In vitro detection of hepatitis C virus in platelets from uninfected individuals exposed to the virus

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INTRODUCTION

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Abstract

Introduction: Despite hepatocytes being the target cells of hepatitis C virus (HCV), viral ribonucleic acid RNA has been detected in other cells, including platelets, which have been described as carriers of the virus in the circulation of infected patients. Platelets do not express cluster differentiation 81 CD81, the main receptor for the virus in hepatocytes, although this receptor protein has been found in megakaryocytes. Still, it is not clear if HCV interacts with platelets directly or if this interaction is a consequence of its association with megakaryocytes. The aim of this study was to evaluate the interaction of HCV with platelets from non-infected individuals, after in vitro exposure to the virus. Methods: Platelets obtained from 50 blood donors not infected by HCV were incubated in vitro at 37oC for 48h with serum containing 100,000IU/mL of genotype 1 HCV. After incubation, RNA extracted from the platelets was assayed for the presence of HCV by reverse transcription – polymerase chain reaction RT-PCR. Results: After incubation in the presence of virus, all samples of platelets showed HCV RNA. Conclusions: The results demonstrate that, in vitro, the virus interacts with platelets despite the absence of the receptor CD81, suggesting that other molecules could be involved in this association.

Keywords: HCV. Platelets. CD81.

Hepatitis C virus (HCV) infects approximately 170 million people in all the world, where it is one of the principal causes of chronic hepatitis, cirrhosis and hepatocarcinom1-3.

Hepatocytes constitute the principal target cells of the virus4,5 and express cluster differentiation 81 CD81, the main receptor associated with the entrance of HCV6,7. However, studies have demonstrated the presence of viral ribonucleic acid RNA in other cell types4, including platelets8,9. In addition, platelets have been shown to act as carriers of the virus in the circulation of individuals infected by the virus10. However, platelets do not express CD81, although the presence of this receptor has been described in megakaryocytes11.

Still, little is known about the interaction HCV and platelets. It is not known if this process occurs initially in the megakaryocyte, where the virus is consequently transferred to the platelets or if the platelets can interact directly with HCV, even though not expressing CD81.

Thus, the aim of this study was evaluate in vitro the possible interaction of HCV with platelets from non-infected blood donors.

METHODS

Aliquots of ethylenediaminetetraacetic acid EDTA-anticoagulated peripheral venous blood were collected from 50 donors at the Botucatu Medical School’s Blood Transfusion Center. Inclusion criteria were: serology negative for anti-HCV antibody, absence of RNA-HCV confirmed by molecular assays and signed informed consent.

The blood sample was centrifuged for 3min at 1,312xg for plasma separation. The plasma was then centrifuged for 5min at 1,600xg to pellet the platelets. The supernatant was removed and the platelet pellet was washed with 0.9% NaCl five times. At this step, platelet pellet aliquots were separated and used to prepare slides for Leishman staining.

The platelet pellet was incubated with 1mL of serum pool containing 100,000IU/mL of genotype 1 HCV. The incubation was at 37°C in an Incubator Shaker (New Brunswick Scientific, USA) for 48h with horizontal mixing at 30xg.

After incubation, the samples were washed with 0.9% NaCl five times, and the last supernatant was used for HCV amplification for reverse transcription – polymerase chain reaction (RT-PCR), to assure the absence of HCV serum.

The platelet pellet was isolated using Buffer RLT (QIAGEN, Valencia, CA, USA) followed by the QIAamp Viral RNA Mini
kit (QIAGEN, Valencia, CA, USA) protocol and was used as the source for HCV 5'UTR genomic region amplification by RT-PCR\textsuperscript{12,13}. To evaluate the quantification of HCV-RNA in platelets the RNA was used as source for Real Time PCR according methodology described by Dexter et al.\textsuperscript{14}.

Two negative controls were used in the procedure: one with the platelet pellet incubated with pooled HCV seronegative serum and the other consisting of an empty tube incubated with the same serum pool used in test samples, without platelets.

**Ethical considerations**

The study was approved by the Research Ethics Committee of Botucatu School of Medicine (number 3134-2009), Universidade Estadual Paulista (UNESP).

**RESULTS**

The 50 blood donors included in this study had a mean age of 33.5 years, (interquartile range, 24.5 - 42.7), where 13 (26%) were men and 37 (74%) women.

All samples gave a result of not detectable for the presence of the viral RNA of HCV before incubation with the virus.

After incubation with HCV-RNA positive serum, the presence of HCV RNA was detected in all platelet samples, evidenced by the amplification of the 5'UTR region, but when RT-PCR was used 32 (64%) samples presented HCV-RNA upper 18.4UI/mL. All negative controls utilized showed undetectable RT-PCR, validating the experiment.

**DISCUSSION**

The platelets have been described as carriers of HCV in infected patients\textsuperscript{8}, despite that these cells do not express CD81, the principal receptor utilized by the virus for entrance into hepatocytes\textsuperscript{6}. However, previous studies have demonstrated the presence of mRNA coding for CD81 in megakaryocytes\textsuperscript{12}, and expression of the receptor protein as well\textsuperscript{11}. Still, little is known about the direct interaction of HCV with platelets.

The results obtained here showed that in 100% of the platelets tested, HCV RNA was detected after incubation with serum containing viral RNA, suggesting that the virus-platelet interaction does not require the megakaryocyte as the initial step of the process.

In addiction others molecules can be suggest how candidates in interaction HCV-platelets as fibronectin\textsuperscript{10} or others adhesion molecules\textsuperscript{15}. Further studies are needed for a better understanding of these interactions.