DRUGS EXPTL. CLIN. RES. **XXVI**(5/6) 261-266 (2000)

PRELIMINARY RESULTS OF INDIVIDUAL THERAPY OF CHRONIC HEPATITIS C BY UKRAIN AND INTERFERON-α

VOLTCHEK I.,[1] SOLOGUB T.,[2] NOWICKY J.W.,[3] GRIGORYEVA T.,[2] BELOZYOROVA L.,[2] BELOPOLSKAYA M.,[2] SEMENYAKO N.,[2] LAMANOVA E.[2]

1) Terra Medica Ltd., St. Petersburg, Russia.
2) Mechnikov Medical Academy, St. Petersburg, Russia.
3) Ukrainian Anti-Cancer Institute, Vienna, Austria.

Summary: *The effects of Ukrain and recombinant human interferon-α_{2b} (IFN) on the state of the thiol-disulfide ratio (SH/SS) of the blood (Russian Federation patent no. 2150700) were studied* in vitro *using the amperometric titration method. The blood of 73 chronic hepatitis C (CHC) hepatitis C virus (HCV)-RNA-positive patients was examined. Ukrain was tested in doses of 0.05-2.0 μg/ml and IFN in 20-1000 U/ml of blood. After* in vitro *examination, 59 patients were treated: 28 with Ukrain and 31 with IFN. The first group of 16 patients (including eight with HCV genotype 1b) was treated with individually selected optimal doses of Ukrain (0.5-2.5 mg every second day). The second group of 12 patients was treated with doses of 2.5 mg Ukrain independent of* in vitro *test results. The third group of 31 patients was treated with individually selected optimal doses of IFN (0.5-2 MU 3 times a week). It was found that 79.4% of CHC patients were sensitive to Ukrain* in vitro *and 65.1% were sensitive to IFN. CHC patients with genotype 1b were sensitive to IFN only in 16.7% of cases while the figure for Ukrain was 92.3%. CHC patients with other HCV genotypes (3, 1a, 2) were sensitive to Ukrain in 86.7% of cases and to IFN in 70.6%. After 1month of individual therapy with Ukrain, 87.5% of CHC patients, including six of eight cases with HCV genotype 1b, became PCR-HCV negative. In the group receiving the standard dose of Ukrain, virological response was only 33.3%. After 1 month, 74.2% of CHC patients treated with individual doses of IFN became PCR-HCV negative and after 3 months 90.3% were PCR-HCV negative. The prognostic significance of the method for screening preparations for the treatment of CHC patients was 89.8%. Treatment with Ukrain was without serious negative effects and the number of side effects of IFN in individual therapy was significantly reduced. Ukrain can be used in the treatment of CHC patients, alone or in combination with IFN preparations; in the cases with HCV genotype 1b Ukrain seems more promising than IFN. Individual therapy with Ukrain and IFN increased the efficacy of treatment 2.5-fold in comparison with standard monotherapy with the same preparations, significantly decreased the number of side effects and dramatically improved cost-effectiveness.*

Introduction

The treatment of acute and especially chronic hepatitis C (CHC) infection has remained a serious problem for public health throughout the world. For

Address for correspondence: I.V. Voltchek, Terra Medica Ltd., Zanevsky pr., 1, offices 253-255, 195196, St. Petersburg, Russia.
Tel/fax: +7-812-4441051
E-mail: terra_medica@actor.ru

0378-6501/2000/5/6 00261+6 $02.50/0

many years the standard treatment for hepatitis C was monotherapy with interferon-α (IFN-α) preparations in standard doses of 3-5-10 MU. Recently many works have appeared, however, which demonstrate this treatment's poor efficacy against CHC. In one study, the sustained virological response to treatment by 3 MU IFN was only 6% after 6 months of treatment and 13% after 12 months (1). The efficacy of IFN monotherapy of CHC caused by hepatitis C virus (HCV) with genotype 1 (a or b), which is the most common type in Western Europe and the United States, is even lower at 2-7% according to the same authors. At the same time, monotherapy with standard doses of IFN causes side effects and complications in up to 72% of cases (1) and the cost of therapy is rather high.

In recent years, the standard therapy for CHC has become IFN in combination with ribavirin, which increases the efficacy of therapy by 25-45%. However, efficacy in patients with HCV 1 (a or b) is still low at 16-28% (1). Adding ribavirin increases the incidence of side effects and makes therapy even more expensive.

It is therefore necessary to search for new methods and drugs for the therapy of CHC. Ukrain (a semisynthetic compound derived from *Chelidonium majus L.* alkaloids and thiophosphoric acid triaziridide) is a biological response modifier and cancerostatic drug which is useful in complementary medicine for the treatment of cancer patients. In previous investigations we (2) and other authors (3) obtained data on the antiviral properties of Ukrain in experimental infection by influenza viruses. There are works on the cytotoxic effect of Ukrain to HIV-infected cells (4) and the clinical effect in AIDS patients with Kaposi's sarcoma (5). There are no data on the efficacy of Ukrain in hepatitis C infection.

However, in our opinion, the low efficacy of CHC treatment may be explained by the absence of an individual approach to the treatment of patients (6). A method for screening medicines based on measuring the thiol-disulfide (SH/SS) ratio (7) has been developed to optimize the treatment of patients by selecting the best preparation and its dose *in vitro* before therapy. This new method was used in our investigation.

Patients and methods

The effects of Ukrain (NSC-631570, Nowicky Pharma, Austria) and recombinant human interferon-α_{2b} (IFN, Reaferon, Vector, Russia and Intron A, Schering Plough, USA) on the state of the thiol-disulfide ratio (SH/SS) of the blood (Russian Federation patent no. 2150700) were studied *in vitro* by the amperometric titration method (8).

Firstly, 10 ml of venous blood was put in a container with an anticoagulant (heparin). Then, 1 ml of the blood was added to the probes (usually eight depending on the number of test preparations and their doses). The test preparations were added to the probes in the required doses (doses *in vitro* were calculated as 1:5000 of therapeutic doses). The preparations were diluted in an isotonic solution of NaCl and this was added to the control probes. All probes were then incubated at 37 °C for 1 h. After incubation the cell fraction of the blood was hemolyzed in 0.1% solution of Trilon B (Khimreactiv, St. Petersburg, Russia; pH 7.0); 0.2 ml blood cells and 1.8 ml Trilon B were mixed and stored at 4 °C for 30 min. The tubes were then centrifuged at 8000 n/min for 15 min for sedimentation of the blood cell stroma. The surface fluid (hemolysate) was used in the study. Titration was carried out with an Analizator for Thiol Antioxidants (ATA, IVSOVT Ltd., St. Petersburg, Russia). The titration of 0.2 ml hemolysate was carried out in 25-30 ml ammonium buffer by adding 0.05 (0.1) ml 1 x 10^{-3} M solution $AgN0_3$ (Uralsky chemoreactive plant, Russia). The SH content was estimated by the direct amperometric titration method, the SS content by an indirect method after adding Na_2SO_3 to divide the SS connections.

The effect of the preparations and their doses was estimated in comparison with control probes incubated only with the isotonic solution of NaCl (7, 8).

The blood of 73 CHC HCV-RNA-positive patients was examined. Ukrain was tested in doses of 0.05, 0.1, 0.2, 0.5, 1.0 and 2.0 μg/ml of blood corresponding to *in vivo* injections of doses of 0.25, 0.5, 1.0, 2.5, 5.0 and 10.0 mg. IFN was examined in doses of 20, 50, 100, 200, 400, 600 and1000 U/ml corresponding to *in vivo* injections of 0.1, 0.25, 0.5, 1.0, 2.0, 3.0 and 5.0 MU.

Fifty-nine CHC patients aged 33.5 ± 8.2 years were selected for the clinical trial (38 males, 21 females). Ukrain was used for the treatment of 28 patients in doses of 0.25- 2.5 mg by intravenous injection every second day. A first group of 16 patients (including eight cases of HCV 1b) received therapy with Ukrain in individual optimal doses (after *in vitro* testing). A second group of 12 patients was treated with doses of 2.5 mg Ukrain independent of *in vitro* test results. In the first and second treatment groups, 14 patients received Ukrain in doses of 0.25-1.0 mg and the other 14 in doses of 2.5 mg. A third group of 31 patients was treated with individually selected optimal doses of IFN at 0.5-2.0 MU by intramuscular injection three times a week. Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and HCV-RNA by polymerase chain reaction (PCR) were examined before therapy and after 1 and 3 months of therapy. All 59 CHC patients were HCV-RNA-positive before therapy.

All patients were treated with Ukrain and IFN at the Clinic of Infectious and Tropical Diseases, Mechnikov Medical Academy, St. Petersburg, Russia. Full information was given about the kind of treatment to be performed and it was carried out with patients' consent. The study followed the ethical guidelines according to the Declaration of Helsinki.

Results and discussion

A positive response with an increased SH/SS ratio in CHC patients' blood was more often registered after adding Ukrain in doses corresponding to *in vivo* injections of 0.5, 1.0 and 2.5 mg of the preparation (Table I). This is highly significant because larger doses are used in the treatment of cancer patients (from 5-25 mg per injection). The rate of negative responses to various doses of Ukrain was rather high at 40-60%.

Very interesting results were obtained by testing IFN: a positive response was also registered more often with low doses of the preparation, corresponding to *in vivo* injections of 1.0, 2.0 and 0.5 MU (Table II). Standard doses of IFN (3.0 and 5.0 MU) produced a positive response only in 7-20% of cases and a very high rate of negative response in 73-80% of

Table I *Chronic hepatitis C patients' (n = 73) response rates to various doses of Ukrain* in vitro.

Corresponding *in vivo* doses (mg)	0.25	0.5	1.0	2.5	5.0	10.0
Positive response (%)	38.5	47.6	47.6	45.2	37.5	29.2
Negative response (%)	46.2	39.7	42.9	45.2	58.3	41.7

Table II *Chronic hepatitis C patients' (n = 73) response rates to various doses of interferon* in vitro.

Corresponding *in vivo* doses (MU)	0.1	0.25	0.5	1.0	2.0	3.0	5.0
Positive response (%)	26.7	31.3	35.7	43.7	42.5	6.7	20.0
Negative response (%)	73.3	50.0	52.9	45.1	38.4	73.3	80.0

cases. We believe that this is the reason for the low efficacy and high toxicity of IFN in CHC.

In our opinion, the positive response with an increased SH/SS blood ratio leads to biostimulating (including immunostimulating) effects of the preparations on different cells and tissues of the organism (blood, bone marrow and immunocompetent cells, hepatocytes, etc.). A negative response with a decreased SH/SS blood ratio leads to cytotoxic (including immunodepressive) effects of the preparation (9).

The data obtained are in line with investigations by other authors in which the immunomodulating and antiviral effects prevail with low doses of IFN, while in high doses it is the cytostatic and antitumor effects which predominate (10). The same can be applied to Ukrain.

After *in vitro* study of the blood to measure CHC patients' sensitivity to Ukrain and IFN in three of the more frequently working doses, it was revealed that 79.4% of patients were sensitive to Ukrain and 65.1% to IFN (Table III). The sensitivity of patients' blood to Ukrain was almost the same in patients with HCV genotype 1b and those with other genotypes. However, the sensitivity to IFN significantly depended on the genotype of HCV and was more than fourfold less in CHC patients with HCV genotype 1b. The data obtained confirm well-known clinical results showing significantly lower efficacy for both IFN monotherapy and therapy in combination with ribavirin in CHC patients with HCV genotype 1b. The sensitivity of CHC patients with HCV genotype 1b to Ukrain was very high and was more than fivefold higher than the sensitivity to IFN.

Investigation of virological response rates after individual therapy with Ukrain and IFN (Table IV) demonstrated that the efficacy of therapy was very high in both groups of patients, though somewhat higher in the group receiving Ukrain. Virological response was achieved in six of eight CHC patients with genotype 1b after individual therapy with Ukrain. At the same time it is important to note that after 3 months of therapy with IFN the rate of virological response in this group increased to 90.3%.

After analysis of the results of Ukrain therapy it was found that the individual method was more than 2.5

Table III *Distribution of patients according to* in vitro *sensitivity to Ukrain and interferon.*

Sensitivity	Total	Genotype 1b	Other genotypes (3, 1a, 2)
Ukrain	50/63 (79.4%)	12/13 (92.3%)	13/15 (86.7%)
IFN	41/63 (65.1%)**	2/12 (16.7%)*	12/17 (70.6%)

*Significant differences with Ukrain group (genotype 1b) and IFN group (other genotypes) (p <0.01)
**Difference is significant with the same group for Ukrain (p < 0.05)

Table IV *Rates of virological response after therapy.*

Treatment	1 month	3 months
Individual Ukrain (0.25-2.5 mg)	14/16 (87.5%)	—
Individual IFN (0.5-2.0 MU)	23/31 (74.2%)	28/31 (90.3%)
Standard Ukrain (2.5 mg)	4/12 (33.3%)*	—
Ukrain in 0.25-1.0 mg doses	12/14 (85.7%)	—
Ukrain in 2.5 mg dose	5/14 (35.7%)**	

*Difference is significant in the group treated with individual doses of Ukrain (p < 0.05)
**Difference is significant in the group treated with Ukrain in 0.25-1.0 mg doses (p < 0.05)

times more effective in terms of the virological response rate than that of standard therapy at 2.5 mg, independent of the results of preparation testing (Table IV). Treatment with lower doses of Ukrain after measuring individual sensitivity was more effective than treatment with higher doses (Table IV).

The same conclusion was reached after analysis of the results of individual therapy of CHC patients with IFN. In agreement with other authors (1), at the end of standard therapy the rates of virological response were 29 and 24% after 6 and 12 months, respectively, of IFN therapy alone at a dose of 3 MU. These response rates are more than three times lower than those obtained after 3 months of individual therapy with IFN.

Other authors (11) have reported the successful treatment of CHC patients with lower doses of IFN (1.5 MU per injection). There have already been a few CHC cases treated with individual doses of IFN (0.5-2.0 MU) with a constant absence of HCV-RNA in PCR and normal ALT levels over a 12-month period, including some with the unfavorable 1a genotype. Follow-up investigations are to be continued.

During effective therapy with Ukrain, some patients demonstrated an increased ALT level after the beginning of therapy, which then decreased moderately. In some patients the ALT level decreased without rising again. There were no serious side effects during Ukrain therapy. In some patients subfebrile temperature was registered at the beginning of therapy. The number and expression of side effects during individual therapy by IFN also significantly decreased (up to 12.9%).

Such high efficacy of individual therapy by Ukrain and IFN in comparison with standard therapy could be due to the injection of preparations in optimal, biologically active doses which leads to optimization of blood, bone marrow, immune and hepatic cell function and induces the production of endogenous factors of antiviral resistance and immune defense (interferons, interleukins and others). The "hitting the target" effect was observed. In the case of individual therapy, patients with a poor prognosis for therapy with IFN, for example with HCV genotype 1b, were excluded from the IFN-treated group. These patients were included in the group receiving Ukrain, to which they were sensitive. For individual therapy with IFN, patients with genotypes 3, 2 and 1a were selected after *in vitro* tests.

Based on our data, we found that the prognostic significance of the method for screening preparations for the treatment of CHC patients was 89.8% (a correct prognosis of treatment results was made in 53 out of 59 cases). The mistakes in prognosis of positive virological results of treatment were made in five patients with long anamnesis and previous unsuccessful treatment with several courses of IFN preparations. There was only one mistake in prognosis of negative virological result of treatment. Four patients (two received Ukrain and two IFN) relapsed during therapy and became nonsensitive after 1 month of therapy. It is clear that in such cases it is necessary to discontinue treatment until sensitivity to the preparation is recovered or the drug should be changed.

Two important factors of the efficacy of the method for the screening of preparations for individual therapy should be noted: i) the existence of significant differences in sensitivity to IFN in CHC patients with HCV genotype 1b and others, which was proved by clinical data a long time ago; and ii) the existence of significant differences in the efficacy of CHC therapy with Ukrain in the individual program and the standard dose. The same applies to IFN in comparison with the results of other authors.

Conclusion

During our investigation it was revealed that Ukrain can be used in the treatment of CHC patients alone or in combination with IFN preparations; in cases with HCV genotype 1b, Ukrain seems more promising than

IFN. The choice of preparation, its dose and the duration of treatment should be based on the results of individual sensitivity *in vitro* by estimating the SH/SS blood ratio. Individual therapy with Ukrain and IFN increased the efficacy of treatment 2.5-3.0-fold in comparison with standard monotherapy with the same preparations, significantly decreased the number of side effects and dramatically improved cost-effectiveness.

References

(1) McHutchison J.G., Gordon S.C., Schiff E.R., et al. *Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C.* N. Engl. J. Med., **339**, 1485, 1998.

(2) Kamyshentsev M.V., Voltchek I.V., Brailovskaya I.V., et al. *Testing Ukrain as an anti-influenza remedy. Recent advances in chemotherapy.* Proc. 18th Int. Congr. Chemother., Stockholm, 1993, p. 645.

(3) Lozjuk R.M., Lisnyak O.I., Lozjuk L.V. *Theoretical grounds and experimental confirmation of the antiviral effect of the preparation Ukrain.* Drugs Exptl. Clin. Res., **22** (Suppl.), 141, 1996.

(4) Liepins A., Nowicky J.W. *Ukrain is selectively cytostatic and/or cytotoxic to human tumor and HIV-infected cells but not to human normal cells. Recent advances in chemotherapy.* Proc. 17th Int. Congr. Chemother., Berlin, 1991, p. 2660.

(5) Voltchek I.V., Liepins A., Nowicky J.W., Brzosko W.J. *Potential therapeutic efficacy of Ukrain (NSC 631570) in AIDS patients with Kaposi's sarcoma.* Drugs Exptl. Clin. Res., **22** (Suppl.), 211, 1996.

(6) Voltchek I.V., Sologub T.V., Belozyorova L.A., et al. *Possibilities for individual biologically active therapy of viral hepatitis.* Terra Medica Nova, **3**, 14, 1999.

(7) *Method for screening of medicines.* Russian Federation patent no. 2150700, 1999, PCT WO 00/65342, 2000.

(8) Sokolovsky V.V. "Blood Thioldisulfide Ratio as a Proof of the Stateon Organism Nonspecific Resistance." Medical Academy of Postgraduate Studies, MAPS, St. Petersburg, 1996.

(9) Kulik G.I. *Comparative in vitro study of the effects of the new antitumor drug Ukrain and several cytostatic agents on the thiol groups in the tissue of Guerin carcinoma and its resistance to cisplatin variant.* Drugs Exptl. Clin. Res., **14**, 277, 1998.

(10) Zmyzgova A.V. "Interferonotherapy of Viral Hepatitis". Moscow, 1999.

(11) Sanchez-Tapias J.M., Forms X., Ampurdanes S. et al. *Low dose α-interferon therapy can be effective in chronic active hepatitis C. Results of multicentre, randomized trial.* Gut, **38**, 603, 1996.