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Tolerance, safety, and kinetics of the new antineoplastic compound dexniguldipine-HCl after oral administration: a phase I dose-escalation trial

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Abstract Dexniguldipine-HCl is a new dihydropyridine compound that exerts selective antiproliferative activity in a variety of tumor models and, in addition, has a high potency in overcoming multidrug resistance. The purpose of this trial was to determine the toxicity and pharmacokinetics of dexniguldipine and to establish a recommended dose for phase II trials. A total of 37 patients with cancer were treated with oral dexniguldipine in increasing doses for up to 7 days. The main parameters evaluated were subjective tolerance and laboratory and cardiovascular parameters (blood pressure and ECG). Blood samples were drawn for analysis of the drug's pharmacokinetics. Dizziness and nausea were the major adverse events observed in seven patients, but episodes were generally mild and not clearly dose-related. Vomiting occurred in one patient. Hypotensive effects and orthostatic dysregulation were observed in some patients but were not considered to be dose-limiting. Therefore, no dose-limiting toxicity was found and the maximally tolerable dose could not be determined. Pharmacokinetic data showed wide interindividual variation and a dose-dependent increase in steady-state serum concentrations at doses of up to 1,000 mg daily, with no clear further increase being observed at higher doses. Consistently high concentrations were achieved with the 2,500-mg dose. Despite the lack of dose-limiting toxicity, higher doses of dexniguldipine do not appear to be useful for clinical evaluation because of the pharmacokinetic properties of the compound; therefore, 2,500 mg/day is recommended as the daily dose for phase II trials.

Key words Dexniguldipine-HCl · Phase I trial Pharmacokinetics

Introduction

Dexniguldipine-HCl (B8509-035) is the (−)-(R)-enantiomer of a dihydropyridine compound, of which the (+)-(S)-enantiomer shows pronounced cardiovascular activity due to its high affinity for the voltage-dependent Ca^{2+} channel [2]. By enantio-selective synthesis, the more than 99.5% pure (R)-enantiomer dexniguldipine-HCl can be produced. As compared with the (S)-enantiomer, the (R)-enantiomer has a 40-fold lower affinity for the Ca^{2+} channel and, accordingly, only minimal hypotensive activity in animal pharmacology models [9]. Both enantiomers have shown antiproliferative activity in several tumor cell lines, but the concentrations necessary to inhibit growth have varied considerably between cell lines.

In the breast-carcinoma cell line ZR-75-1 the dexniguldipine concentration causing a 50% reduction in cell growth (IC_{50}) was reported to be 0.3 μM [5]. In the neuroendocrine carcinoid cell line NCI-H727 a significant ($P < 0.01$) growth-inhibitory effect for dexniguldipine-treated cells as compared with untreated controls was reported at concentrations as low as 0.001 pM, with a relationship to dose being apparent at levels of < 1 pM. In the same assay, concentrations of 0.1–1.0 μM were necessary to demonstrate significant inhibition of cell proliferation in an adenocarcinoma cell line with features of alveolar type II cells (NCI-H358) [11]. In a series of human tumor xenografts in vitro, dexniguldipine demonstrated selective antiproliferative activity against several tumor types. e.g., melanoma and renal-cell carcinoma [4]. Striking

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results were obtained in a hamster model, in which neuroendocrine lung tumors could be completely eradicated by 20 weeks of oral treatment with 32.5 mg/kg dexniguldipine, whereas Clara-cell-type lung tumors were not affected [10].

On the other hand, dexniguldipine-HCl was found to overcome completely type 1 multidrug resistance (MDR). By overexpression of the *mdr* gene, tumor cells produce a 170-kDaT glycoprotein, the so-called P170 glycoprotein, which is incorporated into the cell membrane and serves as an energy-dependent efflux pump for various cytostatics, especially anthracyclines and vinca alkaloids. This results in low intracellular concentrations of cytostatics and, therefore, resistance of these tumor cells. Dexniguldipine has been shown to overcome MDR1 by binding to and blocking the P170 glycoprotein at concentrations of 0.1–1.0 μM in vitro [6–8]. It may be of special interest that the first metabolite (M1) of dexniguldipine, which is the respective pyridine, is active in in vitro assays such as efflux measurement of rhodamine 123 [7].

An MDR-reversing potency is known for several structurally unrelated compounds such as verapamil and cyclosporine, among others [1, 3]. However, the high potency of dexniguldipine (about 10-fold as compared with that of verapamil in vitro) and its low cardiovascular activity provide the opportunity to achieve blood or tumor concentrations that might be high enough to overcome Mdr 1 resistance in patients without producing dose-limiting cardiovascular effects.

Initial results of preclinical investigations for the evaluation of the mechanism of its antiproliferative activity demonstrate that dexniguldipine interferes with intracellular signal transduction by affecting phosphoinositol pathways, protein kinase C expression, and intracellular Ca^{2+} metabolism [5, 14]. From these investigations one could speculate that continuous treatment rather than a single administration would be required for clinical use.

The dexniguldipine dose producing lethality in 50% of the mice tested (acute LD_{50}) was > 300 mg/kg after oral treatment and 17–20 mg/kg after i.v. administration. After 4 weeks of oral administration to rats in various doses, toxic effects such as reduced growth, reduced potassium excretion, and increased calcium excretion were found virtually exclusively in the highest-dose group (40 mg/kg). A 4-weeks trial in dogs revealed vomiting, weight loss, and reduced food intake as dose-limiting toxicities at a dose of 20 mg/kg but no other abnormal finding. In addition, after oral doses of up to 20 mg/kg, no embryotoxic or teratogenic effect was seen in rats or rabbits. On the basis of the results of these 4-week trials, a 7-day administration period seemed justified.

Patients and methods:

The study protocol was approved by an independent ethics committee. Informed consent was obtained from all patients and the trial was performed according to the Declaration of Helsinki in its revised version (Tokyo, Venice, and Hong Kong) and to the rules of good clinical practice (GCP).

Eligible patients suffered from malignant disease and were not considered for standard antitumor treatment. The time from the last prior chemotherapy or irradiation had to be at least 4 weeks. Sufficient general condition (WHO performance status, 0–1) was required as well as sufficient function of liver and kidney (serum creatinine, < 1.8 mg/dl; bilirubin, < 1.5 mg/dl; values for SGOT, SGPT, AP, and γ-GT, $\leq 150\%$ of normal). Further inclusion criteria were a hemoglobin (Hb) value of > 10 g/dl, a leukocytes count of $> 4{,}000/mm^3$, a platelet count of $> 100{,}000/mm^3$, and normal values for Na^+, K^+, and Ca^{2+} as well as a normal ECG. Exclusion criteria were hypotension, severe myocaridal incompetence or treatment with β-adrenoceptor blockers, Ca^{2+} antagonists, and drugs with potential α-blocking side effects.

Dexniguldipine was given as soft gelatin capsules containing 20, 100, or 250 mg, respectively. Patients in the first two groups (20 and 40 mg) received a single dose; all other patients took dexniguldipine once daily in the morning before breakfast for 7 days. As is usual in phase 1 oncology trials, the dexniguldipine dose was to be increased in groups of at least 3–4 patients until the occurrence of dose-limiting side effects so as to define the maximum tolerated dose (MTD) [12]. Should one instance of dose-limiting toxicity (WHO grade III) occur, then the number of patients in the respective group would be doubled; the same applied to a decrease in resting blood pressure requiring fluid replacement or drug therapy. In case of grade IV toxicity, drug administration in this group would be stopped and the number of patients would be increased in the next lower dose group.

Although dexniguldipine is not a typical cytotoxic drug, toxicity was assessed according to WHO criteria. Special attention was paid to cardiovascular parameters. The blood pressure of patients in the supine position was measured daily before and at 2 and 6 h after drug intake. Ion addition, daily measurement was done after patients had arisen to an upright position and stood for 1 min, at approximately 2 h after drug intake, as a rough test for orthostatic dysregulation. ECG and laboratory parameters were done before the start of drug intake and at regular intervals. Serum concentrations of dexniguldipine and its major metabolite M1 (which is the respective pyridine) were measured by an automated nonchiral high-performance liquid chromatography (HPLC) method [16]. This method employs sample cleanup of 1 ml of plasma by liquid-solid extraction. By using two-dimensional HPLC, initial separation is achieved on a LiChrospher-60-RP-B column. A fraction of this eluate is then collected by solid-phase trapping and the final chromatogram is developed on a narrow-bore Hypersil-CPS column and quantified with UV detection at 230 nm. Linearity was proved in the range of 0.25–100 ng/ml. Figures for precision at these concentrations were 7.4% and 3.3%, and those for accuracy, 8.0% and 1.3%, respectively. For evaluation of pharmacokinetics data, blood was drawn at time points 0, 1, 2, 4, 8, and 24 h after drug intake on days 1 and 7. Adverse vents had to be recorded at any time.

Results

A total of 37 patients were included in the present study; the histological diagnosis included non-small-cell lung cancer ($n = 25$), small-cell lung cancer ($n = 2$), pleuramesothelioma ($n = 2$), renal-cell carcinoma ($n = 2$), adenocarcinomas of the colon ($n = 2$) and pancreas ($n = 1$), cervical carcinoma ($n = 1$), papillary carcinoma ($n = 1$), and carcinoma of unknown origin ($n = 1$). All patients completed drug intake according to the protocol. The doses given and the respective numbers of patients are given in Table 1.

Table 1 Number of patients treated in different dose groups

Group	Dose	Frequency	Number of patients
I	20 mg	Once	4
II	40 mg	Once	4
III	100 mg	7 days	4
IV	240 mg	7 days	4
V	500 mg	7 days	8
VI	1,000 mg	7 days	4
VII	1,500 mg	7 days	3
VIII	2,000 mg	7 days	3
IX	2,500 mg	7 days	3

Toxicity

Adverse events reported were dizziness ($n = 4$), nausea ($n = 3$), inappetence ($n = 2$), headache ($n = 1$), and vomiting ($n = 1$) in a total of 7 patients. Altogether, 30 patients reported no adverse effect at all. No organ or hematological toxicity was found and no clinically relevant change in laboratory parameters was seen in any patient (data not shown). Adverse effects encountered according to dose and WHO toxicity are given in Table 2.

A decrease in blood pressure over time was seen in several patients in various dose groups. However, apart from the observation of a decrease of more than 20 mmHg (systolic) in all patients in the highest-dose (2,500 mg) group (but none in the 2,000-mg group), no relationship between the dose and a decrease in resting blood pressure could be detected. After patients had arisen to an upright position and stood for 1 min, decreases in blood pressure were seen in several cases, reaching systolic values of 70 mmHg in one patient in group VI and two patients in group IX. This was partly combined with dizziness and nausea.

In one patient in group I (20 mg), sporadic ventricular extrasystoles were seen on ECG recordings obtained at 2 and 24 h after drug intake that had not been observed at the basic examination. In all other patients, ECGs were unchanged during and after the study period as compared with the tracings obtained before drug intake.

Pharmacokinetics

In Fig. 1 the mean profiles of serum concentration versus time are given for dexniguldipine and its major metabolite M1 as determined on days 1 and 7 in group V, i.e., after daily intake of 500 mg dexniguldipine. The data obtained for this group are shown because the letter contained the highest number of individuals ($n = 8$). Individual maximal serum concentrations on day 1 as well as on day 7 were found at 1–8 h following drug intake (mean t_{max}, 3.7 h). Mean concentration-time profiles were almost parallel on both study days.

Table 2 Adverse events possibly related to the study medication

Dose	Adverse event/toxicity	Number of patients	WHO grade
500 mg	Nausea	1	I
1,000 mg	Dizziness	1	I
	Nausea and headache	1	I
2,000 mg	Dizziness + nausea + inappetence + vomiting	1	II
2,500 mg	Inappetence	1	I
	Dizziness	2	I

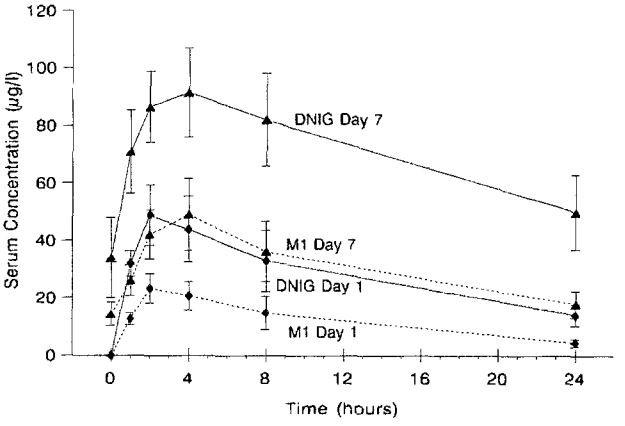

Fig. 1 S—erum concentration-time profiles obtained for dexniguldipine (*DNIG*, —) and its pyridine metabolite M1 (- - -) at days 1 and 7 following once-daily intake of 500 mg DNIG (mean values ± SEM, $n = 8$)

However, steady-state concentrations recorded on day 7 were higher by a factor of about 2. Serum concentration-time profiles obtained for the metabolite M1 showed shapes very similar to those obtained for the parent compound dexniguldipine, but at a lower level. Independent of the delivered dose or frequency of administration, the area under the concentration-time curve (AUC) for the pyridine metabolite accounted on average for almost 40% of the AUC for the parent compound and showed little variation.

For comparison of individual and group data, the average steady-state concentration [$C_{av} = AUC(0\text{–}24\ h)[/24]$] was calculated for each patient after 7 days of drug intake. These data as well as the midean value for each group are given in Fig. 2, showing that the average steady-state concentration is related to the dose in the range of 100–1500 mg, thereafter reaching a plateau.

Discussion

In preclinical investigations, dexniguldipine has been proven to exert two properties that appear to be worthwhile for clinical use in tumor-bearing patients: a selective antiproliferative efficacy in several tumor types (probably tumors depending on neuroendocrine-stimulating

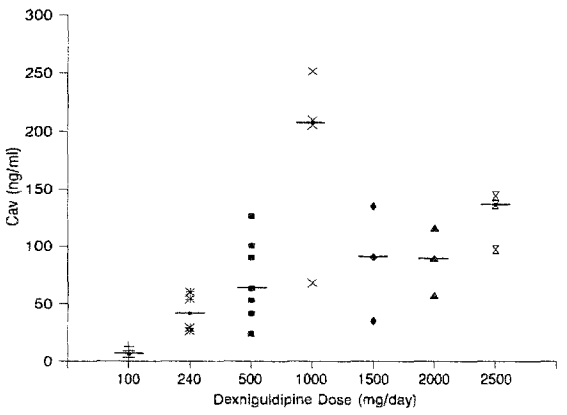

Fig. 2 Average steady-state concentration (C_{av}, ng/ml) measured after 7 days of drug intake; individual and median values for groups receiving doses ranging from 100 to 2,500 mg/day are given

factors) and overcoming of P170-mediated mutlidrug resistance (MDR1).

During the design of this phase I trial, several factors had to be considered:

1. A recommended dose of clinical phase II trials had to be established and, if possible, the MTD should be determined.
2. Pharmacokinetic data on the oral administration of dexniguldipine were to be established and, therefore, a once-daily administration seemed appropriate, especially as animal pharmacokinetic data showed a rather long terminal half-life ($t_{1/2}$, e.g., 15 h after oral administration in the dog).
3. Because dexniguldipine is not a typical cytostatic agent, dose-limiting effects were expected not from bone marrow or organ toxicity but rather from cardiovascular activity, especially lowering of blood pressure or disturbances in atrioventricular conductance. In addition, nausea and vomiting were anticipated according to the results of animal experiments.
4. According to preclinical results and with respect to the proposed mode of action of the compound, single administration or repeated administration (cycles) such as that most common used for cytostatic agents do not seem to be appropriate for the use of dexniguldipine as an antiproliferative drug, but continuous long-term treatment will be desirable. As based on the available preclinical data (especially 4-week toxicology data), a 7-day administration period for this phase I trial seemed justified.

From pharmacology experiments in animals, it was expected that dose-limiting effects would occur at a dose of 1,000 mg/day. However, in this dose range only one patient taking 1,000 mg dexniguldipine/day showed orthostatic dysregulation on day 7 of drug intake. This patient's blood pressure fell to 70/50 mmHg when the patient arose from the resting to the upright position, and this decrease was combined with dizziness and nausea. In interpreting these data, it must be noted that the patient was treated with multiple comedications, including theophylline, isosorbitmononitrate, salbutamol, methyldigoxin, and doxepin. Several of these compounds affect cardiovascular activity and, therefore, the effects observed in this patient were probably due to synergistic effects on peripheral vascular resistance rather than to a single effect of dexniguldipine.

For safety reasons, the number of patients in the 500-mg/day group was increased to eight without observation of other relevant cardiovascular events; therefore, further dose escalation was performed. On the administration of a daily dose of 2,500 mg/day as a single dose in the morning, a moderate decrease in resting blood pressure was observed in all three patients in the highest-dose group. In addition, the systolic blood pressure fell to 70 mmHg in two of these patients as they were rising to an upright position. Thus, cardiovascular effects of dexniguldipine may occur at this dose; however, these effects were not considered to be dose-limiting. In addition, other dose-limiting toxicities were not found. Therefore, an MTD such as that usually defined for cytostatics could not be determined for oral dexniguldipine in the present study.

On the other hand, the determination of a dose for phase II clinical trials may be obtained from the pharmacokinetic properties of a compound, such as its absorption and bioavailability. From the data obtained in this trial the following conclusions can be drawn for dexniguldipine:

1. The interindividual variation is high, as is also known for other dihydropyridine compounds.
2. At doses of up to 1,000 mg/day we found a clear relationship between dose and the average steady-state concentration (C_{av}), although in group VI (1,000 mg/day) the median C_{av} was relatively high. This discrepancy may be explained by the relatively low number of patients per group.
3. At doses above 1,000 mg/day no further relevant increase in C_{av} was found but, notwithstanding the small number of patients involved, consistently high serum concentrations were achieved with the 2,500-mg dose.

Average 24-h serum levels in the range of 100 to 200 ng/ml, as reached in most patients in the upper-dose groups, correspond to approximately 0.2–0.3 μM, and the peak levels measured reached 0.35 μM. From autoradiography data in animals it is known that concentrations attained in most tissues (liver, lung, and kidney, among others) are about 10 to 20-fold those measured in serum (unpublished data). As complete reversal of MDR1 was demonstrated in vitro at concentrations ranging between 0.1 and 1 μM, depending on the assay used, it may be expected that clinically relevant tissue levels can be obtained after oral administration of dexniguldipine. To date, no clinical marker has been found for which a good correlation with the

expression or blockade of 170 glycoprotein can be demonstrated. An increase in serum bilirubin has been suggested as a marker for P170 blockade but has been reported only for cyclosporine A, not for verapamil or other drugs tested. In addition, a correlation between bilirubin increase and clinical response has not been shown [13, 15]. In our trial we did not observe any relevant increase in serum bilirubin.

With regard to the antiproliferative activity of the compound, serum levels reached in our patients compare favorably with those obtained in hamsters at a dose of 32.5 mg/kg (achieved serum levels, approx. 190 ng/ml), which was reproducibly shown to cure neuroendocrine lung tumors in these animals.

In summary, it seems justifiable to start phase II trials with dexniguldipine in both indications, i.e., overcoming of MDR1 and antiproliferative activity in tumor types selected according to preclinical results. With regard to the antiproliferative effect, these studies should aim for long-term continuous administration in patients with hupernephroma, small-cell lung cancer, or melanoma. With regard to MDR modulation, clinical trials of dexniguldipine in combination with MDR-dependent drugs (Especially anthracyclines or vinca alkaloids) should be performed in patients in whom previous treatment with these cytostatics has failed. Primary targets for these trials may be all tumors known to express the *mdr* 1 gene, especially after previous chemotherapy, e.g., acute myeloid leukemia, myeloma, or solid tumors such as breast and ovarian carcinoma (among others). In addition, the daily dosing schedule might be optimized by dividing the dose, e.g., for a three-times-daily schedule. Intravenous administration will be necessary to determine the dose-limiting toxicity and highest plasma levels achievable. Respective trials are currently being conducted.

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