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Viscum album L. preparation *Isorel* modifies the immune response in normal and in tumour-bearing mice

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The *Viscum album* (mistletoe) preparation *Isorel* is able to destroy tumour cells and to modify immune reactivity against a particular antigen in normal and in tumour-bearing animals. CBA/HZgr mice and methylcholanthrene-induced fibrosarcoma were used in these studies. A single dose of *Isorel* M (140 mg/kg or 1400 mg/kg body weight) significantly increased the number of plaque forming cells if applied at the time of injection of sheep red blood cells or 1 day earlier. The application of *Isorel* 1 day after sheep red blood cells did not modify the number of plaque forming cells in comparison to the controls. The higher the dose of *Isorel* the stronger is the immune response to sheep red blood cells. Furthermore, one dose of *Isorel* (140 mg/kg body weight) restored the suppressed immune response of fibrosarcoma-bearing mice to a significant extent. Besides modification of the humoral immune response, the survival time of C57Bl/GoZgr male skin grafts on syngeneic female recipients was significantly shorter if *Isorel* was applied at a particular time after grafting. However, according to plaque forming cell numbers, a prolonged application of *Isorel* was significantly immunosuppressive in normal mice and particularly in tumour-bearing mice. It should be mentioned that the doses of *Isorel* used in this experiment were much higher than generally used in cancer patients. In view of the immunomodulating effects of *Isorel*, the monitoring of the immune response of the patients treated with mistletoe preparations is to be recommended.

Keywords: Immunomodulation, mistletoe, *Viscum album*, mouse fibrosarcoma, skin grafting, plaque forming cells.

Introduction

Several *Viscum album* L. (mistletoe) preparations are commonly used for complementary cancer therapy [1–5]. However, for centuries mistletoe plant extracts have been used to treat patients suffering from very different diseases such as hypertension, epilepsy, debility, or connective tissue disorders [6,7]. This wide area of application and the relatively successful results might indicate its mutual influence on the mechanisms regulating the complex function of the organism. Particular attention is still focused on the immunomodulatory action of mistletoe which, together with its pronounced cytotoxic action against tumour cells, leads to a potent therapeutic approach for treating cancer patients [8–10].

Over the last few years we have studied the influence of the mistletoe preparation *Isorel* on different experimental tumours in mice [11–14]. In our defined experimental models, we observed beneficial effects of *Isorel* applied either alone or in combination with local tumour irradiation, chemotherapy, or surgery [11,13]. These findings support the use of *Isorel* in clinical practice. Furthermore, our previous results in experimental models indicate the use of *Isorel* as a potent immunostimulator if the proper dose and time of application are chosen [11,13]. However, the application of *Isorel* could also result in immunosuppression [11,13,15], and this could be of particular interest for its clinical use in cancer patients. To clarify this problem, we investigated the effects of different single and total doses of *Isorel* in tumour-bearing mice and varied the time of application in normal and tumour-bearing mice. The animals were injected with sheep red blood cells and the intensity of the humoral immune response was determined. The reactivity of *Isorel*-treated mice to foreign skin grafts was also determined. A short review of our results on the immunomodulating action of *Isorel* is presented in this article.

Materials and methods

Animals

CBA/HZgr and C57Bl/GoZgr mice of both sexes were used in the experiments. The animals were about 3 months old, weighing 20–22 g at the beginning of the experiment. The mice were maintained in standard conditions with unrestricted access to food and water.

Isorel

The commercial preparation *Isorel* M, strength 60, was used in the experiments. According to the instructions, this mistletoe extract was produced by a special method in aqueous solution avoiding any denaturating manipulation. The extract from 60 mg of 'planta tota' (whole plant) in 1 ml sterilized sodium chloride was supplied in individual ampoules by Novipharm, Austria.

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Table 1. Plaque forming cell values in normal and fibrosarcoma-bearing CBA/HZgr mice immunized with sheep red blood cells and treated with different single doses of *Isorel* or saline applied at the indicated time

Animals injected with SRBC	Treatment	Time of treatment	PFCV (means ± SD ×10³)
Normal mice	*Isorel* (140 mg/kg bw)	1 day prior to sheep red blood cell injection	27.2 ± 3.2*
		On the day of sheep red blood cell injection	31.4 ± 4.1*
		1 day after sheep red blood cell injection	21.0 ± 2.8
	Isorel (1400 mg/kg bw)	1 day prior to sheep red blood cell injection	38.4 ± 4.1**
		On the day of sheep red blood cell injection	41.7 ± 3.1**
		1 day after sheep red blood cell injection	20.5 ± 4.2
	Saline	On the day of sheep red blood cell injection	20.8 ± 1.0
Mice bearing fibrosarcoma	*Isorel* (140 mg/kg bw)	On the day of sheep red blood cell injection	21.3 ± 3.0
(volume approximately 300 mm³)	Saline	On the day of sheep red blood cell injection	9.5 ± 2.0**
Mice bearing fibrosarcoma	*Isorel* (140 mg/kg bw)	On the day of sheep red blood cell injection	18.1 ± 1.7
(volume approximately 2000 mm³)	Saline	On the day of sheep red blood cell injection	7.8 ± 1.8**

SRBC, sheep red blood cells; PFCV, plaque forming cell values; bw, body weight. *$P < 0.02$, **$P < 0.001$.

Tumour

The tumour used in these experiments was a fibrosarcoma induced in the CBA/HZgr female mouse by injecting methylcholanthrene into the subcutaneous tissue of the flank. Fragments from this original tumour had been transplanted to syngeneic hosts and the first-generation specimens were then kept in liquid nitrogen [11]. By injecting these specimens into the recipient, source mice, second-generation transplant tissue was obtained. From these specimens, growing tumour cell suspension was made as described previously [11]. Each of the experimental mice received 10^6 viable tumour cells subcutaneously into the right thigh. By measuring three tumour diameters, the volume was calculated as described elsewhere [11].

Skin grafting

Full-thickness grafts of tail skin were transplanted onto the dorsolateral side on the recipient trunk as described previously [11].

Plaque forming cell assay

Sheep red blood cells were injected intraperitoneally and the number of haemolytic antibodies producing cells in the spleen determined 4 days later [11].

Statistics

Mann-Whitney and χ^2 tests were used.

Results

The influence of single doses of *Isorel* on the intensity of the immune reaction to sheep erythrocytes in normal and tumour-bearing mice

CBA/HZgr mice (8–12 per group) were injected intraperitoneally with sheep red blood cells and treated with *Isorel* (140 mg/kg or 1400 mg/kg body weight) 1 day prior to sheep red blood cell injection, on the day of the injection, or 1 day after the injection. Furthermore, CBA/HZgr mice bearing CMC-1 fibrosarcoma transplanted in the right thigh were injected with sheep red blood cells and treated with *Isorel*. As shown in Table 1, the mice injected with 140 mg/kg of *Isorel* 1 day before or on the day of sheep red blood cell injection produced a significantly higher ($P < 0.02$) number of plaque forming cells than the other animals (untreated controls or these treated 1 day after sheep red blood cell injection). Moreover, the higher dose of *Isorel* (1400 mg/kg) was more effective and the numbers of plaque forming cells obtained were significantly higher than in the control group ($P < 0.001$) and the group treated with 140 mg/kg of the drug ($P < 0.02$). *Isorel* was further able to restore the immune reaction of tumour-bearing mice. As presented in Table 1, the immune reaction to sheep red blood cells is significantly ($P < 0.001$) suppressed, but only one injection of 140 mg/kg *Isorel* completely restored the reactivity of tumour-bearing mice, regardless of tumour size.

The influence of multiple doses of *Isorel* on the intensity of the immune reaction to sheep erythrocytes in normal and tumour-bearing mice

CBA/HZgr mice (8–12 per group) were treated with repeated doses of *Isorel* and injected with sheep red blood cells. As shown in Table 2, the mice receiving 14 mg/kg *Isorel* daily, over 14 consecutive days, reacted significantly ($P < 0.01$) stronger to sheep red blood cells than control animals. After the addition of 140 mg/kg, no influence was observed on plaque forming cell numbers, i.e. on the intensity of immune response to sheep red blood cells. However, the prolonged application of *Isorel* was significantly suppressive. As shown in Table 2, either 14 mg/kg or 140 mg/kg *Isorel* was significantly ($P < 0.001$) suppressive in mice treated with a daily injection over 5 weeks (except at the weekends, i.e. a total of 25 doses). However, in tumour-bear-

Table 2. Plaque forming cell values in normal and fibrosarcoma-bearing CBA/HZgr mice treated with different doses of *Isorel* or with saline over indicated time intervals immunized with sheep red blood cells on the day of the last *Isorel* or saline injection

Animals injected with SRBC	Treatment intervals	Daily dose	PFCV (means ± SD ×10^3)
Normal mice	14 consecutive days (total 14 injections)	*Isorel* (14 mg/kg bw)	120.8 ± 23.2*
		Isorel (140 mg/kg bw)	76.6 ± 19.8
		Saline	83.1 ± 34.2
	Daily over 5 weeks (except weekends, total of 25 injections)	*Isorel* (14 mg/kg bw)	7.2 ± 2.7**
		Isorel (140 mg/kg bw)	30.7 ± 19.8**
		Saline	108.2 ± 30.0
Mice bearing fibrosarcoma (volume approximately 300 mm^3)	14 consecutive days (total 14 injections)	*Isorel* (14 mg/kg bw)	41.4 ± 26.9
		Isorel (140 mg/kg bw)	17.6 ± 7.8*
		Isorel (1400 mg/kg bw)	9.4 ± 5.4*
		Saline	50.2 ± 9.6

SRBC, sheep red blood cells; PFCV, plaque forming cell values; bw, body weight. *$P < 0.01$, **$P < 0.001$.

Table 3. The influence of *Isorel* on C57Bl/GoZgr male skin graft survival on syngeneic female recipients

Treatment	Treatment time	Days of graft survival (means ± SE)
Saline	Days 1–4 before grafting	25.6 ± 1.5
	Days 2–5 after grafting	33.7 ± 4.4
	Days 8–11 after grafting	26.6 ± 1.9
Isorel (60 mg/kg body weight daily)	Days 1–4 before grafting	30.2 ± 2.0
	Days 2–5 after grafting	31.6 ± 3.8
	Days 8–11 after grafting	18.8 ± 1.9*
Control mice	No treatment	29.1 ± 1.8

*$P < 0.05$.

ing mice, 14 consecutive injections of *Isorel*, 14 mg/kg each, did not significantly change the immune reactivity, while higher concentrations significantly suppressed the reactivity ($P < 0.01$).

The influence of *Isorel* application on foreign skin survival time

C57Bl/GoZgr female mice were grafted with the skin from syngeneic males. There were 39 mice in the control group and 10–14 mice in each of the experimental, i.e. *Isorel* or saline-treated, groups. The animals were injected at the inguinal and the axillary areas with either 15 mg/kg *Isorel* in 0.05 ml saline, or with 0.05 ml saline. The daily dose of *Isorel* was 60 mg/kg and the animals were treated for 4 consecutive days, so that the cumulative drug concentration was 240 mg/kg body weight. *Isorel* was given from 1–4 days before grafting, on days 2–5 after grafting, or on days 8–11 after grafting. The grafts were studied twice a day and were considered to be rejected when they did not show epithelium. As shown in Table 3, a significant ($P < 0.05$) shorter graft survival was observed only in the group receiving *Isorel* from days 8–11 following transplantation.

Discussion

Our results confirm that *Isorel* injected at the proper dose and time interval increases the immune response of normal mice and restores the suppressed immune reactivity in tumour-bearing animals, according to the reactivity to sheep red blood cells [11,13]. Besides the modulation of the humoral immune response, a significant stimulation of cell-mediated immune response, i.e. foreign skin graft rejection, occurred if *Isorel* was applied at the critical time. However, a pronounced immunosuppression, determined by plaque forming cell assay, occurred in normal and in tumour-bearing mice exposed to repeated *Isorel* applications. It should be mentioned that the doses applied in these experiments are much higher than those used in clinical practice. Nevertheless, the monitoring of immune reactivity in cancer patients treated with repeated applications of mistletoe extracts is recommended, in spite of the findings that the aqueous mistletoe preparation *Eurixor* is reported to stimulate the immune response in patients receiving regular subcutaneous applications [16].

The influence of the mistletoe preparation *Isorel* on the immune response of the organism is probably very complex, since several biologically active components are present in the plant extract. A modulation of antigen presentation and/or T and B cell co-operation during the immune reaction to an antigen might be possible. T-cell dependent B-cell responses are initiated in the T-cell zone of the lymph nodes to which antigen-activated B cells specifically migrate, an appropriate response for B cells seeking T-cell help [17,18]. Activated B cells may have different fates; they may differenti-

ate into plasma cells, proliferate and form germinal centres, or, if they receive the 'wrong' kind of T-cell help, they die [19–21]. A possible influence of the mistletoe preparation, i.e. its particular components, on these pathways remains to be clarified. The negative regulation of both B- and T-cell responses, as demonstrated in particular experiments on mice, is of outstanding importance [22,23]. It is suggested that B cells are responsible for evoking the appropriate help from T cells, although it is not clear what are the precise molecular signals [24]. Obviously, complexity must exist at levels of receptor structure and signal transduction in order to accommodate the complexity of immunoregulatory ligands. Many different steps are involved in the immune reaction, including the antigen, its presentation by professional cells, activation of T and B cells via specific recognition and the development of a barrage of both non-specific and specific effector cells and products which may help to get rid of the antigen, whether it is free or cell associated [22]. Each of these steps and events occurring in the immune response are regulated by signals impinging on the cells of the immune system from their environment, involving hosts cell products or some other components such as particular applied drugs. Unfortunately we are far from understanding all the complex interacting molecular and cellular processes that underlie immunity [22]. Thus, the complexity of the mistletoe preparation with different biologically active components could, besides its potential therapeutic effect, contribute to defining particular steps in the immune reaction.

Conclusion

The mistletoe preparation *Isorel* stimulates the immune response in normal and in tumour-bearing organisms. However, prolonged application of higher doses resulted in immunosuppression. The influence of the mistletoe preparation on the immune response is probably very complex and is suggested to modulate antigen presentation and/or T and B cell co-operation during the reaction of an organism to an antigen.

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