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Photodynamic inactivation of enveloped virus in protein plasma preparations by solid-phase fullerene-based photosensitizer

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Blood products;
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Summary
Background: The problem of transfution-transmitted infections still remains serious and actual for health care despite the detailed testing of donors. Human immunodeficiency virus, hepatitis B and C viruses and human cytomegalovirus are among the most dangerous pathogens that can be transmitted with blood. Previously, a composition consisting of fullerene layer applied on silica gel particles was shown to inactivate influenza virus up to complete loss of infectivity.
Methods: In the present study the unit has been developed with source of irradiation whose spectrum is appropriate for solid-phase fullerene. The ability of the unit to inactivate the enveloped influenza virus in protein fraction of donor blood has been studied.
Results: It was shown that at optimized conditions complete inactivation of enveloped virus of extremely high initial titer (7.0—9.5 $\log_{10}EID_{50}$/0.2 mL) in the solution of albumin was achieved after as short time as 30 min of irradiation. This process did not affect the oxidative metabolism of neutrophils and membranes of erythrocytes evaluated by NBT reduction test and morphological analysis of erythrocytes, respectively.
Conclusion: The data obtained suggests that the method described can be recommended for further development and optimization of the procedure of inactivation of viruses in the preparations of the plasma of donor blood.

Introduction

The problem of transfution-transmitted infections still remains serious and actual for health care despite the

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detailed testing of donors. Human immunodeficiency virus, hepatitis B and C viruses and human cytomegalovirus are among the most dangerous pathogens that can be transmitted with blood [1,2].

Even after the control for virus contamination, blood and blood-derived products can infect recipients because several months can pass since infecting of donor and appearance of detectable levels of antibodies in the serum [3,4]. In addition, intensive migration of people widens the spectrum of transfution-transmitted infections that are not tested currently in donors at blood sampling.

Having in mind all mentioned above, WHO has approved several inactivating procedures that allowed to decrease the risk of infection in the course of transfusion of blood and plasma components.

The methods of elimination and inactivation of viruses are not virus-specific. For this reason there is no need to perform a specific procedure for each infectious pathogen. Moreover, these methods are potentially able to eliminate the viruses that are not known to present in the blood or plasma. From another hand, all methods of virus' inactivation have their limits of efficacy and should be considered as compromises between their ability to kill the virus and necessity to avoid the destruction of blood components such as cells and plasma proteins. There are many known methods of virus inactivation [5—7]. Nevertheless, only few are permitted for use in manufacturing of blood products. Currently, there is no universal method that can be applied for producing of all blood plasma components.

For obtaining of virus-inactivated protein preparations of blood plasma, solvent/detergent (S/D) method is of wide use. The main principle of S/D method is treatment of blood plasma with 1% tri(n-butyl) phosphate and 1% Triton X-100, or other detergent. This inactivates the viruses by destructing their lipid envelope that, in turn, prevents the interaction of the virus with plasma membrane of target cell thus decreasing its infectivity. After that, specific protein fractions are obtained by alcohol plasma fractionation according to Cohn [8].

Despite high efficiency of his method of inactivation, it has several disadvantages. First, the process of inactivation is complicated and labor-consuming. It takes about 10 h due to necessity to remove the chemical reagents used in the process. The main amount of reagents is removed by long-term extraction to oil phase following by the removal of the rest of the reagents by means of ultrafiltration, diafiltration or column affinity chromatography. This method of inactivation allows to treat large volumes of plasma with further isolation of limited number of target products by ethanol fractionation. Having in mind that the ethanol treatment itself destroys up to 99% of pathogens, and can be considered as first stage of inactivation, it would be reasonable to carry out the stepwise alcohol fractionation with further inactivation of significantly smaller volumes of target products.

Previously it was shown [9] that enveloped viruses can be effectively inactivated by reactive oxygen species (ROS), in particular, by singlet oxygen, that can be obtained by irradiation of photosensitizers (PS's). Among them, the most widely used are water-soluble PS's, such as phtalocyanins, phenocyanins, derivatives of porphyrins, etc. At the same time, the use of water-soluble PS's for photodynamic inactivation of blood-derived protein preparations is undesirable due to difficulties of further removal of photosensitizers and products of their decomposition from large volumes of biological fluids.

In this connection, the use of insoluble PS's should be much more productive and convenient. These PS's must efficiently produce ROS, be resistant to irradiation and separated easily from the reactive medium.

Currently, fullerenes can be considered as such PS's due to their ability to generate ROS [10,11], their photostability [12] and insolubility on water and biological fluids. Effective inactivation of enveloped viruses Semliki forest virus and vesicular stomatitis virus by crystal fullerene had demonstrated by Kaserman and Kempf [13] and confirmed by our experiments studying the kinetics of photodynamic inactivation of influenza virus in allantoic fluid of chicken embryos [14,15]. Unfortunately high efficiency of influenza virus inactivation was accompanied by formation of protein and fullerene agglomerates leading to delay of virus inactivation.

It was shown that the use of composition consisting of fullerene layer applied on silica gel particles could solve the problem of agglomerates and inactivate influenza virus up to complete loss of infectivity [16]. Additional factor that influences on the efficacy of ROS production by PS's is correspondence of spectrum of absorbance of PS to spectrum of emission of the source of irradiation. In this connection, in the present study we developed the unit with source of irradiation whose spectrum is appropriate for solid-phase fullerene. Using this unit we have studied the inactivation of enveloped viruses in protein fraction of donor blood in the model system containing albumin and solid phase-based fullerene-containing photosensitizer.

Materials and methods

Materials

Fullerene C_{60} (99.85% purity) was prepared in CJSC ILIP (St. Petersburg). Spherical silica gel (Fluka), albumin (for medical purposes, 10% solution), and toluene (Aldrich) were used in experiments.

Preparation of solid phase fullerene-based PS (SPFPS)

The substance of C_{60} in toluene (2.4 mg/mL) was stirred on the magnet vortex in argon flow until completely dissolved. After that, 40 g of silica gel that was previously heated for 3 h at 120 °C in vacuum oven was added to of fullerene solution and mixed for 5 h. The deposit was filtered and air-dried following by 24-h incubation in vacuum at 80 °C. The resulting photosensitizer was placed in dark glass container and stored in dark until use.

Virus propagation

Influenza virus A/Puerto Rico/8/34 (H1N1) was propagated in allantoic cavity of chicken embryos for 48 h at 36 °C followed by overnight cooling at +4 °C. Virus-containing