

CEREBRAL ISCHAEMIC ATTACK IN ASYMPTOMATIC HEPATITIS C VIRUS INFECTION

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Summary: We tried to correlate the onset of the cerebral ischaemic attack accompanied by mixed cryoglobulinaemia with the asymptomatic hepatitis C virus infection. We studied a group of 12 patients with asymptomatic hepatitis C virus infection associated with ischaemic stroke (antihepatitis C virus antibodies-ELISA). Ischaemic stroke occurred in equal proportion in women and men. The patients were not aware of infection at that moment. All patients had type II mixed monoclonal cryoglobulinaemia, hypergammaglobulinemia with polyclonal IgG and monoclonal IgM, blood hyperviscosity, high level of cryocrit, rheumatoid factors-positive, and normal serum alanine aminotransferase levels, despite the replication of hepatitis C virus in infected liver cells and detection of HCV-RNA in serum, HBs antigen-negative, anti-HBs antibody-negative; anti-HCV antibody-positive, antinuclear antibodies-false positive. Immunological tests for autoantibodies, namely, anti-Sm antibody, anti-RNP antibody, anti-SS-A and -B antibodies, and anti-Scl antibody were not detectable due to a high level of serum cryoglobulin. Cryoglobulin was washed several times with an isotonic chloride solution for purification, then subjected to immunological analyses. The purified cryoglobulin contained monoclonal IgM-kappa, polyclonal IgG. Cryoglobulinemia is a risk factor of ischaemic stroke, it causes arterial thrombosis. The long-term exposure to antigens and the autoimmune background may have developed IgM anti-idiotypic auto-antibody against the high level of anti-HCV IgG.

Key words: cerebral ischaemic attack, asymptomatic hepatitis C virus infection, normal aminotransferase level, immunological disorders

Introduction

Stroke is a leading cause of death worldwide but our knowledge of underlying mechanisms is still limited. Ischaemic stroke comprises 85% of all strokes and occurs when a blood clot causes a blockage in a brain artery. It is now recognized that many strokes are linked to specific »psychological triggers« including, possibly, infectious/inflammatory processes. Inflammatory infection appears to be an important predisposing risk of ischaemic stroke (85% are thromboembolic in origin), but little is known regarding underlying molecular mechanisms. Ischaemia is a physiological term indicating insufficient blood flow for normal cellular function. Therefore, we hypothesized that patients with cryoglobulinaemia and with asymptomatic hepatitis C virus infection were more likely to have cerebral ischaemic attack (1). We conducted this study in order to establish this hypothesis.

The aim of this study was to clarify the relationship of cerebral ischaemic attack and HCV-related mixed cryoglobulinaemia in patients with asymptomatic hepatitis C virus infection.

Material and Method

We studied a group of 12 patients with cerebral ischaemic attack associated asymptomatic persistent hepatitis C virus infection. Information about symptoms during cerebral ischaemic attack was obtained from the patients or their families. Diagnosis of cerebral ischaemic attack was made by neurologist based on Stroke Classification.

Patients with chronic hepatitis C have normal serum alanine aminotransferase (ALT) levels, even when tested on multiple occasions. In this situation, the diagnosis should be confirmed by testing of HCV-

RNA. The presence of HCV RNA indicates that the patient has an ongoing viral infection despite normal ALT levels.

Cryoglobulin was obtained from 5 mL of patient's serum by cooling the serum at 0 °C for 12 hours. The precipitate was washed five times with ice-cold Tris-buffered saline (TBS:10 mmol/L tri-HCL/150 mmol/L NaCl, pH 7.5), dissolved in 1 mL of 0.2 mol/L glycine-HCl buffer (pH 3.0 at 37 °C for 5 minutes and neutralized by adding Tris base (2, 3).

Results

The cerebral ischaemic attack occurred in equal proportion in women and men. The patients were not aware of infection at that moment. All patients had type II mixed monoclonal cryoglobulinaemia, hypergammaglobulinemia with polyclonal IgG and monoclonal IgM, blood hyperviscosity, high level of cryocrit, rheumatoid factors-positive and normal serum alanine aminotransferase (ALT) levels, despite the replication of hepatitis C virus (HCV) in infected liver cells and detection of HCV-RNA in serum, HBs antigen-negative, anti-HBs antibody-negative, anti-HCV antibody-positive, antinuclear antibodies-false positive. Type II cryoglobulins, as detected in those cases, consisted of two components of immunoglobulin: mixed monoclonal (IgM-kappa) and polyclonal (IgG) components. Immunological tests for autoantibodies, namely anti-Sm antibody, anti-RNP antibody, anti-SS-A and -B, and anti-Scl antibody were not detectable due to a high level of serum cryoglobulins. It was noted that IgG and IgM serum cryoglobulin levels were very elevated. Serum protein electrophoresis and immunoelectrophoresis demonstrated a monoclonal peak of IgM-kappa type. The purified cryoglobulins contained monoclonal IgM-kappa, polyclonal IgG.

Discussion

Hepatitis C is most readily diagnosed when serum aminotransferases are elevated and anti-HCV is present in serum. The diagnosis is confirmed by the finding of HCV RNA in serum. Testing for HCV RNA (by PCR) confirms the diagnosis and documents the presence viraemia; all patients with chronic infection have the viral genome detectable in serum by PCR. This study examined the hypothesis that the subjects with asymptomatic hepatitis C infection and cryoglobulins present cerebral ischaemic attack. Cryoglobulinaemia is a risk factor of ischaemic attack, it causes arterial thrombosis. The relationship between hepatitis C virus infection and selected immunohaematologic variable in subjects with cerebral ischaemic attack has been fully investigated. Cryoglobulinemia shows the presence of immunoglobulins in serum that precipitate on cooling; this may occur as a primary or secondary disorder to another disease (4). Although a

precise etiologic role is still unknown, antigenic stimulation in hepatitis C virus infection may play a primary role in the production of cryoglobulins. The long-term exposure to antigens and the autoimmune background may develop IgM anti-idiotypic autoantibody against the high level of anti-HCV IgG (5). The main systemic disorders which may be complicated with cerebral arteritis: polyarteritis nodosa, Churg-Strauss syndrome, temporal arteritis, Takayasu's arteritis, SLE, Sjögren's syndrome, mixed cryoglobulinemia, granulomatous arteritis. Cryoglobulinaemia, especially mixed cryoglobulinaemia, is associated with ischaemic attack, caused presumably by cerebral vasculitis or hyperviscosity syndrome (6–8). Another possible mechanism that may be a relevant factor in stroke triggered by infection is an increase in fibrinogen concentration during infection, which increases blood viscosity (9). The most widely accepted hypothesis is as follows: the formation of immune complexes is followed by their deposition in the vessel wall and by inflammation leading to the development of small vessel vasculitis via activation of complement (10). Other factors such as elevations in plasma C4bp may further compromise the protein C system in infection-associated attack patients. Protein S circulates in a free active form and in an inactive complex when bound to C4bp, a regulatory protein in the classic complement pathway. Elevation in C4bp, an acute-phase protein, often accompanies infection/inflammatory states and may foster an acquired free protein S deficiency.

The clinical features are caused by the obstruction of small blood vessels, ischaemia and possible infarction of the tissues which they subtend. In the present study, patients were routinely evaluated for asymptomatic hepatitis C virus infection and cryoglobulins (12–14). However, it was not our intention to study the differences between patients with cryoglobulins and those without cryoglobulins.

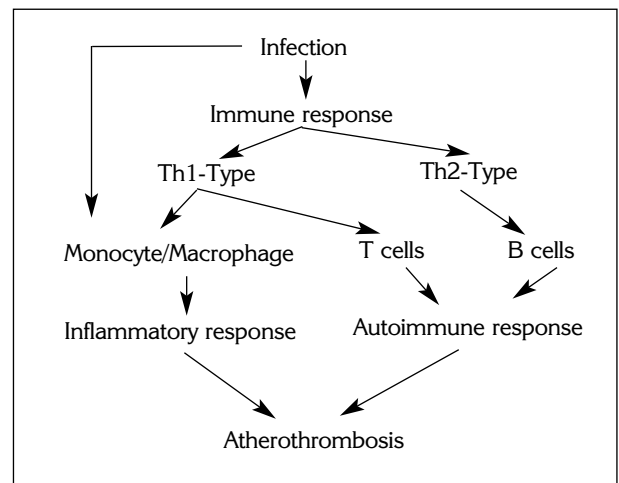


Figure 1. Scheme of pathogenesis in infection-associated atherothrombosis (11)

Further studies will be necessary to elucidate the role of hepatitis C virus infection with cryoglobulins in production of cerebral ischaemic attack.

Based on the findings outlined above, screening of cryoglobulins in ischaemic attack patients is recommended warmly. Validation of an aetiological association between hepatitis C virus infection and ischaemic attack requires additional classification (15).

Conclusion

Cerebral ischaemic attack has been caused by a small-vessel vasculitis or by a small vascular occlusion associated with cryoglobulins.

The findings of the present study showed the presence of cryoglobulins in sera of patients with cerebral ischaemic attack and asymptomatic hepatitis C virus infection. Our results suggest that persistent hepatitis C infection may act as a trigger and may increase the risk of ischemic attack.

CEREBRALNI ISHEMIJSKI NAPAD PRI ASIMPTOMATSKOJ INFEKCIJI HEPATITIS C VIRUSOM

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Kratak sadržaj: Ispitivana je korelacija cerebralnog ishemijskog napada praćena mešovitim krioglobulinemijom sa asimptomatskom infekcijom hepatitis C virusom. Proučavana je grupa od 12 pacijenata sa asimptomatskom infekcijom hepatitis C virusa praćena ishemijskim šlogom, koji se podjednako javio kod muškaraca i žena. Pacijentima nije bilo poznato da imaju infekciju. Svi pacijenti su imali tip II mešovitu monoklonalnu krioglobulinemiju, hipergamaglobulinemiju sa poliklonalnim IgG i monoklonalnim IgM antitelima, hiperviskoznost, visok nivo kriocita, pozitivan reumatodini faktor i normalnu aktivnost serumske alaninaminotransferaze, uprokos replikaciji hepatitis C virusa u ćelijama jetre i HCV-RNK u serumu, negativnog Hbs antigena, negativnih anti-Hbs antitela, pozitivnih anti-HCV antitela i lažno pozitivnih antinulearnih antitela. Zbog visokog nivoa krioglobulina nisu otkrivena sledeća autoantitela, npr. anti-Sm antitelo, anti-RNP antitelo, anti-SS-A i -B antitela i anti-Scl antitela. Krioglobulin je ispiran nekoliko puta fiziološkim rastvorom radi prečišćavanja, a zatim je analiziran. Prečišćeni krioglobuli sadržali su monoklonalna IgM-kapa i poliklonalni IgG. Krioglobulinemija je faktor rizika za ishemijski insult pošto izaziva arterijsku trombozu. Dugotrajno izlaganje antigenima i autoimunosti može da dovede do razvoja IgM anti-idiotopskim autoantitelina usled visokog nivoa anti-HCV IgG.

Ključne reči: cerebralni ishemijski insult, asimptomatska infekcija hepatitis C virusom, normalni nivo aminotransferaza, imunološki poremećaji

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Received: November 15, 2003

Accepted: March 9, 2004