

Perioperative Application of the *Viscum album* Extract Isorel in Digestive Tract Cancer Patients

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Abstract. *Background:* It is assumed that perioperative immunomodulation of cancer patients can attenuate cellular and humoral deficiencies thus improving their overall health status. Mistletoe (*Viscum album* L.) anticancer drugs are likely candidates for such adjuvant therapy, because they do not have major adverse side-effects but have dual desirable activities; immune-modulating effects and relatively selective cytotoxicity for cancer cells. *Materials and Methods:* We used the aqueous extract Isorel, which is produced from the entire plant and is validated for batch consistency. The study involved 70 cancer patients, divided into two groups: Isorel-treated group of 40 patients who received Isorel for 2 pre- and 2 post-operative weeks (1 esophageal, 16 gastric, 2 pancreatic and 21 colorectal carcinomas) and the age- and sex-matched control group of 30 patients that did not receive Isorel (2 esophageal, 9 gastric, 3 pancreatic, 1 ileac and 15 colorectal carcinomas). Blood samples were obtained to study parameters of the immune system before the surgery and the drug administration (D0) and on the postoperative days 1 and 14 (D1, D14). The overall health status was evaluated after 60 days by the Karnofsky Performance Index and by the Analogic Scale of Anxiety. The results were compared by Student's *t*-test and one-way ANOVA test. *Results:* Isorel significantly attenuated the immunosuppressive effects of surgery observed for the Isorel-treated group, increasing the number of NK cells, the T and B cells, in particular T-helper cells, complement, IgA, IgG and IgM values also in comparison to the respective D0 values. Both the

Karnofsky status and the Anxiety Scale improved remarkably in Isorel-treated patients in comparison to the control. *Conclusion:* The results of this study indicate that perioperative use of the mistletoe drug Isorel can improve immune competence and the overall health status of cancer patients undergoing surgery.

Immunomodulation has an important role in modern surgery. Perioperative care should consider the immune system as a functional, integrated system and to give it the same consideration as is given to any other vital system (1). This is especially important in oncology, because cancer often causes immune depression, while surgery (anesthesia, hemorrhage, stress response, ischemia/reperfusion) can act further as an immune suppressive (2-6). Thus, the combined immunosuppressive effects of surgery and cancer may augment the risk of postoperative infections and the dissemination of malignancy.

The perioperative period, when the tumor burden is strongly decreased, but tumor emboli may be discharged mechanically, might be an important focus for the use of immunotherapeutic drugs. Consequently, we can assume that, with the correction of perioperative immune deficiencies by immunomodulation of the cancer patients, we can achieve an augmented immune status, which can contribute to the antitumoral defense. Hence, applying immunotherapy perioperatively might improve immune system function and increase resistance to cancer itself, in particular in protection against implantation of circulating tumor emboli.

To achieve this aim, we chose adjuvant biotherapy with the mistletoe extract Isorel. Mistletoe (*Viscum album*) has a long history as a plant source for the preparation of different extracts used for cancer therapy (7-13). Several data point to a cytotoxic action of these extracts against various tumor cell lines, while their *in vitro* toxicity for normal cells is less pronounced, indicating relatively selective toxicity primarily

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Table I. Immune parameters analyzed – range of respective normal values.

Parameter	Method of determination	Normal values
Cellular immunity		
White cell count	Coulter	4800-10800/mm ³
Lymphocytes absolute count	Coulter	1300-2900/mm ³
B lymphocytes	Rosette EAC	30-35%
T lymphocytes	Rosette E	40-45%
CD ₂ (T lymph.+NK)	Flow cytometry	53-73%
CD ₃ (T lymphocyte)	Flow cytometry	51-69%
CD ₁₉ (B lymph.)	Flow cytometry	3.8-12.8%
CD ₄ (T-helper)	Flow cytometry	34-52%
T-helper/T-suppressor	Flow cytometry	1.2-2.6
CD ₈ (T-suppr./T cyto.)	Flow cytometry	22-38%
NK	CD ₂ -CD ₃	6-19%
Humoral immunity		
IgG	Nephelometry	694-1618 mg%
IgA	Nephelometry	68-400 mg%
IgM	Nephelometry	40-263 mg%
Complement C ₃	Nephelometry	88-201 mg%
Complement C ₄	Nephelometry	16-47 mg%

against the tumor cells (13-19). On the other hand, the application of *Viscum album* extracts *in vivo* might have immunomodulating effects, mainly stimulating the cytotoxic activity of the lymphocytes (18-24). The dual activity of mistletoe preparations, *i.e.* cytotoxicity for tumor cells and the enhancement of the immune defense against malignant cells, supports the use of these preparations in cancer patients. It is generally believed that the mistletoe lectins (complex proteins of high molecular weight [MW=30-120 kDa]), exert most of the medical effects of mistletoe drugs, while bioactive mistletoe peptides (viscotoxins) and low molecular weight substances (alkaloids, flavonoids, oligosaccharides, *etc.*) increase the overall activity of the plant extracts in comparison to the pure lectins (23-27).

Recent clinical trials showed that postoperative Isorel biotherapy combined with chemotherapy resulted in increased survival of patients with advanced colorectal carcinomas (28).

The purpose of this study was to evaluate if perioperative therapy with Isorel can influence the immunity of patients with digestive cancer and their quality of life.

Materials and Methods

Viscum Album extract Isorel. The original extract Isorel A (*abietis* – fir-tree) (Novipharma GmbH, Pörtlach, Austria) prepared from the fresh mistletoe plant grown on fir-tree was used. Three major components of mistletoe (lectins, alkaloids and viscotoxins) may be responsible for its biological effects (29-30). The preparation is

Table II. The analogic scale of anxiety.

Symptoms	Grade of anxiety
Without anxiety	1
Occasional discreet anxiety	2
Anxious mood, rejection tendency	3
Temporary anxiety, rejection tendency of evidence without vegetative symptoms	4
Transitory signs of anxiety with vegetative symptoms	5
Strong anxiety with vegetative signs	6
Strong anxiety with defending symptoms	7
Very strong anxiety that can be compensated for without drugs	8
Extremely strong anxiety that can be compensated for without drugs	9
Unbearable anxiety that can not be compensated for; needs psychotherapy, drugs, crisis intervention	10

produced by cold-water extraction of 60 mg of the entire plant per ml water, without homogenization of the plant or the extract fermentation. The drug production is standardized according to the good manufacturing practice and additionally validated by batch consistency MTT bioassay (30). The drug has been used for adjuvant cancer biotherapy for more than two decades, with no serious side-effects described.

Patients and the study design. We studied 70 prospective and randomised clinically controlled patients undergoing digestive tract cancer surgery. None of these patients had been treated with immunosuppressive agents or anticancer medication. They were divided into two groups: the Isorel-treated group of 40 patients and the control group of 30 patients who did not receive Isorel.

The Isorel-treated patients suffered from: 1 esophageal, 16 gastric, 2 pancreas and 21 colorectal carcinomas. They were 23 males and 17 females with a mean age of 62 years (range 37-87 years). These patients received Isorel vials containing 60 mg plant/ml extract *s.c.* for 2 weeks preoperatively and 2 weeks postoperatively at a dosage of 6 vials per week as follows: in the first pre-operative week, 1 vial, then 2 vials, then 3 vials every second day; the second pre-operative week the same dosage, but in descending order, 3, then 2, then 1. Postoperative treatments were repeated in the same manner. This rhythmic protocol is based on the recommendation of the manufacturer (Novipharma) and is most frequently used in clinical practice.

The control patients presented with the following types of digestive tract tumors: 2 esophageal, 9 gastric, 3 pancreas, 1 ileac and 15 colorectal carcinomas.

There were no sex and age differences between the two groups, and informed consent was obtained from all patients before starting the study.

Parameters monitored. Peripheral blood samples were obtained before the drug administration (D0) and on the postoperative days 1 (D1) and 14 (D14). The following parameters of cellular and humoral immunity were analyzed by standard clinical laboratory

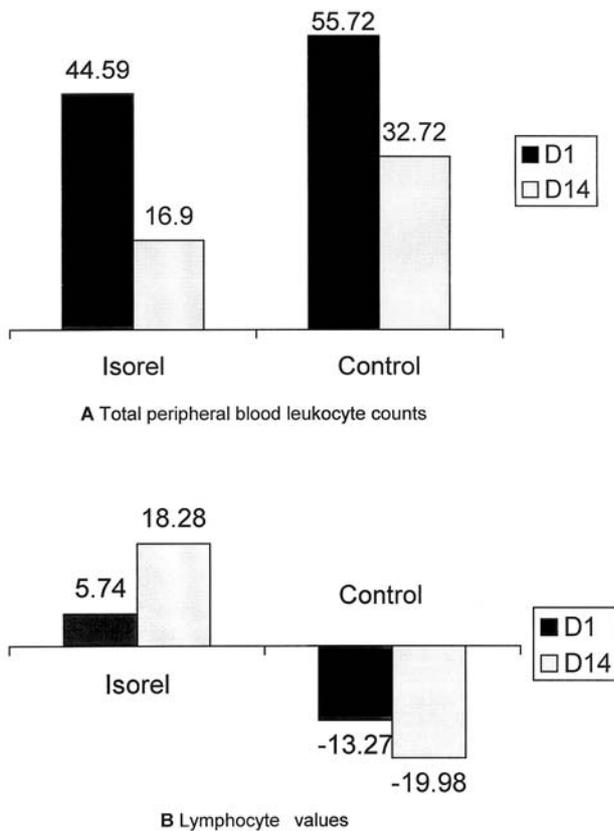


Figure 1. White blood cell counts presented as relative (%) change from respective initial (D0) values.

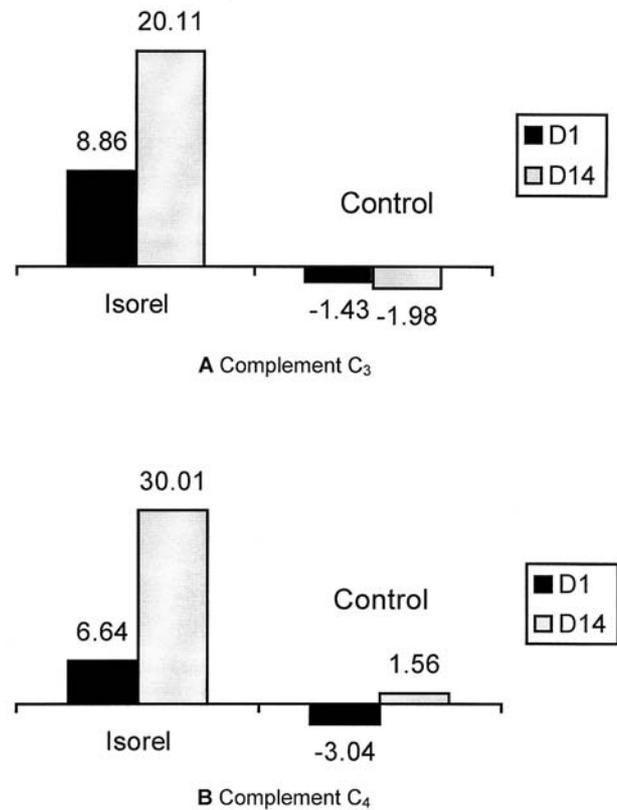


Figure 2. Complement values presented as relative (%) change from respective initial (D0) values.

procedures (flow cytometry and nephelometry): cellular immunity parameters, including leukocyte count and subsets CD₂, CD₃, CD₁₉, CD₄, CD₈, NK (CD₂-CD₃); humoral immunity parameters, including IgG, IgA, IgM, complement C₃ and C₄ (Table I).

The overall health status was evaluated 60 days after beginning of hospitalization by the Karnofsky score and by the psychosomatic status evaluated with the Analogic Scale of Anxiety (Table II).

Statistics. The SPSS program, version 7.5, was used. For each laboratory analysis, descriptive statistical processing was used that included the statistic mean (average), standard deviation and the standard error of mean. The Student's *t*-test was applied to evaluate the differences of mean values, the statistic variation, tendency and statistical significance of null hypothesis or the correlation of the pairs of values (one-way ANOVA) for the tendency and statistical significance of each pair of values.

Results

The number of white cells in the Isorel-treated patients had risen significantly in the first postoperative determination ($p < 0.001$) and was also significantly higher at the second postoperative determination when compared to the

pretreatment values (Figure 1A). The increase of white cells in the control patients was also significant ($p < 0.001$) in both determinations.

For the patients receiving Isorel, we noticed a trend of an increasing number of lymphocytes (Figure 1B) on D1, which was not significant ($p > 0.05$). This trend was significant ($p < 0.001$) on D14, compared with the D0. For the control patients at the same time, we observed a significant decrease of the number of lymphocytes at D1 and D14 ($p < 0.001$).

The value of complement C₃ (Figure 2A) on D14 was increased in Isorel-treated patients when compared to the controls ($p < 0.001$), while in the control patients a slightly decreasing trend was observed for C₃. Similarly, the values of complement C₄ increased significantly ($p < 0.01$) for Isorel-receiving patients, while it did not change in the controls (Figure 2B).

As can be seen in Figure 3, the immunoglobulin values, in particular for IgA and IgM, increased in the postoperative period for Isorel-treated patients at D14 ($p < 0.05$). In the control patients the values were not

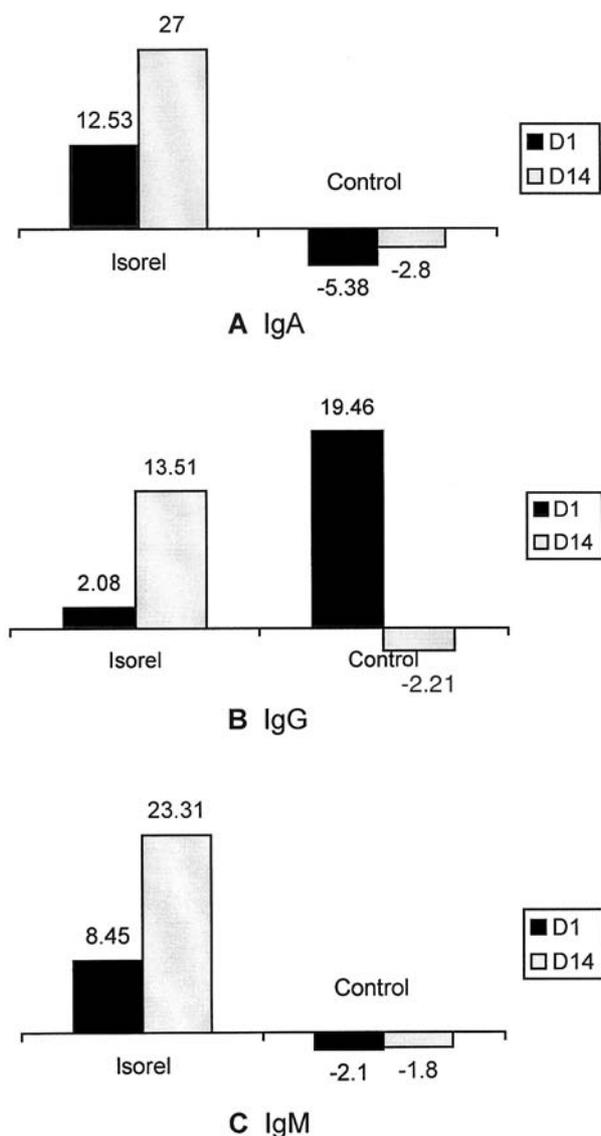


Figure 3. Immunoglobulin values presented as relative (%) change from respective initial (D0) values.

changed, with the exception of the transient increase of IgG at D1, in comparison to the respective values at D0.

The changes in lymphocyte values are shown in Figures 4, 5 and 6. In the Isorel-treated patients the T lymphocyte counts showed a mild decrease on D1, followed by a significant rise at D14 (Figure 4A), while in the control patients a significant decrease on D1 returned by D14 to values equal to those determined on D0. The values of B lymphocytes in the Isorel-receiving patients showed a gradual rise (Figure 4B), while in the control patients we saw a significant decrease ($p < 0.05$) on D1 followed by a slight increase on D14. In the CD₂ (T lymphocytes + NK

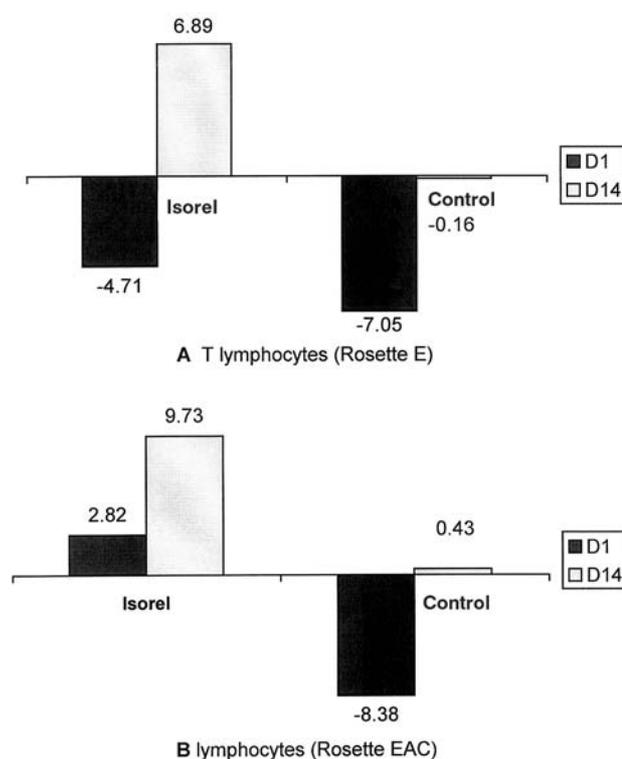


Figure 4. Lymphocyte change presented as relative (%) change from respective initial (D0) values.

cells) determinations (Figure 5A), a significant rise in both postoperative determinations in the patients receiving Isorel was observed ($p < 0.05$). For the control patients a decreasing trend in both determinations was seen, but was not significant. For the CD₃ T lymphocyte determinations, (Figure 5B) we saw a significant rise in both postoperative time-points for the Isorel-treated patients ($p < 0.001$). In the control, we observed non-significant decreases in both determinations. For the CD4 (T-helper) (Figure 5C) we observed a significant rise for both determinations ($p < 0.05$) in the Isorel-treated patients. As in the case of some other lymphocyte subsets, T-helper cells of the control patients showed only indices of non-significant decrease.

In contrast to CD4 (T-helper) values, the values of CD8 (T-suppressor) (Figure 5D) showed a non-significant tendency to rise at D1 ($p > 0.1$) in the Isorel-treated patients and a significant decrease at D14 ($p < 0.05$). For the control patients, there were signs of a slight but non-significant increase of T-suppressor cells ($p > 0.05$).

Consequently, the CD4/CD8 ratio (Figure 6A) increased from the D0 ratio for the Isorel-treated patients on D1 and D14 ($p < 0.05$). In the control patients, the mean values decreased ($p < 0.05$). The NK determinations (CD₂-CD₃) (Figure 6B) in the Isorel-treated patients showed a gradual

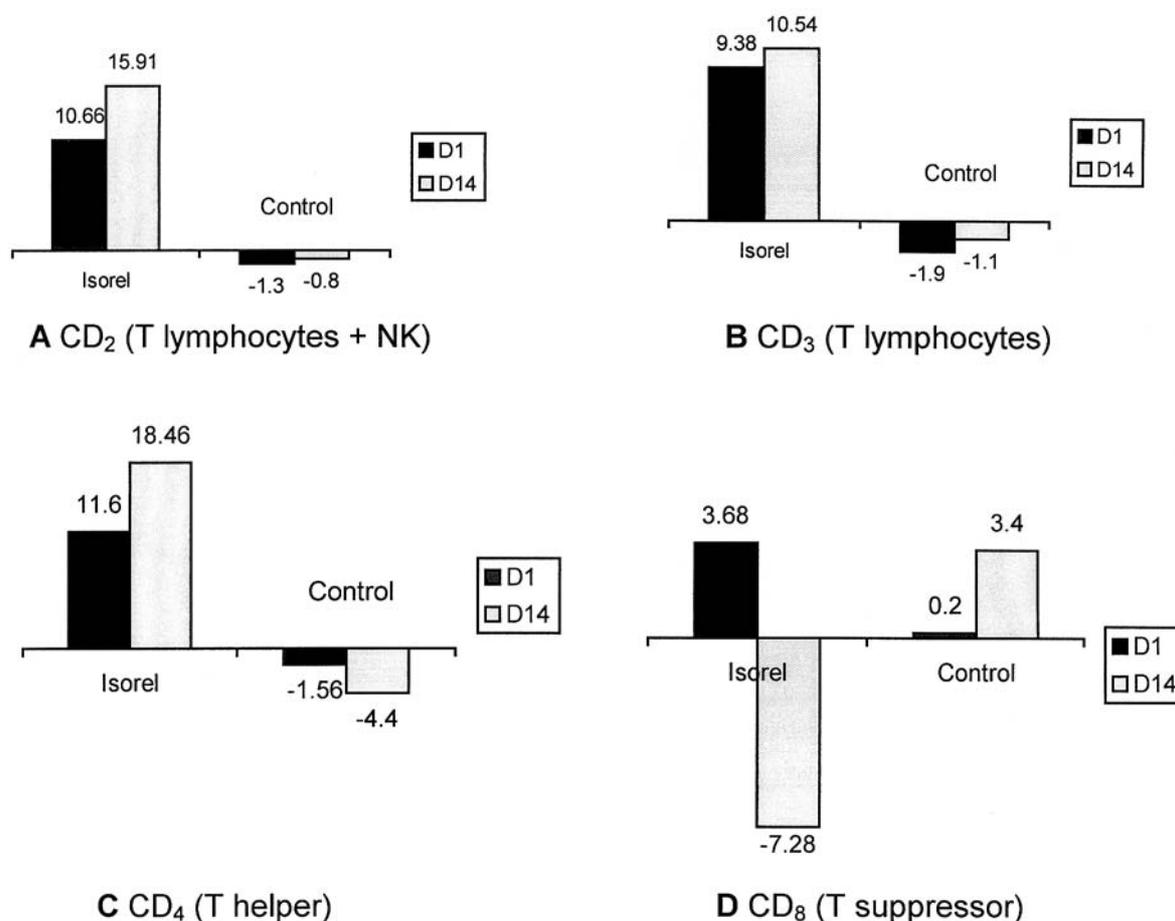


Figure 5. Lymphocyte subsets presented as relative (%) change from respective initial (D0) values.

and strong increase ($p < 0.001$). For the control patients, the indices of non-significant decreases of NK values were observed in both determinations.

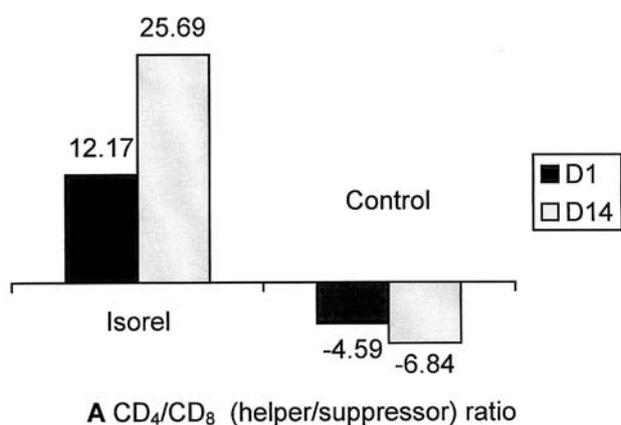
Differences between patients receiving Isorel and the control patients were also observed regarding the overall health status. The Karnofsky performance index (Figure 7A) increased significantly ($p < 0.01$) in the Isorel-treated patients and decreased significantly ($p < 0.05$) in the control patients after 60 days from the beginning of the hospitalization. At the same time, the score on the Anxiety Scale (Figure 7B) decreased significantly for the Isorel-treated group ($p < 0.01$), while it increased significantly for the control group ($p < 0.01$).

Discussion

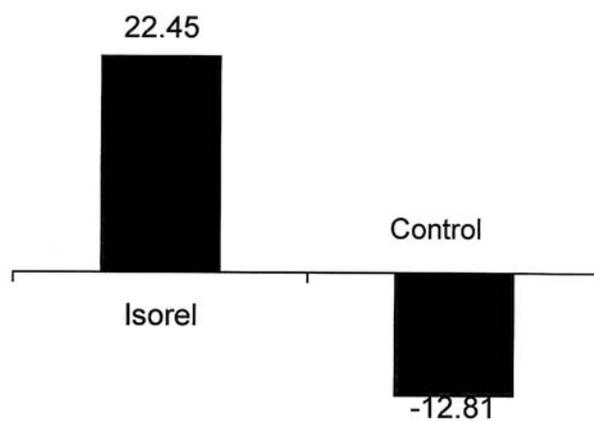
Aqueous mistletoe extracts are used by a majority of cancer patients, and the most recent clinical studies confirm their beneficial effects in various types of cancer (28, 31-38). Taken together with extensive preclinical studies carried out over

two decades, these findings have made complementary cancer therapy by mistletoe extracts one of the most attractive approaches in adjuvant cancer therapy, as acknowledged by the National Cancer Institute and the National Institute of Health, although these drugs are not yet registered by the Food and Drug Administration and are not commercially available in the USA. However, different types of drugs and protocols used could be reasons for inconsistent conclusions obtained by some clinical trials (39, 40).

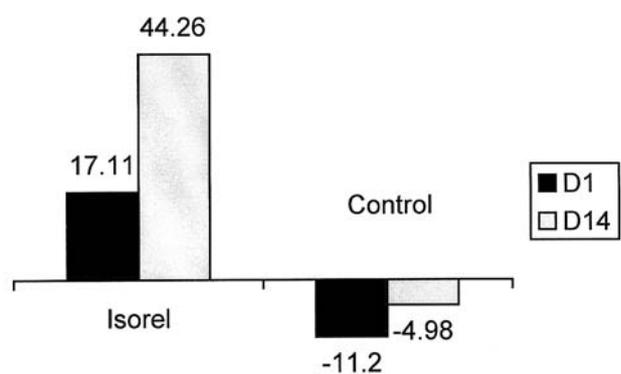
Mistletoe extracts, in particular their lectins, induce both necrosis and apoptosis of the tumor cells, increase immune competence of peripheral blood lymphocytes (in particular NK and T-helper cells) stimulating production of IL₁, IL₆, TNF, IFN- γ , IL₂, IL₁₀ and GM-CSF, that can increase serum levels of β -endorphin and improve quality of life (41-47). Complementary to mistletoe lectins, basic bioactive mistletoe peptides, termed "viscotoxins", are responsible for the additional cytotoxic activities against malignant cells. They also activate the attack of the NK cells and macrophages on the tumor (47, 48). Thus, it is not surprising that the affinity



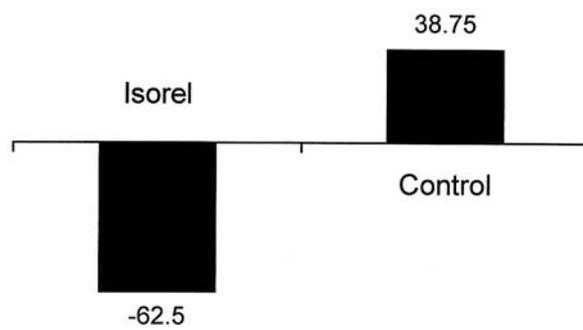
A CD₄/CD₈ (helper/suppressor) ratio



A Karnofsky performance index.



B NK determinations (CD₂-CD₃)



B The analogic scale of anxiety.

Figure 6. T₄/T₈ ratio and NK determinations presented as relative (%) change from respective initial (D₀) values.

Figure 7. Effects of Isorel on quality of life presented as relative (%) change from respective initial (D₀) values.

of tumor cells to bind mistletoe lectins is in reverse correlation with the tumor progression, in particular because these lectins show stronger affinity to bind to tumor cells than their non-malignant counterparts, due to the differences in the glycoproteins of the membrane (12-19, 49).

For the prospective evaluation of immune stimulation, we used the immunomodulating drug Isorel, a total aqueous extract of mistletoe. Studies performed during the last decade revealed that Isorel could inhibit the growth of malignant cells and increase the effectiveness of radio- and chemotherapy, while stimulating host defense against cancer cells and perhaps modulating non-specific immunity (11, 18, 19). The activity of Isorel appears to be "lectin-like", but fractionating the drug into different molecular weight components revealed that none of these fractions was as efficient as the entire drug, indicating the "multifactorial" activity principle (29, 30, 41, 42).

In a previous clinical study, Isorel showed beneficial effects in combined therapy of colon carcinoma patients when used

for several months of postoperative treatment (28). In the current study, which represents the first trial on perioperative treatment done with any mistletoe extract, we noticed significant enhancement of the number of immunocompetent cells and an augmentation of complement factors C₃, C₄, indicating activation of the acute phase reaction. Since initiation and development of the acute phase reaction is induced by cytokines (IL₁, IL₆, TNF-α), we assume that this was the effect of Isorel due to its pro-inflammatory *i.e.* immunostimulating effects (there was no sign of any infection in our patients).

The enhancement of T lymphocytes is an indirect proof of the enhancement of IL₂, a central cytokine in the initiation and maintenance of the immune response. The normalization of the number of T cells through immunotherapy is considered to significantly prolong the survival time (48). Among the lymphocytes, we noticed that the T-helper subset (CD₄) was particularly increased in Isorel-treated patients. Together with an observed decrease

of T-suppressor cells in Isorel-treated patients the Th/Ts ratio had an obvious tendency to normalize in these patients in remarkable contrast to the control group.

Moreover, the unspecific cellular immunity was enhanced after Isorel treatment. Hence, we consider the augmentation of NK cells as the most important result of our trial, which is in agreement with the majority of previous preclinical and clinical studies, irrespective of the type of mistletoe drug used.

Our findings regarding the improvement of both the quality of life and the psycho-emotional status in all patients treated with Isorel compared with the control patients are in agreement with other clinical trials. These findings probably reflect activities of β -endorphin, an endogenous opioid substance with analgesic effects as well as the power to enhance the mood. It is known that mistletoe drugs stimulate production of β -endorphin (47), and we also observed a rise in the blood levels of β -endorphin in the "responder" patients, which overlapped with the enhancement of the immunocompetent cells in the blood stream (data not presented).

The results of this study, on the perioperative use of the mistletoe extract, are the first reported and are in agreement with those obtained in extensive preclinical studies and with clinical experience that support the use of Isorel and related drugs in adjuvant cancer therapy.

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