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CASE REPORT

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Adenovirus-induced liver necrosis in a case of AIDS

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Abstract Adenovirus-induced liver necrosis is rare. Before the era of AIDS (acquired immunodeficiency syndrome) this entity was seen predominantly in infants suffering from inborn immunodeficiency syndromes or from iatrogenic immunosuppression because of bone marrow or liver transplantation. Here, we report a case of a 30-year-old woman with AIDS who developed fever and rapidly progressing liver failure. A frozen section from a needle biopsy of the liver allowed a quick diagnosis of viral liver necrosis. The light-microscopic and electron microscopic aspects were typical of adenovirus infection and should be known to the surgical pathologist. The diagnosis was confirmed by immunohistochemistry and DNA hybridization analysis.

Key words Adenovirus · Liver pathology · Electron microscopy · AIDS

Introduction

Adenovirus infection of the upper respiratory tract is common in childhood. Adenoviruses are also known to induce epidemic keratoconjunctivits. Few cases of predominant infection of the liver have been reported [2–5, 7, 8, 12], and most of these patients were children with severe immunodeficiency of different causes. In adults, liver failure induced by adenovirus infection of the liver is very uncommon.

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Clinical history

A 30-year-old female patient with AIDS (absolute $CD4^+$ lymphocyte count of 3/μl) was admitted with intermittent fever up to 40°C and right upper quadrant pain. The diagnosis of HIV infection had been known for 8 years. Her risk factor for HIV was drug abuse. Initial ultrasound revealed hepatomegaly and a thickened gall bladder wall. The suspected diagnosis was acalculous cholecystitis. Antibiotic regimens did not improve the clinical symptoms. Four days after admission an abdominal CT scan visualized multiple hypodense lesions up to 5 cm in size in the liver. The clinical and radiological differential diagnoses were abscesses and tumour. The patient's general condition deteriorated further, and a needle biopsy of the liver was taken. Although an antiviral therapy with acyclovir and gancyclovir was given, she died 10 days later of liver failure with severe coagulopathy. An autopsy was refused.

Materials and methods

Owing to the poor general condition of the patient and the urgency of diagnosis, frozen sections of the liver biopsy (four cores 1 cm in length and 1 mm in diameter) were made in spite of a possible contamination of the cryostat. The remnants of the biopsy were fixed with formaldehyde (3.8% solution) and embedded in paraffin. Sections of 2–4 μm thickness were stained with haematoxylin & eosin, periodic acid–Schiff reaction, Prussian blue and pico sirius red.

Immunohistochemical staining of sections for viral antigens was performed using biotin-labelled secondary antibodies followed by detection with horseradish peroxidase-coupled streptavidin. Antibodies to the following viruses were used: herpes simplex virus types 1 and 2, hepatitis B virus surface and core antigens (polyclonal rabbit antibodies from DAKO, Hamburg) and two different cytomegalovirus (CMV) early antigens (monoclonal mouse antibodies clone E13 from Paesel & Lorei, Frankfurt/Main, and clone DDG9 from DAKO, Hamburg), and a monoclonal antibody (clone 6–23, J.C. deJong, Bilthoven, The Netherlands) recognizing a genus-specific antigen of all known human adenoviruses (types 1–49) [6].

For transmission-electron-microscopic examination a small part of the specimen was deparaffinized, postfixed with osmium tetroxide and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and Pb-citrate.

From the rest of the liver biopsy (less than 1 mm in size) DNA was prepared by SDS-proteinase-K treatment, phenol/chloroform extraction and ethanol precipitation. About 4 μg of DNA was digested with the endonuclease *Bam*H I (Boehringer, Mannheim). The DNA fragments were separated by electrophoresis in a 0.8%

agarose gel and blotted onto a nylon membrane by capillary transfer. Then hybridization with ^{32}P-labelled adenovirus type 2 DNA was performed [1].

Pathological findings

The cryostat section showed acidophilic confluent necrosis of hepatocytes without inflammatory cell infiltration. The nuclei of the acidophilic and the surrounding hepatocytes were slightly enlarged and of homogeneous opaque structure (Fig. 1a). The frozen section diagnosis was: "virus-induced liver necrosis, possibly herpes simplex". After fixation and paraffin embedding the haematoxylin & eosin-stained sections also showed, in addition to these findings, some multinuclear hepatocytes and longitudinally distorted hepatocytic nuclei (Fig. 1b, c).

On electron microscopy many hepatocyte nuclei were seen to contain abundant viral particles (about 70 nm in size) with an electron-dense core. A hexagonal arrangement of the viral particles was typical (Fig. 2b). In addition, crystalline electron dense bundles were found within deformed hepatocytic nuclei (Fig. 2c). Adenovirus infection was suspected. The negative immunohistochemical results for herpes simpex virus types 1 and type 2, hepatitis B virus core antigen, hepatitis B virus surface antigen and CMV early antigens and the positive immunohistochemistry for adenovirus confirmed the diagnosis of adenovirus-induced liver necrosis.

Comparative analysis of the hybridization blot with the DNA-fragment pattern of adenovirus prototypes revealed an adenovirus type 2 or type 6 (both subgenus C) [10]. No further differentiation between these two adenovirus types was possible, owing to a lack of biopsy material.

Discussion

The opaque nuclei of the hepatocytes undergoing necrosis and the intranuclear crystals made a rapid diagnosis of viral infection possible. Since necrosis but no inflammatory infiltration was seen in our case, infection by typical hepatitis virus species can be excluded, because these viruses induce necrosis only indirectly via immunological reactions. Immunodeficient patients may show massive replication of typical hepatitis viruses in nearly 100% of the hepatocytes without hepatocellular necrosis [9]. Thus, the main differential diagnosis in this case is herpes simplex virus-induced necrosis. The deformation of the hepatocytic nuclei by longitudinal crystals is less typical for herpes simplex virus than for adenovirus-induced necrosis [4, 8, 12]. Furthermore, in the electron microscope herpes simplex virus particles are larger (about 200 nm for complete particles and about 100 nm without capsid and tegument) than adenovirus particles (70–100 nm). The hexagonal arrangement of viral capsids in the nucleus, which is typical for the adenovirus particles, is seen only exceptionally in cases of the herpes virus infection. Furthermore, the cores of the herpes simplex virus particles show a more condensed centre with a bright halo, while the cores of adenoviruses are homogeneously electron dense (Fig. 2a–c). A CMV in-

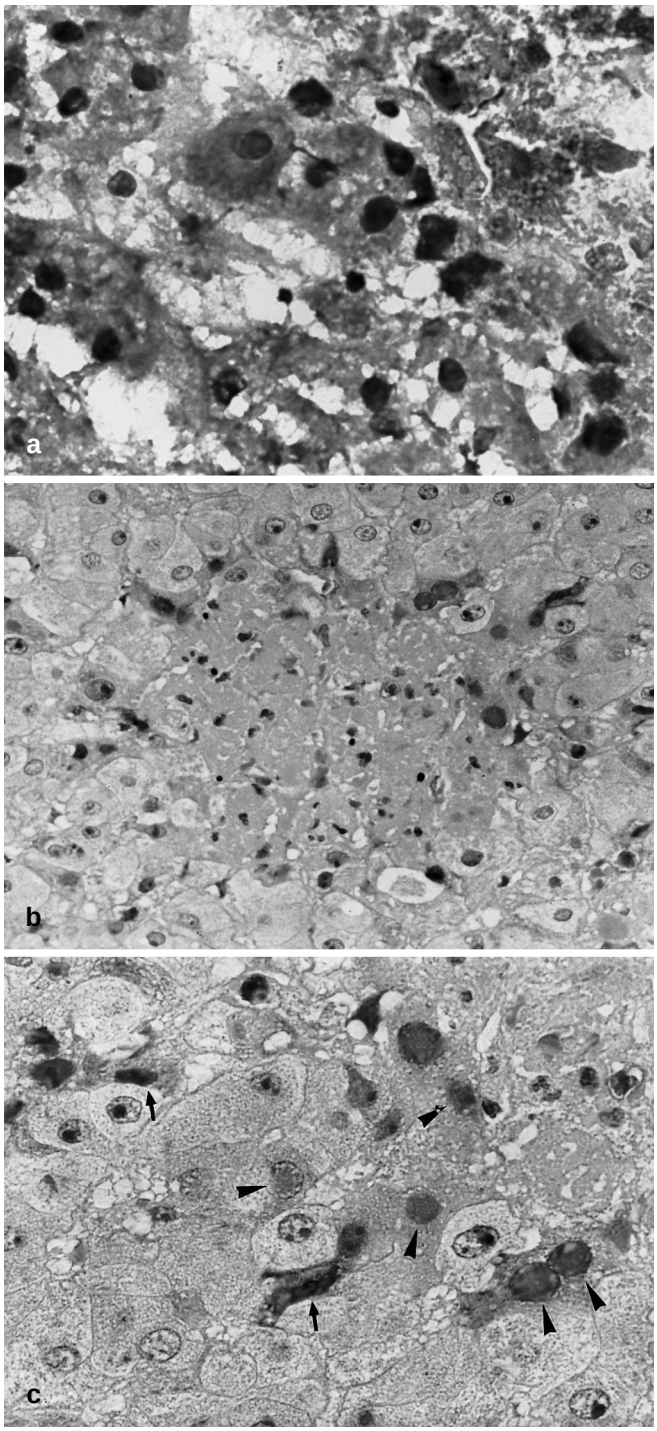

Fig. 1a–c Light microscopic aspects of adenovirus-induced liver necrosis. No inflammatory cells are seen in the necrotic liver tissue (**a** on *right*, **b** in centre). The surrounding hepatocytes show enlarged, opaque nuclei. **c** At higher magnification inclusion bodies can be identified in these nuclei (*arrowheads*). Also, some hepatocytic nuclei are longitudinally deformed (*arrows*). **a** Cryostat section; **b, c** paraffin sections

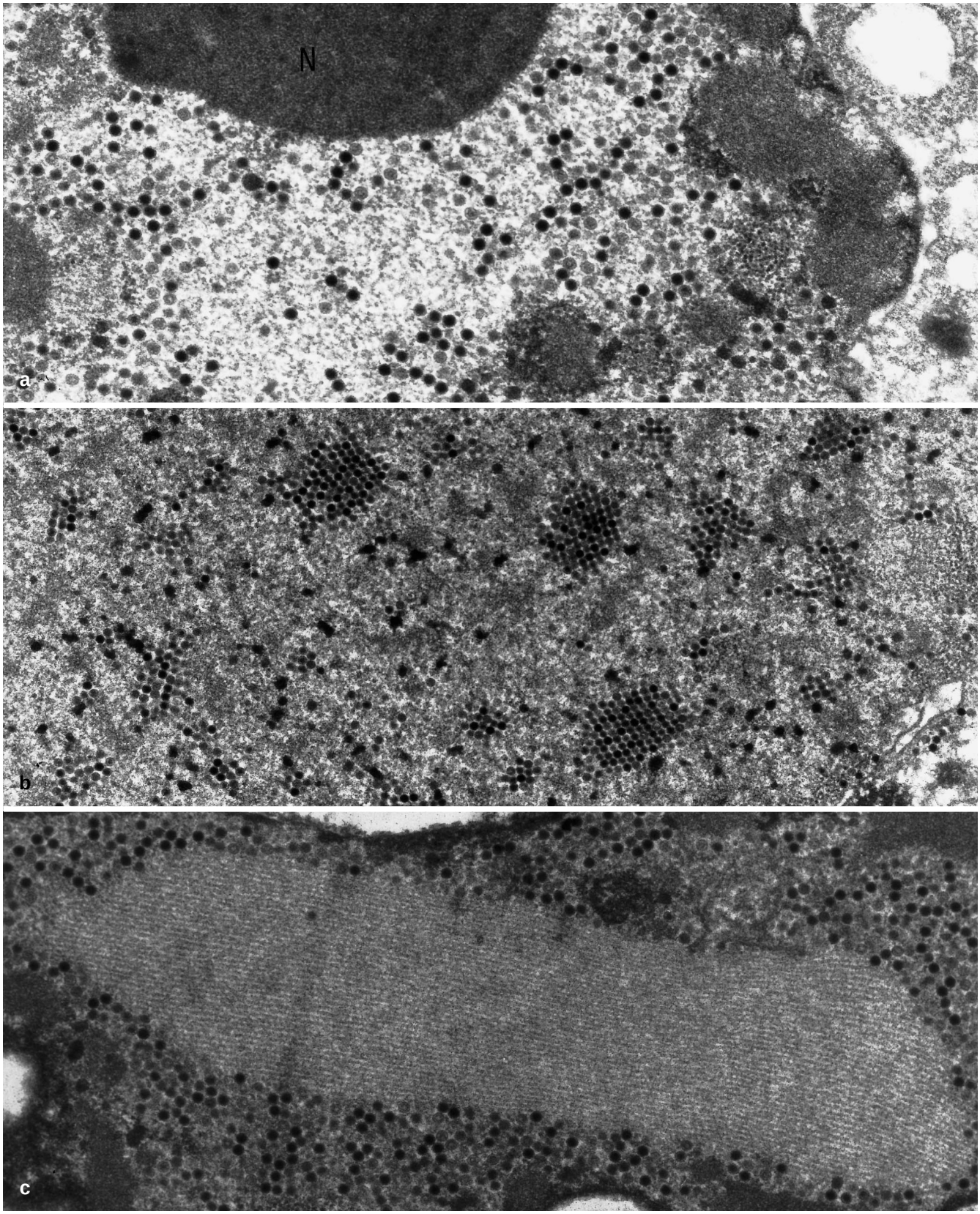

Fig. 2a–c Electron microscopic aspects of hepatocytes infected by adenoviruses. **a** Multiple electron-dense virus particles in a hepatocytic nucleus (*N* nucleolus). ×27,900. **b** Hexagonal arrangement of the viral particles and the mean particular size of 70 nm are typical for adenoviruses. ×21,600. **c** Crystalline electron-dense bundles are typical for infection with adenoviruses and were found within deformed hepatocytic nuclei (cf. Fig. 1c). ×28,600

fection was unlikely because of confluent necrosis, which is not typical for CMV hepatitis, and because of lack of any cytomegalic cytopathology. The negative immunohistochemical reaction against herpes simplex virus types 1 and 2 and two different CMV early antigens excluded these two viruses.

With the clinical history of immunosuppression and liver failure, a presumptive diagnosis of adenovirus-induced liver necrosis can be made from frozen sections or rapid paraffin sections, so that antiviral therapy can be started immediately. Virostatic drugs may improve the poor prognosis of adenovirus-induced liver necrosis, but there are no antiviral agents with proven efficacy against adenoviruses; thus, therapy is experimental [10, 11, 13]. The light microscopic diagnosis has to be confirmed by immunohistochemistry and electron microscopy.

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