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Interleukin-12 in the treatment of chronic hepatitis B and C

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Abstract

Interleukin-12 plays a central role in mounting an effective cellular immune response directed towards elimination of intracellular pathogens. In two open label, multicenter, dose-escalation phase I/II studies tolerability, pharmacokinetics, pharmacodynamics, and efficacy of subcutaneously administered recombinant human interleukin-12 (rHuIL-12) was assessed in the treatment of chronic hepatitis B and C. Forty-six patients with chronic hepatitis B and 60 patients with chronic hepatitis C were treated for 12 and 10 consecutive weeks, respectively. rHuIL-12 was generally well tolerated, but was associated with temporary decreases in neutrophils and lymphocyte counts, and with elevations in serum transaminases and bilirubin. Serum IL-12 levels observed were higher at 0.5 μg/kg compared with 0.25 μg/kg doses, suggesting a dose-related increase in systemic exposure of IL-12. Measurable levels of interferon-γ were also observed at the highest dose of 0.5 μg/kg. At the end of treatment HBV DNA clearance was greater in patients treated with 0.50 μg/kg (25%) or with 0.25 μg/kg (13%) compared with those given 0.03 μg/kg. In patients with chronic hepatitis C, HCV RNA remained detectable in all patients. A more than 50% decrease in pretreatment HCV RNA levels was observed in 3/16 patients (0.03 μg/kg), in 3/14 (0.10 μg/kg), in 6/15 (0.25 μg/kg), and in 8/15 patients of the 0.5 μg/kg dose group. In conclusion, antiviral activity of rHuIL-12 in patients with chronic hepatitis B or C does not appear to be advantageous in comparison to other currently available treatments.

Keywords: HBV DNA levels; HCV RNA levels; Interleukin-12; Interferon-γ; Interleukin-10

1. Introduction

Recombinant human interleukin-12 (rHuIL-12) is a heterodimeric cytokine that promotes cell-mediated immunity by facilitating type 1 helper T lymphocyte responses, including the secretion of interferon-γ from both T and natural killer cells, enhancing the lytic activity of natural killer cells, and augmenting specific cytolytic T lymphocyte

Abbreviations: ALT, alanine aminotransferase; CTL, cytotoxic lymphocytes; HBeAg, hepatitis B virus e antigen; HBsAg, hepatitis B virus surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IL, interleukin; IFN, interferon; NK, natural killer; rHu, recombinant human; TNF, tumor necrosis factor; ULN, upper limit of normal.

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responses (Trinchieri, 1994; Scott, 1993). In addition, interleukin-12 can increase the production of some subclasses of IgG antibodies (Germann et al., 1995). Interleukin-12 has been shown to have potent therapeutic effects in a number of animal models of tumors and infectious diseases, including several viral infections (Brunda et al., 1993; Gately and Mulqueen, 1996; Hendrzak and Brunda, 1995).

Because of the central role of interleukin-12 in mounting an effective cellular immune response directed towards elimination of intracellular pathogens, two open label, international multicenter, dose-escalation phase I/II studies were designed to assess tolerability, pharmacokinetics, pharmacodynamics, and efficacy of subcutaneously administered rHuIL-12 in the treatment of chronic hepatitis B and C.

2. Patients and methods

2.1. Study design in chronic hepatitis B

A total of 116 male and female patients aged 18–65 years were screened for this study. Detailed inclusion and exclusion criteria are given elsewhere (Carreno et al., 2000). Forty-eight patients were enrolled: they were HBsAg- and HBV DNA-positive in serum (viremia levels from 10 to 500 pg/ml); they persisted with abnormal ALT values (within five times the upper limit of the normal range documented at least three times within the 6 months screening period); and had chronic hepatitis B without cirrhosis documented in a liver biopsy obtained within 6–18 months prior to inclusion. The patients had to have stable disease as determined by screening HBV DNA and ALT levels not varying more than 50 and 20%, respectively, to avoid spontaneous responses. Two patients who were randomized did not begin treatment: one refused to give consent and another patient was withdrawn from the study due to a very low HBV DNA level at baseline. Eligible patients were randomly assigned to receive rHuIL-12 (Hoffmann La Roche, Nutley, NJ) subcutaneously once weekly for 12 consecutive weeks. Forty-six patients began treatment: group 1 ($n = 15$) was treated with 0.03 μg rHuIL-12/kg body weight; group 2 ($n = 15$) received 0.25 μg/kg; group 3 ($n = 16$) was administered 0.50 μg/kg. There were no significant differences in the baseline characteristics of the patients among rHuIL-12 dose levels. Following completion of 12 weeks of treatment, the patients were monitored without further therapy for an additional 12 weeks (follow-up period).

2.2. Study design in chronic hepatitis C

In eligible subjects serum ALT had to be elevated at least 1.5 times the upper limit of normal, documented for at least 24 weeks before initiation of treatment. A liver biopsy obtained within the previous 12 months before starting treatment, consistent with chronic hepatitis without cirrhosis as determined by the local pathologist, was required. Seventy-two subjects were screened, and 61 patients met the criteria and were enrolled into the study (Zeuzem et al., 1999). Patients with chronic hepatitis C who met the inclusion criteria were assigned to receive rHuIL-12 (Hoffmann La Roche, Nutley, NJ) given subcutaneously once per week for 10 consecutive weeks. Cohorts of 15 patients were entered at each of the four dose levels (0.03, 0.10, 0.25, and 0.5 μg per kg body weight). Cohort I was treated with a fixed dose of 0.03 μg/kg rHuIL-12 weekly. The maximum doses in cohorts II, III, and IV were reached by slow up-titration over the first 3 weeks as follows: cohort II: 0.03, then 0.1 μg/kg weekly; cohort III: 0.03, 0.1, then 0.25 μg/kg weekly; cohort IV: 0.1, 0.25, then 0.5 μg/kg weekly. Following completion of 10 weeks of treatment, patients were monitored without further therapy for an additional 12 weeks.

2.3. Ethics approval

Both studies were approved by the ethics committees at the participating centers according to the Declaration of Helsinki, and all patients gave written informed consent before enrollment.

2.4. HBV study objectives

The primary efficacy parameter was serum HBV DNA levels at the end of treatment as assessed by liquid hybridization assay (Abbott Labs., North Chicago, IL). Patients were considered to be responders to the study medication if there was either a loss of detectable serum HBV DNA or a 50% or greater decrease in the level of HBV DNA as compared to the baseline, where the baseline HBV DNA was defined as the mean of the three screening levels (at weeks from −13 to −7; week −4; and at the baseline). The secondary study parameters were ALT levels and changes in viral markers (HBeAg, anti-HBe).

2.5. HCV study objectives

The primary efficacy parameter was the response rate at the end of treatment, based on serum HCV RNA levels (Amplicor Monitor HCV™, Roche Diagnostic Systems, Branchburg, NJ). Patients were considered to be responders to the trial medication if there was a 50% or greater decrease in the level of HCV RNA as compared with baseline, where the baseline HCV RNA was defined as the mean of both screening levels. As a secondary efficacy parameter, serum ALT levels were evaluated.

2.6. Pharmacokinetic and -dynamic parameters, anti-rHuIL-12

Further objectives of both studies were to determine the pharmacokinetic and pharmacodynamic profile of rHuIL-12 and the formation of anti-interleukin-12 antibodies during and after subcutaneous administration of rHuIL-12. Serum pharmacodynamic markers included interferon-γ and interleukin-10, which were analyzed by enzyme or radio immunoassays in a central laboratory (ANAWA Lab., Wangen, Switzerland). Anti-rHuIL-12 antibody was measured by enzyme immunosorbent assay (Covance Lab., Vienna, VA).

3. Results

3.1. Efficacy of rHuIL-12 in chronic hepatitis B

No decrease in HBV DNA concentrations was observed in patients treated with 0.03 μg/kg/week rHuIL-12 (Fig. 1). In contrast, HBV DNA concentrations were significantly lower at the end of treatment with respect to the basal values in patients given rHuIL-12 doses of 0.25 and 0.5 μg/kg/week ($P < 0.05$; Fig. 1). At the end of treatment, the virological response was greater, although not statistically significant, in the patients administered 0.50 μg/kg (10/16, 62%) or 0.25 μg/kg (6/15, 40%) compared with those administered 0.03 μg/kg (5/15, 33%). This trend remained the same at the end of follow-up.

Before treatment, 37 patients had HBeAg and 9 anti-HBe. At the end of rHuIL-12 treatment, 3 patients had lost HBeAg (1 in the 0.03 μg/kg dose group and 2 in the 0.50 μg/kg dose group). At the end of follow-up HBeAg was undetectable in 5 patients (14%); all of them seroconverted to anti-HBe: 2/15 from the 0.25 μg/kg group and 3/16 from the 0.50 μg/kg group; HBeAg reappeared during the follow-up in the

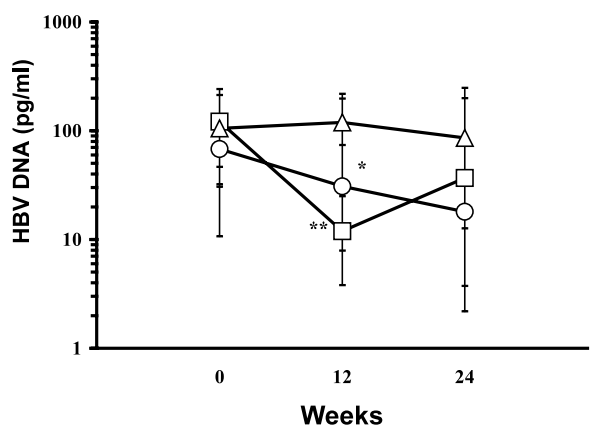

Fig. 1. Serum HBV DNA levels (expressed as the median and the 25th through 75th percentile) at the start, at therapy cessation (12 wks.), and at the end of follow-up (24 wks.) in the three rHuIL-12 dose levels of treatment: △, 0.03 μg/kg/week ($n = 15$); ○, 0.25 μg/kg /week ($n = 15$); □, 0.50 μg/kg/week ($n = 16$). *$P = 0.036$ with respect to the baseline level. **$P = 0.015$ with respect to the baseline level.

patient treated with 0.03 μg/kg who lost the antigen during therapy. According to the HBe status at entrance, HBV DNA clearance at the end of follow-up occurred in 6/37 (16%) patients with HBeAg and 1/9 (11%) with anti-HBe.

No significant differences were observed when comparing ALT levels at the baseline and at the end of rHuIL-12 therapy (mean + S.D.: $3.0 + 1.3 \times$ ULN vs. $2.7 + 1.9 \times$ ULN). ALT normalization was observed in four patients at the end of rHuIL-12 treatment: two in the 0.03 μg/kg group and two in the 0.5 μg/kg group. At the end of the follow-up period, seven patients had normal ALT values: 2/15 (13%) from the 0.03 μg/kg group; 1/15 (7%) from the 0.25 μg/kg group; and 4/16 (25%) from the 0.5 μg/kg group. ALT normalization together with a sustained clearance of HBV DNA and HBeAg was observed at the end of the follow-up in three patients (one in each rHuIL-12 dose level); two of them seroconverted to anti-HBe.

3.2. Efficacy of rHuIL-12 in chronic hepatitis C

The median pretreatment viremia in the 0.03 μg/kg dose group ($n = 16$) was 4.7×10^5 copies/ml. Four weeks after initiation of treatment, at the end of treatment and after 12 weeks of follow-up HCV RNA levels were 3.7×10^5, 5.8×10^5 ($P = 0.81$ vs. baseline), and 3.0×10^5 copies/ml, respectively. In the higher rHuIL-12 dose groups pretreatment, 4 and 10 weeks treatment and follow-up HCV RNA levels were 3.2×10^5, 3.0×10^5, 3.7×10^5 ($P = 0.83$ vs. baseline), and 4.4×10^5 copies/ml (0.1 μg/kg, $n = 14$), 5.1×10^5, 3.1×10^5, 3.7×10^5 ($P = 0.02$ vs. baseline), and 4.1×10^5 copies/ml (0.25 μg/kg, $n = 15$), and 5.6×10^5, 1.6×10^5, 2.8×10^5 ($P = 0.13$ vs. baseline), and 1.6×10^5 copies/ml (0.5 μg/kg, $n = 15$), respectively (Fig. 2). At the end of treatment a more than 50% decrease in pretreatment HCV RNA levels was observed in 3/16 patients of 0.03 μg/kg dose group, in 3/14 of the 0.10 μg/kg dose group, in 6/15 of the 0.25 μg/kg dose group, and in 8/15 patients of the 0.5 μg/kg dose group.

Although in several cases serum ALT levels decreased either during or after treatment, ALT normalization was observed in only four patients at the end of treatment and in five patients at the end of follow-up.

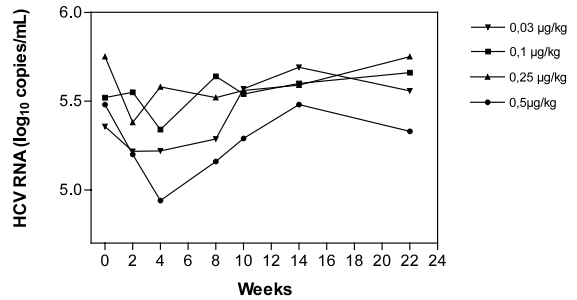

Fig. 2. Mean serum HCV-RNA levels in patients treated with human recombinant interleukin-12. Patients with chronic hepatitis C received either 0.03, or a maximum of 0.10, 0.25 or 0.5 μg/kg rHuIL-12 subcutaneously once weekly for 10 consecutive weeks. Subsequently, patients were monitored without further therapy for an additional 12 weeks.

3.3. Pharmacokinetics of rHuIL-12

The pharmacokinetics of rHuIL-12 was evaluated in 18 patients: 8 were administered the 0.25 μg/kg dose and 10 were administered the 0.50 μg/kg dose. The maximum serum concentration (C_{max}) of IL-12 in patients receiving the 0.25 μg/kg dose ranged from 55 to 130 pg/ml. The corresponding C_{max} at the 0.50 μg/kg dose ranged from 41 to 315 pg/ml occurring at about 8–12 h after rHuIL-12 administration. The estimated serum half-life of rHuIL-12 was at least 12 h.

3.4. Pharmacodynamics of rHuIL-12

Serum interferon-γ was undetectable at the 0.25 μg/kg dose in six of eight patients in whom it was measured. In the 0.25 μg/kg cohort interleukin-10 was not detectable at baseline. The maximum IL-10 increase ranged from < 26 to 54 pg/ml and < 26 to 87 pg/ml in weeks 3 and 10 after administration, respectively. At the dose of 0.5 μg/kg, serum interferon-γ was measurable in seven of ten patients studied. The maximum concentration ranged from 17 to 50 pg/ml at 24 h after dosing. In three patients of the 0.5 μg/kg cohort, interleukin-10 was detectable at baseline (approx. 30 pg/ml). The maximum IL-10 increase ranged from 30 to 158 pg/ml and 38 to 153 pg/ml after administration in weeks 3 and 10, respectively.

3.5. Antibodies to rHuIL-12

Only one HBV-infected patient who did not respond to the 0.25 μg/kg dose developed low level anti-rHuIL-12 antibodies. In two HCV-infected patients an increase of anti-rHuIL-12 antibody levels by one dilution factor from first to last sample was observed.

3.6. Adverse events

The most frequent clinical adverse events seen during treatment with rHuIL-12 were fever, fatigue, chills, headache, myalgia, dizziness, nausea, coughing and rhinitis. In addition, vomiting, insomnia and buccal mucosal ulceration were seen in the higher dose groups. Local reactions that were dose-unrelated were observed in several patients at the site of injection. The most frequent laboratory abnormalities were transient decreases in leukocyte counts and transient increases of aminotransferases and occasionally bilirubin, most of which returned to baseline during treatment.

Three HBV-infected patients discontinued rHuIL-12 treatment, including a female administered five injections of 0.50 μg/kg after an 11-fold increase in ALT values compared with baseline ($40 \times$ ULN); the event was considered as related to rHuIL-12 therapy. Another patient discontinued treatment prematurely due to buccal mucosal ulceration after the 10th dose of rHuIL-12. A third patient discontinued treatment after 8 weeks, due to an episode of atrial fibrillation, which was treated and resolved.

In a 48-year-old HCV-infected female, rHuIL-12 treatment (0.03 μg/kg) was prematurely discontinued due to hematuria. Urological examinations were unremarkable and hematuria resolved. Two additional patients with chronic hepatitis C experienced serious adverse events. A 57-year-old male treated with 0.5 μg/kg rHuIL-12 developed a coughing fit and breathlessness, which resolved after antihypertensive and antiobstructive treatment. The second patient with a serious adverse event was a 36-year-old male treated with 0.03 μg/kg for 10 weeks who experienced abdominal pain 1 week after the end of therapy.

4. Discussion

The antiviral effects of IL-12 have been studied in mice infected with lymphocytic choriomeningitis virus (Orange et al., 1994, 1995a), murine cytomegalovirus (Orange et al., 1995b), herpes simplex virus type 1 and type 2 (Gately and Mulqueen, 1996), vesicular stomatitis virus (Bi et al., 1995), encephalomyocarditis virus (Ozmen et al., 1995), and in a model of retrovirus-induced murine acquired immunodeficiency syndrome (Gazzinelli et al., 1994). In each of these models, IL-12, when used at an optimal dose, had beneficial effects, as measured by reduced viral load, improved survival, or both.

The CTL response against HBV plays a major role in controlling the infection. It is directed towards a diversity of virus epitopes and its vigor is crucial for the resolution of HBV infection (Chisari and Ferrari, 1995). Current therapies are aimed at reducing the virus load and at the same time at improving the patient's immune system. The CTL activity is weak or absent in the peripheral blood of patients chronically infected by HBV (Rehermann et al., 1996) and the intrahepatic T cells show a mixed Th1/Th2 (Th0) cytokine profile (Bertoletti et al., 1997). In this context, IL-12 may contribute to upregulate Th1 cell responses; and, to exert antiviral effects through HBV antigen-specific CTL responses and the liberation of virucidal substances, such as interferons (Fernández et al., 1997) and TNFα (Barnaba et al., 1994). In HBeAg-transgenic mice, IL-12 treatment following immunization with an HBe peptide was able to decrease anti-HBe antibody production and to break HBeAg T-cell tolerance (Milich et al., 1995), thus promoting potent immunotherapeutic activity (Shimizu et al., 1998). Furthermore, virus replication is suppressed in the liver of HBV-transgenic mice via a non-cytolytic mechanism that is mediated by IFNγ and TNFα as a result of CTL recognition of HBV antigen (Cavanaugh et al., 1997). Moreover, the importance of IL-12 in HBV clearance has been reported recently in patients with chronic hepatitis B undergoing treatment with IFNα (Rossol et al., 1997).

In the present phase I/II study serum HBV DNA levels decreased significantly at the end of rHuIL-12 treatment and after the follow-up compared with the baseline in patients treated with 0.50 μg/kg or 0.25 μg/kg, but not in those given 0.03 μg/kg. Moreover, loss of HBeAg and seroconversion to anti-HBe was only noted at the end of the follow-up in patients from the 0.25 μg/kg and the 0.50 μg/kg dose groups. However, the results obtained in this pilot study with rHuIL-12 seem less in comparison with those achieved with IFNα or with nucleoside analogues.

A striking clinical feature of hepatitis C virus infection is that approximately 70–80% of patients with acute hepatitis C will develop chronic infection. Data show that specific $CD4^+$ T-cell clones from patients with self-limited infection predominantly secreted Th1-type cytokines (Diepolder et al., 1995; Tsai et al., 1997) and that activation of Th2 responses in acute hepatitis C patients may play a role in the development of chronicity (Tsai et al., 1997). Virus-specific CTL recognizing epitopes in the variable regions of either the envelope or non-structural proteins have also been isolated from the livers of patients with chronic hepatitis C (Koziel et al., 1992). The ability of IL-12 to enhance cell-mediated immunity through its effects in promoting Th1 responses, inducing IFN-γ production by both T and NK cells, and augmenting specific CTL responses suggested an utility in the treatment of chronic hepatitis C. Furthermore, interferon-α treated patients with a virological end-of-treatment response revealed higher IL-12 serum levels and produced more IL-12 by peripheral blood mononuclear cells than non-responders (Quiroga et al., 1998). Because of the lack of an appropriate animal model, the efficacy of IL-12 for treatment of HCV could only be investigated by a clinical trial.

Despite evidence that the in vivo antiviral activity of interleukin-12 is at least in part mediated by IFN-γ, no profound antiviral effect of rHuIL-12 was observed in patients with chronic hepatitis C. The decline of serum HCV-RNA at week 10 of treatment compared with baseline was statistically significant in the 0.25 μg/kg dose cohort. However, the median decline from 5.1×10^5 to 3.7×10^5 copies/ml is not clinically relevant. The reasons for the response failure remain unknown. Interestingly, HCV quasispecies analyses by single-strand conformation polymorphism in patients treated with rHuIL-12 showed substantial changes in the band pattern indicating that rHuIL-12 exerts limited antiviral activity against certain HCV quasispecies in vivo (Lee et al., 2000).

In general, subcutaneous administration of rHuIL-12 was well tolerated. Flu-like symptoms were the most frequent adverse clinical events seen during treatment. The most frequent laboratory abnormalities were transient decreases in leukocyte counts and transient increases of aminotransferases and bilirubin. Histological examination of livers from IL-12-treated animals revealed randomly distributed, multifocal parenchymal infiltrates composed of cells of lymphocytic (i.e. mainly NK cells and $CD8^+$ T cells) and/or monocytic lineages. The mononuclear infiltrates were very often associated with liver necrosis (Gately et al., 1994a,b; Sarmiento et al., 1994). However, in toxicology studies in chimpanzees infected with HBV or HCV, administration of IL-12 did not result in acute fulminant hepatitis (Gately, 1997).

In conclusion, the level of antiviral activity of rHuIL-12 in patients with chronic hepatitis B or C does not appear advantageous over other currently available treatments. To improve efficacy, other treatment schedules (two or three times per week) should be studied in future trials. However, application of higher interleukin-12 doses may be restricted by potential side effects including liver toxicity. The recent observation that interferon-α up-regulates the expression of IL-12 receptor on Th1 cells raises the question of whether additional trials investigating the potential antiviral synergy of interleukin-12 and interferon-α may be worthwhile.

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