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CGP 6809, a sugar-containing nitrosourea derivative: pharmacological and physicochemical properties

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Summary. CGP 6809 is a water-soluble nitrosourea derivative with quite distinct chemical and biological properties as compared with the well-known representatives of this class of compounds. It is related to the antibiotic streptozotocin, from which it is distinguished in the structure of the sugar moiety and the position of the methylnitrosourea residue. CGP 6809 possesses practically the same alkylating potential as streptozotocin; however, its carbamoylating activity is comparable with that of CCNU. In contrast to other nitrosourea derivatives, CGP 6809 showed relatively little activity in murine leukemias but was markedly active in solid transplantable melanomas (Harding-Passay, B16), in the 11095 prostate carcinoma, and in a substrain of Yoshida hepatoma (AH 7974) resistant to BCNU and CCNU. In the Ehrlich and Yoshida ascitic tumors complete responses were seen with no toxic death. Dose-dependent activity was found in the human lung carcinoma MBA 9812 and almost complete growth inhibition was achieved in the human melanoma WM 47 by both the oral and parenteral routes of administration. However, mammary tumor lines (Ca 755, 2661/61, R-3230AC), the Guerin-T8 uterus epithelioma, and the Rous sarcoma/S-R proved to be relatively refractory to this drug. This was also the case for the Lewis lung carcinoma implanted i.m. or s.c. However, development of lung metastases was markedly inhibited. Combination therapy using CGP 6809 with cyclophosphamide, 5-fluorouracil, or chlorambucil in the same model led to partial responses of the primary tumor as well as almost total eradication of lung metastases.

Introduction

Since the observation in the early 1960s that N-nitrosourea (NMU) can increase the life span of mice with i.p. or intracerebrally implanted leukemia L1210 [17], nitrosoureas have become a family of antitumor drugs. Through structure variation and optimization of NMU, more lipophilic and alkylating chloroethylnitrosoureas such as BCNU, CCNU, and MeCCNU were developed, which have shown considerable antitumor activity in a variety of experimental [13] and human malignancies [10]. Unfortunately, delayed and cumulative hematologic toxicity has limited the clinical use of these drugs.

The discovery of the antibiotic streptozotocin, with reduced myelosuppression and pronounced antitumor activity in experimental models [21], led to the synthesis of a new series of hydrophilic nitrosoureas with and without sugar carriers. Some of them, such as ACNU, GANU, RFCNU, TA-077, and chlorozotocin have shown marked activity against a broad spectrum of transplantable solid and ascites tumors and leukemias in mice and rats [1, 12, 16], proving to be active in clinical trials [10]. These nitrosourea derivatives differ from each other in their therapeutic activity in animals and humans. However, they all share the alkylating 2-chloroethyl group as the active moiety and can cross-link nucleic acids [3].

In this paper we report on the antitumor activity, tolerability, and physicochemical properties of CGP 6809, a water-soluble methylnitrosourea derivative. Parts of these results have been presented in a preliminary form [14].

Materials and methods

Chemicals. CGP 6809 (ethyl-6-deoxy-3,5-di-O-methyl-6-[3-methyl-3-nitrosoureido]-α-D-glucofuranoside) (Fig. 1) was synthesized in the chemical research laboratories of Ciba-Geigy Ltd. (Basel, Switzerland). BCNU and CCNU were obtained by courtesy of Bristol Laboratories (Syracuse, N.Y.). Streptozotocin (Sigma), cyclophosphamide (Endoxan; Asta), 5-fluorouracil (Fluoro-Uracil; Roche) and chlorambucil (Leukeran; Wellcome) were purchased from commercial sources.

Determination of stability in aqueous solution. The stability of CGP 6809 in aqueous solutions was determined in anal-

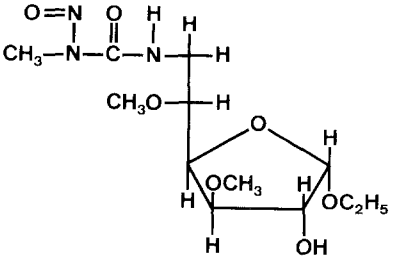

Fig. 1. Structural formula of CGP 6809: $C_{12}H_{23}N_3O_7$; ethyl-6-deoxy-3,5-di-O-methyl-6-(3-methyl-3-nitrosoureido)-α-D-glucofuranoside

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ogy to a previously published procedure of Wheeler et al. [23]. Aliquots (60 mg/3.0 ml) of aqueous solutions of CGP 6809 were diluted with 25 ml buffer solutions (Titrisol; Merck) of pH 2.0–8.0 and kept at 30°–80° C. Periodically, UV absorption was measured or samples were subjected to high-pressure liquid chromatography on a Nucleosil 10 C 18, 200 × 6 × 4 mm column, with 0.5% ammonium sulfate in water/acetone (750/250) as mobile phase. The intact compound was detected by UV absorbance at 228 nm and the half-lives were calculated from the drug concentrations at various times.

Determination of carbamoylating activity. The carbamoylating activity of CGP 6809 was determined in comparison with CCNU against 2-phenylethylamin instead of the commonly used L-lysine [11]. For preparation of the buffer solution, 2.83 ml 2-phenylethylamin (Fluka, purum) was stirred into 430 ml phosphate buffer (pH 6.0) to adjust the pH to 7.4. This mixture was diluted with 570 ml ethyl alcohol (Fluka puriss. p.a., Ph. Helv.) and made up to 1,000 ml with approximately 40 ml double-distilled water; 20 ml buffered solution was transferred to each of two white 50-ml pill vials equipped with magnetic stirring devices and heated to 37° C. Under stirring, 145 mg (0.45 mmol) CGP 6809 was added to one vial and 105 mg (0.45 mmol) CCNU to the other. CGP 6809 dissolved immediately, and CCNU, within 5 min. The solution was stirred for 24 h at 37° C and freed of alcohol. The resulting products were extracted with chloroform, crystallized, and analyzed.

Determination of alkylating activity. The alkylating activity of CGP 6809 was determined by color reaction with 4-(4-nitrobenzyl)-pyridine according to the procedure of Wheeler et al. [23]. A mixture of 2.0 ml water, 1.0 ml acetate buffer (0.025 *M*, pH 6.0), 1.0 ml solution of the test substance (10 µmol) in acetone, and 0.4 ml solution of 4-(4-nitrobenzyl)-pyridine was left to react in a water bath at 37° C for 1–3 h. The reaction solution was then cooled in an ice bath and 2.0 ml acetone, 5.0 ml ethyl acetate, and 1–5 ml 0.25 *N* NaOH was added (color development did not begin until NaOH was added). The solution was shaken 20 times and the phases were allowed to separate. A part of the upper phase was removed with a Pasteur pipette and transferred to a dry 1-cm cuvette. The extinction was measured at 540 nm.

Mouse toxicity studies. Male and female Ha/ICR mice (4–8 weeks of age, 20–30 g body wt.) were treated with single and multiple (daily for 5 consecutive days) i.p. or p.o. doses of CGP 6809 dissolved in distilled water; 10–15 animals were used per dose and all were monitored for 60 days. The LD_{10}, LD_{50}, and LD_{90} of CGP 6809 were determined from the survival data. Confidence limits (95%) of LD_{50} were determined by the method of Wilcoxon and Litchfield [24]. Swiss mice were used for the determination of myelosuppressive effects. Blood cell counts [red blood count (RBC), white blood count (WBC), platelets] as well as tibial bone marrow (TBM) tests were carried out individually on six and three mice in the treated and control groups, respectively, at time points indicated in the tables.

Tumors and animals. CGP 6809 was tested for antitumor activity in mice and rats in various ascitic and solid tumors. The mice and rats, tumors, transplantation sites and number of tumor cells injected are listed in the result tables. For experiments with leukemias and ascites tumors, ascites was taken from the passage animals 7 days after transplantation, counted in a TOA CC-108 Microcell Counter, and diluted with Hanks' solution to appropriate concentrations. For experiments with solid tumors, tumors of about 20–40 mm diameter were excised from the passage animals under sterile conditions about 2 weeks after transplantation. They were minced, diluted with Hanks' solution, and injected s.c. into the animals' left flank or i.m. into the left hind leg. The human xenografts, lung carcinoma MBA 9812 and melanoma WM 47, were excised from the passage animals 2–3 weeks after transplantation, depending on the individual tumor line. Pieces of tumor of about 25 mg each were transplanted by trocar into the left flank of athymic BALB/c nude mice.

Experimental conditions. After tumor transplantation, the animals were allotted at random to treatment and control groups of 10 each. They were kept in macrolon cages with free access to food and water in air-conditioned rooms at a temperature of 23° ± 2° C and a relative humidity of 55% ± 5%. Unless otherwise stated, treatment of mice with test compounds or placebo was started 4 h and that of rats, 24 h after tumor transplantation and continued for a time period as indicated in the result tables. Treatment of xenograft-bearing nude mice was started 3 days after transplantation and continued for 15 consecutive days. To speed up solubilization, CGP 6809 was solubilized with a few drops of ethanol and adjusted to final concentrations with double-distilled water immediately before use.

Parameter calculation and statistics. The antitumor activity of CGP 6809 was evaluated according to NCI Instruction 14 [9]. The mean tumor weight or ascites volume or the mean survival of drug-treated animals was multiplied by 100 and divided by the mean tumor weight or the mean survival of placebo-treated controls; the results were expressed as %T/C. The metastatic lung nodules in the experiments with solid Lewis lung carcinoma were evaluated according to a method described by Wexler [22]. The statistical analysis of the tumor data was carried out by means of the parametric Bartlett and Dunnett tests [6], or the nonparametric Steel test [18]. The test chosen depended on the result of the Shapiro-Wilk test [15], which discriminated data distributed normally (parametric) from nonparametric data.

Results

Physicochemical properties of CGP 6809

The structural formula of CGP 6809 is given in Fig. 1. It is a colorless to slightly creamy crystalline compound with a molecular weight of 321.3, a melting point of 89°–90° C, an optical rotation [α] +42° ±1° (chloroform, c = 1.022), and an UV absorption λ max. of 227.0 nm/log ε 3.827. This agent is soluble in most organic solvents and up to 2.5% in water. The pure compound has remarkably higher stability than other nitrosoureas such as BCNU, streptozotocin, or chlorozotocin and can be stored at room temperature for several months with no indication of decomposition. At 21° C, CGP 6809 shows maximal stability between pH 4.0 and 6.0, with half-lives of 4,591 and 2,853 h, respectively. At a temperature of 37° C (pH 6.0), the half-life

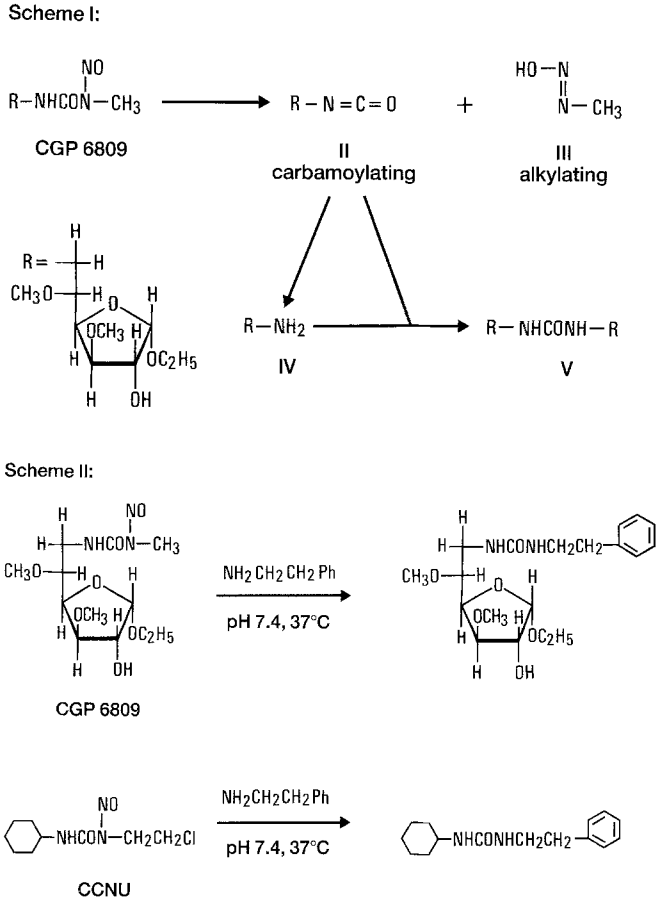

Fig. 2. Proposed reaction scheme of CGP 6809

of CGP 6809 dropped to approximately 45 h; this was still about 5 and 10 times longer than that observed for streptozotocine and BCNU, respectively. The analysis of the reaction mixture showed that CGP 6809 decomposed according to a known reaction [19] (Fig. 2, Scheme I), yielding as reactive intermediates the carbamoylating and alkylating moieties ‹II› and ‹III›. The intermediate ‹III› gave volatile products, whereas one part of the isocyanate ‹II› hydrolyzed with water to the amine ‹IV›, which was further carbamoylated to the symmetrical urea ‹V›. This last compound and a trace of the amine ‹IV› were the only detectable products.

Under analogous reaction conditions (37° C, pH 7.4) in the presence of 2-phenylethylamine (Fig. 2, Scheme II), 46% of the 2-phenylethylamine was found to be carbamoylated with CGP 6809 and 32%, with CCNU within 24 h. Streptozotocin, which is known to have practically no carbamoylating activity [25], was not included in this experiment. CGP 6809 displayed alkylating activity comparable with that of streptozotocin, but which was weaker than that of CCNU or BCNU. Setting BCNU at 100%, the alkylating activity [23] of CCNU, streptozotocin, and CGP 6809 after 1 h at 37° C (pH 6.0), was 50%, 18%, and 19%, respectively.

Mouse toxicity studies

Table 1 shows a summary of lethality data. The LD_{10} was found to be nearly identical for male and female mice after a single i.p. treatment. The LD_{10} for five consecutive daily i.p. doses was roughly 3 times lower. A comparison of the total doses to induce death in 50% of female mice after a single i.p. dose or total multiple i.p. dose indicated

Table 1. Lethality of CGP 6809 in Ha/ICR mice

Sex	Route of administration	LD_{10}	LD_{50} (mg/kg)	LD_{90}	Confidence limits (95%) of LD_{50}[a]
			Single-dose study		
Male	i.p.	780	920	1,125	844–1,003
Female	i.p.	750	890	1,050	816– 970
Male	p.o.	1,075	1,450	1,975	1,306–1,610
Female	p.o.	600	1,125	2,150	953–1,328
			Multiple-dose study (daily × 5)		
Male	i.p.	245	375	580	305– 461
Female	i.p.	285	375	500	310– 454
Male	p.o.	685	970	1,375	844–1,116
Female	p.o.	670	790	930	561–1,113

[a] Statistical evaluation according to Wilcoxon and Litchfield [24]

Table 2. Hematological parameters in male Swiss mice after single or multiple i.p. doses[a] of CGP 6809

Treatment	Single dose[b]				Multiple dose (× 5)[b]			
	TBM (× 10^7)	RBC (× 10^6)	WBC (× 10^3)	Platelets (× 10^5)	TBM (× 10^7)	RBC (× 10^6)	WBC (× 10^3)	Platelets (× 10^5)
CGP 6809	0.22 ± 0.08	7.59 ± 1.04	1.60 ± 0.55	4.35 ± 0.54	1.17 ± 0.21	7.52 ± 0.30	7.56 ± 3.88	4.01 ± 0.72
Controls	1.64 ± 0.37	8.07 ± 0.95	6.90 ± 2.11	4.66 ± 0.35	1.50 ± 0.24	7.88 ± 0.71	7.21 ± 1.50	5.03 ± 0.99

[a] Corresponding to LD_{10} doses in male mice (see Table 1)
[b] Determined 4 or 7 days, respectively, after the last doses

a less than cumulative pattern, as did the same data for male mice. The LD_{10} was somewhat higher for males than for females after single p.o. administration, but in the multiple-dose study nearly identical values were obtained for males and females. The multiple oral dose study indicated a less than cumulative pattern of lethality, as did the multiple i.p. study.

Myelosuppression induced by CGP 6809 was studied in Swiss mice using 1 × and 5 × treatment regimens at the corresponding LD_{10} dose level (Table 2). Since male and female mice behaved quite similarly, only values for male mice are shown. At 4 days after a single injection, a decreased bone marrow cellularity and a lowered WBC count were readily apparent. By day 29 this myelosuppressive effect was fully reversed (data not shown). Daily injections on 5 consecutive days were – surprisingly – only marginally suppressive. No significant effects were observed on days 14 and 29 of the postobservation period.

Antitumor activity

Leukemias. The effect of CGP 6809 on the survival of female $B_6D_2F_1$ mice with leukemia $L1210/S_2$ is shown in Table 3. CGP 6809 given p.o. or i.p. from days 1 to 4 significantly increased the survival of the mice. Intermittent treatment (days 0/3/6/9) also significantly increased the survival of mice with leukemia $L1210/S_2$. Both the p.o. and i.p. routes of administration were effective. CGP 6809 had no effect on the survival of mice with lymphatic leukemia CA55 or lymphoma $6C_3HED/S$, nor on that of rats with Dunning leukemia R3323 treated on a 4-day schedule (starting on day 1) with 250 or 125 mg/kg p.o. or i.p. (data not shown).

Ascites tumors. The pronounced activity of CGP 6809 after oral or i.p. treatment of mice and rats with various ascites tumors is shown in Table 4. The growth of Ehrlich ascites carcinoma in Tif:MAGf mice and of mastocytoma P815 in $B_6D_2F_1$ mice was markedly inhibited by both oral and i.p. administration of CGP 6809. Yoshida ascites hepatomas AH 66 (Table 4) and AH 130 (data not shown) in male Wistar rats were found to be extremely sensitive to CGP 6809 over a wide dose range. Oral administration of 31.3 mg/kg to rats with Yoshida hepatoma AH 66 or AH 330 inhibited the tumor growth completely.

Dose-response relationships showed a linear pattern. The smallest fully effective i.p. dose against both Yoshida hepatomas AH 66 and AH 130 was 6.25 mg/kg; again, linear dose-response relationships were observed. Much higher doses were needed to inhibit the growth of the Yoshida hepatoma AH 7974 (Table 4). This result is particularly interesting since Ichimura [8] has reported that this tumor is resistant to BCNU and CCNU and only marginally sensitive to ACNU.

Solid tumors. Table 5 summarizes the activity of CGP 6809 against various solid tumors in mice and rats. Pronounced

Table 3. Effect of CGP 6809 on survival of $B_6D_2F_1$ mice with leukemia $L1210/S_2$

Dose (mg/kg)	Schedule (days)[a]	Survival (% T/C)
250 p.o.	1–4	143*
125 p.o.	1–4	129*
250 i.p.	1–4	187*
125 i.p.	1–4	133*
625 p.o.	0/3/6/9	163*
400 i.p.	0/3/6/9	163*

[a] Days after i.p. transplantation of 1×10^6 cells
* $2\ P < 0.001$, Dunnett test

Table 4. Effect of CGP 6809 on ascites tumors in mice and rats

Tumor	Dose[a] (mg/kg × 4)	Ascites volume[b] (% T/C)
Ehrlich carcinoma (MAG : Tif mice)	250 p.o.	34*
	250 i.p.	0*
	100 i.p.	46*
Mastocytoma P815 ($B_6D_2F_1$ mice)	250 p.o.	38*
	125 p.o.	55*
	100 i.p.	41*
	50 i.p.	56*
Yoshida hepatoma AH 66 (Wistar rats)	31.3 p.o.	0*
	15.6 p.o.	5*
	7.8 p.o.	50*
	3.9 p.o.	91
	6.3 i.p.	0*
	3.1 i.p.	30*
	1.6 i.p.	80
Yoshida hepatoma AH 7974 (Wistar rats)	250 p.o.	32*
	125 p.o.	55*
	100 i.p.	19*
	50 i.p.	30*

[a] Treatment: 4 h after i.p. transplantation of 1×10^6 cells and once daily for 3 consecutive days
[b] 10 days after transplantation
$N = 10$ per treatment or control group
* $2\ P < 0.01$, Dunnett test

Table 5. Effect of CGP 6809 on various solid tumors in mice and rats

Tumor	Dose[a] (mg/kg)	Tumor weight (% T/C)
Harding-Passey melanoma (BALB/c mice)	125 × 8 p.o.	18*
	62.5 × 8 p.o.	48*
	125 × 8 i.p.	11*
	62.5 × 8 i.p.	23*
B16 melanoma ($C_{57}Bl/6$ mice)	250 × 15 p.o.	10*
	125 × 15 p.o.	40*
	250 × 15 i.p.	28*
	125 × 15 i.p.	71
11095 prostate carcinoma (F344 rats)	250 × 4 p.o.	1*
	50 × 4 i.p.	72
Guerin T8 uterus epithelioma (Wistar rats)	250 × 4 p.o.	55*
	125 × 4 i.p.	64
Walker carcinosarcoma 256 (Wistar rats)	125 × 4 p.o.	69
	125 × 4 i.p.	27*
R3230 AC mammary carcinoma (F344 rats)	125 × 10 p.o.	78
	125 × 10 i.p.	64*
Rous sarcoma/S–R (Wistar rats)	250 × 8 p.o.	40*
	125 × 8 i.p.	79

[a] Start of treatment: 24 h after s.c. transplantation of 0.2 ml (mice) or 1.0 ml (rats) tumor suspension in Hanks' solution 1 : 10
$N = 10$ per treatment or control group
* $2\ P < 0.01$, Dunnett test

effects were seen against the Harding-Passey melanoma and the B16 melanoma in mice. CGP 6809 was only marginally active against the mouse mammary adenocarcinomas 775 and 2661/61 (data not shown). CGP 6809 also inhibited the growth of the androgen-independent prostate carcinoma 11095 but was only moderately active against the uterus epithelioma Guerin T8, Walker carcinosarcoma 256, mammary adenocarcinoma R3230 AC, and Rous sarcoma/S-R in rats.

Human xenografts. The human lung carcinoma MBA 9812 was significantly inhibited in a dose-dependent manner by oral treatment with CGP 6809 (Fig. 3). Almost complete arrest of the growth of human melanoma WM 47 was obtained by oral and i.p. treatment (Fig. 4). After the cessation of treatment, the tumor growth remained arrested during the postobservation time of 10 days.

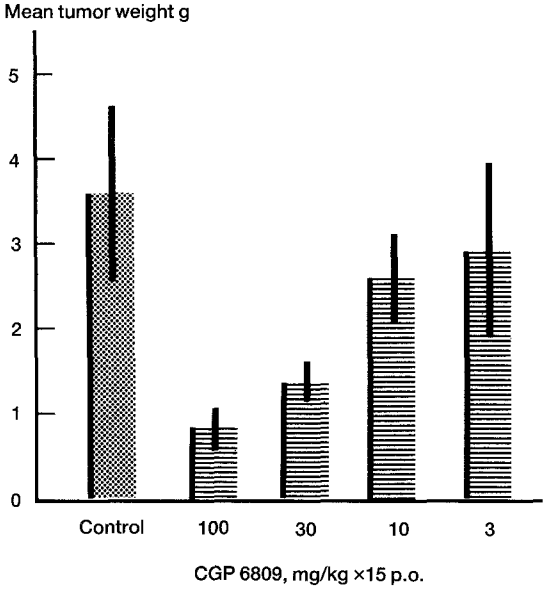

Fig. 3. Mean tumor weight of s.c. transplanted human lung carcinoma MBA 9812 after a 15-day oral treatment course with various doses of CGP 6809

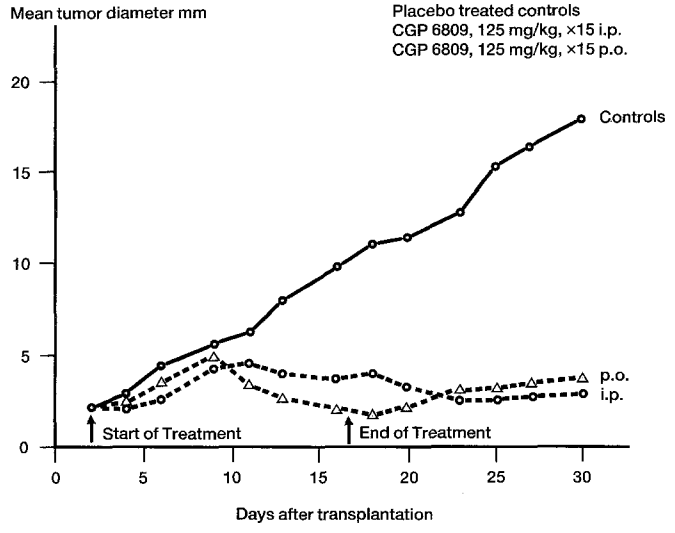

Fig. 4. Effect of CGP 6809 on the growth of s.c. transplanted human melanoma WM47 in female BALB/c nu/nu mice

Table 6. Effect of CGP 6809 and various cytostatics, alone or in combination, on tumor growth and lung metastases in $C_{57}Bl/6$ mice with i.m. transplanted Lewis lung carcinoma

Compound	Dose (mg/kg)	Tumor weight[a] (% T/C)	Number of metastatic lung nodules, median (range)
Control	–	100	100 (70–139)
CGP 6809	125 × 10 p.o.	101	0 (0)
Cyclophosphamide	100 × 2 i.p.	53*	0 (0–3)
CGP 6809	125 × 10 p.o.		
+	+	11*	0 (0–2)
Cyclophosphamide	100 × 2 i.p.		
Control	–.	100	123 (40–187)
CGP 6809	125 × 10 p.o.	97	1 (0–4)
5-Fluorouracil	100 × 2 i.p.	51*	0 (0–11)
CGP 6809	125 × 10 p.o.		
+	+	33*	0 (0)
5-Fluorouracil	100 × 2 i.p.		
Control	–.	100	134 (91–195)
CGP 6809	125 × 10 p.o.	97	7 (0–19)
Chlorambucil	20 × 2 i.p.	71	32 (2–65)
CGP 6809	125 × 10 p.o.		
+	+	33*	0 (0–2)
Chlorambucil	20 × 2 i.p.		

[a] On day 21 after tumor transplantation. Treatment: CGP 6809, 5 times weekly for 2 weeks; cyclophosphamide, 5-Fluorouracil or chlorambucil, day 1 + 7 after tumor transplantation
* 2 $P < 0.01$, Dunnett test

Combination chemotherapy: Female $C_{57}BL/6$ mice with Lewis lung carcinoma were treated with CGP 6809 alone or in combination with cyclophosphamide, 5-fluorouracil, or chlorambucil. As shown in Table 6, the growth of Lewis lung carcinoma was not inhibited by oral treatment with CGP 6809 alone, whereas a pronounced inhibition of lung metastasis was noted. However, a combination of CGP 6809 with cyclophosphamide caused a significantly more pronounced growth inhibition of the primary tumors than either drug alone. In a survival experiment with Lewis lung carcinoma (Fig. 5), this drug combination

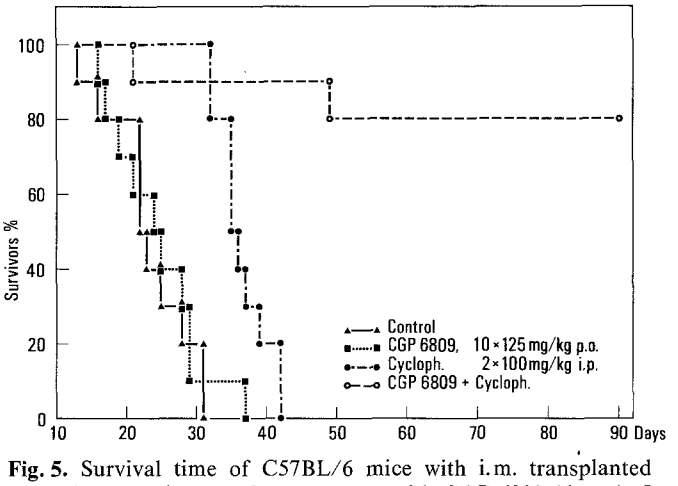

Fig. 5. Survival time of C57BL/6 mice with i.m. transplanted Lewis lung carcinoma after treatment with CGP 6809 (days 1–5 and 8–12) and cyclophosphamide (days 1 and 7) alone and in combination

caused 80% long-term survivors (>90 days) in contrast to the single-drug application. Increased inhibition of tumor growth was also seen in the same model (Table 6) after treatment with CGP 6809 combined with 5-fluorouracil or chlorambucil; however, long-term survival was not achieved with these combinations.

Discussion

CGP 6809 is a nitrosourea compound that is structurally and functionally different from known clinically investigated nitrosoureas such as CCNU, MeCCNU, ACNU, GANU, and RFCNU. As a methylnitrosourea derivative, it is related to streptozotocin, from which it differs in the structure of the sugar moiety and the position of the nitrosourea residue. This structural property may explain some of the qualities that were noted with respect to both the biological activity and the tolerability of this compound.

The biological activity of CGP 6809 is distinct from that of compounds such as BCNU and ACNU. It is inferior compared with the latter drugs in achieving long-time survival in murine leukemia models. However, in the Ehrlich and Yoshida ascites tumors, complete responses at tolerated doses were achieved; partial responses were seen in a resistant substrain of Yoshida hepatoma, AH7974, where BCNU and CCNU were ineffective [8]. The compound was also surprisingly active at tolerated doses in solid transplantable murine melanomas such as the Harding-Passey and B16 melanoma, and it possessed remarkable activity against the human melanoma WM47. Good activity was also seen in the solid rat 11095 prostate carcinoma, whereas mammary carcinoma lines as well as the Guerin-T8 uterus epithelioma and the Rous sarcoma/S-R were relatively insensitive to CGP 6809.

No effect was seen on the primary tumor in the solid Lewis lung carcinoma implanted i.m. or s.c. However, a marked and dose-dependent effect on the development of metastases was readily apparent with a treatment schedule starting 24 h after inoculation of the tumor. In the same model, several compounds (cyclophosphamide, 5-fluorouracil, chlorambucil) showed markedly increased efficacy when combined with CGP 6809, resulting in partial responses of the primary tumor and almost total eradication of metastases. Also, survival of mice treated with a combination of cyclophosphamide and CGP 6809 was far longer (resulting in 80% long-term survivors) than that of mice treated with either of the single agents. These additive therapeutic effects of the drug combinations may be clinically exploitable since CGP 6809 has a different toxicity profile than its combination partners (see below). CGP 6809 was also significantly active against the human lung carcinoma MBA9812 as well as various human tumor xenografts, as previously reported by Fiebig et al. [7].

To shed some light on the potential mechanism of action of CGP 6809, its chemical decomposition rate in aqueous solution and its alkylating and carbamoylating activities were determined in comparison with those of BCNU, CCNU, and streptozotocin. It could be shown that CGP 6809, due to a partially substituted sugar residue, possesses a significant carbamoylating activity, comparable with that of CCNU.

Classically, the alkylating property of nitrosoureas is believed to be mainly responsible for their antitumor effects, whereas their carbamoylating activity is believed to determine their toxic qualities [2, 23]. These conclusions, which were mainly based on findings in ascites tumor models, cannot be generalized, as shown with CGP 6809. The present study showed that this type of compound, despite its low alkylating activity, exerted strong antitumor activity in a broad spectrum of transplantable solid murine tumors as well as human xenografts.

As demonstrated in the toxicity studies, this antitumor activity was achieved at fully tolerated doses. Myelosuppression was relatively modest, particularly (and surprisingly) on the multiple-dose regimen, and readily reversible. Several other characteristics add to these attractive properties. First, CGP 6809 appeared to be devoid of diabetogenic activity. Single doses of 500 mg/kg i.p. or 1,000 mg/kg p.o. were well tolerated in this respect (data not shown); this finding confirms results previously reported by Bernacki et al. [2]. Second, the compound was also practically devoid of mutagenic activity when tested on strains TA98, TA98/TCTO, and TA1537 of *Salmonella typhimurium* (data not shown). Thirdly, no symptoms of embryotoxicity or teratogenicity were detected when the compound was given s.c. to pregnant Sprague-Dawley rats during days 8–13 post coitum at a dose of 6×150 mg/kg body wt.

The overall profile of CGP 6809 warrants clinical development, which until now has led to phase I clinical trials in terminal cancer patients. These trials confirmed the generally good tolerability of the drug and established dose-limiting hepatotoxicity at 4,500 mg/m^2 when given i.v. every 6 weeks. One colon carcinoma patient responded to the highest dose tested. Further trials using lower doses at 2-week intervals are under way [4, 5].

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