ANTICANCER RESEARCH *25*: 4733-4736 (2005)

Adjuvant Intravesical Treatment with a Standardized Mistletoe Extract to Prevent Recurrence of Superficial Urinary Bladder Cancer

URSULA ELSÄSSER-BEILE[1], CHRISTIAN LEIBER[1], ULRICH WETTERAUER[1], PATRICK BÜHLER[1], PHILIPP WOLF[1], MARTIN LUCHT[2] and ULRICH MENGS[3]

[1]Department of Urology and [2]Center of Clinical Studies, University of Freiburg, D-79104 Freiburg; [3]Madaus AG, D-51101 Köln, Germany

Abstract. *Background: Adjuvant intravesical Bacillus Calmette-Guerin (BCG) treatment after resection of non invasive superficial bladder cancer has been shown to significantly decrease tumor recurrence. However, the serious local and systemic side-effects of this treatment have promoted the use of other immunoactive substances, which, to date, have all failed to show efficacy equal to BCG therapy. Patients and Methods: In the present phase I/II clinical trial, an aqueous mistletoe extract, standardized to mistletoe lectin, was applied intravesically to 30 patients with superficial urothelial bladder carcinomas of stages pTa and pT1, grades 1 to 2. After transurethral resection, each patient received 6 instillations at weekly intervals of 50 ml of the extract with mistletoe lectin concentrations between 10 ng/ml and 5,000 ng/ml. This was retained in the bladder for 2 hours. Three patients per group received a dose, which was then doubled in the next group. The clinical follow-up consisted of examinations by cystoscopy, cytology and random biopsies. Results: Within the observation time of 12 months, 9 patients had tumor recurrence, while 21 patients remained tumor-free. This recurrence rate was comparable to that of local historical controls with superficial bladder cancer of the same stages and grades that had been treated with adjuvant BCG. The tolerability of the intravesically-administered mistletoe extract was very good. None of the study patients had local or systemic side-effects according to the WHO classification 1-4. Conclusion: From these results, it is concluded that the standardized mistletoe extract could be a potential adjuvant therapy for superficial bladder cancer. Further studies may show the optimal intravesical treatment regimen.*

Correspondence to: Prof. Dr. U. Elsässer-Beile, Department of Urology, University of Freiburg, Hugstetterstr. 55, D-79106 Freiburg, Germany. Tel: +49-761-270-7182, Fax: +49-761-270-7191, e-mail: ursula.elsaesser@uniklinik-freiburg.de

Key Words: Superficial bladder cancer, adjuvant intravesical treatment, mistletoe extract, mistletoe lectin.

The adjuvant intravesical use of Bacillus Calmette-Guerin (BCG) after surgical resection of superficial bladder cancer has been shown to decrease tumor recurrence from about 70% to 30% in several studies and, therefore, has become an established therapy for this tumor entity (1, 2). However, its benefits are outweighed by its sometimes severe side-effects of cystic disorders, fever and single cases of miliary tuberculosis, as well as death (3-5). These side-effects promoted the use of therapies with other immunomodulatory substances, which, however, to date have all failed to show an efficacy equal to that of BCG therapy.

Aqueous extracts of the European mistletoe (*Viscum album L.*) have been widely used for more than 70 years as an alternative therapy in the treatment of patients with malignant disease (6-8). However, there is an obvious discrepancy between the popularity of mistletoe extracts and their classification as a non-conventional treatment modality with unproven efficacy in oncology. The reliable evaluation of the effects of extract preparations is seriously hampered by the fact that the extract composition is complex and greatly depends on the different methods of preparation, the time of harvest and the type of host tree. In search of the biologically active constituents of the extracts, mistletoe lectins are regarded as most important and were documented to exert a remarkable immunomodulating capacity *in vitro* and *in vivo*, *e.g.,* enhancing the cytotoxicity of natural killer cells against model targets (9) and stimulating the secretion of cytokines such as interleukin-1 (IL-1), IL-6, interferon-γ (IFN-γ), tumor necrosis factor (TNF), and colony stimulating factors (10-12). Additionally, mistletoe lectins have been shown to possess cytotoxic and apoptosis-inducing properties against a large number of human tumor cell lines *in vitro* (13). Also, *in vivo* an

antitumoral effect was shown (14). According to these findings, a direct application of mistletoe extract to the tumor surroundings in the bladder is expected to be superior to systemic application. Therefore, the present clinical study was designed to evaluate the tolerability, as well as antitumoral and immunological effects, of a standardized mistletoe extract in patients with superficial bladder cancer.

Patients and Methods

Patients. A total of 30 patients with superficial bladder cancer and histopathologically verified pTa G1 (n=6, recurrent or multilocular), pTa G2 (n=14) or pT1 G2 (n=10) lesions, which were treated with complete transurethral resection, were entered into this prospective phase I/II trial according to the inclusion/exclusion criteria. Before admission to the study, patients were physically examined to determine their clinical WHO performance score. Possible local and systemic adverse events due to the application of the mistletoe extract were described to the patients and all the included patients provided informed consent. The majority of patients were male (n=23) aged between 35 and 80 years (median 70 years).

Methods. For intravesical treatment an aqueous mistletoe extract (1:1.1-1.5 Madaus AG, Köln, Germany) was used, which was standardized to the content of mistletoe lectin by a binding assay, as described by Vang *et al.* (15). Therapy was started between 2 and 7 weeks after transurethral resection. Each patient received 6 instillations at weekly intervals of 50 ml of the extract with mistletoe lectin concentrations between 10 ng/ml and 5,000 ng/ml. Three patients per group received a dose which was then doubled in the next group. After instillation into the bladder, the extract was retained for 2 hours.

Recurrences were confirmed by cytology, ureterocystoscopy and histopathological assessment, which were repeated at follow-up 3, 6, 9 and 12 months after the beginning of the instillation therapy.

The local historical control group consisted of 18 patients with bladder carcinomas of stages pTa G2 (n=5) and pT1 G2 (n=13), who had undergone transurethral resection and were then treated with 6 instillations of adjuvant BCG at weekly intervals.

Results

Of the 30 patients incluced in the study, 6 had recurrent or multilocular pTa G1 tumors, 14 had pTa G2 tumors and 10 had pT1 G2 tumors. These patients were distributed among the 10 therapy groups. Table I summarizes the staging and grading of the 30 patients in the 10 therapy groups.

The tolerability of the intravesically-administered mistletoe extract was very good at all applied concentrations. In the 30 patients, no local or systemic side-effects according to the WHO grades 1 - 4 were noted.

After a follow-up of 12 months, 9 of the 30 patients (30%) developed recurrences, while 21 patients remained without evidence of disease. The highest rate of tumor recurrence was found in the groups treated with the relatively low lectin concentrations of 80 ng/ml and 160 ng/ml, however, no clear correlation between dosage and recurrence could be shown. For comparison with the local historical BCG-treated control group, those 6 patients with pTa G1 tumors were excluded, because such patients have a better prognosis and are normally not treated with an adjuvant intravesical therapy.

The recurrence rate in the remaining 24 patients of the study group with pTa G2 and pT1 G2 tumors was 8 out of 24 cases (33%). This was comparable to the recurrence rate of 5 out of 18 patients (28%) in the historical control group with pTaG2 and pT1G2 tumors, which were treated with adjuvant BCG. These data are given in Table II.

Discussion

The primary purpose of adjuvant therapy after transurethral resection of superficial bladder cancer is to prevent a possible recurrence. Intravesical chemotherapy and, even more, the use of BCG have been shown to be effective in reducing the recurrence rate in patients with pTa and pT1 tumors. Numerous studies have been conducted in this group of tumors in the last 20 years and an overall reduction of the recurrence rate from about 70% (transurethral resection alone) to about 30% (transurethral resection and adjuvant BCG therapy) has been achieved. Therefore, the adjuvant intravesical use of BCG has become an established therapy for this tumor entity. Yet, its benefits are outweighed by its sometimes severe local and systemic side-effects, which have necessitated the search for alternative therapy modalities.

In our study, for the first time a standardized mistletoe extract was used intravesically for the adjuvant treatment of superficial bladder cancer. In contrast to BCG, this treatment was excellently tolerated and none of the patients had local or systemic side-effects, even at the highest dose of 5,000 ng/ml mistletoe lectin.

The recurrence rate of the patients included in our study was about 30%. According to the phase I/II study, successively increasing concentrations of the mistletoe extract were used. However, no correlation between dosage and recurrence was found. Additionally, no correlation was observed between tumor stage, grading and recurrence rate. We are aware that the included groups of superficial bladder tumors were not completely homogeneous and that pTa G1 tumors, even if recurrent or multilocular, have a somewhat different prognosis. Therefore, only those patients with pTa G2 and pT1 G2 tumors were compared with the matched local historical control group treated with BCG.

The results of this study are in agreement with two preceding animal model studies, which also showed not only a very good tolerability, but also antitumoral effects of

Table I. *Patient data and therapy groups.*

Group	Patient No.	Sex	Age	Tumor stage	Mistletoe lectin dose (ng/ml)	Recurrence	Toxicity WHO grade 1-4
1	1	m	77	Ta G2	10	no	no
	2	m	67	Ta G2	10	no	no
	3	m	70	Ta G1	10	no	no
2	4	m	67	Ta G1	20	no	no
	5	m	71	Ta G2	20	no	no
	6	m	41	Ta G2	20	yes	no
3	7	m	74	Ta G2	40	no	no
	8	m	76	Ta G2	40	no	no
	9	m	80	Ta G1	40	no	no
4	10	m	74	Ta G2	80	no	no
	11	m	54	Ta G1	80	yes	no
	12	m	71	T1 G2	80	yes	no
5	13	m	65	T1 G2	160	yes	no
	14	m	78	T1 G2	160	yes	no
	15	f	35	Ta G2	160	yes	no
6	16	f	69	Ta G1	320	no	no
	17	m	71	T1 G2	320	no	no
	18	m	66	T1 G2	320	yes	no
7	19	f	70	T1 G2	640	no	no
	20	m	72	T1 G2	640	no	no
	21	m	67	Ta G1	640	no	no
8	22	f	77	T1 G2	1,280	yes	no
	23	m	61	Ta G2	1,280	no	no
	24	m	74	Ta G2	1,280	no	no
9	25	f	72	T1 G2	2,500	no	no
	26	m	71	Ta G2	2,500	no	no
	27	f	39	Ta G2	2,500	no	no
10	28	m	48	Ta G2	5,000	yes	no
	29	m	56	Ta G2	5,000	no	no
	30	f	60	T1 G2	5,000	no	no

intravesically-applied mistletoe extract or pure mistletoe lectin of doses up to 1,500 ng/ml (14, 16).

To date, no controlled clinical data are available on the influence of intravesically-applied mistletoe extracts on the recurrence rate of superficial bladder cancer. In our study, the low recurrence incidence after adjuvant mistletoe extract treatment is encouraging and is not the result of patient selection, since the study patients and the local historical controls were comparable with respect to staging and grading. Further studies may help to find the optimal dose and confirm the role of standardized mistletoe in patients with superficial bladder cancer.

Table II. *Comparison of recurrence after adjuvant intravesical mistletoe extract therapy and adjuvant intravesical BCG therapy.*

Stage/Grade	Recurrences after adjuvant mistletoe extract	Recurrences after adjuvant BCG
TaG1	1/6	
Ta G2	3/14	2/5
T1 G2	5/10	3/13
Ta G2 + T1 G2	8/24=33%	5/18=28%

References

1 Kurth KH, Bouffioux C, Sylvester R, van der Meijden AP, Oosterlinck W and Brausi M: Treatment of superficial bladder tumors: achievements and needs. The EORTC Genitourinary Group. Eur Urol *37 Suppl 3*: 1-9, 2000.

2 Amling CL: Diagnosis and management of superficial bladder cancer. Curr Probl Cancer *25*: 219-278, 2001.

3 Rawls WH, Lamm DL, Lowe BA, Crawford ED, Sarosdy MF, Montie JE, Grossman HB and Scardino PT: Fatal sepsis following intravesical bacillus Calmette-Guerin administration for bladder cancer. J Urol *144*: 1328-1330, 1990.

4 Deresiewicz RL, Stone RM and Aster JC: Fatal disseminated mycobacterial infection following intravesical bacillus Calmette-Guerin. J Urol 144: 1331-1333; discussion 1333-1334, 1990.

5 Lamm DL, van der Meijden PM, Morales A, Brosman SA, Catalona WJ, Herr HW, Soloway MS, Steg A and Debruyne FM: Incidence and treatment of complications of bacillus Calmette-Guerin intravesical therapy in superficial bladder cancer. J Urol *147*: 596-600, 1992.

6 Ernst E, Schmidt K and Steuer-Vogt MK: Mistletoe for cancer? A systematic review of randomised clinical trials. Int J Cancer *107*: 262-267, 2003.

7 Kienle GS, Berrino F, Bussing A, Portalupi E, Rosenzweig S and Kiene H: Mistletoe in cancer – a systematic review on controlled clinical trials. Eur J Med Res *8*: 109-119, 2003.

8 Stauder H and Kreuser ED: Mistletoe extracts standardised in terms of mistletoe lectins (ML I) in oncology: current state of clinical research. Onkologie *25*: 374-380, 2002.

9 Hajto T, Hostanska K and Gabius HJ: Modulatory potency of the {beta}-galactoside-specific lectin from mistletoe extract (Iscador) on the host defense system *in vivo* in rabbits and patients. Cancer Res *49*: 4803-4808, 1989.

10 Hajto T, Hostanska K, Frei K, Rordorf C and Gabius HJ: Increased secretion of tumor necrosis factor α, interleukin 1, and interleukin 6 by human mononuclear cells exposed to β-galactoside-specific lectin from clinically applied mistletoe extract. Cancer Res *50*: 3322-3326, 1990.

11 Gabius HJ, Walzel H, Joshi SS, Kruip J, Kojima S, Gerke V, Kratzin H and Gabius S: The immunomodulatory beta-galactoside-specific lectin from mistletoe: partial sequence analysis, cell and tissue binding, and impact on intracellular biosignalling of monocytic leukemia cells. Anticancer Res *12*: 669-675, 1992.

12 Ribereau-Gayon G, Dumont S, Muller C, Jung ML, Poindron P and Anton R: Mistletoe lectins I, II and III induce the production of cytokines by cultured human monocytes. Cancer Lett *109*: 33-38, 1996.

13 Mengs U, Gothel D and Leng-Peschlow E: Mistletoe extracts standardized to mistletoe lectins in oncology: review on current status of preclinical research. Anticancer Res *22*: 1399-1407, 2002.

14 Mengs U, Schwarz T, Bulitta M and Weber K: Antitumoral effects of an intravesically applied aqueous mistletoe extract on urinary bladder carcinoma MB49 in mice. Anticancer Res *20*: 3565-3568, 2000.

15 Vang OPL, K and Bog-Hansen TC: A New Quantitative and Highly Specific Assay for Lectinbinding Activity, 5. Berlin - New York: W. de Gruyter, pp. 637-644, 1986.

16 Elsasser-Beile U, Ruhnau T, Freudenberg N, Wetterauer U and Mengs U: Antitumoral effect of recombinant mistletoe lectin on chemically induced urinary bladder carcinogenesis in a rat model. Cancer *91*: 998-1004, 2001.

Received June 22, 2005
Accepted September 1, 2005