

Inhibition of HBV gene expression and replication by stably expressed interferon- α 1 via adeno-associated viral vectors

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Abstract

Background Interferon- α 2 (IFN α 2) is routinely used for anti-hepatitis B virus (HBV) treatment. However, the therapeutic efficiency is unsatisfactory, particularly in East Asia. Such inefficiency might be a result of the short half-life, relatively low local concentration and strong side-effects of interferons. Frequent and repeated injection is also a big burden for patients. In the present study, a single dose of vector-delivered IFN α 1 was tested for its anti-HBV effects.

Methods Adeno-associated viral vector (AAV-IFN α 1) was generated to deliver the IFN α 1 gene into hepatocytes. IFN α 1, hepatitis B surface (HBsAg) and e (HBeAg) antigens were measured by enzyme-linked immunosorbent assay and/or western blotting. The level of viral DNA was measured by quantitative real-time polymerase chain reaction.

Results AAV-IFN α 1 effectively transduced HBV-producing cells (HepAD38) and mouse hepatocytes, where IFN α 1 was expressed in a stable manner. Both intracellular and extracellular HBsAg and HBeAg were significantly reduced *in vitro*. In the HBV-producing mice, the concentration of IFN α 1 in the liver was eight-fold higher than that in plasma. Compared with control groups, HBeAg/HBsAg antigen levels were reduced by more than ten-fold from day 1–5, and dropped to an undetectable level on day 9 in the AAV-IFN α 1 group. Concurrently, the level of viral DNA decreased over 30-fold for several weeks.

Conclusions A single dose administration of AAV-IFN α 1 viral vector displayed prolonged transgene expression and superior antiviral effects both *in vitro* and *in vivo*. Therefore, the use of AAV-IFN α 1 might be a potential alternative strategy for anti-HBV therapy. Copyright © 2008 John Wiley & Sons, Ltd.

Keywords adeno-associated virus; gene therapy; hepatitis B virus; interferon- α 1; hydrodynamic transfection

Introduction

Hepatitis B virus (HBV) infection is one of the major threats to public health worldwide [1–3]. More than 10% of the Chinese population suffers from chronic HBV infection. Of these individuals, approximately 25% develop HBV-associated diseases, including liver failure, cirrhosis and hepatocellular carcinoma (HCC). Nowadays, HCC is the second leading cause of cancer related

death in China [4]. Based on comparison of the HBV genome sequence, eight genotypes of HBV (A to H) have been classified [5–8]. These genotypes have distinct geographical distributions [9–11]. Genotypes A and D have a higher prevalence in Europe and North America whereas genotypes B and C are prevalent in East Asia, including China, Japan, and other countries in South Asia. In China, genotypes C and B account for approximately 60% and 40% of infected individuals, respectively, whereas the other genotypes are very rare. Patients with genotype C HBV are at greater risk of advanced HCC than those with other genotypes.

Interferons (IFNs) are a family of cytokines produced by mammalian cells when they are invaded by viruses. Depending on their cellular origin, stimulus nature and antigenic specificity, two major classes of IFN in humans have been designated as type I IFNs (α , β , ϵ , κ , ω and τ) and as type II IFNs (γ), each of which contains multiple subtypes [12]. Under normal/healthy conditions, only a low level of IFNs are expressed, yet they increase when the body is attacked by viral infections, or exposed to double-stranded RNA [12]. Therefore, IFNs have been considered as a natural medical resource for antiviral agents since the 1960s. However, due to the high cost of development, only IFN α 2 is approved by the Food and Drug Administration and is widely applied in therapeutic use against specific types of leukemia [13–15], multiple sclerosis [16,17], hepatitis B [18–20] and C [21–23], genital warts [24–26] and cancers [27–30].

Contrary to our expectations, the level of IFNs was found to be extremely low or even undetectable in chronic HBV patients [31]. IFN α 2 has been routinely used as an antiviral agent to treat chronic HBV (CHB) infection in the last two decades. Unfortunately, the therapeutic outcomes for many Asian patients, who account for 75% of CHB carriers and are infected by genotype B or C virus, are far from satisfactory [32,33]. It has been found that genotype B and C HBV predisposed the patients to a higher risk of complications, including liver cirrhosis and HCC [34–36]. Several factors, including short half-life, limited possible dosage and a low local hepatic concentration of IFN, may cause the therapeutic inefficiency. On the other hand, IFN has many subtypes, and each of them bears different specificities and efficacies against viruses [12]. It has been shown that CHB patients with genotype A respond to IFN α 2 treatment markedly better than those with genotype C [37–39]. The antiviral effects of other IFN α subtypes for genotype C HBV are yet to be tested. Our previous studies demonstrated that the anti-SARS coronavirus activities of IFN α 1 were over 20-fold stronger than those of IFN α 2 (He *et al.*, unpublished data). Therefore, it is worthwhile to investigate the anti-HBV activities of IFN α 1, particularly against the genotype C virus.

Adeno-associated virus (AAV) is a nonpathogenic human parvovirus with a 4.7 kb single-stranded genome. The recombinant AAV (rAAV) is now recognized as one of the most promising vectors for delivery of therapeutic genes [40,41]. In previous studies, rAAV was shown to

induce low inflammatory response, to transduce both dividing and nondividing cells, and to sustain long-term expression *in vivo* [40,42]. In the USA, the use of AAV2 vectors in Phase I and II clinical trials for different diseases has confirmed the feasibility, tolerance and absence of undesirable side-effects in the trial groups [43–47].

Since HBV can only infect human beings and chimpanzees *in vivo*, cell culture experiments have been widely used to investigate HBV virology and to screen anti-HBV drugs. HepAD38 has been generated to consistently reproduce a high level of HBV under tetracycline control [48]. This cell line has been recognized as a good *in-vitro* model to mimic CHB infections. Recently, a hydrodynamic transfection mouse model was developed for anti-HBV studies [49]. In the present study, we adopted both HepAD38 cell line and the hydrodynamically transfected mice to investigate the inhibitive ability of prolonged expression of IFN α 1 both *in vitro* and *in vivo*.

Materials and methods

Cells and cell culture

HEK293 and HepAD38 cells were grown in Dulbecco's modified Eagle's medium, supplemented with 10% fetal bovine serum (Sigma, St Louis, MO, USA), 1% penicillin/streptomycin, and 1% glutamine. They were incubated at 37°C under 5% CO₂. For HepAD38 cells, the medium was supplied with 400 µg/ml G418 and 0.3 µg/ml tetracycline. To begin an assay, confluent cells were washed with prewarmed phosphate-buffered saline (PBS) and fed with medium without tetracycline. The replication of HBV in the HepAD38 cells was facilitated by the withdrawal of tetracycline from the culture media.

Production of rAAV

A cDNA encoding human IFN α 1 was amplified by the reverse transcription-polymerase chain reaction (RT-PCR) using the total RNA isolated from peripheral blood mononuclear cells of a Chinese patient. The primers were 5'-CGGGGA TCCATGGCCTCGCCCTTTGCTTTA-3' (forward) and 5'-CGGGAATTCATGAAAGCGTGACC TGGT-3' (reverse). The purified PCR products were inserted into an AAV2 vector and confirmed by DNA sequencing. The AAV-enhanced green fluorescent protein (EGFP) vector was used as the control.

The rAAV vectors were produced in HEK 293T cells using a helper virus-free system [50,51]. The rAAVs were purified as previously described with minor modifications [52]. The viral genome copies (GCs) were determined by quantitative real-time PCR (qPCR). It was carried out with a set of primers and a probe targeting the woodchuck HBV (WHBV) posttranscriptional regulatory element (WPRES) region. The primers used in the qPCR reaction were 5'-CGGCTGTTGGGCACTGA-3' (forward) and 5'-CCGAAGGGACGTAGCAGAAG-3' (reverse), and

the probe was 5'-FAM-ACGTCCTTTCCATGGCTGCTCGC-TAMRA-3'. The PCR reaction was carried out using the ABI 7500 real time PCR machine (Applied Biosystems, Foster City, CA, USA). Aliquots of viral stock (5×10^{12} GCs/ml) were stored at -80°C until use. To measure the transduction efficiency, HepAD38 cells were transfected with AAV-EGFP at 10^2 , 10^3 , 10^4 , or 10^5 GCs per cell. The corresponding fluorescent signals were captured and recorded using a fluorescent microscope.

Western blotting, enzyme-linked immunosorbent assay (ELISA) and quantification of HBV DNA

HepAD38 cells were harvested at different time points (i.e. 24, 48, and 72 h) after rAAV infection. Electrophoresis and blotting were performed as described previously [53]. Briefly, the standardized amounts of cell extracts (50 μg) were separated by using SDS-PAGE, transferred onto a poly(vinylidene difluoride) membrane, and incubated with an monoclonal antibody against human IFN α 1 (Calbiochem, Darmstadt, Germany). The HBsAg and HBeAg were measured by ELISA using the Murex HBsAg Version 3 Kit (Abbott Murex, Dartford, UK), according to manufacturer's instructions. The DNA of HBV in the culture cells was isolated from the viral particles and quantified by qPCR as described previously [54,55].

Animal studies

The ethics approval was obtained from the University Ethics Committee (UEC). The animals were taken care and treated according to the guidance of UEC. We used 3–4-week-old female BalB/C and nude mice. The mice were divided into three groups (six mice per group). One group was administrated with PBS, and the other two with AAV-EGFP and AAV-IFN α 1 vectors, respectively. For the later groups, treatment was made by injecting 1×10^{12} GCs of AAV into the tail veins. One week later, 40 μg of pHBV (Adr subtype, genotype C) [55] was injected into the tail vein of each mouse. The injected volume was equivalent to 10% of their body mass (i.e. 1 ml for a mouse of 10 g) and the total volume was delivered within 7–10 s. On days 1, 3, 5, 9, and 21, blood samples were collected for the determination of the expression level of the surface and e antigens, as well as the viral genomic DNA. The employed protocols have been described previously [55].

Immunocytochemical studies

The liver tissues or HepAD38 cells were fixed with 4% paraformaldehyde at 4°C . Direct observation of EGFP was performed under a fluorescence microscope. To examine the expression level of IFN α 1 and HBsAg, samples were treated with 0.1% Triton X-100 in PBS for another 10 min, and exposed to 0.3% hydrogen peroxide in methanol for

30 min at 4°C . They were blocked with normal bovine serum, and incubated with the primary monoclonal antibody against IFN α 1 or HBsAg (anti-HbsAg; Zymed Laboratories, San Francisco, CA, USA). After hybridization with FITC or Cy3 conjugated secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA), images were photographed.

Statistical analysis

A two-tailed Student's *t*-test was used for inter-group comparison. The results are displayed as the mean \pm SD. $p < 0.05$ was considered statistically significant.

Results

The transduction of hepatocyte-derived cells by rAAV

We inserted a cDNA encoding IFN α 1 into the multiple cloning sites of an AAV2 vector to generate pAAV-IFN α 1. We cotransfected pAAV-IFN α 1 or a plasmid expressing the enhanced green fluorescence protein (pAAV-EGFP) with AAV package vector pDG into HEK293 cells to produce the rAAV vectors.

To determine the transduction efficiency, we infected HepAD38 cells with AAV-EGFP vector at 10^2 , 10^3 , 10^4 , or 10^5 GCs per cell. It was noted that both the transduction efficiency and the EGFP expression level were dramatically elevated with an increase in viral vector levels. When GCs/cell increased to 10^4 – 10^5 , most cells displayed strong fluorescence at 48 h post-transduction and the strong fluorescence could last for several passages (data not shown).

Expression of IFN α 1 by rAAV-mediated gene transfer in hepatoma cells

We examined the expression level of IFN α 1 in both the HepAD38 cells and the growth medium at different time points by western blotting and ELISA. Our results indicated that both the extracellular (culture media, Figure 1A) and intracellular (Figure 1B) IFN α 1 appeared at 24 h post-infection, and the expression level of extracellular IFN α 1 remained unchanged until 72 h. No detectable IFN α 1 protein was observed in the cells infected with AAV-EGFP vectors by western blotting analysis. The concentration of IFN α 1 in the culture media was significantly increased at 48 h and maintained over a week (Figures 1A and 1C). The concentration of IFN α 1 reached up to 27 ng/ml on day 8 in the culture media (Figure 1C).

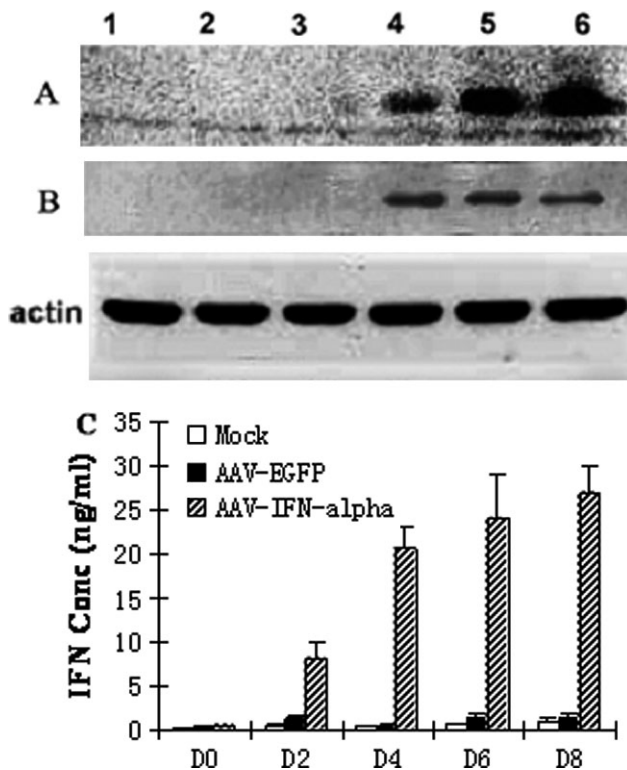


Figure 1. IFN α 1 expression after rAAV-IFN α 1 transduction of HepAD38 cells. IFN α 1 protein was detected both in the culture media (A) and intracellular extracts (B) by western blotting. Cells were transduced with AAV-EGFP (lanes 1, 2 and 3) or AAV-IFN α 1 (lanes 4, 5 and 6). The samples were collected at 24 h (lanes 1 and 4), 48 h (lanes 2 and 5) and 72 h (lanes 3 and 6) post-transduction, respectively. (C) The IFN- α 1 protein level in the culture media was measured by ELISA

Inhibition of HBV gene expression and replication in cultured cells

We investigated both HBsAg and HBeAg levels in HepAD38 cells after administration of rAAV vectors. The cells were transduced with rAAV and cultured with complete media without tetracycline for 48 h. Compared

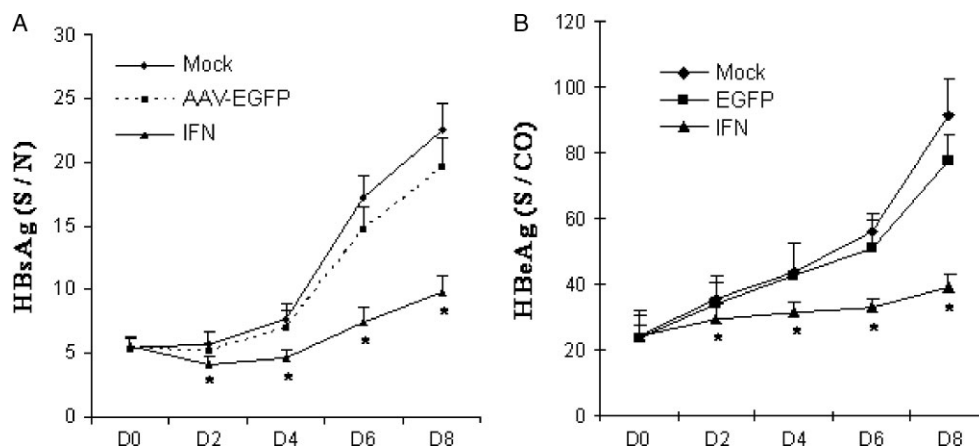


Figure 2. Reduction of HBsAg and HBeAg in the culture media. HepAD38 cells were transduced with AAV-EGFP, AAV-IFN α 1 viruses or nontransduced (Mock). Relative HBsAg (A) and HBeAg (B) levels were measured by ELISA. Values are expressed as the mean \pm SD (sample to cut-off ratio) at each time point ($n = 5$, * p < 0.01). The experiments were performed at least three times, and consistent results were obtained

with the cells transduced with AAV-EGFP vectors or the mock (i.e. nontransduced), both HBsAg and HBeAg levels were significantly reduced in the culture media of cells transduced with AAV-IFN α 1 vectors from day 2 to day 8 ($n = 5$, $p < 0.01$; Figure 2). On day 8, the HBsAg and HBeAg levels were reduced more than 60%. The reduction of the intracellular HBsAg level was also examined and confirmed by using immunocytochemical studies (data not shown).

Inhibition of HBV gene expression and viral replication in mice

We first followed the protocols described by Yang *et al.* to establish a mouse model for this study [49]. In immunocompetent Balb/C mice, the HBsAg and HBeAg levels in the plasma peaked at 24 h after hydrodynamic transfection of a HBV-producing plasmid (pHBV), whereas both HBsAg and HBeAg dropped rapidly to undetectable levels (by ELISA) on day 7 (data not shown). This indicated that Balb/C mice were not suitable for anti-HBV gene therapy studies. In nude mice, both HBsAg and HBeAg levels peaked at 24 h after administration of pHBV and maintained for at least 1 week (Figure 3A). Thereafter, a lower level of HBsAg was maintained for another week. The expression level of HBeAg was similar to that of HBsAg (Figure 3B). The viral load remained at 4.4 logs/ml in the plasma on day 21 (Figure 3C). As nude mice supported longer HBV gene expression and replication, they were chosen to prove the principle of this study.

The rAAV-mediated transgene expression level in the liver both in immunocompetent Balb/C and nude mice was also examined. As shown in Figure 4, in AAV-EGFP and AAV-IFN α 1 groups, EGFP and IFN- α 1 were strongly expressed, respectively, in most of the hepatocytes near the blood vessels on day 7 (Figure 4) and maintained at similar levels for at least over 2 months (data not

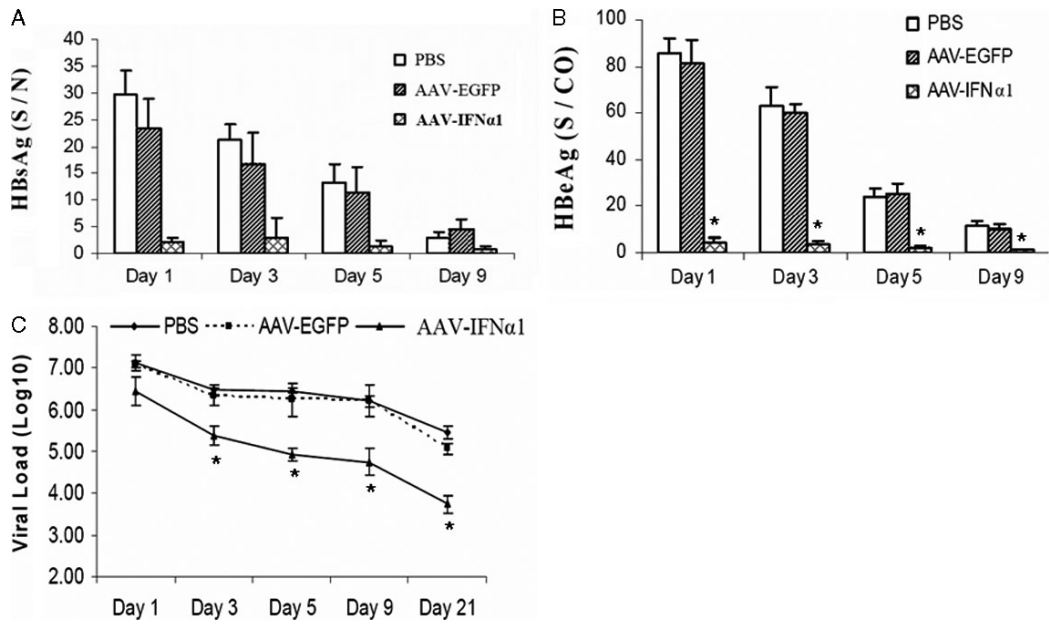


Figure 3. Significant reductions of HBV antigens and viral loads in the plasma after administration of AAV vectors. Both (A) HBsAg level and (B) HBeAg levels were determined by ELISA. The viral DNA was isolated from plasma and the viral load was measured by qPCR (C). * $p < 0.01$ indicates statistical significance

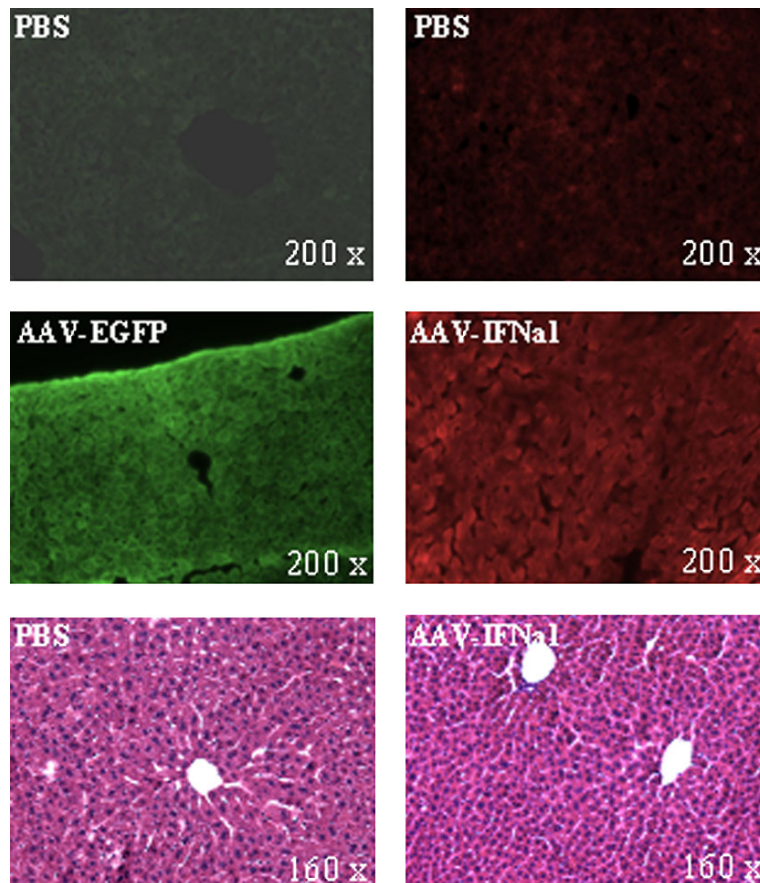


Figure 4. Highly effective gene transfers by rAAV and rAAV-mediated high-level gene expression in hepatocytes. Liver sections were examined under a fluorescence microscope (upper and middle panels) or a light microscope (lower panels). Upper panel: mice were administrated with PBS (control); middle panels: mice were administrated with AAV-EGFP (left) and AAV-IFN α 1 (right). EGFP was directly detected under fluorescence microscopy; whereas IFN α 1 was detected by indirect immunostaining with Cy3-cojugated secondary antibody. Lower panel, liver sections were subjected to H&E staining

shown). This was consistent with our previous findings [56]. By contrast, neither EGFP, nor IFN α 1 was detected in PBS-treated group. Also, no obvious liver damage or necrosis was observed in the AAV-IFN α 1-transfected group as revealed by the hematoxylin and eosin (H&E) staining (Figure 4). Our results show that the mean value of IFN α 1 concentration in the liver was eight-fold higher (126.7 pg/g) than that in the plasma (14.6 pg/ml). No obvious elevations of both alanine aminotransaminase (ALT) and aspartate aminotransaminase (AST) activities were observed in all groups (Figure 5).

By the use of the immunohistochemical assay, we observed that the HBsAg was present in the livers of the AAV-EGFP group, whereas a very faint signal was detected in the AAV-IFN α 1 group (Figure 6). We also used ELISA to examine HBsAg and HBeAg levels in the

plasma. The levels of both antigens were reduced by over ten-fold, particularly in AAV-IFN α 1 group compared to the PBS and AAV-EGFP group on days 1 and 3 (Figures 3A and 3B; $P < 0.01$). On day 5, no detectable level of HBsAg or HBeAg was found in AAV-IFN α 1 group, whereas HBsAg still remained at a high level in other groups.

Finally, the potential of IFN α 1 in inhibiting HBV replication was also demonstrated. The viral load reached 7 logs/ml in the plasma of PBS or AAV-EGFP groups, whereas the viral load was only 6 logs in the AAV-IFN α 1 group on day 1. The viral load of AAV-IFN α 1 group dropped to 6.4 logs on day 3 and day 5, and further to 6.2 logs on day 9. No significant anti-HBV effect was observed in AAV-EGFP group (Figure 3C). Compared with the PBS and AAV-EGFP group, the mean value of viral loads in

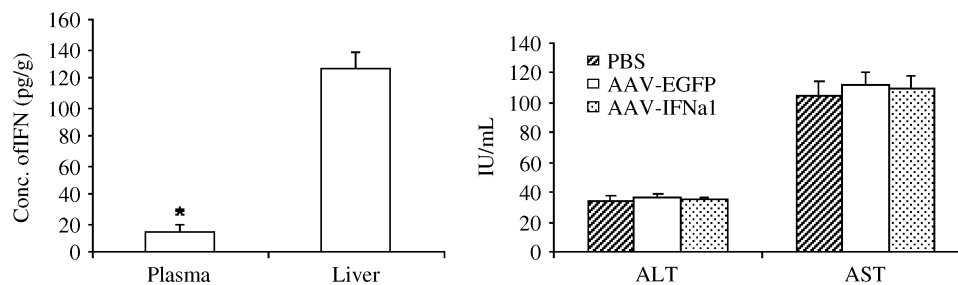


Figure 5. IFN α 1, ALT and AST levels in plasma. Total proteins were extracted to measure: (A) the IFN α 1 level and (B) ALT and AST levels in the plasma, after administration of AAV vectors ($n = 5$, $*p < 0.01$)

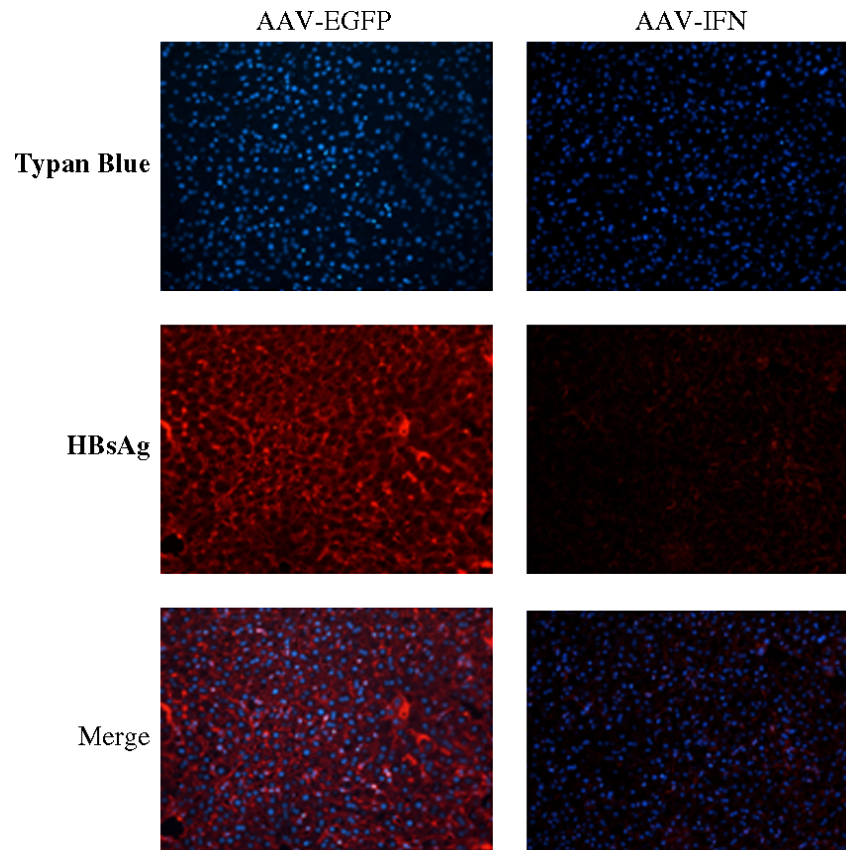


Figure 6. Local expression of IFN α 1 reduced HBsAg level in the liver. The HBsAg was demonstrated by Cy3 fluorescence (middle panels); the position of cell nucleus was revealed by trypan blue staining (top panels)

AAV-IFN α 1 group decreased by over 30-fold on days 5, 9, and 21 (Figure 3C).

Discussion

IFN α 2 has been widely employed to combat chronic HBV infection worldwide, yet its clinical outcome has been unsatisfactory, especially in Chinese patients. Apart from this, the frequent and repeated injection of IFN during anti-HBV treatment has burdened patients even more. According to previous studies, an effective anti-HBV or anti-WHBV therapy could be achieved by expressing IFN α stably via SV40 or AAV-mediated gene transfer [57,58]. Although SV40 vector may not be suitable for human gene therapy whereas WHBV is different from human HBV, these results suggest that gene therapy would be a good alternative for anti-HBV treatment. In the present study, we developed an AAV-IFN α 1 vector that demonstrated the therapeutic potential of IFN α 1 in anti-HBV therapy.

AAV2, a vector that has been used extensively in clinical trials, has demonstrated great potential for gene therapy applications. Its ability to mediate long-term transgene expression in the liver is one of the major advantages. Our previous study showed that angiostatin could last for at least 6 months in the liver of immunocompetent mice when it was introduced by AAV2 [56]. A similar observation was revealed in the present study, in which the IFN α 1 could last for at least 2 months in the liver in either immunocompetent or nude mice. Although AAV8 displayed better transduction efficiency to hepatocytes than AAV2, no clinical trial data concerning the efficacy and safety of AAV8 are available. In the present study, a better transduction ratio (i.e. similar to that of AAV8) was achieved by administration of high-dose AAV2 via hydrodynamic transfection (Figure 4). For clinical application, drugs or rAAV2 could be administered through the portal vein or localized perfusion so as to extend their dwelling time and to improve their therapeutic effects (e.g. high level transduction) [59,60]. In order to simulate the clinical condition, we used AAV2 as the gene delivery vector in the present study. Our results showed that rAAV-IFN α 1 reduced the expression of both HBsAg and HBeAg in a sustained manner, and suppressed the viral replication without detectable liver toxicity (Figure 3).

However, an obvious delay of transgene expression is known to be one of the shortcomings of AAV2. In our study, a delay of EGFP or IFN expression was noted. The EGFP expression level and the transduction efficiency were increased with an elevation of the multiplicity of infection, whereas strong fluorescent signals lasted for several passages (data not shown). Prolonged IFN α 1 gene expression mediated by rAAV in hepatocytes was observed (Figure 1). Furthermore, we performed pre-administration of rAAV before hydrodynamic transfection of pHBV plasmid to resolve the difficulty of importing HBV-carrier animals (i.e. HBV transgenic mice), and

also the problems of short-term gene expression and replication of HBV in the hydrodynamic transfection mouse model. In the present study, strong EGFP and IFN α 1 signals were demonstrated in the liver cells (Figure 4). High levels of IFN α 1 were observed in the liver for at least 4 weeks (data not shown). The concentration of IFN α 1 in the liver was approximately eight-fold higher than that in the plasma. Since the half-life of IFN is short (i.e. approximately 2 h) in a healthy circulation system and the repeated injection of IFN was one of the major concerns, a sustained level of IFN in the liver is certainly an advantage. A constant and prolonged local expression of IFN would surely benefit the anti-HBV treatment by reducing side-effects or by enhancing efficacy. As no obvious IFN induction or toxicity was observed in both *in vitro* and *in vivo*, rAAV appears to be a suitable vector in gene transfer or delivery of IFN α 1.

In the present study, both intracellular and extracellular antigens were significantly reduced after transduction of rAAV-IFN α 1. Both the HBsAg and HBeAg levels were significantly decreased by more than five-fold in the culture media. This finding is of prime significance because a minimal dosage has already achieved a similar antiviral effect as a high-dosage (1000 units/ml) of recombinant IFN γ in Huh6 and HB611 cells [61]. Moreover, such an antiviral effect was equivalent to commercially available IFN α (50 000 units/ml), which is isolated from leukocytes and composed of multiple interferon isoforms (see Supplementary Figure S1). The drastic decrease (i.e. over ten-fold) of HBsAg, HBeAg, and viral load (i.e. by over 30-fold) in the plasma was another achievement with the use of AAV-IFN α 1. Since little effect could be contributed by the animal immune system, IFN α 1 might somewhat stimulate other biological mechanisms *in vivo* that further synergize its antiviral activity. This is because IFNs are capable of acting directly (i.e. against infected cells), and indirectly (i.e. against viral infections by extending the innate and adaptive immune responses), thus boosting the overall antiviral ability [62].

In recent years, the use of short hairpin RNA (shRNA) to target HBV transcripts has become another notable application of AAV vectors [63]. The major advantage of shRNA is that it displays sequence-specific targeting where one can design multiple effective shRNAs to target various transcripts. Previously, our team showed that shRNA displayed synergistic antiviral effects with lamivudine [54]. However, when rAAV-shRNA was compared with rAAV-IFN α 1, the latter offers more advantages. First, IFN α is a secreted protein, which could spread from one cell to the other via circulation. Effective antiviral effects could be easily achieved by transducing a portion of liver cells using rAAV-IFN α vectors. For shRNAs, they expressed in an intracellular manner, which could not be transferred from one cell to another. This limits its antiviral efficacy and increases the difficulty for gene delivery. The effectiveness of shRNA delivery is one of the major concerns in anti-HBV gene therapy. Second, the efficacy and safety of IFN α have been proven clinically for decades, yet these issues remain to be elucidated for microRNA/shRNA. In an animal

study, oversaturation of cellular microRNA/shRNA has been shown to lead to fatality in mice. Therefore, safety issues of using microRNA/shRNA based gene therapy are still a concern [50]. Our team has investigated the feasibility of AAV2-shRNA vector for the treatment of HBV. In that study, no obvious liver damage or mortality was observed in nude mice (He *et al.*, unpublished data). However, off-target shRNAs and the rapid generation of shRNA-resistant mutants [64] raise other concerns with respect to shRNA based gene therapy. Taken together, the advantage of IFN α lacking resistance selection gives confidence to the proposed application of rAAV-IFN α 1 vector for anti-HBV therapy.

In conclusion, we demonstrated that AAV-IFN α 1 vector is a potential alternative for anti-HBV therapy. With the flexibility of this system, one can easily also test other isoforms of IFN, or other therapeutic genes, as well as shRNAs for various disorders such as colon cancer [65]. IFN is already being used clinically to treat HCC metastasis [66,67]. Moreover, the use of IFN α in synergy with other drugs (e.g. nucleotide analogs) and their summative antiviral or anti-HCC effects are well studied [68]. These findings provide a foundation for the feasibility of using AAV-IFN α 1 vector in anti-HBV gene therapy. The combination of gene therapy and chemotherapy or immunotherapy, on the other hand, could also offer better antiviral effects with reduced drug dosage and side-effects. Nevertheless, further investigations on the use of rAAV-IFN α 1 could provide benefits beyond CHB patients, since such a system might promote an improved clinical outcome for other diseases.

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