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Research paper

Zilascorb(^{2}H), a low-toxicity protein synthesis inhibitor that exhibits signs of anticancer activity in malignant melanoma

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Zilascorb(^{2}H) is a benzaldehyde derivative giving rise to strong protein synthesis inhibition. It has shown antitumor activity against human malignant melanoma grown as xenografts in nude mice. The effect was manifest only after prolonged daily treatment and was quickly reversible when treatment was stopped. Drug-induced fever was the dose-limiting toxicity observed during clinical phase I studies of zilascorb(^{2}H). The object of the present study was to assess antitumor activity, safety and tolerability of the drug in melanoma patients. Sixteen patients with disseminated malignant melanoma were included, all presenting with WHO performance status 0–2 and adequate organ functions. Previous chemo- or radiotherapy was accepted, while patients with known CNS metastases were excluded. Due to its low solubility and quickly reversible activity, zilascorb(^{2}H) 1400 mg was infused by the patients twice daily through a venous access port for up to 12 weeks. Induction of tumor regression was demonstrated in one patient, who was, however, withdrawn from treatment after 2 weeks because of recurrent fever and fatigue. All the 12 patients evaluable for antitumor activity had progressive disease. Zilascorb(^{2}H) was well tolerated, except for fever reactions and reversible liver toxicity. Most patients learned quickly how to handle a venous access port, but daily self-administration of i.v. infusions became too cumbersome to justify further patient inclusion despite the tumor regression observed. We conclude that zilascorb(^{2}H) seems to have the potential for antitumor activity in metastatic malignant melanoma and is well tolerated. Daily self-administration of drug infusions is not desirable for long periods and zilascorb(^{2}H) tablets have been developed. Because of its favorable toxicity profile, especially compared to other protein synthesis inhibitors, zilascorb(^{2}H) may be particularly interesting for combinations with other anticancer drugs. [© 1998 Lippincott Williams & Wilkins.]

Key words: Clinical trial, malignant melanoma, protein synthesis inhibitor, zilascorb(^{2}H).

Study drug was supplied free of charge from Pronova AS.

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Introduction

More than 50 years ago Boyland screened different aldehydes for anticancer activity, concluding that several of them had significant *in vivo* efficacy against solid murine tumors.[1] Benzaldehyde (BA), which is isolated from fig fruit (*Ficus carica* L), and its derivatives benzylidene glucose (BG) and sodium benzylidene ascorbate (SBA) have later shown *in vitro* cytotoxicity in human cervical carcinoma cells (NHIK 3025),[2-4] and antitumor activity against a variety of murine tumors.[5-8] Early clinical trials of aldehyde derivatives have been characterized by more than 50% objective response rates achieved in large groups of patients with advanced carcinomas,[9-11] except for one report of lacking antitumor response in colorectal cancer patients treated with BG.[12] Remarkably, no acute toxicity was observed in patients or animals in any of the referred studies.[2-4,6,12-14]

The above substances all give rise to strong *in vitro* protein synthesis inhibition,[2-4,14] which is enhanced by the exchange of one hydrogen atom with deuterium.[14-18] Furthermore, SBA is capable of generating extracellular free radicals through ascorbate oxidation and it induces apoptosis *in vitro*.[19-21] Hence, the cytotoxicity of the BA derivatives can probably be explained by more than one mechanism.

Zilascorb(^{2}H) (5,6-benzylidene-d_1-L-ascorbic acid) (Figure 1) is the deuterated derivative of SBA. Its protein synthesis inhibitory activity is reversible, as surviving cells retain their previous protein synthesis rate and morphological appearance within 1 h after drug removal.[14] The substance has shown antitumor efficacy against human tumor xenografts of melanoma (EE) and ovarian carcinoma (OVCAR-3 and SKOV) carried in nude mice.[14] Growth inhibition could be

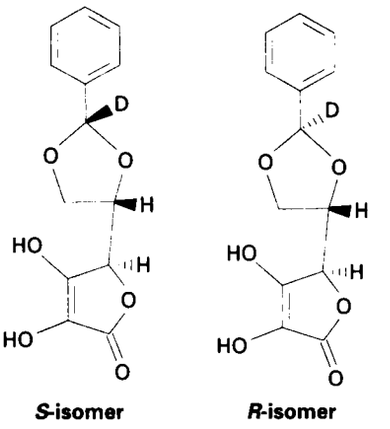

Figure 1. Chemical structure of zilascorb(^{2}H) (5,6-benzylidene-d_1-L-ascorbic acid).

detected after 4 days of zilascorb(^{2}H) injections[14] and normal growth rate was regained shortly after treatment cessation.[13] Drug-induced fever was the dose-limiting toxicity reported from initial clinical trials of zilascorb(^{2}H), but otherwise the agent was well tolerated.[22,23]

The purpose of the present study was to examine the antitumor activity and toxicity of zilascorb(^{2}H) in patients with disseminated malignant melanoma. Since BA and its derivatives induce cell death only following prolonged treatment,[2-4] the drug was administered daily over a period of 3 months. The recommended dose from phase I dose-escalating studies was 40 mg/kg/day.[22,23] In order to simplify self-administration of treatment in the patient's home, we used a fixed dose of zilascorb(^{2}H) 1400 mg twice daily in this study.

Patients and methods

Patients

Males and females aged 18–75 years with WHO performance status 0–2 entered the study. Eligibility criteria included written informed consent, histologically confirmed diagnosis of metastatic malignant melanoma, measurable or evaluable disease, life expectancy > 3 months, serum creatinine < 125 μmol/l, serum bilirubin < 30 μmol/l, serum ASAT/ALAT < 80 U/l, serum albumin > 25 g/l, total serum protein > 50 g/l, WBC < 3000 mm^3, thrombocytes > 75 000mm^3 and hemoglobin > 9.0 g/dl. Patients with CNS metastases, history of major cardiovascular disease or previous malignancy were excluded, as well as pregnant or lactating women. Prior chemo- or radiotherapy was accepted, provided a treatment-free interval of 4 weeks (6 weeks for nitrosoureas or mitomycin C or extensive radiotherapy).

Number of patients

Based on Gehan's rule,[24] evaluation of 14 patients was planned before rejecting zilascorb(^{2}H) as ineffective, i.e. not likely to provide a 20% response rate with 5% rejection error. In case of one or more objective responders among the 14, an additional 11 patients would be included to estimate zilascorb(^{2}H)'s true response rate.

Drug administration

Zilascorb(^{2}H) was supplied as a freeze-dried substance (350 mg/vial) from Pronova (Oslo, Norway). Each drug dose was reconstituted in 100 ml normal saline solution. Zilascorb(^{2}H) was infused during 30 min through a venous access port that was implanted s.c. on the patient's chest wall prior to treatment start. The patient or a relative was trained in the technique of i.v. infusion before discharge from hospital.

Treatment schedule

All patients infused zilascorb(^{2}H) 1400 mg twice daily for up to 3 months. Out-patient visits were arranged after 2, 4, 8 and 12 weeks of treatment, during which physical examination as well as hematological and serum chemistry tests were performed. Before start of treatment and in case of fever, chest X-ray was carried out, and plasma levels of IgE, C-reactive protein (CRP), and the cytokines IL-6 and IFN-γ were measured. Tumor response was evaluated according to WHO criteria after 3 months of zilascorb(^{2}H) treatment or at withdrawal from study. Patients receiving at least 4 weeks of therapy were considered evaluable for antitumor activity.

The trial was approved by the official Ethics Committee and The Norwegian Medicines Control Authority, and conducted according to The Declaration of Helsinki. Serious and unexpected adverse events were reported according to the Norwegian Regulations and European Guidelines for Good Clinical Practice.

Results

Patients characteristics

These are presented in Table 1. Sixteen patients were included and 12 (eight males and four females) were evaluable for antitumor activity. Five patients had previously received chemotherapy, i.e. DTIC (n=2), DTIC and eldesine (n=1), DTIC and fotemustine (n=1), and fotemustine, cisplatin and tamoxifen (n=1). One patient had received palliative radiotherapy for painful bone metastases.

Four patients were withdrawn from the study within 4 weeks of zilascorb(^{2}H) treatment; two of them due to recurrent drug induced fever and fatigue, one due to rapidly progressing disease, and another for the reason of hyperglycemia and drug fever.

Antitumor activity

One previously untreated patient achieved a rapid partial response in axillary lymph node metastases on day 35, although the zilascorb(^{2}H) treatment had been withdrawn from day 18 due to recurrent drug fever (see 'Toxicity' for details). The antitumor activity was not formally verified 4 weeks later as required, since further treatment with DTIC was instituted shortly after. The axillary metastases continued to regress during DTIC therapy and a residual tumor was eventually resected. The patient died due to disseminated disease 55 months after withdrawal from zilascorb(^{2}H) treatment.

The study was closed following evaluation of 12 patients, as the necessary self-administration of i.v. infusions twice daily was undesirable. Most patients handled the venous access port correctly and neither infections nor extravasal drug infusions were reported. Anyhow, the majority of patients reported the procedure to be too cumbersome to perform at home for such a long time. We considered that further patient inclusion could not be justified despite the observation of one objective partial response.

Table 1. Patient characteristics

No. of patients	16
Sex	
male	11
female	5
Age (years)	
median	57
range	35–70
Performance status (ECOG)	
0	11
1	5
Previous therapy	
surgery	16
chemotherapy	5
radiotherapy	1
Primary site of tumor	
cutaneous	12
ocular	2
unknown	2
Distribution of metastatic sites (no. of patients)	
lung	9
lymph nodes	12
liver	7
skin	2
pancreas	1

Toxicity

All patients were considered evaluable for toxicity and the main side effects observed during zilascorb(^{2}H) treatment are listed in Table 2. Only one patient experienced nausea and vomiting (WHO grade II). Nine patients experienced fever of WHO grade II (38–40.0°C) and one of WHO grade III (>40.0°C), mostly accompanied with fatigue. Three patients were eventually withdrawn solely for this reason, as neither dose reductions nor temporary treatment discontinuation prevented recurrent fever reactions. Paracetamol 1–2 g/day was administered before reintroduction of zilascorb(^{2}H) in three patients, but fever reoccurred in two of them. However, in another five patients paracetamol and/or naproxen were administered as analgetics, and it is remarkable that none of these patients experienced fever.

The patient with the rapid tumor regression experienced fever (WHO grade II) and fatigue after each dose of zilascorb(^{2}H) from day 14. Concomitant

Table 2. Maximal grade of toxicity observed in 16 melanoma patients during zilascorb(^{2}H) treatment

Toxicity	WHO grade				
	0	I	II	III	IV
Fever	6	0	9	1	0
Nausea/vomiting	15	0	1	0	0
ASAT/ALAT	9	3	3	1	0
LDH	10	4	0	2	0
ALP	11	4	1	0	0

ASAT, aspartate amino-transferase; ALAT, alanine amino-transferase; LDH, lactate dehydrogenase; ALP, alkaline phosphatase.

increases in the infection parameters CRP (70–136 U/l) and IL-6 (45 pg/ml) prompted administration of antibiotics, but the fever still reoccurred after each zilascorb(^{2}H) dose even following dose reductions of 25 and 50%. Blood cultures were negative and bacterial contamination of the venous access port was ruled out. Body temperature normalized within 24 h following the final discontinuation of zilascorb(^{2}H) on treatment day 18 strongly indicating the fever reaction to be drug related. Serum analyses revealed an elevation of IFN-γ from 0 pg/ml pretreatment to 822 pg/ml during a fever episode in this particular patient, but there was otherwise no overall correlation between fever reactions and levels of CRP, IgE, IL-6 or IFN-γ.

Zilascorb(^{2}H) induced moderate hepatic toxicity (Table 2). All patients with hepatic toxicity of WHO grade III had liver metastases. In three patients, hepatic toxicity of WHO grade II–III was temporarily observed during fever episodes. Another patient experienced a decline in albumin and total protein, with concomitant elevations of transaminases, alkaline phosphatase and lactate dehydrogenase (WHO grade I–III).

An itching but quickly resolving exanthema was reported by two patients. One patient presented a similar exanthema with concomitant fever, hyperglycemia (fasting p-glucose 10.8 mmol/l), and elevations of transaminases, alkaline phosphatase and lactate dehydrogenase (WHO grade I–II). Upon retreatment with zilascorb(^{2}H) 350 mg/day all biochemical tests and symptoms normalized except for hyperglycemia, for which reason the patient was eventually withdrawn from the study.

Discussion

We report our experiences with the benzaldehyde derivative zilascorb(^{2}H) in 16 patients with disseminated malignant melanoma. The compound is a reversible protein synthesis inhibitor that induces antitumor activity after prolonged daily treatment.[2–4] One of the 12 evaluable patients achieved a rapid tumor regression and, although not a formal objective response, inclusion of additional patients up to 25 was planned. The patients learned impressingly fast how to administer their daily zilascorb(^{2}H) infusions at home through a venous access port, without experience of infections or extravasal drug injections. Nevertheless, the administration principle was not feasible for a treatment duration of several months, the reason for which we ultimately decided to close the study.

Zilascorb(^{2}H) has shown antitumor efficacy against human melanoma xenografts in nude mice;[14] however, anticancer activity in animals does not always predict clinical efficacy accurately. It could be argued that disseminated melanoma, which is quite often a rapidly progressing disease, is not the optimal tumor type for testing antitumor activity of a slow-acting agent. This was furthermore illustrated by the fact that 10 of the 12 evaluable patients progressed before completion of the scheduled 12 weeks of zilascorb(^{2}H) therapy, in spite of good pretreatment performance status (Table 1).

Drug fever was the toxicity that most frequently caused drug withdrawal in the present study, in accordance with reports from previous clinical trials of zilascorb(^{2}H).[22,23] The known ability of zilascorb(^{2}H) to enhance levels of IFN-γ and tumor necrosis factor from stimulated human lymphocytes suggests a connection between cytokine secretion, temperature elevations and antitumor activity (Pronova, unpublished results). In this study, the administration of antipyretics to three patients before zilascorb(^{2}H) reintroduction prevented fever in only one. However, five patients receiving paracetamol and/or naproxen as analgetics did *not* experience fever, and it cannot be excluded that the drugs prevented an elevation of body temperature in these patients.

In addition, zilascorb(^{2}H) caused moderate liver toxicity. This study is too small to draw firm conclusions, but it can be speculated whether this toxicity was somehow connected to protein synthesis inhibition.

One problem arising with the BA derivatives is their low solubility. The large volume of each zilascorb(^{2}H) dose prevents use of portable drug pumps for long-term treatment. To simplify drug administration, zilascorb(^{2}H) *tablets* were recently formulated, and a phase I clinical and bioavailability study has been performed.[25] The tablets were quickly absorbed and slowly eliminated, their bioavailability was satisfactory, and the drug well tolerated. Oral administration of zilascorb(^{2}H) was feasible for long-term treatment and one patient with metastatic colorectal carcinoma obtained an objective response lasting for 15 months.[25]

Compared to other protein synthesis inhibitors, zilascorb(^{2}H) and similar compounds are principally attractive anticancer drugs due to their favorable clinical toxicity profile. The plant protein ricin, also a potent protein synthesis inhibitor, caused fatigue and muscular pain,[26] and the marine compound giroline induced hypotension, shock and severe asthenia, precluding its further clinical evaluation.[27] The BA derivatives should be suitable for combina-

tion with other anticancer drugs. Preclinical experiments have demonstrated that the protein synthesis inhibitors zilascorb(^{2}H), sparsomycin, histidinol and anguidine are able to potentiate the cytotoxicity of several anticancer drugs, among them cisplatin.[28-35] The mechanism of synergism between protein synthesis inibitors and cisplatin is not fully understood, but it has been speculated that the synthesis of intracellular cisplatin antagonists or DNA-repairing enzymes is inhibited.[28,31]

In conclusion, zilascorb(^{2}H) seems to have ability to induce tumor regression in disseminated malignant melanoma. In addition it has unusually low toxicity compared to other antineoplastic agents including several protein synthesis inhibitors and the agent may be suitable for combinations with other anticancer drugs. The present trial was prematurely closed due to practical obstacles with long-lasting self-administration of i.v. infusions; however, zilascorb(^{2}H) *tablets* have been developed and proved to simplify drug administration.[25]

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