Local Reactions to Treatments with *Viscum album* L. Extracts and their Association with T-Lymphocyte Subsets and Quality of Life

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Abstract. *Background: In a previous study a decline of T-lymphocyte function was observed within a 6-month period of Viscum album extract (VA-E) application which did not occur in those patients with dose adaptation in response to strong local reactions (LR) or in those with moderate LR. To further investigate the immunological prerequisites of these differences in the VA-E susceptibilities, an analysis was carried out of the pre-existing differences in the tumor patients' lymphocyte subsets, and of whether the LR pattern (none, moderate, strong) might be associated with distinct aspects of the patients' quality of life. Patients and Methods: Seventy-one cancer patients were subcutaneously treated with VA-E (Iscador®) at increasing concentrations and their lymphocyte subsets measured by flowcytometry during a 6 month observation period; quality of life was assessed with the HLQ questionnaire. Results: The occurrence of stronger LR was associated with a primarily higher level of T-cells and their $CD4^+$ T-helper/inducer subset, and $CD25^+$ respectively HLA-DR^+ (activated) T-cells. Moreover, counts or proportions of T-cells, CD4+ T-helper/inducer cells and $CD8^+$ $CD28^+$ cytotoxic cells were lower, while the relative proportions of $CD8^+$ $CD28^-$ suppressor cells, B- and NK-cells were the highest in the group with moderate LR. In particular, this latter group had a significantly higher quality of life. Conclusion: Our results indicate that the induction of moderate LR in response to VA-E application was associated with better T cell function and quality of life.*

Extracts from *Viscum album* (VA-E) are widely used as a complementary cancer treatment, particularly in Germany and Switzerland. Several clinical studies, including historical, retrospective, prospective and randomized trials, reported extended survival times, improved quality of life, and tumor regression with mistletoe therapy (for review see (1-3)). The pharmacological effects of VA-E are defined not only by the induction of an apoptotic respectively necrotic cell death (4-8), but also by indirect immune activation (for an overview see 1,2,9,10). In a recent study, we observed that within a 6-month period of VA-E application, the stimulated T-lymphocyte function may decline in some of the tumor patients, but not in patients with dose adaptation in response to strong local reactions (10). In fact, the dose applications of VA-E remain a matter of discussion. Strong local reactions after subcutaneous injection of VA-E, which are in most cases due to a rapid increase of applied VA-E concentrations, may call forth a physiological "reaction reduction" from T-cells in some patients. With respect to the T-cell function, we thus suggested that a long-term VA-E application should be paused periodically to allow recovery of T-cell reactivity (10). Because we have noticed that the local reactions in response to VA-E cannot be fully explained by the applied concentration (10), differences in individual immunological reactivity towards the antigens of the applied VA-E are suggested. With respect to the local reactions, in this evaluation, we thus aimed first to investigate preexisting differences in the tumor patients' lymphocyte subsets which could explain differences in the responses to VA-E, and second to investigate whether these differences in local reaction might be associated with distinct aspects of quality of life.

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Patients and Methods

Design. The primary aim of this observational study which was registered according the German law and was carried out according to the ICH-GCP guidelines (10), was the functional competence of stimulated T-lymphocytes, and these findings have already been published in a recent issue of this journal (10). In an attempt to

explain the observed effects, we now focuss on the secondary aims of the study, *i.e.* the course of peripheral lymphocyte subsets and the quality of life of the patients.

Patients. All patients were informed of the purpose of the study and gave informed consent to participate. They were recruited consecutively between March 2002 and November 2004 at the tumor outpatient clinic of the Gemeinschaftskrankenhaus Herdecke, which was run by two highly experienced medical doctors. The patients were not selected and were enrolled consecutively as they attended the clinics of the two medical doctors, which followed different dosage schemes.

According to the inclusion and exclusion criteria (10), 71 patients were enrolled. However, 4 patients were not analysed because they left the study within the first 3 months. Thirty-six patients had breast, 14 prostate, and 17 colorectal (8 colon, 8 rectum, 1 sigma) cancer. Forty-four of them were female and 23 male. All further details were given in (10).

Mistletoe extract. As described (10), women received a commercially available and standardized fermented drug extract from mistletoes grown on apple trees: Iscador® M (Weleda AG, Schwäbisch Gmünd, Germany), while men received a fermented drug extract from mistletoes grown on oak trees: Iscador® Qu. All patients received the VA-E by injection subcutaneously twice per week in the morning at increasing concentrations. The observation period for each patients lasted 6 months.

Local reactions. To analyse the local reactions in response to the subcutaneous application of VA-E more precisely, the patients received a "side-effect diary". They documented the concentration of each Iscador® ampoule and the observed local reactions (size, redness, swelling, itching) at the injection side.

Peripheral blood lymphocytes. Peripheral blood lymphocytes from the patients were analysed diferentially each month using monoclonal antibodies (CD3, CD4, CD8, CD16, CD19, CD25, CD28, CD56, CD62L, HLA-DR; Coulter-Immunotech, Krefeld, Germany, and Becton Dickinson, Heidelberg, Germany) by flow cytometry (EPICS XL-MCL; Coulter, Krefeld, Germany) according to standard procedures. To minimize the problem of multiple testing and individually variable courses of immune cells within the observation period, the cell counts were pooled over 6 months.

Quality of Life. To investigate different aspects of quality of life, the HLQ-questionnaire was used (11, 12), which, in its primary version, differentiates the following factors: Physical Complaints, Vitality, Mental Behaviour, Presence of Personality and Social Environment.

Data management. Data were documented in case report forms. After monitoring by an independent institution, the data were transferred into an ACCESS database twice by two different typists. A plausibility control of each parameter was performed. The monitor (Wilfried Tröger and Petra Siemers from the Department of Clinical Research, Freiburg) corrected all errors by reviewing the source data. Missing data were not replaced.

Statistics. Data were presented as mean values (MV)±standard deviations (SD). To address differences between the respective groups, analyses of variance (ANOVA) were performed. Differences with $p<0.001$ were regarded as highly significant and differences with an F-value >10 as highly relevant. Data analysis was performed using SPSS 12.0 for Windows (SPSS GmbH Software, München).

Table I. *Peripheral blood lymphocytes prior to any VA-E application.*

LR within the first 8 weeks	% $CD3^+$	$CD3^+$/µl	$CD4^+$/µl	% $CD25^+$ in $CD3^+$	% HLA-DR^+ in $CD3^+$
None					
MV	72.23	1138	691	14.00	6.66
SD	8.69	488	262	9.63	4.04
Moderate					
MV	65.97	932	558	18.29	8.15
SD	8.72	328	189	10.45	4.13
Large					
MV	75.21	1512	931	22.93	12.16
SD	9.37	1080	677	8.11	6.78
Overall					
MV	70.89	1151	699	17.21	8.28
SD	9.40	646	389	10.10	5.14
F-value	**5.289**	3.672	4.263	4.392	**6.519**
p-value	**0.007**	0.031	0.018	0.016	**0.003**

Only significant differences are presented ($p<0.05$; ANOVA).

Results

In 32 cases (48%) no local reactions (LR) were observed in response to subcutaneous VA-E application within the first 8 weeks, while in 21 cases (31%) moderate LR (1-3 cm) and in 14 cases (21%) strong reactions (>3 cm) occurred. Patients without LR were significantly ($F=30.2$; $p<0.001$) older (64±8 years) as compared to patients with moderate (58±10 years) or strong LR (58±8 years). Most men had no LR (63%), 14% had moderate and 23% strong LR; in contrast, 40% of women had no LR, 40% had moderate and 20% strong LR. These gender-specific differences were not significant ($p=0.08$; Pearson's Chi^2). The patients did not differ with respect to TNM/G staging (data not shown). As one may suggest that the occurrence of LR in response to the VA-E application is dependent on the reactivity of the patient's immune system, the mean initial numbers of peripheral lymphocytes were analysed with respect to the LR as an 'indicator'. As shown in Table I, the stronger the LR, the higher the initial (pre-VA-E) number and proportion of $CD25^+$ and HLA-DR^+ (activated) T-cells was. This means that the reactivity is in fact depending on the pre-existing T-cell activity. Moreover, we found significant differences with respect to the relative proportion and number of T-cells, and their $CD4^+$ subset, *i.e.* the lowest values were found in the group with moderate LR.

Table II. *Lymphocyte subsets and local reactions.*

LR within the first 8 weeks	Leucocytes $\times 10^3$/µl	Lymphocytes /µl	% Lymphocytes	% $CD3^+$ T	$CD3^+$ T /µl	% $CD4^+$ Th	$CD4^+$ T /µl	% $CD8^+$ $CD28^-$ Ts	$CD8^+$ $CD28^-$ Ts /µl	% $CD8^+$ CD28+ Tc	$CD8^+$ CD28+ Tc /µl	% $CD19^+$ B	$CD19^+$ B /µl	% $CD16^+$/ $CD56^+$ NK	$CD16^+$/ $CD56^+$ NK /µl
None															
MV	5.66	1609	27.03	71.38	1159	44.71	705	14.98	252	11.77	195	11.14	175	14.08	221
SD	1.85	561	7.45	8.93	470	8.95	264	7.08	185	5.86	120	4.31	83	6.66	128
Moderate															
MV	5.71	1442	24.26	65.54	955	40.32	584	17.17	258	10.84	166	15.41	211	15.47	220
SD	1.68	480	6.91	8.90	385	10.18	240	9.40	194	4.35	121	9.10	113	7.51	128
Large															
MV	6.48	1961	28.23	74.94	1520	46.65	944	15.58	347	14.21	252	11.03	198	10.75	187
SD	2.29	1036	8.76	8.48	997	7.53	629	7.82	339	7.19	135	6.03	120	4.99	113
Overall															
MV	5.85	1632	26.41	70.30	1172	43.73	718	15.80	274	12.00	198	12.47	191	13.80	213
SD	1.93	695	7.73	9.48	635	9.39	389	8.08	232	5.88	127	6.82	103	6.84	126
F-value	6.73	**17.47**	9.24	**35.78**	**25.45**	**16.32**	**27.89**	3.23	6.20	**10.20**	**14.07**	**21.23**	5.57	**14.97**	2.74
p-value	**0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.041	**0.002**	**<0.001**	**<0.001**	**<0.001**	**0.004**	**<0.001**	n.s.

Pooled results between the LR groups differed significantly with $p<0.05$ (ANOVA). n.s., not significant.

Next, lymphocyte subsets were analysed with respect to the LR over the pooled treatment time course. Here several highly significant differences were found (Tables II and III). The strongest variances (F>10) between the LR groups were found with respect to lymphocyte counts, number and proportion of $CD3^+$ T-cells, $CD4^+$ T-helper/inducer cells, $CD8^+CD28^+$ cytotoxic cells, and the relative proportion of B- and $CD16^+/CD56^+$ NK-cells, and $CD25^+$ in $CD3^+$ T-cells (Tables II and III). Again, the activation marker expression (CD25 and HLA-DR) of T-cells correlated with the size of LR.

As reported previously, the functional competence of T-cells from the tumor patients decreased with time, however, not in the group with moderate LR (10). In fact, in this moderate LR group, the lowest counts of $CD3^+$ T-cells, $CD4^+$ T-helper/inducer cells and $CD8^+CD28^+$ cytotoxic cells and CD4/CD8 ratio were found, while the relative proportion of $CD8^+CD28^-$ suppressor cells, $CD19^+$ B-cells and $CD16^+/CD56^+$ NK-cells was the highest in this group (Tables II and III). As we have assumed that this latter, more stable course of T-cell function could be of benefit for patients, we investigated aspects of the quality of life in these patients. Highly significant differences were found between the three LR groups (Table IV). The aspects Physical Complaints, Vitality, Mental Behaviour and Social Environment were the highest in the group with moderate LR; only the aspect Presence of Personality was the highest in the patients with strong LR. The strongest variance was found, however, for Mental Behaviour (Table IV), the lowest

Table III. *Lymphocyte subsets and local reactions.*

LR within the first 8 weeks	% $CD25^+$ in $CD3^+$	% HLA-DR$^+$ in $CD3^+$	% $CD28^+$ in $CD8^+$	% $CD62L^{high}$ in $CD8^+$	% $CD62L^{low}$ in $CD8^+$	CD4/CD8
None						
MV	11.29	7.11	44.73	34.68	47.23	2.01
SD	9.13	4.47	15.50	13.14	9.15	1.24
Moderate						
MV	17.10	7.63	41.55	37.49	45.28	1.61
SD	13.11	4.58	17.06	16.63	12.64	0.78
Large						
MV	20.16	8.88	48.55	36.54	46.08	1.74
SD	10.20	4.72	18.72	19.28	13.14	0.67
Overall						
MV	15.04	7.66	44.55	35.97	46.37	1.83
SD	11.35	4.60	16.88	15.76	11.27	1.02
F-value	**26.40**	5.04	5.08	1.45	1.32	7.41
p-value	**<0.001**	**0.007**	**0.007**	n.s.	n.s.	**0.001**

Pooled results between the LR groups differed significantly at $p<0.05$ (ANOVA). n.s., not significant.

Table IV. *Quality of life aspects and local reactions.*

LR within the first 8 weeks	Physical complaints	Vitality	Mental behavior	Presence of personality	Social environ -ment	HLQ-Score
None						
MV	47.90	49.97	52.66	46.56	50.10	49.80
SD	10.20	10.28	11.18	6.47	5.86	4.99
Moderate						
MV	50.76	53.32	58.40	47.62	51.68	52.61
SD	9.32	9.89	11.45	5.98	6.44	5.02
Large						
MV	46.58	49.77	51.72	48.35	49.03	49.46
SD	8.72	7.85	7.96	4.97	5.61	3.80
Overall						
MV	48.53	51.00	54.30	47.28	50.37	50.63
SD	9.73	9.79	11.02	6.05	6.07	4.95
F-value	6.19	6.04	**15.80**	3.21	5.98	**18.12**
p-value	**0.002**	**0.003**	**<0.001**	0.041	**0.003**	**<0.001**

Pooled results between the LR groups differed significantly at $p<0.05$ (ANOVA).

Table V. *Predictors of local reactions (stepwise regression model).*

	Unstandardized coefficients		Standardized coefficients		
Model	B	Std. Error	Beta	t	*p*-value
(constant)	–0.975	1.535		–0.635	0.528
Doses escalation (PP)	0.669	0.173	0.455	3.871	0.000
Age	–0.034	0.016	–0.242	–2.157	0.036
T-cell function (PHA)	0.048	0.018	0.331	2.743	0.009
NK-cells/µl	–0.003	0.002	–0.259	–2.138	0.038

Excluded variables: Gender, TNM, G, liver enzymes, C-reactive protein, quality of life, other lymphocyte subsets.

for Presence of Personality. Possible predictors of LR (stepwise regression model of the data prior to VA-E application) were evaluated and the dose escalation model (slow, swift, reduction) was found to be of outstanding importance (Table V). Also of significant relevance was the capacity of T-cells to respond to mitogens *in vitro* (T-cell activation), and as negative predictors, age and NK-cell number (Table V).

Discussion

As VA-E is widely used in complementary cancer treatment, it is highly important that the applied concentrations are optimal for patients. Phytotherapeutical companies recommend one constant VA-E concentration for a few months, while companies with an anthroposophical background recommend increasing concentrations for several months. In a previous study, we reported that VA-E should be applied in a more individual basis (particularly in adaptation to LR) than on the basis of fixed escalation schemes, because the most stable course of T-cell function was found in the group with dose adaptation on the grounds of strong LR and in tumor patients with moderate LR (10). We now confirm that the occurrence of a strong LR in response to the VA-E application is associated with a pre-existing difference in distinct lymphocyte subpopulations, particularly a higher level of T-cells and their $CD4^+$ T-helper inducer subset, and of activated T-cells with higher expression of interleukin-2 receptors (CD25) and HLA-DR molecules on their surface. Moreover, we found lower cells counts and proportions of distinct subsets, *i.e.* $CD3^+$ T-cells, $CD4^+$ T-helper/inducer cells and $CD8^+CD28^+$ cytotoxic cells, while the relative proportion of CD8+CD28- suppressor cells, $CD19^+$ B- and $CD16^+/CD56^+$ NK-cells was the highest in this group. This could thus be a rationale to explain the observed differences, particularly the more stable course of stimulated T-cell function in the group with moderate LR (10). As positive LR predictors (apart from VA-E concentration), the dose escalation model and the capacity of T-cells to respond to mitogens *in vitro* were confirmed, while age and NK-cell number were found to be negative predictors. Although as yet we cannot determine whether these differences may have an effect on the survival of the patients (who will be followed for at least 5 years for outcome), we nevertheless found a better quality of life in patients with moderate LR.

Conclusion

Taken together our results indicate that VA-E should be applied more individually (particularly in adaptation to the local reaction) rather than on the basis of any fixed escalation schemes. The induction of moderate LR was associated with better T cell function and quality of life, while patients with strong LR and even those without LR (which were significantly older) had a lower quality of life.

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Conflict of Interest

The study was supported by a grant of Software AG foundation, a non-governmental and non-pharmaceutical organization. The medical doctors recruiting and treating the patients did not receive any financial support from pharmaceutical companies.

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