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Weekly 24 h infusion of aviscumine (rViscumin): A phase I study in patients with solid tumours

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Abstract

Aviscumine is a ribosome-inactivating protein with potent antitumour activity *in vitro* and *in vivo* and is an *Escherichia coli*-derived recombinant counterpart of natural mistletoe lectin-I. The current study was performed to determine the safety profile, dose-limiting toxicity (DLT) and maximum tolerated dose (MTD) of a prolonged infusion of aviscumine in cancer patients. Aviscumine was given once weekly as a 24 h central intravenous infusion in patients with advanced, refractory progressive solid malignant tumours. Fourteen fully eligible patients (11 male, 3 female) with a median age 58 yrs (range 41–77) were enrolled. They had histologically verified disease, were $\geqslant$18 yrs old, had an ECOG PS $\leqslant$2 and adequate bone marrow, liver and renal function. DLT was defined as any non-haematological grade 3–4 toxicity (Common Toxicity Criteria [CTC] version 2.0), neutrophil count <500/μl for $\geqslant$7 days, febrile neutropenia or thrombocytopenia grade 4. The MTD was defined as the dose level below the dose at which $\geqslant$2 patients per dose level experienced a DLT during the first treatment cycle. Colorectal cancer, soft tissue sarcoma and pancreatic cancer were the most common tumour types. Dose levels of aviscumine ranged from 4 to 6 μg/kg. The median number of cycles was 2.8 (range, 2–8). Common side effects in cycle 1 were fatigue, fever, nocturia, urticaria, erythema and pruritus. DLTs occurred in 2/3 patients on the 6 μg/kg dose level and consisted of increases in ASAT grade 3, ALAT grade 3, γGT grade 3/4, hypokalemia grade 3 and fatigue grade 3. No DLTs were observed on dose levels 4 and 5 μg/kg. The best response (RECIST) was stable disease in 4 pts, lasting for 4–8 cycles. Pharmacokinetics indicated that potentially active plasma levels of the compound were maintained during the entire infusion. We conclude that the recommended dose for weekly 24 h infusions of Aviscumine should be 5 μg/kg.

Keywords: Aviscumine; CD75s; Phase I study; Recombinant mistletoe lectin; Ribosome-inactivating proteins; Solid tumours

1. Introduction

Mistletoe lectin-I (ML-I, *Viscum album*-agglutinine I) is the major active constituent of natural mistletoe extract [1]. The lectin component induces cell death in various transformed cell lines [2–6]. ML-I is a 66-kDa heterodimer that consists of a toxic A-chain, a site-specific type II ribosome-inactivating N-glycosidase [7], and a carbohydrate-binding-subunit B responsible for cellular lectin uptake [8–10].

The ML-I gene was sequenced in 1999 and the production of pure, biochemically defined ML-I was achieved by cloning and separate expression of the A- and B-chain in *E. coli* BL21DE3 by recombinant DNA techniques. Active heterodimeric protein rViscumin (INN: aviscumine) was produced [9,10] in an aqueous, buffered solution. The protein was linked by

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disulfide bond and remained stable at pH 7.0–9.0 (Fig. 1) [10].

Extract preparations from natural mistletoe (*Viscum album*) have been widely used as alternative therapy in the management of patients with malignant disease for several decades, based on presumed immunostimulatory and antineoplastic effects [1,11]. There was, however, an obvious discrepancy between the popularity of mistletoe extracts among cancer patients and a striking lack of evidence-based data to support their use in oncology [12–15]. The availability of pure, homogeneous protein allowed structural and mechanistic studies, in order to gain a better insight into the mode of action of this product on tumour and immune cells, as the isolation of the glycosylated proteins from natural mistletoe extract yielded only heterogeneous material. The recombinant drug was studied extensively in preclinical models and it was found to possess antineoplastic and immunomodulatory properties *in vitro* and *in vivo* and was tolerated well in animals [16–20]. Furthermore, the cellular receptor involved in the binding of the heterodimer was identified [21,22]. Aviscumine preferentially binds to terminally α2-6-sialylated ganglioside structures. In contrast to another well-known ribosome-inactivating agent, ricin, aviscumine was characterised as a sialic acid-specific type II ribosome-inactivating protein. Neolacto-series gangliosides with a Neu5Acα2-6Galβ1-4GlcNAc-terminus (CD 75s) were defined as the aviscumine receptors for the B-chain, leading to internalisation of the holoprotein [21]. In further mechanistic studies, the A-chain was confirmed to be a potent hydrolase that selectively cleaves the N-glycosidic bond of the adenine-4324 residue in eukaryotic 28S ribosomal RNA. This effect leads to a catalytical inactivation of ribosomes and thereby inhibits translation and protein synthesis which is the presumed aviscumine mode of action [7,9].

In vitro, aviscumine was found to induce apoptosis in a low concentration range (fM to pM) and necrosis at higher concentrations (nM to mM). The mean aviscumine concentration required to eliminate 70% of human tumour cell lines *in vitro* (Freiburg tumour panel) was 0.4 and 2 ng/ml to inhibit tumour xenograft colony formation by 70%. On a molar basis, aviscumine was about 5000 times more potent than doxorubicin and 1500 times more potent than paclitaxel against some human tumour cell lines. Aviscumine was more active against doxorubicin-resistant breast tumour cells and vindesine-resistant pleural mesothelioma cells than on the respective drug-sensitive parental cell lines, indicating that the drug is not affected by common resistance mechanisms. *In vitro*, the drug enhanced the cytotoxic effects of vincristine, mafosfamide, idarubicin and cisplatin in the human leukaemia cell lines K562 and KG1a.

The intraperitoneal (ip.), subcutaneous (sc.) or intravenous (iv.) administration of the recombinant protein was shown to have growth inhibitory action in various heterotopic tumours and metastasis mouse models [19]. A significant inhibitory effect on experimental urothelial carcinogenesis was seen after intravesical instillation into rats. Intratumoural treatment of human ectopic CXF 280, and ip. administration in 60444 and 5776 colon cancer in immunodeficient mice led to a strong and dose-dependent inhibition of tumour progression. Combination therapy of aviscumine with

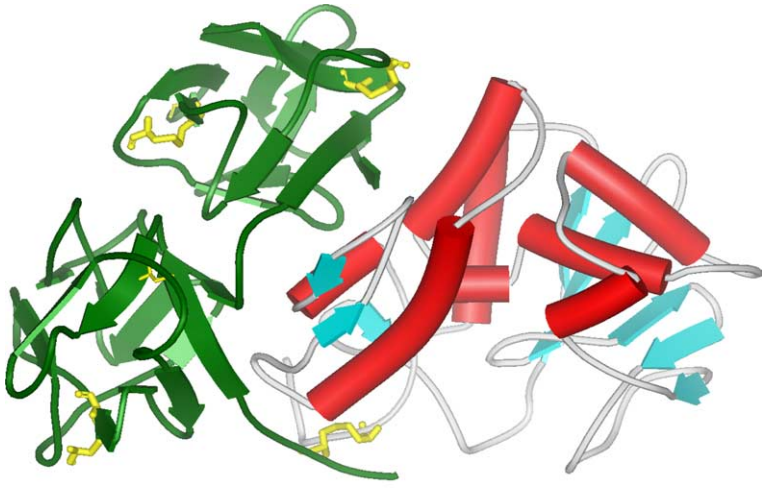

Fig. 1. Composition of aviscumine.

doxorubicin was found to be superior to the effect of each single agent alone. The ip. administration of aviscumine into human ovarian cancer-bearing SCID mice led to a significant prolonging of survival [18].

Immunological studies *in vitro* showed that aviscumine stimulated the release of IL-1α and IL-6 from human keratinocytes and fibroblasts, of IL-12, IFNγ and TNFα from peripheral blood mononuclear cells as well as the expression of the IL-2 receptor alpha chain and HLA-DR on peripheral blood T lymphocytes. Additionally, aviscumine increased the activity of human natural killer cells against lymphoma cells *ex vivo* [16].

Toxicology studies showed that aviscumine can be safely applied in animals and lacks relevant genotoxic or mutagenic effects up to dosages of 0.001 mg/kg iv. Repeated iv. or sc. dosing did not reveal any specific target organ toxicity in rats and dogs up to 1000 mg/kg. Bleeding complications were observed on much higher doses. Local reactions at sc. injection sites were observed at concentrations from 50 ng/ml onwards.

A first human dose-finding study was performed by the New Drug Development Group of the European Organization for Research and Treatment of Cancer (EORTC). The drug was given twice weekly as a 1 h iv. infusion to 41 patients with refractory cancers [17]. Dose ranges of 10–6400 ng/kg were visited. Liver toxicity was dose limiting, as reversible grade 3 increases in transaminases, γGT and/or alkaline phosphatase were observed. Further toxicities attributed to the study drug included fatigue, fever, nausea, vomiting and allergic reactions. A number of patients with progressive disease achieved disease stabilisation for up to 8 treatment cycles. Using the 1 h infusion schedule, the drug was found to have a short human alpha half life of only 13 min, most likely related to rapid proteolysis. The pharmacokinetic and clinical findings of the EORTC trial were the scientific rationale for designing this second iv. dose-finding trial of aviscumine in patients with refractory solid tumours. We performed a single centre study investigating the safety and toxicity of a weekly prolonged infusion of the recombinant anticancer agent.

2. Patients and methods

2.1. Objectives

This Phase I, open-label, multiple dose, dose-escalation study was conducted in Hannover, Germany. The primary objective was to assess the safety and toxicity profile of a weekly 24 h iv. infusion of aviscumine, to evaluate the dose-limiting toxicity (DLT) and maximum tolerated dose (MTD) and to establish a recommended dose for further clinical trials. Secondary objectives were to study the pharmacokinetics of this schedule and to assess if adequate plasma exposure can be achieved.

2.2. Patient selection criteria

Patients with a histologically or cytologically confirmed diagnosis of a progressive solid malignant tumour were eligible for the trial, provided the disease was refractory to further conventional treatment. Standard selection criteria included age $\geqslant$18 yrs, ECOG performance status 0–2, no central nervous system involvement, adequate bone marrow (white blood count $\geqslant 3 \times 10^9$/l, neutrophils $\geqslant 1.5 \times 10^9$/l, platelets $\geqslant 100 \times 10^9$/l), liver (serum bilirubin within 1.5 times the upper limit of normal [ULN], serum ASAT, ALAT and alkaline phosphatase <2.5 times ULN) and renal function (serum creatinine <120 μmol/l). In contrast to the previous EORTC trial, this study did not exclude patients with previous exposure to natural mistletoe preparations. Prior chemotherapy, hormone and/or radiation treatment had to be completed at least 4 weeks prior to study entry. Patients were not allowed to receive any immunostimulating substances, biological response modifiers, colony stimulating factors, systemic steroids or monoclonal antibodies during the study. Patients had to use effective contraception if of reproductive potential and were not allowed to be pregnant or lactating. Written informed consent was obtained from all participating patients according to applicable German laws. Patient insurance was provided by the study sponsor, VISCUM AG (Bergisch Gladbach, Germany). The study was conducted in accordance with the Good Clinical Practice Guidelines as issued by the International Conference on Harmonisation and the Declaration of Helsinki.

2.3. Study logistics

After obtaining informed consent, patients were screened for potential trial participation. Eligible candidates were registered by a fax procedure at the involved Clinical Research Organization prior to the start of treatment and after verification of the eligibility criteria and selection of the target lesions for response assessment. The treatment started within one week after registration. Aviscumine was given weekly until one of the following withdrawal criteria occurred: disease progression, unacceptable toxicity, patient's refusal or patient's best interest according to the treating physician.

2.4. Study treatment

Aviscumine concentrate was supplied in 10 ml vials by the sponsor and kept in the hospital pharmacy at 2–8 °C. The drug was formulated as a pyrogen free, phosphate buffered solution at pH 8.0 and did contain sodium chloride, polysorbate 80 and glutamic acid. The appropriate volume of aviscumine was calculated according to the body weight of the patient, diluted with

physiological saline up to a total volume of 500 ml and administered with portable elastomeric pumps (Baxter Intermate, Baxter Healthcare) within 8 h after preparation of the infusion. Aviscumine was given weekly as a 24 h iv. infusion via port catheters. One cycle was arbitrarily defined as three consecutive weeks of treatment (three infusions). The treatment was given continuously without interruption between the treatment cycles. After the end of each cycle the safety and tolerability of the treatment was assessed in detail. Patients with progressive disease were removed from the study, patients achieving stable disease or an objective response could remain on treatment. Supportive care was at the discretion of the investigator.

2.5. Dose escalation procedure

Based on pharmacokinetic data from the first iv. phase I trial performed by the EORTC, a dose of 6 μg/kg body weight given over 24 h was considered to be safe in man and was chosen as starting dose in this study. It was planned to increase the dose levels in steps of 66% (first escalation), 50% (second escalation) and 33% (each further escalation) in cohorts of 3–6 patients. The protocol was later amended, as DLT occurred on the starting dose level. After review by the local ethics committee, two lower dose levels were visited and the study was successfully completed.

2.6. Definition of DLT, MTD and recommended dose

DLTs were defined as any non-haematological grade 3–4 toxicity (with the exclusion of nausea and vomiting), an absolute neutrophil count <500/μl lasting for ⩾7 days, febrile neutropenia (defined as an absolute neutrophil count <1000/μl lasting for ⩾3 days associated with fever ⩾38.5 °C for 24 h) or thrombocytopenia grade 4 CTC. In case that more than one patient experienced DLT in cycle 1 at a given dose level, this dose was regarded as not tolerable and the dose below this level was defined as MTD and recommended for the further development of this schedule.

2.7. Clinical evaluation, laboratory tests and follow-up

Within two weeks prior to study treatment, the following baseline tests were performed: Tumour evaluation, tumour markers (if applicable), complete medical history, ECOG performance status, vital signs, body temperature, physical examination, pregnancy test in fertile female patients, weight, height, complete blood count, serum chemistry (total protein, albumin, Na^+, K^+, Ca^{2+}, creatinin, bilirubin, ASAT, ALAT, ALP, γGT) and ECG. Immediately prior to the first administration of aviscumine the following parameters were obtained: full blood count, serum chemistry, tumour markers, physical examination, ECOG performance status, body temperature and vital signs. On a weekly basis, the following parameters were assessed: Toxicity, vital signs, body temperature, complete blood count and serum chemistry. The toxicity evaluation according to CTC was performed prior to each treatment cycle. Tumour imaging was repeated every six weeks.

2.8. Criteria for evaluation of toxicity and response

All adverse events were graded according to CTC (version 2.0). The causality of clinical events was categorised as unrelated, unlikely, possible, probable, definitely related or not assessable. Diseases and clinical symptoms already known at the beginning of the study were not classified as adverse events if they were encountered again at subsequent examinations, except in the case of a relevant increase in intensity or incidence. All patients who had started the treatment were included in the overall toxicity assessment. Serious adverse events were defined according to the ICH Good Clinical Practice Guidelines and reported within 24 h to the sponsor of the trial. This included all events occurring during or within 30 days of termination of the study treatment. The response to the treatment with aviscumine was assessed according to the RECIST criteria [23].

2.9. Pharmacokinetic analysis

Pharmacokinetic samples were collected in all patients who were enrolled after the first observation of a DLT. Samples were taken prior to the start of the first infusion in cycle 1 as well as 4 h, 6 h, 24 h, 24 h 15 min, 24 h 30 min, 25 h, 26 h, and 28 h after start of the first infusion of aviscumine. The samples were shipped on dry ice to the sponsor for analytical purposes. Pharmacokinetic analyses with monoclonal anti aviscumine (A- and B-chain) antibodies were performed using a immuno-PCR method as previously described [24,17].

3. Results

3.1. Patient characteristics

Between 02/2003 and 04/2004, 14 fully eligible patients entered the trial. The median age was 58.3 years. Colorectal cancer, soft tissue sarcoma and pancreatic cancer were the most common tumour types. The vast majority of patients were pre-treated with surgery and chemotherapy, others had received radiotherapy, hormone treatment and/or immunotherapy (see Table 1). All patients were refractory to standard treatment. There were no relevant protocol deviations, and all patients were included in the trial analysis.

Table 1
Patient characteristics

Gender (no. of patients)	
Female	3
Male	11
Age (years)	
Median	58
Range	41–77
ECOG PS (no. of patients)	
0	9
1	4
2	1
Primary tumour (no. of patients)	
Colorectal cancer	7
Soft tissue sarcoma	3
Pancreatic cancer	2
Prostate cancer	1
Urothelial bladder cancer	1
Previous treatment (no. of patients)	
Surgery	13
Systemic therapy	13
Radiotherapy	4

3.2. Treatment administration

The median number of treatment cycles administered was 2.8, with a range of 1–8 (see Table 2). Two patients discontinued the treatment after the first administration of aviscumine due to anaphylactic reactions. There were no further dose or schedule modifications in cycle 1. The dose levels visited were ranging from 4 to 6 μg/kg. Dose limiting events were observed in 2 out of 3 patients on the starting dose level of 6 μg/kg. Further patients were enrolled on the 4 and 5 μg/kg level after protocol amendment and ethics board approval.

The majority of patients discontinued the treatment due to disease progression. Two patients were taken off study due to liver toxicity, two patients went off due to anaphylactic reactions and were replaced.

Table 2
Treatment administration

	No. of patients
No. of cycles per patient	
1	5
2	5
4	2
5	1
8	1
No. of patients per dose level	
4 μg/kg	3
5 μg/kg	8
6 μg/kg	3
Reasons for trial discontinuation	
Disease progression	10
Liver toxicity and progression	2
Anaphylaxis	2

3.3. Toxicity

All patients were evaluated for toxicity, including four patients who went off study during the first treatment cycle due to toxicity (2) or allergic reactions (2). In general, aviscumine was well tolerated. The most frequent clinical toxicities were fatigue, high urinary frequency/nycturia, fever, and pruritus and with few exceptions the observed toxicities were grade 1/2 events (see Table 3). Three patients who were pretreated with natural mistletoe extracts had local reactions at previous sc. injection sites (erythema, pruritus and urticaria). Two patient had an anaphylactic reaction after the first administration of the compound with generalised urticaria, one of them also complained of dysphagia and swelling of the larynx. Both patients were taken off study and replaced. Haematological toxicity was uncommon. The 24 h iv. infusion of aviscumine was not associated with neutropenia, granulocytopenia or thrombocytopenia. There was no evidence of cumulative toxicity in patients continuing treatment beyond a first treatment cycle. There were no toxic deaths.

3.4. Dose limiting events, maximum tolerated dose and recommended dose

Dose limiting events were observed in the first treatment cycle in 2 patients in the 6 μg/kg cohort (see Table 4). One of them had an increase in ASAT and γGT grade 3 CTC. A further patient had fatigue grade 3 and an increase in ALAT grade 3 and γGT grade 4, associated with an increase in alkaline phosphatase grade 2 and ASAT increase grade 2, as well as hypokalemia grade 3.

With 2 out of 3 patients on the 6 μg/kg having reversible grade 3/4 toxicity, the starting dose level had to defined as being the MTD. No dose limiting events were seen on the 4 and 5 μg/kg dose levels, so 5 μg/kg can be recommended for further clinical evaluation of this schedule.

3.5. Response assessment

The tumour evaluation was done every other cycle. 4 patients were evaluable for response after cycle 2, 2 patients were evaluable after cycle 4. The best overall response during the conduct of the trial per patient is shown in Table 5. Four patients achieved disease stabilisation for 2–8 cycles, but there were no minor, partial, complete or tumour marker responses. The time to progression varied between 18 and 149 days. There was no further evidence of clinical or tumour marker responses.

3.6. Binding of aviscumine to archived tumour tissue

Immunohistochemical tumour binding studies were performed with archived material from three patients

Table 3
Treatment-related adverse events (maximum CTC grade, all cycles)

	4 μg/kg, $n = 3$	5 μg/kg, $n = 8$	6 μg/kg, $n = 3$	Total, $n = 14$
Grade CTC	1/2/3	1/2/3	1/2/3/4	1–4
Fatigue	2/0/0	2/0/0	1/1/1/0	7
Urinary frequency	0/0/0	0/1/0	0/3/0/0	4
Allergic reaction	0/0/0	0/1/1	1/0/0/0	3
Fever	1/0/0	1/0/0	1/0/0/0	3
Pruritus	0/0/0	1/1/1	0/0/0/0	3
Diarrhea	1/0/0	1/0/0	0/0/0/0	2
Nausea	0/0/0	1/0/0	1/0/0/0	2
SGOT	0/0/0	0/0/0	0/1/1/0	2
SGPT	0/0/0	0/0/0	0/1/1/0	2
Sweating	0/0/0	1/0/0	0/1/0/0	2
Alkaline phosphatase	0/0/0	0/0/0	0/1/0/0	1
Anorexia	0/0/0	0/0/0	1/0/0/0	1
Bilirubin	0/0/0	0/0/0	0/1/0/0	1
Skin	0/0/0	1/0/0	0/0/0/0	1
Dysphagia	0/0/0	0/0/1	0/0/0/0	1
Gamma-GT	0/0/0	0/0/0	0/0/0/1	1
Headache	0/0/0	0/0/0	1/0/0/0	1
Hypernatremia	0/0/0	0/0/0	1/0/0/0	1
Hypocalcemia	0/0/0	0/0/0	0/1/0/0	1
Insomnia	1/0/0	0/0/0	0/0/0/0	1
Hypoproteinemia	0/0/0	0/0/0	1/0/0/0	1
Mouth dryness	1/0/0	0/0/0	0/0/0/0	1
Sensory neuropathy	0/0/0	0/0/0	1/0/0/0	1
Rigors, chills	0/0/0	0/0/1	0/0/0/0	1
Taste disturbance	1/0/0	0/0/0	0/0/0/0	1
Tumour pain	0/0/0	0/0/0	1/0/0/0	1

Table 4
Dose limiting events (all patients, cycle 1, related events only)

Dose level	Patient no.	Dose-limiting toxicity	Grade CTC
6 μg/kg	1	Increase ASAT	3
		Increase γ-GT	3
6 μg/kg	3	Increase ALAT	3
		Increase γ-GT	4
		Hypokalemia	3
		Fatigue	3

In addition to these DLTs, patient no. 1 had grade 2 increases in ALT and bilirubin, patient no. 3 also had grade 2 elevations of AST, alkaline phosphatase and hypocalcemia. Both patients also complained of increased urinary frequency.
Two further patients (patients no. 9 and 13) had anaphylactic reactions after the first iv. administration of aviscumine and were replaced. These patients had previously been exposed to natural mistletoe extracts. The allergic reaction started at sites previously used for sc. mistletoe injections.

Table 5
Best overall response (RECIST)

Response	No. of patients
Complete response	0
Partial response	0
Stable disease/no change	4
Progressive disease	5
Early progression	3
Not evaluable	2

treated in this trial. In patient no. 8 (colorectal cancer), who had the longest time to progression (149 days) on study, 80% of tumour cells bound aviscumine with ++ to +++ staining intensity. In patient no. 10, a further case of colorectal carcinoma who received 5 cycles and showed progression after 76 days, 80% of the primary tumour cells stained with +++ intensity. In contrast, the primary tumour of patient no. 12 with bladder cancer, who progressed within only 36 days of treatment, did not stain with aviscumine.

3.7. Pharmacokinetics

Plasma concentrations of aviscumine were provided by chimera biotec GmbH (Dortmund, Germany). They were determined in 10 patients. Plasma levels above 2 ng/ml (mean IC70 of aviscumine in cell lines) were maintained throughout the infusion in the majority of cases. Maximum concentration was 5.4 ng/ml (median) for 6 evaluable patients on the 5 μg/kg dose level (recommended dose). The median time with a plasma concentration above 2 ng/ml was 24 h. At the end of the infusion, plasma concentrations of aviscumine decreased rapidly with an initial alpha half-life of 0.23 h. The median beta half-life was 3.78 h. The total half-life was calculated as 2.81 h. AUC(0-inf) was 19 h * ng/ml (median) for 6 evaluable patients at the 5 μg/kg (range, 58–140 h * ng/ml). Further details on

Table 6
Pharmacokinetics of aviscumine at recommended dose (dose level 5000 ng/kg, cycle 1, $n = 6$ evaluable patients)

Parameter	Arithmetic mean	Standard deviation	Median	Unit
C_{max}	4826	1312	5356	pg/ml
T_{max}	11.4	9.95	5.98	h
AUC 0-inf	113811	30693	119311	h*pg/ml
Tau	23.7	2.77	24.0	h
T1/2α	0.26	0.16	0.25	h
T1/2β	4.85	2.15	4.21	h
T1/2 total	3.12	1.31	3.01	h
Cl total	3.87	1.74	3.34	l/h
Clss	4.50	2.22	3.72	l/h
MRTss	3.94	1.79	4.62	h
Vss	19.64	14.76	16.72	l

Tau: Duration from start of the infusion exceeding threshold of 2 ng/ml.

the pharmacokinetic evaluation of aviscumine are shown in Table 6.

4. Discussion

Aviscumine is an innovative antineoplastic compound with interesting pharmacology and a unique mode of action. The recombinant heterodimer enters the cell via specific binding of the B-chain to CD75s, a membraneous ganglioside with unknown physiological function that is frequently expressed in common solid tumours. This binding leads to internalisation of the holoprotein. The A-chain of the drug catalyses the inactivation of ribosomes and thus inhibits translation and protein synthesis.

In an EORTC Phase I study, the administration of a twice weekly 1 h iv. infusion of aviscumine was found to be safe up to a dose of 5600 ng/kg body weight. 6400 ng/kg was the MTD, associated with reversible hepatotoxicity as dose-limiting event in 2 out of 5 patients. The toxicity profile in that clinical trial was quite acceptable, and according to the safety data it was concluded that the recommended dose for further clinical trials is 5600 ng/kg. Aviscumine was found to stimulate the immune system with a secretion of cytokines in plasma independent of the administered dose of the drug [17].

In this follow-up Phase I dose-finding study a total of 14 patients with refractory solid tumours were treated with aviscumine, here administered as a weekly 24 h infusion. The starting dose of 6 μg/kg body weight was associated with grade 3/4 dose-limiting events. Further dose levels assessed were 4 and 5 μg/kg. These dose levels were found to be safe. Dose limiting events were liver toxicity, hypokalemia and fatigue, which basically confirmed the experience of the EORTC with this compound. In addition, allergic reactions were observed in patients previously exposed to natural mistletoe extracts, ranging from mild urticaria to severe anaphylactic events, the latter leading to treatment discontinuation in two patients. Interestingly, allergic reactions started in most cases in anatomic sites were patients had previous injections of natural mistletoe preparations. The recombinant lectin should be given with caution to patients with previous mistletoe exposure. Interestingly, the previous EORTC study did not reveal allergic reactions; in that trial, however, patients with previous exposure with mistletoe were excluded from trial participation.

There was no clinical evidence of antitumour activity of aviscumine, but 4 out of 12 evaluable patients with tumour progression at baseline achieved disease stabilisation, which lasted between 76 and 149 days. There was a trend that patients with longer lasting disease stabilisation had primary tumours which were actively binding aviscumine, according to a subgroup analysis in a very limited number of patients. This finding suggests a targeted antineoplastic action of the compound, which is known to bind to CD75s on the cell surface.

The pharmacokinetic assessment was of importance in this trial, as the previous EORTC study had demonstrated a very short half-life of the compound after the 1 h infusion. As expected, the infusional schedule achieved continuous plasma concentrations above 2 ng/ml, the threshold of antitumour activity in preclinical models, during the entire infusion. This could potentially explain why our starting dose level was dose limiting and could potentially translate into improved antitumour efficacy of this schedule.

In summary, a dose of 5 μg/kg aviscumine per kg body weight as a weekly 24 h intravenous infusion is recommended for further studies. The dosing regimen is well-tolerated and aviscumine plasma concentrations above 2 ng/ml are maintained during the entire infusion.

Conflict of interest statement

The Phase I study was performed for and funded by VISCUM AG. One co-author of this research paper is currently employed by the sponsor of this trial (H. Lentzen). One co-author of this article was previously employed by the manufacturer of the compound (K. Wilhelm-Ogunbiyi). The first author of this article has served on the advisory board of the sponsoring company (P. Schöffski).

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