DOI 10.1007/s10517-019-04555-4

Effect of Glycosaminoglycans on Pathogenic Properties Far-Eastern Tick-Borne Encephalitis Virus

G. N. Leonova and S. I. Belikov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 167, No. 4, pp. 467-470, April, 2019
Original article submitted December 20, 2018

We studied the effect of sulfated glycosaminoglycan on the infection properties of high-virulence Dal'negorsk strain and low-virulence Primorye-437 of tick-borne encephalitis virus. Differences in reproductive activity of these strains and their tropism to the target cells were revealed. Glycosaminoglycan reduced pathogenetic activity of high-virulence strain *in vitro*, but had no effect on low-virulence strain. The interaction of imperfect virus particles of non-pathogen strain with the glycosaminoglycan led to their accumulation in cell, but in the culture medium of SPEV cells infected with experimental and control samples, accumulation of virus particles did not differ. The results on activity of glycosaminoglycan binding with strains differing by their biological and molecular genetic characteristics can be used to assess their pathogenic potential.

Key Words: *tick-borne encephalitis virus strains; pathogenic properties; GAG inhibition*

The interaction of viral particles with cell surface receptors is the initial phase of virion penetration into the cell, determines tropism of the virus, its virulence and pathogenicity. The primary stage of flavivirus penetration into the target cells is determined by the interaction of the surface viral glycoprotein E with cell surface receptors. Numerous studies have demonstrated that flaviviruses contact with the host cell by binding with glycosaminoglycans (GAGs), *e.g.* with heparan-sulphate proteoglycans or syndecan on the surface of the target cell [2,4,6,7,11]. Syndecans primarily act as a factor of attachment and concentration of flavivirus particles on the surface of the target cell prior to their interaction with primary receptors and receptor-mediated endocytosis. Pretreatment of tick-borne encephalitis virus (TBEV) with GAGs can inhibit attachment of flavivirus particles to the cell surface thus attenuating their pathogenic properties.

Here we demonstrate manifestation of the pathogenic properties of TBEV strains during their interaction with glycosaminoglycans inhibiting their binding to the cell surface.

G. P. Somov Research Institute of Epidemiology and Microbiology, Vladivostok, Russia. ***Address for correspondence:*** galinaleon41@gmail.com. G. I. Leonova

MATERIALS AND METHODS

Two strains of the Far-Eastern subtype of TBEV, Dal'negorsk (Dal') strain isolated from the brain of a deceased patient with a focal TBE form (GenBank number FJ402886) and Primorye-437 strain (P-437) isolated from the blood of a patient with inapparent infection (GenBank number JQ825162). The initial virus titer in SPEV cell culture for Dal′ strain was 8 lg TCD_{50}/ml and for P-437 5 lg TCD_{50}/ml [8].

Sulfated GAG, chondroitin sulfates sodium salt (Sigma-Aldrich), was used in a dose of 100 μg; activity of this GAG against TBEV was previously studied [3]. The rate of inhibition of virus binding upon addition of GAG was determined as follows: [1-(number of viral plaques in the experiment/number of viral plaques in the absence of GAG)]×100%.

The dynamics of accumulation of TBEV strains Dal' and P-437 in SPEV cell culture was analyzed. To this end, 9 tubes with 24-h cell monolayer were infected with TBE (control) and 9 test tubes were in-

fected with a mixture of TBE+GAG (experiment). The accumulation of the virus was assessed in cells and culture medium. Experimental samples were formed from 3 tubes and examined in 0, 6, 24, and 48 h after infection of SPEV cells using indirect immunofluorescence assay (IFA) and plaque-forming test. All experiments were performed in triplicates.

The antigen in SPEV culture infected with control and experimental samples was identified by IFA. The cells from 3 tubes were collected in 6, 24, and 48 h and slides were prepared. TBEV antigen in cells was detected by applying immune serum against TBE and fluorescent FITC-labeled immunoglobulins in working dilution specified by the manufacturer (MEDGAMAL Branch, N. F. Gamaleya Research Institute of Epidemiology and Microbiology). The slides were examined under an MC-200 TF fluorescence microscope. The relative number of cells containing fluorescent TBEV antigen was calculated relative to the total number of cells in the field of view. The control and experimental samples were compared using non-parametric Mann—Whitney *U* test. The differences were significant at $p \leq 0.05$.

RESULTS

We revealed differences in the inhibitory action of GAG on the studied TBEV strains (Fig. 1). For Dal' strain, the rate of GAG-inhibition was high: the relative number of non-inhibited virus particles was 18%. P-437 strain was significantly less inhibited by GAG (the number of non-inhibited viable viral particles was 83%, $p \leq 0.01$).

The number of large plaques of Dal strain in the experimental samples decreased by 5 times in comparison with the control. The number of small plaques of P-437 strain in the experimental and control samples was almost the same.

A comparative study of the intracellular virus accumulation was carried out using IFA to clarify the features of the infectious process (Fig. 2). In case of Dal' strain (control), native viral particles quickly entered the cells and in 6 h, infected cells constituted 63%; while viral particles treated with GAG penetrated the cells more slowly and in 48 h, just few cells contained viral particles. The presence of cells containing specific TBEV antigen suggests that GAG did not completely inhibit virions.

Low rates of cell infection (13%) for strain P-437 at the same observation term attested to its slow penetration into cells ($p \leq 0.05$). Different GAG-dependent replicative activity of TBEV strains was revealed. Strain P-437 was characterized by more intensive intracellular replication in the experiment in comparison with the control (Fig. 2). Intracellular accumulation of Dal' strain antigen in the experiment was significantly lower ($p \leq 0.01$) than in the control, and only in 48 h, the percentage of cells containing specific antigen slightly increased, which attested to high, though not complete GAG-inhibition of virions.

In addition, we studied the dynamics of TBEV accumulation in the culture medium of SPEV cells infected with Dal' and P-437 strains (control and experimental samples). Strain Dal' was used in a dose of 2.5 lg PFU/ml (Fig. 3). Infectious activity of Dal' strain (control) increasing from 0 (6 h) to 1.5 lg PFU/ml (24 h) and 3.5 lg PFU/ml (48 h). After treatment with GAG, infectious activity of Dal' strain decreased (Fig. 3, *a*). Virus replication rates in the control and

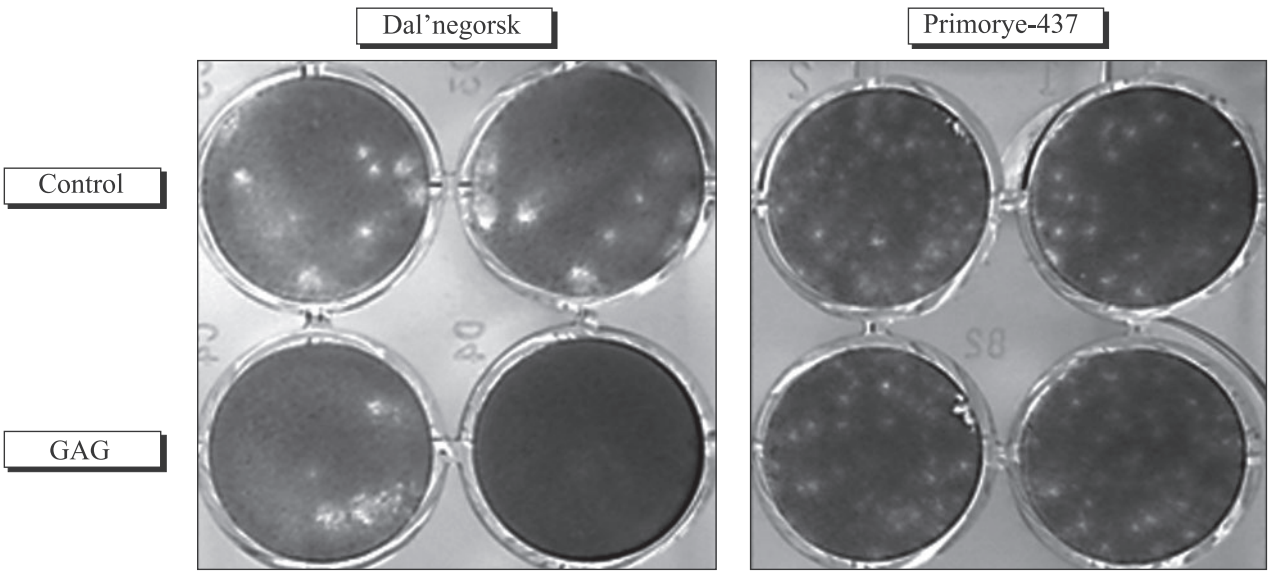

Fig. 1. GAG-inhibition of TBEV strains isolated from patients with focal form (Dal'negorsk) and inapparent form (Primorye-437). The morphology of plaques is shown on SPEV cells.

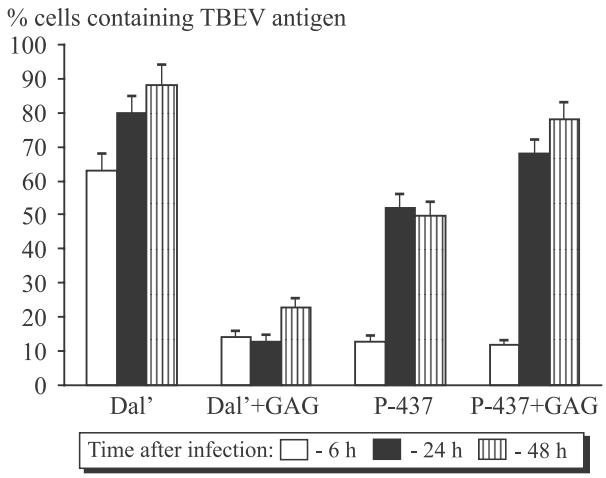

Fig. 2. Detection of antigens of Dal'negorsk (Dal') and Primorye-437 (P-437) strains in SPEV cells by IFA in dynamics.

experiment in 48 h differed significantly ($p \geq 0.05$). In the test sample, the number of virus particles at that time reached 2 lg PFU/ml indicating virus reproduction. Treatment with GAG did not reduce the titer of strain P-437 virus at infection dose of 2 lg PFU/ml. In the culture medium of SPEV cells infected with control and experimental samples, virus accumulation was similar (Fig. 3, *b*).

To understand the differences in the infectious activity of TBE strains characterized by different pathogenicity, their full-genome molecular genetic characteristics were compared. Strain P-437 has 24 amino acid substitutions relative to strain Dal': Q32R, K64N, K69R, D100N, and L111Del in capsid protein; I130V, V137A, and A151V in prM; A431T and V463A in protein E; S141G in NS1; K52R and T168I in NS2A; F108V in NS2B; R16K, S45S, and S261A in NS3; M95V, V179A, and A213V in NS4B; S634T, R677K, I692V, and A724S in NS5 [1]. Flavivirus particles adhere to the surface of target cells via interaction of surface viral protein E with cell receptors. Protein prM, a precursor of membrane protein M, is cleaved by cell protease furin during maturation of virus particles, which leads to reorganization of the virus surface. As a result, mature viral particles carry only surface protein E that interacts with the surface of the infected cells; protein M is located beneath protein E and does not participate in binding to cell receptors. Incomplete cleavage of prM protein leads to the appearance of defective "rough" virus particles poorly binding the target cells. It can be assumed that amino acid substitutions in the membrane regions of proteins prM and E in P-437 strain contribute to the formation of immature, defective, imperfect virus particles with a reduced ability to bind to target cells.

GAG treatment of viral particles of the Dal' strain prior to their interaction with cells prevents their internalization and leads to virus inhibition, probably due to competition of GAG with cell receptors [3,5,9,10]. Strain P-437 that presumably has rough surface, poorly binds GAG; therefore, its activity little changes upon addition of GAG preparation.

Thus, the obtained results indicate that strains of TBE virus with different pathogenicity for humans can differ by the surface structure of mature virus particles. Identified substitutions in the low virulent strain can lead to the formation of imperfect "rough" viral particles that poorly bind with cell receptors. Preliminary added GAG preparation can modulate the formation of the infectious process in different ways: it reduced infectious activity of virulent strain Dal' and had practically no effect on strain P-437 isolated from the blood of a patient with subclinical TBE. The described approach to the study of binding of GAG

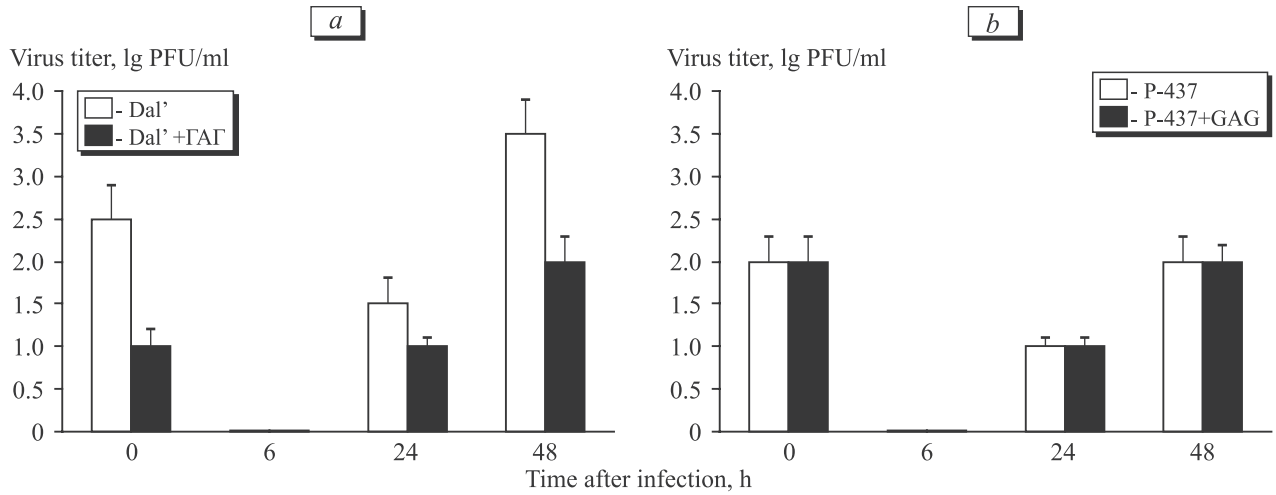

Fig. 3. Dynamics of tick-borne encephalitis virus accumulation in culture medium of SPEV cells. *a*) Dal'negorsk strain (Dal'); *b*) Primorye-437 strain (P-437).

preparations with strains with different biological and molecular genetic characteristics can be used to assess their pathogenic potential.

The study was supported by the research project 0545-2014-0011 of the Federal State Budgetary Scientific Institution.

REFERENCES

1. Belikov SI, Kondratov IG, Potapova UV, Leonova GN. The relationship between the structure of the tick-borne encephalitis virus strains and their pathogenic properties. PLoS One. 2014;9(4). ID e94946. doi: 10.1371/journal.pone.0094946.
2. Germi R, Crance JM, Garin D, Guimet J, Lortat-Jacob H, Ruigrok RW, Zarski JP, Drouet E. Heparan sulfate-mediated binding of infectious dengue virus type 2 and yellow fever virus. Virol. 2002;292(1):162-168.
3. Goto A, Hayasaka D, Yoshii K, Mizutani T, Kariwa H, Takashima I. A BHK-21 cell-culture-adapted tick-borne encephalitis virus mutant is attenuated for neuroinvasiveness. Vaccine. 2003;21(25-26):4043-4051.
4. Hilgard P, Stockert R. Heparan sulfate proteoglycans initiate dengue virus infection of hepatocytes. Hepatology. 2000;32(5):1069-1077.
5. Kozlovskaya LI, Osolodkin DI, Shevtsova AS, Romanova LIu, Rogova YV, Dzhivanian TI, Lyapustin VN, Pivanova GP, Gmyl AP, Palyulin VA, Karganova GG. GAG-binding variants of tick-borne encephalitis virus. Virology. 2010;398(2):262-272.
6. Kroschewski H, Allison SL, Heinz FX, Mandl CW. Role of heparan sulfate for attachment of tick-borne encephalitis virus. Virology. 2003;308(1):92-100.
7. Lee E, Wright PJ, Davidson A, Lobigs M. Virulence attenuation of Dengue virus due to augmented glycosaminoglycan-binding affinity and restriction in extraneural dissemination. J. Gen. Virol. 2006;87(Pt 10):2791-2801.
8. Leonova GN, Maystrovskaya OS, Kondratov IG, Takashima I, Belikov SI. The nature of replication of tick-borne encephalitis virus strains isolated from residents of the Russian Far East with inapparent and clinical forms of infection. Virus Res. 2014;189:34-42.
9. Luat le X, Tun MM, Buerano CC, Aoki K, Morita K, Hayasaka D. Pathologic potential of variant clones of the oshima strain of far-eastern subtype tick-borne encephalitis virus. Trop. Med. Health. 2014;42(1):15-23.
10. Mandl CW, Kroschewski H, Allison SL, Kofler R, Holzmann H, Meixner T, Heinz FX. Adaptation of tick-borne encephalitis virus to BHK-21 cells results in the formation of multiple heparan sulfate binding sites in the envelope protein and attenuation in vivo. J. Virol. 2001;75(12):5627-5637.
11. Okamoto K, Kinoshita H, Parquet Mdel C, Raekiansyah M, Kimura D, Yui K, Islam M.A, Hasebe F, Morita K. Dengue virus strain DEN2 16681 utilizes a specific glycochain of syndecan-2 proteoglycan as a receptor. J. Gen. Virol. 2012;93(Pt 4):761-770.