INT. J. IMMUNOTHERAPY **XIX**(2-4) 55-59 (2003)

EFFICACY AND SAFETY OF THE DRUG UKRAIN IN CHRONIC HEPATITIS C PATIENTS

SOLOGUB T.V.,[1] VOLTCHEK I.V.,[2] KIVISEPP N.A.,[1] GRIGORYEVA T.[1]

1) Department of Infectious Diseases, St. Petersburg State Medical Academy, St. Petersburg, Russia.
2) Terra Medica Ltd., St. Petersburg, Russia.

Summary: *The aim of this study was to compare the therapeutic effect of the drug Ukrain, a semisynthetic compound derived from* Chelidonium majus L. *alkaloids and thiophosphoric acid triaziridide, with that of recombinant human interferon (IFN)-alpha$_{2b}$ in the treatment of chronic hepatitis C. Seventy-five chronic hepatitis C patients received Ukrain at a single dose of 0.5 mg, 1.0 mg, 2.5 mg or 5.0 mg intravenously, or 1.0 mg subcutaneously, while two control groups of 25 patients each were treated with either 3 million IU IFN-alpha$_{2b}$ or with only basic therapy. Results suggest that Ukrain therapy was more effective than IFN-alpha$_{2b}$ monotherapy, and that smaller doses were the most effective.*

Introduction

There are more than 350 million chronic viral hepatitis B (HBV) carriers and 170 million chronic viral hepatitis C (HCV) carriers in the world (1). The epidemiological characteristics of HBV and HCV, including mechanism, modes and factors of transmission, and risk groups, *etc.,* allow simultaneous infection and a combined disease course (mixed infection), often transforming into the chronic form (2). Chronic inflammation caused by hepatitis B or hepatitis C viruses or, especially, by mixed infection, leads ultimately to chronic diseases of the liver (CDL): chronic hepatitis (HC), cirrhosis of the liver (LC) or liver cell carcinoma (LCC) (3, 4).

Numerous publications in recent years have presented data on the use of antiviral drugs to suppress viral replicative activity (5, 6). It is common to use interferon (IFN) preparations in standard therapeutic dosage in cases of HCV infection. However, at present nearly all researchers note the low efficacy of IFN monotherapy and the high incidence of toxicity (7-9).

Resistance to IFN in a considerable proportion of patients with HC also prompts a search for non-IFN therapy (10, 11). In a previous study we revealed the

Address for correspondence: I.V. Voltchek, Terra Medica Ltd., Shpalernaya Str., 32-19, St. Petersburg, 191123, Russia.
Tel: +7 812 2726974 Fax: +7 812 2740862
E-mail: ivoltchek@terramedica.spb.ru

0255-9625/2003/2-4 00055+4 $02.50/0

antiviral efficacy in chronic hepatitis C of the drug Ukrain, a semisynthetic compound derived from *Chelidonium majus L.* alkaloids and thiophosphoric acid triaziridide (12). All the above considerations encouraged us to explore further the possible use of Ukrain in the therapy of chronic hepatitis C.

Patients and methods

The treatment center for the study was the Department of Hepatology of the S.P. Botkin Infectious Diseases Hospital, St. Petersburg, Russia. Ukrain (Nowicky Pharma, Vienna, Austria) was administered intravenously or subcutaneously at various doses to patients in the study groups. Recombinant human IFN-alpha$_{2b}$ (Reaferon®: Vector, Novosibirsk, Russia) was administered to one control group at the standard dose. The effects of therapy were monitored by clinical examination, biochemical testing, polymerase chain reaction (PCR) testing and ultrasound investigation.

Full information about the proposed nature of the treatment was given, and written informed consent was obtained from each patient. The study followed ethical guidelines set down in the Helsinki Declaration.

Patients with verified chronic hepatitis C included in the investigation ranged in age from 18 to 55 years, and included both males and females.

The groups of patients receiving both traditional therapy and Ukrain (the study groups) totaled 75 patients. Of these, 15 patients received Ukrain intravenously at a dose of 0.5 mg (group A); 15 received a dose of 1.0 mg i.v. (group B); 15 received a dose of 2.5 mg i.v. (group C); 15 received a dose of 5.0 mg i.v. (group D); and 15 received a subcutaneous dose of 1.0 mg (group E). The control groups of patients not receiving Ukrain comprised: 25 treated with Reaferon® (group 1); and 25 receiving basic therapy (group 2) (Table I).

Exclusion criteria were: (i) age $<$ 18 years or $>$ 55 years; (ii) immunosuppressive therapy, steroid hormones in immunosuppressive dosage, immune response modifiers, IFN drugs or inducers, or other antiviral remedies in the 4-month period prior to inclusion in the clinical study; (iii) concomitant diseases: alcoholism, diabetes, tuberculosis, cancer of any stage, AIDS or mental disorders.

Patients received Ukrain at doses of 0.5, 1.0, 2.5 or 5.0 mg by intravenous injection three times a week for 3 months, or at a dose of 1.0 mg administered by subcutaneous injection three times a week for 3 months. Control group 1 received therapy with Reaferon® administered in a 3 million IU dose by intravenous injection three times a week for 3 months. Basic therapy in the control group 2 consisted of enzymes and vitamins.

Table I *General patient characteristics by treatment group*

Group	Treatment	No. patients	Male	Female	Age
Study group A	Ukrain 0.5 mg i.v.	15	9 (60%)	6 (40%)	31.0 ± 1.48
Study group B	Ukrain 1.0 mg i.v.	15	9 (60%)	6 (40%)	32.1 ± 1.8
Study group C	Ukrain 2.5 mg i.v.	15	10 (67%)	5 (33%)	34.3 ± 1.12
Study group D	Ukrain 5.0 mg i.v.	15	9 (60%)	6 (40%)	28.8 ± 2.5
Study group E	Ukrain 1.0 mg s.c.	15	10 (67%)	5 (33%)	29.1 ± 1.8
Control group 1	Reaferon® 3 mln IU	25	18 (72%)	7 (28%)	33.3 ± 1.4
Control group 2	Basic therapy i.v	25	23 (92%)	2 (8%)	29.0 ± 1.2

Patients in both the study and control groups were comparable in age, sex and presence of concomitant diseases. Safety of the study drug was estimated on the following parameters: clinical signs during treatment; local and systemic symptoms such as local irritation, temperature reactions, skin condition, general well-being, *etc.*; patient's opinion of the drug safety; blood count (white blood cell [WBC], red blood cell [RBC], platelets, blood sedimentation rate [BSR]); and biochemistry (bilirubin, alanine aminotransferase [ALT], aspartate aminotransferase [AST], *etc.*).

HCV-RNA was measured by qualitative reverse transcriptase-polymerase chain reaction (RT-PCR) using diagnostic kits Amplisense HCV-240/VKO-440 (Central Research Institute of Epidemiology, Ministry of Health of Russian Federation, Moscow, Russia) and amplificator Tercyc (DNA-Technology, Moscow, Russia) in accordance with the suppliers' instructions.

Clinical response to Ukrain therapy was estimated on the basis of clinical, biochemical and immunological parameters as follows: (i) complete response: AST and ALT levels decrease or no longer increase, no clinical symptoms of disease and no HCV-RNA in PCR assay; (ii) partial response: AST and ALT initial levels decrease, or no HCV-RNA in PCR assay; (iii) no response: AST and/or ALT levels do not stabilize (and may increase), and replicative viral activity is shown by PCR assay.

Fisher's exact test was used for statistical calculation.

Before treatment was started, histopathological assessment of liver inflammation had been performed. No patients had clinical signs of liver cirrhosis.

Results

In most cases patients of all groups had no complaints before treatment began. However, some patients had periodic malaise, anorexia and dull pain in the right upper abdomen. Following the first injections of Ukrain, nearly all patients were already reporting marked subjective improvements in general well-being (Table II).

In study group A, mean bilirubin levels before the start of therapy were 17.6 $\pm$ 2.71 μmol/l, and after treatment 18.42 $\pm$ 0.84 μmol/l; the activity of serum ALT changed from 159.24 $\pm$ 25 to 141.0 $\pm$ 32.9 IU/l. Complete positive response to treatment, according to the specified criteria, was observed in seven patients (46.7%), including three cases of genotype 1b (Table IV). Partial response, with a negative PCR HCV assay, was observed in two patients (13.3%), both of genotype 1b (Table IV). In two PCR HCV positive patients, the ALT level decreased. In four patients (26.7%) there was no response to treatment. Intravenous Ukrain injection at the smallest dose (0.5 mg) was therefore sufficient to achieve a favorable virologic response (60.0%) (Table III). Deterioration of biochemical parameters occurred in patients with active chronic hepatitis and ALT levels exceeding upper limits 5- to 7-fold. Biochemical deterioration was not observed in patients with low initial inflammation activity.

In study group B, treated with 1.0 mg single dose of Ukrain, a complete positive response to treatment was observed in six patients (40.0%). Partial positive virologic response was observed in three patients (20.0%). No response to therapy was noticed in five

Table II *Changes in clinical symptoms in patients with HCV infection*

	Number of patients with clinical symptoms	
Group	Before treatment	After treatment
Study group A	18	9
Study group B	22	11
Study group C	25	17
Study group D	10	10
Study group E	22	11
Control group 1	24	16
Control group 2	20	10

Table III *Response to therapy according to the HCV-RNA assay value*

Group	Patients with negative HCV-RNA value after treatment (%)
A	60.0*
B	60.0*
C	40.0**
D	40.0**
E	33.3
Control 1	24.0
Control 2	8.0

*$p < 0.01$ and **$p < 0.05$ *versus* control group 1 (Fisher's exact test).

patients (33.3%), including one patient whose ALT activity increased during treatment. The reason for the increase in ALT activity in this one case is not known (Table IV).

Analyzing the data from study group C, in which Ukrain was injected at a single dose of 2.5 mg, it is noticeable that a complete response was achieved in only four patients (26.7%). A partial response was observed in five patients (33.3%): the PCR HCV assay of two patients was negative, and ALT activity decreased in three patients. Six patients (40%) showed no response (Table IV). Mean bilirubin value in the group was 16.0 ± 2.0 μmol/l before treatment and 16.5 ± 3.0 μmol/l after treatment. The ALT index was considerably changed during therapy: within 1 month of the start of therapy, the ALT value decreased in half of the patients by 36%, from 153.5 ± 19.0 IU/l, to 98.3 ± 17.5 IU/l after therapy. ALT value increased in 16.6% of patients, by an average of 20%. ALT activity remained at approximately the same level in 33.3% of patients. These data allow us to conclude that using Ukrain at a single dose of 2.5 mg does not cause the development of immune cytolytic activity in the majority (83.3%) of patients with chronic viral hepatitis C.

In study group D, receiving Ukrain at a single dose of 5.0 mg, a complete positive response was achieved in five patients (33.3%). A partial response, manifesting only as normalization of ALT activity, was observed in three (20%) patients. The ALT value increased 2-fold in one patient. In six patients (40%), no positive response was achieved (Table IV). In this group, the mean level of ALT before treatment was 186.1 ± 28.7 IU/l, and after treatment 143 ± 34.1 IU/l. Analysis of the ALT data revealed that, after 3 months' therapy, ALT had decreased in 60.0% of patients by an average of 1.6-fold, increased in 6.7%

Table IV *Summary of drug efficacy evaluation*

		HCV-RNA response		ALT response		Complete response		No response	
Treatment	No. patients	*n*	%	*n*	%	*n*	%	*n*	%
Ukrain									
0.5 mg i.v.	15	2	13.3*	2	13.3	7	46.7*	4	26.7*
1.0 mg i.v.	15	3	20.0*	1	6.7*	6	40*	5	33.3*
2.5 mg i.v.	15	2	13.3*	3	20.0	4	26.7	6	40.0*
5.0 mg i.v.	15	1	6.7	3	20.0	5	33.3*	6	40.0*
1.0 mg s.c.	15	1	6.7	5	33.3*	4	26.7	5	33.3*
Reaferon® i.v.	25	1	4.0	4	16.0	5	20.0	15	60.0
Basic therapy i.v.	25	0	0	12	48.0	2	8.0	11	44.0

*$p < 0.05$ *versus* control group 1 (Fisher's exact test).

by an average of 1.4-fold, and in 33.3% had remained at approximately the same level.

In the patients of study group E, receiving a subcutaneous injection of Ukrain at a single dose of 1.0 mg, complete response was observed in four cases (26.7%), and a partial response with decreased ALT activity in five patients (33.3%); in one patient, the HCV genotype was not detectable after treatment, but the ALT level had not decreased. In five patients (33.3%), no response was observed. The mean activity of ALT before treatment was 168.0 ± 28.9 IU/l, and after therapy 125.0 ± 31.2 IU/l. Three months after the start of therapy, ALT values had decreased in 60.0% of patients by an average of 2.1-fold, increased in 16.6% by an average of 1.2-fold, and remained unchanged in 33.3% of patients.

Discussion

Analyzing the therapeutic efficacy of Ukrain at the different doses, it is possible to conclude that the optimum single dose at the high activity of the inflammation, estimated according to serum ALT activity, is 1.0 mg. The data of PCR assay (Table III) also suggests that low-dosage Ukrain therapy is preferable.

In comparison with the study groups, the rate of PCR HCV negative results in control group 1 after 3 months of treatment was lower (24.0%). Complete positive response in this group occurred in five cases (20%), and partial response in five cases (20%): ALT decreased in four patients, while in one patient PCR HCV became negative, although ALT did not decrease. There was no response in 60% of cases. Mean levels in biochemical tests did not differ from other groups. In 36% of patients, ALT decreased 2.4-fold after therapy, in 52% ALT increased 2-fold, and in 12% ALT was the same as before the treatment. The rate of PCR HCV negative blood tests of patients in control group 2 (basic therapy only) after 3 months' treatment was 8% lower than in control group 1 (Table III).

Our study revealed Ukrain to be an effective and safe drug in the treatment of chronic viral hepatitis C.

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