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The Influence of Iscador on the Course of Hepatitis C

Part I: Turjanov MCh, Tomkevich MS, Malyshev NA, Blochina NP, Bajdun LV, Gracheva LA, Lobyshev VI,

Part II: Kogan EA, Demura SA, Azov AG, Tomkevich MS, Turjanov MCh, Malyshev NA, Blochina NP

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Part I

Hepatitis C has been spreading among the population for several decades. Considering this spread, the chronic course of the disease and the fact that it often affects young patients in whom liver cirrhosis and hepatocellular carcinoma are possible late complications, hepatitis C must be counted among the diseases that have an urgent need for maximally effective treatment.

Viral persistence leads to immunodeficiency, which in turn promotes the progress of the viral infection. In addition, the virus is only weakly immunogenic and exhibits great variability, an impediment to the development of specific antiviral drugs. Of particular importance in the combination therapy of hepatitis C are drugs of natural origin such as Marinol, Silybor or Silymarin from the fruits of the holy thistle. Great use is being made of vegetal preparations containing everlasting, chicory, Saint-John's-wort, etc.(3, 4.5, 8, 20).

The European specialized literature of recent years discusses the results of the treatment of hepatitis B and C by means of white-berry mistletoe preparations, namely ISCADOR and ABNOBAviscum. Long-term therapy (6 to 18 months) with these preparations leads to a fall and normalization of serum transaminases (after an initial increase), a fall in the viral load, and a marked improvement of general status and quality of life (7, 11, 19).

The white-berry mistletoe is a plant that has been known for ages and has always had its place in traditional medicine. Nowadays, its main components (**viscotoxins** and **lectins**) are well known and recognized to influence diverse phases in the cycle of damaged cells. Experimentally, proof has been obtained of their effects on natural killer cells, T helper cells and T suppressor cells, on the phagocytic activity of granulocytes, on the formation of interferon and antibodies, and of their virostatic potential (10).

Results of a study of Iscador on the course of chronic hepatitis C will be described in the present paper.

Material and methods

Forty patients with positive HCV RNA and positive HCV antibody tests, aged 18-40 years, were included in the study. From the beginning they stopped the intake of preparations containing synthetic active agents. None of the patients had a history of a previous hepatitis B. The kinetic determination of transaminases relied on Coba Mira Plus. The immunoenzyme analysis with determination of the markers of an HCV infection was made by means of the test systems Hepanostika and LIA-TEK. The determination of HCV RNA after PCR resorted to the «Amplicor» systems of Hoffmann-La-Roche, and the genotype analysis to «Ampli-Sens-50-R HCVgenotype» reagents.

The phenotypic modifications of T cells caused by Iscador were studied: T helper cells (CD4), T suppressor cells (CD8), CD4/CD8 ratio, and the numbers of natural killer cells (CD16, CD56), activation markers (CD28), Fas/APO-1-apoptose receptors (CD 95) and B lymphocytes (CD19). In addition, the cytokine (TNF-α, INF-γ, and IL-2) content of lymphocytes was determined. For this part of the study, reagents from R&D, Dako, Coulter, Beckman and others were used.

The treatment with Iscador (Weleda AG, Germany) consisted of 2 to 3 subcutaneous injections a week, the dosage being adapted to the individual case on the basis of the improvement in general status and an increase of transaminases (ALT and AST) and C-reactive protein to no more than twice the initial values. The 12-month treatment was followed by a 6-month follow-up period.

The results achieved were grouped according to the investigated characteristics. The means and the variances before treatment and at different stages of treatment were calculated. Because of the differences between patients and the resulting large dispersion of variables, the sign test was made use of, for it is remarkably responsive to individual courses, as well as the F-test. We considered results with p=0.25 to be clinically reliable, i.e. to be different from the initial values in 75% of cases.

Results

The treatment with Iscador was well tolerated. Almost from the beginning the patients experienced an improvement in their general status, which remained present throughout treatment and after the 6-month follow-up period. No unwanted side effects were registered.

The initial values of ALT and AST were higher than 150 units (Table 2) in 61% of patients. As the Table shows, in the treated group patients with a tendency to normalization of enzymatic values became progressively more numerous over time. Yet, it must be mentioned that the initial response to Iscador was an elevation of the two transaminases, even though — as seen in the course of the study — this elevation had a positive prognostic value. The dynamics of ALT are described in more detail below.

Table 2 shows that, within 18 months of the beginning of treatment (12 months ▷

Table 1. Results of genotype determinations of the patients included in the study.

1b	2b	1a	2a	3a	1a3a	1b3a	1b2b
10%	5%	5%	15%	10%	10%	15%	5%

The genotype was not determined in 30% of the patients.

Table 2. Quantitative distribution of Iscador-treated patients according to their ALT values (percentages).

Time from treatment onset	**Up to 40 units**	**41-100 units**	**101-150 units**	**Over 150 units**
Beginning	0%	17%	22%	61%
1 month	5,5%	39%	39%	16,5%
3 months	17%	28%	22%	33%
6 months	11%	45%	22%	22%
8 months	28%	28%	0%	44%
10 months	11%	34%	11%	44%
12 months	12%	47%	12%	29%
15 months	35%	29%	18%	18%
18 months	33%	47%	20%	0%

Table 3. Quantitative distribution of Iscador-treated patients according to their lymphocyte subpopulations (percentages).

T lymphocytes	**Up to 1000**	**1000-1500**	**1500-2000**	**Over 2000**
Beginning	23%	45%	22%	11%
1 month	6%	47%	29%	18%
6 months	5%	28%	56%	11%
12 months	0%	76%	18%	6%
18 months	40%	27%	27%	6%
T helper cells	**Up to 400**	**400-600**	**600-800**	**Over 800**
Anfang	18%	23%	29,5%	29,5%
1 month	0%	18%	29%	53%
6 months	0%	16,5%	16,5%	67%
12 months	0%	25%	50%	25%
18 monthse	33%	7%	20%	40%
T suppressor cells	**Up to 400**	**400-600**	**600-800**	**Over 800**
Beginning	17%	45%	33%	5%
1 month	0%	35%	53%	12%
6 months	6%	18%	47%	29%
12 months	0%	18%	47%	35%
18 months	47%	27%	20%	6%
NK cells	**Up to 200**	**200-400**	**400-600**	**Over 600**
Beginning	17%	39%	11%	33%
1 month	11%	33%	28%	28%
6 months	17%	50%	33%	0%
12 months	41%	29%	30%	0%
18 months	47%	27%	13%	13%
B lymphocytes	**Up to 100**	**100-200**	**200-300**	**Over 300**
Beginning	24%	47%	6%	23%
1 month	22%	45%	33%	0%
6 months	22%	50%	23%	5%
12 months	0%	24%	35%	41%
18 months	0%	24%	35%	41%

▷

of treatment and 6 months of follow-up), there was a constant increase in the number of patients with normal ALT values (0-33%) and a decrease (61-0%) in the number of patients with elevated values (>150 units). AST behaved in a similar manner. Fig. 1 summarizes the changes in mean enzymatic values over 18 months; absolute values are provided for the elevation of transaminases 3 months after the beginning of treatment.

Table 3 shows some constants in the change of immunologic variables. Most of them (CD3, CD4, CD8, CD16) increased in 57% of patients during the first month of treatment, with a concomitant decrease of the absolute number of B lymphocytes in 29% of patients (no change in 14%). In some patients, an increase in the immunologic variables was seen 6 months after the beginning of treatment. Near the end of the treatment period, the values returned more frequently to their initial levels or even remained lower. In many patients, the positive changes of the immunologic variables were maintained during follow-up.

It is easily seen that most of these variables in patients with hepatitis C move to a medium-level range during treatment, whereas many of the initial and final values are distributed in the superior or inferior range. Diagram 2 shows the evolution of phenotypic features of lymphocytes (in absolute values) during treatment, with a maximum during months 1-6 after the beginning of treatment.

The variables CD28 and CD95 are activation markers; especially in patients with hepatitis C, they undergo changes, with first a rise and thereafter a constant decrease. Table 4 and Diagram 3 show that, during treatment, cytokine-producing lymphocytes undergo changes similar to those of the other variables, fluctuating changes usually going through a maximum 1 to 3 months after the onset of treatment.

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The viral load was determined before treatment was started and at intervals of 6 months for 18 months. It tended to decrease. Yet, in spite of the good general status of the patients and the positive changes in the biochemical markers of hepatitis C, no virus eradication was observed.

The non-parametric statistical evaluation of all the data indicates a high reliability of the results regarding the changes of both the biochemical markers and the immunological variables ($p<0.1$). It must be stressed that the beneficial results obtained were still present during follow-up in most of the investigated patients.

Discussion of the results

Many inconsistent theories accounting for the chronic course of hepatitis C are mentioned in the literature.

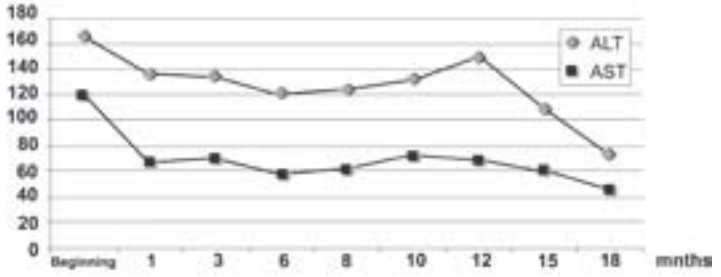
A possible cause is the direct cytotoxic effect of the virus on the infected cell, which can lead to the latter's death without the immune system being involved (13, 18). On the other hand, one may think of a role for the immune system. For instance, in patients with a long-term remission the immune response of the T cell system is less marked (16). Also, a clear correlation can be observed between the presence of cytotoxic T lymphocytes and the inflammatory activity in the liver (15, 21). Most authors point to the occurrence of a Th1 immune response in hepatitis C, a prognostically more favorable variant than a Th2 response (12). By producing cytokines, macrophages and T helper cells initiate the effector phase of the immune response, i.e. the cellular immune response.

Other findings underline that, besides their positive effects, cytokines released as a defense against the virus may also, when present in high concentrations and by virtue of their inflammation-promoting action, have adverse effect on the target of the hepatitis C virus, namely the liver cell (9).

Table 4. Average dynamics of cytokine production by circulating lymphocytes in Iscador-treated patients (percentages).

	Interleukin-2	Interferon-γ	Tumor necrosis factor α
Beginning	4,58	6,87	20,27
After 1 month	16,36	19,05	30,39
After 6 months	11,17	9,9	18,78
After 12 months	2,16	4,94	3,91
After 18 months	0,61	3,77	1,46

Figure 1. Dynamics of ALT and AST averages during Iscador treatment.



The results of the present study convincingly demonstrate the positive effect of the preparation under study on the **initiation and development of immune responses** in patients suffering from chronic hepatitis C. After only one month of treatment there is in part of the patients an increase in the production of CD4, CD8 and NK cells, with a different tendency emerging after the treatment is stopped, viz. that of an increase in CD19+ cells (B cells). Concomitantly, the raised pretreatment production of inflammation-promoting cytokines (of the Th1 type) is raised further in the lymphocytes after 1 month of treatment but is subsequently reduced by continued Iscador treatment.

Also the basic biochemical variables of hepatitis activity (ALT, ASP, and C-reactive protein) show a tendency to decrease after an initial inflammatory increase. The transaminase dynamics have a great importance for the determination of the optimal therapeutic regimen, for they allow one to establish the minimum and the adequate dosages individually. More and more, the literature points to the necessity for the individual dosage of biologic preparations. Some authors (1) are of the opinion that the individual dosage of interferon enhances the effectiveness of therapy. Since the administration of Iscador initially causes a certain increase in enzyme values in response, it is advisable to prescribe it mainly to patients with low or slightly increased values.

On the strength of the data presented here, Iscador can be regarded as a promising preparation for the treatment of hepatitis C. According to our experience and taking into consideration the individual characteristics of the patients and the duration of life of hepatic cells (12 months), we believe that the duration of treatment should not be less than 12 months; in some patients it can be raised to 18 months.

Summary

1. In patients with hepatitis C, Iscador caused a decrease and normalization of serum transaminases 8-12 months after the beginning of treatment.

2. Iscador had a clearly positive effect on the production of cytokines in the peripheral blood lymphocytes.

3. In all patients, an improvement of general status was observed during treatment with Iscador.

4. An eradication of viral RNA was not observed under Iscador treatment.

5. Iscador therapy can be administered for up to 18 months.

Turjanov MCh, Tomkevich MS, Russian Medical Academy of Postgraduate Studies, Moscow

Malyshev NA, Blochina NP, Hospital for Infectious Diseases No. 1, Moscow

Bajdun LV, Gracheva LA, Research Institute for Pediatric Hematology, Moscow

Lobyshev VI, Moscow State University

Figure 2. Dynamics of lymphocyte subpopulations during Iscador treatment.

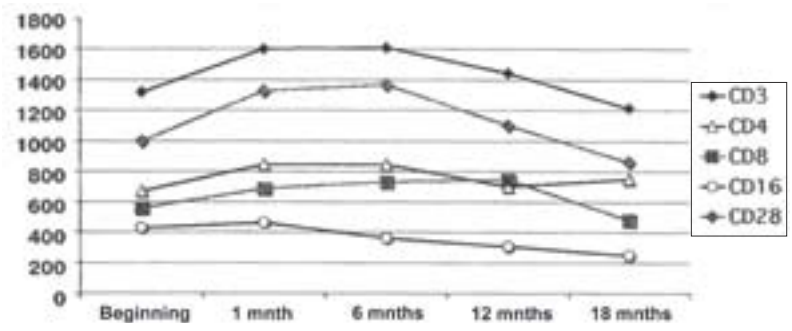
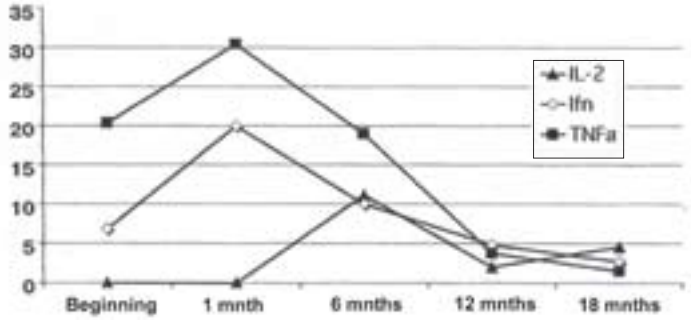


Figure 3. Dynamics of cytokine production by lymphocytes during Iscador treatment.



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Part II

Morphological and immunohistochemical study

This second part is devoted to the results of morphological and immunohistochemical investigations on the effects of Iscador on the course of chronic hepatitis C. The morphological findings are of critical importance insofar as the clinical disease course and the laboratory data often remain unremarkable and the histologic changes described in the literature are partly unspecific.

The morphological diagnosis, including immunohistochemistry and in situ hybridization for evidencing apoptosis, provides the most reliable means to evaluate not only the extent and correlation of the adverse process but also the inflammatory and cirrhotic changes that occur as a result of hepatitis C. To this extent, the morphological and immunohistochemical assessment of the efficacy of Iscador (Weleda) is of interest because the preparation has shown positive effects on the clinical and laboratory variables relative to the course of the disease (see Part I).

Diverse conceptions of the cytopathogenic effect of the hepatitis C virus on hepatocytes and other liver cells are to be found in the literature. Some types of damage to the hepatic tissue are well known, e.g. focal necrosis, apoptosis, and fatty and hydropic degeneration of hepatocytes. The available data on the role of cytokines in viral hepatitides are contradictory. Even though most cytokines defend the body against the virus, their disproportional accumulation has markedly harmful effects on the liver. Among the cytokines that are able to foster inflammatory and cirrhotic processes, particular attention has been drawn to the transforming growth factor TGB-β the fibroblast growth factor FGF-β fibronectin, tumor necrosis factor TNF-α which causes the transformation of hepatic stellate cells into myofibroblasts, the insulin-like growth factor IGF-1 and its binding proteins IGFBPs, the platelet-derived growth factor PDGF and the transforming growth factor TGF-α among others.

An important role in the protection of the body against the virus is that of the immune cells of lymphocytic and monocytic origin, including Kupffer cells. Depending on the cytokine profile, they foster the appearance of a given type of immune response. When present in the human body, the hepatitis C virus interacts with the immunocompetent cells, the T helper cells (CD4), which start to produce IL-2, INF-γ and TNF and thus trigger a Th1 cellular response, and the NK cells, which produce INF-γ too.

The aim of the study was to assess the extent of apoptosis, necrosis, dystrophic, inflammatory, and cirrhotic changes, and the expression of inflammation- and cirrhosis-promoting cytokines in the liver tissue of patients with chronic hepatitis C, thus enabling an evaluation of the therapeutic effects of Iscador to be made.

Material and methods

An extensive morphological examination was carried out on liver biopsies of 29 patients with newly diagnosed and untreated hepatitis C and representative biopsies with not less than 5 portal spaces per preparation. Biopsies were repeated after the end of treatment in 19 patients.

In paraffin sections stained with hematoxylin-eosin and van Gieson's solution of trinitrophenol and acid fuchsin, the inflammatory activity and the degree of fibrosis were assessed by means of the PAS reaction. The histologic activity index after Knodell was calculated.

In the paraffin sections first placed in a microwave oven for 20 minutes at 600 W, the following cytokines were determined immunohistochemically by means of the peroxidase reaction and the use of monoclonal antibodies: TGF-β (DAKO), TNF-α (DAKO), b-FGF (DAKO), fibronectin (DAKO), collagen type IV (DAKO after treatment with trypsin), and CD95 (Russian Institute of Immunology). Positive and negative control reactions were performed.

The results of the immunohistochemical reactions were evaluated in points (weak reaction 1-2, moderate reaction 3-4, strong expression 5-6) separately in various cell types and extracellularly.

The in situ detection of apoptotic cells was carried out by means of the TUNEL assay (ApopDetek, Enzo Diagnostic, USA). The apoptotic index was calculated on 300 cells as the ratio of apoptotic to healthy cells.

Results

Morphological analysis

The histological observation of liver biopsies performed before treatment showed the lobules to be well preserved as a rule, and trabecular structures were not always present (Fig. 1).

There was a combination of dystrophic, necrotic, and apoptotic damage

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in the hepatocytes. Fatty degeneration with large globules was seen in 47% of patients, with (40%) or without (7%) hydropic dystrophy. In 53% of patients, only pronounced dystrophy of hepatocytes was observed. Piecemeal necrosis was present in the peripheral areas of lobules, associated with lymphohistocytic infiltration. In virtually all patients, Councilman's bodies were found in various numbers, both between the trabecular cells and in the lumen of sinusoids. Generally there was no cellular infiltration in the vicinity of these bodies.

The inflammatory infiltrate in the hepatic tissue was present mainly along the portal spaces and in the vicinity of the necrotic foci; it was composed of lymphocytic and histiocytic elements. The inflammatory infiltrate often destroyed the integrity of the limiting membrane, so that elements of the infiltrate and bands of connective tissue penetrated the hepatic lobules.

Very characteristically, hypertrophy and hyperplasia of Kupffer cells was present to a various extent in different patients, ranging from mild to severe. In all cases, cholangiolitis (often productive) at different stages was seen.

The chronic course of the hepatitis was evidenced by a perturbed regeneration of hepatocytes and by cirrhotic changes. In the area of the limiting membranes of the lobules, rosette formation and a polymorphism of the (sometimes binucleate) hepatocytes was observed.

The portal spaces were dilated, longer than normal, and usually fibrotic. A characteristic finding was the transformation of sinusoids into capillaries.

In the majority of cases, chronic active hepatitis was present (histologic activity after Knodell: 4-10). There were but little differences between patients with regard to the degree of inflammatory infiltration and the periportal fibrosis, whereas there were clear differences with regard to the damage to hepatocytes due to dystrophy and necrosis.

Microscopic examination of the liver biopsies with positive morphological dynamics showed a reduction of the area and dissemination of necrosis, a reduction of the intensity of inflammation in the periportal infiltrates and a diminution of Kupffer cells hyperplasia after 12 months of Iscador treatment (Fig. 2). In isolated cases, a fall in the intensity of inflammation in the bile ductules and a tendency to less hydropic and fatty degeneration were seen, the fibrotic changes in the portal spaces remaining unchanged. A consequence of the lesser liver cell necrosis was a fall in the transaminase levels, which proceeded in phases and underwent excursions from one extreme to the other (see Part I).

Apoptosis

Initially, apoptotic bodies were present mainly in the liver parenchyma, in the peripheral areas of the lobules, generally uncorrelated to the inflammatory infiltrate. One may assume that the apoptosis of hepatocytes is caused by the direct cytopathogenic effect of the hepatitis C virus. Apoptosis was more conspicuous in patients with a histologic activity index greater than 6 (Fig. 3)

Generally, the degree of apoptosis was considerably reduced at the end of treatment, with a smaller number of apoptotic corpuscles in the sinusoid cells, especially in the Kupffer cells. It is remarkable that the reduction of the degree of apoptosis in Kupffer cells was accompanied with a reduction of their hyperplasia.

The apoptotic index (AI) fell considerably in Iscador-treated patients, i.e. by a factor of several times 10-1000. The reduction of the AI correlated with that of necrosis and serum transaminase values. Viremia was generally not reduced concomitantly.

Tumor necrosis factor (TNF-α)

Initially, the expression of TNF-α was high in hepatocytes, Kupffer cells, the endothelium of sinusoids, and the macrophages of the infiltrate (Fig. 5).

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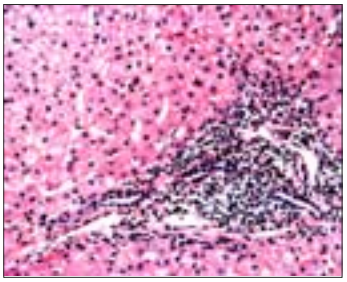
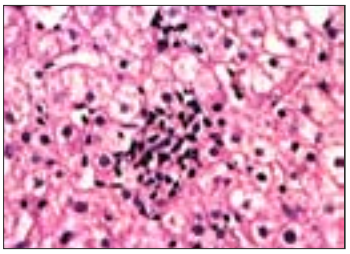
Figure 1



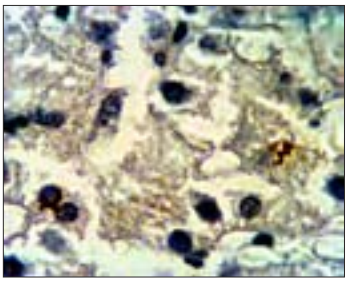
Liver of a patient with chronic hepatitis before Iscador treatment: bridging necrosis, marked hyperplasia of Kupffer cells, lymphoid infiltration and fibrosis in the portal spaces, and hydropic and fatty dystrophy of hepatocytes. Staining: hematoxylin-eosin (magnification: 200 x).

Figure 2



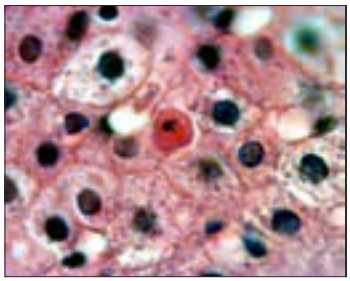
Liver of a patient with chronic hepatitis C improving its course on Iscador. There are still small necrotic foci and signs of cholangiolitis and hydropic hepatocyte dystrophy. Staining: hematoxylin-eosin (magnification: 400 x).

Figure 3



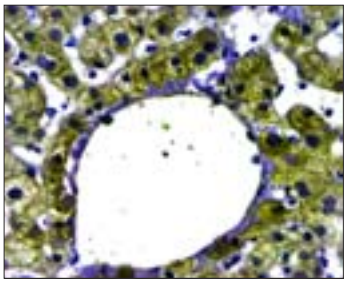
Apoptosis in liver tissue during hepatitis C. Apoptotic corpuscles in the liver tissue before therapy. TUNEL assay (magnification: 1000 x).

Figure 4



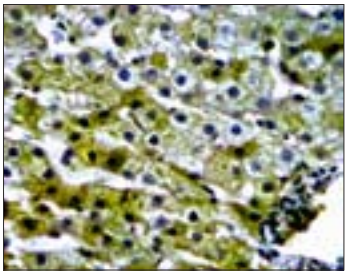
Apoptosis in liver tissue during hepatitis C. Councilman's bodies in the liver tissue of a patient with a positive course on Iscador treatment. Staining: hematoxylin-eosin (magnification: 1000 x).

Figure 5



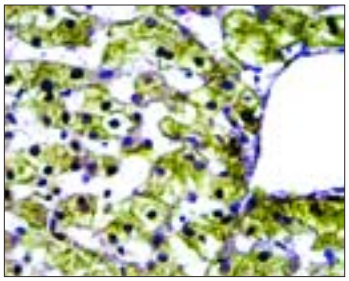
TNF-α in hepatocytes during chronic hepatitis before Iscador treatment. High TNF-α expression in hepatocytes in the Kupffer cells. Staining: immunoperoxidase (magnification: 400 x).

Figure 6



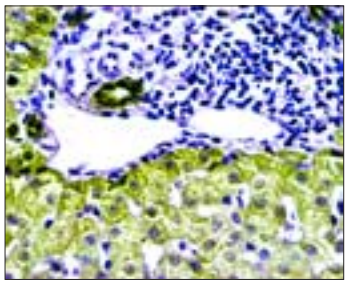
TNF-α in hepatocytes during chronic hepatitis responsive to Iscador treatment. TNF-α expression in isolated Kupffer cells and hepatocytes. Staining: immunoperoxidase (magnification 400 x).

Figure 7



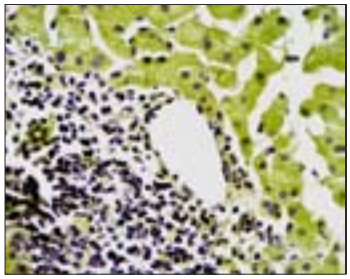
CD95 expression in liver tissue during chronic hepatitis before Iscador treatment. High CD95 expression in the hepatocytes. Staining: immunoperoxidase (magnification 400 x).

Figure 8



CD95 expression in liver tissue during chronic hepatitis after favorable response to Iscador treatment. Medium CD95 expression in hepatocytes and high expression in cholangiocytes. Staining: immunoperoxidase (magnification 400 x).

Figure 9



FGF-β expression in liver tissue during chronic hepatitis C. High FGF-β expression in hepatocytes and fibroblastic cells before beginning treatment. Staining: immunoperoxidase (magnification 250 x).

After the 12 months of Iscador treatment, TNF-α (expression was reduced in most patients (Fig. 6). A diminution of TNF-α expression was observed in Kupffer cells, hepatocytes, and the vascular endothelium. The reduction of TNF-α production in these cells was associated with that of the TNF-α concentration in the extracellular compartment too.

Fas / APO-1 receptor (CD95)

CD95 was detected in the membranes and cytoplasm of hepatocytes, in Kupffer cells, and in the epithelium of bile ductules (Fig. 7). Its highest expression was found in the epithelium of bile ductules and in peripheral hepatocytes. CD95 was expressed in Kupffer cells in 75% of cases. After the termination of treatment, a change in the expression and distribution of CD95 had taken place in the liver tissue, and CD95 expression was considerably diminished in Kupffer cells and in the epithelium of bile ductules (Fig. 8).

Fibroblast growth factor (FGF-β)

FGF-β was seen to be expressed in hepatocytes, hepatic fibroblasts and myofibroblasts (the main production site of this growth factor), and Kupffer cells. Besides, FGF-β accumulated in the extracellular matrix of the stroma of both the portal spaces and the sinusoids (Fig. 9).

As a rule, Iscador treatment caused a reduction in the production of FGF-β by liver cells, which was particularly marked in hepatocytes and Kupffer cells. By contrast, a slight accumulation of FGF-β remained in the extracellular matrix of 50% of patients.

Transforming growth factor (TGF-β)

TGF-β was expressed in the hepatocytes, the Kupffer cells, the endothelium of sinusoids, and the macrophages of the infiltrate (Fig.10). Besides, TGF-β accumulated in the extracellular matrix of the portal fields and in the basal membrane of the sinusoids. After the end of therapy, a reduction of TGF-β

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expression was seen, mainly in the Kupffer cells, the vascular endothelium, the hepatocytes, and the macrophages of the infiltrate.

Fibronectin (FN)

Fibronectin was expressed in the cytoplasm of hepatocytes, Kupffer cells, macrophages, and stroma fibroblasts. In addition, it accumulated in the extracellular matrix of the portal spaces and the sinusoids. In the livers of Iscador-treated patients, FN expression by hepatocytes, Kupffer cells, and fibroblasts was strongly diminished (Fig. 11). FN was present in very small concentrations in the extracellular matrix.

Collagen type IV

Collagen type IV was detected in the basal membrane of sinusoids transformed into capillaries and in the vascular structures and bile ductules of the portal spaces. There were irregular deposits of collagen IV along the sinusoids, located on segments transformed into capillaries. In 25% of Iscador-treated patients, there was a reduction of collagen IV in the basal membrane of sinusoids and in the structures of portal spaces (Fig. 13). Possibly there is a correlation with the expression of collagen-degrading enzymes by liver cells.

Discussion and conclusions

The morphological and immunohistochemical study of liver tissue of patients with chronic hepatitis C given Iscador treatment evidenced a positive effect of the preparation in most patients.

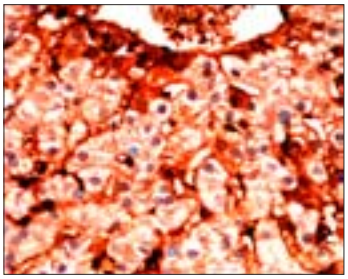
The positive effect of Iscador is associated with its effects on three essential pathologic processes of hepatitis C, responsible for the liver damage and the chronic course of the disease:

1. Death of hepatocytes through necrosis and apoptosis;

2. Inflammation as an immune response to the virus and the damage to hepatocytes;

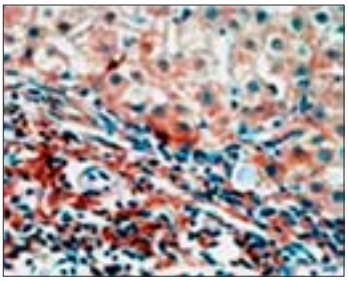
3. Fibrotic processes eventually leading to the development of cirrhosis.

Figure 10



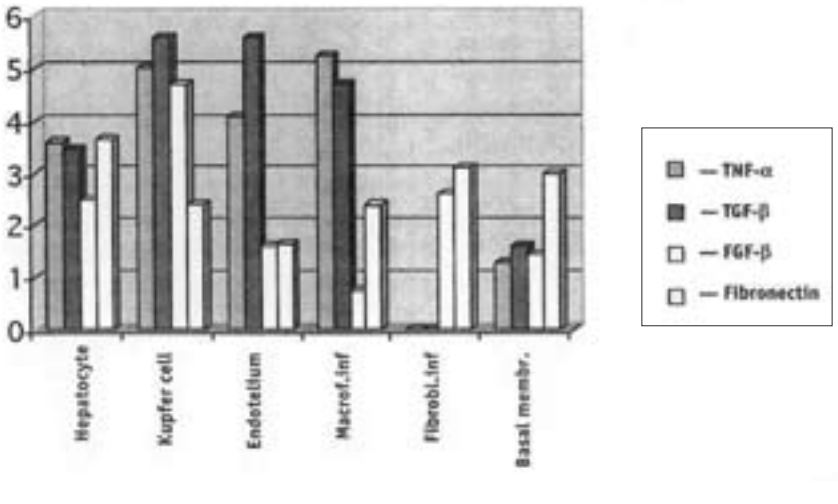
TGF-β expression in liver tissue during chronic hepatitis and before Iscador treatment. High TGF-β expression in hepatocytes and Kupffer cells of centrolobular areas. Staining: immunoperoxidase (magnification 400 x).

Figure 11



Fibronectin expression in liver tissue during chronic hepatitis after Iscador treatment. Fibronectin in the Kupffer cells and fibroblastic cells. Staining: immunoperoxidase (magnification 400 x).

Figure 12 Cytokine expression in liver cells after Iscador treatment.



Iscador limits hepatocyte death: their necrosis and apoptosis is reduced, the apoptosis index falls, as does the expression of such apoptosis-promoting factors as TNF-α CD95; and TGF-β (Figs 12 and 14).

The reduction of the inflammatory immunologic activity was demonstrated by morphological criteria and by reduction of the expression of inflammation-promoting factor TNF-α. There was a tendency to stabilization of cirrhotic processes, as shown by histology, an effect that, together with a low production of the fibrosis-promoting cytokines FGF-β, FN, TGF-β and TNF-α leads one to assume that Iscador slows down the cirrhotic process.

The observation of a degradation of collagen type IV in some patients was new. It is probably due to an activation of collagenase type IV in the liver cells.

Our clinical, biological, and morphological observations, evaluated statis-

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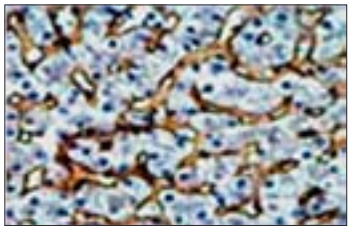
tically, lead to the conclusion that, as a rule, Iscador is well suited for the treatment of hepatitis C. The totality of morphological and histochemical data shows that Iscador improved the course of the disease in 83% of patients. Serum transaminase levels were lowered in 100% of patients and remained lowered for the whole period of observation including a 6-month follow-up: 18 months after the beginning of treatment the transaminases were in the normal range in 33% of patients and near the normal range in 47% (a total of 80%). Cytokine production diminished in both the liver and the lymphocytes. However, viremia remained unchanged.

Figure 13

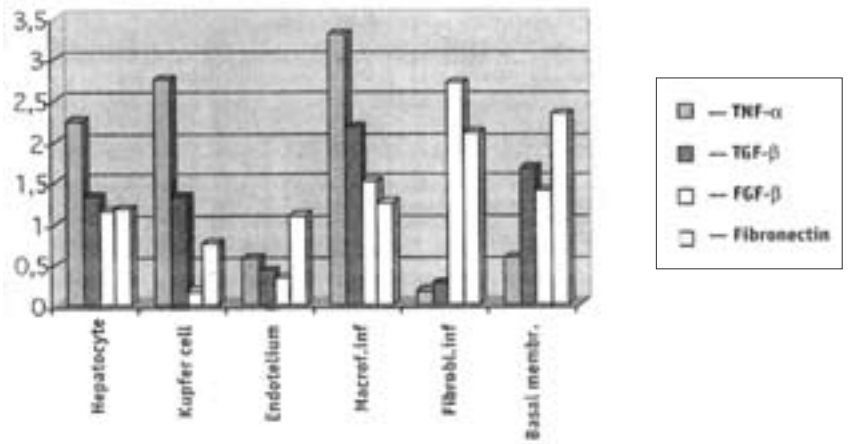


Expression of collagen type IV in liver tissue during chronic hepatitis and after Iscador treatment. Collagen type IV in the extracellular matrix along the sinusoids. Staining: immunoperoxidase (magnification 400 x).

Immunohistochemistry showed that, in hepatitis C, Kupffer cells are the main hepatic production sites of the most important cytokines. Hyperplasia of these cells is attended with their producing greatly increased levels of apoptosis- and fibrosis-promoting cytokines such as TGF-β, TNF-α, and FGF-β. This hyperactivity of the Kupffer cells probably plays an important role in the progression of the disease. In the course of Iscador therapy we observed the hyperplasia of the Kupffer cells and their production of TGF-β, TNF-α, and FGF-β to be reduced, changes that were correlated with the positive clinical dynamics.

From the clinical, biologic, immunologic, and morphologic points of view, it has been observed that Iscador exerts a clear-cut positive effect on the course of hepatitis C. Further studies are necessary to ascertain the optimal duration and regimen of Iscador treatment in chronic hepatitis C.

Figure 14 Expression of apoptosis-promoting factors in the liver cells after Iscador treatment



Kogan EA, Demura SA, Azov AG, Medical Academy I.M. Sechenov, Moscow

Tomkevich MS, Turjanov MCh, Russian Medical Academy of Postgraduate Studies, Moscow

Malyshev NA, Blochina NP, Hospital for Infectious Diseases No. 1, Moscow

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ANNEX

The study described in the above papers was carried out in 1999-2001 in Moscow with the collaboration of O. Titze, MD (Schwäbisch-Gmünd) and J. Klasen, MD (Asklepios Westklinikum Hamburg). Below, excerpts from the study protocol of M. Tomkevich (Moscow 2001) are reproduced; they contain important details and additional information relative to the study design.

Aim of the study

The study aimed at exploring the efficacy and tolerability of Iscador Qu series 0 and Iscador Qu Spezial on the progressive course of hepatitis C in groups of patients classified according to the presence of viral markers and other signs of activity.

Site of the study

The study was carried out at the Moscow Center of Hepatology in collaboration with the Hospital for Infectious Diseases No. 1 (Moscow) and the Russian Medical Academy of Postgraduate Studies (Moscow). The laboratory analyses and the histopathologic evaluations were performed in leading institutes of Moscow and other Russian institutes.

Conditions of the study

The efficacy of Iscador was assessed according to the clinical disease course, laboratory measurements (specific disease-related and immunologic variables), and the results of biopsies.

Every side effect mentioned by a patient or observed by his physician was registered.

Laboratory analyses and functional tests were performed at times 0 and on months 0.5, 1, 3, 6, 8, 10, and 12 of treatment (8 times). The duration of therapy was 12 months, and there was a 6-month follow-up period. All changes in laboratory values and in functional status were analyzed.

Inclusion criteria

- Patients of both sexes aged 18-55 years;
- Detection of HCV RNA and anti-HCV antibodies;
- ALT and AST values at least twice the normal.

Exclusion criteria

- Treatment with corticosteroids or immunosuppressants during the last three months before the beginning of Iscador treatment;
- Chronic liver diseases of other origin;
- Alcoholism, drug addiction;
- Diabetes;
- Treatment with interferon in the last 6 months before the beginning of Iscador treatment;
- Detection of HBsAg, anti-HBe, or anti-HBc;
- Tuberculosis;
- Cancer;
- AIDS;
- Pregnancy;
- Severe cardiovascular disease, airway disease, kidney disease, gastrointestinal disease, hematologic disease and other diseases;
- Severe system diseases such as Crohn‘s disease;
- Increased risk of infections for occupational reasons.

Patient information

Before the study was started, the information for patients and the consent to participate in the study were signed by all the patients.

Dosage, route of administration and regime

In the initial phase, Iscador Qu series 0 was injected subcutaneously 2 to 3 times a week in slowly increased doses. If there was the expected local skin reaction, the dose attained was used for the maintenance phase, in which 1 mg of Iscador Qu Spezial was injected subcutaneously 3 times a week. There were checks of the transaminases, C-reactive protein, other parameters of inflammation and possible side effects at regular intervals according to a precise plan, and the results of these investigations were registered.

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Title of the Russian original:
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