Sulfated Polyanions Do Not Inhibit Duck Hepatitis B Virus Infection

WOLF-BERNHARD OFFENSPERGER, SILKE OFFENSPERGER, EIKE WALTER, HUBERT E. BLUM AND WOLFGANG GEROK

Department of Medicine, University of Freiburg, 7800 Freiburg, Germany, and Gastrointestinal Unit and Molecular Hepatology, MGH Cancer Center, Massachusetts General Hospital, Harvard Medical School, Charlestown, Massachusetts 02129

Received 30 July 1991/Accepted 13 September 1991

On the basis of the antiviral action of sulfated polyanions in human immunodeficiency virus and other viral infections, we studied the effect of dextran sulfate and heparin on duck hepatitis B virus infection. These agents do not affect viral uptake and replication in liver cells in vitro or in vivo. Sulfated polyanions, therefore, appear to have no potential for the treatment of hepadnavirus infections.

Dextran sulfate, heparin, and other sulfated polysaccharides have been shown to be potent antiviral agents (5, 11, 25). They primarily act via inhibition of viral absorption to target cells, such as the binding of the human immunodeficiency virus (HIV) to CD4-positive cells (4, 16). In addition, inhibitory effects on HIV replication, mediated through interference with RNase H, have been described (17). Sulfated polyanions are widely used as antilipemic agents and anticoagulants. Newer sulfated polysaccharides with marked antiviral and reduced antithrombin activity have been developed (3).

Duck hepatitis B virus (DHBV) belongs to a group of hepatotropic DNA viruses (hepadnaviruses) which includes the human hepatitis B virus, the woodchuck hepatitis virus, the ground and tree squirrel hepatitis viruses, and the heron hepatitis B virus. Because of their close relatedness to retroviruses and because an effective therapy for hepadnavirus infections is not available to date, we studied the effect of sulfated polyanions on DHBV infection in vitro and in vivo.

Primary duck hepatocytes and ducklings were used as in vitro and in vivo models, respectively, of DHBV infection. Hepatocytes were isolated from DHBV-infected and uninfected ducks as described previously (19, 23). Hepatocytes from uninfected ducks were infected in vitro by the addition of 30 μl of DHBV DNA-positive serum to a plate containing 2.5 ml of medium and 2 × 10⁶ cells. After 2 h at 37°C, the cells were washed and incubated with fresh medium. Uninfected hepatocytes were routinely preincubated with the agent to be studied at day 2 after plating and infected with DHBV DNA-positive serum in the presence of the drug 24 h later. Cell viability was determined by trypan blue exclusion.

As shown in Fig. 1A, primary duck hepatocytes can be efficiently infected in vitro (lane 1). Also, in vivo-infected hepatocytes can be kept in culture over prolonged periods of time (lane 3). The DHBV DNA hybridization pattern observed reflects replicative intermediates with the most prominent species in the 1.3-kbp position, representing full-length minus-strand molecules, and in the 3.0-kbp position, representing full-length virion DNA; the intermediate band at about 1.8 kbp represents covalently closed circular (supercoiled) DHBV DNA. In addition to demonstrating the suitability of the in vitro and in vivo models to study DHBV infection, the data demonstrate that chloroquine, a lysosomotropic agent, blocks DHBV uptake into primary hepatocytes but not viral replication in already infected liver cells (lanes 2 and 4). By comparison, phosphonoformate, a known inhibitor of HBV and DHBV DNA polymerase and reverse transcriptase, efficiently blocks viral replication in hepatitis B virus-infected duck hepatocytes infected in vitro (Fig. 1B, lane 2), and in already infected hepatocytes (Fig. 1B, lane 4). These data demonstrate that primary duck hepatocyte cultures can serve as a useful in vitro system for the study of antiviral agents targeted to different stages of the life cycle of DHBV.

The in vitro model of DHBV infection was extended to young ducklings, which can be efficiently infected by DHBV-positive serum. In this in vivo model, the application of suramin, a known inhibitor of viral uptake (20), results in an almost complete suppression of DHBV infection (data not illustrated), demonstrating the suitability of this system for the in vivo assessment of antiviral strategies.

By using the model systems described above, the effect of sulfated polyanions on DHBV infection was first assessed in vitro. As shown in Fig. 2 for noninfected primary duck hepatocytes, dextran sulfate and heparin do not inhibit DHBV infection at concentrations up to 500 μg/ml. Similarly, in primary duck hepatocytes isolated from DHBV-infected ducks, viral replication was not affected by either drug. Also, polycations, such as poly-L-lysine (8 μg/ml) and DEAE-dextran (12 μg/ml), were without effect on viral uptake (data not illustrated).

For in vivo analyses, DHBV-negative ducklings were infected by DHBV in the presence of dextran sulfate or heparin. Dextran sulfate and heparin were given intravenously twice daily, starting 2 days after hatching at a dose of 50 μg/g of body weight. One day later, the ducklings were infected by intravenous injection of 100 μl of DHBV DNA-positive serum (approximately 10¹⁰ virions per ml), followed by the application of the polyanions until day 14, when the ducklings were sacrificed. As shown in Fig. 3, dextran sulfate and heparin do not inhibit DHBV infection at a dose approximately corresponding to the maximal oral dosage given for treatment of AIDS patients (1).

Sulfated polyanions are pivotal in many forms of cell recognition and adhesion (8). Their antiviral effect has been demonstrated for herpes simplex virus (18, 22, 26), cytomegalovirus, vesicular stomatitis virus, Sindbis virus, arenav-
FIG. 1. (A) Effect of chloroquine on DHBV infection and replication in vitro. Southern blot analysis of DNA isolated from primary duck hepatocytes after 2 weeks in culture was carried out as described earlier (19). Lanes 1 and 2, primary hepatocytes isolated from DHBV DNA-negative ducks, infected with DHBV DNA-positive serum; lanes 3 and 4, primary hepatocytes isolated from DHBV DNA-positive ducks. Lanes 1 and 3, no chloroquine added; lanes 2 and 4, 50 μM chloroquine added. (B) Effect of phosphonoformate on DHBV infection and replication in vitro. Lanes 1 and 2, primary hepatocytes isolated from DHBV DNA-negative ducks, infected with DHBV DNA-positive serum; lanes 3 and 4, primary hepatocytes isolated from DHBV DNA-positive ducks. Lanes 1 and 3, no phosphonoformate added; lanes 2 and 4, 500 μg of phosphonoformate per ml added. Size markers are HindIII-digested lambda DNA and 10 pg of DHBV DNA. Autoradiographic exposure time was 2 days at −80°C.

FIG. 2. Effect of dextran sulfate (A) and heparin (B) on DHBV infection in vitro. Primary hepatocytes were isolated from DHBV DNA-negative ducks, followed by infection with DHBV DNA-positive serum. Lane 1, no drug added; lanes 2 to 4, 100 (lane 2), 250 (lane 3), and 500 (lane 4) μg of the respective drug added per ml. For further experimental details, see the legend to Fig. 1.

FIG. 3. Effect of dextran sulfate and heparin on DHBV infection in vivo. Southern blot analysis of DNA isolated from duck liver was performed as described earlier (19). Sample sources: lanes 1 and 2, two control ducklings; lanes 3 and 4, two ducklings treated with dextran sulfate (50 μg/g of body weight); lanes 5 and 6, two ducklings treated with heparin (50 μg/g of body weight). For further experimental details, see the legend to Fig. 1. Molecular size markers are shown to the left in kilobase pairs.

DHBV from ducks, and particularly ducks, was described (7). The virus was isolated from primary hepatocytes of ducks and was studied in vitro and in vivo. DHBV DNA-positive serum was injected into ducklings, which became infected with the virus. The infected ducklings had DHBV DNA-positive cells. Furthermore, the virus was isolated from the liver cells of infected ducklings. This indicates that DHBV can be transmitted from cell to cell in vivo. DHBV DNA-positive serum was also used to infect primary hepatocytes of ducks in vitro. The infected cells were found to have DHBV DNA-positive cells. This indicates that DHBV can be transmitted from cell to cell in vitro.

Furthermore, the virus was isolated from the liver cells of infected ducklings. This indicates that DHBV can be transmitted from cell to cell in vivo. DHBV DNA-positive serum was also used to infect primary hepatocytes of ducks in vitro. The infected cells were found to have DHBV DNA-positive cells. This indicates that DHBV can be transmitted from cell to cell in vitro.
fore, hold little promise for the treatment of hepatadnavirus infections.

The study was supported by a grant from the Deutsche Forschungsgemeinschaft (SFB 154 TP-A2 and TP-A3) and a Hermann-and-Lilly-Schilling professorship from the Stifterverband für die deutsche Wissenschaft to H.E.B.

The expert technical assistance of Birgit Hockenjos is gratefully acknowledged.

REFERENCES


