Available online at www.sciencedirect.com
SCIENCE DIRECT
Vaccine 24 (2006) 4343–4353
Vaccine
www.elsevier.com/locate/vaccine

Immunogenicity and safety of a novel therapeutic hepatitis C virus (HCV) peptide vaccine: A randomized, placebo controlled trial for dose optimization in 128 healthy subjects ☆

Christa Firbas [a], Bernd Jilma [a,*], Erich Tauber [b], Vera Buerger [b], Sandra Jelovcan [b], Karen Lingnau [b], Michael Buschle [b], Jürgen Frisch [b], Christoph S. Klade [b]

[a] *Department of Clinical Pharmacology, Medical University of Vienna, Währinger Gürtel 18-20, A-1090 Vienna, Austria*
[b] *Intercell AG, Vienna Biocenter Campus, A-1030 Vienna, Austria*

Received 2 August 2005; received in revised form 17 February 2006; accepted 2 March 2006
Available online 20 March 2006

Abstract

As interferon/ribavirin-based standard therapy is curative in only about half of HCV patients, there remains an important need for alternatives including vaccines. The novel peptide vaccine IC41 consists of five synthetic peptides harboring HCV T cell epitopes and poly-L-arginine as synthetic adjuvant. In this randomized, placebo-controlled trial, 128 HLA-A2 positive healthy volunteers received four s.c. vaccinations of seven different doses IC41, HCV peptides alone, poly-L-arginine alone or saline solution, every 4 weeks. IC41 was safe and well tolerated. Mild to moderate local reactions were transient. Immunogenicity was assessed using T cell epitope specific [^{3}H]-thymidine proliferation, IFN-gamma ELIspot and HLA-tetramer assays. IC41 induced responses in all dose groups. Higher responder rates were recorded in higher dose groups and increasing number of vaccinations were associated with higher responder rates and more robust responses. Poly-L-arginine was required for the aimed-for Th1/Tc1-type immunity (IFN-gamma secreting T cells).

Keywords: Hepatitis C; Peptide vaccine; Randomized controlled trial; Human

1. Introduction

Hepatitis C virus (HCV) is responsible for the majority of both parenterally transmitted and community acquired non-A, non-B hepatitis. An estimated 170 million humans, or 1–3% of the world population, are infected with HCV [1] with an even higher prevalence in the third world. About 10 Million Europeans, 3.9 million Americans and 2 million Japanese are infected with HCV, and 35,000 new infections occur in the US alone each year (WHO, Weekly Epidemiologic Report, No. 3, 2000, 75). Since the current gold standard of treatment with pegylated interferon and ribavirin is curative in only about half of HCV patients [2], there remains an important need for alternative therapies and effective vaccines. The high unmet medical need is underscored by the fact that each year 8000–10,000 deaths and 1000 liver transplantations are due to HCV in the US (NIH Consensus Development Conference Statement, Management of Hepatitis C, June 2002; CDC: Fact Sheet Hepatitis C, http://www.cdc.gov).

HCV is a positive-stranded enveloped RNA virus belonging to the family of flaviviridae. Its 10 kilobase genome contains a single open-reading frame giving rise to a polyprotein

☆ Presented in part at the American Association for the Study of Liver Disease (AASLD) meeting in Boston, MA, October 29–November 2, 2004, and at the 40th Annual Meeting of the European Association for the Study of the Liver (EASL) in Paris, April 13–17, 2005. The protocol and all amendments were approved by the Ethics Committee of the Medical University,Vienna. The study was carried out in compliance and in accordance with good clinical practice (GCP), and all relevant guidelines of the international conference for harmonization (ICH). Written informed consent was obtained from all participants prior to study entry at the Department of Clinical Pharmacology, Vienna.

* Corresponding author. Tel.: +43 1 40400/2981; fax: +43 1 40400/2998.
E-mail address: bernd.jilma@meduniwien.ac.at (B. Jilma).

0264-410X/$ – see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.vaccine.2006.03.009

which is posttranslationally processed into structural (Core, E1, and E2/NS1) and non-structural proteins (NS2, NS3, NS4, and NS5) [3,4].

Infection leads to viral persistence and chronic disease in ~80% of the cases [5], and sequels contribute significantly to morbidity and mortality. Chronic HCV infection over years leads to liver fibrosis and cirrhosis. Moreover, it is a leading cause of hepatocellular carcinoma (HCC) and liver transplantation, which often occurs despite humoral and cellular immune responses against the structural proteins of the virus [6,7].

Primary HCV infection causes broad and multispecific $CD4^+$ and $CD8^+$ T cell responses. It has been reported that stronger, broader and more sustained Th1/Tc1 (IFN-gamma) responses are associated with resolving infection [7–18]. Indeed T cell responses can readily be detected in humans in the absence of viremia many years after clearing infection [14,19–23]. Although chronically infected patients also show some IFN-gamma responses, these tend to be weaker and directed against less epitopes [21,23]. In addition HCV specific T cells appear impaired in chronic infection [15,24,25]. The high mutation rate of an RNA virus and the existence of quasispecies in the same individual facilitate immune escape mechanisms that can undermine productive T cell responses [16,26–33]. Additional potential immune deviations in chronic HCV include dysfunction of dendritic cells [34–38] and suppressor T cells [39–44].

The role of specific antibodies against HCV is more controversial. Envelope antibodies would be the prime candidates for virus neutralization, but their presence in chronically infected patients as well as in animal experiments argue against efficient humoral virus neutralization in vivo [45,46]. Further, the existing antibodies are mostly specific against HVR1 of the envelope protein 2. This is disadvantageous, because the heterogeneity in the envelope HVR [47] may be accompanied by the failure of the immune system to mount an antibody response to the dominant strain [48] and also to respond to interferon therapy. Finally, antibody-mediated immune pressure seems to directly correlate with an evolution of viral escape mutants during the course of infection [49].

In light of the above we hypothesized that eliciting an anti-HCV immune response based on the induction of epitope-specific $CD8^+$ cytotoxic T lymphocytes (CTLs) and $CD4^+$ responses may be highly beneficial. We also reason that it is advantageous to concentrate on well-conserved proteins using well-characterized epitopes spread over a large percentage of the population e.g. HLA A2-restricted epitopes.

This is reflected in the design of IC41 that contains at least four HLA-A2 restricted CTL epitopes and three highly promiscuous $CD4^+$ Helper T cell epitopes, all of which have been shown to be targeted in patients responding to standard treatment or spontaneously recovering from HCV (own unpublished data). The synthetic HCV peptides in IC41 are adjuvanted with poly-L-arginine, which has been shown to augment Th1/Tc1 (IFN-gamma) responses in animal studies [50–54].

Here, IC41 was investigated in a randomized, placebo controlled trial enrolling 128 HLA A2 positive healthy volunteers. This study was designed to examine the immunogenicity of different doses of IC41 with or without poly-L-arginine as adjuvant. An immunization schedule of four s.c. vaccinations, each 4 weeks apart was chosen on the basis of a previous phase 1 trial (own unpublished data). Here we report on the clinical outcome of this study (safety, overall immunological responses to vaccination), a detailed immunological analysis will be published elsewhere.

2. Methods

2.1. Vaccines

The IC41 HCV vaccine (Intercell AG, Vienna, Austria) consists of defined components: peptide antigens and poly-L-arginine, both synthesized by chemical means to high purity and consistency. In order to minimize viral escape, a pool of five different peptides (Ipep 83, 84, 87, 89, 1426) conserved in the most prevalent HCV genotypes 1a (100%, 100%, 83%,100%, 100% for the respective five peptides), 1b (98%, 90%, 15%, 94%, 88%) and 2 (91%, 96%, 13%, 91%, 87%) was employed. As IC41 harbours besides highly promiscuous T-helper epitopes, HLA-A2 restricted CTL epitopes, only individuals positive for HLA-A2 were enrolled in the study. The prevalence of this marker is 45–50% within Caucasians [55]. For the current study, several doses of IC41, HCV peptide vaccine only, poly-L-arginine only and saline were applied (see Table 1).

2.2. Study design and interventions

This was a single (subject) blind, randomized, controlled parallel group study for dose optimization and to assess safety of a HCV peptide vaccine in healthy subjects. Twelve

Table 1
Description of treatment groups

5 HCV peptides (mg)	Poly-L-arginine (mg)	No. of subjects
Control groups		
0	0	20
0	2.00	12
5.00	0	12
Treatment groups		
0.50	0.25	12
0.50	0.50	12
2.50	0.25	12
2.50	1.25	12
2.50	2.00	12
5.00	0.50	12
5.00	2.00	12
Total number of subjects		128