Antiviral Research 96 (2012) 333–339

Contents lists available at SciVerse ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

# Anti-hepatitis B virus activities of α-DDB–FNC, a novel nucleoside–biphenyldicarboxylate compound in cells and ducks, and its anti-immunological liver injury effect in mice

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ARTICLE INFO

*Article history:*
Received 29 July 2012
Revised 13 October 2012
Accepted 15 October 2012
Available online 23 October 2012

*Keywords:*
α-DDB–FNC
HepG2.2.15
Duck hepatitis B virus (DHBV)
Liver injury

ABSTRACT

Infection with hepatitis B virus (HBV) continues to be a major global cause of acute and chronic liver disease with high mortality. Herein, we examined both the anti-HBV and hepatoprotective activity of α-DDB–FNC. In human HBV-transfected liver cell line HepG2.2.15, α-DDB–FNC effectively suppressed the secretion of HBV antigens in a time and dose-dependent manner with 25.11% inhibition on HBeAg and 43.68% on HBsAg at 2.5 μM on day 9. Consistent with the HBV antigen reduction, α-DDB–FNC (2.5 μM) also reduced HBV DNA level by 77.74% extracellularly and 78.94% intracellularly on day 9. In the duck hepatitis B virus (DHBV) infected ducks, after α-DDB–FNC was given once daily for 10 days, the serum and liver DHBV DNA levels were reduced markedly with 96.81% and 97.21% at 10 mg $kg^{-1}$ on day 10, respectively. In Con A-induced immunological liver-injury mice, α-DDB–FNC significantly inhibited the elevation of serum ALT, AST, TBiL and liver MDA, NO levels. Furthermore, significant improvement of the liver was observed after α-DDB–FNC treatment both in ducks and mice, as evaluated by the histopathological analysis. In conclusion, our results demonstrated that α-DDB–FNC possesses both antiviral activity against HBV and hepatoprotective effect to Con A-induced liver-injury mice.


## 1. Introduction

Chronic hepatitis B virus (HBV) infection continues to be a major public health problem worldwide, with more than 350 million people being chronically infected (Lau and Bleibel, 2008). During the course of HBV persistent infection, continuous intrahepatic inflammation maintains a cycle of liver cell destruction and regeneration that often terminates in hepatocellular carcinoma (HCC) (Li et al., 2005a,b; Dandri and Locarnini, 2012). It has been demonstrated that liver injury is mostly caused by repeated attempts of the host's immune responses to control HBV infection. In the process of liver injury, hepatocellular apoptosis induced by the proapoptotic molecules of T cells activated following antigen recognition triggers a cascade of antigen nonspecific effector systems and causes necroinflammatory disease (Nakamoto and Kaneko, 2003). Accordingly, new agents for the treatment of hepatitis B are expected to control HBV persistent infection and prevent the development of HCC. However, there is still no such kind effective treatment for millions of chronically infected individuals. Current treatments may be accompanied by adverse effects and drug resistance following by prolonged administration (Levine et al., 2002; Perrillo, 2005). Therefore, the discovery and development of novel antiviral drugs with both anti-HBV activity and hepatoprotective effect for the treatment of HBV is urgently needed.

In order to develop effective drugs against viral hepatitis, our institute have synthesized a series of FNC (2′-deoxy-2′-β-fluoro-4′-azidocytidine) derivatives (Wang et al., 2011) with anti-cancer, anti-HCV and anti-HBV activities (Zheng et al., 2012; Klumpp et al., 2008). These compounds were achieved for the first time by the Department of Chemistry at Zhengzhou University. Through pharmacological screening, it was found that 4′-azido-2′-deoxy-2′-β-fluoro-3′-(4,4′-dimethoxy-2′-methoxycarbonyl-5,6,5′,6′-bis(methylenedioxy)-1,1′-biphenyl-2-carboxyl)cytidine (α-DDB–FNC) has potential antiviral and hepatoprotective effects, which was accomplished by esterification of α-DDB (Jin et al., 2007) monomethyl ester and 3′-hydroxyl group of FNC. The structure of α-DDB–FNC is as shown in Fig. 1. In this study, we used HepG2.2.15 cell line and duck HBV-infected

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http://dx.doi.org/10.1016/j.antiviral.2012.10.003

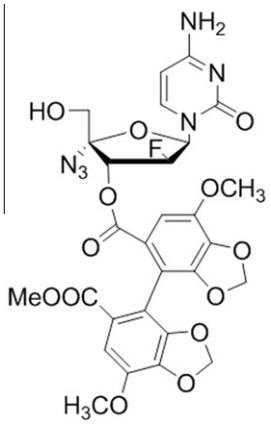


**Fig. 1.** Chemical structure of α-DDB–FNC.

duck models to evaluate anti-HBV activity of α-DDB–FNC (Li et al., 2008), and used Concanavalin A (Con A)-induced liver injury model in mice to investigate the protective efficacy of α-DDB–FNC (He et al., 2011). The results revealed that α-DDB–FNC can efficiently inhibit the HBV replication and the expression of HBeAg and HBsAg in HepG2.2.15 cells and inhibit the DHBV DNA replication *in vivo*. In addition, the results showed that α-DDB–FNC has a hepatoprotective effect to Con A-induced liver injury in mice.

## 2. Evaluation of α-DDB–FNC for *in vitro* anti-HBV activity

### 2.1. Materials and methods

#### 2.1.1. Materials

RPMI 1640 medium was obtained from Solarbio science & technology Co. Ltd. (Beijing, China). Fetal bovine serum (FBS), G418 and MTT were purchased from Sigma (St Louis, MO, USA). HBeAg and HBsAg enzyme immunoassay (ELISA) kits were purchased from Kehua (Shanghai, China). Viral DNA Extraction Kit and Tissue DNA Extraction Kit were obtained from Omega (Bio-Tek, USA). Lamivudine (3TC) was purchased from GlaxoSmithKline (Suzhou, China). α-DDB–FNC was synthesized by a laboratory of department of chemistry at Zhengzhou University with purity of 97.89%.

#### 2.1.2. HepG2.2.15 cells culture

HepG2.2.15 cells were provided by the Wuhan Institute of Virology and cultured in RPMI 1640 medium with 10% FBS, 200 μg/mL G418. The cells were maintained in a humidified incubator at 37 °C in 5% $CO_2$. The medium was changed every 3 days.

#### 2.1.3. Analysis of cellular toxicity

MTT assays were used to detect the survival rates of HepG2.2.15 cells (Ferrari et al., 1990). Briefly, HepG2.2.15 cells were seeded at a density of $2 \times 10^3$ cells per well on 96-well plates. After incubation for 24 h, the cells were treated with medium containing α-DDB–FNC of different concentrations (4, 20, 100, 500, 1000 μM). 3TC (20 μM) was served as the positive control. The culture medium was replaced with a fresh one on third day or sixth day during 9-day experiment. On day 9, MTT (5 mg/mL) was added 20 μL per well. After incubation routinely for 4 h, the supernatants were discarded and 180 μL of DMSO was added into each well to solubilize the formazan. The absorbance (*A*) at 490 nm was measured by using an automatic plate reader (BIO-RAD, 168-1000XC, USA). The survival ratio of HepG2.2.15 cells (%) was calculated [1-(A490 of experimental group/A490 of negative control) × 100%] (Lin et al., 2009).

#### 2.1.4. Detection of HBeAg and HBsAg

The HepG2.2.15 cells were plated at a density of $2 \times 10^4$ cells per well on 24-well plates and routinely cultured for 24 h. Then, HepG2.2.15 cells were treated with freshly prepared medium containing α-DDB–FNC (0.1, 0.5, 2.5 μM) or 3TC (20 μM). Nomal control cells were treated with RPMI 1640 only. Media were changed every 3 days, both the supernatants and cells were collected. The collected supernatants were detected using an ELISA Kit for the detection of HBeAg and HBsAg according to the manufacturer's instructions. The absorbance (*A*) at 450/630 nm was measured by using an automatic plate reader (Su et al., 2009; Cui et al., 2010). Inhibition ratio to HBeAg or HBsAg (%) = [1 − (A450/630 of experimental group/A450/630 of negative control) × 100%] (Zhou et al., 2007).

#### 2.1.5. Quantification of HBV DNA by FQ-PCR

The extracellular and intracellular HBV DNA was extracted by Viral DNA Extraction Kit according to the manufacturer's instructions. The concentration of extracted HBV DNA was determined by real-time fluorescent quantitative Polymerase Chain Reaction (FQ-PCR), which was performed in Light-Cycler 1.5 (Roche, Mannheim, Germany) using the HBV Fluorescent Quantitative PCR Detection Kit (Piji Biotechnology Development, Shenzhen, PR China) according to the manufacturer's protocol. Inhibition ratio to HBV DNA (%) = [1 − (HBV DNA concentration of experimental group/HBV DNA concentration of negative control) × 100%] (Guo et al., 2007; Kim et al., 2001; Gao et al., 2008).

## 3. Assay of antiviral activity of α-DDB–FNC in duck hepatitis

### 3.1. Animals

Young ducks (males and females were not distinguished) were Ma Ya from the Henan province. All animals were treated according to the procedures outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health.

### 3.2. DHBV infection and drug treatment experiment

Each duck, aged 3 days, was injected into its tibial vein with 0.2 mL of serum from ducks with positive DHBV DNA serology (Srivastav et al., 2010). On seventh day, their blood was drawn, DHBV DNA of serum samples was extracted and PCR was performed to screen out positive ducks.

Drug treatment experiment was carried out on the seventh day after ducks were infected with DHBV. The positive ducks were randomly divided into five groups with 16 ducks in each group. All the five groups were observed: The drug groups were administered with doses of 0.4, 2, 10 mg $kg^{-1}$ of α-DDB–FNC suspended in 0.5% CMC-Na, positive control group with 3TC (20 mg $kg^{-1}$), and blank control group with 0.5% CMC-Na. Drugs were administered orally once daily for 10 days continuously.

### 3.3. Detection of DHBV DNA

The antiviral activity of α-DDB–FNC was assessed by comparing the serum and liver DHBV DNA levels after α-DDB–FNC-treated at initiation of treatment (T0), the fifth day of treatment (T5), the tenth day of treatment (T10) and the third day (P3) of post-treatment follow-up (Chen et al., 2004; Li et al., 2005a,b). The duck serum DHBV DNA was extracted using Viral DNA Extraction Kit. The duck liver tissue DHBV DNA was extracted using Tissue DNA Extraction Kit. FQ-PCR was performed in Light-cycler (Roche) using SYBR Green I (Zheng et al., 2012).

### 3.4. Histopathological examination of duck liver

The DHBV-positive ducks were treated with α-DDB–FNC and 3TC once daily for 10 days. The animals were sacrificed and the liver tissues were removed, fixed in 10% of formalin solution and embedded in paraffin, stained with hematoxylin and eosin, and examined by light microscopy.

## 4. Assay of protection from Con A-induced liver injury of mice

### 4.1. Animals

Male KM mice weighing 20 ± 2 g were reared at 23 ± 3 °C in a 12 h light/dark cycle. Animals were fed with a standard laboratory chow and water *ad libitum*. All animals were treated according to the procedures outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health.

### 4.2. Methods

Con A (obtained from Sigma) was dissolved in 0.9% saline. Biphenyldicarboxylate (DDB) (obtained from Zhejiang Wanbang) and α-DDB–FNC were dissolved in 0.5% CMC-Na for animal experiments. Mice were randomly divided into six groups with ten animals in each group. The groups included normal control, model (injected Con A only), standard reference and treatment with α-DDB–FNC at three concentrations. Standard reference group was given DDB at 150 mg $kg^{-1}$ $d^{-1}$ (Gao et al., 2005) and the α-DDB–FNC groups were given α-DDB–FNC at 300, 150 or 75 mg $kg^{-1}$ $d^{-1}$ for five consecutive days. The normal control group and the model group were given 0.5% CMC-Na at the same volume.

On the third day, all groups were given saline with Con A (20 mg $kg^{-1}$, 0.2 ml, i.v.) except that the normal control group was given 0.2 ml 0.9% saline only (i.v.). On the fifth day, four hours after DDB or α-DDB–FNC administration, all groups were given Con A again except that the normal control group was given saline only. No food was provided immediately after Con A administration but tap water was available *ad libitum*. Eight hours after Con A administration, blood was withdrawn from the eye socket. All blood samples were centrifuged at 1000 rpm and 4 °C for 10 min to obtain the serum. Aspartate aminotransferase (AST), serum glutamate pyruvate transaminase (ALT) and total bilirubin (TBiL) were assayed using automatic assay kits (Italy Vialicenza, Roneoo156). The liver of each mice was promptly removed and used to determine the tissue level malondialdehyde (MDA) and nitric oxide (NO). The level of MDA and NO was determined using the MDA and NO detection kits, respectively (Jiancheng Bio-engineering, Jiangsu, China). All the procedures were performed according to the manufacturer's instructions (Itoh et al., 2009; Liu et al., 2010).

### 4.3. Histological examination of mice

The left liver lobes were cut out and fixed in 10% formalin solution. After pathological sectioning and HE staining, liver histopathologic changes were examined by light microscopy.

## 5. Statistical analysis

Results are means ± SD of the indicated number of independent experiments. Statistical significance was determined using analysis of variance (ANOVA). $P < 0.05$ was considered statistically significant.

## 6. Results

### 6.1. Cytotoxicity of α-DDB–FNC

We firstly investigated the cytotoxicity of α-DDB–FNC on the cell viability of HepG2.2.15 cells. The results from the MTT test showed that α-DDB–FNC inhibited the growth of HepG2.2.15 cells with $CC_{50}$ of 965.4 μM (Fig. 2), indicating low toxicity of the compound. The cytotoxicity of α-DDB–FNC was measured to determine the treatment concentrations in the HepG2.2.15 cell culture.

### 6.2. Effects of α-DDB–FNC on HBeAg and HBsAg in HepG2.2.15 cells

The HBeAg and HBsAg in the supernatant were determined by ELISA. The results indicated that α-DDB–FNC had effect on the expression of HBV antigens at a low concentration and it could inhibit the secretion of HBeAg and HBsAg dose-dependently in HepG2.2.15 cells at certain concentration with enough time (Fig. 3). At 2.5 μM, the inhibition rates of α-DDB–FNC on HBeAg and HBsAg were 25.11% and 43.68% on day 9, respectively. 3TC (20 μM) had 24.91% and 24.23% inhibition on day 9. These results suggested that α-DDB–FNC was much more potent than 3TC on inhibiting HBeAg and HBsAg.

### 6.3. Effect of α-DDB–FNC on HBV DNA replication

To further confirm the antiviral activity of α-DDB–FNC in HepG2.2.15 cells, the HBV DNA levels were evaluated after α-DDB–FNC treatment. Consistent with the inhibitory effects on HBeAg and HBsAg secretion, the treatment of HepG2.2.15 cells with α-DDB–FNC at various concentrations for 3, 6 or 9 days resulted in the reduction of the extracellular and intracellular HBV DNA levels in a dose-dependent manner, as compared with the no drug control group. The mean inhibition percentage of HBV DNA level with α-DDB–FNC at the dosages of 0.1, 0.5 and 2.5 μM was 46.96%, 53.57% and 77.74% extracellularly and 48.18%, 65.31% and 78.94% intracellularly, on day 9, respectively. The inhibition rates of 3TC (20 μM) on extracellular and intracellular HBV DNA levels were 90.48% and 82.66% on day 9, respectively. The results showed that α-DDB–FNC had inhibitory effect on HBV DNA in HepG2.2.15 cell line at a low concentration extracellularly and intracellularly. As shown in Fig. 4.

### 6.4. Effect of α-DDB–FNC on DHBV replication in vivo

The antiviral activity of α-DDB–FNC was further evaluated in DHBV-infected ducks model. Serum and liver tissues DHBV DNA levels were measured by FQ-PCR. Compared with the control

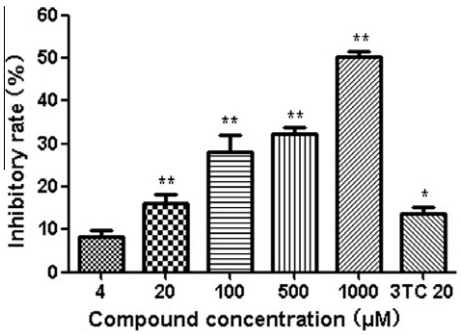


**Fig. 2.** Cytotoxicity assay of α-DDB–FNC. HepG2.2.15 cells were cultured in the presence of α-DDB–FNC at various concentrations for 9 days. The cell viability was measured by MTT method. Data represent the mean ± SD ($n = 4$), $^{*}p < 0.05$ and $^{**}p < 0.01$ compared to control.

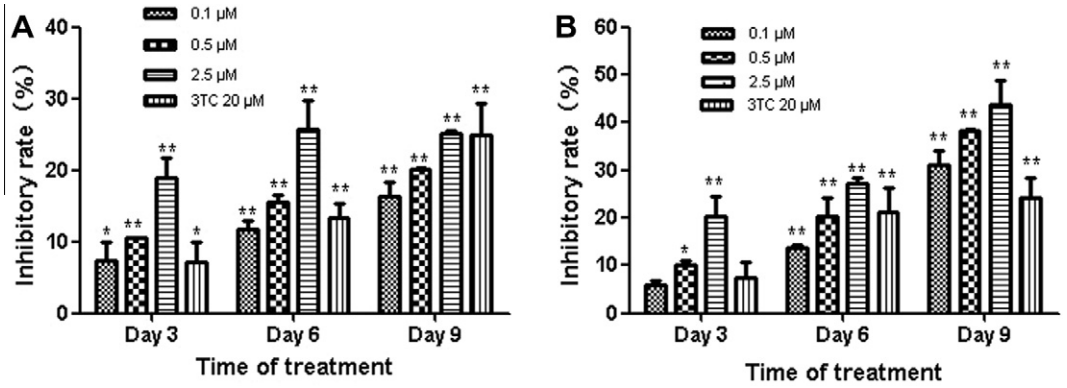


**Fig. 3.** Inhibitory effect of α-DDB–FNC on HBeAg and HBsAg in HepG2.2.15 cells. The (A) HBeAg and (B) HBsAg in the supernatants were quantified using specific ELISA kits. Data were presented as mean ± SD of three experiments. $^{*}p < 0.05$ and $^{**}p < 0.01$ compared with the no drug control group.

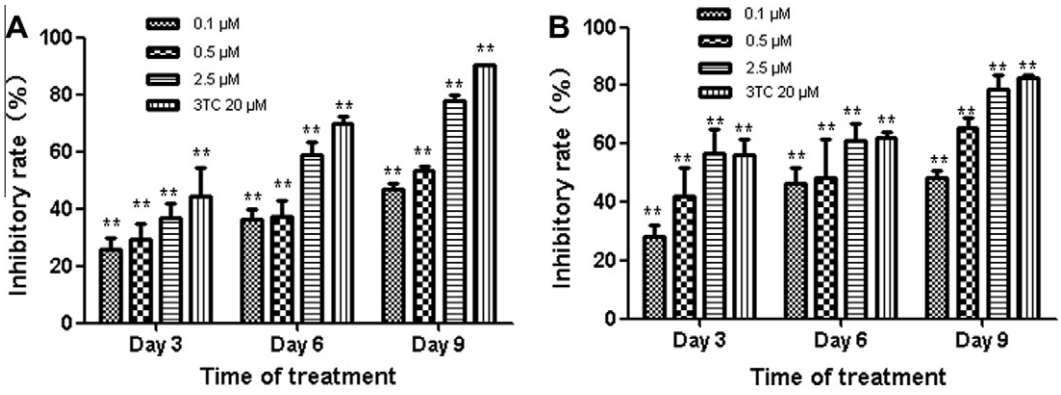


**Fig. 4.** Inhibitory effect of α-DDB–FNC on HBV DNA level. The inhibition ratio of α-DDB–FNC for HBV DNA in (A) extracellular and (B) intracellular. HBV DNA level was quantified by FQ-PCR on days 3, 6 and 9. The experiments were performed three times, and data were presented as mean ± SD of all experiments. $^{**}p < 0.01$ compared with control.

group, serum and liver tissues levels of DHBV DNA of each group decreased to different extents after treatment with α-DDB–FNC and 3TC on days 5 and 10, respectively. The average inhibition percentage of serum DHBV DNA levels with α-DDB–FNC at the dosages of 0.4, 2.0 and 10 mg $kg^{-1}$ $d^{-1}$ was 61.98%, 87.11% and 95.51% for day 5 and 81.04%, 89.76% and 96.81% for day 10, respectively; 3TC-treated (20 mg $kg^{-1}$ $d^{-1}$) groups resulted in 78.07% and 97.10% inhibition on days 5 and 10, respectively. The mean inhibition percentage of livers DHBV DNA levels with α-DDB–FNC at the dosages of 0.4, 2.0 and 10 mg $kg^{-1}$ $d^{-1}$ was 45.84%, 66.55% and 87.47% for day 5 and 75.26%, 84.02% and 97.21% for day 10, respectively. 3TC-treated (20 mg $kg^{-1}$ $d^{-1}$) groups resulted in 86.21% and 95.46% inhibition on days 5 and 10, respectively. α-DDB–FNC significantly reduced serum and liver tissue DHBV DNA levels in a time and dose-dependent manner. The levels of both serum and liver tissue DHBV DNA had a lower degree of rebound after withdrawal of the drug for 3 days. These results suggested that α-DDB–FNC (10 mg $kg^{-1}$ $d^{-1}$) had a stronger inhibitory effect on DHBV DNA than 3TC (20 mg $kg^{-1}$ $d^{-1}$), as shown in Fig. 5.

### 6.5. Histopathological examination of the duck livers

Typical photographs of liver sections by light microscopy are as shown in Fig. 6. Tenth-day treatment with α-DDB–FNC at all doses, particularly 10 mg/kg, significantly improved the necrosis, inflammatory cell infiltration and massive ballooning degeneration of the hepatic cytoplasm. In addition, histopathological profiles of the liver from the high dosage group of α-DDB–FNC resulted in more significant improvement than that with 3TC at 20 mg $kg^{-1}$.

### 6.6. Protection against Con A-induced immunological liver injury in mice

We examined the hepatoprotective effect of α-DDB–FNC on Con A-induced liver injury in mice. In the Con A intoxicated group, serum ALT, AST and TBiL and liver MDA, NO level were significant increased as compared to the untreated control ($p < 0.05$) (Table 1). When administered orally for 5 days before the Con A treatment, α-DDB–FNC at 150 or 300 mg $kg^{-1}$ significantly suppressed the levels of serum ALT, AST and TBiL and liver MDA, NO ($P < 0.01$). These results indicate that α-DDB–FNC has a protective effect against Con A-induced liver injury.

### 6.7. Histopathological examination of the mice livers

To obtain histological evidence for the protection from liver injury of α-DDB–FNC in mice, liver sections were prepared and stained with hematoxylin and eosin, representative images are as shown in Fig. 7. Histology of the liver sections of control animals showed normal hepatic cells with prominent nucleus and visible central veins (Fig. 7A). The hepatic cells of Con A-intoxicated mice were mostly found to have fatty degeneration, necrosis and cytoplasmic vacuolization (Fig. 7B). The disorganisation caused by the Con A treatment was decreased in the sections from mice treated with α-DDB–FNC at all doses.

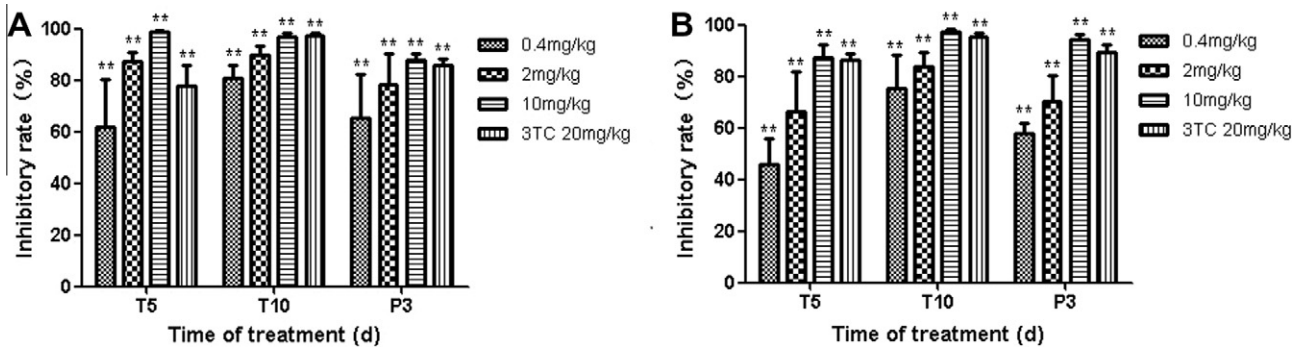


**Fig. 5.** Inhibitory effect of α-DDB–FNC on DHBV DNA level. The inhibition ratio of α-DDB–FNC for DHBV DNA in (A) duck serum and (B) duck liver. DHBV DNA level was quantified by FQ-PCR on days 5 (T5), 10 (T10) and 13 (P3). 3TC (20 mg $kg^{-1}$ $d^{-1}$) was used as the positive control. Data were expressed as mean ± SD ($n = 4$), and were statistically analysed using Dunnett's multiple comparison test. α-DDB–FNC treatment (0.4, 2.0 and 10 mg $kg^{-1}$ $d^{-1}$) significantly inhibited DHBV DNA in duck serum and liver. $^{**}P < 0.01$ compared with the control.

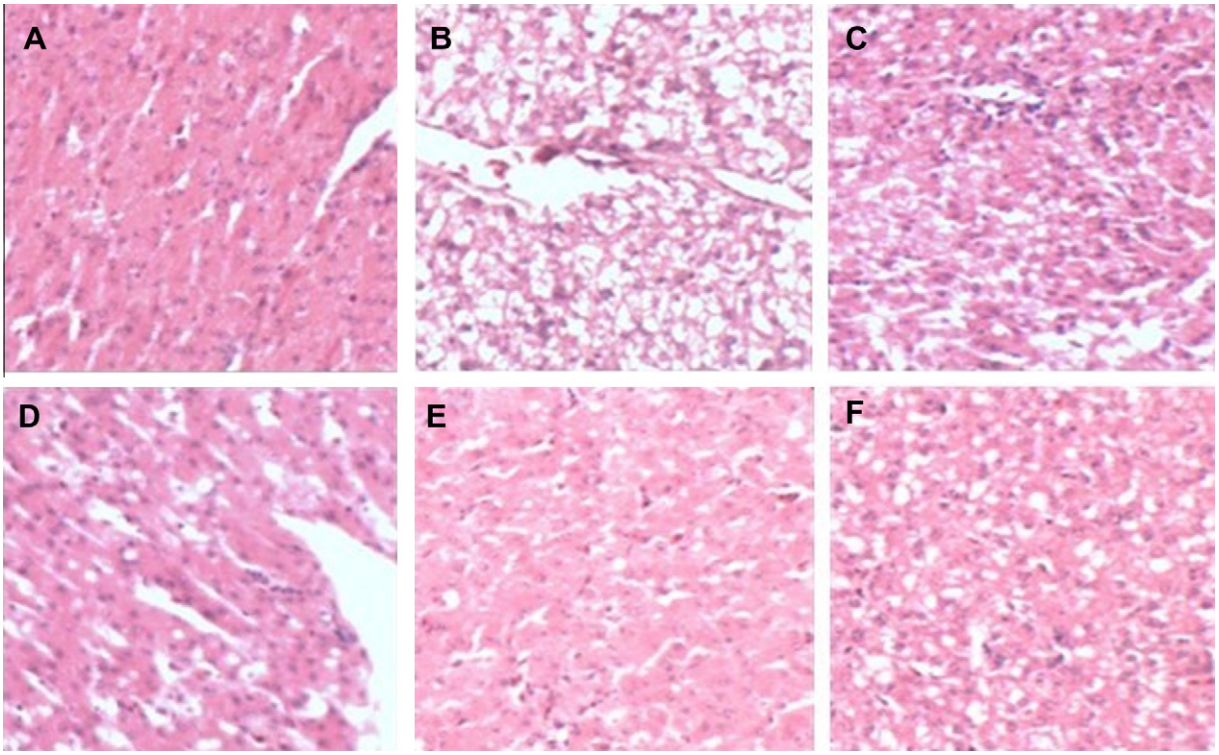


**Fig. 6.** Histopathological changes in duck livers. (A) A section of normal duck liver. Ducks were treated with α-DDB–FNC at (B) 0, (C) 0.4 mg $kg^{-1}$, (D) 2 mg $kg^{-1}$and (E) 10 mg $kg^{-1}$ or with (F) 3TC at 20 mg $kg^{-1}$ once a day for 10 days. Liver sections were stained with haematoxylin and eosin, and examined by light microscopy. Representative photographs are presented (magnification ×200).

**Table 1**
Effect of α-DDB–FNC on serum ALT, AST and TBiL level and liver MDA, NO level against ConA induced liver injury in mice.

| Groups | ALT/U $L^{-1}$ | AST/U $L^{-1}$ | TBiL/μm $L^{-1}$ | MDA/nm $mgprot^{-1}$ | NO/μm $gprot^{-1}$ |
|---|---|---|---|---|---|
| Control | 53.27 ± 2.52 | 141.50 ± 4.42 | 8.83 ± 1.16 | 5.61 ± 0.33 | 4.78 ± 0.78 |
| ConA alone | 115.27 ± 9.46$^{\Delta\Delta}$ | 257.63 ± 29.10$^{\Delta\Delta}$ | 11.40 ± 0.87$^{\Delta}$ | 8.09 ± 0.24$^{\Delta\Delta}$ | 11.69 ± 1.58$^{\Delta\Delta}$ |
| DDB (150 mg $kg^{-1}$) | 62.74 ± 3.64$^{\blacktriangle\blacktriangle}$ | 142.00 ± 6.14$^{\blacktriangle\blacktriangle}$ | 8.56 ± 1.06▲ | 5.16 ± 0.27$^{\blacktriangle\blacktriangle}$ | 5.91 ± 1.07$^{\blacktriangle\blacktriangle}$ |
| α-DDB–FNC (75 mg $kg^{-1}$) | 76.76 ± 6.29$^{\blacktriangle\blacktriangle}$ | 180.22 ± 15.11$^{\blacktriangle\blacktriangle}$ | 10.93 ± 0.61 | 6.22 ± 0.36$^{\blacktriangle\blacktriangle}$ | 10.35 ± 1.41▲ |
| α-DDB–FNC (150 mg $kg^{-1}$) | 69.01 ± 3.42$^{\blacktriangle\blacktriangle}$ | 168.16 ± 12.13$^{\blacktriangle\blacktriangle}$ | 7.13 ± 0.57$^{\blacktriangle\blacktriangle}$ | 6.23 ± 0.29$^{\blacktriangle\blacktriangle}$ | 7.76 ± 1.78$^{\blacktriangle\blacktriangle}$ |
| α-DDB–FNC (300 mg $kg^{-1}$) | 59.19 ± 2.72$^{\blacktriangle\blacktriangle}$ | 163.29 ± 6.70$^{\blacktriangle\blacktriangle}$ | 5.85 ± 0.53$^{\blacktriangle\blacktriangle}$ | 5.79 ± 0.39$^{\blacktriangle\blacktriangle}$ | 5.99 ± 1.51$^{\blacktriangle\blacktriangle}$ |

Data are expressed as mean ± SD in each group ($n = 10$). Compared with the control group $^{\Delta}P < 0.05$ and $^{\Delta\Delta}P < 0.01$; compared with the model group ▲$P < 0.05$ and ▲▲$P < 0.01$.

## 7. Discussion

Currently, there are three categories of nucleosides available as anti-HBV antiviral agents: L-nucleosides (lamivudine and telbivudine), acyclic phosphonate derivatives adefovir and tenofovir, and the cyclopentane deoxyguanosine analogue entecavir (Yuen and Lai, 2011; Papatheodoridis et al., 2002). These agents have strong antiviral effect against HBV, however, the clinician still faces

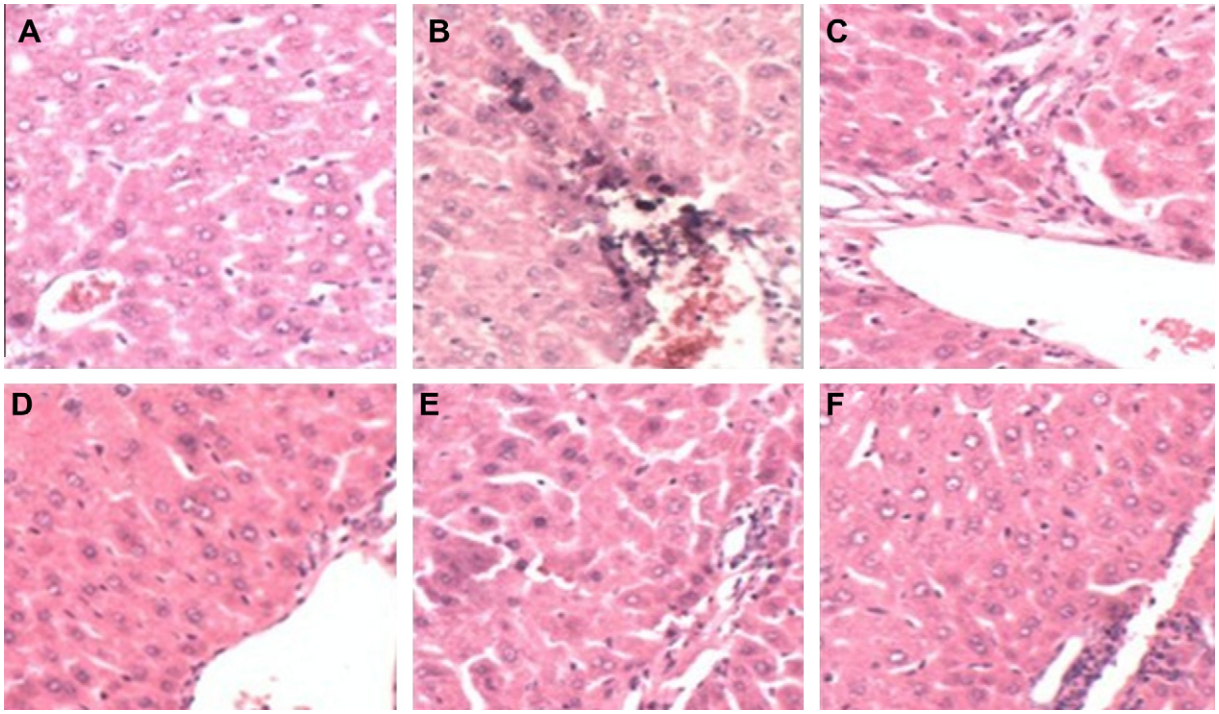


**Fig. 7.** Effect of α-DDB–FNC on the histological changes in the livers of mice. Sections of paraffin-embedded liver tissue were stained with hematoxylin and eosin. The liver was excised from normal (A), Con A-injured control (B), DDB(150 mg $kg^{-1}$) treated (C), and α-DDB–FNC (75 mg $kg^{-1}$) treated (D), α-DDB–FNC (150 mg $kg^{-1}$) treated (E) and α-DDB–FNC (300 mg $kg^{-1}$) treated (F). Magnification for all photographs, ×200.

several challenges in treating this relatively complex disorder. In the treatment of hepatitis B patients, many of them have different extents of liver damage and some other side effects, and most importantly, viral rebound with exacerbation of liver pathology after cessation of therapy. Therefore, an ideal agent for HBV not only can inhibit HBV replication of, but also can arrest the progression of liver injury and prevent the development of hepatic complications such as liver failure and HCC (Sung et al., 2008). α-DDB–FNC has a novel chemical structure, which was synthesized from α-DDB and FNC. As so far, there is no report on the anti-HBV and hepatoprotective activity of α-DDB–FNC. In this paper, we examined the anti-HBV activities of α-DDB–FNC both *in vitro* and *in vivo*. For the *in vitro* study, we took HepG2.2.15 cells, a widely used model for the evaluation of anti-HBV drugs (Xie et al., 2007; Li and Wang, 2006). Our results indicate that α-DDB–FNC exhibited a time and dose-dependent inhibitory effect on the secretion of HBeAg and HBsAg antigens in the range from 0.5 to 2.5 μM. The anti-HBV activity of α-DDB–FNC was further confirmed by its inhibitory effects on the levels of HBV DNA in HepG2.2.15 in a time and dose-dependent manner. In addition, the duck DHBV model represents a suitable and a widely used system for the study of *in vivo* activity of anti-HBV agents (Delmas et al., 2002). In DHBV-infected duck, α-DDB–FNC reduced serum and liver DHBV DNA levels in a dose-dependent manner and the anti-HBV activity was also confirmed by histopathological improvement. The high dosage group of α-DDB–FNC (10 mg $kg^{-1}$) resulted in more significant anti-HBV activity and histopathological improvement than that with 3TC at 20 mg $kg^{-1}$.

It has been recently reported that the liver damage observed in chronic hepatitis B virus infections is mainly mediated by the immune response against the virus (Kajiya et al., 2009). Thus, we used the Con A-induced liver-injury mice, which is regarded as an appropriate model of human immunomediated liver diseases to investigate the hepatoprotective activity of α-DDB–FNC (Shi et al., 2012; Liu, 2009). Our results indicated that α-DDB–FNC significantly inhibited the elevation of serum ALT, AST and TBiL and liver MDA, NO levels. The histopathological study suggested that α-DDB–FNC can prevent against Con A-induced liver injury in mice.

Summarily, we discovered the first evidence that α-DDB–FNC can efficiently inhibit both HBV replication and expression of HBeAg and HBsAg in HepG2.2.15 cells, and also inhibit the DHBV DNA replication in ducks *in vivo*. In addition, the study also showed that α-DDB–FNC had a hepatoprotective effect against Con A-induced liver injury in mice. All these studies provide a strong support for the development of α-DDB–FNC and its analogues as a potential alternative or complementary therapy for the treatment of HBV infection.

## Acknowledgements

This work is supported by grants from Outstanding Young Scholarship from National Science Foundation of China (NSFC) (JC;30825043) and NSFC (JC; 21172202), the major projects of National Science and Technology Creation of New Drugs (JC;2012ZX09102101-003) and the Special Funding of Technological Innovation Talent of Henan Province (Liyun Zheng; 4076).

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