Administration of Intradermal Autoleukocyte Immunization as a Reduction Method of Cryoglobulin Synthesis in Patients with Chronic Hepatitis C

Abstract
Possibilities of cryoglobulin synthesis inhibition in patients with chronic hepatitis C by the method of intradermal immunization with inactivated autoleukocytes, isolated from heparinized venous blood, have been presented in the article. Immunization was conducted in three groups of patients with chronic hepatitis C with mixed cryoglobulinemia. The first group comprised 28 patients, who followed antiviral therapy with pegylated interferon and ribavirin; the second – 12 patients with mild and asymptomatic course of chronic hepatitis C (detected accidentally). Besides, autoleukocyte immunization was performed to 8 patients with severe vasculitis and contraindications for interferon therapy. Autoleukocyte immunization causes decrease in cryoglobulin amount in all patients (100%); in the majority of them a significant reduction of clinical manifestations of cryoglobulinemia syndrome occurred. The degree of positive influence of autoleukocyte immunization on clinical manifestations of cryoglobulinemia syndrome had individual character and depended on the severity of vasculitis. The method is recommended for introduction into clinical practice.

Keywords: chronic hepatitis C, cryoglobulinemia, intradermal autoleukocyte immunization

Introduction
After identification of hepatitis C (HCV) virus, it was established that it is the main trigger factor of mixed cryoglobulinemia (MCG). In patients with 1st type of cryoglobulinemia HCV-infection was rarely detected, however, in MCG of the 2nd and 3rd types, incidence of HCV-infection detection, according to literature data, ranges from 30 to 85-90% [1-4].

HCV lymphotropism, manifested by stimulation and activation of B-lymphocytes, is considered to be the key factor in pathogenesis of HCV-associated MCG. It leads to synthesis of wide spectrum antibodies (particularly, monoclonal rheumatoid factor) and promotes formation of cryoglobulinemia syndrome. Proliferation of B-cells may lead to the development of B-non-Hodgkin lymphoma [2, 5-7].

Cryoglobulinemic syndrome (CS) is a systemic vasculitis of tiny (rarely middle) vessels, which may result in disturbance of functions of various organs and systems. Accordingly, CS is characterized by variety of clinical manifestations, which usually depend on the degree of severity and localization of damaged vessels. In patients with chronic hepatitis C the syndrome of cryoglobulinemia is most often manifested by skin purpura, arthralgia, myalgia, damaged peripheral (rarely central) nervous system, and kidney diseases – from latent nephritis to severe renal failure. At the same time, such vasculitis may cause damage to gastrointestinal tract, lungs, organs of vision, etc. [3, 8, 9].

In some patients with chronic hepatitis C the development of severe cryoglobulinemic vasculitis with mesangiocapillary glomerulonephritis and non-Hodgkin lymphoma and can be the cause of death even before the development of liver cirrhosis and its complications. Malignant hypertension, caused by kidney damage, in its turn, leads to cardiac insufficiency.

Since cryoglobulinemic vasculitis is a cause of autoimmune process, it is evident that such patients are often administered immunosuppressive medicines, aimed at inhibition of synthesis of immunoglobulins and formation of immune complexes. In certain
patients, usually those without severe vasculitis, such measures (especially with simultaneous conduction of plasmapheresis) lead to development of remission. However, immunosuppressive therapy promotes intensification of virus replication, liver inflammation and development of other complications of infectious process. With improvement of antiviral therapy these drugs are rarely prescribed, only in severe forms of vasculitis.

Nowadays antiviral therapy is considered to be a basic method of cryoglobulinemia treatment in patients with chronic hepatitis C. It is proved by correlative connections between efficacy of antiviral therapy and CS. However, clinical manifestations persist in some patients with stable virological response [10-13].

Limitation of cryoglobulinemia treatment in patients with chronic hepatitis C to antiviral drugs is possible only in non-severe forms of vasculitis (without evident renal failure). Chimeric monoclonal antibodies to CD20 antigen of B-lymphocytes – drug rituximab are administered in severe cases (renal failure, skin ulcerative-necrotizing hemorrhagic vasculitis). Mechanism of rituximab influence on autoimmune processes is explained by destruction of B-cells’ activity and, accordingly, weakening of autoimmune process, which is manifested in laboratory by decreased activity of rheumatoid factor and cryocrit. But this process is not stable, since following resuming of B-lymphocyte activity leads to exacerbation of cryoglobulinemia. Besides, influence of rituximab on the condition of immune system may promote intensification of HCV RNA replication [14,15].

Preventive measures of development of severe clinical manifestations of CS in patients with chronic hepatitis C, in whom they are at present absent or manifested by mild form of skin purpura, moderate general weakness and intensification of cold feeling, especially in the extremities, are also actually not elaborated.

AIM

Thus, the aim of our investigation was an attempt to extend the system of treatment of cryoglobulinemia syndrome in patients with chronic hepatitis C. Thus, non-medicated therapy in the form of intradermal immunization with inactivated autoleukocytes was tested. We took into consideration that autoleukocyte immunization is used in the treatment of oligozaospermia, caused by cryoglobulinemia [16,17], and other autoimmune conditions, particularly those pertaining to the thyroid [18,19]. Besides, in case of psoriasis it was shown that the method of intradermal autoleukocyte immunization leads to inhibition of intensive TNFα synthesis [20,21].

Materials And Methods

Patients with chronic hepatitis C with cryoglobulins in blood serum receiving antiviral therapy with pegylated interferon and ribavirin were involved in the research (first group). Totally 28 patients aged 18-63 with MCG were examined, among them 17 (60.71%) women, 11 (39.29%) men. In most patients (20) HCV genotype was 1st type (25.0%) and in one patient – 2nd type (3.57%). There were no contraindications for antiviral therapy in patients; individuals with liver cirrhosis were not included in the research. Severe vasculitis and renal insufficiency were not detected. However, most of them experienced cold extremities (18; 64.29%); arthralgia was periodically observed in 12 patients (42.86%), skin purpura without itching, usually on lower extremities, was seen in 8 patients (28.57%).

The second group comprised 12 patients with mild or clinically asymtomatic form of chronic hepatitis C (hepatitis was detected in blood donors or during scheduled examination of medical workers). Cryoglobulinemia was not clinically manifested in such individuals.

Besides, autoleukocyte immunization was conducted to 8 patients with severe forms of chronic hepatitis C and contraindications for conduction of interferon therapy. These patients had severe vasculitis with evident renal failure, one patient had non-Hodgkin lymphoma.

Autoleukocyte immunization in the first group of patients with chronic hepatitis C was commenced 2-3 weeks before the beginning of antiviral therapy, further – individually, depending on peculiarities of increase in cryoglobulin amount. Immunization of patients of the 2nd and 3rd groups was performed once or several times with 30-40 day interval, a number of procedures depended on reaction, namely consideration of the achieved effect and duration of remission.

Analysis of blood for cryoglobulins

For detection of cryoglobulins, blood from peripheral vein in amount not less 10 ml was taken with warm syringe (37°C), incubated at 37°C until formation of clot. A part of separated serum was dissolved with veronal-medinal buffer (pH 8.6) in correlation 1:10; its optic density was determined by the method of spectrophotometry (device Immnochem-2100 – photometric system, manufacturer High Tecnology, USA was used, wave length – 500 nm). The rest of the serum was incubated at 4°C for 7 days, after that it was also dissolved in buffer, and optic density was determined. If the difference in optic density of serum before and after cooling exceeded 10%, the result was considered positive.

The difference in optic density (%) was considered as a result of investigation and was expressed in conditional units (for example, if the difference in optic density of serum before and after cooling was 15 %, the result equaled 15 conditional units).

To prove that increase in optic density of cooled serum is associated with presence of cryoglobulins, the serum was warmed at 37°C for an hour. Decrease in optic density proved the result of investigation.

The type of cryoglobulins was determined by the method of historesistant curves, thus, optic density of serum was compared before and after cooling in different periods of cold incubation (+ 4°C).

Intradermal immunization with inactivated autoleukocytes

It includes 2 stages: isolation of leukocytes from the peripheral blood and their intradermal introduction.

Leukocytes are isolated by precipitation of heparinized venous blood of a patient. For this 80-100 ml of venous blood is taken
with previously warmed syringe (37°C) into a vial with heparin (Heparini-Richter) in correlation 50 units of heparin per 10 ml of blood (volume depends on the amount of leukocytes in 1 ml of blood). Then the blood is poured into test-tubes, 10 ml each and incubated at the angle 45° in thermostat at 37°C for 90-140 minutes. Blood plasma is carefully, without mixing, aspirated, leukocytes are washed twice in 5-10-multiple volume of 0.9% sodium chloride solution by centrifugating at 200 g. The suspension of autocells is injected intradermally in the dose 0.1 ml into 8-10 points of the skin in the back (between scapulae) (Table 1).

The results of immunization were estimated according to content of CG in blood serum before and in 10-12 days after immunization, further with the interval 1-2 months for a year. If necessary, manifestation of skin vasculitis, intensity of arthralgia, functional conditions of the kidneys were addressed, results of hematological and biochemical investigations were compared.

All experiments were accepted by the local Ethical Committee of Danylo Halytsky Lviv National Medical University.

Results of investigation

Results of single intradermal autoleukocyte immunization of patients with chronic hepatitis C and MCG, which was performed on patients of the 1st group 2-3 weeks before antiviral interferon therapy, are presented in table 1. From the data presented in table 1 it is seen that in the majority of patients amount of cryoglobulins in blood serum significantly decreased after single autoleukocyte immunization. Thus, in 20 patients with chronic hepatitis C with CG (from 28) precipitating cold-shock proteins were not detected already in 10-12 days after immunization (71.43 %). In 8 other patients with chronic hepatitis C a considerable decrease in the amount of cryoglobulins occurred, thus, all patients (100 %) showed rapid and positive response to intradermal autoleukocyte immunization. Duration of the achieved effect was individual and ranged within 1 to 6 months. Longer period of remission (3-6 months) was in the patients, in whom CG were not detected after single immunization. In patients, who showed decrease in CG synthesis, but the amount exceeded 30 conditional units, their previous level was observed in 1.5-2 months and pathological manifestations of MCG syndrome partially repeated, though they were weaker. Especially immunization had a positive impact on disappearance (or a significant reduction) of increased sensitivity to the cold.

Patients with recurrent amount of cryoglobulin in blood serum were subjected to booster immunizations during antiviral therapy with an interval of 1-3 months. Under the influence of immunization patients’ condition improved, they responded to therapy better. It was confirmed by the fact that patients were satisfied with the procedure and the majority of them asked to repeat it. Anyway, among 12 patients with chronic hepatitis C, who periodically experienced arthralgia signs, they disappeared completely in 5 patients and during therapy (48 weeks) they did not recur (41.67%); from 8 patients with chronic hepatitis C with skin purpura, it disappeared in 3 of them in 10-15 days after the first immunization and did not appear again, in other patients it decreased significantly. Cryoglobulinemia had a positive influence on the condition of cold intolerance.

Connection between efficacy of antiviral therapy and duration of the achieved decrease in cryoglobulin amount was not established. Probably, it is caused by insufficient number of examined patients, though a sequel of side effect of interferon therapy is not excluded.

In most patients with clinically absent signs of cryoglobulinemia, amount of cold-shock pathological proteins completely normalized (in 9 from 12; 75.0%), in others – considerably decreased. However, since etiological factor was not eliminated (patients did not receive antiviral therapy), amount of cryoglobulins gradually increased (during 3-9 months), though it remained lower than before immunization.

Concerning the influence on the course of systemic cryoglobulinemic vasculitis, efficacy of treatment depended on the severity of the disease. Thus, we could not save a female patient with severe necrotic hemorrhagic vasculitis (over 15% of skin was affected) and non-Hodgkin lymphoma. The best result was achieved in patients with non-severe signs of vasculitis: insignificant purpura, moderate arthralgia, primary manifestations of sensor neuropathy (efficacy of treatment approximated 90%).

Treatment was less effective in patients with severe kidney damage (cryoglobulinemic mesangiocapillary glomerulonephritis), although amount of cryoglobulins in these patients’ blood also decreased. Among 8 such patients, remission was observed in 3, however, stable remission – only in one individual.

Thus, method of autoleukocyte immunization leads to decrease in cryoglobulin synthesis and may be used for treatment of

<table>
<thead>
<tr>
<th>Primary amount of CG</th>
<th>Amount of cryoglobulins after autoleukocyte immunization</th>
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<tbody>
<tr>
<td></td>
<td>0*</td>
</tr>
<tr>
<td>10-30</td>
<td>4** (100%)</td>
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<tr>
<td>31-50</td>
<td>2 (66.67%)</td>
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<td>51-60</td>
<td>3 (75.0%)</td>
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<tr>
<td>61-70</td>
<td>4 (66.67%)</td>
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<tr>
<td>&gt; 70</td>
<td>7 (63.64%)</td>
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<tr>
<td>Total patients</td>
<td>20</td>
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Table 1: Amount of cryoglobulins in blood serum of patients with chronic hepatitis C before and after intradermal autoleukocyte immunization (the first group of patients)

Note: * – amount of CG; ** – number of patients with the corresponding amount of CG.
patients with signs of MCG; it is highly effective in vasculitis of mild and severe severity.

**Discussion**

Reduction of cryoglobulin amount in blood serum can, in our opinion, be used not only for weakening clinical signs of MCG syndrome, but also as a prophylactic measure for prevention of deterioration of patients’ clinical condition.

The suggested method is worth attention, because treatment of MCG syndrome, especially in patients with chronic hepatitis C, is not sufficiently elaborated. Difficulty is associated with many factors, among them the important ones are: polymorphism of clinical signs, caused by simultaneous damage to various organs and systems; 2) presence of other, besides cryoglobulinemia, autoimmune processes, which often appear in chronic hepatitis C; 3) viral liver damage, resulting in violation of metabolism processes and limiting possibility of drug administration. It is important to note that pathogenetic connection between these conditions exists, which can not be always taken into consideration. However, autoleukocyte immunization has a wide spectrum of action, because it influences different autoimmune processes [22,23], may inhibit excessive synthesis of TNFα [20,21]. Due to these peculiarities of MCG syndrome, it is expedient to use autoleukocyte immunization method, which actually does not have contraindications.

Efficacy of autoleukocyte immunization may be explained by complex influence of autoreactive cells on mechanisms of immune reaction. T-lymphocytes are known to carry autoimmune diseases. Thus, in case of immunization, autoreactive cells may cause activity of cellular lymphocyte-mediated immune response, that is, trigger immune surveillance generating cytotoxic leukocytes. The process of correction of Jerne’s network – idiotype-anti-idiotype regulation of the immune response is also important, which is confirmed by increase in anti-idiotype antibodies in a patient’s blood serum after leukocyte immunization. One more mechanism may be involved in this manipulation: activation of CD3+, CD8+, CD25+ - lymphocytes, as well as CD3+, CD8+, CD28+ - lymphocytes, simultaneously with blockage of Fc-receptors and glycoprotein, lectin receptors on B-lymphocytes [24]. Possibly, the level of loading leukocytes with antigens of a disease agent has an important meaning. Anyway, immunization with leukocytes, which contain virus and its antigens, may intensify virological surveillance. It is confirmed by the efficacy of treatment of frequently recurrent chronic herpes [25]. Theoretically, presence of a disease agent in leukocytes reduces likelihood of immune system weakening, even in case of inhibition of autoimmune processes. This issue requires further investigation.

**Conclusion**

1. Intradermal autoleukocyte immunization of patients with chronic hepatitis C with MCG, even along with antiviral interferon therapy leads to inhibition of CG synthesis and promotes weakening of its clinical manifestations. Thus, autoleukocyte immunization of patients with chronic hepatitis C is expedient before and during interferon therapy.

2. Autoleukocyte immunization is worth implementation into chronic hepatitis C therapy, because it reduces intensity of pathological clinical signs, caused by autoimmune processes, especially in patients with non-severe forms of cryoglobulinemic vasculitis. Though efficacy of immunization of patients with severe forms of kidney damage is insufficient, its administration in complex therapy is expedient, since it causes inhibition of CG synthesis.

3. It is also expedient to use autoleukocyte immunization in patients with chronic hepatitis C without clinical signs of vasculitis for prevention of possible exacerbation.
References