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RESEARCH OF BIOLOGICAL PROPERTIES OF ENTEROVIRUS STRAINS ASSOCIATED WITH ISCHEMIC STROKE

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ABSTRACT

Introduction: The research of biological properties of enteroviruses associated with ischemic stroke (IS) allows us to identify their intratypic differences.

The aim: to identify genetic markers of strains of enteroviruses associated with IS.

Materials and methods: 11 strains of enteroviruses isolated from the serum of patients with IS were identified in the virus neutralization test. Genetic markers of isolated strains (A_{bent}, marker S, marker rct_{40}) were determined.

Results: Eleven strains of enteroviruses were isolated from the serum of patients with IS. Eight viruses: Coxsackie B viruses (serotypes 2, 3, 4) and ECHO viruses (serotypes 6, 9, 27 (two strains), 29) were identified in these strains. Other three strains of enteroviruses were unidentified.

Different combinations of genetic markers were found. Seven strains of enteroviruses (Coxsackie B2, B3, ECHO 6, ECHO 9, ECHO 27 (two strains) and one unidentified virus) had virulence markers: A_{bent}–, rct_{40}+ and S–. Three strains (Coxsackie B4, ECHO 29, one unidentified virus) had markers: A_{bent}–, rct_{40}+, S+. Another one unidentified virus had markers: A_{bent}+, rct_{40}+, S –.

Conclusions: All 11 isolates of enteroviruses associated with IS had rct_{40}+ marker, 10 of the 11 isolates had marker A_{bent}– and 8 of 11 isolates had marker S–. The research of genetic markers allows to perform typic and intratypic differentiation of strains of enteroviruses associated with the IS.

KEYWORDS: ischemic stroke, enterovirus, biologic properties of enteroviruses

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INTRODUCTION

Scientific interest in enteroviruses is dictated by an annual increase in the number of isolated types of enteroviruses, their poliorganic tropism as well as an expansion of the spectrum of diseases caused by them. Scientists have proved the pathogenetic role of enteroviruses in pathologies such as myocardial infarction, myocarditis, pericarditis, dilated cardiomyopathy, atherosclerosis, glomerulonephritis, appendicitis, hepatitis, pancreatitis, juvenile diarrhea [1, 2, 3].

As of today the role of enterovirus infections in the development of vascular pathology of the brain is urgent since these diseases compose from 30% to 50% of diseases of the cardiovascular system, among which the ischemic stroke (IS) plays a leading role [4]. Important risk factors for the development of the IS are the infectious and inflammatory processes that initiate the development of atherosclerosis [5]. The role of representatives of herpes simplex viruses of 1 and 2 types, varicella virus, cytomegalovirus, Epstein-Barr virus, adenoviruses, influenza viruseses, enteroviruses, Chlamidia pneumonie and their associations in the development of the IS has been proven. At the same time, the role of enteroviruses as a trigger factor in the development of the IS, their epidemiologically relevant serotypes and biological properties remains urgent and needs a further in-depth research [6, 7, 8, 9].

According to researchers findings, there are differences between pathogenic and apatogenic strains of enteroviruses [10]. Genetic markers of enterovirus virulence allow to identify their intratypic differences. After consideration of various genetic markers for determination of the enterivirus pathogenicity, we have chosen the following frequently used markers: the bentonite marker reflecting the affinity degree to bentonite (A_{bent}+ or A_{bent}–), the magnitude of enterovirus plaques under the bentonite nutrient coating (marker S) and a decrease in the ability of attenuated strains to rct_{40} (at 40 °C). Integrated use of genetic markers to determine the phenotypic properties and virulence of isolated strains of viruses is more rational method than the use of any marker alone [11, 12]. Such a phenotypic characteristic is required for the grouping of viruses associated with the IS.

THE AIM

The purpose of our research was to identify the genetic markers of strains of enteroviruses isolated from the sera of patients with the IS.

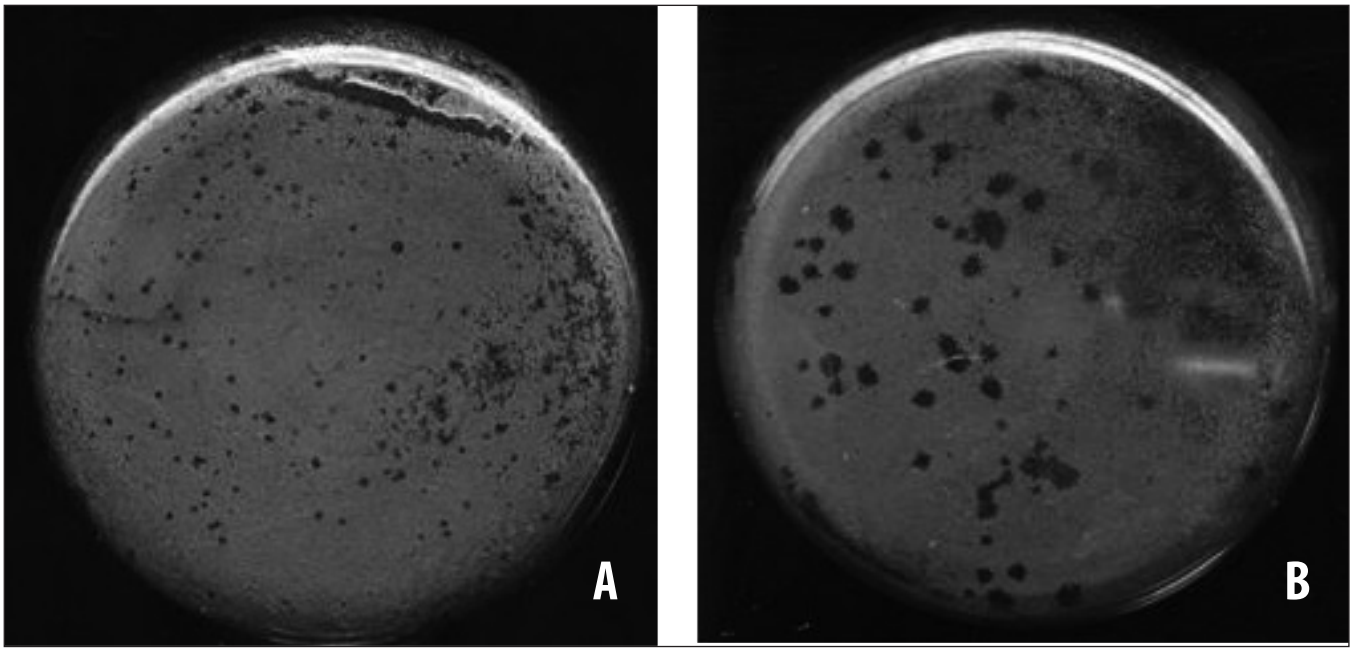

Figure 1. The reaction of plaque formation under bentonite nutrient coating in HEp-2 cell culture:
a) small plaques caused by the Coxsackie B3 virus;
b) large plaques caused by the Coxsackie B4 virus.

MATERIAL AND METHODS

The materials for the reserch were 11 isolates of enteroviruses isolated from the sera of patients with various forms of the IS, who were hospitalized in the neurological department and the department of cerebrovascular pathology of the Alexander Clinical Hospital in Kyiv in 2009-2016. Virus isolation was performed using the WHO recommended cell cultures: RD, HeLa and HEp-2. The identification of isolated viruses was performed using diagnostic sera for poliomyelitis of I-III types, Coxsackie B viruses of 1-6 types and ECHO of 1-34 types in the virus neutralization reaction. The genetic markers of isolated strains of enteroviruses were determined, namely: the bentonite marker A_{bent} which determines the affinity degree to bentonite; marker S determined by the size of viral plaques under the bentonite coating; marker rct_{40} which plays a role of an indicator of the viruses ability to be reproduced at elevated temperature (40 °C) according to the common method [10, 13].

RESULTS

11 cytopathic viral agents were isolated from the sera of patients with the IS in cell cultures by the classic virologocal method. The enterovirus genome was detected in polymerase chain reaction (PCR) [9].

The isolated virus strains were identified in the virus neutralization reaction as Coxsackie B viruses (serotypes 2, 3, 4) and ECHO viruses (serotypes 6, 9, 27 (two strains), 29). Other three strains were unidentified but detected as enteroviruses by PCR.

The selected virus strains had the following genetic markers: 10 out of 11 viruses had the A_{bent}– bentonite marker (90.9% of the strains) and only one of the unidentified viruses had the A_{bent}+ marker (9.1%). The greater virulence of the A_{bent}– of enteroviruses than A_{bent}+ variant was reconfirmed by the data obtained. A positive marker rct_{40}+ was detected in all strains of viruses (100%) isolated from the sera of patients with the IS. Virus-derived plaques were divided into two groups depending upon size: small plaques up to 1.5 mm in diameter (marker S–, 72.7%) and large plaques from to 1.5 mm in diameter (marker S+, 27.3%) (Figure.1)

When studying the genetic marker S under the bentonite coating, we isolated strains of ECHO 29, Coxsackie B4 and one unidentified virus strain. The mentioned three viruses induced the appearance of large plaques with festoon edges (marker S+). Majority of virus strains, namely: Coxsackie B2, Coxsackie B3, ECHO 6, ECHO 9, ECHO 27 (both strains) viruses, unidentified viruses (two strains) induced the appearance of small plaques with more even edges. That is to say, these viruses had the genetic marker S–. Hence, the strains of enteroviruses we have isolated induce the appearance of small plaques under the bentonite coating. According to V.P. Shyrobokov and V.N. Girin such appearance of small plaques is associated with the virulence of these strains and gives grounds to suggest about their role in the development of the IS [14].

It was proved by other researchers that marker rct_{40} has the highest correlation with enterovirus virulence while the bentonite marker A_{bent} and the marker S have a slightly less correlation [15]. According to our data, 7 strains of enteroviruses (63.6%) isolated from the sera of patients with the IS, namely: Coxsackie B2, B3, ECHO 6, ECHO 9, ECHO 27 (both isolates) and one unidentified virus, had all three positive virulence markers: A_{bent}–, rct_{40}+ , S– (induced

Table I. Distribution of genetic markers of virulence of enteroviruses isolated from the sera of patients with the IS

Viruses	Number of strains	Number of strains with genetic markers of virulence		
		A_{bent}–	rct_{40}+	S–
Coxsackie B	3	3	3	2
ECHO	5	5	5	4
Unidentified virus strains	3	2	3	2
Total	11	10	11	8

the appearance of small plaques in cell culture under a bentonite coating). Other strains of isolated enteroviruses were characterized by different combinations of virulence markers. In particular, Coxsackie B4, ECHO 29 and one unidentified strain had A_{bent}–, rct_{40}+, S+ markers; another one unidentified strain had A_{bent}+, rct_{40}+, S– markers (Table I).

Based on the results obtained, we can conclude about the phenotypic characteristics of the strains of selected enteroviruses by the genetic markers A_{bent}–, rct_{40}+, and S–. Therefore, the following characteristics are distinctive for strains of enteroviruses associated with the IS: the ability to reproduce at 40 °C (positive marker rct_{40}+), low affinity to bentonite (marker A_{bent}–) and the formation of small plaques under the bentonite coating (marker S–).

DISCUSSION

Bentonite marker (A_{bent}), first discovered and described by V.P. Shyrobokov, characterizes the degree of affinity of viral particles to bentonite. Particularly, it has been shown that A_{bent}– variant is the most virulent in the population of I type polioviruses and Coxsackie viruses while A_{bent}+ is more virulent in the polioviruses of II and III types [14]. Further studies revealed a dependence of bentonite affinity upon the type of viruses: polioviruses of all three types (vaccine strains), Coxsackie A7, A8, A18, Coxsackie B3, B4, B6 viruses have high affinity to bentonite. Coxsackie A10, Coxsackie B1 and B2 viruses have low affinity to bentonite [14]. A_{bent}+ and A_{bent}– dissociants differ in virulence, antigenicity and immunogenicity, organotropism and environmental stability [11, 16]. Highly virulent and immunogenic strains of enteroviruses have a low degree of affinity to bentonite at low alkaline pH and thus they have A_{bent}–. Our data is completely consistent with this finding. The bentonite test for intratypic differentiation of I type polioviruses also was recommended by L.V. Kopanitsa (2003). 84.9% of field isolates of polioviruses of I type which are of vaccine origin have a genetic marker A_{bent}+; and strains of polioviruses of I type with wild characteristics are represented by the variant A_{bent}– (100%) [16].

Therefore, the researchers consider the bentonite marker as an integral indicator that testifies a number of biological properties of isolated enterovirus strains and is used in the study of both clinical isolates and isolated enteroviruses from environmental objects (e.g. waste water) [12, 13].

The marker S was first described as a property of attenuated strains of polioviruses to form small plaques under agar coating. Method for detecting enterovirus plaques under a bentonite nutrient coating offered by V.P. Shyrobokov has appeared to be the most sensitive, faster, easier and more accessible in use than one under agar coating [14].

Thus, when studying the genetic marker S under the bentonite coating, we detected S+ marker in the strains of ECHO 29, Coxsackie B4 and one unidentified virus, whereas we detected the genetic marker S– in the other strains, namely: Coxsackie B2, B3, ECHO 6, ECHO 9 strains, ECHO 27 (both strains), unidentified viruses (two strains).

According to V.P. Shyrobokov, enteroviruses under bentonite coating can induce plaques that are heterogeneous in size. The differences in size of plaques induced by wild strains of polioviruses of II and III types and of plaques of their attenuated variants was proven [14]. Therefore, it can be stated that the marker S can also be used for intratypic differentiation of isolated enteroviruses.

Our data on the low ability of sorbtion to bentonite (A_{bent}–) in 10 of the 11 viruses (90.9%) isolated from the sera of patients with the IS are consistent with those of L.M. Grytsenko (2011), according to which a majority of strains of ECHO viruses isolated from patients (63.2%) had a low ability to absorb on bentonite (A_{bent}–) [17]. O.I. Yevtushenko proved that Coxsackie B viruses are mainly associated with the A_{bent}– genetic marker, which is able to overcome the placental barrier in pregnant mice and cause fetal and neonatal mouse deaths (91.2%), while variant A_{bent}+ causes the death of mice in 30.3% [18]. According to V.A. Ponyatovsky (2015), vaccine strains of polioviruses isolated from waste water had all negative virulence markers, the positive marker of S+ was most frequently detected in Coxsackie B; marker rct40+ was most frequently detected in ECHO; marker A_{bent}– was most frequently detected in the untyped viruses [12].

At the same time, according to our data, all 11 viruses isolated from the sera of patients with the IS had the marker rct_{40}+. This is consistent with the data of V.P. Shyrobokov who proved that more virulent Coxsackie B1 and poliovirus of II type MEF1 are more actively reproduced at higher temperatures in comparison with the low virulent Coxsackie B6 viruses and vaccine strain of poliovirus of II Sebin type [14]. L.M. Grytsenko (2011) found that ECHO virus strains isolated from healthy individuals (72.2%) were characterized by the rct_{40}– marker, and only 16.7% of the viral strains were characterized by the

rct_{40}+ marker. The majority of viral strains isolated from the patients had predominantly rct_{40}+ marker (73.7%), 10.5% of the strains had the rct_{40}– variant, and the remaining (15.8%) strains had the intermediate variant [17]. According to V.M. Girin, a high correlation between bentonite marker A_{bent} and rct_{40} marker was detected in 100% of strains isolated from waste water polioviruses as well as in 60.5% of ECHO viruses and 55.0% in Coxsackie viruses [16].

According to our data, the rct_{40}+ marker (all 11 isolates) was most frequently detected in the enterovirus isolates tested; the A_{bent}– bentonite marker (10 out of 11 isolates) and the S– marker (8 out of 11 isolates) were frequently detected.

Therefore, based on analysis of literature and the study of the genetic markers of enteroviruses isolated in sera of patients with the IS, we can confirm not only the virulence of isolated viruses, but also the presence of common phenotypic characteristics of viruses involved in IS. This allows us to recommend the determination of genetic markers for the intratypic differentiation of enteroviruses isolated from the sera of patients with the IS.

CONCLUSIONS

It was found that 11 isolates of enteroviruses were identified as Coxsackie B viruses (serotypes 2, 3, 4), ECHO viruses (serotypes 6, 9, 27 (two strains), 29) and three unidentified strains. They have certain genetic characteristics: the marker rct_{40} + was detected in all 11 isolates; 10 out of 11 isolates had the A_{bent}– bentonite marker; 8 of 11 isolates had the marker S– had. These common features can be considered as secondary characteristics for study of the enteroviruses role in development of the IS. The use of the virological diagnostic method with study of genetic markers allows to perform typic and intratypic differentiation of isolated strains of enteroviruses associated with the IS.

PROSPECTS FOR FURTHER RESEARCH

Further studies will focus on a comprehensive study of the strains of enteroviruses we have identified, with the identification of those that play an etiopathogenetic role in the emergence and development of the IS. In addition, we consider it expedient to point out the prospects for the prevention of the IS with a vaccine on the basis of the enteroviruses that would be most relevant from the epidemiological and etiopathogenetic point of view.

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Conflict of interest:

The Authors declare no conflict of interest.

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