

Study of the Antiviral Activity and Toxicity of Dextrin. In Vitro Study

Arthur K Melkonian¹ and Gagik V Hakobyan^{2*}

Abstract

Objective: This study was to in vitro study of the antiviral effect of "Dextrin" on the replication of the EMCV virus in HeLa cells and toxicity of "Dextrin".

Methods: For this purpose, the virus at a dose of 100 TCD₅₀ is applied to a 24-hour monolayer of HeLa cells. The experiment is carried out under conditions of 5% CO₂ saturation and 100% humidity. The virus dose used was expressed as a tissue cytopathic dose of 100 TCD₅₀/ml.

Cultivation of HeLa cells, to study toxicity, was performed on 96-well plates under conditions of 5% CO₂ saturation and 100% humidity. The toxic effect of "Dextrin" on HeLa cells was studied by the degree of destruction of the monolayer and at the population level.

Results: Peak levels of the virus reached after 48 hours of replication accompanied by complete destruction of the cell monolayer (7-8 cycles of replication). As follows from the graph, "Dextrin significantly" (significance is indicated by asterisks.) reduces the dose of the virus when introduced into the medium with HeLa cells to, or at the early stages of EMCV virus replication (-1,0 and 1 hour). Based on the data obtained, it was decided to use a dose of 1:100 (1%), which is the highest non-toxic dose.

Conclusion: "The investigated Dextrin has an antiviral effect against the EMCV virus, when applied in the early stages of infection. This may open up the prospect of studying the antiviral spectrum of Dextrin in more future studies".

Keywords: Dextrin; Antiviral activity; Toxicity; Antiviral; Replication.

¹Chief Executive Officer at Promtest, Armenia

²Professor, Head of Department of Oral and Maxillofacial Surgery, University of Yerevan State Medical University, Yerevan, Armenia

***Corresponding Author:** Gagik Hakobyan, Professor, Head of Department of Oral and Maxillofacial Surgery, Yerevan State Medical University, 65 Yerevan, Armenia.

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Introduction

For decades, a variety of pandemics associated with viruses have been recorded in the world health care. Effective therapy of viral diseases, quite common, is one of the urgent problems of practical medicine.

Treatment of viral diseases is not an easy task as the emergence of new viruses, drug resistance and the frequent lack of specific treatment complicates antiviral therapy. Scientists, clinicians in various research centers are working hard for the clinical development and implementation of antiviral drugs.

The current search for antiviral drugs that have fewer side effects on the patient's body is aimed at obtaining them from natural resources. The development of antiviral drugs from natural resources is very important for increasing the therapeutic efficacy of drugs and reducing their toxic effects.

Currently, research on the use of materials derived from natural starch as a potential antiviral drug is relevant.

Dextrin is a widely used in food and medicine glucose-containing saccharide polymer having the same general formula of starch, but smaller and less complex [1,2].

Dextrins, in terms of molecular concentration per linear, branched dextrin and cyclodextrin, have the same physicochemical and biological characteristics [3]. Cyclodextrins are more resistant to non-enzymatic hydrolysis. Dextrins consist of D-glucose, which includes predominantly α -(1,4)-

glycosidic bonds [4] and branched segments encompass α (1,6)-glycosidic bonds [5].

Cyclodextrins are of interest because of their ability to improve drug bioavailability. Dextrin reduces the side effect of the drug, stabilizes the therapeutic agent, the action the uptake of dextrin nanoparticles by cellular systems occurs through a process known as endocytosis [6].

Dextrin has various properties, hygroscopicity, fermentability, sweetness, stability, gelation, solubility, bioavailability [7].

Dextrin is a cheap, available resource for biomedical purposes, dextrin-protein and dextrin-drug conjugates, peritoneal dialysis solutions have proven themselves in medical applications.

Viruses reproduce using the molecular mechanism of a living cell, and cannot reproduce otherwise, therefore, the action of antiviral drugs is based on the mechanism of disruption of the virus replication process.

The main stages of viral replication include [8]

- Attachment in which proteins on the surface of the virus interact with receptors on the surface of the host cell.
- **Penetration:** In this stage, usually the viral and cell membranes fuse.
- **De-enveloping:** This is the stage in which the viral genes are released into the cell.

- **Replication:** In this stage, the cell synthesizes viral components, mRNA, proteins and DNA/RNA, depending on the type of virus.
- **Assembly:** This stage accumulates enough viral nucleic acids and proteins to form virions (viral particles).
- **Release:** Budding or lysis of the cell surface releases the virions from the host.
- Each stage is a potential target for

In this perspective, the focus will be on polymers' antiviral activity.

1958 Gerber, et al., discovered that polysaccharides isolated from *Gelidium cartilagenium* (red algae) had an inhibitory effect on influenza A and mumps A viruses [9]. After dextran sulfate was discovered as an anti-HIV material, researchers began to investigate a wide range of sulfated polysaccharides as potential antiviral materials [10].

Evidence suggests that, due to their safety, biocompatibility, and unique structure, cyclodextrins can provide a non-toxic virucidal effect, including on coronavirus and influenza virus, mediated by virus binding to cell receptors and fusion of the viral envelope with the host cell membrane. Studies have shown that cholesterol, which is present in the microdomains of the viral envelope and cell membrane, plays an important role for the successful entry of enveloped viruses into the host cell. Cyclodextrins can cause the destruction of the lipid raft and subsequent structural deformation of the viral envelope [11].

Cyclodextrins can also deplete host cell membrane cholesterol, making them less susceptible to viral infection and could potentially be used to contain infection or as virucidal agents to fight viral infection [12,13].

The study showed the antibacterial, antiviral, immunomodulatory effects of iodine-lithium-alpha-dextrin (ILalphaD) [14,15].

This study focuses on the potential use of natural starch as an alternative antiviral drug.

Objective of this study was to in vitro study of the antiviral effect of "Dextrin" on the replication of the EMCV virus in HeLa cells and toxicity of "Dextrin".

Method

Study of the antiviral effect of "Dextrin" on the replication of the EMCV virus in HeLa cells.

For this purpose, the virus at a dose of 100 TCD₅₀ is applied to a 24-hour monolayer of HeLa cells. After adsorption of the virus (after 40-50 minutes), the supernatant medium is drained and a new supporting medium with "Dextrin" in the maximum dose that did not cause toxic effects is applied to the cells. The experiment is carried out under conditions of 5% CO₂ saturation and 100% humidity. The experiment is repeated three times, the arithmetic means and spreads are presented in the report.

Encephalomyocarditis virus EMCV belongs to the genus *Cardiovoviridae*, family *Picornaviridae*. A virus with an extremely wide host spectrum. Adapted to HeLa culture. The virus dose used was expressed as a tissue

cytopathic dose of 100 TCD₅₀/ml. The virus dose was calculated according to the Kärber method (Finney J. 1952).

HeLa cell culture: Cells are cultured at 37°C in Eagle DMEM medium supplemented with 10% bovine serum. Seeding dose 2 × 10⁵ cells/ml.

All experiments on antiviral action were carried out 3 times and their summarized data are presented below. The studies were carried out in terms of 2,6,12,24 and 48 hours. In control populations, the EMCV virus begins replication immediately after introduction onto the cell monolayer. The first signs of virus replication were noted by 6 hours after infection (taking into account the time of virus replication, this should be expected).

To study the effect of the timing of Dextrin administration on virus replication, the drug was administered 1 hour before infection (-1 hour), simultaneously with infection (0 hours), 1,2,3,4,5,6 hours after infection (1 hour, 2 hours, 3 hours, 4 hours, 5 hours and 6 hours respectively).

Studying the toxicity of "Dextrin"

Cultivation of HeLa cells, to study toxicity, was performed on 96-well plates under conditions of 5% CO₂ saturation and 100% humidity. Before the start of the experiment, Dextrin was passed through 0.8 μm micropore filters (Gelman Sciences) to ensure sterility in the samples under study.

1. Add 0.2 ml of the studied dilutions of the drug to all wells of the panel, with

a monolayer of cells except for the wells in row H (control).

2. Add 0.2 ml of maintenance medium to all wells in row H (control).

The toxic effect of "Dextrin" on HeLa cells was studied by the degree of destruction of the monolayer and at the population level.

Statistical analysis: Considering the non-parametric distribution of the studied traits, the Wilcoxon-Mann-Whitney u-test was used using the SPSS 13.0 program (SPSS, Inc., Chicago, IL).

Result

Peak levels of the virus reached after 48 hours of replication accompanied by complete destruction of the cell monolayer (7-8 cycles of replication).

As follows from the (Figure 1), "Dextrin" significantly (significance is indicated by asterisks.) reduces the dose of the virus when introduced into the medium with HeLa cells to, or at the early stages of EMCV virus replication (-1,0 and 1 hour).

The presence of the toxic effect of "Dextrin" was expressed by the degree of destruction of the cell monolayer: "-"- no destruction, "□" rarefaction of the cell layer, "+"-destruction of 25% of the monolayer, "++"-destruction of 50% of the monolayer, "+++ » -destruction of 75% of the monolayer, « ++++ » -destruction of 100% of the cells of the monolayer.

The summarized results of 3-fold experiments on the effects of various concentrations of "Dextrin" are presented in (Table 1).

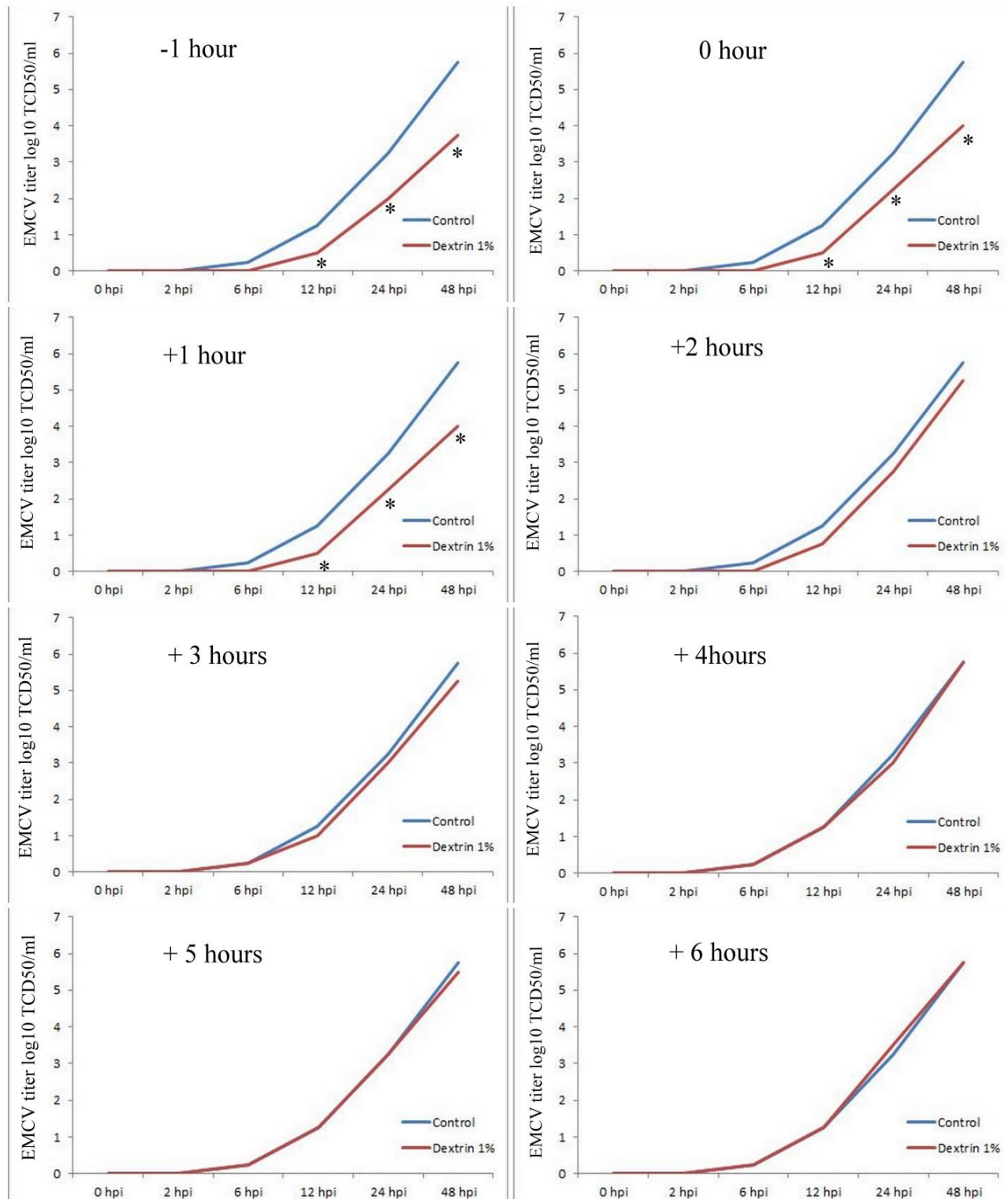


Figure 1: The value of "Dextrin" virus when introduced into the medium with HeLa cells before or at the early stages of EMCV virus replication (-1,0 and 1 hour).

Dilutions of stock solution "Aa"	Degree of damage to the monolayer (24 hours)	Determination of the toxicity of "Dextrin" on a monolayer of HeLa
1:1	++++	++++
1:2	++++	++++
1:4	++++	++++
1:8	++++	++++
1:16	++++	++++
1:32	+++	++++
1:64	±	+
1:128	-	-
1:256	-	-
1:512	-	-

Table 1: Degree of damage to the monolayer (48 hours).

As follows from Table 1, "Dextrin" at a dilution of 1:1-1:32 caused complete destruction of the cell culture monolayer, within 24 hours. At a dilution of 1:64, about half of the cells were destroyed, and starting from a dilution of 1:128, there was no destruction of the monolayer at 24 hours of incubation and almost absent at 48 hours. Based on the data obtained, it was decided to use a dose of 1:100 (1%), which is the highest non-toxic dose.

Discussion

Effective control and treatment of viral diseases remains a global public health goal. Despite the constant growth in the production of antiviral drugs and vaccines in

the pharmaceutical market, there is an increased risk of the spread of various viral epidemics with high incidence rates in the world.

A viral disease is a difficult task for drug therapy, since the virus can live and reproduce only inside the host cell, changing its metabolic processes, therefore, the scientific development of antiviral drugs is aimed at developing such drugs that act at different stages of the virus life cycle-they prevent the virus from attaching to cell, penetration into and exit of mature viral particles from the cell, will disrupt the reproduction (replication) of the virus. The action of these drugs in a therapeutic dose is

detrimental to the virus and is practically safe for the body.

The search for new tools that can help solve even part of the above problems is an urgent problem of modern biomedical science.

Current trends in the development of antivirals are focused on finding effective drugs from natural resources that have fewer side effects on the patient's body and are often cost-effective in terms of production.

Recent studies have shown that sulfated polysaccharides have various antiviral properties. Numerous advantages over other classes of antiviral drugs, such as relatively low manufacturing cost, wide range of antiviral properties, low cytotoxicity, low induction of resistance to viral drugs, high lyophilicity, safety, suggest them as promising candidates as antiviral drugs in near future [16-18].

In recent years, many natural compounds have been found to have antiviral activity in cell culture, and some of these compounds are currently in preclinical or clinical trials. Among these antiviral substances, naturally occurring carbohydrates should be noted.

Starch is the most common natural storage carbohydrate in plants, (wheat, rice, corn, barley, potatoes, sweet potatoes, bean seeds and peas). There are scientific studies which have reported on the use of starch in biomedical applications [19,20].

Natural sugars act as immunomodulators that determine the body's energy potential. Carbohydrates may be present in the form of

mono-, oligo-and/or polysaccharides. It is known that carbohydrates, together with proteins and lipid components of biological membranes, divide the space between the contents of the cell and the external environment [21].

Mono-, oligo- and polysaccharides in the product play an important role in intracellular cognition [22]. The specific effects of polysaccharides are associated with the activation of macrophages and T-lymphocytes, stimulation with interferon and immunomodulatory effects at the cellular level.

The polysaccharides contained in the product have a positive effect on inflammation, on the effects of ionizing radiation, enhance the regeneration of nerve and muscle tissues, inhibit the growth of connective tissue, and have some antitumor activity [23].

In the literature, there are many publications discussing various aspects of the application of polysaccharides (for drug delivery and regenerative engineering, for the development of polysaccharide-based nanomaterials for biomedical applications, the use of polysaccharide-based scaffolds only for bone marrow regeneration) [24-26].

Among materials derived from starch, dextrin is widely used. "Dextrin" is a split starch, an oligosaccharide obtained by heat treatment of corn and potato starch. "Dextrin" is well proven, has huge potential for biomedical applications.

In this study, we studied the antiviral effect of "Dextrin" representing potential interest for

practical medicine. *In vitro* studies have shown the antiviral activity of the drug "Dextrin", which serves as the basis for further clinical studies primeneie Dextrin in the prevention and treatment of various viral diseases. *In vitro* study has given encouraging results and the next step of the research is to investigate the possible antiviral therapeutic effect of "Dextrin" in vivo in a Syrian hamster model. The studies have already begun and

after receiving and processing the results, they will be published.

Conclusion

The investigated "Dextrin" has an antiviral effect against the EMCV virus, when applied in the early stages of infection, which opens the prospect of studying the antiviral spectrum "Dextrin".

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