka, 2010). The liver plays a key role in the metabolism and detoxification of DON (Wu et al., 2013). However, various investigations into DON in piglet liver have generated inconsistent results, with some showing hepatotoxicity of DON, and others not. For example, DON at 3.1 mg/kg feed for 37 d had no impact on pig liver (Stanek et al., 2012). However, growing pigs given DON-contaminated feed (6 mg/kg for 21 d) increased the values of ALT and AST in serum (Wu et al., 2013). A diet containing 8 mg/kg DON fed for 4 weeks disrupted the immune-related processes in the liver of piglets (Reddy et al., 2018). In the present study we selected the administration dose of DON of 1 and 3 mg/kg feed and fed piglets for 4 weeks. Herein, compared with the control group, DON elevated ALT, AST and GGT activity, and the ratio of AST to ALT was greater than 1, demonstrating that DON caused severe liver damage (Wang et al., 2020; Zhang et al., 2020a).

DNA methylation could be used as a sensitive molecular indicator of DON-induced liver injury. Regarding DNA methylation, DNMT1, DNMT3A and DNMT3B, maintain synergistically the stability of DNA methylation (Liu et al., 2019a). Mycotoxins can lead to changes in gene DNA methylation, thereby changing gene expression (Liu et al., 2019a, 2019b). It was found that 10 mM DON increased the percentage of 5-mC in DNA from 4.5% to 9% in Caco-2 cells (Kouadio et al., 2007). In the current study, DON exposure raised the genomic 5-mC level in piglet livers. We also observed that with the higher concentration of DON the effect was also greater. This might indicate that the dynamic changes of 5-mC level may be closely related to the degree of liver injury induced by DON (Ye et al., 2016). Additionally, DNA methylation is a molecular switch that regulates CYP450 enzymes expression of DON-exposed piglet liver. In the present study, it was found that DON at 3 mg/kg could decrease the methylation level of the promoter region of enzymes, including CYP1A1, CYP1A2, CYP2D25, CYP2E1 and CYP3A29, and thus increase their mRNA expression levels. Strangely, the results found that DON at 3 mg/kg significantly increased the methylation level of the CYP2B22 gene promoter, but the expression of CYP2B22 was significantly increased, which suggested that DON also affected CYP2B22 expression through other transcriptional factors (Gray and Squires, 2013; Messina et al., 2009). There were differences in the regulation of DNA methylation on the expression of different CYP450 enzymes, which may be related to the sequence of gene promoter, specific transcription factors and gene polymorphisms of different individuals (Tang and Chen, 2015).

DON also affected the expression of genes related to animal feeding and growth. NNMT is closely related to ALT release and liver inflammation (Sternak et al., 2010). In our study, DON significantly reduced the expression of NNMT, which may be an important factor in the weight loss of piglets. GALP is involved in regulating appetite and inflammation and energy metabolism (Dungan Lemko et al., 2008; Kageyama et al., 2005; Man and Lawrence, 2008). A high dose of DON (3 mg/kg) significantly increased the expression of GALP in the liver, which may be due to the rapid weight loss, malnutrition and increased leptin secretion of piglets fed with long-term high dose of DON (Lawrence and Fraley, 2011). Meanwhile, DON at 3 mg/kg could demethylate CpG sites in the promoter region of the GALP gene, leading to a sharp increase in the expression of GALP, which is the first time this has been discovered.

5. Conclusion

DON significantly raised the levels of AST, ALT and GGT, probably reflecting liver injury. In vivo research also found that DON increased the expression level of CYP450 enzymes and disturbed the expression of growth-related genes *NNMT*, *GALP*, *IGF-1* and *IGFBP2*, in which DNA methylation mediated the expression of these genes. In vitro experiments showed that DON exposure increased the expression of metabolic enzymes CYP3A4, CYP1A1 and CYP2E1, and inhibited cell growth in a dose-dependent manner and led to cell necrosis. The silence CYP1A1 or CYP2E1 could slow down DON-caused cell growth inhibition by elevating IGF-1 expression. These results suggest that CYP450 enzymes may be therapeutic targets to alleviate hepatoxicity and growth delay under DON exposure. In the future, this needs to be further confirmed in different models.

Author contributions

Xu Wang and **Lv-hui Sun:** financial support, experimental guidance and manuscript editing. **Aimei Liu:** concept and design, performed research, analysed data, manuscript writing. **Yaqin Yang, Jingchao Guo, Yan Gao, Qinghua Wu** and **Ling Zhao:** participate in experiments, animal breeding and statistical data.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

Acknowledgments

This work was supported by National Natural Science Foundation of China (NSFC) (32072925 and 31972741) and the Fundamental Research Funds for the Central Universities (266202 0DKPY020).

Suppplementary Tables

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aninu.2021.11.009.

References

Amuzie CJ, Pestka JJ. Suppression of insulin-like growth factor acid-labile subunit expression–a novel mechanism for deoxynivalenol-induced growth retardation. Toxicol Sci 2010;113:412–21.
Antonissen G, Devreese M, De Baere S, Martel A, Van Immerseel F, Croubels S. Impact of Fusarium mycotoxins on hepatic and intestinal mRNA expression of cytochrome P450 enzymes and drug transporters, and on the pharmacokinetics of oral enrofloxacin in broiler chickens. Food Chem Toxicol 2017;101:75–83.
Balakina AS, Aksenov IV, Trusov NV, Guseva GV, Avrenyeva LI, Kravchenko LV, et al. The influence of separate and combined supplementation with curcumin and quercetin on the protective capacity in rats. Vopr Pitan 2017;86:14–22.
Bou-Fakhredin R, Dia B, Ghadieh HE, Rivella S, Cappellini MD, Eid AA, et al. CYP450 mediates reactive oxygen species production in a mouse model of beta-Thalassemia through an increase in 20-HETE activity. Int J Mol Sci 2021:22.
Cong YM, Chi QR, Teng XH, Li S. The protection of selenium against cadmium-induced mitochondrial damage via the cytochrome P450 in the livers of chicken. Biol Trace Elem Res 2019;190:484–92.
Danicke S, Brussow KP, Valenta H, Ueberschar KH, Tiemann U, Schollenberger M. On the effects of graded levels of Fusarium toxin contaminated wheat in diets for gilts on feed intake, growth performance and metabolism of deoxynivalenol and zearalenone. Mol Nutr Food Res 2005;49:932–43.
Dungan Lemko HM, Clifton DK, Steiner RA, Fraley GS. Altered response to metabolic challenges in mice with genetically targeted deletions of galanin-like peptide. Am J Physiol Endocrinol Metab 2008;295:E605–12.
Gerez JR, Pinton P, Callu P, Grosjean F, Oswald IP, Bracarense APFL. Deoxynivalenol alone or in combination with nivalenol and zearalenone induce systemic histological changes in pigs. Exp Toxicol Pathol 2015;67:89–98.
Gray MA, Squires EJ. Effects of nuclear receptor transactivation on boar taint metabolism and gene expression in porcine hepatocytes. J Steroid Biochem 2013;133:110–9.
Guo P, Lu Q, Hu S, Martinez MA, Lopez-Torres B, Martinez M, et al. The NO-dependent caspase signaling pathway is a target of deoxynivalenol in growth inhibition in vitro. Food Chem Toxicol 2021;158:112629.
Kageyama H, Takenoya F, Kita T, Hori T, Guan JL, Shioda S. Galanin-like peptide in the brain: effects on feeding, energy metabolism and reproduction. Regul Pept 2005;126:21–6.
Kouadio JH, Dano SD, Moukha S, Mobio TA, Creppy EE. Effects of combinations of Fusarium mycotoxins on the inhibition of macromolecular synthesis, malondialdehyde levels, DNA methylation and fragmentation, and viability in Caco-2 cells. Toxicon 2007;49:306–17.
Lawrence C, Fraley GS. Galanin-like peptide (GALP) is a hypothalamic regulator of energy homeostasis and reproduction. Front Neuroendocrinol 2011;32:1–9.
Li H, Kong R, Wan B, Yang L, Zhang S, Cao X, et al. Initiation of PI3K/AKT pathway by IGF-1 decreases spinal cord injury-induced endothelial apoptosis and microvascular damage. Life Sci 2020;263:118572.
Liu A, Sun Y, Wang X, Ihsan A, Tao Y, Chen D, et al. DNA methylation is involved in pro-inflammatory cytokines expression in T-2 toxin-induced liver injury. Food Chem Toxicol 2019a;132:110661.
Liu A, Xu X, Hou R, Badawy S, Tao Y, Chen D, et al. DNA methylation and RASSF4 expression are involved in T-2 toxin-induced hepatotoxicity. Toxicology 2019b;425:152246.
Liu M, Zhang L, Chu XH, Ma R, Wang YW, Liu Q, et al. Effects of deoxynivalenol on the porcine growth performance and intestinal microbiota and potential remediation by a modified HSCAS binder. Food Chem Toxicol 2020a;141:111373.
Liu A, Hu S, Wu Q, Ares I, Martinez M, Martinez-Larranaga MR, et al. Epigenetic upregulation of galanin-like peptide mediates deoxynivalenol induced-growth inhibition in pituitary cells. Toxicol Appl Pharmacol 2020b;403:115166.
Man PS, Lawrence CB. Galanin-like peptide: a role in the homeostatic regulation of energy balance? Neuropharmacology 2008;55:1–7.
Marin S, Ramos AJ, Cano-Sancho G, Mycotoxins Sanchis V. Occurrence, toxicology, and exposure Assessment. Food Chem Toxicol 2013;60.
Marques MF, Martins HM, Costa JM, Bernardo F. Co-occurrence of deoxynivalenol and zearalenone in crops marketed in Portugal. Food Addit Contam B 2008;1:130–3.
Messina A, Nannelli A, Fiorio R, Longo V, Gervasi PG. Expression and inducibility of CYP1A1, 1A2, 1B1 by beta-naphthoflavone and CYP2B22, 3A22, 3A29, 3A46 by rifampicin in the respiratory and olfactory mucosa of pig. Toxicology 2009;260:47–52.
Molina A, Chavarria G, Alfaro-Cascante M, Leiva A, Granados-Chinchilla F. Mycotoxins at the start of the food chain in Costa Rica: analysis of six Fusarium toxins and ochratoxin A between 2013 and 2017 in animal feed and aflatoxin M-1 in dairy products. Toxins 2019;11.
Ouni M, Castell AL, Linglart A, Bougneres P. Genetic and epigenetic modulation of growth hormone sensitivity studied with the IGF-1 generation test. J Clin Endocrinol Metab 2015;100:E919–25.
Paravidino VB, Mediano MFF, Crochemore-Silva I, da Cruz VL, Antunes MML, Beaulieu K, et al. The compensatory effect of exercise on physical activity and energy intake in young men with overweight: the EFECT randomised controlled trial. Physiol Behav 2021;229:113249.
Pallares N, Carballo D, Ferrer E, Fernandez-Franzon M, Berrada H. Mycotoxin dietary exposure assessment through fruit juices consumption in children and adult population. Toxins 2019;vol. 11.
Peng Z, Liao Y, Chen L, Liu S, Shan Z, Nussler AK, et al. Heme oxygenase-1 attenuates low-dose of deoxynivalenol-induced liver inflammation potentially associating with microbiota. J Appl Toxicol 2019a;374:20–31.
Peng Z, Liao Y, Wang X, Chen L, Wang L, Qin C, et al. Heme oxygenase-1 regulates autophagy through carbon-oxygen to alleviate deoxynivalenol-induced hepatic damage. Arch Toxicol 2019b;94(2):573–88.
Peng Z, Chen L, Nussler AK, Liu L, Yang W. Current sights for mechanisms of deoxynivalenol-induced hepatotoxicity and prospective views for future scientific research: a mini review. J Appl Toxicol 2017;37:518–29.
Pestka JJ. Deoxynivalenol: mechanisms of action, human exposure, and toxicological relevance. Arch Toxicol 2010;84:663–79.
Reddy KE, Jeong JY, Lee Y, Lee HJ, Kim MS, Kim DW, et al. Deoxynivalenol- and zearalenone-contaminated feeds alter gene expression profiles in the livers of piglets. Asian Austr J Anim 2018;31:595–606.
Santana AM, Silva DG, Clemente V, Pizauro LJL, Bernardes PA, Santana CH, et al. Blood gas and serum biochemical RIs for healthy newborn Murrah buffaloes (Bubalus bubalis). Vet Clin Pathol 2018;47:94–9.
Stanek C, Reinhardt N, Diesing AK, Nossol C, Kahlert S, Panther P, et al. A chronic oral exposure of pigs with deoxynivalenol partially prevents the acute effects of lipopolysaccharides on hepatic histopathology and blood clinical chemistry. Toxicol Lett 2012;215:193–200.
Sternak M, Khomich TI, Jakubowski A, Szafarz M, Szczepanski W, Bialas M, et al. Nicotinamide N-methyltransferase (NNMT) and 1-methylnicotinamide (MNA) in experimental hepatitis induced by concanavalin A in the mouse. Pharmacol Rep 2010;62:483–93.

Streit E, Schatzmayr G, Tassis P, Tzika E, Marin D, Taranu I, et al. Current situation of mycotoxin contamination and co-occurrence in animal feed–focus on Europe. Toxins 2012;4:788–809.
Strobl JS, Dannies PS, Thompson EB. Rat growth hormone gene expression is correlated with an unmethylated CGCG sequence near the transcription initiation site. Biochemistry 1986;25:3640–8.
Suman M, Generotti S, Cirlini M, Dall'Asta C. Acrylamide reduction strategy in combination with deoxynivalenol mitigation in industrial biscuits production. Toxins 2019;11.
Tang X, Chen S. Epigenetic regulation of cytochrome P450 enzymes and clinical implication. Curr Drug Metabol 2015;16:86–96.
Tardivel C, Airault C, Djelloul M, Guillebaud F, Barbouche R, Troadec JD, et al. The food born mycotoxin deoxynivalenol induces low-grade inflammation in mice in the absence of observed-adverse effects. Toxicol Lett 2015;232:601–11.
Van Le Thanh B, Lemay M, Bastien A, Lapointe J, Lessard M, Chorfi Y, et al. The potential effects of antioxidant feed additives in mitigating the adverse effects of corn naturally contaminated with Fusarium mycotoxins on antioxidant systems in the intestinal mucosa, plasma, and liver in weaned pigs. Mycotoxin Res 2016;32:99–116.
Wang S, Yang J, Zhang B, Wu K, Yang A, Li C, et al. Deoxynivalenol impairs porcine intestinal host defense peptide expression in weaned piglets and IPEC-J2 cells. Toxins 2018;10:E541.
Wang S, Liang C, Zhao L, Meng Z, Zhang C, Jia Q, et al. Influence of radioactive iodine therapy on liver function in patients with differentiated thyroid cancer. Nucl Med Commun 2018a;39:1113–20.
Wang T, Wang N, Zhang R, Huang S, Qiu H, Long F, et al. Jie-du-hua-yu granules promote liver regeneration in Rat models of acute liver failure: miRNA-mRNA expression analysis. Evid Based Complement Alternat Med 2020;2020:8180959.
Wang X, Liu Q, Ihsan A, Huang L, Dai M, Hao H, et al. JAK/STAT pathway plays a critical role in the proinflammatory gene expression and apoptosis of RAW264.7 cells induced by trichothecenes as DON and T-2 toxin. Toxicol Sci 2012;127:412–24.
Wang XD, Yang X, Xu HB, Cao P, Gao P, Liang J. Exposure status and health risk assessment of deoxynivalenol from cereals in Chinese population in different regions. Chin J Prevent Med 2019;53:394–7.
Wettstein FE, Bucheli TD. Poor elimination rates in waste water treatment plants lead to continuous emission of deoxynivalenol into the aquatic environment. Water Res 2010;44:4137–42.
Wu L, Wang W, Yao K, Zhou T, Yin J, Li T, et al. Effects of dietary arginine and glutamine on alleviating the impairment induced by deoxynivalenol stress and immune relevant cytokines in growing pigs. PLoS One 2013;8:e69502.
Xie JB, Dong WY, Liu R, Wang YM, Li YB. Research on the hepatotoxicity mechanism of citrate-modified silver nanoparticles based on metabolomics and proteomics. Nanotoxicology 2018;12:18–31.
Ye C, Tao R, Cao Q, Zhu D, Wang Y, Wang J, et al. Whole-genome DNA methylation and hydroxymethylation profiling for HBV-related hepatocellular carcinoma. Int J Oncol 2016;49:589–602.
Yu M, Peng Z, Liao YX, Wang LL, Li D, Qin CY, et al. Deoxynivalenol-induced oxidative stress and Nrf2 translocation in maternal liver on gestation day 12.5 d and 18.5 d. Toxicon 2019;161:17–22.
Zhang J, Tang LL, Li LY, Cui SW, Jin S, Chen HZ, et al. Gandouling tablets inhibit excessive mitophagy in toxic milk (TX) model mouse of wilson disease via pink1/parkin pathway. Evid Based Complement Alternat Med 2020a;2020:3183714.
Zhang L, Ma R, Zhu MX, Zhang NY, Liu XL, Wang YW, et al. Effect of deoxynivalenol on the porcine acquired immune response and potential remediation by a novel modified HSCAS adsorbent. Food Chem Toxicol 2020b;138:111187.
Zhou Y, Wu S, Wang F, Li Q, He C, Duan N, et al. Assessing the toxicity in vitro of degradation products from deoxynivalenol photocatalytic degradation by using upconversion nanoparticles@TiO2 composite. Chemosphere 2020;238:124648.