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Short report

Zilascorb(^{2}H), a new reversible protein synthesis inhibitor: clinical study of an oral preparation

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The new anti-cancer drug zilascorb(^{2}H) has shown promising activity in preclinical models. Its putative mechanism of action is reversible protein synthesis inhibition and long-term treatment is required. As a clinical treatment modality, long-term daily zilascorb(^{2}H) infusions, as used in previous studies, are not regarded feasible. Therefore, an oral formulation of the drug was developed, and pharmacokinetic profile, toxicity and antitumor activity of zilascorb(^{2}H) *tablets* were studied. Thirteen patients with advanced solid cancer not amenable to established therapy, but with adequate performance status and organ functions, were included. The treatment was given as a daily i.v. zilascorb(^{2}H) infusion for 5 days, followed by zilascorb(^{2}H) tablets twice daily for 3 months. Blood and urine sampling was performed when estimated plasma steady-state level was reached for each formulation, respectively. Analyses of drug concentrations in plasma and urine were performed by high performance liquid chromatography. Zilascorb(^{2}H) in tablet formulation had a bioavailability of 32%, was quickly absorbed and slowly eliminated. Concomitant use of the H_2-blocker ranitidine possibly enhanced bioavailability. Zilascorb(^{2}H) was well tolerated. Two patients experienced drug-related fever, disturbing the treatment schedule for one of them. Moderate nausea was reported. One objective response was obtained. The bioavailability of zilascorb(^{2}H) tablets was satisfactory. The principle of oral administration of zilascorb(^{2}H) is feasible for long-term treatment and the side effects are acceptable. The mechanisms of action and the very low toxicity of the drug makes it a candidate for combination with other anticancer agents.

Key words: Bioavailability, clinical trial, oral administration, pharmacokinetics, protein synthesis inhibitors, zilascorb(^{2}H).

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Introduction

In 1940 Boyland screened 30 different aldehydes and their related compounds for antitumor potential. He found that several of them had significant activity against spontaneous mammary carcinomas and sarcomas in mice.[1] Later, benzaldehyde isolated from fig fruit (*Ficus carica* L), and its derivatives benzylidene glucose and sodium benzylidene ascorbate showed antitumor activity *in vitro* against NHIK 3025 human cervical carcinoma cells[2–5] and *in vivo* against the murine tumors AC 755, Ehrlich carcinoma and hepatocellular carcinoma.[4,6–8]

In patients with advanced head-and-neck, lung, breast and gastrointestinal carcinoma treated with benzaldehyde,[9] benzylidene glucose[10] or sodium benzylidene ascorbate, Kochi *et al.* reported overall objective response rates of more than 50%.[11] Some of the responses lasted up to 27 months[10] with no toxicity observed with any of the derivatives. In a later study; however, Tanum *et al.* reported no responders among 14 patients with metastatic colorectal cancer treated daily with benzylidene glucose for 8 weeks.[12]

The substances benzaldehyde, benzylidene glucose and sodium benzylidene ascorbate are regarded as reversible protein synthesis inhibitors, i.e. the protein synthesis quickly resumes to normal levels after removal of the drug.[2–5,12] In attempts to increase antitumor activity, deuterization of the formyl group of different aldehydes was performed by Pettersen *et al.*[13,14] One of the products, 5,6-benzylidene-d_1-L-ascorbic acid [zilascorb(^{2}H)] (Figure 1), proved to be more effective than benzaldehyde, benzylidene glucose and sodium benzylidene ascorbate with respect to cell inactivation and protein

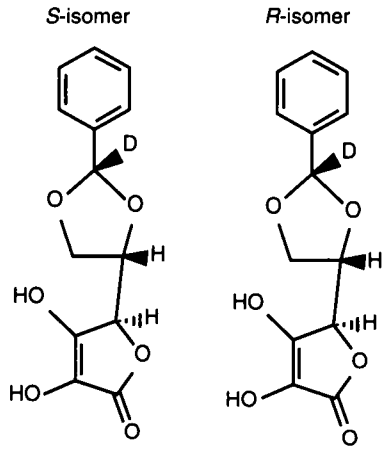

Figure 1. The chemical structure of *S*- and *R*-isomers of 5,6-benzylidene-d_1-L-ascorbic acid, i.e. zilascorb(^{2}H).

synthesis inhibition.[15] The growth inhibitory effect became manifest after 4 days when the drug was administered by daily i.v. injections in nude mice carrying xenografts of human malignant melanoma (EE) or ovarian carcinoma (OVCAR-3).[16] Extensive tumor necrosis was seen in sections of most of the xenografts, even when no reduction in tumor size was present. Normal growth rate was regained within approximately 1 day after the treatment was stopped.

The initial half-life of the related compound benzylidene glucose is of the order of less than 1 h.[6,13,17] In previous clinical trials, patients were treated with zilascorb(^{2}H) i.v. infusions twice daily for several weeks, which was a very uncomfortable regimen.[18,19] The drug caused fever and unacceptable fatigue in 25% of the patients, but had otherwise low toxicity. An oral formulation resulting in a long terminal half-life was warranted. In the present study, the pharmacokinetic parameters, toxicity and antitumor activity of zilascorb(^{2}H) 500 mg tablets was evaluated.

Patients and methods

Patients

Males and females 18–75 years of age with a WHO performance status of 0–2 entered the study. Eligibility criteria included written informed consent, histologically confirmed diagnosis of malignancy, life expectancy >3 months, serum creatinine <125 μmol/l, serum bilirubin <30 μmol/l, serum ASAT/ALAT <80 U/l, serum albumin >30 g/l, total serum protein >60 g/l, WBC $>4000/mm^3$, thrombocytes $>100\,000/mm^3$ and hemoglobin >11.0 g/dl. Patients with metastases to the central nervous system, history of major cardiovascular disease or previous malignancy were excluded. Prior chemotherapy was accepted provided a treatment-free interval of more than 4 weeks, (for nitrosoureas, mitomycin C or extensive radiotherapy, more than 6 weeks). At every visit, weight, performance status, blood pressure, heart rate and body temperature were measured, and hematology and serum chemistry tests were performed. Before the start of treatment and in the case of fever, chest X-ray, ECG and blood analyses of IgE, C-reactive protein (CRP), cytokines and differential leukocyte count were performed.

The trial was approved by the official Ethics Comittee and The Norwegian Medicines Control Authority and was conducted according to The Declaration of Helsinki.

Drug administration

The drug was supplied by Pronova (Oslo, Norway) as a freeze-dried substance (700 mg/vial) for i.v. infusion and as 500 mg tablets for oral administration.

All patients received a fixed dose based on data from previous phase I i.v. studies[18,19] and bioavailability data from oral administration of zilascorb(^{2}H) to dogs (unpublished data, Pronova). During the first 5 days, zilascorb(^{2}H) was infused rapidly as 2.8 g q.d. Blood sampling for pharmacokinetic studies was performed during 48 h starting on day 5. From day 7 zilascorb(^{2}H) tablets 3.0 g b.i.d. were administered during a 3 months total treatment time, leaving out three doses during 48 h blood sampling from day 20.

Attempts were made to collect urine during the blood sampling. Due to low patient compliance, the data are incomplete and will not be presented in full.

During the study, benzaldehyde analyses (see HPLC analyses) were performed when all sampling procedures in three patients had been completed. A dose change was considered if analyses suggested bioavailability to be less than 30% or greater than 70%.

Toxicity was graded due to WHO criteria and treatment response was evaluated by WHO criteria after 3 months.

Blood sampling

Blood was collected in heparinized (150 IU Li-heparine) glass tubes (Terumo, Leuven, Belgium) and immediately centrifuged (4000 *g*, 10 min). Plasma was stored in 3.6 ml Cryotubes (Nunc, Intermed, Denmark) at −20°C until analysis. On days 5 and 20, i.e. before pharmacokinetic blood sampling, zilascorb(^{2}H) was administered after overnight fasting and no food was allowed the first hour after drug intake.

Blood samples (10 ml) were obtained prior to the infusion on days 1–4. On day 5 samples were drawn at 0, 5, 20, 40 and 60 min, and 2, 4, 8, 12, 24, 36 and 48 h after the end of infusion. The first zilascorb(^{2}H) *tablet* dose was administered immediately after completion of blood sampling. On day 20, samples were drawn prior to and 20, 40, 60, 80 and 100 min and 2, 2.5, 3, 4, 6, 8, 12, 24, 36 and 48 h after the oral morning dose. The next zilascorb(^{2}H) tablet dose was administered immediately after completion of blood sampling.

HPLC analyses

Lacking a direct method for plasma zilascorb(^{2}H) measurement, zilascorb(^{2}H) and benzaldehyde containing metabolites were hydrolyzed to benzaldehyde on a 1:1 molar ratio basis before analysis. The following scheme was used: 200 μl plasma was mixed with 100 μl 7.6 M HCl (Merck, Darmstadt, Germany) and 200 μl 3.3 M NH_4OH (Merck). Then 500 μl HPLC-grade methanol (Rathburn, Walkerburn, UK) was added, the samples vortex mixed, stored at −20°C overnight for protein precipitation and centrifuged (15 000 *g*, 15 min) the next day. Plasma samples were filtered through 0.45 μm HPLC filters (Millipore) and volumes of 20 μl were injected by a Waters 715 or 717 Ultra WISP sample processor on a Supelcosil^{TM} LC-18 DB 4.6 × 250 mm (5 μm) column protected with a 2 cm Pelliguard^{TM} column (Supelco, Bellefonte, USA). The mobile phase consisted of 0.01 M phosphate buffer (pH 7) (Riedel-de Haën, Seelze, Germany) mixed 1:1 with HPLC grade methanol at a flow rate of 1.5 ml/min and was delivered by a Waters 600E system controller. Detection was performed by a Waters 468 or 490 detector. Benzaldehyde was detected at 250 nm. Data management and storage of chromatograms were performed by a Waters Expert Ease program. Zilascorb(^{2}H) from Norsk Hydro Research Centre (Porsgrunn, Norway) was used as a standard substance for calibration.

Pharmacokinetic calculations

The bioavailability (F) of zilascorb(^{2}H) was determined by comparing the area under the plasma curve (AUC) within the dose intervals after i.v. infusion and oral administration at steady-state; $F = AUC_{oral}/AUC_{i.v.}$. F can also be determined from cumulative urine data;

$$F = [(Ae_{\tau ss})B/(Ae_{\tau ss})A][(\text{dose}/\pi)A/(\text{dose}/\pi)B]$$

where A is the i.v. dose, B is the oral dose, π is the dosing interval and Ae_{ss} is the drug excreted unchanged during a dosing interval at steady-state. The elimination half-life ($t_{1/2}$) of zilascorb(^{2}H) was determined by the slope of the *log* plasma elimination curve. The elimination rate constant (k_{el}) was calculated from the equation $k_{el} = \ln 2/t_{1/2}$. The absorption rate constant (k_a) was determined from plasma concentration data by the method of residuals. The total clearance of zilascorb(^{2}H) (Cl_{total}) was determined from the following relationship; $Cl_{total} = \text{dose} \times F/AUC_{\tau ss}$ and renal clearance (Cl_{renal}) from $Cl_{renal} = Ae_{\tau ss}/AUC_{\tau ss}$. The volume of distribution (V_d) was estimated from the equation $V_d = Cl_{total}/k_{el}$. Concerning single dose i.v. administration, calculations were made from the equations above replacing $AUC_{\tau ss}$ with $AUC_{0-\infty}$ and $Ae_{\tau ss}$ with $Ae_{0-\infty}$. $AUC_{0-\infty}$ was estimated by extrapolation of the plasma curve while $Ae_{0-\infty}$ corresponds to the total amount of zilascorb(^{2}H) excreted unchanged in the urine after a single i.v. dose. All pharmacokinetic calculations were performed by software SIPHAR/WIN (SIMED, France).

Results

Patient characteristics are presented in Table 1. Thirteen patients entered the study. They suffered from a variety of malignancies and were beyond the scope of cure with established therapy. All patients had previously received antitumor treatment.

Data from six of the patients are incomplete due to adverse events and interference of the analyses by concomitant drugs. Seven patients completed at least 3 months of treatment and were evaluable for assessment of tumor response.

In addition, one patient had achieved stable disease through treatment with zilascorb(^{2}H) *capsules* for 4 months in an unpublished phase I. The size of the capsules precluded their further clinical use. Due to her improving performance status she was offered further treatment with zilascorb(^{2}H) *tablets*. No pharmacokinetic blood sampling was

Table 1. Patient characteristics

Patient characteristics	Number
Number of patients	13
Males/females	8/5
Age (years)	
median	47
range	34–73
ECOG status	
0	2
1	9
2	2
Previous therapy	
surgery	12
chemotherapy	10
radiotherapy	3
Primary tumors	
colorectal carcinoma	7
lung carcinoma	3
prostate carcinoma	1
bladder carcinoma	1
malignant melanoma	1

done from this patient, but she was evaluable for toxicity and for antitumor activity.

Pharmacokinetics

Pharmacokinetic results are presented in Table 2. The plasma curves had a peak corresponding to a t_{max} of approximately 1 h, indicating rapid absorption. The initial half-life was short, suggesting a rapid distribution of the drug from the central to the second compartment. The terminal half-life was satisfactorily long. Looking closer at the individual data, $t_{1/2\beta}$ of one patient is an obvious outlier (1648 h), partly explaining the great variability.

The maximal concentration of benzaldehyde achieved during steady-state, C_{maxss}, was one-fourth of the value seen after i.v. administration (data not shown). The oral formulation has a high volume of distribution, which is in agreement with the magnitude of the terminal half-life and total clearance. The bioavailability of zilascorb(^{2}H) 500 mg tablets was 32%, which was slightly lower than what was seen in our previously mentioned capsule study.

Due to the low toxicity of the drug, the dose was experimentally increased by 50 or 100% in four patients after completion of the blood sampling. Two patients received zilascorb(^{2}H) 4.5 g b.i.d. for 1 and 2 months, respectively. The plasma steady-state level of benzaldehyde was thereby elevated by 30%. Two patients received 6.0 g b.i.d., *both* for 2 months, increasing the plasma steady-state level of benzaldehyde by 70% (data not shown).

Pharmacokinetic data from a patient in our capsule study suggested that *ranitidine* enhanced the bioavailability of oral zilascorb(^{2}H), possibly through decreasing hydrolysis of zilascorb(^{2}H) due to increased gastric pH. For that reason, one patient received zilascorb(^{2}H) tablets 4.5 g b.i.d. and two received 6.0 g b.i.d., both in combination with ranitidine 150 mg b.i.d. The steady-state plasma level of benzaldehyde increased by approximately 40% when ranitidine was added. One patient experienced transitory fever episodes when receiving 6.0 g b.i.d., otherwise the elevated doses were well tolerated. However, for obvious practical reasons a daily intake of 24 tablets is not desirable.

Toxicity

No major hematological, nephro- or neurotoxicity was observed in any of the patients. No significant elevations of the cytokines tumor necrosis factor (TNF)-β, interferon (IFN)-γ, interleukin (IL)-1, IL-2 and IL-6 or IgE were seen during fever episodes. Mean performance status and body weight were stable or decreased during treatment for all patients.

Two patients experienced body temperatures of greater than 38°C, possibly related to the oral zilascorb(^{2}H) treatment. One patient had a body temperature of up to 39.4°C and fatigue from day 11. CRP increased from 25 mg/dl pretreatment to 188 mg/dl, otherwise there were no signs or symptoms of an ongoing infection. Zilascorb(^{2}H) treatment was stopped for 3 days and naproxen 1.0 g q.d. introduced before drug re-exposure. No further fever episodes were seen and naproxen was withdrawn after 3 weeks.

Another patient experienced several episodes with asthenia and a body temperature of more than 40°C. Neither paracetamol nor prednisone 40 mg q.d. prevented fever occurring after each zilascorb(^{2}H) intake. Dose titration over 1 month (500 mg q.d. to 6.0 g b.i.d.) with prednisone 10 mg q.d. was successful, i.e. the patient experienced a few fever episodes resolving within 12 h. A concomitant slight increase of s-urea (9.3 mmol/l) and non-fasting s-glucose (9.8 mmol/l) normalized during further treatment.

One patient had two outbursts of an itching exanthema localized to the cubital fossa, evaluated by a dermatologist to be a drug exanthema. Liver enzymes levels were elevated (ASAT = 124 U/l,

Table 2. Pharmacokinetic parameters derived from plasma level of benzaldehyde and urine level of zilascorb(^{2}H) after administration of zilascorb(^{2}H) as two different oral formulations

Zilascorb(^{2}H) formulation and dose	F_{pl}[a] (%)	F_u[a] (%)	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	C_{maxss} (mM)	t_{max} (h)	C_{ss} (mM)	V_{dss} (l)	Cl_{total} (l/h)	Cl_{renal} (l/h)
Tablets at 500 mg dose 3.0 g	32 ± 12 (n = 7)	34 ± 13 (n = 7)	1.35 ± 0.56 (n = 10)	299 ± 485 (n = 10)	0.14 ± 0.04 (n = 10)	1.03 ± 0.12 (n = 10)	0.03 ± 0.01 (n = 10)	—	—	4.8 ± 1.4 (n = 7)
IV_{ss} 2.8 g	—	—	0.94 ± 0.31 (n = 9)	64 ± 23 (n = 9)	0.87 ± 0.17 (n = 9)	—	0.02 ± 0.01 (n = 9)	622 ± 429 (n = 9)	6.2 ± 1.9 (n = 9)	4.8 ± 1.4 (n = 8)

Results are given as mean ± SD.

[a]Bioavailability of zilascorb(^{2}H) tablets is estimated by comparison to an i.v. dose of 2.8 g. F = bioavailability, pl = plasma, u = urine, $t_{1/2\alpha}$ = initial half-life, $t_{1/2\beta}$ = terminal half-life, C_{maxss} = maximum plasma concentration at steady state, t_{max} = time to reach C_{maxss}, C_{ss} = plasma concentration at steady-state, V_{dss} = volume of distribution at steady-state, Cl_{total} = total clearance, Cl_{renal} = renal clearance, n = number of patients, IV_{ss} = i.v. dose at steady-state, IV_{sd} = i.v. single dose.

ALAT = 100 U/l), but like the exanthema the values normalized within a few days on continuous treatment.

One patient received 2.8 g zilascorb(^{2}H) extravascularly due to erroneous position of the cannula. There was an immediate local reaction with increased temperature, redness, tenderness and swelling. All reactions subsided within 2 days, leaving no sequelae.

Antitumor activity

One patient with metastatic rectal carcinoma had stable disease of her lung metastases after treatment with zilascorb(^{2}H) *capsules* for 4 months. Due to improving general condition she was offered further treatment with the tablets. The tablet dose was gradually increased to 4.5 g b.i.d. and 6.0 g b.i.d. with no side effects. After a total treament time of 5 months, she experienced an objective partial response in her bilateral lung metastases, lasting for 15 months. This patient also had liver metastases, but as they were first interpreted as hemangiomas, their development was not followed accurately.

Another patient with metastatic rectal carcinoma achieved a 40% size reduction of his liver metastases after 2 months of treatment, lasting for 2 months. Because of fever reactions he only received three tablet doses at 3.0 g during the first month of treatment (see Toxicity for details).

Discussion

The reversible protein synthesis inhibitor zilascorb(^{2}H) represents a new type of compound in cancer therapy. With its remarkably low toxicity and gradual onset of antitumor effect, it differs from most drugs currently employed in the management of cancer patients. To overcome problems associated with long-term daily i.v. treatment, an oral tablet formulation of the drug was developed. Pharmacokinetic parameters and toxicity of zilascorb(^{2}H) tablets were investigated. The tablets were easy to administer, were quickly absorbed and slowly eliminated. Although incomplete, urine analyses suggest the drug to be mainly eliminated through the kidneys.

From animal studies it is known that LD_{50} of zilascorb(^{2}H) is approximately 2200 mg in mice (about 110 g/kg body weight) (internal report, Pronova). In this study it seemed difficult to reach an MTD without exceeding the number of tablets possible to administer orally. The study was not primarily designed to establish the MTD of the substance; however, when doses of zilascorb(^{2}H) tablets were increased by 100% it was well tolerated. Dose limitation of zilascorb(^{2}H) seems to be connected to formulation rather than toxicity. Animal data suggest the antitumor effect of zilascorb(^{2}H) to be dose independent at doses above 5 mg/kg, as neither 20, 40 nor 113 mg/kg induced any stronger anti-tumor effect than 5 mg/kg.[16] This suggests that the most effective dose of zilascorb(^{2}H) possibly is reached at a lower dose than its MTD. Similar observations were done in animal studies with the closely related deuterated benzaldehyde derivative benzylidene-d_1-glucose (P-1013).[20] When P-1013 was given orally daily for several weeks to nude mice with s.c. tumors, it was shown that tumor growth was better suppressed with a dose of 90 than 200 mg/kg.

In our study only one patient had difficulties in completing zilascorb(^{2}H) tablet treatment due to fever and fatigue. In the initial studies, zilascorb(^{2}H) infused in doses ranging from 3 to 45 mg/kg/day caused fever and fatigue in one-fourth of the patients,[18,19] even at the lowest dose. It was speculated whether endogenous cytokines could be responsible for the fever reactions, as zilascorb(^{2}H) *in vitro* enhanced the serum level of IFN-γ by 200% and TNF by 10–30% (unpublished results). No systematic elevations of cytokine levels were seen in our study (data not shown).

The mechanism behind the effect of zilascorb(^{2}H) is basically unknown. *In vitro* studies suggest the drug to act as a protein synthesis inhibitor,[2,3,14,15] and generation of extracellular free radicals to be involved in cytotoxicity and inhibition of protein synthesis.[21,22] In contrast to zilascorb(^{2}H), several other protein synthesis inhibitors are highly toxic in clinical settings. The plant protein ricin caused dose-dependent fatigue and muscular pain in a phase I study.[23] In another study, giroline caused severe hypotension, asthenia, nausea and vomiting in most patients, precluding further clinical evaluation of the drug.[24]

By serendipity, we discovered that the H_2-blocker ranitidine may enhance the bioavailability of zilascorb(^{2}H) given as tablets. This effect may be exploited, as zilascorb(^{2}H) has low toxicity and it is warranted to reduce the number of tablets required daily for an effective dose. It is known that H_2-blockers cause interactions with several drugs through changes of intragastric pH or inhibition of gastric emptying.[25,26]

Through this study, the novel principle of long-term oral administration of an anticancer drug was

shown to be tolerable and realistic. Fever was observed less frequently during zilascorb(^{2}H) tablet treatment than during infusions. Zilascorb(^{2}H) tablets showed a satisfactory bioavailability and a beneficial toxicity profile.

The tolerability of the drug makes it interesting for combination with other anticancer drugs with different mechanisms of action. It has been shown *in vitro* that zilascorb(^{2}H) has the ability to increase the cytotoxic effect of cisplatin on human NHIK 3025 cells.[27] Also the protein synthesis inhibitors sparsomycin[28] and L-histidinol[29] have shown ability to augment the activity of cisplatin on murine tumors (L1210 leukemia and B16 melanoma, respectively). Further work to establish the mechanisms underlying zilascorb(^{2}H)'s antitumor effect is warranted and the potential of drug combinations with zilascorb(^{2}H) in the clinic should be exploited.

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