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Usutu virus infection in a patient who underwent orthotropic liver transplantation, Italy, August-September 2009

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We report a case of Usutu virus (USUV)-related illness in a patient that underwent an orthotropic liver transplant (OLT). Post transplant, the patient developed clinical signs of a possible neuroinvasive disease with a significant loss of cerebral functions. USUV was isolated in Vero E6 cells from a plasma sample obtained immediately before the surgery, and USUV RNA was demonstrated by RT-PCR and sequencing. This report enlarges the panel of emerging mosquito-borne flavivirus-related disease in humans.

Introduction

In recent years, several mosquito-borne flaviviruses were identified as new emerging pathogens in animals and humans worldwide. The widespread occurrence of flaviviruses, such as West Nile virus (WNV), Dengue virus (DENV), Japanese encephalitis virus (JEV), yellow fever virus (YFV) and tick-borne encephalitis virus (TBEV) represents an important global health problem [1]. In the past ten years, infections with Usutu virus (USUV), a mosquito-borne flavivirus of the JEV serogroup and related to WNV, has been detected in a variety of birds in central European areas such as Austria, Hungary and Italy [2,3,4]. To date, USUV did not show considerable pathogenicity for humans [5]. In particular, no clinically evident USUV-related infections have so far been documented in humans.

Here we report a case of USUV-related disease in a female patient who, during a viraemic episode caused by USUV, received an orthotropic liver transplant (OLT) as a final consequence of a thrombotic thrombocytopenic purpura (TTP). This patient developed a neurological disease with severe impairment of the cerebral functions within the first days after OLT.

Case report

On 10 August 2009, a few days after returning to Italy from a holiday in Egypt, a woman in her 40s developed a TTP and received 18 plasma exchanges until 4 September 2009. Two weeks later, on 14 September, the patient presented with fever of 39.5°C, headache, skin rash, mild increment of cytolitic liver enzyme, without signs of TTP relapse, and was treated with antibiotics (moxifloxacin and amoxicillin clavulanate) without any response. On 18 September, the patient was admitted to hospital for persisting fever and headache. Any sign of TTP was excluded by total body computed tomography (CT) scan, and a peripheral blood smear did not show schistocytes or other fragmented red blood cells. Within a few days, a fulminant hepatitis and impairment of neurological functions were observed and rapidly developed into a coma. The molecular and serological laboratory diagnosis for the most common viruses associated with hepatitis (hepatitis A, B and C virus, cytomegalovirus and Epstein-Barr virus) gave negative results.

Two weeks after the OLT the patient slowly regained a low level of consciousness as well as some motor function of cranial nerves and limbs, and an intensive rehabilitative programme was started.

Virological analysis

Since 3 September 2009, systematic screening has been performed on blood, tissue, stem cell and organ donations from individuals living in the Emilia Romagna region in Italy, where WNV transmission was observed in summer 2009 [6]. This screening activity was undertaken following the data about WNV circulation in wildlife, horses and mosquitoes obtained from the regional integrated surveillance system that was in place from 15 June to 31 October. Screening for WNV was done using a nucleic acid amplification test (NAAT-Transcription-Mediated Amplification (TMA): PROCLEIX WNV, Novartis Diagnostics).

On 24 September, a plasma specimen obtained from the above patient immediately before surgery, was positive in the WNV NAAT assay. The test was repeated twice and the results were confirmed. A second sample was obtained from the patient one day after the OLT and the WNV NAAT was again positive.The level of positivity obtained with the two specimens was quite low, suggesting either an extremely low concentration of WNV RNA in the blood or a false positive reaction. Additional blood samples obtained during the following 15 days gave negative results.

The liver's donor was also investigated. The donor had been living in the area of Parma and her plasma, obtained before liver donation, was NAAT-negative for WNV.

The NAAT result was further investigated by real-ime RT-PCR targeting the WNV envelope (env) gene [7]. Surprisingly, the result was negative. Consequently we extended the investigation to additional members of the Flaviviridae family, including at first TBEV, because this agent was already reported in Italy and because the illness caused by this virus can involve the central nervous system with a possible association with liver injury [8]. The plasma specimens were analysed by real-time RT-PCR specific for the 3' non-coding region of the TBEV genome [9], and resulted negative.

A further step in the aetiological investigation was the use of a heminested RT-PCR with primer pairs which amplify the NS5 region of the Flavivirus genus. This method was developed for the detection by PCR of the principal pathogenic flaviviruses (including DENV, JEV, USUV, WNV YFV, and Zika virus) and subsequent identification by sequencing [3]. We performed the heminested RT-PCR as reported by Scaramozzino et al. [10] with minor modifications (details available on request) and obtained a single amplicon of the expected size (220 bp). Both strands of the amplicon were sequenced using the PCR primers and analysed by BLAST (http://www.ncbi.nlm.nih.gov/blast). This analysis revealed 98% sequence identity (over 203 nt) to the USUV genome sequences available in GenBank, and no higher homology with any other published DNA sequence. Low homologies were observed to the WNV genome sequence (80 % identity) and to the JEV genome sequence (79% identity); this partial homology is very likely due to the fact that these flaviviruses are closely related.

In conclusion, the sequencing results demonstrated the presence of USUV in the clinical samples of our patient. Additional confirmation of USUV viraemia was obtained by a PCR assay specific for USUV, performed as reported by Weissenbock et al. [11]. USUV was subsequently isolated in Vero E6 cells, and the identity of this isolate was confirmed by the heminested RT-PCR test reported above. As expected, the sequence obtained from the cultured virus isolate was identical to the one obtained from the amplified plasma sample. Complete sequencing of this human pathogenic USUV isolate is in progress.

Discussion

The results presented in this report, demonstrate USUV viraemia in an immunocompromised OLT recipient suffering from severe neurological impairment caused by an encephalitis. It is noteworthy that the NAAT test PROCLEIX WNV was capable of detecting a WNV-related virus, which indicates a potential problem with the specificity of this method.

The clinical findings observed closely resemble those reported in an animal model of USUV-related neurological disease [10]. To our knowledge, this report is the second description of the involvement of USUV in a human disease. Before, USUV-related infections had been reported as a cause of disease in animals, mainly birds, with no demonstrated pathogenicity for humans. Recently, it has been observed that USUV is circulating in owls and blackbirds in the North Eastern part of Italy, suggesting the possibility of USUV transmissions to humans in that area [12].

We are currently involved in an extensive serological investigation for USUV antibodies in the blood donors that were used for the plasma exchanges for our patient in order to define whether this therapy could have been the source of the infection or whether it was acquired naturally through a mosquito bite. In addition, a study is in progress to identify the presence of USUV in additional plasma and tissue specimens obtained from the same patient in order to quantify the viral load and the persistence of the USUV viraemic stage and to assess the possible involvement of USUV in the original liver disease. This case of USUV-related illness in humans has added this virus to the list of those that can be transmitted to humans by local mosquitoes and can cause severe diseases in immunocompromised individuals.

Authors' correction

On the request of the authors, the name of GE Gerunda was corrected on 13 September 2012.

References

1. Poidinger M, Roy AH., and Mackenzie SJ. Molecular Characterization of the Japanese Encephalitis Serocomplex of the Flavivirus Genus. Vir. 1996; 218(2): 417–21.
2. Bakonyi T, Erdélyi K, Ursu K, Ferenczi E, Csörgo T, Lussy H, et al. Emergence of Usutu virus in Hungary. J Clin Microbiol. 2007; 45(12): 3870-74.
3. Chvala S, Kolodziejek J, Nowotny N, Weissenböck H. Pathology and viral distribution in fatal Usutu virus infections of birds from the 2001 and 2002 outbreaks in Austria. J Comp Pathol. 2004; 131(2-3): 176-85.
4. Lelli R, Savini G, Teodori L, Filipponi G, Di Gennaro A, Leone A, et al. Serological evidence of USUTU virus occurrence in north-eastern Italy. Zoonoses Public Health. 2008; 55(7): 361-67.
5. Weissenböck H, Hubálek Z, Bakonyi T, Nowotny N. Zoonotic mosquito-borne flaviviruses: Worldwide presence of agents with proven pathogenicity and potential candidates of future emerging diseases. Vet Microbiol. 2009; in Press.
6. Rizzo C, Vescio F, Declich S, Finarelli AC, Macini P, Mattivi A, et al.. West Nile virus transmission with human cases in Italy, August - September 2009. Euro Surveill. 2009;14(40):pii=19353.
7. Lanciotti RS, Kerst AJ, Nasci RS, Godsey MS, Mitchell CJ, Savage HM, et al. Rapid Detection of West Nile virus from human clinical specimens, field-collected mosquitoes and avian samples by a TaqMan reverse transcriptase-PCR assay. J. Clin. Microbiol. 2000; 38(11): 4066-71.
8. Misić-Majerus L, Bujić N, Madarić V, Avsić-Zupanc T. Hepatitis caused by tick-borne meningoencephalitis virus (TBEV)--a rare clinical manifestation outside the central nervous system involvement. Acta Med Croatica. 2005; 59(4):347-52.
9. Schwaiger M, Cassinotti P. Development of a quantitative real-time RT-PCR assay with internal control for the laboratory detection of tick borne encephalitis virus (TBEV) RNA. J. Clin. Virol. 2003; 27(2): 136-45.
10. Scaramozzino N, Crance JM, Jouan A, Debriel DA, Stoll F, Garin D. Comparison of Flavivirus universal primer pairs and development of a rapid, highly sensitive heminested reverse transcription-PCR assay for detection of flaviviruses targeted to a conserved region of the NS5 gene sequences. J. Clin. Microbiol. 2001; 39(5): 1922-27.
11. Weissenböck H, Bakonyi T, Chvala S, Nowotny N. Experimental Usutu virus infection of suckling mice causes neuronal and glial cell apoptosis and demyelination. Acta Neuropathol.2004; 108, 453–60.
12. Manarolla G, Bakonyi T, Gallazzi D, Crosta L, Weissenböck H, Dorrestein GM et al. Usutu virus in wild birds in Norther Italy. Vet Microbiol. 2009; Aug 8. [Epub ahead of print].

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