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CGP 6809 – A new nitrosoureido-sugar derivative with activity in human tumor xenografts*

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Summary. CGP 6809 [ethyl-6-deoxy-3,5-di-O-methyl-6-(3-methyl-3-nitrosoureido)-α-D-glucofuranoside] is a new methylnitrosoureido-sugar derivative that has been shown to be active against a broad spectrum of transplantable tumours in mice and rats [14]. We investigated the anti-tumour effect of CGP 6809 in ten selected, human tumour xenograft lines growing s.c. in nude mice. The p.o. administration of 125 mg/kg per day for 10–15 days was less toxic (lethality 12% in tumour-bearing nude mice) than the i.p. injection of 62.5 mg/kg per day (lethality 22%). The anti-tumour effect was similar for both application routes; two large bowel cancers responded to treatment with CGP 6809, rectal cancer CXF 158 showed a remission, and the rapidly growing, undifferentiated colonic cancer CXF 280 exhibited a transient no-change. Furthermore, remissions were observed in the epidermoid lung cancer LXF 322 and in thyroid cancer 117. Tumour progression was found in another epidermoid lung cancer and in three stomach cancers, one melanoma, and one soft tissue sarcoma. CGP 6809 is a promising new agent for clinical trials, especially for large bowel and epidermoid lung cancer.

Introduction

CGP 6809 [ethyl-6-deoxy-3,5-di-O-methyl-6-(3-methyl-3-nitrosoureido)-α-D-glucofuranoside; Fig. 1] is a new methylnitrosourea-sugar derivative that lacks the strongly alkylating 2-chloroethyl group at the N^1 position that is characteristic for BCNU, CCNU, MeCCNU, and chlorozotocin [10, 11, 13]. This new compound can be considered to be an analogue of streptozotocin. The substituent at the N^3 position is glucofuranose, as opposed to glucose for streptozotocin.The substituent at the N^3 position is glucofuranose, as opposed to glucose for streptozotocin [9] and chlorozotocin. Streptozotocin is a naturally occurring product of *Streptomyces achromogenes* [18]; its two unique pharmacological properties are its potent diabetogenicity, mediated by the destruction of pancreatic islet beta cells, and anti-tumour activity without bone marrow toxicity [12, 13].

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The structural modification of CGP 6809 may be responsible for its being relatively well tolerated in mice and rats as well as its lack of diabetogenicity and teratogenic potential in rats [1; Schieweck and Schmidt-Ruppin, personal communication 1984]. CGP 6809 shows very limited alkylating or mutagenic activity, and its carbamoylating activity is similar to that of CCNU [15]. A high anti-tumour activity has been demonstrated in transplantable tumours in mice and rats, including Rauscher leukemia, Harding-Passey melanoma, B16 melanoma, prostate carcinoma 11095, and Yoshida hepatoma. A s.c. human melanoma in nude mice was also sensitive [14].

We report the results of our present study testing the effectiveness of CGP 6809 in ten human tumours established as continuous xenograft lines in nude mice. Promising anti-tumour activity, with partial remissions in three tumours, was observed.

Materials and methods

We used 6- to 8-week-old athymic nude female NMRI mice purchased from the Zentralinstitut für Versuchstiere, Hannover. The animals were housed in macrolon cages set in laminar flow rackets and maintained under conditions previously described by Fortmeyer and Bastert [7].

The effect of CGP 6809 on ten human tumours grown s.c. in serial passages in nude mice was studied. The experiments were carried out on tumours that had undergone between 8 and 30 passages; all were frozen in liquid nitrogen. The human origin of the tumours was demonstrated by an isoenzymatic technique. Tumour models were selected from a panel of 70 regularly growing tumours. Characteristics of the selected models are shown in Table 1. All of the tumours responded to at least one drug [5].

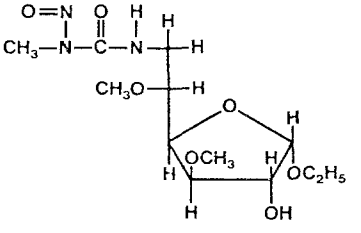

Fig. 1. Structure of CGP 6809: ethyl-6-deoxy-3,5-di-O-methyl-6-(3-methyl-3-nitrosoureido)-α-D-glucosofuranoside ($C_{12}H_{23}N_3O_7$)

Table 1. Selection of ten responsive human xenografts as tumor models for the secondary screening of new drugs

Tumor designation	Origin	Histology	Transplantation month/year	Doubling time (days)[a]	Start of therapy after weeks[b]	Effective reference drugs[c]
GXF 97	Stomach	moderately differentiated adenocarcinoma	5/79	9.1	3–4	MITO, CCNU, PLAT
GXF 209	Stomach	poorly differentiated adenocarcinoma	8/80	15.6	4	MITO, PLAT
GXF 218	Stomach	poorly differentiated adenocarcinoma	10/80	7.8	3–4	MITO, VIND
CXF 158	Rectum	poorly differentiated adenocarcinoma	12/79	12.1	3–4	CCNU
CXF 280	Colon	undifferentiated cancer	7/81	8.8	3	MITO, CCNU
LXF 211	Lung	epidermoid cancer	9/80	11.9	3–4	PLAT, VIND
LXF 322	Lung	epidermoid cancer	12/81	12.6	4	MITO, CCNU
SXF 81	Soft tissue	undifferentiated sarcoma	3/79	3.0	2–3	CY, CCNU
XF 117	Thyroid	anaplastic cancer	8/79	3.5	2	DTIC
XF 154	Skin	melanoma	11/79	6.2	3	

[a] In serial passage
[b] Median tumor area (a × b) 30–50 mm^2
[c] Partial response or complete remission

Tumour slices averaging 3 × 3 × 0.5–1 mm in diameter were implanted s.c. in both flanks of the animals. Tumour growth was recorded weekly by measuring two perpendicular diameters; the product of the two diameters was taken as the measure for tumour size. Relative tumour size values were calculated according to tumour size on day x divided by tumour size on day 0 at the time of randomisation. Mice were randomised to the untreated control or the test groups after 17–34 days, when the median product of the tumour diameters was 38 mm^2. Individual tumours suitable for randomisation had to demonstrate the following properties: a product of the two tumour diameters of at least 10 mm^2, and an estimated depth at least half that of the smaller diameter. In addition, tumours with a yellow colour reflecting a high amount of fibrous tissue were excluded. Using these criteria, we did not observe spontaneous regression or stationary growth behaviour in the untreated control groups after 3–4 weeks [6]. The tumour-take rate was about 90%. Each test group consisted of 5–6 animals displaying 6–10 evaluable tumours.

The anti-tumour effect of CGP 6809 was evaluated following maximal tumour regression; in non-regressing tumours, after 3–4 weeks. The effect of treatment was classified as remission (product of the two diameters, < 50% of the initial value), minimal regression (51%–75%), no change (76%–124%) and progression (≥ 125% of initial values). Furthermore, tumour size in the test/control group and tumour growth delay [17] were evaluated.

CGP 6809 was obtained from K. H. Schmidt-Ruppin, Ciba-Geigy Ltd. (Basel, Switzerland). It was synthesised in the Chemical Research Laboratories, Ciba-Geigy Ltd. (Basel, Switzerland) according to a described procedure (European Patent Specification EP 126; 6. 12. 1977). This agent has a molecular weight of 321.3, a melting point of 89°–90° C, an optical rotation (α) of ±42° ±1° (chloroform, c = 1.0122), and a UV absorption maximum of 227.0 nm (log P 0.31). The pure compound is stable and can be stored in a refrigerator.

The compound was freshly prepared daily (100 mg dissolved in 0.5 ml absolute ethanol and made up to volume with 9.5 ml aqua destillata). The LD_{50} values were rather high: 900 mg/kg after a single i.p. injection and 1,483 mg/kg after a single oral treatment (Canter and Mihich, personal communication). In rodent tumours, repeated daily applications were more effective than a high single-dose schedule. In the present study enteral and parenteral treatments were investigated, with a daily dose of 62.5 mg/kg injected i.p. 10–15 times over a period of 2 or 3 weeks; oral administration was carried out via stomach tube at a dose of 125 mg/kg per day over the same period.

Results

Toxicity

Oral therapy (125 mg/kg per day) with CGP 6809 was less toxic than i.p. injection (62.5 mg/kg per day). After 21 days, the lethality in tumour-bearing nude mice was 12% after oral and 22% after i.p. administration. The mortality of CGP 6809 in immuno-competent non-tumour-bearing NMRI mice was somewhat lower. The oral administration of 125 mg/kg per day given 15 times over 3 weeks resulted in the death of 2/16 mice (12%) and the i.p. treatment, in that of 1/16 (6%).

Anti-tumour activity

The anti-tumour activity of CGP 6809 is summarised in Table 2. Three of ten xenografts underwent remission (Figs. 2 and 3) and one tumour remained unchanged. The effect on both large bowel cancers was noteworthy, with one remission and one no-change. Partial remissions were also obtained in an epidermoid lung cancer and a thyroid cancer. In six xenografts the effectiveness of oral vs i.p. administration was compared: both treatment routes were equally effective. No statistical differences were found in

Table 2. Effectiveness of CGP 6809 given orally against human tumor xenografts compared with that of established drugs

Tumor designation	Tumor type	Median doubling time (days)	CGP 6809	ADR	MITO	CDDP	VIND	CCNU	CY
GXF 97	Stomach	9.1	−	−	++++	+++	++	++	+
GXF 209	Stomach	15.6	−	+	+++	++	±	+	−
GXF 218	Stomach	7.8	−	−	++	−	+	−	ND
CXF 158	Rectal	12.1	++	−	−	−	−	++	−
CXF 280	Colonic	8.8	±	−	+++	+	−	+++	+
LXF 211	Lung, epidermoid	11.9	−	−	ND	+	++	++	−
LXF 322	Lung, epidermoid	12.1	+++	−	++	−	−	++	−
XF 117	Thyroid	3.5	++	−	ND	±	+	−	−
XF 81	Soft tissue sarcoma	3.0	−	−	ND	−	−	+++	+++
XF 154	Melanoma	6.2	−	ND	ND	−	±	−	ND
Total complete + partial remissions			3/10	0/9	5/6	2/10	2/10	6/10	1/8

−, progression; ±, no change; +, minimal regression; ++, partial remission; +++, complete remission; ++++, complete remission for >4 months; ND, not done

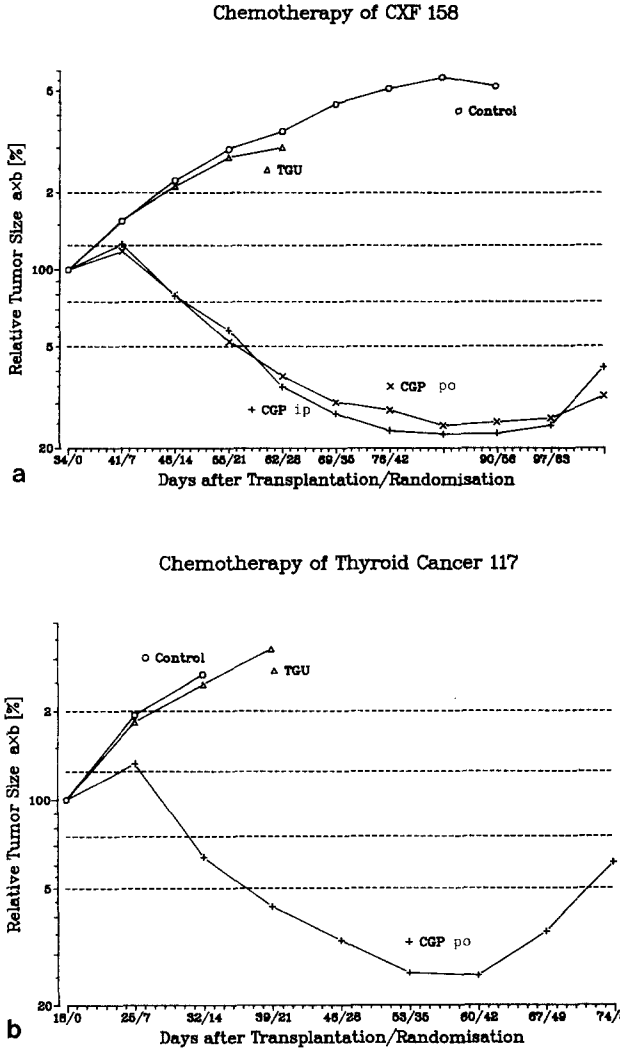

Fig. 2. Effect of CGP 6809 against rectal cancer CXF 158 (*a*) and thyroid cancer 117 (*b*). TGU (1,2,4-triglycidyl-urazol) is another experimental drug. The number of tumors on days 0 and 28, respectively, were: (*a*) for i.p. CGP 6809, 10 and 8 and for p.o. CGP 6809, 8 and 8; (*b*) for p.o. CGP 6809, 8 and 8

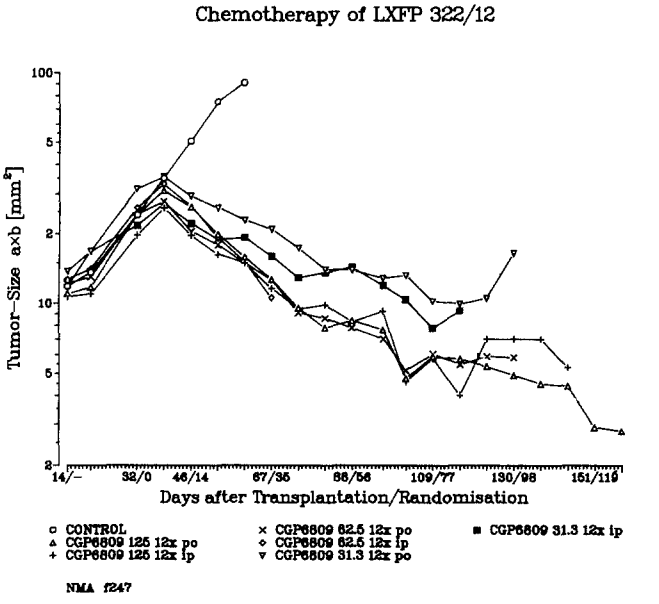

Fig. 3. Dose-response effect of CGP 6809 against the epidermoid lung cancer LXF 322. Treatment was given daily for 12 days; doses between 31.3 and 125 mg/kg effected remissions after p.o. and i.p. administration. The number of tumors ranged from 7 to 8 on day 0 and from 6 to 8 on day 28

either tumour size or growth delay. However, a dose double that used for i.p. injection was necessary to obtain a similar effect via the oral route.

Cross-resistance and collateral sensitivity

The response of the selected tumour models to six established drugs in comparison with CGP 6809 is shown in Table 2. No complete cross-resistance or collateral sensitivity was determined with any of the six agents. Of the four tumours responding to CGP 6809, three were also responsive to CCNU; of the six tumours resistant to CGP 6809, four responded to CCNU.

Discussion

The new nitrosourea derivative CGP 6809 was highly effective in several experimental tumours in mice and rats as well as in three of ten human tumour xenograft lines. In the responding tumours, daily treatment over 2–3 weeks was very effective. A single-dose schedule was not studied. The equitoxic dose for the oral route was about twice that for i.p. injection. Both treatment routes produced similar toxic and anti-tumour effects, suggesting a sufficient resorption of the compound. Clinical studies should be undertaken to investigate an i.v. and oral formulation.

The drug produced regressions in very rapidly as well as slowly growing tumours, suggesting that it does not react only on the rapidly growing fraction. It displayed no complete cross-resistance or collateral sensitivity with any of the six established drugs. In particular, its lack of complete cross-resistance with CCNU was noted; the thyroid cancer was sensitive to CGP 6809 and resistant to CCNU. Differences in alkylating and carbamoylating activity might be responsible for these varying effects.

With 2-phenyl-ethylamine as a substrate, CGP 6809 showed a clearly weaker alkylating activity than BCNU and CCNU that was comparable with that of streptozotocin. The carbamoylating activity of CGP 6809 with the same substrate was comparable with that of CCNU [15]. These differences in chemical reactivity may be responsible for the variations in LD_{50} as well as experimental antitumour activity in comparison with those of chloroethyl-nitrosoureas.

CGP 6809 and streptozotocin are structurally related to methyl-nitrosourea (MNU). MNU, streptozotocin and the chloroethyl-nitrosoureas BCNU and CCNU exhibit carcinogenic effects [16]. Differences in the degree of carcinogenic activity of chloroethyl-nitrosoureas were detected by Eisenbrand et al. [4]. In contrast to streptozotocin [8], the lack of a mutagenic effect for CGP 6809 by the Ames test (Oesch, personal communication 1984) suggests that the latter may not have carcinogenic potential; however, this question has not yet been studied experimentally.

The parent compound MNU exhibits side effects in clinical application similar to those of the chloroethyl-nitrosoureas, including delayed bone marrow and gastrointestinal toxicity [3]. An anti-tumour activity has been demonstrated in lung cancer, Hodgkin's disease, and melanomas [2] and seems to be similar to that of BCNU and CCNU. Streptozotocin has a different toxicity spectrum, with renal toxicity and severe nausea and vomiting being dose-limiting; however, bone marrow suppression has not been observed [3, 8]. The anti-tumour activity differed as well, with no activity in brain and large bowel cancer but high activity in islet cell pancreatic cancer and some activity in Hodgkin's disease. However, its lack of diabetogenic potential in rats and absence of mutagenic activity in comparison with that of streptozotocin suggest that CGP 6809 may have a different toxicity and anti-tumour spectrum.

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