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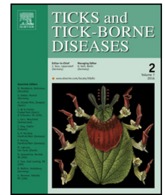

Original article

Tick-borne encephalitis virus neutralization by high dose intravenous immunoglobulin

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ABSTRACT

Tick-borne encephalitis (TBE) is a potentially lethal neuroinfection in humans, caused by TBE virus (TBEV). Currently, there are no approved therapeutic agents to treat TBE. Previously, it was suggested that application of high dose intravenous immunoglobulin (IVIG) may pose potentially successful treatment for severe cases of TBE. In this study, we determined the titers of TBEV-neutralizing antibodies in two IVIG lots originating from the same manufacturer, and tested their ability to treat a lethal TBEV-infection in a mouse model. Using an *in vitro* assay, more than 100-fold difference in TBEV-neutralizing capacity was demonstrated between the two individual IVIG lots. High TBEV-neutralizing activity of IVIG containing TBEV-specific antibody was confirmed in two different human neural cell lines, but IVIG without TBEV-specific antibodies had no or little effect on virus titers in the culture. In TBEV-infected mice, 90% of protection was achieved when the mice were treated with IVIG containing higher titers of TBEV-specific antibodies, whereas no immunotherapeutic effect was seen when mice were treated with IVIG without TBEV-specific antibodies. No antibody-dependent enhancement of TBEV infectivity induced by cross-reactive antibodies or by virus-specific antibodies at neutralizing or sub-neutralizing levels was observed either in cell culture or in TBEV-infected mice treated with any of the IVIG preparations. The results indicate that IVIG lots with high TBEV antibody titers might represent a post-exposure prophylaxis or first-line effective therapy of patients with a severe form of TBE.

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1. Introduction

Tick-borne encephalitis virus (TBEV) (family *Flaviviridae*, genus *Flavivirus*) is a causative agent of tick-borne encephalitis (TBE), a severe human neuroinfection manifesting as meningitis, meningoencephalitis, meningoencephalomyelitis or meningoencephalomyeloradiculitis. The infection is fatal in approximately 1% of cases, but in up to 46% of cases it may result in long-lasting or permanent neurological damage, known as post-encephalitic syndrome. This include neuropsychiatric problems, balance disorders, headache, dysphasia, hearing defects, spinal paralysis, tremor, etc. (Haglund and Günther, 2003). The most severe cases are reported in elder (Jelenik et al., 2010) or immunocompromised patients (Chmelík et al., 2016). However, severe courses of TBE with accompanying post-encephalitis syndrome do also occur in children (von Stülpnagel et al., 2016) Although an effective vaccine against TBEV is available, a vaccination coverage remains still low in several endemic countries (Süss et al., 2010). During the last 30 years, there has been a continuous increase in the numbers of human cases of TBE in Europe. Currently, between 10,000 and 15,000 TBE cases per year are reported in Europe and Asia (Suss, 2008; Bogovic and Strle, 2015). There are no approved therapeutic agents to treat TBE. The use of specific anti-TBEV immunoglobulins for TBE therapy was discontinued in Europe due to the concerns of antibody-dependent enhancement (ADE) of the infectivity after post-exposure prophylaxis in children (Kluger et al., 1995; Waldvogel et al., 1996). However, ADE was not confirmed in TBEV-infected mice after passive pre- or post-exposure prophylactic administration of the specific anti-TBEV antibodies (Kreil and Eibl, 1997). Moreover, spe-

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cific anti-TBEV immunoglobulins are still in use in Russia, where a single post-exposure administration an anti-TBEV immunoglobulin in a dose of 0.05 ml/kg body weight ensures protection on average in 79% of TBE cases (Pen'evskaia and Rudakov, 2010). Based on an increasing number of case reports on the successful treatment of other arboviral infections, including Japanese encephalitis, Eastern equine encephalitis, West Nile fever, or chikungunya, with high dose intravenous immunoglobulins (IVIG) (Ben-Nathan et al., 2003, 2009; Srivastava et al., 2015; Planitzer et al., 2007; Caramello et al., 2006; Rajapakse, 2009; Golomb et al., 2001; Chusri et al., 2011; Hamdan et al., 2002; Shimoni et al., 2001), we suggested using IVIG also for the treatment of severe cases of TBE (Růžek et al., 2013). IVIG is a commercial preparation of purified human IgG manufactured from pooled plasma from thousands of healthy donors and is approved for clinical use (Rhoades et al., 2000). IVIG has a broad repertoire of antibodies neutralizing various pathogens and neutralization is commonly assumed to be the main mechanism of action. Moreover, IVIG has important immunomodulatory effects, which include activation/blockade of Fc receptors, attenuation of complement-mediated damage, induction of anti-inflammatory cytokines, anti-inflammatory effect by cytokine-specific, or CD4 and MHC class I-specific autoantibodies, autoantibodies against the Fas receptor, etc. (Boros et al., 2005).

In this study, the potency of IVIG for TBEV neutralization was tested *in vitro* in two human neural cell lines, and *in vivo* using a lethal mouse model mimicking severe cases of TBE in humans. Our results demonstrate that individual IVIG lots even from the same manufacturer can differ significantly in the titers of TBEV-neutralizing antibodies. The passive administration of IVIG containing sub-neutralizing levels of TBEV-specific antibodies has no effect on the survival of TBEV-infected mice, but IVIG containing anti-TBEV antibodies can effectively prevent or ameliorate the development of the disease.

2. Material and methods

2.1. Human serum and IVIG preparations

Pooled human serum was obtained from Sigma-Aldrich (St. Louis, MO, USA). Two lots of OCTAGAM® 10% [100 mg/ml] (Octapharma, Manchester, UK) were used; i.e., lot No. A125C8534 (further referred as IVIG1), and lot No. C343A8541 (further referred as IVIG2). The IVIG1 and IVIG2 preparations represented a pooled material from more than 3500 donors, and contained human normal IgG > 95%, with a broad spectrum of antibodies against various infectious agents. IgA content was ≤ 0.2 mg/ml (http://www.octapharma.com.au/fileadmin/user_upload/octapharma.au/product_docs/octagam_10__PI.pdf).

2.2. Cell cultures

Human neuroblastoma cells and human glioblastoma cells (kindly provided by Professor T. Eckschlager, 2nd Faculty of Medicine, Charles University in Prague) were grown at 37 °C/5% CO_2 in Iscoveís modified Dulbeccoís medium (IMDM) supplemented with 10% fetal calf serum (FCS) and 1% mixture of antibiotics/antimycotics (penicillin, streptomycin; Biosera). Porcine kidney stable (PS) cells (Kozuch and Mayer, 1975) were grown at 37 °C in Leibovitz's L-15 medium supplemented with 3% newborn calf serum and 1% mixture of antibiotics/antimycotics (penicillin, streptomycin; Biosera).

2.3. ELISA and plaque reduction neutralization test

Specific anti-TBEV antibodies in the pooled human serum, IVIG1 and IVIG2 were determined by sandwich-type ELISA (IMMUNOZYM FSME (TBE) IgG; PROGEN Biotechnik, Germany). The assay was performed according to the instruction of the manufacturer. The IgG antibody titer was expressed in Vienna Units per ml (VIEU/ml).

Presence of specific neutralizing anti-TBEV antibodies in IVIG preparations and pooled human serum was determined by plaque reduction neutralization test (PRNT) as described previously (Bárdos et al., 1983), with slight modifications. Ten mg/ml of protein content of the IVIG1, IVIG2, and pooled human serum were used for PRNT. Inactivation of complement was done by incubation at 56 °C for 30 min. Two fold serial dilutions of the samples were incubated with 1×10^3 pfu of TBEV strain Hypr for 90 min at 37 °C. After that, 5×10^4 of PS cells was added to the wells of 96-well plate and the suspension was overlaid with carboxymethylcellulose. After incubation for 5 days, the cells were fixed and stained as described previously (De Madrid and Porterfield, 1969). The last dilution of the sample that caused 80–100% reduction of cytolysis was regarded as the endpoint titer.

2.4. Virus stocks, infection of cell cultures, and virus titrations

Low-passage TBEV strains Neudoerfl (kindly provided by Professor F. X. Heinz, Medical University in Vienna; passaged four times in brains of suckling mice) and Hypr (passaged six times in brains of suckling mice) were used in the study. TBEV strain Neudoerfl was isolated in 1971 from an *Ixodes ricinus* tick in Burgenland, Austria; Hypr strain was isolated in 1953 from the blood of a deceased child in Moravia, Czech Republic.

Human neuroblastoma or human glioblastoma cells were seeded in 96-well plates at concentration of 1×10^4 cells/well and cultured overnight. The other day, the cells were infected with TBEV strain Neudoerfl at a multiplicity of infection (m.o.i.) = 5. The cells were treated with either IVIG1 or IVIG2 under three regimens. One group was pretreated with 10 mg/ml of either IVIG1 or IVIG2 for 4 h before the infection. The second group was treated with the same concentrations of the preparations after the infection throughout the whole experiment. The third group represented a combination of both; i.e., combined pretreatment as well as treatment post-infection. A control group represented TBEV-infected cells without any treatment. Cell supernatants were collected daily from day 1 to day 4 (neuroblastoma), and 1 to day 4 and on day 6 (glioblastoma) post-inoculation, and subjected to a plaque assay.

The titer of the virus was assayed on PS cell monolayers based on a modified protocol by De Madrid and Porterfield (1969). Briefly, tenfold dilutions of the virus suspension were placed in 96-well plates and 1×10^4 of PS cells per well was added. After 4 h incubation at 37 °C and 0.5% CO_2, a carboxymethylcellulose (1.5% in L-15 medium) overlay was added to each well. Following 5 day incubation at 37 °C and 0.5% CO_2, the cells were stained with naphtalene black. Infectivity was expressed as pfu/ml.

Viral RNA was isolated from cell culture supernatants by QIAamp Viral RNA Mini Kit (Qiagen), and viral genome copies were quantified using RT-qPCR for TBEV (Path-TBEV-EASY; Primerdesign) on a Rotor Gene-3000 (Corbett Research).

Cell cytotoxicity of both IVIG preparations was tested by Annexin V Apoptosis Detection Kit FITC (eBioscience, CA, USA). Briefly, human neuroblastoma or glioblastoma cells were seeded in 24-well plates in concentration of 3×10^5 of cells/ml. The cells were subsequently incubated in the presence of IVIG preparations or the pooled human serum at concentrations 0 mg/ml, 0.1 mg/ml, 1 mg/ml, and 10 mg/ml for 24 h. Cell viability was tested 72 h after the IVIG/pooled human serum addition according to the instructions in the kit manual. The analysis was performed using BD FACS Canto II flow cytometer with BD FACS Diva Software.

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