ORIGINAL ARTICLE

Effects of Iscador preparations on the reactivity of mouse immune system

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Abstract

OBJECTIVES: Anticancer preparations made from plants have been an object of scientific interest for many years. It is worth noting that as many as 25% of cytostatics used in the anticancer chemotherapy are obtained from plants. One of the medical preparations which significantly influences cell metabolism is Iscador. Iscador preparations are used as complementary therapy in the conventional anticancer treatment. These are aqueous extracts of mistletoe (*Viscum album L.*). One repeatedly finds that mistletoe (*Viscum album L.*) extracts show immune-modulating effects.

THE AIM at the present work was to study the influence of iscador Qu, M, P at a dose 5 mg/kg b.w., on the total protein concentration in blood serum and proportions of blood protein fractions determined by electrophoresis. Additionally leukocyte activity was estimated, which, served as indicators of the immune system reactivity in mice treated with anticancer preparations of vegetable origin.

RESULTS: The experiment indicated statistically significant increase in albumin fraction level and lymphocyte count. Moreover, decrease of the total protein content, protein fractions globulins α2, β, γ and neutrophil, monocyte count in mouse serum was observed.

INTRODUCTION

Immune system plays significant role in the development of many diseases, including cancer. Numerous experimental and clinical data show, that the formation of tumours coincides with changes in the immune system arrangement. It is, therefore, assumed that the reversal of those changes may be beneficial in the treatment of neoplastic diseases. Since the very invasive character of those diseases, a quick and strong support of the immune system re-arrangement seems necessary. Iscador preparations are used as complementary therapy in the conventional anticancer treatment (Klopp *et al.* 2005). These are aqueous extracts of mistletoe (*Viscum album L.*) parasitizing apple tree (Iscador M), oak (Iscador Qu) or pine tree (Iscador P). They contain a variety of bioactive substances. The greatest therapeutic effect, particularly in the anticancer therapy, has been associated with viscumins and viscotoxins. As shown on various cells lines in culture, Iscador preparations are cytotoxic to cancer cells but have little effect on normal cells (Hubert *et al.* 2002). This substantial selectivity towards transformed cells is apparently due to viscumines (lectins) MLI, MLII, MLIII. They are cytotoxic glycoproteins with molecular mass of 56-64 kDa, able to distinguish between the cell membranes of normal and cancerous cells. This is due to the ability of the lectins to form specific bonds with sugar moieties of the cell membrane proteins, known to be different in normal and

cancer cells. According to Fritz *et al.* (Fritzet *et al.* 1999) viscumins are able to form bonds with approximately 70 % of cancer cells.

Viscum album L. contains a group of basic proteins called viscotoxins (A1, A2, A3, B, 1-PS and U-PS) (Coulon *et al.* 2002). They also contain large proportions of cysteine residues and, therefore, are classified as thionins. The most commonly observed effect of viscotoxins on the cell is a destruction of the plasma membrane integrity. The mechanism of this effect is yet unknown. There are also data demonstrating immunomodulatory effects of viscotoxins (Klein *et al.* 2002; Mengs *et al.* 2002; Maier & Fiebig 2002; Chernyshov *et al.* 2000; Buelow *et al.* 2008; Elluru *et al.* 2006) through activation of NK cells, lymphocytes T and cytokines. *In vitro* studies by JANSSEN *et al.* (1993) showed inhibition of the growth of neoplastic cells. Viscumines inhibit protein synthesis what leads to cell death by apoptosis, while viscotoxins damage plasma membranes causing cell death by necrosis (Büssing *et al.* 2005; 2007).

In the present work, the total protein concentration in blood serum and proportions of blood protein fractions determined by electrophoresis as well as leukocyte activity, served as indicators of the immune system reactivity in mice treated with anticancer preparations of vegetable origin.

MATERIAL AND METHODS

The experiments were carried out on 40 mice males, average body weight 25–26 g, bred in the constant light conditions LD 12:12 and fed with standard diet with unlimited access to water. The animals were divided into four groups: a control and three experimental groups. Control animals were given a single intraperitoneal injection of 100 μl of saline. Animals of the first experimental group were treated with 5 mg/kg b.w. of iscador Qu (100 μl), the second with iscador M and the third with iscador P at the same doses. Twenty four hours after injection animals were anaesthetized and decapitated. The blood samples were collected from the carotid artery. The total protein content in blood sera was determined according to a modification of Lowry's method Kirsche and Wiederanders, (1984). Serum protein fractions were measured by the method of Laemmli (Laemmli *et al.* 1970) . The results were statistically evaluated with the Student's t-test.

RESULTS

Iscador preparations Qu, M and P lowered the total protein content in sera of treated animals by 8.5, 4.4 and 3.1%, respectively (***Table 1***). The changes were statistically significant ($p \leq 0.001$ or $p \leq 0.01$).

The question arouse whether those preparations also influence proportions of the individual serum protein fractions. Therefore, it has been decided to study the concentration of albumin and globulin fractions in sera of the control and treated mice.

Treatment with Iscador preparations increased albumin and decreased globulin content in the blood sera of experimental animals (***Tables 2-4***). The changes were statistically significant ($p \leq 0.001$). The most pronounced effect was that of Iscador Qu (nearly 18% increase in the content of albumin). Among globulin sub-fractions, the levels of α2, β and γ significantly decreased; the only exception was β-globulin in mice treated with Iscador P (that was unchanged). On the contrary, α1-globulin that was undetectable in the blood sera of control animals appeared in small amounts (approximately 2 %) in sera of treated animals.

In the next series of experiments we traced the reactivity of white blood cells with special attention to the pool of lymphocytes, monocytes and neutrophils. All three Iscador preparations elevated the count of lymphocytes in the blood of treated animals (***Table 5***). The changes were statistically significant ($p \leq 0{,}001$). Iscador Qu exerted the greatest and Iscador P the smallest effects. Simultaneously the contents of neutrophils and monocytes were significantly reduced ($p \leq 0.001$) and again, Iscador Qu and P were the most and the least effective, respectively (***Tables 6 and 7***).

DISCUSSION

Increase of albumin concentration has been observed in dehydration and anaphylactic shock. The important function of albumin is its ability to bind and transport a large variety of ligands. Among them there are free fatty acids, calcium, steroid hormones, bilirubin, and some of the tryptophan present in plasma. The observed increase in the albumin fraction after treatment with Iscador, may be important for the therapy since many pharmaceuticals, including sulphonamides, penicillin G, dicoumarol and aspirin, are bound and transported by this protein. Furthermore, Szaroma *et al.* (2006), reported an increase in calcium ion concentration in mouse blood serum after administering of Iscador. It seems that an increase in albumin fraction could be also a response to hypercalcemia and could be viewed as an attempt by the organism to maintain the balance between the free and protein-bound calcium ions. The concentration of proteins in blood serum varies in different pathological conditions like inflammation, neoplastic diseases or infections. Also, many protein functions activate only under such conditions. It is worth noticing that in the present work, the α1-globulin fraction could only be demonstrated after the administration of Iscador. This fraction usually consists of alpha 1-acid glycoprotein (AGP), α1-antitrypsin and α1-lipoprotein. AGP is a typical acute-phase protein; its level increases 3-4 times under inflammatory conditions (Eap *et al.* 1993) and it was found to react with the lymphocyte surface (Kushner *et al.* 1993). Another component of α1-globulin fraction, α1-lipoprotein may

Table 1. Total protein content in blood sera of mice treated with Iscador preparations

Group	Total protein [g/l]	Change (%)	Sd	Test-t
Control	57.4 ± 0.2	-	0.8	-
Iscador Qu	52.5 ± 0.3	↓8.5	1.0	11.7*
Iscador M	54.8 ± 0.3	↓4.3	0.9	6.1*
Iscador P	55.6 ± 0.4	↓3.1	1.2	13.6**

Statistically significant at: *$p \leq 0.001$; **$p \leq 0.01$

Table 2. Proteins fractions in blood sera of mice after administration of Iscador Qu

FRACTION	Control (%)	Iscador Qu – 5 mg/kg m.c. (%)	Change (%)
Albumin	57.4 ± 0.21	67.7 ± 0.09*	↑17.9
Globulin α1	-	1.9 ± 0.08	-
Globulin α2	16.1 ± 0.19	12.6 ± 0.08*	↓21.7
Globulin β	16.7 ± 0.09	13.6 ± 0.07*	↓18.5
Globulin γ	9.8 ± 0.05	4.2 ± 0.02*	↓57.1

* statistically significant at $p \leq 0.001$

Table 3. Proteins fractions in blood sera of mice after administration of Iscador M

FRACTION	Control (%)	Iscador M – 5 mg/kg m.c. (%)	Change (%)
Albumin	57.4 ± 0.21	63.8 ± 0.06*	↑11.1
Globulin α1	-	2.0 ± 0.03	-
Globulin α2	16.1 ± 0.09	15.5 ± 0.05*	↓3.7
Globulin β	16.7 ± 0.09	14.1 ± 0.03*	↓15.5
Globulin γ	9.8 ± 0.05	4.6 ± 0.05*	↓53.1

*statistically significant at $p \leq 0.001$

Table 4. Proteins fractions in blood sera of mice after administration of Iscador P

FRACTION	Control (%)	Iscador P - 5 mg/kg m.c. (%)	Change (%)
Albumin	57.4 ± 0.21	62.9 ± 0.25*	↑9.5
Globulin α1	-	1.4 ± 0.05	-
Globulin α2	16.1 ± 0,09	13.9 ± 0.09*	↓13.6
Globulin β	16.7 ± 0.09	16.9 ± 0.08^{ns}	↑1.1
Globulin γ	9.8 ± 0.05	4.9 ± 0.02*	↓50.0

* statistically significant at $p \leq 0.001$

bind and transport many drugs as well as endogenous compounds, such as steroids and autocoids (Eap *et al.* 1993). The observed increased amounts of albumin and alpha 1-globulin may suggest that one of these serum protein fractions (or both) participate in the transport of various Iscador components.

Growing body of indirect evidence indicates that human tumour cells contain specific antigens able to elicit immune response. Observed some times, spontaneous tumour regression usually occurs with inflammatory reactions. This suggests possible immune mechanism. There are two types of immune response, cellular and humoral. Antigens induce activation and proliferation of lymphocytes B. This proceeds in cooperation of lymphocytes T and leads to the differentiation of B-cells into effector B-cells (plasma cells

Tab. 5. Lymphocyte count in the blood of mice treated with Iscador preparations

Group	Lymphocytes (%)	Change (%)	Sd	Test-t
Control	55.8±0.3	-	1.2	-
Iscador Qu	74.9 ± 0.2	↑34.2	0.7	42.1*
Iscador M	68.3 ± 0.5	↑22.4	1.6	19.3*
Iscador P	62.5 ± 0.4	↑12.0	1.3	11.5*

*statistically significant at $p \leq 0.001$

Tab. 6. Neutrophil count in the blood mice treated with Iscador preparations

Group	Neutrophils (%)	Change (%)	Sd	Test-t
Control	41.0 ± 0.3	-	1.1	-
Iscador Qu	21.6 ± 0.5	↓ 47.3	1.7	29.7*
Iscador M	28.0 ± 0.3	↓31.7	1.2	24.1*
Iscador P	34.1 ± 0.5	↓ 16.8	1.6	10.7*

*statistically significant at $p \leq 0.001$

Tab. 7. Monocyte count in the blood mice treated with Iscador preparations

Group	Monocytes (%)	Change (%)	Sd	Test-t
Control	2.7 ± 0.05	-	1.1	-
Iscador Qu	2.0 ± 0.06	↓ 25.9	0.2	8.5*
Iscador M	2.1 ± 0.04	↓ 22.2	0.1	8.51*
Iscador P	2.4 ± 0.04	↓ 11.1	0.1	4.5*

*statistically significant at $p \leq 0.001$

or plasmocytes) that produce immunoglobulins (antibodies). Thus, the T-dependent stimulation of B cells proliferation is crucial to humoral immune response. Antibodies are present in blood and tissue fluids where they interact with antigens, either soluble or located on cell surfaces. Lymphocytes B can also directly bind free antigens and internalize them by receptor mediated endocytosis. These processes also depend on helper T4 lymphocytes.

Our results indicate that immune stimulating properties of Iscador preparations include activation of certain types of immune cells and promote specific immune defense mechanisms leading to increased lymphocytes proliferation in vivo and in vitro. According to Stoss *et al.* (1999) iscador stimulates the immunity system and is cytotoxic to tumor cells. Moreover, they can speculate that iscador was shown to increase the number and cytotoxicity of NK cells and to induce antitumor response in animals (Fernandez-Botran 1991; Luci *et al.* 2008).

Lowered count of neutrophils and monocytes observed during this work in mice treated with Iscador, is apparently a result of the engagement of this cell fractions in phagocytic reactions. Depletion of these cells has been frequently observed after treatment with other pharmaceuticals.

In conclusion, Iscador induces humoral immune response. Stimulation of lymphocytes involves specific metabolic changes driving these cells into the cell division cycle. Resting lymphocytes undergo stimulation after binding appropriate ligands. These are predominantly antigens but also exogenous pharmaceuticals or mitogens like plant pectin.

Thus, the observed increase of lymphocytes under the influence of Iscador preparations results from the stimulation of resting lymphocytes mainly by viscumins introduced during the treatment. Relatively lower effect of Iscador P may be explained by the composition of this preparation. Its main components are viscotoxins which do not affect hematologic parameters (Hubert *et al.* 2006).

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