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Influence of Complementary *Viscum album* (Iscador®) Administration on Microcirculation and Immune System of Ear, Nose and Throat Carcinoma Patients Treated with Radiation and Chemotherapy

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Abstract. *With the techniques of vital microscopic and reflection spectrometric imaging, representative characteristics of microcirculation and immunology of white blood cells were evaluated before, during and after radiotherapy and chemotherapy of patients suffering from ear, nose and throat carcinomas. Adverse effects of radiotherapy and chemotherapy on the microcirculation and the immune system were decreased and reconstitution processes were accelerated by complementary administration of a standardized mistletoe extract (Iscador®).*

Surgery, radiotherapy and chemotherapy are evidence-based treatment modalities in oncology. Complementary therapies, *e.g.* with standardized mistletoe extract, are based on the opinion that not only the local cellular transformation but also the general health should be considered, since both obviously enable or inhibit tumor growth (24).

A series of experimental and clinical trials are available which evaluated the effectiveness of complementary mistletoe therapy in improving quality of life. Recently, various components of mistletoe extract and their pharmacological effects have been investigated scientifically (1, 2, 5-9, 14-19, 22, 23).

A great body of investigations has shown that a therapeutically relevant immunomodulation can be induced by administration of mistletoe extract (11,12). The characteristic changes in microcirculation and immunological capacities of white blood cells suggest an improvement of the general condition. This might be due to optimization of the regular microcirculatory system and enhancement of resistance mechanisms, which obviously are of prime importance for cancer patients. Thus, surgery and subsequent radiotherapy and chemotherapy induce temporary but significant side-effects on the microperfusion and immune system (20,21).

This study was performed to evaluate whether the generalized adverse effects of radiotherapy and chemotherapy can be influenced by complementary administration of a standardized mistletoe extract.

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Materials and Methods

Male patients with squamous cell carcinomas of the larynx and pharynx were investigated during an observation period of about 12 weeks. All patients were treated after surgery with radiotherapy and chemotherapy (radiation with 50 - 60 Gy; chemotherapy with cisplatin and 5-fluorouracil/5-FU; dosage as recommended in international treatment protocols).

From a total of 20 patients, two groups were randomized: *Control group (n=10 patients):* radiotherapy and chemotherapy without additional administration of mistletoe extract.
Treatment (verum) group (n=10 patients): radiotherapy and chemotherapy with additional administration of mistletoe extract.

The patient characteristics were: age ~60 years, height ~172 cm, weight ~73 kg.

Patients of the treatment group received subcutaneous injections of commercially available standardized mistletoe extract in the abdominal region as complementary medication: Iscador® Qu Series 0 (ampoule with 1 ml), Iscador® Qu 5 mg special (ampoule with 1 ml), (WELEDA AG, Schwaebisch Gmuend, Germany).

The administration scheme for the standardized mistletoe extract was based on the treatment guidelines discussed in the literature (2,23,24).

Administration scheme for the complementary medication with mistletoe extract: Iscador® Series 0: 8th d - 0.01 mg; 10th d - 0.01 mg; 12th d - 0.1 mg; 15th d - 0.1 mg; 17th d - 1.0 mg; 19th d - 1.0 mg; 22nd d - 1.0 mg.

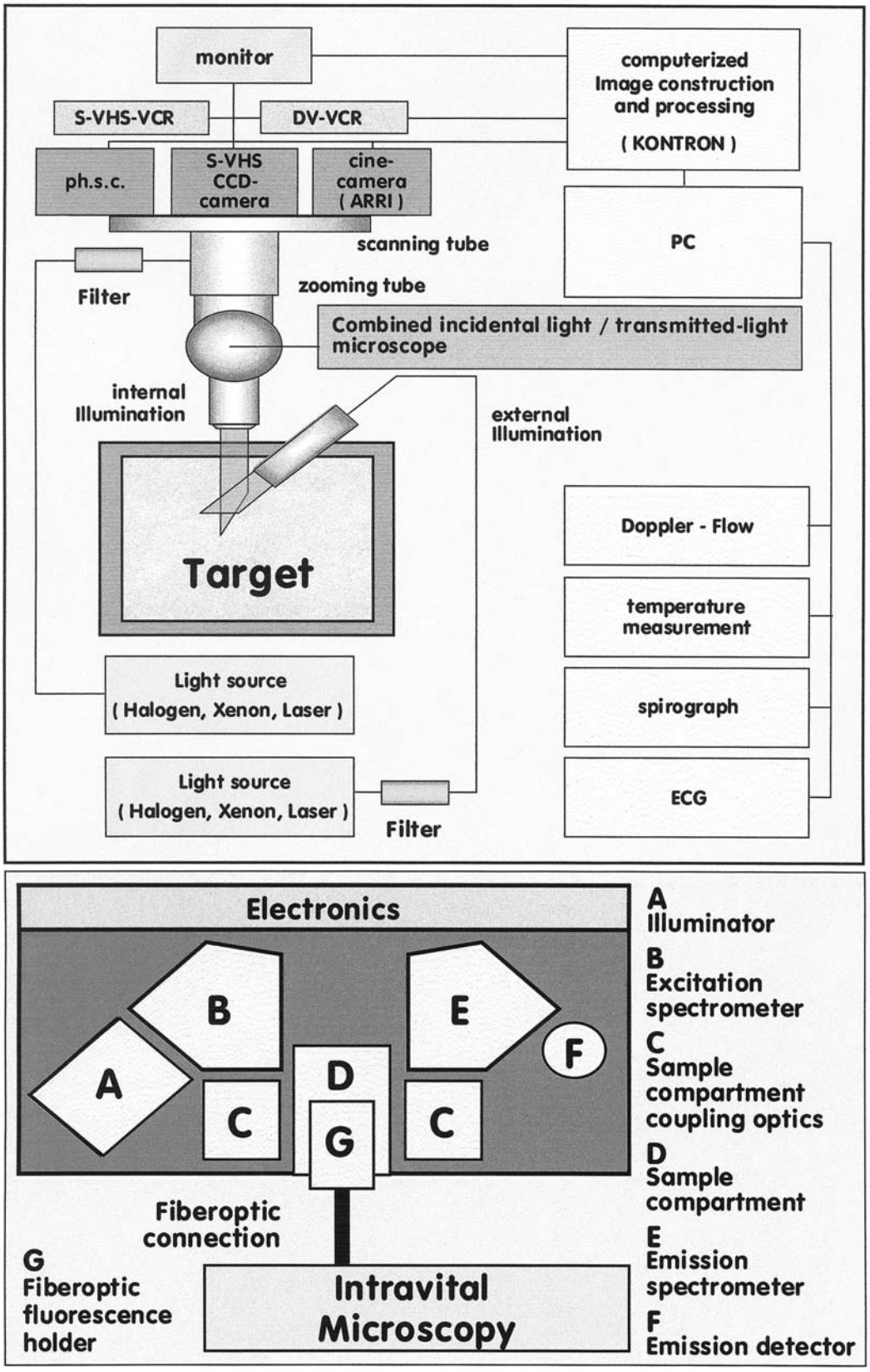

Figure 1. *Block diagrams of the vital microscopic and reflection spectrometric investigation units.*

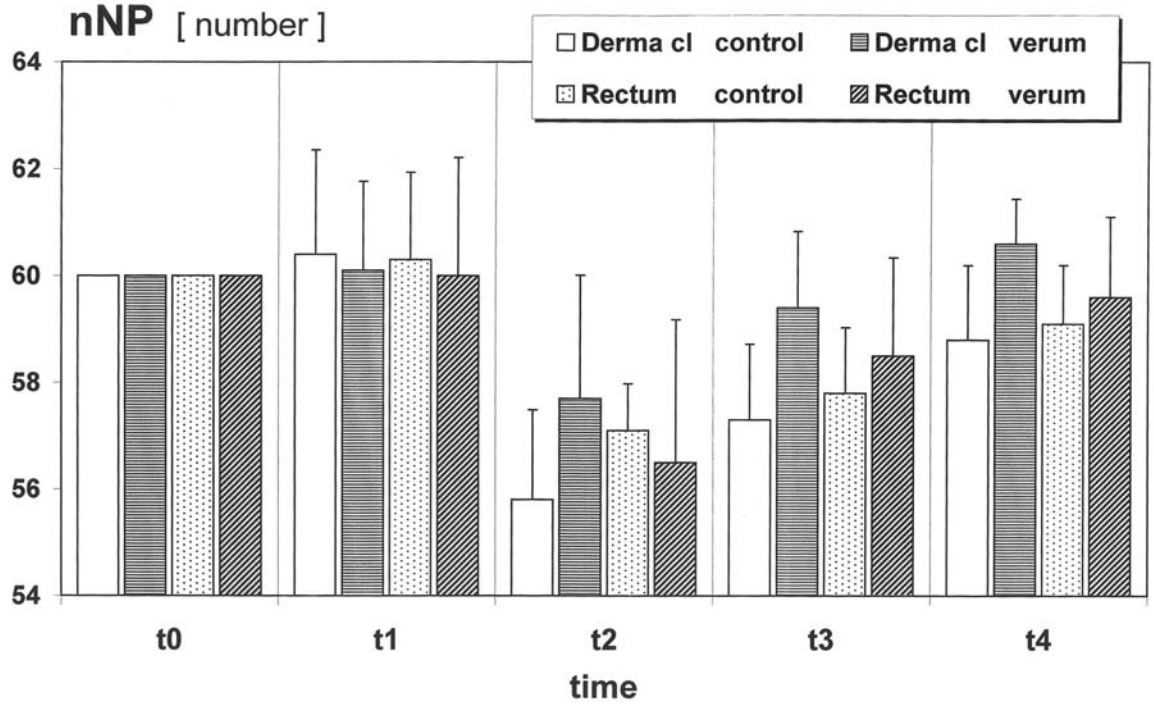

Figure 2. *Number of blood cell perfused nodal points nNP from the target tissues in patients of the control and treatment groups during the observation period. Ordinate: number in a defined microvascular network unit. Abscissa: measuring time period (t0 - before surgery; t1 - before the start of radiation and chemotherapy (1st week); t2 - after the start of radiation and chemotherapy (3rd week); t3 - (6th week); t4 - (9th week). Selected target tissue: Derma cl (cutis / subcutis contralateral to the derma near the tumor), rectum. All characteristic differences between the control and verum groups after the time period t2 are significant.*

Iscador® Series 0: 24th d - 0.01 mg; 26th d - 0.01 mg; 29th d - 0.1 mg; 31st d - 0.1 mg; 33rd d - 1.0 mg; 36th d - 1.0 mg; 38th d - 1.0 mg.

Iscador® Qu special: 40th d - 2.5 mg; 43rd d - 2.5 mg; 45th d - 2.5 mg; 47th d - 5.0 mg; 50th d - 5.0 mg; 52nd d - 5.0 mg; 54th d - 5.0 mg; 57th d - 5.0 mg; 59th d - 5.0 mg; 62nd d - 5.0 mg.

Data were collected at 6 defined times during an observation period of 90 days:
Time t0 - before surgery,
Time t1 - before the start of radiation and chemotherapy (1st week),
Time t2 - after the start of radiation and chemotherapy (3rd week),
Time t3 - (6th week),
Time t4 - (9th week),
Time t5 - (12th week).

Measurements of the microcirculation and white blood cells were performed in various organs or tissue regions of the patients almost simultaneously: subcutis - injection location, gingiva - (upper jaw, labial), rectum - (muscularis, penetration 45 mm.), cutis - near the tumor (distance 6 mm), cutis - at a distance of 35 mm from the edge of the tumor, cutis - contralaterally to the cutis near the tumor.

The target tissues were defined stereotactically. Measurements were taken from all the patients in comparable tissue regions.

Investigation methods comprised: Intravital microscopic investigation unit in a combined illuminating and radiating process with computer-aided image processing (ZEISS, ARRI, KONTRON); Vital microscopic reflection spectrometry (SPEX).

Additional information about the methods employed and special measuring guidelines can be found in the literature (4, 10-13). Figure 1 shows block diagrams of the investigation units used. The following characteristics were determined with the aid of the vital microscopic and reflection spectrometry units: Target tissue volume $V=1200\ \mu m^3$, microvascular network units (arterioles, capillaries, venules) with diameters $d \leq 80\ \mu m$ and the initial lymph stream.

Number of perfused nodal points (nNP) in a defined tissue volume unit (n=60 nNp).

Tube hematocrit (Hkt), detected as the percentage of change in comparison to pretreatment values.

Streaming flow of the initial lymph (QL), detected as percentage of changes.

Number of adhering white blood cells on a defined venule wall surface (nWBC/A). Defined inner surface of the venule=18000 μm^2.

Number of migrating white blood cells in a defined tissue volume unit (nBC/V), number of cells.

Documentation of the primary images: Microphotographic momentary images (mf) in KB-, RF- or plate format (NIKON, LINHOF with ARRI adapter; up to 1/8000 seconds); up to 36 DIN.

35mm cinefilm (color or black and white), ARRI complete camera and steering system (up to 120 pictures / second), Agfa special film; automatic Agfa film development system.

Video imaging (U-matic, super-VHS; JVC, PANASONIC, BLAUPUNKT, SIEMENS).

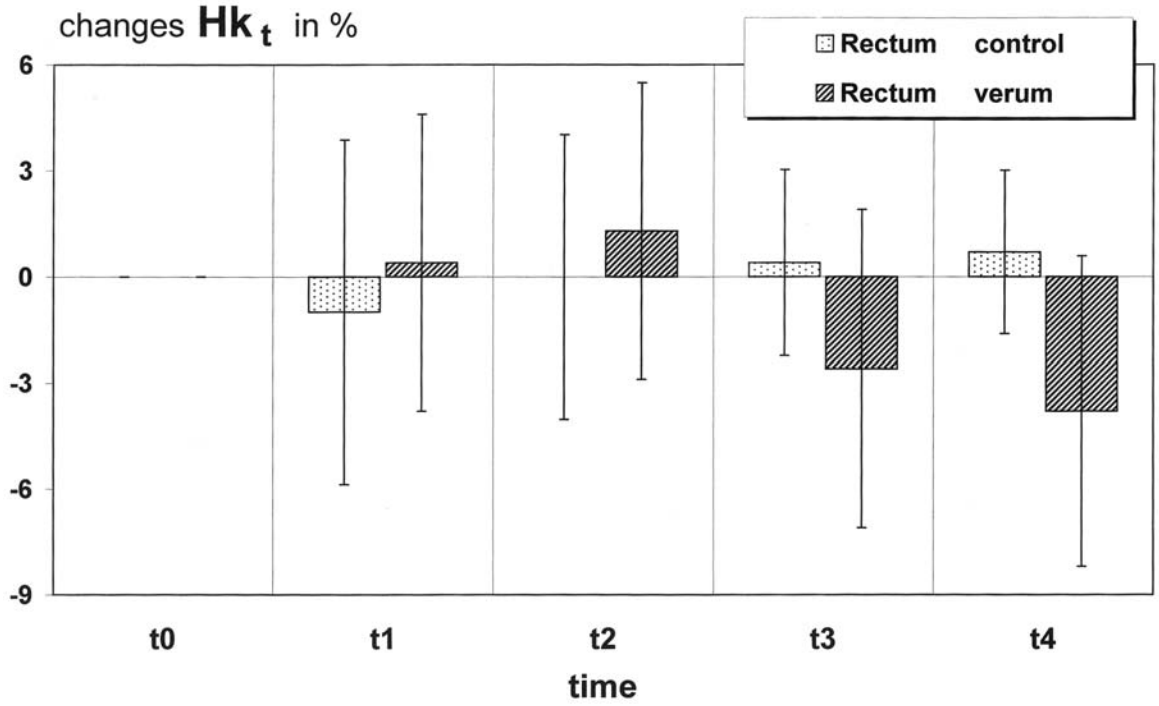

Figure 3. *Tube hematocrit Hkt from the target tissue rectum in patients of the control and treatment groups during the observation period. Ordinate: changes in %. Abscissa: measuring time period (t0 - before surgery; t1 - before the start of radiation and chemotherapy (1st week); t2 - after the start of radiation and chemotherapy (3rd week); t3 - (6th week); t4 - (9th week). All characteristic differences between the control and verum groups after the time period t2 are significant.*

Measurement of various geometric and dynamic characteristics of the microcirculation was done by computerised picture-to-picture analysis, partially in pseudocolor transformation and computer identification of the primary images (computer-aided histological findings). Macrocirculatory conditions have to be kept strictly constant and have be controlled simultaneously for the intravital microscopic measuring.

Local concentration of the adhesion molecule ICAM-1 were detected in relative units from 0 to 10.

The WILCOXON rank sum test was used for the statistical analysis of the data. Statistical analysis was performed with significance level of 5% (two-sided test).

Results and Discussion

A local reaction at the injection site of standardized mistletoe extract was found in all patients of the treatment group, however, the effects on the dermal surface did not warrant interruption of the mistletoe administration or reduction of the dosage. There were no other adverse effects. Recent trials with healthy volunteers characterized the local reaction as a response to the stimulation of the mistletoe injection. A similar reaction was observed in cancer patients in earlier trials (11,12).

Data of the characteristic blood distribution and flow of the microcirculation in selected target tissues of patients of the control and treatment groups are summarized in Figures 2 and 3. Obviously, a deterioration of the microcirculation in the target tissues begins with radiotherapy and chemotherapy (the interval between measuring periods t1 and t2). This can be shown in the reduced distribution of blood in the microvascular networks (characteristic nNP). The flow characteristics of blood in the microcirculation (rheological characteristic Hkt) deteriorate simultaneously with the start of radiotherapy and chemotherapy. Despite the comparatively low values of these changes, a microhemodynamically relevant impediment of the microcirculation must be anticipated (4,11,12).

The consequence of those characteristic changes is a reduction of the local microcirculation during the time interval of radiotherapy and chemotherapy. However, patients of the verum group treated with mistletoe extract were affected relevantly less by these restrictions than patients of the control group. The compensation for disturbances of the microcirculation lasted for several weeks. At the end of the 90-day observation period, the patients of the control group had not yet returned to

Figure 4

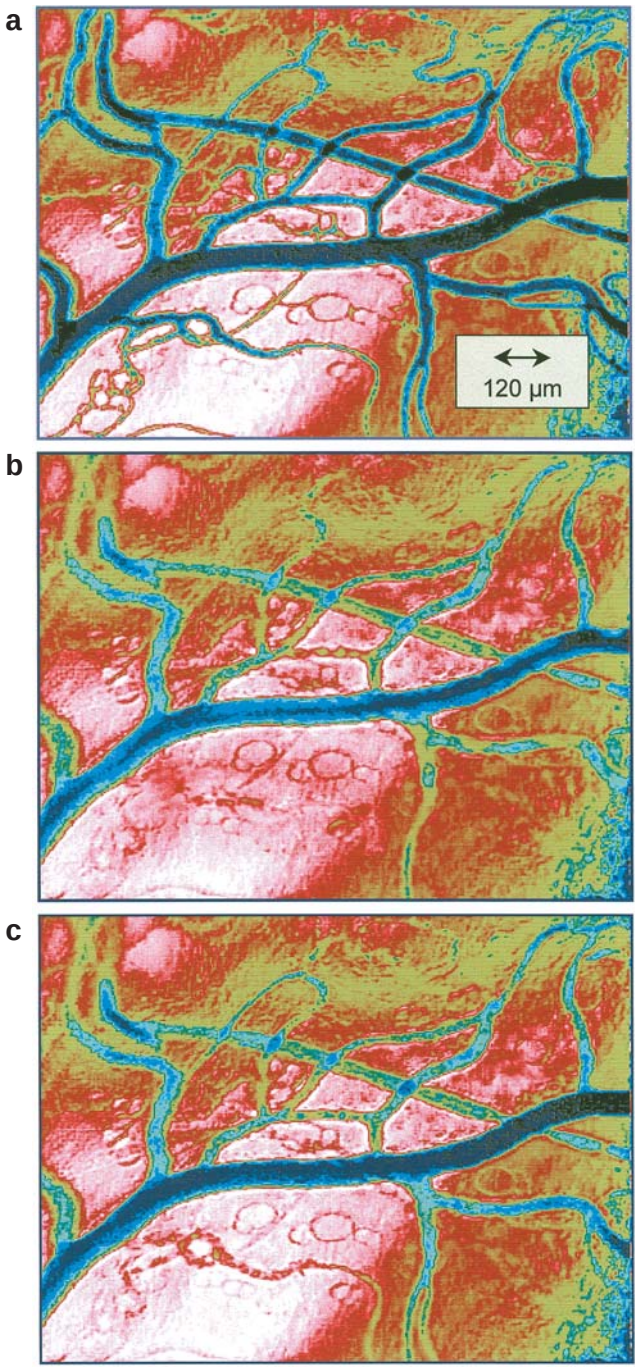

Figure 5

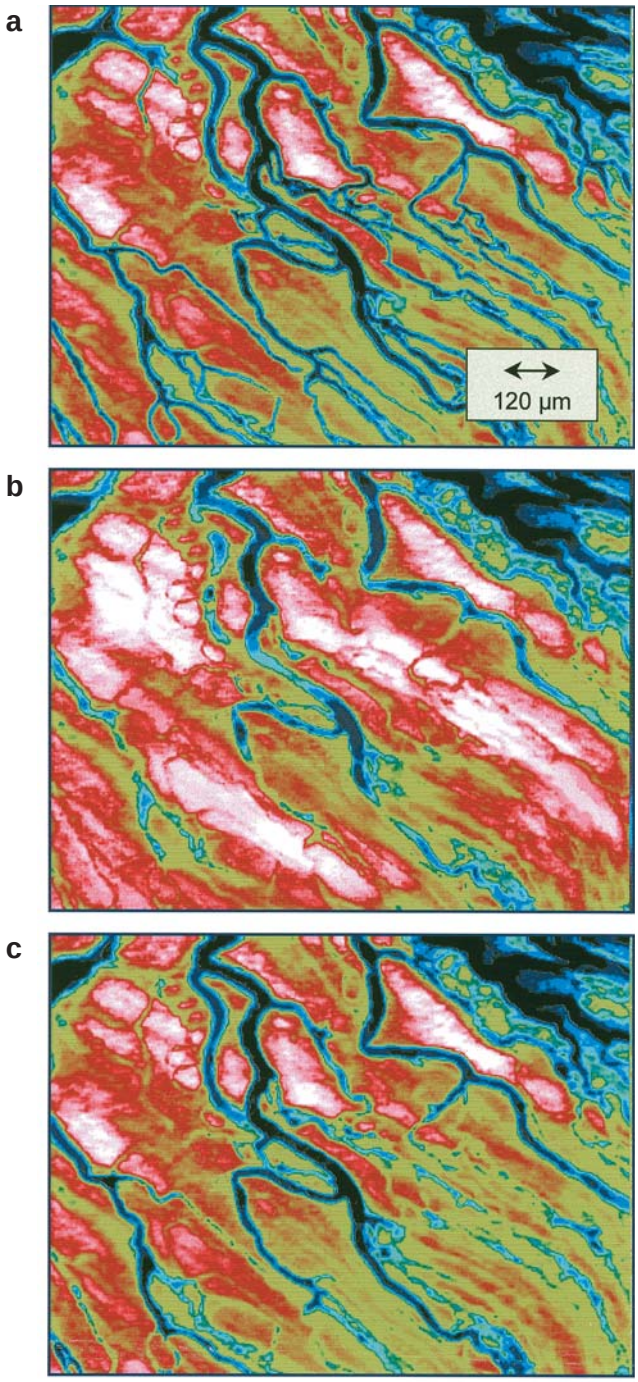

Figure 4a, b, c / Figure 5a, b, c. *Vital microscopic findings of the same microvascular region of a patient of the control group (Fig. 4) / a patient of the verum group (Fig. 5) during various observation periods.*
(Rectum [muscularis]; capillaries, arterioles, venules; mf 1/2000 s; computer transformed primary illustrations in pseudocolor transformation - the cell perfused microvessels are marked in dark blue).
a: Time t0 - before surgery;
b: Time t2 - after the start of radiation and chemotherapy;
c: Time t5 - at the end of the observation period, 12th week.
The differing functional condition of the microcirculation (distribution condition) at the end of the observation period (t5) in the patient from the control group and the patient from the verum group is particularly obvious.

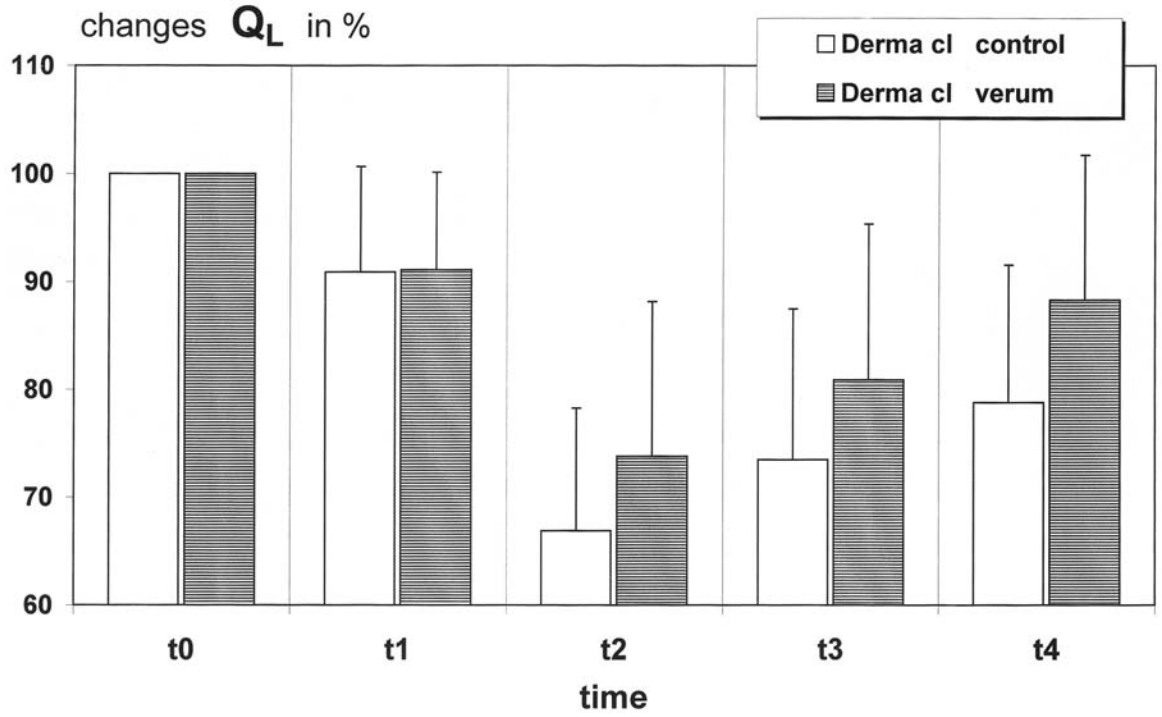

Figure 6. *Flow of the initial lymph QL in the target tissue derma cl (cutis / subcutis contralateral to the derma near the tumor) in patients of the control and treatment groups during the observation period. Ordinate: changes in %. Abscissa: measuring time period (t0 - before surgery; t1 - before the start of radiation and chemotherapy (1st week); t2 - after the start of radiation and chemotherapy (3rd week); t3 - (6th week); t4 - (9th week). All characteristic differences between the control and verum groups after the time period t2 are significant.*

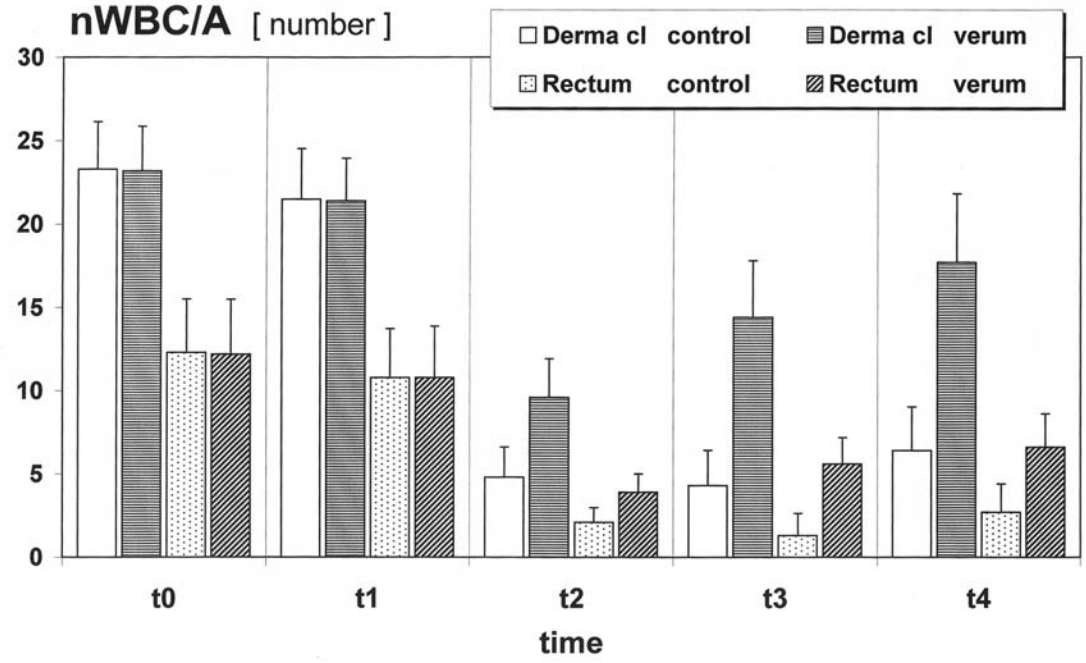

Figure 7. *Number of adhering white blood cells on a defined venular surface nWBC/A from the target tissue areas in patients of the control and treatment groups during the observation period. Ordinate: number. Abscissa: measuring time period (t0 - before surgery; t1 - before the start of radiation and chemotherapy (1st week); t2 - after the start of radiation and chemotherapy (3rd week); t3 - (6th week); t4 - (9th week). Target tissue: derma cl (cutis / subcutis contralateral to the derma near the tumor), rectum. All characteristic differences between the control and verum groups after the time period t2 are significant.*

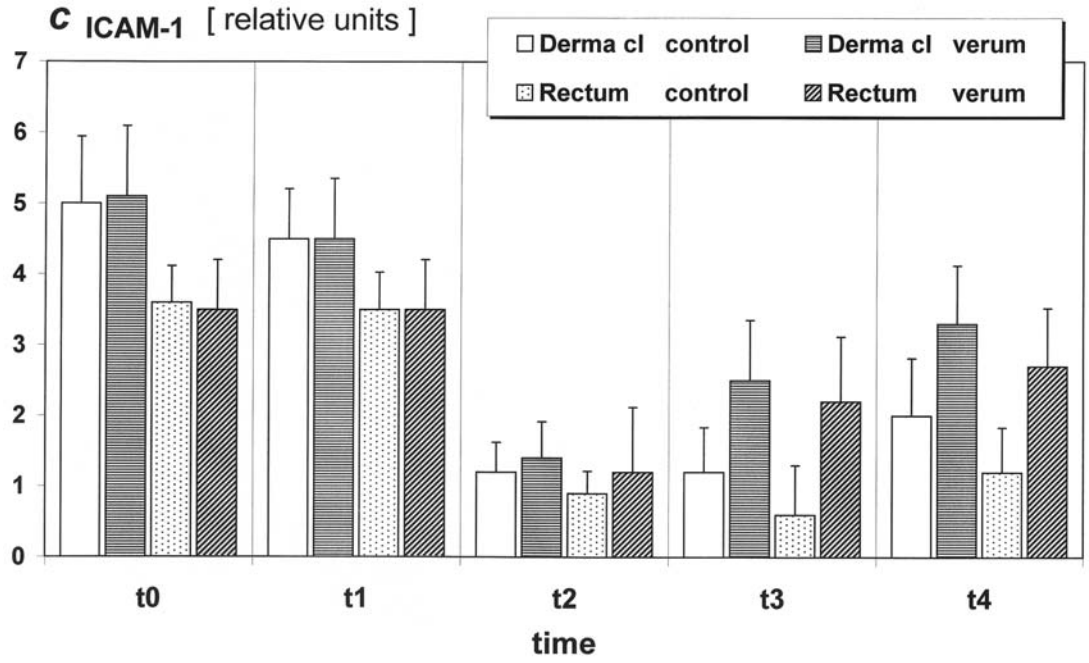

Figure 8. *ICAM-1 from the target tissue areas in patients of the control and treatment groups during the observation period. Ordinate: relative units (0 - 10). Abscissa: measuring time period (t0 - before surgery; t1 - before the start of radiation and chemotherapy (1st week); t2 - after the start of radiation and chemotherapy (3rd week); t3 - (6th week); t4 - (9th week). Target tissue: derma cl (cutis / subcutis contralateral to the derma near the tumor), rectum. All characteristic differences between the control and verum groups after the time period t2 are significant.*

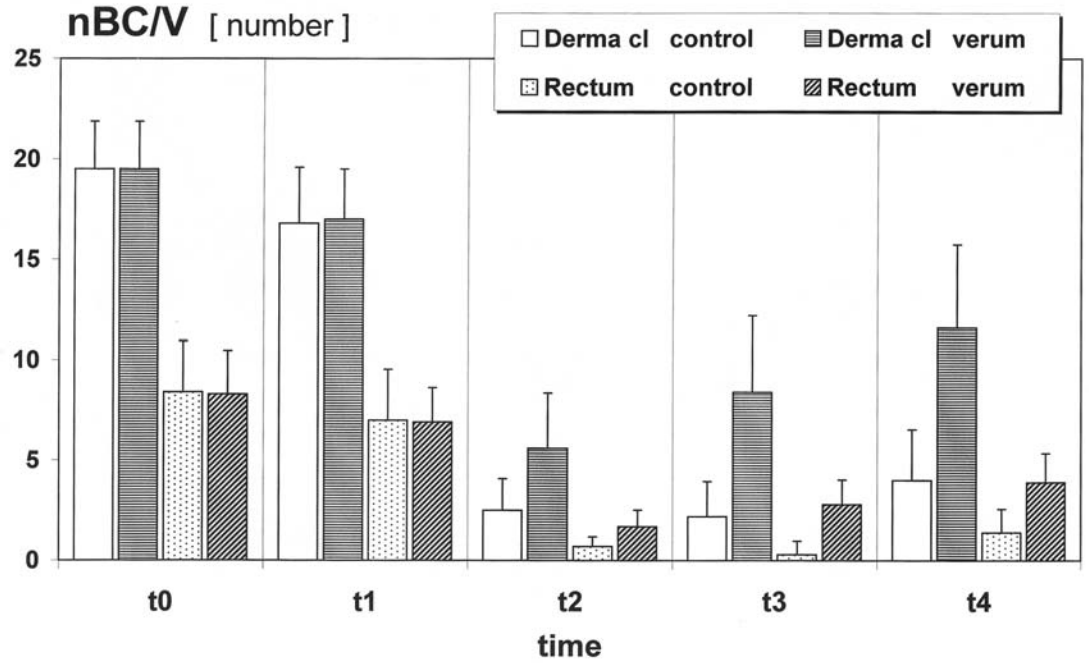

Figure 9. *Number of migrating white blood cells nBC/V from the target tissue areas in patients of the control and treatment groups during the observation period. Ordinate: number in a defined tissue volume unit. Abscissa: measuring time period (t0 - before surgery; t1 - before the start of radiation and chemotherapy (1st week); t2 - after the start of radiation and chemotherapy (3rd week); t3 - (6th week); t4 - (9th week). Target tissue: derma cl (cutis / subcutis contralateral to the derma near the tumor), rectum. All characteristic differences between the control and verum groups after the time period t2 are significant.*

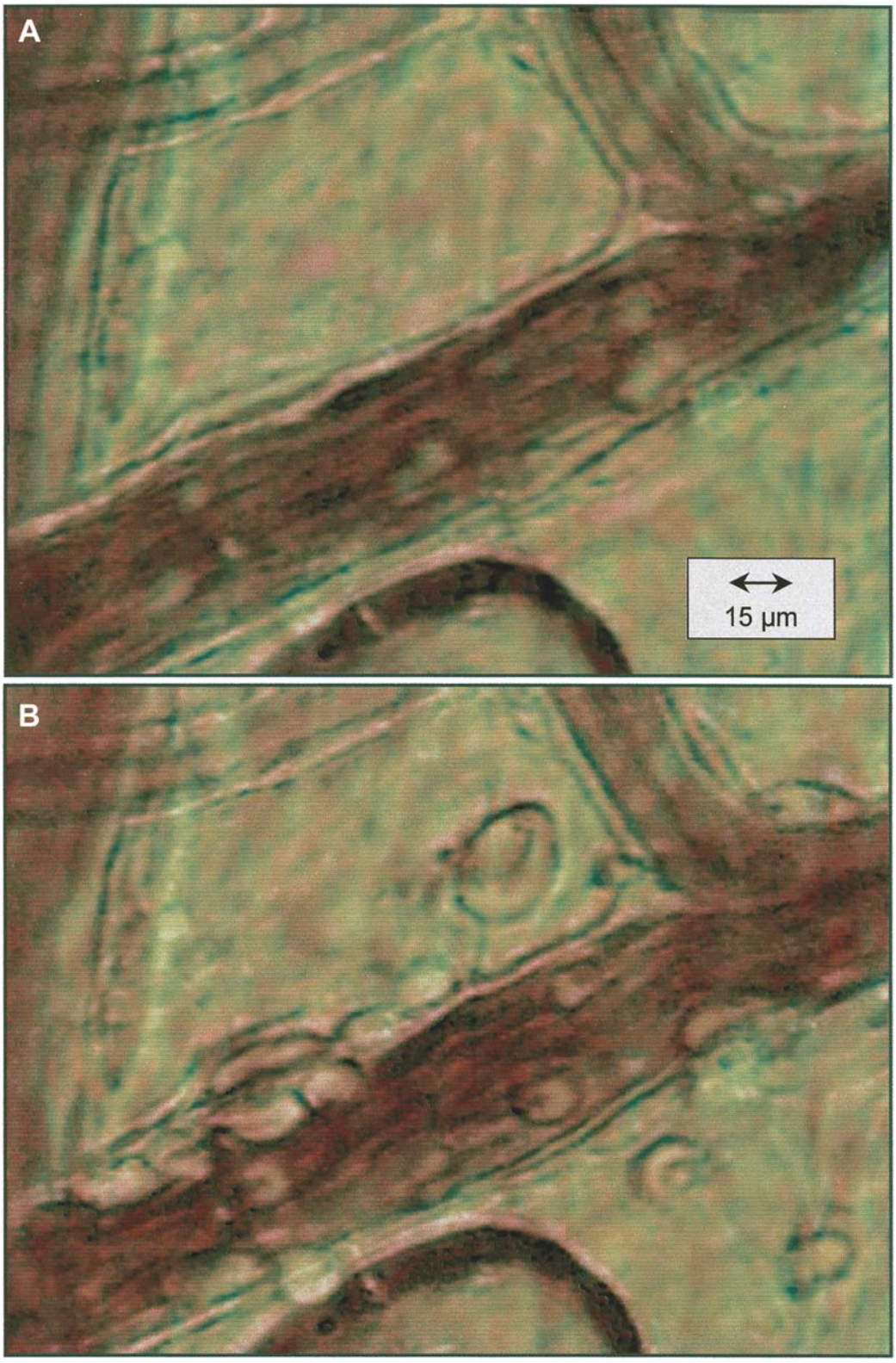

Figure 10A and B. *Example of vital microscopic images of a patient of the treatment group (mf, 1/8000s: venular microvessel in the cell layer muscularis of the rectum at various observation periods). A: After the start of radiation and chemotherapy (Time t2) only limited transmigration of white cells can be observed. In this example there is no recognizable transmigration. B: With the administration of mistletoe the migration of the white blood cells changes (the same microvascular section at Time t4).*

pretherapeutic values (measuring period t0 before surgery). Patients of the verum group reached a balanced microcirculation at the end of the observation period that was close to that of the pretherapeutic condition.

Figures 4a - c and 5a - c show examples of vital microscopic findings from one patient each of the control group and the treatment group as an image sequence at different observation periods (period t0 before surgery, period t2 after the start of radiation and chemotherapy, period t5 at the end of the observation period).

Recent investigations have shown that there is a close correlation between the function of the microcirculation and the first steps of an immunological reaction. Enrichment, roll-off and adhesion phenomena as well as the migration of leukocytes are only possible under defined microhemodynamic conditions (3,4,11,12). It is to be expected that changes in the function of the microcirculation after initiation of radiotherapy and chemotherapy influence the behavior and characteristics of the white blood cells in patients in the control and verum groups. The question to be asked was whether the immunological behavior in the patients of the treatment group differed significantly from that of the patients of the control group. To evaluate these data, we refer to a series of basic findings (1-5, 7, 11, 12, 2 0, 21, 23, 24).

Besides characteristics of the microcirculation of blood, (dynamic) characteristics of the initial lymph stream are important, especially in realizing the migration phenomena of white blood cells and their further motion (3,4). The corresponding behavior of the characteristic flow of the lymph stream (QL) and the characteristic changes of the microcirculation of blood in target tissues are summarized in Figure 6.

The characteristic changes found in the adhesion and migration of white blood cells (characteristics nWBC/A, ICAM-1, nBC/V) are of great importance for the evaluation of the therapeutic success of complementary mistletoe extract treatment. Figures 7, 8, and 9 show that suppression of the immunological reaction begins at the start of radiotherapy and chemotherapy and cannot be avoided, even for patients of the treatment group. However, it developed in a milder form in comparison to the control group. In addition, it must be emphasized that the patients of the treatment group displayed a restitution process of this disturbed behavior by the end of the observation period as opposed to those patients of the control group.

Figures 10A and B show vital microscopic images of a patient of the treatment group concerning changes of the migration of white blood cells on a venule wall at different observation periods before and after administration of standardized mistletoe extract.

Evaluation of the therapeutic success of complementary mistletoe treatment of patients with ear, nose and throat carcinomas demonstrated that patients of the verum group showed an optimized microcirculation and advantageous conditions for the first steps of immunological reactions at the end of the 90-day observation period after surgery, radiation and chemotherapy as compared to patients of the control group. In agreement with other scientists, it is recognized that the direct effects of radiation and chemotherapy on the microcirculation and immune system are serious and apparently unavoidable. They can, however, be decreased by appropriate complementary measures, *e.g.* administration of standardized mistletoe extract. Furthermore, this treatment results in acceleration of the restitution processes (20, 21, 23, 24).

Complementary administration of standardized mistletoe extract demonstrated microhematological and immunological success. These data are encouraging for further studies.

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