Pharmacokinetics of natural mistletoe lectins after subcutaneous injection

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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, monocentric 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 immunoperoxidase chain reaction (PCR) technique (Imperacer®, Chimera Biotec) after single subcutaneous injection of a mistletoe extract (abnobaVISCOM® 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 abnobaVISCOM® 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 AbnobaVISCOM · Anthroposophical medicine · Healthy volunteers · Phytotherapy · Safety · NK cells

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 not 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. Ingestion of mistletoe extracts are mainly natural mistletoe lectins (nML), viscosities, 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].

nML 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 viscous albumin 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/m², with normal values for blood pressure, pulse rate, body temperature, hematochemical, 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 precancerses. 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 made.
necessary, as the inclusion criteria limited BMI to 18.5–
28 kg/m². 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 col-
lection 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 (Imper-
acer®) [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, Dortmud,
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 ebML. Briefly, the antigen was immobilized on
capture-antibody-coated microparticle 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 calibra-
tion curve to quantify the antigen and test robustness and
specificity. Each calibration curve (6,000–93.75 pg/ml anti-
gen) 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 Imper-
cer® Kit (No. 11–030, Chimera Biotech, Germany). Mono-
clonal mouse anti-ML antibody 5F5, anti-ML-I-(A-Chain)
and 5H8 Anti-ML-A (Institut für Immunpräparate 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 subtrac-
ting 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 con-
centration 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 con-
centration of 6.5 ng nML/ml was expected to be measured if
100% of injected nML was distributed equally and immedi-
ately 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 phycocerythrin (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 mini-
um 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 sufficient number to enable evaluation of
the single-dose pharmacokinetics of abnobaVISCUM®
Fraxini 20 mg.

For statistical analyses, two populations of trial partici-
pants 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 partici-
pants 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
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

Table 1 Volunteer demographics

<table>
<thead>
<tr>
<th>Demographic characteristic (n=15)</th>
<th>Arithmetic mean</th>
<th>SD</th>
<th>Minimum</th>
<th>Median</th>
<th>Maximum</th>
<th>95% confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower limit</td>
</tr>
<tr>
<td>Age (years)</td>
<td>31.4</td>
<td>6.3</td>
<td>18.0</td>
<td>30.0</td>
<td>42.0</td>
<td>18.0</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>181.3</td>
<td>6.4</td>
<td>171.0</td>
<td>180.0</td>
<td>193.0</td>
<td>171.0</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>78.0</td>
<td>10.4</td>
<td>65.0</td>
<td>74.0</td>
<td>99.0</td>
<td>65.0</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.7</td>
<td>2.4</td>
<td>20.3</td>
<td>23.5</td>
<td>27.7</td>
<td>20.3</td>
</tr>
</tbody>
</table>

SD standard deviation, BMI body mass index

![Individual natural mistletoe lectin concentration-time profiles (n=15)](image_url)

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 nM/L/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_{0} and t_{1/2} (t_{1/2} = ln2/λ_{0}) 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 nM/L 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 nM/L concentrations decreased below detection threshold within the time period of blood sampling. For volunteers in whom the nM/L 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_{e}(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 (nM/L)

<table>
<thead>
<tr>
<th>Parameters after a single s.c. dose of abeoabVISCUM® Fraxini 20mg</th>
<th>Number</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Arithmetic mean</th>
<th>Standard deviation</th>
<th>Lower quartile</th>
<th>Median</th>
<th>Upper quartile</th>
<th>Lower limit 95% confidence interval</th>
<th>Upper limit 95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{max} (pg/ml)</td>
<td>15</td>
<td>188.7</td>
<td>3738</td>
<td>1043</td>
<td>1162</td>
<td>283</td>
<td>594</td>
<td>1029</td>
<td>189</td>
<td>3738</td>
</tr>
<tr>
<td>t_{max} (h)</td>
<td>15</td>
<td>0.3</td>
<td>366.0</td>
<td>26.4</td>
<td>85.8</td>
<td>1.0</td>
<td>2.0</td>
<td>10.0</td>
<td>0.3</td>
<td>336</td>
</tr>
<tr>
<td>AUC(0-t_{last}) (h×pg/ml)</td>
<td>15</td>
<td>1401</td>
<td>123405</td>
<td>34652</td>
<td>35786</td>
<td>4533</td>
<td>20984</td>
<td>58116</td>
<td>1401</td>
<td>123405</td>
</tr>
<tr>
<td>AUC(0-∞)* (h×pg/ml)</td>
<td>7</td>
<td>1401</td>
<td>20914</td>
<td>8395</td>
<td>8173</td>
<td>2552</td>
<td>4533</td>
<td>19084</td>
<td>1401</td>
<td>20914</td>
</tr>
<tr>
<td>CL_{e}(0-72 h) (L/h)</td>
<td>15</td>
<td>428</td>
<td>14279</td>
<td>3819</td>
<td>3543</td>
<td>1510</td>
<td>2739</td>
<td>4589</td>
<td>428</td>
<td>14279</td>
</tr>
<tr>
<td>CL_{e}(0-336 h) (L/h)</td>
<td>15</td>
<td>160</td>
<td>14279</td>
<td>2779</td>
<td>4001</td>
<td>344</td>
<td>953</td>
<td>4412</td>
<td>160</td>
<td>14279</td>
</tr>
<tr>
<td>CL_{e}(0-336 h)* (L/h)</td>
<td>15</td>
<td>953</td>
<td>14279</td>
<td>5412</td>
<td>4001</td>
<td>1048</td>
<td>4412</td>
<td>7837</td>
<td>953</td>
<td>14279</td>
</tr>
</tbody>
</table>

Maximum plasma concentration C_{max}. Time to reach maximum plasma concentration t_{max} (h) and elimination rate constant λ_{0} (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(t_{last}-∞) 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 (last) 

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

B CL_{e}(0-72 h) and CL_{e}(0-336 h): because the apparent subcutaneous clearance CL_{e} [CL_{e}(0-∞)] could not be determined in most volunteers, the apparent subcutaneous clearances for the time periods from 0 to 72 h [CL_{e}(0-72 h)] and from 0 to 336 h [CL_{e}(0-336 h)] were determined in all volunteers
Table 3 Individual pharmacokinetic parameters of natural mistletoe lectins

<table>
<thead>
<tr>
<th>Volunteer</th>
<th>Pharmacokinetic parameters of natural mistletoe lectins after one single sc dose of <em>Viscum</em> Fraxini 20 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
</tr>
<tr>
<td>1</td>
<td>715</td>
</tr>
<tr>
<td>2</td>
<td>2970</td>
</tr>
<tr>
<td>3</td>
<td>3738</td>
</tr>
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<td>4</td>
<td>2916</td>
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<td>5</td>
<td>593</td>
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<tr>
<td>6</td>
<td>660</td>
</tr>
<tr>
<td>7</td>
<td>1028</td>
</tr>
<tr>
<td>8</td>
<td>959</td>
</tr>
<tr>
<td>9</td>
<td>368</td>
</tr>
<tr>
<td>10</td>
<td>447</td>
</tr>
<tr>
<td>11</td>
<td>307</td>
</tr>
<tr>
<td>12</td>
<td>188</td>
</tr>
<tr>
<td>13</td>
<td>283</td>
</tr>
<tr>
<td>14</td>
<td>222</td>
</tr>
<tr>
<td>15</td>
<td>250</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lower quartile</th>
<th>Median</th>
<th>Upper quartile</th>
<th>Lower Limit 95% confidence interval</th>
<th>Upper Limit 95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>-0.9</td>
<td>-0.2</td>
<td>0</td>
<td>-1.5</td>
<td>1.4</td>
</tr>
<tr>
<td>White blood count/μl</td>
<td>70</td>
<td>640</td>
<td>1220</td>
<td>-990</td>
<td>2680</td>
</tr>
<tr>
<td>Platelet count/μl</td>
<td>58000</td>
<td>108000</td>
<td>136100</td>
<td>3000</td>
<td>18000</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0</td>
<td>0</td>
<td>0.1</td>
<td>-0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>-0.3</td>
<td>0.3</td>
<td>0.9</td>
<td>-1.0</td>
<td>1.2</td>
</tr>
<tr>
<td>Sodium (mmol/l)</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Potassium (mmol/l)</td>
<td>0.1</td>
<td>0.3</td>
<td>0.7</td>
<td>-0.3</td>
<td>1.2</td>
</tr>
<tr>
<td>Calcium (mmol/l)</td>
<td>0</td>
<td>0.1</td>
<td>0.2</td>
<td>0</td>
<td>0.3</td>
</tr>
<tr>
<td>Creatine kinase (U/l)</td>
<td>-39</td>
<td>1</td>
<td>19</td>
<td>-80</td>
<td>119</td>
</tr>
<tr>
<td>Alanine aminotransferase (U/l)</td>
<td>1</td>
<td>5</td>
<td>20</td>
<td>-5</td>
<td>31</td>
</tr>
<tr>
<td>Aspartate aminotransferase (U/l)</td>
<td>-1</td>
<td>2</td>
<td>4</td>
<td>-6</td>
<td>11</td>
</tr>
<tr>
<td>Lactate dehydrogenase (U/l)</td>
<td>11</td>
<td>22</td>
<td>27</td>
<td>-10</td>
<td>48</td>
</tr>
<tr>
<td>Gamma glutamyl transferase (U/l)</td>
<td>0</td>
<td>3</td>
<td>8</td>
<td>-2</td>
<td>30</td>
</tr>
<tr>
<td>Bilirubin (mg/dl)</td>
<td>-0.3</td>
<td>-0.1</td>
<td>0</td>
<td>-1.3</td>
<td>0.4</td>
</tr>
<tr>
<td>Alpha amylase (mg/dl)</td>
<td>-2</td>
<td>4</td>
<td>11</td>
<td>-5</td>
<td>25</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>0</td>
<td>0.5</td>
<td>0.8</td>
<td>-0.4</td>
<td>1.2</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>-0.1</td>
<td>0.4</td>
<td>0.6</td>
<td>-0.4</td>
<td>0.9</td>
</tr>
<tr>
<td>C-reactive protein (mg/dl)</td>
<td>0</td>
<td>0.2</td>
<td>0.5</td>
<td>0</td>
<td>0.7</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>-7</td>
<td>2</td>
<td>14</td>
<td>-35</td>
<td>32</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>-19</td>
<td>5</td>
<td>52</td>
<td>-62</td>
<td>103</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>-3</td>
<td>-1</td>
<td>4</td>
<td>-4</td>
<td>21</td>
</tr>
</tbody>
</table>

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 abnoBaVISICUM® 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 abnoBaVISICUM® 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.

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References

19. ADVINUS Therapeutics Private Limited (2009) Mistletoe extract AbnobaViscum Fraxini 20 mg: 90 day study in Sprague-Dawley rats by subcutaneous route with toxicokinetics and 4 week

