Cancer Chemother Pharmacol (1985) 15 : 88–90

Cancer Chemotherapy and Pharmacology

Short communication

Effectiveness of P-aminobenzoyl-O-phenylenediamine (Goe 1734) against mouse, rat, and human tumour cells

P. Lelieveld, R. J. F. Middeldorp, and L. M. van Putten

Radiobiological Institute TNO, P.O. Box 5815, 2280 HV Rijswijk, The Netherlands

Summary. *The new N-phenylbenzamide derivative Goe 1734 was tested for its antitumour effects against mouse, rat, and human tumours. The preparation showed marginal activity against leukaemia L1210, moderate activity against Lewis lung carcinoma, and high activity against osteosarcoma C22LR and Brown Norway myeloid leukaemia. In the subrenal capsule assay the drug was active against four (cisplatin: 2) of nine human tumours. An in vitro clonogenic assay did not reveal any activity of Goe 1734 when mouse osteosarcoma or human tumour cells were exposed for only 1 h. However, continuous exposure led to 70% or greater inhibition of colony formation at concentrations of 0.1-1 μg/ml (osteosarcoma) or 0.2–2 μg/ml (human tumours).*

Introduction

P-Aminobenzoyl-O-phenylenediamine Goe 1734; NSC 328786) is a new N-phenylbenzamide derivative with a relatively simple structure (Fig. 1). It was originally developed by the Gödecke chemical and biological research groups (Freiburg, W. Germany) as a potential anticonvulsive agent. In rats, a marked inhibition of peripheral blood cells and of spermatogenesis was observed. A possible cytostatic effect was suggested. No effect was shown when it was tested against mouse leukaemia P388 in the standard NCI screen. The compound was offered to us for testing against a variety of mouse, rat, and human tumours with various administration schedules. The results are presented in this communication.

Materials and methods

Drug treatment. Since Goe 1734 is insoluble in water, it was suspended in 0.8% methocel (Methocel E4M Prem., Dow Chemical GmbH Schwingewerk, Stade-Brunshausen) and administered PO within 1 h after preparation of the suspension. Fresh suspensions were made daily before treatment. The volume of administration was 0.01 ml/g body weight (mice) or 0.002–0.005 ml/g body weight (rats). The drug was administered daily for 9 or 10 days or until early toxic death.

Tumour models. The effectiveness of Goe 1734 was determined against a variety of mouse, rat, and human tumour cells. The mouse models used were the experimental tumour models leukaemia L1210, osteosarcoma C22LR, and Lewis lung carcinoma. In rats, the Brown Norway myeloid leukaemia (BNML) was used [3]. Human ovarian, colon, and lung tumours available as early passage xenografts in nude donor mice were tested for sensitivity in irradiated normal mice by the subrenal capsule assay [1, 2].

Lymphocytic leukaemia L1210 is maintained as a cell line growing alternately in vitro and in vivo. Mice received 10^5 L1210 cells IP from an in vitro culture. Groups of five mice were treated with different doses of the drug. Control mice (10 mice per group) were treated with 0.8% methocel PO. Cages were checked for dead mice twice daily. The end point was the median survival time (MST), which was expressed as percent T/C (treated over control group).

Brown Norway myeloid leukaemia (BNML) cell suspensions were prepared from spleens of highly leukaemic BNML rats. Rats (4 per treatment group and 8 as controls) received 10^7 leukaemic spleen cells by IV injection. Treatment was started on day 7 and consisted of ten oral drug administrations in 2 weeks. The end point was the MST. In vivo, tumour cells increase in number by a factor of 10 in about 4 days. Consequently, an increase in life span of 8 days corresponds to two decades of cell kill [3].

Osteosarcoma C22LR grows SC in donor mice. Cell suspensions were prepared by enzymatic treatment [6]. Tumour cells (10^6 cells per locus) were inoculated SC into each flank of recipient mice. Tumours were measured in three dimensions three times per week. Treatment was started when the tumour volume (i. e., the product of the diameters in 3 dimensions) reached 100–200 mm^3. Per group, 10 tumours in five mice were used. Changes in the body weight of the mice were also recorded. The end point for effectiveness was tumour growth delay. This is defined as the difference in time (mean value) between treated and control tumours at the time when the tumour volumes were four times the mean volume at the start of treatment.

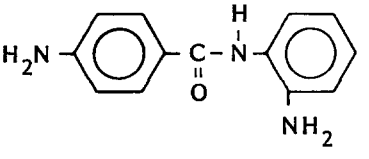

Fig. 1. Chemical structure of Goe 1734

This study was supported by the Gödecke Research Institute, Freiburg, Federal Republic of Germany. The supply of methocel by Stokvis, Rotterdam, The Netherlands is gratefully acknowledged.

For the Lewis lung carcinoma, the procedure was similar to that for the osteosarcoma, except that the difference in tumour volume of treated and control tumours (i. e., tumour growth delay) was calculated when tumour volumes reached a value of 800 mm^3. Per group, four to five mice bearing four to ten tumours were used.

Human tumours. The slightly modified [2] subrenal capsule assay [1] was used to determine drug effectiveness against four ovarian, two colon, and three bronchial tumours. None of these tumours was derived from patients treated with cytostatic drugs. Small pieces of human tumour obtained from early-passage transplants in nude mice (passage 8 or earlier) were placed under the kidney capsules of mice [1] irradiated with 4 Gy whole-body γ-irradiation [2]. The tumours were accurately measured in two dimensions by use of an operation microscope fitted with an ocular micrometer grid. Six days later, the kidneys were taken out and the size of the graft was again determined. The change in mean diameter 1/2 (a+b) between day 0 and day 6 was calculated. Suspensions of Goe 1734 were administered PO on days 1 and 5 at a dose of 150 mg/kg. In view of the location of the tumour, IP administration is not considered suitable, since this might have a local effect. Effectiveness was compared to that of cisplatin, which was administered IV, also on days 1 and 5. A significant antitumour effect was assumed if the change in diameter of the treated tumours differed significantly (Student's *t*-test) from the change in the control tumours.

In vitro studies. Goe 1734 was studied in vitro with the aid of a soft agar clonogenic assay [4]. The preparation was dissolved in a small volume of DMSO and diluted further with saline. Cells of the osteosarcoma C22LR and of human tumours (1 ovarian, 1 colon, and 2 lung tumours) were exposed to different concentrations of the drug either for 1 h or continuously. The end point for assessment of effectiveness was the ID70, i. e., the dose which caused 70% inhibition of colony formation.

Results

In tumour-bearing mice, depending on the strain a maximum dose of 9 × 60–80 mg/kg PO was tolerated. Higher doses, even when administered less frequently, were toxic and led to early death. The cause of death was not investigated. In rats, 10 daily doses of 40 and 30 mg/kg were not tolerated. When low daily doses were used it seemed that a total dose of 200 mg/kg caused about 50% mortality.

The results for leukaemia L1210 and for the BNML are presented in Table 1. In the L1210 model there was modest but just significant activity at only one dose level (9 × 60 mg/kg). Lower doses were inactive, while higher doses were toxic. Goe 1734 was very effective in prolonging the life span of BNML rats. Early toxic death occured shortly after the last of 10 doses of 20 mg/kg. Several rats that survived the treatment with 10 ×20 and 5 × 30 mg/kg died later from recurrent disease, as confirmed by histopathology. Growth of the BNML could be shown in bone marrow areas invading surrounding structures such as the epidural space of the vertebral canal, spinal roots, and arachnoidea of the brain.

The results for osteosarcoma C22LR and for Lewis lung carcinoma are presented in Table 2. In the osteosarcoma

Table 1. Effect of Goe 1734 on lymphocytic leukaemia L1210 in mice and Brown Norway myeloid leukaemia in rats

Treatment	Median survival time (days)	T/C (%)
L1210 lymphocytic leukaemia		
Control (methocel only)	8	100
20 mg/kg daily for 9 days	8	100
40 mg/kg daily for 9 days	9	113
60 mg/kg daily for 9 days	11	138
80 mg/kg daily for 9 days	8	100
90 mg/kg daily for 9 days	6	Toxic
120 mg/kg daily for 7 days	6	Toxic
180 mg/kg daily for 4 days	5	Toxic
BN myeloid leukaemia		
Control (methocel only)	23	100
10 mg/kg on days 1–5 for 2 weeks	47	204
20 mg/kg on days 1–5 for 2 weeks	70	304
30 mg/kg on days 1–5 for 1 week	99	430
30 mg/kg on days 1–5 for 2 weeks	17	Toxic
40 mg/kg on days 1–5 for 2 weeks	15	Toxic

Table 2. Effect of Goe 1734 on osteosarcoma C22LR and Lewis lung carcinoma in mice

Treatment	No. of tumours for evaluation	Tumour growth delay (days) (mean ± SE)	Body weight change (g) (T–C)
Osteosarcoma C22LR			
20 mg/kg daily for 9 days	10	3.9 ± 0.5	−1.8
40 mg/kg daily for 9 days	10	8.5 ± 0.5	−3.2
60 mg/kg daily for 9 days	10	7.8 ± 0.8	−2.5
80 mg/kg daily for 9 days	10	13.3 ± 0.9	−8.1
90 mg/kg daily for 9 days	4	12.3 ± 0.4	−8.8
Lewis lung carcinoma			
20 mg/kg daily for 9 days	5	4.9 ± 2.6	NE
40 mg/kg daily for 9 days	6	3.3 ± 1.6	NE
60 mg/kg daily for 9 days	6	5.8 ± 1.8	NE
80 mg/kg daily for 9 days	4	5.3 ± 1.8	NE
240 mg/kg (single dose)	10	3.5 ± 1.1	NE

NE, not evaluated

Table 3. Effect of Goe 1734 and cisplatin on human ovarian, colon, and lung tumours, assessed by the subrenal capsule assay

Tumour type and code	Change in tumour diameter (mm)		
	Control	Goe 1734	Cisplatin
Ovary			
S	0.185	−0.20*	−0.15*
S[a]	0.10	−0.09*	−0.13*
C	0.165	−0.07	−0.17
30	0.00	0.07	0.04
P	0.57	0.58	0.49
Colon			
F	0.225	−0.20*	−0.13
K	0.025	−0.05	−0.063
Lung			
1	−0.033	−0.20*	−0.09
2	0.54	0.03*	0.17
3	0.04	0.025	−0.09

[a] Confirmation experiment
* Significantly different ($P < 0.05$) from control values (Student's *t*-test)

model Goe 1734 showed good activity even at doses which caused only a moderate relative weight loss of the mice. The observed values for tumour growth delay are similar to those found with cisplatin (data not presented). In the Lewis lung model a modest activity, which seemed to be independent of dose and schedule, was observed. A single dose of 240 mg/kg resulted in a tumour growth delay similar to that following treatment with nine doses of 20 or 40 mg/kg. In the human tumour systems (Table 3), the preparation was active against one (confirmed) of four ovarian tumours and one of two colon tumours. In these tumours, Goe 1734 and cisplatin showed similar activity. In contrast to cisplatin, Goe 1734 was effective against two of three lung tumours. Although limited experience with the modified subrenal capsule assay must lead to caution in interpreting these results, they suggest that activity of Goe 1734 against human tumours might be assumed.

In the soft agar clonogenic assay, exposure to Goe 1734 for 1 h did not have any apparent inhibitory effect up to the highest dose tested (20 μg/ml). Continous exposure, however, was effective. Inhibition of colony formation by $\geqslant 70\%$ was observed at doses of 0.1–1 μg/ml for osteosarcoma and 0.2–2 μg/ml for the human tumours tested.

Discussion

Goe 1734 has been shown to be a potent anticonvulsive agent (Herrmann, unpublished data). In rhesus monkeys, skin grafts from two unrelated donors showed prolonged survival times (Van Vreeswijk, unpublished observation), a result later confirmed in rats (Weiershausen et al., paper in preparation). Further, marked leucocytopenia and an inhibition of rat spermatogenesis were observed (Fritschi and Wiegleb, unpublished data). Such effects on rapidly proliferating cells might indicate a possible cytostatic potential of the drug.

In the standard NCI screen, IP administration of Goe 1734 was ineffective against mouse leukaemia P388. In the present study, repeated PO administration was effective against mouse osteosarcoma, BN myeloid leukaemia, and several human tumours. The degree of effectiveness of Goe 1734 in the mouse osteosarcoma and the human tumours was similar to that of cisplatin. Using nude mice, Fiebig studied the antitumour effects of Goe 1734 against nine human tumours, including ovarian tumour S, which in our hands showed (confirmed) sensitivity. Drug treatment was given on days 1, 5, 9, and 14 after tumour grafting. This may explain why Fiebig, in contrast to our results, found absolutely no tumour growth inhibition with these nine xenografts (personal communication).

The mechanism of action of Goe 1734 is unknown. The compound is an analogue of benzamide. It was recently found that benzamide, and also 3-aminobenzamide, not only inhibited the synthesis of poly(adenosine diphosphate-ribose) but also reduced glucose metabolism and methionine incorporation into DNA and effected cell survival [5].

In conlusion, Goe 1734 had a high antitumour effectiveness in some animal and human tumour models after repeated oral administration. In vitro activity was observed only after continuous drug exposure. Our in vivo and in vitro results indicate that the drug must be administered on a continuous basis for maximum effectiveness. This makes Goe 1734 an interesting compound which, in our opinion, warrants further development.

References

1. Bogden AE, Cobb WR, Lepage DJ, Haskell PM, Gulkin TA, Ward A, Kelton DE, Esber HJ (1981) Chemotherapy responsiveness of human tumors as first transplant generation xenograft in the normal mouse: six-day subrenal capsule assay. Cancer 48: 10
2. Edelstein MB, Smink T, Ruiter DJ, Visser W, Van Putten LM (1984) Improvements and limitations of the subrenal capsule assay for determining tumour sensitivity to cytostatic drugs. Eur J Cancer Clin Oncol 20 : 1549
3. Hagenbeek A (1977) Growth regulation in the BN myelocytic leukaemia. Leuk Res 1: 149
4. Hamburger AW, Salmon SE (1977) Primary bioassay of human tumor stem cells. Science 197 : 461
5. Milan KM, Cleaver JE (1984) Inhibitors of poly(adenosine diphosphate-ribose) synthesis: effect on other metabolic processes. Science 223: 589
6. Reinhold HS (1965) A cell dispersion technique for use in quantitative transplantation studies with solid tumours. Eur J Cancer 1: 67

Received September 24, 1984/Accepted November 2, 1984