ABSTRACT

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Development of new animal models is critically important to study the biology of hepatitis B virus (HBV) and evaluate the intrahepatic effects of therapeutics aimed at curing chronic HBV infection. Currently, chimpanzees are the only animals that are infectable by HBV and recapitulate the entire HBV replication cycle. However, the use of chimpanzees and other great apes in biomedical research has been banned in a number of countries. As a consequence, there is an urgent need to develop new models of HBV infection and replication. This study aimed to use adeno-associated viruses (AAVs) to model HBV replication in cell culture and in vivo. To achieve this, recombinant AAVs bearing greater-than-genome-length sequences of the A1 HBV subgenotype (AAV-A1) or Subgenotype D3 (AAV-D3) were produced, whereby AAV-D3 served as a positive control. Subgenotype A1 infection is of particular concern to South Africa where it has the highest prevalence and is associated with a much greater risk of developing HBV-related hepatocellular carcinoma. Vectors packaged in AAV2 (AAV2-A1 and AAV2-D3) or AAV8 (AAV8-A1 and AAV8-D3) capsid were produced in large scale for in vitro or in vivo studies, respectively. Following transduction of liver-derived cells with AAV2-A1 or AAV2-D3, significant levels of HBV surface antigen (HBsAg) were detected in culture supernatants. Evaluation of the in vivo safety of AAV vector dose used to deliver HBV genome sequences was carried out using Cytometric bead array and RT-qPCR. Administration of AAV8-A1 or AAV8-D3 to mice did not induce expression of inflammatory markers as assessed by cytometric bead array analysis (IL-12p70, IL-6, TNF, IL-10, MCP-1, INF-γ) or RT-qPCR (*IFN-β*, *OAS-1* and *IFITI*). In addition, alanine transaminase enzyme levels remained within the normal range in animals receiving AAV8-A1 or AAV8-D3, indicating no liver toxicity. Sustained expression of HBsAg and HBcAg were detected in mice infected with AAV8-A1 or AAV8-D3 for a period of 6 months. The HBcAg levels in the liver of mice injected with AAV8-A1 were higher in the nucleus, whereas AAV-D3 injected mice livers had more cytoplasmic HBcAg localisation. The relaxed circular (RC) and double stranded linear HBV DNA intermediates were detected in mouse livers using Southern blot. The Northern blot analysis revealed the 3.5 kb, 2,4kb and 2, 1 kb transcripts. These DNA intermediates and RNA transcripts serve as indicators of HBV replication. Detection of viral particle equivalents using qPCR confirmed HBV replication in mice injected with AAVs bearing HBV genomes. There was no significant liver inflammation or cirrhosis detected as a result of infecting the mice with HBV genome bearing AAVs.

The possibility of using the AAV-HBV A1 murine model to screen anti-HBV therapeutics was explored. AAV8 expressing artificial primary-microRNA that target the HBV *X* region (scAAV8 miR 3/5,8,9) efficiently silenced viral gene expression from AAV-A1 or AAV-D3, both in cell

culture and in mice. These data showed for the first time that AAVs can be successfully used to model HBV subgenotype A1 replication. Most importantly, this model can be applied to screening novel anti-HBV therapeutics to enhance development of innovative treatments of chronic HBV infection.