Survival of a hepatitis C virus surrogate in anaesthetic and analgesic drugs

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Abstract

The re-use of solutions used in surgical procedures provides an opportunity for transmission of infectious agents should a breakdown in good work practices occur. Agents that are blood-borne are particularly important in this respect. We examined whether a hepatitis C virus surrogate (bovine viral diarrhoeal virus) could survive exposure to Propafol and Fentanyl, drugs commonly used for induction of anaesthesia and analgesia, respectively. Testing involved the spiking of ampoules of these solutions with a high-titred preparation of virus. Following incubation of this mixture at ambient temperature for various periods of time, attempts were made to isolate the virus in cell cultures. Our results showed that the surrogate virus survived for up to 2 hours without loss of titre in these solutions, suggesting that the hepatitis C virus would also survive and could also be transmitted in a surgical setting under some circumstances.

Introduction

Even though some intravenous anaesthetic agents are distributed as single-use products, their re-use in more than one patient is a possibility if a volume of solution remains following the procedure performed on the first patient. Potential contamination may occur if a drawing-up needle and syringe is re-used to access additional doses from an ampoule containing residual drug. The practice of aspirating an intravenous cannula to ensure venous patency may contaminate the syringe and, subsequently, the contents of the ampoule if it is re-accessed. If the remaining contents of the ampoule are administered to another patient, infectious agents could be subsequently transmitted. Agents of particular concern include the human immunodeficiency virus (HIV) and the human hepatitis B and C viruses. In particular in Australia, hepatitis C virus (HCV) is common relative to the other blood-borne viruses, and is highly transmissible by the parenteral route.

Nosocomial and occupational transmission of HCV has been reported in a number of settings, including haemodialysis units [1], surgeon to patients [2] and patient to surgical staff [3]. None of these investigations has proven anaesthetics or analgesics as the source of transmission, although multidose vials of local anaesthetics, saline, heparin and other solutions stored between patients in a paediatric oncology ward have been implicated as the likely source of transmission of HCV in one outbreak [4]. With this in mind, we investigated whether two solutions, Propafol and Fentanyl, used commonly as intravenous anaesthetic agents, would support the maintenance of infectivity of an HCV surrogate during the time normally taken to carry out a number of sequential surgical procedures. Propafol is used for induction of anaesthesia; Fentanyl is a narcotic-based analgesic.

Methods

Because HCV does not replicate in conventional cell culture systems, the bovine viral diarrhoeal virus (BVDV), which has similar morphology, genome organisation and likely RNA replication strategy to HCV [5], was used as a surrogate virus. This strategy has been used previously to assess the stability of HCV to biochemical and/or biophysical inactivation procedures [6]. Aliquots of Fentanyl (as fentanyl citrate 50µg/ml, David Bull Laboratories, Melbourne) and Propafol (as 1% Diprivan, Zeneca Ltd, Cheshire, UK) were spiked with a stock of BVDV to yield a final virus concentration of 1,000 50% tissue culture infectious doses (TCID50) per ml. The concentrations of Fentanyl and Propafol spiked with virus were the same as those provided in ampoules for clinical use. The concentration of virus used was approximately 1000-fold the amount of virus needed to infect a culture of susceptible cells with a standard volume of inoculum.

The BVDV-spiked solutions were then held at room temperature (21°C) for 1 min, 1 hour and 2 hours. Following this incubation time, each solution was made up to 10mls with

5% TES solution (10mM Tris-HCl, 1mM EDTA, 150mM NaCl) and ultracentrifuged at 120,000g for 1.5 hours. The viral pellet was resuspended in 100µL of culture medium consisting of Minimum Essential Medium (Eagle) (ICN Biomedicals Inc. Ohio, USA) containing 5% bovine calf serum and inoculated in quadruplicate into Madin Darby bovine kidney cells.

Following 10 days' incubation at 37°C in an atmosphere containing 5% CO_2, the cells were harvested and dried onto individual wells on glass slides. After fixation in acetone, evidence of BVDV-specific antigens was sought by immunofluoresence using mouse-derived BVDV-specific monoclonal antibodies and sheep anti-mouse immunoglobulin conjugated to fluoroscein isothiocyanate (Silenus-Amrad Operations, Melbourne). The titre of stock virus recovered from an untreated control (virus incubated in 5% w/v sucrose in TES) was compared to that recovered from virus that had been in contact with either of the two drugs.

Results

The results in Table 1 indicate that virus incubated in the presence of a sucrose-based saline solution retained its titre over a 2 hour incubation period at room temperature (21°C). There were no significant changes in titre of the same preparation of BVDV exposed to either Fentanyl or Propafol for periods of up to 2 hours compared to an untreated control.

Table 1. Results of a typical experiment examining the recovery of an HCV surrogate (BVDV) from Fentanyl and Propafol after contact times of up to 2 hours.

Treatment	Contact time (minutes)	Titre of BVDV recovered
Control*	–	3.25
	120	3.25
Fentanyl	1	3.00
	60	3.00
	120	2.75
Propafol	1	3.75
	60	3.75
	120	3.50

** Virus incubated for 120 minutes in 5% w/v sucrose in TES.*

Discussion

Our results demonstrate that exposure of BVDV to Fentanyl or Propafol for up to 2 hours does not result in a significant reduction in virus concentration compared to an untreated control. Although these experiments were carried out only at 21°C, we would not expect any change to this observation at temperatures within a few degrees either side of it. In fact, because temperatures in operating theatres are often several degrees lower than 21°C, increased survival of the virus is a likely outcome.

This is the first time, to our knowledge, that the likely survivability of this virus in these commonly used drugs has been reported. On the basis of the similar biological properties of BVDV and HCV, we expect that HCV would also retain infectivity following exposure to either of these products under the same conditions. We used an infectious surrogate (BVDV) in these experiments so that infectivity, or otherwise, could be demonstrated after exposure of virus to Fentanyl or Propafol. Exposure of a high-titre preparation of HCV to these solutions, followed by detection of residual viral RNA by molecular methods, would not have demonstrated conclusively that infectivity remained.

We have previously reported the survival of HIV in multi-dose local anaesthetic vials[7]. Our current data extends this to HCV, albeit under a different set of circumstances in that we have demonstrated that transmission might be possible from solutions intended for single-use only that are instead re-used in multiple patients. These observations highlight the need for continued review of these practices. Factors needing particular attention in this regard include the administration, inadvertent or otherwise, of any residual volume of drug in single-use ampoules, and the re-use of needles and syringes to transfer these solutions. In the event that a breach of good work practice occurs, the potential exists for transmission of HCV through the administration of intravenous anaesthetic agents.

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