Resistant and dependent mutants of the Coxsackie B1 virus to the picornaviral inhibitor disoxaril

Abstract-summary

INTRODUCTION

Human enteroviruses, and in particular Coxsackie viruses, cause a wide range of diseases in children and adults. They mainly affect the central nervous system and the heart. One of the main, and for the time being, insurmountable problems for the creation and implementation of effective chemotherapy for the diseases caused by these viruses is the rapid emergence of resistance to the relevant chemotherapeutics. For the past 5-10 years, the list of these drugs has been continued with the first chemotherapeutics against hepatitis B virus and papillomaviruses. Virus-encoded drug resistance has been established for almost all compounds with antiviral activity, and the genetic basis of resistance is already known. In RNA-containing viruses, and in particular in picornaviruses such as Coxsackie B1 virus, random genetic mutations occur at a high frequency because genetic control is weak and RNA polymerase makes errors about a million times more often than DNA - polymerase. Therefore, in natural conditions the picornaviral population is quite heterogeneous. These large numbers of mutants are the result of point mutations occurring during viral replication.

In recent years, substances that interact with viral capsid proteins have been the subject of intense research among picornaviral inhibitors. The question of the development of resistance to this type of compound remains open.

The results of our studies support the view that determining the genetic markers of mutants should be a prerequisite for their study. It is important to investigate the molecular basis of the antiviral effect - targets attacked by inhibitors, by comparing the genomic sequences of the wild-type virus with the mutants obtained. This is a significant step in the development of antiviral chemotherapeutics.

AIM AND TASKS

We set out to clarify the phenomenon of resistance to the capsid inhibitor - disoxaril of the Coxsackie B1 model virus, including the phenotypic characteristics and the molecular genetic basis of resistance.

To accomplish this, the following tasks were set:

1. Isolation of disoxaril-resistant mutants of Coxsackie B1 virus from the body of disoxaril-treated newborn mice during experimental infection with Coxsackie B1 virus.

2. Isolation of Coxsackie B1 disoxaril-resistant mutants in FL cell cultures.

3. Isolation of a disoxaril-dependent mutant of Coxsackie B1 virus in FL cell cultures.

4. Phenotype characteristic (determination of genetic markers) of disoxaril mutants of Coxsackie B1 virus: size and shape of agar plates; thermostability and pathogenicity for newborn mice.

5. Determination of the kinetics of the effect of disoxaril on the replication of the disoxaril mutants of the Coxsackie B1 virus.

6. Sequential analysis of the gene encoding VP1 for the disoxaril mutants of the Coxsackie B1 virus.

CONCLUSIONS

The following conclusions can be drawn from the studies conducted:

1. The administration of a treatment course with the picornaviral inhibitor disoxaril in experimental Coxsackie B1 virus infection in mice results in the emergence of a drug-resistant population in the mouse brain.

2. A Coxsackie B1 disoxaril-dependent mutant was obtained as a result of serial passages of the disoxaril-resistant mutant in the presence of disoxaril.

3. There are significant differences in phenotypic characteristics between the Coxsackie B1 disoxaril mutants (plaque size and shape, heat sensitivity and animal pathogenicity). Resistant mutants show some increased pathogenicity in newborn mice.

4. Using a single-step replication cycle, disoxaril was found to inhibit the early stages (undressing) of the disoxaril-sensitive mutant, and in the case of the disoxaril-dependent mutant, its absence blocked the assembly process of infectious virion erythrocytes. .

5. Sequential analysis of the RNA of the disoxaril mutants revealed a change in the locus of the VP1 in the resistant and dependent mutants compared to the baseline (insertion of AAA instead of AAC (ntt 2749-2751 from baseline wild strain / ins AAAnt)). Resistant and dependent mutants have been shown to have a high degree of identity with respect to the portion of the genome encoding the VP1 protein and a sharply lower rate compared to the original disoxaryl-sensitive laboratory strain.

CONTRIBUTIONS

As a result of the work done, the following contributions of original character (described for the first time in the literature) have been made:

1. The development of a resistant enterovirus population to chemotherapy in the brain of experimental animals infected with Coxsackie B1 virus and undergoing treatment with a WIN compound (disoxaril) has been demonstrated. This explains the moderate curative effect of the WIN compound in vivo.

2. A WIN-dependent (disoxaril) mutant of model enterovirus (Coxsackie B1 virus) was obtained.

3. The reversal of drug mutants resistant and dependent on the WIN compound (disoxaril) of the model enterovirus (Coxsackie B1 virus) was investigated.

4. Genetic markers of the WIN-resistant (disoxaril) enterovirus population (size and shape of plaques, heat sensitivity and pathogenicity in animals) have been investigated.

5. A set of genetic markers has been developed, proposed as a requirement for the study of enterovirus inhibitors.

6. Data on the mechanism of action of disoxaril on the replication of the disoxaril-dependent mutant of Coxsackie B1 virus have been obtained - inhibition of the assembly process of infectious virions.

7. A molecular genetic analysis (sequential analysis) of the genomic region encoding VP1 of the coxsackie B1 disoxaril mutants was performed.

SCIENTIFIC PAPERS IN RELATION TO THE DISSERTATION

PUBLICATIONS:

1.Nikolova, I., Galabov, A. S. (2002): The Virus Mutants Resistant to Chemotherapeutics - A Main Problem in the Treatment of Viral Infections. Biotechnol. & Biotechnol. Eq. 16/2002/2, 65-71.

2.Nikolova, I., Galabov, A.S. (2003): Development of resistance to disoxaril in Coxsackievirus B1 infected newborn mice. Antiviral Res. 60, p. 35-40.

3.Nikolova, I., Galabov, A. S. (2002): Isolation of Disoxaril-Dependent Mutant of Coxsackievirus B1. Proceedings of the Tenth Congress of the Bulgarian Microbiologists, Plovdiv, Oct 9-12, 2002 (in press).