Eur J Clin Pharmacol (2010) 66:889–897
DOI 10.1007/s00228-010-0830-5

PHARMACOKINETICS AND DISPOSITION

Pharmacokinetics of natural mistletoe lectins after subcutaneous injection

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Received: 21 December 2009 / Accepted: 20 April 2010 / Published online: 14 May 2010

Abstract
Purpose Knowledge of natural mistletoe lectins (nML) pharmacokinetics can be regarded as essential for further rational studies with mistletoe preparations. Studies with intravenous application of a recombinant type II ribosome inactivating protein (rML) analogous to nML revealed a short half-life of about 13 min in cancer patients. This open-label, phase I, monocenter clinical trial was performed in order to describe the pharmacokinetics of nML.
Methods In 15 healthy male volunteers aged 18–42 years, nML were detected with a modified sandwich immuno-polymerase chain reaction (PCR) technique (Imperacer®, Chimera Biotec) after single subcutaneous injection of a mistletoe extract (abnobaVISCUM® Fraxini 20 mg) with marketing authorization containing about 20 µg nML/ml. Secondary objectives were safety and the number of activated natural killer cells ($CD54^+/CD94^+$).
Results In none of the volunteers were nML detectable before the injection, and in all volunteers, nML were detected in serum samples after injection. Individual variability, however, was large. Mean and median peak concentrations were reached 1 and 2 h after injection, respectively. In some volunteers, nML were still detectable at the final investigation 2 weeks after injection. The injection resulted in fever and flu-like symptoms in all volunteers, but no serious adverse events occurred. All symptoms and local reactions at the injection site completely disappeared within a range of 4–95 days. The number of activated natural killer (NK) cells did not change.
Conclusions Natural ML from abnobaVISCUM® Fraxini 20 mg are detectable in serum after a single subcutaneous injection. Detectability is considerably longer compared with intravenously administered rML. The subcutaneous injection of this preparation without usual pretreatment with lower doses results in short-lasting fever and other flu-like symptoms.

Keywords AbnobaVISCUM · Anthroposophical medicine · Healthy volunteers · Phytotherapy · Safety · NK cells

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Introduction

Mistletoe preparations have been used for decades for supportive cancer treatment within the concept of anthroposophical medicine. Despite >40 randomized clinical trials, the efficacy of mistletoe treatment in cancer therapy is non yet clear and discussed controversially [1]. Reasons for this unsatisfactory situation are that different mistletoe preparations with different ingredients in different concentrations have been tested and that the pharmacology of mistletoe

extracts is unclear. Active ingredients of mistletoe extracts are mainly natural mistletoe lectins (nML), viscotoxins, and polysaccharides. From these, nML are the most interesting substances for anticancer activity. In vitro and in animal models, they have been demonstrated to have distinct cytotoxic properties [2, 3]. In doses below cytotoxicity levels, nML stimulate the unspecific and specific immune system in humans [4].

ML are glycoproteins and occur naturally in two types: ribosome inactivating proteins class 2, which are divided in the three subtypes nML-I,-II, and-III; and viscum album chitin-binding ML (cbML). The molecular weight of nML I-III is about 63 kDa. They have very similar biological properties and are composed of an N-glycosidase (A-chain) and a galactoside-recognizing lectin (B-chain) connected by a disulfide bridge [5, 6]. The A-chain inhibits protein synthesis [7, 8]. The B-chain binds to carbohydrate residues on the cell surface, thus entering the cell by receptor-mediated endocytosis and inducing apoptosis of the cell [8, 9]. The cbML belongs to a different class of lectins with a different structure, low antigenicity, and a molecular weight of only about 11 kDa [10]. It is far less toxic than nML I–III and is not included in our analysis.

Recently, the technique to detect nML I–III in nanogram ranges in human serum has been developed [11, 12]. A recombinant type II ribosome inactivating protein (rML) analogous to nML I revealed a short half-life of about 13 min in cancer patients [12]. Knowledge of nML pharmacokinetics can be regarded as important to optimize clinical use and further rational studies with mistletoe preparations. For the first time, we therefore investigated the pharmacokinetics of nML from a commercially available mistletoe preparation in humans.

Patients and methods

The study was performed as an uncontrolled, nonrandomized, open-label, phase I, monocenter clinical trial. Primary outcome was the pharmacokinetics of nML following a single dose (1 ml) of subcutaneously administered abnobaVISCUM® Fraxini 20 mg in healthy male volunteers. Secondary outcomes were safety and activation markers ($CD54^+/CD94^+$) on natural killer (NK) cells. This marker was selected because treatment of patients with metastatic colorectal cancer and lung cancer with NK cells ($CD54^+/CD94^+$) activated by heat-shock proteins showed promising antitumor effects [13]. We wanted to test the hypothesis that mistletoe-induced fever activates NK cells.

The study comprised a screening (examination 1), a period of hospitalization (examination 2–6) from the night before until 72 h after s.c. injection of the investigational medicinal product (IMP), and a final follow-up (examination 7) on day 14 ± 3 after injection of the IMP. Natural ML concentrations in volunteers' sera were analyzed before and 0.3, 0.7, 1.0, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 18, 24, 36, 48, 72, and 336 h after IMP injection. Safety laboratory parameters [creatinine, urea, uric acid, sodium, potassium, chloride, calcium, creatine kinase, alanine amino transferase (ALT), aspartate amino transferase (AST), lactate dehydrogenase, alkaline phosphatase, gamma-glutamyl transferase, total bilirubin, total protein, albumin, alpha-amylase, C-reactive protein (CRP), cholesterol, triglycerides, glucose] were determined at examinations 1, 2, and 7. $CD54^+/CD94^+$ NK cells were determined before and 6, 24, and 72 h after injection.

Inclusion criteria were 18- to 45-year-old, nonsmoking, healthy men; body mass index (BMI) 18.5–28 kg/m^2, with normal values for blood pressure, pulse rate, body temperature, hematological, biochemical, coagulation parameters, and electrocardiogram (ECG). Exclusion criteria were signs of any clinically significant disease, regular use of medication, drug abuse, positive urine screening for drugs, positive blood test for ethanol, participation in another clinical trial, previous therapy with mistletoe preparations, history of allergy to a medicinal product, allergic diseases unless the investigator considered them as clinically irrelevant for the purpose of this clinical trial, positive HIV, hepatitis B or C serology, regular intake of more than 20 g ethanol per day, donation of blood within 3 months prior to study entry, difficult peripheral venous access, or inability to understand the nature and the extent of the trial. Only volunteers who gave written informed consent and met all eligibility criteria were included. The relevant ethics committee provided a favorable opinion on the clinical trial prior to study start, and the study was performed in compliance with the principles of Good Clinical Practice and the Declaration of Helsinki.

Medication

AbnobaVISCUM® Fraxini 20 mg is an injectable, endotoxin-free plant extract from the European mistletoe species *Viscum album L.* for the treatment of malignant tumors, tumor recurrences, and defined precanceroses. As abnobaVISCUM® Fraxini 20 mg has the highest content of nML (approximately 20,000 ng/ml) of all commercially available mistletoe preparations, this preparation was chosen in order to detect nML in the nanogram range after subcutaneous injection. Mistletoes from deciduous trees such as the ash tree, from which abnobaVISCUM® Fraxini is derived, have relatively high proportions of nML-I in relation to nML-II/III [14], but due to methodological difficulties, no differentiation of the MLs could be performed in the commercial extract. The amount of cbML in abnobaVISCUM® Fraxini 20 mg is approximately 1 μg/ml [15]. Each volunteer was given the same single dose (1 ml) of subcutaneously administered abnobaVISCUM® Fraxini 20 mg. No dose adjustments were

necessary, as the inclusion criteria limited BMI to 18.5–28 kg/m^2. Intratumoral injections with abnobaVISCUM® Fraxini 20 mg resulted in highly significant tumor reductions in human pancreatic cancer xenografts [3].

Quantification of nML and activated NK cells

Each blood sample was put on ice immediately after collection and centrifuged for 10 min at 4°C and 2,500×g. Thereafter, at least 2 ml of serum was immediately frozen and stored at −80°C in the clinical trial center. Natural ML in sera of volunteers was measured by an ultrasensitive immuno-polymerase chain reaction (PCR) method (Imperacer®) [11, 12, 16], which combines protein detection through enzyme-linked immunosorbent assay (ELISA) with the exponential signal amplification typical for PCR. The method was validated by Chimera Biotech GmbH, Dortmund, Germany, for natural mistletoe lectins of abnobaVISCUM® in human serum. It does not discriminate between nML subtypes I–III. Due to the completely different structure, it does not detect cbML. Briefly, the antigen was immobilized on capture-antibody-coated microplate surfaces directly from the serum samples as delivered for analysis without additional purification. To minimize background effects, samples were diluted 1:3 in a detergent-containing sample dilution buffer. Parallel to the samples, a dilution series of the antigen was studied. AbnobaVISCUM® Fraxini 20 mg, batch no. 407B04; and abnobaVISCUM® Mali 20 mg, batch no. 502B33 (both ABNOBA GmbH, Germany) were used as reference substance. Spiked samples were used as a calibration curve to quantify the antigen and test robustness and specificity. Each calibration curve (6,000–93.75 pg/ml antigen) was prepared in antigen-free individual serum taken prior to antigen application. This dilution series was additionally frozen to simulate the effect of freezing on the antigen-containing samples for analysis. Following incubation of the samples and the calibration curve, the immobilized antigen was coupled with a specific antibody-DNA conjugate. The assay was carried out with a lectin-specific tailored Imperacer® Kit (No. 11–030, Chimera Biotec, Germany). Monoclonal mouse anti-ML antibody 5F5, anti-ML-I-(A-Chain) and 5H8 Anti-ML-A (Institut für Immunopräparate und Nährmedien GmbH, , Germany) were used as detection and capture antibody, respectively.

After washing, the DNA marker was amplified by real-time PCR. In data analysis, a baseline correction was applied. The instrument software calculates the threshold cycle (Ct), which represents the first PCR cycle at which the reporter signal exceeds the signal of the baseline (threshold) and sets it in the phase where the signal increases linearly. Baseline correction and threshold were identical in all validation measurements. Next, ΔCt values were calculated by subtracting Ct values obtained for each signal from the total number of cycles carried out in the experiment. This purely mathematical conversion facilitates data comparison with conventional ELISA data, as ΔCt values are directly proportional to the antigen concentration.

Assay precision, sensitivity, specificity, and robustness were suitable for this pharmacokinetic study. A linear concentration range of the Imperacer® assay was validated from 0.1–100 ng nML/ml. Regarding a dose of 20,000 ng nML and approximately 3,000 ml serum in humans, a maximum concentration of 6.6 ng nML/ml was expected to be measured if 100% of injected nML was distributed equally and immediately in the blood circulation without being metabolized. With a linear measurement range starting at 0.1 ng, it was possible to measure a quantitative nML concentration if only 5% of the injected amount of 1 ml abnobaVISCUM® Fraxini 20 mg appeared in the blood circulation at one sample time point. This was expected to be low enough to successfully detect kinetic parameters. The cutoff value, above which nML concentrations were positive, was 0.1 ng nML/ml. To identify NK cells, heparinized blood was used, and peripheral blood mononuclear cells (PBMC) were isolated by Ficoll gradient. After staining with phycoerythrin (PE)-conjugated anti-CD56, peridinin-chlorophyll protein (PerCP)-conjugated anti-CD45, and fluorescein isothiocyanate (FITC) conjugated anti-CD94 antibodies (all obtained from Becton-Dickinson, San Jose, CA, USA), PBMCs were incubated with the respective antibodies or immunoglobulin G (IgG) isotype control antibodies (BD Biosciences Pharmingen). A minimum of 10,000 lymphocytes was counted. Quadrants were set based upon the isotype controls for each antibody.

Statistics

No comparison with a control group was planned, and no data for comparison existed. As the study was to be conducted for exploratory purposes, no statistically based sample size calculation was performed. It was planned to include $n=16$ (two groups of $n=8$) healthy male volunteers into the trial, because this is a sufficient number to enable evaluation of the single-dose pharmacokinetics of abnobaVISCUM® Fraxini 20 mg.

For statistical analyses, two populations of trial participants were defined:

1. The pharmacokinetic population, which included all participants who provided evaluable and interpretable pharmacokinetic results during the 72 h after dosing of abnobaVISCUM® Fraxini 20 mg.
2. The safety population, which encompassed all participants who met all eligibility criteria and were included in the clinical trial.

Analyses of nML pharmacokinetics and immunological activation marker were performed on the pharmacokinetic

Table 1 Volunteer demographics

	Demographic characteristics ($n=15$)						
	Arithmetic mean	SD	Minimum	Median	Maximum	95% confidence Interval	
						Lower limit	Upper limit
Age (years)	31.4	6.3	18.0	30.0	42.0	18.0	42.0
Height (cm)	181.3	6.4	171.0	180.0	193.0	171.0	193.0
Weight (kg)	78.0	10.4	65.0	74.0	99.0	65.0	99.0
BMI (kg/m^2)	23.7	2.4	20.3	23.5	27.7	20.3	27.7

SD standard deviation, *BMI* body mass index

population. Evaluation of safety and tolerability was performed on the safety population. Missing or manipulated data were corrected, if possible. If this was not possible, the data were treated as missing.

Results

Thirty-five volunteers were screened, and 20 were found eligible. Deviating from the originally planned sample size of 16, only 15 were administered IMP; five were excluded before administration due to newly developed exclusion criteria between screening and hospitalization. Volunteer demographics are shown in Table 1. Natural ML were detectable in none of the volunteers before and in all volunteers after injection of the IMP. Although all volunteers received s.c. injection of the IMP from the same experienced investigator individual serum concentration-time profiles varied considerably: Volunteers 1–9 and 14 [10/15 (67%)] showed a fast increase with high concentrations of nML, followed by a slow decrease. A second increase was observed in volunteers 2, 5, 9, and 14 (27%). The remaining 5/15 (33%), i.e., volunteers 10–13 and 15, had an undulating course with low levels of nML concentrations. At the final examination (day 14 ± 3 after injection), serum nML concentrations were not detectable in 5/15 volunteers, but 9/15 (60%) still had measurable nML concentrations. For one volunteer, the last scheduled nML concentration could not be determined because he did not appear for the last examination. The course of nML concentrations is shown in Fig. 1 and pharmacokinetic data

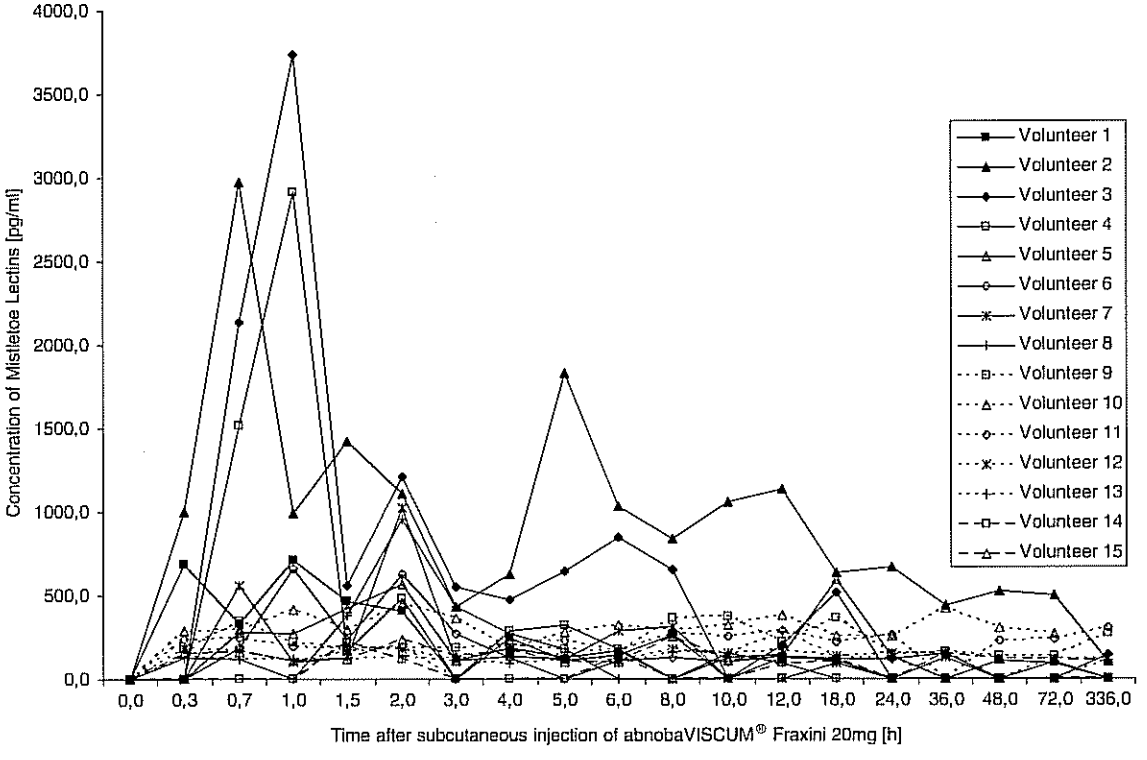

Fig. 1 Individual natural mistletoe lectin concentration-time profiles ($n=15$)

are presented in Tables 2 and 3. The mean serum peak concentration was observed at 01:00 h postdose. By the end of the study, the curve had not —but had almost—returned to predose values.

The arithmetic means of maximum plasma concentration C_{max} and area under the plasma concentration time curve from zero to infinity (AUC(0-∞) amounted to 1,043 pg/ml and 8,395 h*pg/ml, respectively. T_{max} ranged from 0.3 to 336.0 h ,with a median of 2.0 h. A calculated concentration of 3.7 ng nML/ml for the highest signal of the study as determined in volunteer 3 at 1 h after injection corresponds to 56% serum availability for this time point (100%= 6.6 ng/ml). Calculation of λ_z and $t_{1/2}$ ($t_{1/2}$=ln2/ λ_z) was not possible in any volunteer due to nonlinear concentration-time profiles. The AUC(0-t_{last}) was determined by trapezoidal analysis. In volunteers 2, 3, 9–13, and 15, AUC(t_{last}-∞) could not be determined because the nML concentration-time curves could not be extrapolated to infinity due to nonlinearity. In volunteers 1, 4–8, and 14, AUC(t_{last}-∞) did not need to be determined because nML concentrations decreased below detection threshold within the time period of blood sampling. For volunteers in whom the nML serum concentrations had not decreased below the detection threshold of 100 pg/ml in the time period of blood sampling, i.e., 10/15 volunteers (67%), AUC(0-∞) and CL_{sc}(0-∞) were not determinable. Compared with baseline, activated NK cells ($CD54^+$/$CD94^+$) as proportion of total NK cells (CD16+/CD56+) did not change significantly after IMP injection.

There were no serious adverse events (AE). Fifty-three of 55 treatment-emergent AEs were assessed by the investigator to be at least possibly related to the IMP, and all 15 volunteers experienced one or more AE with at least possible causal relationship to the IMP. Only 3/55 treatment-emergent AEs observed in 2/15 volunteers (13 %) were of severe intensity (flu-like symptoms in two, nausea in one). As expected, a local inflammatory reaction at the injection site was observed in almost all participants (14/15, 93%) and 15/15 had an increase of body temperature to >37.5°C (Fig. 2), which was accompanied by flu-like symptoms in all and nausea in eight. Eleven volunteers (73.5%) took concomitant medication because of pain at the injection site during hospitalization, and 3/15 also after discharge from the clinical trial center. In 12 volunteers (80%), the inflammatory reaction persisted beyond the final study examination, the longest being 95 days.

With regard to laboratory evaluations, no clinically significant deviations from the normal range of hematological, biochemical, and urinalysis parameters range were observed at the final examination (day 14 ± 3 days) after IMP administration (Table 4). None of the volunteers showed a clinically significant ECG abnormality throughout the study. Apart from the local inflammatory reactions at the injection site, no clinically significant abnormalities were found during physical examination.

Table 2 Summary of pharmacokinetic parameters of natural mistletoe lectins (nML)

	Parameters after a single s.c. dose of abnobaVISCUM® Fraxini 20mg									
	Number	Minimum	Maximum	Arithmetic mean	Standard deviation	Lower quartile	Median	Upper quartile	Lower limit 95% confidence interval	Upper limit 95% confidence interval
C_{max} (pg/ml)	15	188,7	3738	1043	1162	283	594	1029	189	3738
t_{max} (h)	15	0,3	336,0	26,4	85,8	1,0	2,0	10,0	0,3	336
AUC(0-tlast) (h[a]pg/ml)	15	1401	125405	34652	35786	4533	20984	58116	1401	125405
AUC(0-∞)[a] (h[a]pg/ml)	7	1401	20984	8395	8173	2552	4533	19084	1401	20984
CLsc(0-72 h)[b] (L/h)	15	428	14279	3819	3543	1510	2739	4589	428	14279
CLsc(0-336 h)b (L/h)	15	160	14279	2779	4001	344	953	4412	160	14279
CLsc(0-∞)* (L/h)	15	953	14279	5412	4001	1048	4412	7837	953	14279

Maximum plasma concentration C_{max}. Time to reach maximum plasma concentration t_{max} (h) and elimination rate constant λz (1/h): could not be determined in any of the volunteers due to nonlinear course of the concentration-time profiles. Area under the plasma concentration-time curve AUC(tlast-∞) could not be determined, as extrapolation of the concentration time curves from last data point to infinity was not possible in volunteers 2,3, 9–13, and 15 due to nonlinear run of the curves and was not necessary in volunteers 1, 4–8, and 14 because mistletoe lectin serum concentrations had already decreased to or below predose values within the time period of blood sampling, i.e., within the time point of last blood sampling (tlast)

[a] AUC(0-∞) and apparent subcutaneous clearance (CLsc) (0-∞): the summary statistics for AUC(0-∞) and CLsc(0-∞) included only volunteers 1, 4–8, and 14, in whom the total AUC was equal to the AUC(tlast-∞). Volunteers 2,3, 9–13, and 15 could not be taken into account for the summary statistics of AUC (0-∞) and CLsc(0-∞), because in these cases, AUC(tlast-∞) could not be determined (see above)

[b] CLsc(0–72 h) and CLsc(0–336 h): because the apparent subcutaneous clearance CLsc [CLsc(0-∞)] could not be determined in most volunteers, the apparent subcutaneous clearances for the time periods from 0 to 72 h [CLsc(0–72 h)] and from 0 to 336 h [CLsc(0–336 h)] were determined in all volunteers

Table 3 Individual pharmacokinetic parameters of natural mistletoe lectins

Volunteer	Pharmacokinetic parameters of natural mistletoe lectins after one single sc dose of abnobaVISCUM® Fraxini 20 mg									
	C_{max} [pg/ml]	t_{max} [h]	λ_z [1/h]	$t_{1/2}$ [h]	AUC (0–t_{last}) [h[a]pg/ml]	AUC (t_{last}–∞) [h[a]pg/ml]	AUC (0–∞) [h[a]pg/ml]	CL_{sc} (0–72 h) [L/h]	CL_{sc} (0–336 h) [L/h]	CL_{sc} (0–∞) [L/h]
1	715	1.0	[a]	[a]	19083	[b]	19083	3084	1048	1048
2	2970	0.7	[a]	[a]	125405	[a]	[a]	428	159	[a]
3	3738	1.0	[a]	[a]	30141	[a]	[a]	1719	663	[a]
4	2916.	1.0	[a]	[a]	3049	[b]	3049	6559	6559	6559
5	593	18.0	[a]	[a]	7161	[b]	7161	2792	2792	2792
6	660	1.0	[a]	[a]	20984	[b]	20984	2739	953	953
7	1028	2.0	[a]	[a]	4533	[b]	4533	4411	4411	4411
8	959	2.0	[a]	[a]	2552	[b]	2552	7836	7836	7836
9	368	10.0	[a]	[a]	66726	[a]	[a]	1510	299	[a]
10	447	2.0	[a]	[a]	58115	[a]	[a]	890	344	[a]
11	307	336.0	[a]	[a]	85601	[a]	[a]	1429	233.	[a]
12	188	0.3	[a]	[a]	39024	[a]	[a]	2585	512	[a]
13	283	12.0	[a]	[a]	18971	[a]	[a]	4588	1054	[a]
14	222	1.5	[a]	[a]	1400	[b]	1400	14279	14279	14279
15	250	8.0	[a]	[a]	37028	[a]	[a]	2432	540	[a]

[a] No determination possible due to nonlinear run of curve; [b] no extrapolation necessary as serum concentration decreased to or below predose values within the time period of blood sampling

Discussion

This study investigated for the first time pharmacokinetics of naturally occurring MLs from a commercially available nML-rich mistletoe extract. The main findings were:

1. Natural ML from mistletoe extracts can be detected in human serum after a single subcutaneous injection.
2. Detectability of nML in serum is considerably longer than that of recombinant type II ribosome inactivating protein (rML) analogous to mistletoe lectin.
3. Pharmacokinetics of nML after subcutaneous injection is subject to considerable interindividual variability.

Due to nonlinear kinetics, the half-life of nML could not be determined, as calculation of half-life is meaningless in

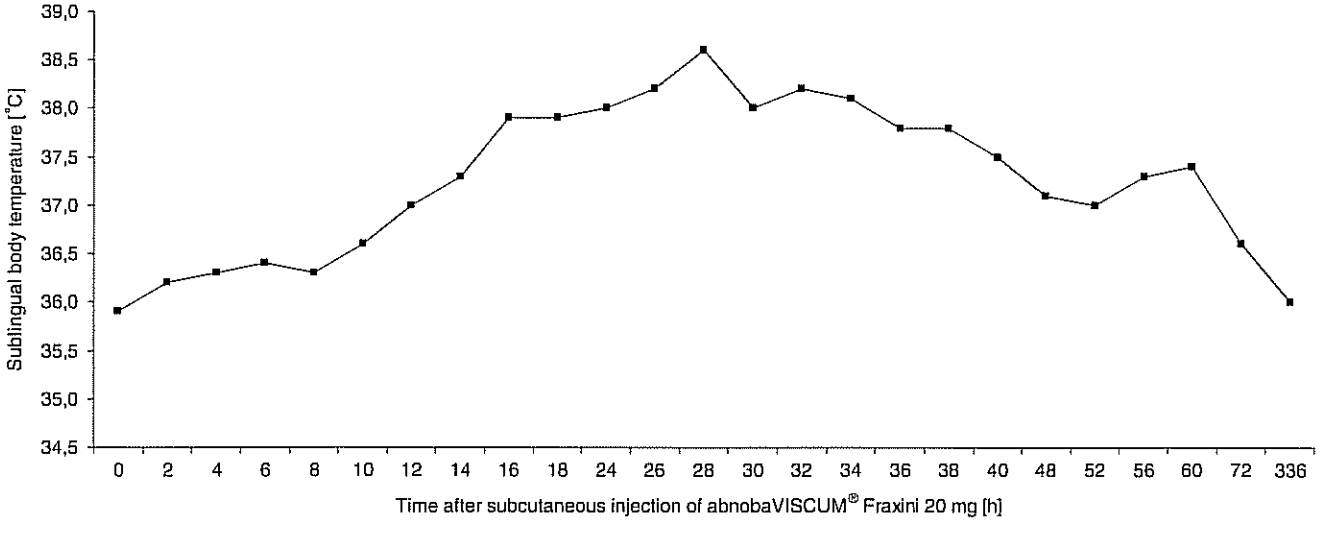

Fig. 2 Arithmetic mean of sublingual body temperature after investigational medicinal product injection (n=15)

Table 4 Safety laboratory parameters: difference from before to 14±3 days after subcutaneous application of 1 ml abnobaVISCUM® Fraxini 20 mg (n=15)

Parameter	Lower quartile	Median	Upper quartile	Lower Limit 95% confidence interval	Upper Limit 95% confidence interval
Hemoglobin (g/dl)	−0.9	−0.2	0	−1.5	1.4
White blood count/µl	70	640	1220	−990	2680
Platelet count/µl	58000	108000	136000	3000	18000
Creatinine (mg/dl)	0	0	0.1	−0.1	0.3
Uric acid (mg/dl)	−0.3	0.3	0.9	−1.0	1.2
Sodium (mmol/l)	−1	1	2	−5	3
Potassium (mmol/l)	0.1	0.3	0.7	−0.3	1.2
Calcium (mmol/l)	0	0.1	0.2	0	0.3
Creatine kinase (U/l)	−39	1	19	−80	119
Alanine aminotransferase (U/l)	1	5	20	−5	31
Aspartate aminotransferase (U/l)	−1	2	4	−6	11
Lactate dehydrogenase (U/l)	11	22	27	−10	48
Gamma glutamyl transferase (U/l)	0	3	8	−2	30
Bilirubin (mg/dl)	−0.3	−0.1	0	−1.3	0.4
Alpha amylase (mg/dl)	−2	4	11	−5	25
Total protein (g/dl)	0	0.5	0.8	−0.4	1.2
Albumin (g/dl)	−0.1	0.4	0.6	−0.4	0.9
C-reactive protein (mg/dl)	0	0.2	0.5	0	0.7
Cholesterol (mg/dl)	−7	2	14	−35	32
Triglycerides (mg/dl)	−19	5	52	−62	103
Glucose (mg/dl)	−3	−1	4	−4	21

case of nonlinear concentration-time-profiles. Because rML half-life is only 13 min [12] and in 9/15 of our volunteers nML was detectable even 2 weeks after injection, it can be conclusion that there is longer detectability of nML in serum, even though the application route was intravenous in the study by Schöffski et al. [12] and subcutaneous in our study. In contrast to rML, nML is glycosylated and has different kinetics of association and dissociation with glycoconjugates. Furthermore, B chains of rML and nML differ in binding specificity to carbohydrates [17]. This may cause different uptake and distribution in blood and tissue and could explain the longer detectability of nML in serum. Cytotoxicity of nML and rML were similar in human acute lymphoblastic leukemia cell line (MOLT-4) cell cultures [17]. In vitro studies using human peripheral blood mononuclear cells found, however, significant differences between rML and nML on cell viability and immunomodulation [18]. A long detectability in serum regarded as advantageous for therapeutic use might be opposed by the strong interindividual differences of nML pharmacokinetics after subcutaneous injection.

The high interindividual differences observed—although all volunteers were injected by the same investigator in the same abdominal quadrant—may be attributed to different patterns of nML binding to carbohydrates and release from the subcutaneous tissue. The observed nonlinear pharmacokinetics of the large nML molecules (50–63 kDa) would support this hypothesis. Pharmacokinetic investigations in rats, performed within a subchronic toxicity study with abnobaVISCUM® Fraxini 20 mg, also showed high interindividual differences between the animals under good laboratory practice conditions [19]. Cross-reactivities could potentially have affected the test results but are unlikely. Cross-reactivity to cbML can be excluded because of its completely different structure and because comparing experiments with antibodies to nML I–III and cbML in healthy volunteers and tumor patients showed no cross-reactivity [10]. Because nML I–III is highly antigenic after parenteral injection—almost 100% of nML-exposed individuals develop anti-nML antibodies—and anti-nML antibodies are absent in individuals without previous exposure to parenteral mistletoe preparations [20], a cross-reactivity to environmental factors such as diet or inhaled environmental antigens can also widely be excluded. Metabolites of nML are, to our knowledge, not known.

We chose s.c. application because it is the common form of mistletoe preparations and because abnobaVISCUM® Fraxini 20 mg has marketing authorization for s.c. injection

only. Also, pharmacokinetics of other molecules, such as soluble recombinant interleukin-4 receptor (sIL-4R) [21] and erythropoietin [22], with a size comparable to nML of 140- and 34 kDa, respectively, were investigated after s.c. injection. Murine sIL-4R elimination half-life was 2.3 h following intravenous injection and 6.2 h after s.c. injection. Also, sIL-4R blood level was lower after s.c. injection but bioavailability was comparable. Subcutaneous application of erythropoietin in 48 volunteers resulted in considerable interindividual differences of C_{max} from 40 to 95 IU/l. The half-life was about three times longer than after intravenous application. Despite comparability between these endogenously occurring substances is limited, the findings of a longer detectability and higher interindividual differences are, in principle, in accordance with our results.

Initial high doses of s.c. abnobaVISCUM® Fraxini 20 mg have a variety of side effects, especially fever and related symptoms, and strong local reactions. The manufacturer recommends, therefore, lower doses for initial therapy. In one publication, however, even higher doses (two ampoules of abnobaVISCUM® Fraxini 20 mg) were applied initially and had beneficial effects in 23 patients with advanced hepatocellular carcinoma [23]. The non-hematological toxicity included fever, erythema, and pain at the injection site, as in our volunteers. No hematological toxicity was observed. This published clinical experience encouraged us to regard one ampoule abnobaVISCUM® Fraxini 20 mg as safe for this phase I pharmacokinetic study. The hypothesis that the percentage of activated NK cells ($CD54^+/CD94^+$) increases after a single injection of abnobaVISCUM® Fraxini 20 mg could not be confirmed. As it is now known that nMLs are absorbed into the blood after s.c. injection of abnobaVISCUM® Fraxini 20 mg in healthy volunteers, pharmacokinetic considerations should also be addressed in subsequent clinical trials with mistletoe preparations for oncological patients.

Acknowledgment The study was sponsored by Abnoba GmbH, Pforzheim, Germany.

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