PRZEGL EPIDEMIOL 2007; 61: 739 - 745

Agnieszka Bednarska[1], Andrzej Horban[1], Marek Radkowski[2],

CENTRAL NERVOUS SYSTEM AS A POSSIBLE SITE OF HCV REPLICATION

[1]Wojewódzki Szpital Zakaźny w Warszawie
Dyrektor: Andrzej Horban
[2]Zakład Immunopatologii Instytutu Chorób Zakaźnych i Pasożytniczych Akademii Medycznej w Warszawie
Kierownik zakładu: Marek Radkowski

There is mounting evidence of HCV neuroinvasion on the clinical and molecular level. It seems that microglia cells, representing a resident CNS macrophages population, are the main target for the virus. It can be also speculated that the macrophages carry HCV to CNS compartment. Non-specific inflammation and changes in the metabolic pathway of infected cells can play a role in pathogenesis of neurological symptoms which are observed in HCV infected patients.

Key word: HCV, CNS, macrophages, extrahepatic replication
Słowa kluczowe: HCV, OUN, makrofagi, replikacja pozawątrobowa

INTRODUCTION

Hepatitis C virus (HCV) is a small, enveloped, positive – strand RNA virus belonging to the *Flaviviridae* family (1). HCV is a cause of the disease recognized for the first time in the 1970s, after the development of diagnostic tests of hepatitis A and hepatitis B viruses. It appeared that most cases of transfusion – associated hepatitis were not caused by either of these viruses and the term : non-A, non-B hepatitis virus was created for the new agent at that time (2 ,3). In the late 1980s genome of this virus was cloned and later termed hepatitis C virus (4).

Primarily HCV was regarded as strictly hepatotropic since replicative form of genom negative – strand RNA was detected only in the liver of patients with chronic hepatitis C infection (5). However, considering extrahepatic clinical manifestation of HCV infection, cells other than hepatocytes were investigated as a potential place of the virus replication. These results become highly controversial when a publication of *R. Lanford* and colleagues (6) questioned earlier findings. They proved that standard RT - PCR method using Taq poly-

merase lacks sufficient strand specificity in detection of negative strand HCV –RNA while rTth method of RT-PCR appeared highly strand specific. Employing Tth based method no negative strand RNA was detected in PBMC and other extrahepatic tissues in HCV positive subject (6). Now it is known that this study had serious limitation - the tissue samples was obtained from a single chimpanzee that had a low level of HCV replication (6).

A new approach was to conduct a study on a material collected during postmortem examination of intravenous drug users died due to AIDS. HCV infection in this group is very frequent and viral load in persons with severe immunodeficiency is high, thus they provide a better model for extrahepatic HCV replication (7). In these studies a frequent presence of negative strand HCV-RNA was detected in lymph nodes and occasionally in pancreas, thyroid, adrenal gland, spleen, bone marrow. (8).

The replication of RNA viruses is an error-prone process, primarily because the viral RNA polymerase lacks of proof-reading 3' –5' exonuclease activity. Consequently RNA viruses are characterized by a high mutation rate (9) and like other RNA viruses, e.g. HIV, HCV exists in infected subject as a population of different but closely related genomes – *quasispecies* (10). One genome called master genome is usually quantitatively predominant and represents the best fitting to the actual host conditions strain. Thus the genetic divergence may stem from virus adaptation to growth in different tissue and cell types (11). This compartmentalization can be also a result of infection with different variants with different cell tropism. At present genetic divergence is often regarded as another line of evidences of independent virus replication in particular compartment, and thus extrahepatic HCV replication.

CLINICAL PICTURE

Patients with chronic hepatitis C are more likely to have significant changes in their physical and mental well being, such as fatigue and depression, than patients with liver disease of other etiology. The symptoms remit following successful antiviral therapy, indicating that the presence of the virus plays a role in their etiology. Similarly, liver transplant recipients, who suffered from HCV recurrent hepatitis, had significantly lower sense of quality of life, greater depression, higher psychological distress and lower physical functioning than patients without recurrence of infection (12,13).

HCV can influences the neuropsychiatric profile of infected individuals. In the study conducted by *Ryan* and colleagues the neuropsychiatric profile of individuals with advanced HIV coinfected with hepatitis C was compared to similarly advanced HIV patients without HCV coinfection.. Coinfected subjects had significantly greater rates of past depression symptoms. (14)

Recently it was also demonstrated that HCV infection is associated with cognitive dysfunction (15). HCV-infected patients were impaired on more cognitive tasks than those who cleared the virus. Detailed analysis showed impairments in concentration, speed of working memory, depression and fatigue, independent from a history of intravenous drug usage (IVDU) or symptoms of HCV hepatitis severity. (15). There are evidences of biological basis of this dysfunction: elevations of choline/creatine ratios were observed in proton magnetic resonance spectroscopy in the basal ganglia and white matter in patients with histologically

mild hepatitis C (15,16). These abnormalities were not present in healthy volunteers as well as in patients with hepatitis B and were unrelated to hepatic encephalopathy or history of intravenous drug abuse. Moreover, patients who were impaired on 2 or more cognitive tasks had a higher mean choline/creatine ratio compared with the unimpaired patients (15,16).

In a prospective study performed on 40 HCV positive patients with type II mixed cryoglobulinemia , which is a typical extrahepatic clinical manifestation of HCV infection, abnormal MRI findings were significantly more frequent in persons with HCV infection and mixed cryoglobulinemia than in patients with HCV infection only or healthy controls. The number of examined impaired cognitive functions was also higher in patients with HCV infection and cryoglobulinemia than in healthy controls. Altogether this can suggest inflammatory involvement of CNS, probably vasculitis of small cerebral vessels (17)

HCV REPLICATION IN THE CNS

On the molecular level the neurocognitive dysfunctions remain still unclear.

HCV was found in CSF and brain of anti HCV positive patients (18,19,20). Negative strand was present in three of six brain samples in one study and 2 of 13 CSF cell pellets in another study 18,19).

Viral sequences amplified from cerebrospinal fluid (CSF) were significantly different from those found in serum and they were classified as belonging to a different genotype. However they were of the same genotype as sequences derived from PBMC (18). Similarly HCV-RNA sequences amplified from brain tissue and serum were different and belonged to different genotypes. In this case HCV negative strand was also detected in lymph nodes and its sequences were identical to those found in brain while different from amplified from serum derived RNA (19).

This provides a strong evidence that HCV variants invading CNS are of extrahepatic, predominantly "*lymphotropic*" origin.

In another study brain, liver, lymph node tissue samples and serum were examined and viral IRES (*internal rybosomal entry site*) clones from each anatomical compartment were sequenced. A majority of brain clones were not represented in serum quasispecies pool and contained specific mutations associated with extrahepatic replication of HCV. Analysis of HVR1 (*hypervariable region 1*) sequences revealed that brain clones were most closely related to lymph node origined (21).

As mentioned above there are several evidences that symptoms of cognitive as well as motor disabilities found in patients with hepatitis C are the consequence of virus replication within central nervous system and its influence on brain cells functions.

An attempt was made to determine whether HCV replication could be found in brain among patients with hepatitis C and psychiatric symptoms. Tissue samples of central nervous system obtained during autopsy after unsuccessful liver transplantation from two patients with recurrent hepatitis C, who also developed severe depression were studied. In both cases HCV-RNA negative strand was present in brain tissue and it differed from viral sequences amplified from serum. (20)

The possible mechanism of HCV neuroinvasion remains unknown. It is speculated that infected macrophages transmit HCV to central nervous system.

Similarly, after liver transplantation from infected donor to infected recipient, donor PBMC migrate from the graft carrying donor HCV strain and spreading it within the new host (22).

Viral replicative activity in maternal PBMC is related to mother-to-infant transmission of HCV. In fact, the presence of a negative strand in PBMC of mothers is highly associated with HCV transmission to newborns (23)

It seems that after being transmitted through the blood-brain barrier, subsequently there is a secondary spread of HCV to permissive cells within the brain.

In a recent study several types of brain cells like microglia, neurons, astrocytes, oligodendrocytes were separated by laser capture microscopy from autopsy brain tissue obtained from HCV – positive patients. Viral positive and negative RNA strands were detected in the microglia cells only (24).

Microglia cells are the main resident immunological cells in the CNS. The resting microglia cells migrate into the CNS during embryological development but they can also be derived from circulating blood monocytes which migrate into the CNS. (25,26}

Both resident microglia and blood-derived monocytes in the CNS express typical macrophage markers like CD40, CD11c, CD68 (27)

POSSIBLE PATHOMECHANISMS

It is widely accepted that microglia contribute to the neurodegeneration through a release of variety of proinflammatory substances. Several studies have established an association of microglial activation in such neurodegenerative diseases as multiple sclerosis, Alzheimer's disease and AIDS dementia complex (28,29,30).

It is also proposed that in many psychiatric disorders, microglia play an essential role, e.g. mononuclear phagocytes accumulated in the cerebrospinal fluid of schizophrenic patients during acute psychotic episodes (31) and microglial activation was often observed in the postmortem pathohistologic examination of patients with schizophrenia (32).

Recently a study based on incubation of macrophages from healthy donors with HCV infected serum has been published. It revealed, that *in vitro* HCV infection of macrophages is associated with their activation and induction of secretion of proinflammatory cytokines like TNF-alpha and IL-8 (33). One can speculate that the virus could induce nonspecific inflammation within the brain tissue providing possible mechanism of nerve cells damage.

CNS demands high aerobic metabolism. Reduction in enzymes of oxidative metabolism is associated with some of the most common disorders of the nervous system: Alzheimer's disease and other dementias. (34,35). The energy production dysfunction based on mitochondrial ATP production impairment can also play a role in the pathogenesis of Huntington disease. (36)

A down-regulation of several oxidative phosphorylation genes in HCV-infected patients compared with controls was observed in a study on an autopsy material. In this study brain tissue samples were obtained from three HCV-positive patients and three HCV – negative subjects. Altogether, 84 gene fragments were down or up regulated. Among them, 9 gene fragments responsible for energy metabolism were down regulated and the fold change

comparing to HCV negative controls was statistically significant in each of that nine cases. (24).

CONCLUSIONS

There are mounting evidences that HCV can affect central nervous system. Negative-strand HCV-RNA was found in CSF and brain of HCV infected patients, including patients with nervo-psychiatric diseases. It seems that microglia cells, representing a resident CNS macrophages population, are the main target for the virus. It can be also speculated that the macrophages carry HCV to CNS compartment. Although it is still unknown what is the cause of the neurological symptoms it seems that non-specific inflammation and changes in the metabolic pathway of infected cells can play a role in their pathogenesis.

A Bednarska, A Horban, M Radkowski

OŚRODKOWY UKŁAD NERWOWY JAKO JEDNO Z MIEJSC POZAWĄTROBOWEJ REPLIKACJI HCV

STRESZCZENIE

Zaobserwowano, że u chorych na przewlekłe zapalenie wątroby typu C częściej niż u pacjentów niezakażonych HCV występują takie objawy jak uczucie zmęczenia, senność, apatia, depresja. Zauważonno również istnienie korelacji między skutewcznym leczeniem antywirusowym a ustąpieniem wymienionych dolegliwości, co może sugerować związek przyczynowy między HCV i występowaniem zaburzeń neropsychicznych. Według najnowszych badań zakażenie HCV jest związane z upośledzeniem funkcji poznawczych, a u osób z potwierdzonym histopatologicznie zapaleniem wątroby wywołanym tym wirusem, spektroskopia protonowa rezonansu magnetycznego wykazuje obecność zaburzeń metabolizmu w obrębie ośrodkowego układu nerwowego. Obecność nici minus HCV RNA w płynie mózgowo-rdzeniowym i mózgu badanych pacjentów także przemawia za możliwością zakażenia ośrodkowego układu nerwowego, prawdopodobnie komórek mikrogleju, tym wirusem.

REFERENCES

1. Shimizu YK, Feinstone SM, Kohara M, i in. Hepatitis C virus: detection of intracellular virus particles by electron microscopy. Hepatology 1996 23(2):205-9.
2. Feinstone SM, Kapikian AZ, Purcell RH, i in. Transfusion-associated hepatitis not due to viral hepatitis type A or B. N Engl J Med 1975 Apr 10;292(15):767-70
3. Prince AM, Brotman B, Grady GF, i in. Long-incubation post-transfusion hepatitis without serological evidence of exposure to hepatitis-B virus. Lancet. 1974;2(7875):241-6
4. Choo QL, Kuo G, Weiner AJ, i in. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science. 1989;244(4902):359-62.
5. Fong TL, Shindo M, Feinstone SM, Hoofnagle JH, Di Bisceglie AM. Detection of replicative intermediates of hepatitis C viral RNA in liver and serum of patients with chronic hepatitis C. J Clin Invest 1991; 88:1058-60.
6. Lanford RE, Chavez D, Chisari FV, i in. Lack of detection of negative-strand hepatitis C virus RNA in peripheral blood mononuclear cells and other extrahepatic tissues by the highly strand-specific rTth reverse transcriptase PCR. J Virol. 1995;69(12):8079-83

7. Sherman KE, O'Brien J, Gutierrez AG, i in. Quantitative evaluation of hepatitis C virus RNA in patients with concurrent human immunodeficiency virus infections. J Clin Microbiol. 1993 Oct;31(10):2679-82.
8. Laskus T, Radkowski M, Wang LF, i in. Search for hepatitis C virus extrahepatic replication sites in patients with acquired immunodeficiency syndrome: specific detection of negative-strand viral RNA in various tissues. Hepatology. 1998;28(5):1398-401.
9. Holland J, Spindler K, Horodyski F, i in Rapid evolution of RNA genomes. Science. 1982 Mar 26;215(4540):1577-85
10. Martell M, Esteban JI, Quer J, i in. Hepatitis C virus (HCV) circulates as a population of different but closely related genomes: quasispecies nature of HCV genome distribution J Virol 1992; 66(5): 3225-3229
11. Maggi F, Fornai C, Morrica A, i in Divergent evolution of hepatitis C virus in liver and peripheral blood mononuclear cells of infected patients. J Med Virol. 1999;57(1):57-63
12. Foster GR Hepatitis C virus infection: quality of life and side effects of treatment. J Hepatol 1999;31 Suppl 1:250-4.
13. Singh N, Gayowski T, Wagener MM, i in. Quality of life, functional status, and depression in male liver transplant recipients with recurrent viral hepatitis C. Transplantation. 1999;67(1):69-72.
14. Ryan EL, Morgello S, Isaacs K, i in. Neuropsychiatric impact of hepatitis C on advanced HIV. Neurology. 2004;62(6):957-62.
15. Forton DM, Thomas HC, Murphy CA, i in. Hepatitis C and cognitive impairment in a cohort of patients with mild liver disease. Hepatology. 2002;35(2):433-9.
16. Forton DM, Allsop JM, Main J, i in. Evidence for a cerebral effect of the hepatitis C virus. Lancet 2001;358(9275):38-9.
17. Casato M, Saadoun D, Marchetti A, i in. Central nervous system involvement in hepatitis C virus cryoglobulinemia vasculitis: a multicenter case-control study using magnetic resonance imaging and neuropsychological tests. J Rheumatol 2005;32(3):484-8.
18. Laskus T, Radkowski M, Bednarska A, i in. Detection and analisis of hepatitis C virus sequences in cerebrospinal fluid. J Virol 2002; 76(19):10064-10068
19. Radkowski M, Wilkinson J, Nowicki M, i in. Search for hepatitis C virus negative-strand RNA sequences and analysis of viral sequences in the central nervous system: evidence of replication. J Virol 2002;76(2):600-608
20. Vargas H, Laskus T, Radkowski M, i in. Detection of hepatitis C virus sequences in brain tissue obtained in recurrent hepatitis C after liver transplantation. Liver Transpl, 2002;8(11):1014-9.
21. Forton DM, Karayiannis P, Mahmud N, i in. Identification of unique hepatitis C virus quasispecies in the central nervous system and comparative analysis of internal translational efficiency of brain, liver, and serum variants. J Virol 2004;78(10):5170-83.
22. Radkowski M, Wang LF, Vargas H, i in. Hepatitis C virus in peripheral blood mononuclear cells from a chronically infected patient receiving liver graft from infected donor. Transplantation 1999;67(4):627-9.
23. Azzari C, Resti M, Moriondo M, i in. Vertical transmission of HCV is related to maternal peripheral blood mononuclear cell infection. Blood 2000;96(6):2045-8.
24. Adair DM, Radkowski M, Jablonska J, i in. Differential display analysis of gene expression in brains from hepatitis C-infected patients. AIDS 2005;19 Suppl 3:S145-50.
25. Ling EA, Wong WC. The origin and nature of ramified and amoeboid microglia: a historical review and current concepts. Glia 1993;7(1):9-18
26. Cuadros MA, Navascues J. The origin and differentiation of microglial cells during development. Prog Neurobiol 1998;56(2):173-89.
27. Gilles J. Guillemin and Bruce J. Brew Microglia, macrophages, perivascular macrophages, and pericytes: a review of function and identification J Leukoc Biol 2004 75: 388-397.

28. Dickson DW, Lee SC, Mattiace LA, i in. Microglia and cytokines in neurological disease, with special reference to AIDS and Alzheimer's disease. Glia 1993;7(1):75-83.
29. Raine CS Multiple sclerosis: immune system molecule expression in the central nervous system. J Neuropathol Exp Neurol 1994;53(4):328-37.
30. Yoshioka M, Bradley WG, Shapshak P, i in. Role of immune activation and cytokine expression in HIV-1-associated neurologic diseases. Adv Neuroimmunol 1995;5(3):335-58.
31. Nikkila HV, Muller K, Ahokas A, i in. Accumulation of macrophages in the CSF of schizophrenic patients during acute psychotic episodes. Am J Psychiatry 1999;156(11):1725-9.
32. Bayer TA, Buslei R, Havas L, i in. Evidence for activation of microglia in patients with psychiatric illnesses. Neurosci Lett 1999;271(2):126-8.
33. Radkowski M, Bednarska A, Horban A, i in nfection of primary human macrophages with hepatitis C virus in vitro: induction of tumour necrosis factor-alpha and interleukin 8. J Gen Virol 2004;85(Pt 1):47-59
34. Blass JP Brain metabolism and brain disease: is metabolic deficiency the proximate cause of Alzheimer dementia? J Neurosci Res 2001;66(5):851-6
35. Gibson G Causes of cell damage in hypoxia/ischemia, aging and Alzheimer's disease. Neurobiol Aging. 1989;10(5):608-9;
36. Milakovic T,Johnson GV Mitochondrial respiration and ATP production are significantly impaired in striatal cells expressing mutant huntingtin J Biol Chem 2005;280(35):30773-82.

Otrzymano: 25.06.2007 r.

Adres do korespondencji:
lek. med. Agnieszka Bednarska
Wojewódzki Szpital Zakaźny, 01-201 Warszawa, ul. Wolska 37
tel 022 3355216
e-mail: basand@mp.pl