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SHORT COMMUNICATION

Heat sensitivity of a SARS-associated coronavirus introduced into plasma products

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Background and Objectives Various measures to inactivate/remove viruses have been implemented for manufacturing plasma-derived products. Here, we examined the heat inactivation ability of an agent of the severe acute respiratory syndrome (SARS), SARS coronavirus (CoV).

Materials and Methods The Frankfurt-1 strain of SARS-CoV was incorporated in manufacturing processes of several products by using samples collected immediately before liquid heat treatment at 60 °C.

Results SARS-CoV was easily inactivated by this treatment for 60 min in all in-process samples. However, the different composition of the tested samples affected the heat sensitivity of the virus strain: the infectivity of the virus in Antithrombin III preparation still remained after heating for 30 min at 60 °C.

Conclusion If by rare chance SARS-CoV contaminates source plasma, there should be no or only minor risk of this virus infection, due to sufficient inactivation by the 60 °C 10 h liquid heating step, although we must pay attention to the composition used for blood product preparation.

Key words: SARS-CoV, plasma-derived products, viral safety, virus inactivation.

Introduction

Various measures are available to inactivate or remove viruses during the manufacture of plasma-derived products. In the present study we evaluated the ability of liquid heat treatment to inactivate a coronavirus (CoV) associated with severe acute respiratory syndrome (SARS). The virus was introduced into several products during their manufacture and processing, immediately before liquid heat treatment at 60 °C.

Materials and methods

We used Vero E6 cells, cultured in minimal essential medium (MEM) containing 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin, for propagation of the Frankfurt-1 strain of SARS-CoV [1]. The Frankfurt-1 strain of SARS-CoV was kindly provided by Dr John Ziebuhr (University of Würzburg, Würzburg, Germany), through Dr Fumihiro Taguchi (National Institute of Infectious Diseases, Tokyo, Japan). For the infectivity assay, Vero E6 cells were seeded in a 96-well microplate (4×10^5 cells/ml, 0·1 ml/well) and, after overnight culture at 37 °C in 5% CO_2/air, the cells were inoculated with serial 10-fold dilutions of the virus stock solution (0·1 ml/well, five wells per dilution). On day 3 of culture, virus infectivity [the tissue culture infectivity dose 50% ($TCID_{50}$/ml), $\log_{10}$] was calculated by using Karber's method [2].

The four products tested (all supplied by the Benesis Corporation, Osaka, Japan) were:

(1) A heat-treated/polyethylene glycol (PEG)-treated intravenous immunoglobulin preparation (Kenketsu Venoglobulin®-IH).

(2) An anti-thrombin III preparation (Neuart®).

(3) A haptoglobin preparation (Haptoglobin Injection-Yoshitomi).

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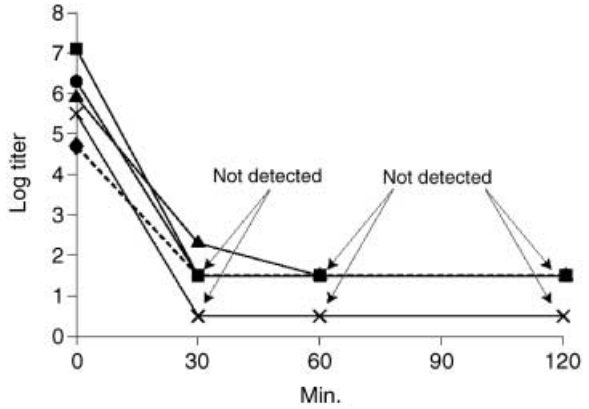

Fig. 1 Inactivation kinetics of a SARS-associated coronavirus (SARS-CoV). The Frankfurt-1 strain was inoculated, at either 10% (v/v, solid line) or 1% (v/v, broken line), into in-process solutions of a heat-treated/polyethylene glycol-treated intravenous immunoglobulin preparation (◆), an anti-thrombin III preparation (▲), a haptoglobin preparation (■), and a 25% human serum albumin preparation (●), each collected immediately before liquid heat treatment. The virus in these products was then treated at 60 °C for up to 2 h. The virus was also inoculated (10% v/v) into minimal essential medium (MEM) containing 2% fetal bovine serum (FBS) and then heat treated as a control (**x**). The data shown represent the virus infectivities remaining after heat treatment.

(4) A 25% human serum albumin preparation (Kenketsu Albumin-Wf).

In-process samples of the plasma-derived products used in the study were collected immediately before the 10-h liquid heat treatment at 60 °C that is used in the manufacture. The samples were inoculated with SARS-CoV, followed by heat treatment in liquid at 60 °C for 0·5–2 h, after which the remaining infectivity was titrated as described above.

Results and discussion

In all four in-process samples, Frankfurt-1 was rapidly inactivated to below the detection limit within 60 min. However, its infectivity in the anti-thrombin III preparation persisted, despite heating for 30 min at 60 °C, although the same amount of heating inactivated the virus in the other three preparations (Fig. 1). This result was confirmed by an independent experiment (data not shown). Furthermore, when Frankfurt-1 was inoculated into the solutions containing the stabilizer alone (without protein), 30 min of heating inactivated the virus to below the detection limit in all four products (data not shown), suggesting that the combination of the blood product preparation and its stabilizer affected the heat sensitivity of the virus. Rabenau and his colleagues also conducted experiments using the SARS-CoV FFM-1 strain. As in our study, they found that while the virus was stabilized by heating at 56 °C for 30 min in the presence of 20% FBS, it was inactivated at 60 °C in the presence and absence of 20% FBS [3]. In this context therefore we must take into consideration the combination of the plasma products and the stabilizer.

References

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