GUINEA PIG (CAVIA PORCELLUS) CAN BE OR NOT USED AS AN EXPERIMENTAL MODEL TO STUDY HEPATITIS A VIRUS INFECTION?

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ABSTRACT

Priorities for new hepatitis A virus (HAV) vaccine include the existence of susceptible animal models to perform studies of infectivity, pathogenesis, humoral and cellular immunity, cytokine responses, antiviral drugs and vaccine efficacy. Previous study demonstrated that guinea-pigs (*Cavia porcellus*) appeared useful for studying some aspects of HAV pathogenesis and for testing safety of vaccines. The experimental infection was studied in twelve guinea-pigs inoculated intraperitoneally; four of them with the Brazilian HAV wild strain (HAF-203 accession: GenBank AF268396) (group 1); four received HAF-203 strain adapted to FRhK-4 cells (group 2) and, four uninfected animals inoculated only with medium 199 (group 3). One animal of each group was killed on days 14, 28, 42 and 56 postinoculation. All parameters investigated were normal, including liver histology, serum ALT/AST levels and absence of RNA HAV in feces or liver samples. The seroconversion, at days 28 and 56 (group 1) confirmed HAV inoculation. However, anti-HAV titer alone did not guarantee immunity or predict susceptibility. We were unable to establish a guinea-pig as model of HAV.

INTRODUCTION

Hepatitis A, caused by a small RNA virus, is an acute disease of very short duration, acquired through ingestion of food or water contaminated with fecal material is believed to have been responsible for infection in more than five billion individuals (Purcell & Emerson 2001). About twenty thousand new cases of hepatitis A virus (HAV) infection were reported during 2000-2005 in Brazil (Vitral et al. 2008). The changing epidemiology in Brazil revealed increasing reported HAV infection incidence among adolescents and adults (Villar et al. 2002, Villar et al. 2004, Villar et al. 2006a, Vitral et al. 1998, Vitral et al. 2006). Vaccination against HAV is, unquestionably, the most important method for the control in countries of intermediate endemicity in which massive childhood vaccination may be considered as public health education and improved sanitation (WHO - World Health Organization 2000).

Numerous attempts have been made to develop animals’ models of HAV. The first documented experimental infection with HAV was done by Deinhardt et al. (1962) in chimpanzees inoculated with serum or feces of hepatitis infected patients.
However, even among nonhuman primates the host range in HAV infection is fairly limited. Identifying and characterizing the best species for studying hepatitis A is always laborious and expensive (Purcell & Emerson 2001).

Although rodents and humans share many anatomic and physiological features of the enterohepatic system, rodents cannot be infected with hepatitis B virus (HBV) or hepatitis C virus (HCV) (Rogers & Fox 2004). However, studies using HBV-and HCV-transgenic mice clearly demonstrate that viral gene products can, a priori, induce tumors (Koike 2009).

It was reported that guinea pigs (Cavia porcellus) could be used in several tests of immunogenicity, effectiveness and safety of vaccines (Binn et al. 1986, Flehmig et al. 1987, Elbert et al. 1992, Mitchell & Galun 2003, Mitchell et al. 2006) and in events related to the proper vaccine of hepatitis A (Hornei et al. 2001, Burnett et al. 2003). HAV adapted to tissue culture was able to infect and replicate in guinea pig cell culture (Dotzauer et al. 1994).

In an article published in 2001 (Hornei et al. 2001) that details an alternative animal model for hepatitis A virus studies, the authors describe that guinea pigs reaction to infection by HAV showed similar pattern responses with a New World non-human primate – Callithrix jacchus (Baptista et al. 1993).

Uncertainty or controversy establishing guinea pig as model for HAV infection conduct us to an experimental research project to answer this question. In this paper guinea pigs were tested to HAV strain HAF-203 (Baptista et al. 2006) to obtain data on the potential suitability for laboratory studies.

MATERIAL AND METHODS

Ethical approving.

The study design was carried out according to the protocol approved by the Institutional Committee for Experimentation and Care of Research Animals (CEUA-Fiocruz: PO 257/05) and follows the regulations for the use of laboratory animals of the Guide for the Care and Use of Laboratory Animals by the US National Institutes of Health (National Research Council 1996).

Experimental animals.

Twelve clinically healthy, 19 days-old, male and female domestic guinea pigs (Cavia porcellus), coat color white (albino), genetically heterogeneous “Fiocruz” strain, supplied by the Center for Laboratory Animal Breeding, Oswaldo Cruz Foundation.
Inocula.

The strain HAF-203 (accession:GenBank AF268396) of hepatitis A virus, was isolated from stool of a Brazilian child with sporadic hepatitis A infection. Briefly, stool samples were diluted at 1% (w/v) in PBS (10 nM of sodium phosphate, 0.15 M of NaCl) with penicillin (100 IU/mL) and streptomycin (100 mg/mL), centrifuged in low speed and then filtered by a 0.45 µm membrane (Gaspar et al. 1992). Later, the viral load was quantified by a real-time polimerase chain reaction (PCR) with $4.35 \times 10^5$ copies of viral RNA/mL (Villar et al. 2006b). HAV was also propagated in fetal rhesus monkey kidney (FRhK-4) cells.

Experimental design.

Guinea pigs were divided into three groups of four animals each. Group 1 received intraperitoneally (ip) HAV strain HAF-203 (wild HAV); Group 2 were inoculated ip with HAV strain HAF-203 adapted for growth in FRhK-4 cells and the Group 3 of controls animals were inoculated (ip) only with medium 199.

The animals were anaesthetized with ketamine hydrochloride (Vetarnacoïn™, 20 mg/kg) administered intramuscularly. Euthanasia was performed by means of complete exsangination through direct cardiac puncture at 14, 28, 42 and 56 days postinoculation (pi).

Detection of HAV RNA by real-time PCR from the liver samples.

Liver extraction of HAV RNA was adapted from the TRIzol® method as described previously (de Paula et al. 2003). The complementary DNA (cDNA) was synthetized and then analyzed by real-time PCR using the GeneAmp 7500 PCR machine (Applied Biosystems).

Detection of HAV RNA by real-time PCR from fecal samples.

Stool samples were collected weekly for RNA molecular analysis. Viral RNA was extracted from feces samples and RNA was eluted using the commercial assay QIAamp viral RNA (QIAGEN, Valencia, California, USA).

Serology and blood biochemistry.

Serum was analyzed by ELISA (enzyme-linked immunosorbent assay) using a commercially kit (Bioelisa HAV 96T Kit, Biokit, S.A., Barcelona, Spain) to detect IgM and total anti-HAV antibodies.
Serum was assessed for alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT) levels by a manual colorimetric procedure (Reitman & Frankel 1957) with the automatized Vitros System Chemistry 750 XRC (Ortho-Clinical Diagnostics Inc., Johnson & Johnson Company, New York, USA) and their reagents.

Statistic analyses were performed on the data collected from enzymatic tests applying Friedman’s non-parametric tests (Conover 1980, Siegel & Castellan 2006) with Minitab 14 software (Minitab Incorporation, USA, 2003).

**Histological analysis.**

Samples of liver, gallbladder, kidney, small and large intestine, submandibular salivary gland, spleen, mesenteric lymph node and tonsils were fixed in 10% neutral formalin. Paraffin sections, 4 µm in thickness were stained with haematoxylin and eosin (H&E). Sections from liver were also stained with Masson’s trichrome, periodic acid-Schiff (PAS) reagent and by Giemsa.

**RESULTS**

**Clinical observation.**

Animals were kept under continuous clinical surveillance. No guinea pigs showed clinical signs during the experiment.

**Serology and blood biochemistry.**

Two guinea pigs (50%) that had been experimentally infected with HAV strain HAF-203 were positive to total anti-HAV antibodies at days 28 and 56 days pi. Of the animals from group 2 and 3 none seroconverted throughout the experiment.

By comparing the obtained results of ALT (p=0.2573), AST (p=0.3554) and GGT (p=0.990) enzyme activities in blood plasma during the experiment statistically significant differences were not recorded as represented.

**Detection of HAV RNA by real-time PCR from fecal and liver samples.**

HAV RNA could not be detected in stool and liver samples from all animals by RT-PCR from day 14 to 56 following inoculation. Linearity of the standard curves were verified in the dilutions from $1.5 \times 10^1$ to $1.5 \times 10^7$ viral RNA copies/mL having the highest coefficient of determination (0.99) and values of -3.38, -3.44 e -3.22 for slopes.

**Histological analysis.**

No abnormalities in livers that could suggest damage or impaired function were found in all guinea pigs euthanized (Figure 1). None of the animals in either experiment
developed any pathological lesions of the gallbladder, kidney, spleen, small and large intestines, mesenteric lymph node, submandibular salivary gland and tonsils.

**Figure 1.** Guinea pig as an experimental animal model to hepatitis A infection. The picture represents a normal liver sample from animal number 4 (Group 1) at 56 days after hepatitis A virus inoculation. The photomicrography of the liver shows: **A.** Hepatocyte plates and sinusoidal capillaries, portal triad (EP) and central vein (VC). Hematoxylin-eosin staining, 320x. **B.** Glycogen in hepatocytes of zone 3. Periodic acid-Schiff staining, 640x. **C.** One bile canaliculus (CB) surrounded by macrophages and lymphocytes. Giemsa staining, 1,200x. **D.** Portal vein (VP) ramification involved with conjunctive tissue. Masson's trichrome staining, 1,200x.

**DISCUSSION**

Initiatives for the development of vaccines against Hepatitis A have been facing an obstacle, namely the lack of a suitable animal model. Non-human primates are considered the principal models for HAV which more closely mimics many of the aspects of the human disease (Purcell & Emerson 2001, Deinhardt et al. 1962, Dotzauer et al. 1994). Due to high economical and moral concerns, the feasibility of guinea pigs
(Cavia porcellus) in the experimental infection by HAV is considered among the replacement options (Binn et al. 1986, Elbert et al. 1992, Hornei et al. 2001).

Because of contradictory previous reports the aim of this study was to evaluate clinical manifestations, liver enzyme elevations, viral shedding, viremia, seroconversion to anti-HAV, and detectable HAV antigen in liver specimens from guinea pigs after inoculation with wild-type and cell culture-adapted HAV strain HAF-203.

No overt clinical signs were detected in any of the animals from the beginning to the end of the experiment. Previous studies demonstrated that HAV also causes subclinical infections in New World non-human primate species including common marmosets (Callithrix jacchus) and squirrel monkeys (Saimiri sciureus) (Pinto et al. 2002) but also in guinea pigs (Hornei et al. 2001).

ELISA results (total anti-HAV antibodies) showed seroconversion in only two of these animals infected strain HAF-203 at 28 and 56 days pi. Report in the literature indicates that occurred significantly differences in circulating antibodies in guinea pigs of different strains after a single immunizing antigen dose (Munoz 1967). Hornei et al. (2001) produced good response in Dunkin-Hartley guinea pigs screened for specific HAV antibodies. In terms of immunogenicity to inactivated HAV particles, guinea pigs have been considered as intermediate producers of HAV antibodies (Elbert et al. 1992).

Any significant increased ALT, AST and GGT enzyme activities aspartate aminotransferase (AST) levels were detected during the follow up. HAV experimentally infected non-human primates causes mild elevations in blood levels of liver enzymes (Pinto et al. 2002, Trahan et al. 1987, Mathiesen et al. 1980, Asher et al. 1995, Vitral et al. 1995, Purcell et al. 2002), evoked by inflammatory reaction in liver parenchyma (Dotzauer et al. 1994, Pinto et al. 2002, Chitambar et al. 2001).

We employed a highly accurate hepatitis A virus RNA quantitative real-time PCR assay that allows equal detection and quantification of. HAV RNA was not detected in stool and liver samples from all animals throughout the time course of this study.

No unexplained mortalities or pathologies were observed in any guinea - pigs. On the contrary, Hornei et al. (2001) identified in Dunkin-Hartley guinea pigs hepatocellular lesions highly suggestive of hepatitis A infection. These authors also described inflammatory lesions and immune reactivity in small intestine and spleen, respectively. There is still considerable controversy about HAV replication of the
extrahepatic sites (Keenan et al. 1984) work all guinea pigs did not show any hepatic or multiorgan inflammatory and destructive changes.

We cannot adequately compare our results using the outbred “Fiocruz” strain guinea pigs as experimental models for hepatitis A virus strain (HAF-203) with the research group from University of Lübeck (Hornei et al. 2001). Experiments were performed with different virus strain (HAV strain HM-175 x HAV strain HAF-203) and therefore, the difference in guinea pigs strain (Dunkin-Hartley x “Fiocruz”).

Analyses of the results of this study do not allow the indication of “Fiocruz” strain guinea pigs as experimental models for hepatitis A virus strain (HAF-203).

Competing interests: None.

REFERENCES


