Immunomodulating effect of adjuvant interferon inducers in patients receiving combined photocoagulation/ strontium-90 brachytherapy organ-saving treatment for uveal melanoma

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Background: *A great deal of evidence has been accumulated over the last decade indicating that a failure of immunologic control mechanisms plays an important role in the tumor initiation and growth. Our experience argues that a systemic approach to the treatment of cancer will allow not only the enhancement of capabilities of the organ-saving treatment, but also the improvement of prognosis for survival time.*

Purpose: *To investigate the immunomodulating effect of adjuvant interferon inducer, tilorone, in patients receiving the combined photocoagulation/ strontium-90 brachytherapy treatment for uveal melanoma.*

Materials and Methods: *This prospective study involved 83 uveal melanoma patients of the Filatov institute oncology department. They were divided into two groups comprising 43 patients who received the combined treatment with adjuvant tilorone therapy, and 40 patients whose disease was managed by the combined treatment alone, respectively. The immunological status was determined at the baseline, and at 3-, 6-, and 9-month time points of the study.*

Results: *In both groups, the baseline immunological features were decreased CD8 (suppressor T cells/ cytotoxic T cells) percentage, increased CD4/ CD8 ratio, and subnormal CD16 (natural killer cells) percentage, whereas the baseline humoral immunity features were increased IgA levels with respect to healthy controls. When used as an adjuvant to the combined treatment for uveal melanoma, tilorone offers benefit beyond the combined treatment alone with respect to normalization of the immunological status of the patients (including normalization of suppressor T cells/ cytotoxic T cells (CD8) percentage, NK cells (CD16) percentage, and CD4/CD8 ratio). Additionally, normalization of IgA levels is observed.*

Conclusion: *Tilorone can be integrated into a complex modality approach for uveal melanoma treatment as an immune correction agent with the anti-tumor resistance-enhancing capacity.*

Introduction

A great deal of evidence has been accumulated over the last decade indicating that a failure of immunologic control mechanisms plays an important role in the tumor initiation and growth.

The current opinion is that the anti-tumor resistance involving both non-specific and specific mechanisms of immunological reactivity plays an important role in the pathogenesis of uveal melanoma [1, 2].

Our experience argues that a systemic approach to the treatment of cancer will allow not only the enhancement of therapeutic capabilities of the organ-saving treatment, but also the improvement of prognosis for survival time [3].

Administration of chemically synthesized and biological medical products with immunotropic activity both along with the main treatment of patients with malignancies and immune system disorders, and to prevent cancer recurrence and metastasis in the remission period, is an essential component of the treatment for uveal melanoma. The primary goal of immunotherapy is to target the defects in the immune system and to eliminate tumor cells. This treatment modality exerts its action on cancer cells both through activation of the immune system as a whole, and through activation of natural killer cells, doing no harm to normal body cells. The effect of immunotherapeutic agents does not depend on the division phase of the cancer cell cycle, and has no linear relationship with time (as opposed to, e.g., radiotherapy).

Currently, interferon and its inducers can be regarded as the most effective non-specific therapy for ocular tumors.

The substances which can induce interferon (IFN) have been generally classified into high molecular weight and low molecular weight inducers, either natural or synthesized compounds. All of them have antitumorigenic, antiviral, immunomodulating and antiproliferative properties.

The use of IFN inducers has a number of advantages as compared to the use of recombinant interferon preparations.

Tilorone, an IFN inducer, promotes the synthesis of IFN-α, IFN-β, and IFN-γ of no antigenicity. Their synthesis is controlled by interleukins and repressor proteins and the production does not rise to the level which can be harmful for the body [4, 5].

Additionally, IFN inducers do not promote non-specific cytotoxicity and do not amplify the autoimmune response of the body [6].

Since malignant tumor growth results in immunodepression, one of the most important tasks of cancer immunotherapy is clinical investigation of the preparations which possess antitumor activity and exert no cytopenic effect on immunocompetent cells. Administration of high doses of recombinant interferon can cause cytopeny [5, 7].

Some investigators [7, 8] have shown that tilorone exhibits antitumor activity against a number of transplantable tumors, including the ascitic Ehrlich carcinoma, and inhibits the development of leukemia and growth of virus-induced tumors without hematopoietic suppressive effect. The species specificity of interferon is determined by the structure of the receptor and can be surmounted by incorporation of interferon into liposomes. The number of receptors on a cell surface may be tens to thousands. The level of receptor expression on the membrane is the cell feature; the expression can be suppressed by administration of high interferon doses (more than 6 mln IU), but restores within 48 hours [7].

In vitro, tilorone induces interferon in cultures of immunocompetent cells. According to different authors, the level of interferon induction varies widely across cell types [9, 10].

Tilorone is a low molecular weight fluorene compound which penetrates the blood–encephalic barrier. When interferon is administered orally, the blood level of IFN peaks within 12 to 18 hours, and disappearance of interferon from the blood stream is observed within 48 hours. The most significant levels of IFN have been found in the intestine and liver. Epithelial cells, hepatocytes, T cells and granulocytes have been found to be the main producers of IFN following oral administration of IFN [5].

A number of studies [11, 12] have shown that administration of interferon contributes to the improved inflammation and increased macrophage activity, amount of proinflammatory cytokines, and lysosomal enzyme secretion.

It has been reported that tilorone can stimulate the cytolytic activity of lymphocytes [13, 14] and antibody production, and restore the ratio of T helpers/T suppressors [5, 8]. Additionally, tilorone promotes the proliferation and differentiation of marrow cells and synthesis of membrane receptors [4, 6].

It has been shown that IL-2, when incubated with lymphocytes, can generate a subset of cytolytic

lymphocytes, lymphokine-activated killers (LAK). A comparative analysis of the proteins secreted by LAK cells and by tilorone induced lymphocytes has shown that the cells of both types can cause both contact lysis and secretory lysis [14, 15].

Within the process of organ-saving treatment for uveal melanoma, the activation of immune system occurs. This has been confirmed by morphological studies which revealed an increase in the amount of immunocompetent cells infiltrating the tumor parenchyma following exposure to physical factors [2].

Ample evidence exists that adjuvant administration of interferon improves the results of antitumor therapy [3, 16, 17]. Currently, interferon inducers are actively used in the treatment of tumors [18].

The purpose of this prospective study was to investigate the immunomodulating effect of adjuvant interferon inducer, tilorone, in patients receiving the combined photocoagulation/strontium-90 brachytherapy treatment for uveal melanoma.

Materials and Methods

The study involved 83 uveal melanoma patients of the ocular oncology department of the Filatov Eye Disease and Tissue Therapy Institute, Odessa.

The uveal melanoma patients were divided into two groups. Group 1 comprised 43 patients who received photocoagulation and strontium-90 brachytherapy with adjuvant tilorone therapy, while group 2 comprised 40 patients whose disease was managed by photocoagulation and brachytherapy without immunological correction.

Tilorone was administered at a dose of 125 mg orally on 2 consecutive days for 5 weeks of a 5-week cycle. The cycles were repeated every 65 days for a total of 5 courses.

Immunological status [19] of uveal melanoma patients was determined at the baseline, and at 3-, 6-, and 9-month time points of the study.

The mean baseline tumor protrusion was statistically significantly higher in uveal melanoma patients receiving adjuvant tilorone therapy than in those not receiving it (7.4 ± 3.1 mm vs. 5.8 ± 2.9 mm, respectively, P = 0.001). Moreover, the mean baseline tumor volume was significantly higher in the uveal melanoma patients of group 1 than in those of group 2 (66.8 ± 32.7 mm3 vs. 52.8 ± 31.2 mm3, respectively, P = 0.05).

Table 1. Indices of T cell cellular immunity, humoral immunity and phagocytic neutrophil activity in the patients (n = 43) receiving combined photocoagulation and strontium-90 brachytherapy with adjuvant tilorone therapy for uveal melanoma, M ± SD

Immunity indices (relative values)	Time points of the study				Control healthy blood donors (N=16)
	baseline	3 months	6 months	9 months	
CD3+ T cells (%)	62.8±12.4	65.6±14.2	67.2±16.2	68.9±17.1	64.4±6.3
CD4+ T helpers (%)	44.6±10.8	46.4±10.2	48.2±12.3	46.8±10.4	42.1±6.8
CD8+ T suppressors/ cytotoxic cells (%)	9.2±4.8↓	11.2±3.9	12.6±8.9	11.6±4.8	16.5±5.1
CD4/CD8 ratio, immuno-regulatory index	4.8±2.4↑	4.0±2.8	3.8±2.4	4.0±2.6	3.0±1.4
CD16+ NK cells (%)	8.0±4.2↓	12.4±3.8*	14.8±4.2*	16.8±4.4*	16.4±5.4
CD19+ B cells (%)	14.4±4.8	13.8±3.6	14.2±3.8	16.2±2.9	16.6±3.4
Phagocytic neutrophil activity (%)	64.0±16.2	68.2±15.4	72.8±14.8*	70.4±14.6*	68.4±8.2
IgA (g/L)	2.2±0.8↑	1.8±0.6	1.9±0.4	2.0±0.6	1.9±0.5
IgM (g/L)	1.2±0.4	0.8±0.4	0.9±0.3	0.8±0.3	0.8±0.2
IgG (g/L)	11.8±4.1	14.3±2.8	15.2±4.1	16.2±4.4	13.9±2.5

Note: *, P < 0.05, compared with the group of uveal melanoma patients not receiving tilorone (Table 2); ↓, reduced values with respect to controls (healthy blood donors); ↑, increased values with respect to controls (healthy blood donors)

Table 2. Indices of T cell cellular immunity, humoral immunity and phagocytic neutrophil activity in the patients (n = 40) receiving the combined treatment alone for uveal melanoma, M ± SD

Immunity indices (relative values)	Time points of the study				Control healthy blood donors (N=16)
	baseline	3 months	6 months	9 months	
CD3+ T cells (%)	60.0±11.2	62.0±12.2	64.2±14.2	62.8±15.4	64.4±6.3
CD4+ T helpers (%)	42.8±14.2	44.9±12.4	40.8±10.4	46.2±11.2	42.1±6.8
CD8+ T suppressors/ cytotoxic cells (%)	8.4±5.4↓	8.2±3.8↓	8.6±4.2↓	9.4±4.0↓	16.5±5.1
CD4/CD8 ratio, immuno-regulatory index	5.4±2.8↑	5.5±2.6↑	4.7±2.4↑	14.9±2.9↑	3.0±1.4
CD16+ NK cells (%)	8.2±5.2↓	8.4±4.2↓	9.2±3.8↓	8.6±5.0↓	16.4±5.4
CD19+ B cells (%)	14.2±4.6	12.8±4.2	12.3±3.7	15.2±4.0	16.6±3.4
Phagocytic neutrophil activity (%)	62.0±15.6	64.1±8.2	60.8±11.0	64.6±10.4	68.4±8.2
IgA (g/L)	2.4±0.9↑	2.8±0.8↑	2.9±0.6↑	2.6±0.9↑	1.9±0.5
IgM (g/L)	0.9±0.4	1.0±0.2	1.2±0.4↑	1.0±0.3	0.8±0.2
IgG (g/L)	12.2±3.6	14.8±3.8	14.6±4.0	12.8±2.8	13.9±2.5

Note: ↓, reduced values with respect to controls (healthy blood donors); ↑, increased values with respect to controls (healthy blood donors)

Results and Discussion

The indices of T cell cellular immunity, humoral immunity and phagocytic neutrophil activity for the patients receiving adjuvant tilorone therapy are shown in Table 1, whereas those for the patients not receiving it are shown in Table 2.

In both groups, the baseline immunological features were decreased CD8 (suppressor T cells/ cytotoxic T cells) percentage and increased CD4/CD8 ratio (immunoregulatory index) with respect to healthy controls, and subnormal CD16 (natural killer cells) percentage, whereas the baseline humoral immunity features were increased IgA levels with respect to healthy controls.

After 3 months of therapy, the CD8 (suppressor T cells/ cytotoxic T cells) percentage normalized in the patients receiving adjuvant tilorone therapy, and remained subnormal in the patients not receiving it. Additionally, the CD16 (natural killer cells) percentage normalized in the patients of group 1, and was significantly increased in those of group 2 (12.4 ± 3.8 vs. 8.4±4.2%, P < 0.05). Moreover, at the 3 month time point, the IgA levels normalized in the patients receiving adjuvant tilorone therapy and were increased in the patients not receiving it.

At 6- and 9 month time points, the CD8 (suppressor T cells/ cytotoxic T cells) percentage

and CD4/CD8 ratio remained in the normal range in the patients of group 1. Additionally, the CD8 (suppressor T cells/ cytotoxic T cells) percentage and CD4/CD8 ratio remained subnormal and was increased, respectively, in the patients of group 2.

At the 6 month time point, the CD16 (natural killer cells) percentage was statistically significantly increased in the patients receiving adjuvant tilorone therapy compared with those not receiving it (14.8 ± 4.2 % vs. 9.2 ± 3.8 %, respectively, P < 0.05). A similar relationship was observed at the 9 month time point.

The NK cell percentage (CD16) was statistically significantly higher in the patients of group 1 (16.8 ± 4.4 % vs. 8.6 ± 5.0 %, respectively, P < 0.05). By the sixth and ninth months of the study, the increase in phagocytic neutrophil activity was statistically significantly higher in the uveal melanoma patients of group 1 than in those of group 2. At all non-baseline (i.e., 3-, 6- and 9-month) time points of the study, IgA levels were above normal in the patients not receiving adjuvant tilorone therapy, and stabilized within a normal range in those receiving it.

Conclusion

1. When used as an adjunct to the combined treatment for uveal melanoma, tilorone offers benefit beyond the combined treatment alone with respect to normalization of the immunological status of the patients (including normalization of suppressor T cells/ cytotoxic T cells (CD8) percentage, NK cells (CD16) percentage, and CD4/CD8 ratio). Additionally, normalization of IgA levels is observed.

2. Tilorone, an interferon inducer, can be integrated into a complex modality approach for uveal melanoma treatment as an immune correction agent with the anti-tumor resistance-enhancing capacity.

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