

CASE REPORT

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Does human bocavirus infection depend on helper viruses? A challenging case report

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Abstract

A case of severe diarrhoea associated with synergistic human bocavirus type 1 (HBoV) and human herpes virus type 6 (HHV6) is reported. The case supports the hypotheses that HBoV infection under clinical conditions may depend on helper viruses, or that HBoV replicates by a mechanism that is atypical for parvoviruses, or that HBoV infection can be specifically treated with cidofovir.

Background

The human bocavirus (HBoV) was discovered in 2005 by the Swedish group of Tobias Allander and colleagues [1]. By now, HBoV was detected in patients suffering from respiratory infections and gastrointestinal diseases, but a proof that HBoV is the causative agent in such cases is missing as it remains impossible so far to fulfil Koch's modified postulates [2]. The latter problem is caused by the fact that HBoV is hardly to propagate in cell culture [3] and that no transmission within an animal model was successful until today.

Since HBoV infections are accompanied by co-pathogens in a very high frequency it was postulated that HBoV is a passenger rather than a pathogen in airway infections [2], despite of the fact that HBoV causes a productive infection with viral shedding, viremia, and putatively persistence in different organs [4-7]. Although HBoV meanwhile was classified as an autonomous parvovirus [3] rather than a Dependovirus like the Adeno-associated virus (AAV) there remains the possibility that HBoV infections depend on helper viruses or at least contributes synergistically to the clinical course. Dependoviruses require a helper virus such as the polyomavirus SV40, herpes viruses, or adenoviruses, of which SV40 and Herpesviruses are known to be able to initiate a rolling circle genome replication [8-12]. A recent study has demonstrated that HBoV in turn is capable to

form head-to-tail DNA-sequences that on the other hand are a typical feature of a rolling circle replication. The presented case report provides evidence that the HBoV infection is probably dependent on herpesvirus replication or sensitive to anti-herpesviral therapy with the nucleotide analogue cidofovir.

Case Report

The patient is a 14 months old Caucasian male with a known history of a myelosarcoma with affected bone marrow, recurrent HHV6 pneumonia, and multiple hospitalisations for several reason. Of note, the patient also suffers from a secondary immunodeficiency with predominant global lack of Immunoglobulins A, G, and M, which origin is not yet determined.

In January 2011 the patient was hospitalized for severe pneumonia accompanied by Norovirus-induced gastroenteritis with severe diarrhoea. Thereby the hospitalisation was required as the symptoms of diarrhoea were recurring since November 2010 and resulted in a stop of weight gain with the risk of dehydration. The patient was released from the hospital 3 days later but was re-hospitalized after four weeks due to aggravation of the gastrointestinal disease.

Despite reduced body weight the patient's general condition was classified as good, however accompanied by moderate anaemic but partially irritated flaked skin, reduced turgor, and wheezing, with radiological signs of an atypical pneumonia (banded shadows in the right upper lobe and moderately reduced ventilation). Antibody levels and the blood lymphocyte count were extremely low, whilst monocytes and eosinophiles were

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significantly increased. Serologically, the patient was tested negative for CMV, EBV, parvovirus B19, and adenoviruses but positive for HHV6 with a viremia increasing from 5.3×10^6 to 7.8×10^6 copies per ml serum. Stool samples were tested negative for rotavirus, adenoviruses, norovirus, *Clostridium difficile* (strains and toxins), *Salmonella*, *Shigella*, *Yersinia*, and *Campylobacter coli/jejuni* as well as enteropathogenic *E. coli*.

Due to the ongoing and severe diarrhoea further analyses and pathological investigations were initiated. Thereby, the human herpes virus 6 (HHV6) infection was confirmed in blood, duodenum, and colon biopsies. Instead, human bocavirus was detected in stool and, surprisingly, in duodenum biopsies but not in blood or colon biopsies. Sequencing analyses and subsequent blastN revealed that HBoV1 was present with an E-value of 5×10^{-68} to 3×10^{-65} and a total matching score ranging between 292 and 297. No further signs for a pathological disorder were detected. Based on the pathological and virological diagnoses antiviral therapy was started with Cidofovir. During this therapy, the HHV6 viremia decreased but remained detectable in both serum and stool samples taken in two intervals of 14 days; thereby, most surprisingly, during the Cidofovir therapy HBoV became undetectable in the stool samples 2 weeks after the onset of the antiviral therapy while HHV6 remained positive.

Discussion

In the recent past human bocavirus has been frequently associated with severe diarrhoea and turned out to be a most frequent agent in this clinical disorder [13-19], leading to the assumption that HBoV is the causative agent despite the missing proof of Koch's postulates for HBoV [2]. Thereby, mainly the genotypes 2-4 but not genotype 1 cause gastrointestinal infections, whilst the latter is mainly found in respiratory secretions. This latter observation is compatible with the fact that a mild respiratory infection with the clinical picture of a beginning atypical pneumonia was observed that was characterized by slight wheezing and radiological signs. The severe diarrhoea may have been a result of a synergistic interaction between the HHV6 infection and the superinfection by human bocavirus. Human herpes virus 6 was described to be a causative agent for prolonged diarrhoea in high risk patients [20]; the HHV6 associated diarrhoea may have been aggravated by the symptoms that are caused by human bocavirus. Although HBoV genotype 1 is mainly associated with respiratory infections it appears possible that in case of immuno-incompetent patients gastro-intestinal symptoms occur that are usually associated with the HBoV genotypes 2-4.

The case described here is interesting and surprising for two additional reasons: First, the presented case

gives raise to the hypothesis that HBoV in fact replicates in the gastrointestinal tract, in particular in the duodenum as confirmed by positive PCR results from the clinical material of duodenum but a negative PCR for the colon samples. Thus, it appears likely that a locus for replication of human bocavirus was identified. Second, the observation that HBoV persisted until the onset of Cidofovir therapy is of major interest. On the one hand this observation leads to the hypothesis that HBoV itself is sensitive to Cidofovir and in turn we would have a specific drug available that could be administered in severe clinical cases. Cidofovir was described to display antiviral activity against DNA viruses beyond the family of herpesviruses [21,22]. On the other hand this observation could lead to a more groundbreaking hypothesis. Recently we have shown that in clinical samples DNA sequences were detectable that are of head-to-tail structure [23]. Head-to-tail sequences of viral genomes are a typical feature of a rolling circle replication [24] rather than of a rolling hairpin as postulated for other parvoviruses [25-27]. The hypothesis that HBoV may replicate in a classical rolling circle mechanism is supported by the fact that HBoV genomes are predominantly of negative sense polarity and that solely a minority of HBoV strains additionally encapsidates positive stranded progeny DNA [28]. Notably, rolling circle replication can be initiated by polyomaviruses and herpesviruses including HHV6 [29-34], both known to be able to act as helperviruses for the dependoviruses, a subfamily of parvoviruses that is not able to replicate autonomously [29-35]. The fact that in the presented case HHV6 accompanied the HBoV infection may consequently give raise to the hypothesis that HBoV in fact replicates in a rolling circle mechanism in the inner sense and that rolling circle replication of HBoV may be triggered by helperviruses. This assumption is supported by the fact that HBoV in the majority of clinical cases was described to be accompanied by at least one co-pathogen which in turn led to the ongoing discussion that HBoV is an innocent bystander rather than a true pathogen [36-41]. In concert with the observation that HBoV can replicate autonomously in primary cell culture [3] the present case report goes beyond and leads to the conclusion that HBoV infection can occur autonomously but can be triggered in its severity by co-infections that in turn may modify the molecular replication strategy of HBoV. Although some of the hypotheses presented in this report remain a matter of speculation until it becomes possible to culture human bocavirus in a simplified culture system or in an animal model the presented observation are of major importance for the treatment of severe infections associated with HBoV and as thus deserve foremost attention.

Ethical Statement

Written informed consent was obtained from the parents to publish the clinical case in anonymized form.

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Competing interests

The authors declare that they have no competing interests.

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