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Characterization of the anti-tumour activity against solid tumours of a new nitrosoureido sugar: Cy 233

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Summary. The anti-tumour properties of Cy 233, a new nitrosoureido sugar, were investigated in two murine solid tumours: B16 melanoma and subcutaneously implanted colon adenocarcinoma. Injected i.v., Cy 233 exerted a strong anti-tumour effect against the established B16 melanoma: long-term survivors were recorded with all schedules of treatment. The drug was even more effective against advanced colon 38 adenocarcinoma: it produced a high percentage of total tumour regression, regardless of the route of administration (i.p., i.v., p.o.). The marked in vivo activity of Cy 233 against advanced colon 38 adenocarcinoma, which is known to be resistant to such major anti-cancer drugs as BCNU and chlorozotocin, its water solubility and its stability in aqueous media are further elements warranting toxicological and clinical studies of this agent.

Introduction

During the last decade, many efforts have been devoted to the development of new analogues of known anti-cancer drugs in the hope of reducing side effects and increasing therapeutic effectiveness. These new analogues include nitrosourea derivatives that are known to be experimentally very active and clinically very toxic. However, agents with similar chemical structures, such as nitrosourea analogues, may differ both quantitatively and qualitatively in their mode of action, toxicity and anti-tumour activity [5, 13, 15].

Lipophilic nitrosourea derivatives such as BCNU, CCNU and methyl-CCNU have shown similar toxicity and anti-tumour activity in animal models [7, 14, 18]. Hydrophilic derivatives such as chlorozotocin, GANU and MCNU share the structural property of a glucose moiety, although the position to which the *N*-(2-chloroethyl)-*N*-nitrosoureido group is attached differs from one molecule to another, which may change their pharmacological characteristics [1, 15]. Other sugar analogues with reduced bone marrow toxicity have shown strong anti-tumour effects in experimental models [3, 10, 20]. Fox et al. [4] have also reported that carbamoylating activity may differ from one derivative to another, which may change the therapeutic index. Recently, large cells of lung carcinoma and adenocarcinoma objectively responded to a hydrophilic nitrosourea derivative, TCNU [16], which apparently possesses unusual pharmacological characteristics [11, 16].

In our laboratories, methyl 3-[3-(2-chloroethyl)-3-nitrosoureido]-2,3-dideoxy-α-D-*arabino*-hexopyranoside (Cy 233; NSC-609224) was found to be very potent against L1210 leukaemia, with a large therapeutic index, and showed stronger alkylating properties than BCNU, CCNU and RFCNU, with a lower carbamoylating potential [8, 12]. Since all nitrosoureas are highly active against L1210 leukaemia, better discriminating models using advanced and refractory diseases should be investigated to characterize their structures. The present report describes the therapeutic effectiveness of Cy 233 against established B16 melanoma and advanced colon 38 adenocarcinoma in mice.

Materials and methods

Drugs. BCNU and TCNU were kindly donated by the Drug Research and Development (DR & D) Division of Cancer Treatment (DCT, NCI, Bethesda, Md). Cy 233 was supplied by Institut Choay, Montrouge, France. Its structure is shown in Fig. 1. Drugs were dissolved in saline and freshly prepared in a volume of 0.01 ml/g body weight prior to their administration to tumour-bearing mice. Schedules and routes of treatment are indicated in Tables 1–6.

Animals and tumours. B16 melanoma (B16) and colon 38 adenocarcinoma (Co 38) were maintained in syngeneic adult C57BL/6 mice. For evaluation of the anti-tumour activity of Cy 233, the B16 tumour was subcutaneously (s.c.) implanted on day 0 in B6C3F1 (C57BL/6 × C3H) male mice weighing 20–23 g. After 1 g tumour was mixed with 9 ml cold balanced salt, the preparation was homogenized and each mouse was inoculated with 0.5 ml tumour homogenate. The Co 38 tumour was also implanted s.c. in male or female BD2F1 (C57BL/6 × DBA/2) mice. A tumour fragment measuring approximately ($3 \times 3 \times 2$ mm^3) was implanted with a 12-gauge trocar into the flank of each mouse on day 0. Standard NCI protocols [6], with minor modifications [2], were followed for maintaining, transferring and implanting the tumours. The number and sex of the mice are indicated in Tables 1–6.

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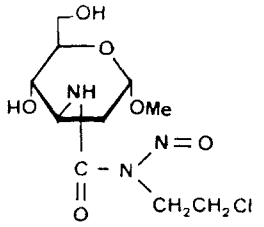

Fig. 1. Structure of Cy 233

Table 1. Anti-tumour activity of Cy 233 against established B16 melanoma

Dose mg/kg)	TI% (tumour-free mice on day 30/total)		ILS %	LTS on day 60/ total
20	toxic	toxic	−38	0/10
15	94	4/10	102	4/10
10	88	0/10	63	2/10
BCNU 20	67	0/10	98	4/10
Controls	0	0/21	0	0/21

The tumour was implanted s. c. on day 0 in male B6C3F1 mice (19–21 g); drugs were given i. v. on days 3, 7 and 11. TI, tumour inhibition; ILS, increase in life span; LTS, long-term survivors

Table 2. Influence of the treatment schedule on the therapeutic effectiveness of Cy 233 on B16 melanoma

Schedule	Optimal dose	Median survival (days)	ILS[a] %	LTS on day 60/ total
q6h × 2; day 3 (9h, 15h)	15	59	88	8/10
q1d × 1; day 3	20	49	56	4/10
q1d × 5 (days 3–7)	5	54	72	4/10
q4d × 4 (days 3, 7, 11, 15)	10	53	69	5/10
q7d × 3 (days 3, 10, 17)	10	53	69	3/10
Controls	0	31.3	0	0/30

Male B6C3F1 mice were implanted s. c. with B16 melanoma on day 0; i. v. treatment started on day 3, when the tumour was measurable.
[a] Long-term survivors (LTS) were excluded in the calculation of the increase in life span (ILS)

Anti-tumour activity. For the survival experiments, the anti-tumour activity of the drugs was assessed from two parameters: (a) the median survival of drug-treated mice *vs* that of one saline-treated control, expressed as the percentage of increase in life span (% ILS = T/C% − 100), calculated from the median survival of treated and control mice; and (b) the incidence of long-term (60 days) survivors.

For the tumour-growth inhibition studies, anti-cancer activity was assessed on the basis of the percentage of tumour inhibition (% TI) calculated from median tumour weights in treated and control mice on the day of evaluation, as indicated in Tables 3–6. Tumour weights were derived from caliper measurements of the tumours, using the formula for a prolate ellipsoid: tumour weight (mg) = $(L \times W^2)/2$, where L and W are the tumour length and width (mm), respectively. The % TI was calculated as 100 − [(median tumour weight in treated mice/median tumour weight in controls) × 100]. The number of tumour-free mice in the group on the day of evaluation was recorded. Tumour regressions were indicated when necessary in the advanced tumour system.

Table 3. Anti-tumour activity of Cy 233 against early-stage colon 38 adenocarcinoma

Drugs	Dose (mg/kg)	Median tumour/ weight on day 20 (mg)	TI[a] (%)	Tumour-free mice on day 20/ total
Cy 233	20	0	100	4/9
	10	600	64	0/9
	5	864	48	0/9
BCNU	20	1,648	0	0/10
5-FU	70	358	79	0/10
Controls	0	1,648	0	0/26

Tumour fragments were implanted s. c. on day 0 in male BDF1 mice weighing 21–24 g; drugs were given i. p. on days 2 and 9
[a] Tumour growth inhibition was evaluated on day 20

Table 4. Comparative anti-tumour effect of Cy 233, TCNU and BCNU against colon 38 carcinoma

Drugs	Optimal dose (mg/kg)	TI[a] (%)	Tumour-free on day 20/ total
Cy 233	20	100	3/10
TCNU	15	100	5/10
BCNU	30	45	0/10
Control[b]	0	0	0/30

Male BDF1 mice were implanted s. c. with Co 38 tumour fragments on day 0; drugs were given i. p. on days 2 and 9
[a] Tumour growth inhibition was evaluated on day 20
[b] The median tumour weight in control mice was 800 mg on day 20

Results

Activity against established solid B16 melanoma

To ascertain whether or not the antineoplastic activity of Cy 233 was limited to murine leukaemia and early treated, i. p. implanted tumour, the effect of a relatively late administration of this compound on s. c. B16 melanoma was investigated. Cy 233 was injected i. v. on days 3, 7 and 11 post-implantation at daily doses of 20, 15 and 10 mg/kg per injection. For comparison, 20 mg/kg BCNU was given by the same route and schedule as was Cy 233. After randomization of the mice, the median tumour weight in treated and control groups at the start of the treatment was 32 mg; that in the control group on the day of evaluation (day 30) was 7,812 mg.

As shown in Table 1, 20 mg/kg Cy 233 given three times was toxic; compared with that in controls, survival in this group was reduced. The administration of

Table 5. Comparative anti-tumour activity of Cy 233 and TCNU against advanced-stage colon 38 carcinoma

Drug doses (mg/kg)		Route of treatment	Median tumour weight (mg) on days		TI% on days	
			20	27	20	27
Cy 233	20	i.p.	2	0	100 (4/8)[a]	100 (5/8)[a]
	15		18	264	97 (0/8)	80 (2/8)
	10		18	196	97 (0/8)	85 (0/8)
Cy 233	20	i.v.	0	0	100 (5/5)	100 (4/5)
	15		4	0	100 (4/8)	100 (6/8)
	10		75	192	87 (7/8)	85 (2/8)
TCNU	20	p.o.	2	6	100 (4/8)	100 (1/8)
	15		88	41	85 (0/8)	90 (0/8)
	10		331	613	42 (0/8)	53 (0/8)
Controls	0		568	1,295	0 (0/24)	0 (0/24)

Tumour fragments were implanted s.c. on day 0 in female CDF1 mice (19–22 g); drugs were given on days 8, 12 and 16. The median tumour weight before randomization on day 8 was 12 mg (range, 8–40 mg)
[a] Numbers in parentheses indicate the number of tumour-free mice/total number in the group on the day of evaluation

15 mg/kg produced a high increase in life span (ILS = 102%), with four of ten mice surviving on day 60. When tumour inhibition was evaluated in this group, four of ten mice were free of tumour on day 30, which indicated tumour regression. BCNU could increase survival (ILS = 98% at 20 mg/kg), but no tumour regression was recorded. We observed only tumour growth inhibition, and all mice in the group had tumours on day 30.

Information on the influence of the schedule of treatment when the drug was given i.v. to mice bearing s.c. B16 melanoma is given in Table 2. CY 233 exerted significant activity on a variety of treatment schedules. The highest number of 60-day survivors was recorded with the i.v. administration of 15 mg/kg twice daily on day 3 post-implantation.

Activity against colon 38 adenocarcinoma

At an early stage of the disease, drug treatment was given i.p. on days 2 and 9 post-implantation. The activity of Cy 233 was compared with that of BCNU and 5-fluorouracil (5-FU). The results of this experiment are shown in Table 3. The median tumour growth inhibition following the administration of 20 mg/kg Cy 233 reached a maximum of 100%, and four of nine mice were tumour-free on the day of evaluation (day 20 post-implantation). BCNU was totally inactive in this experiment. 5-FU given at 70 mg/kg could induce a significant tumour growth inhibition (79%), but all mice in this group had tumours on the day of evaluation.

In a second experiment, the efficacy of Cy 233 against early-stage Co 38 was compared with that of TCNU, a nitrosourea currently undergoing clinical trials. The effectiveness of Cy 233 against Co 38 was confirmed in this experiment, in which TCNU produced similar activity and that of 30 mg/kg BCNU was only marginal (Table 4).

To ascertain that the antineoplastic activity of Cy 233 was not limited to the i.p. route of administration and early treatment, the effect of the drug was investigated following i.v. administration in mice with advanced-stage disease. Cy 233 was injected either i.p. or i.v. on days 8, 12 and 16 post-implantation. For comparison, oral TCNU was also given in this experiment. The results are shown in Table 5.

The median tumour weight in all animals at the start of the treatment was 12 mg; that in control animals was 568 mg on day 20 and 1,295 mg on day 27. When given i.p. at 20 mg/kg on days 8, 12 and 16, Cy 233 completely in-

Table 6. Therapeutic effect of oral Cy 233 against advanced-stage colon 38 carcinoma

Drug doses (mg/kg)		Median tumour weight (mg) on days				TI% on days		
		9	20	27	35	20	27	35
Cy 233	30	52	0	T	T	100 (6/7)[a]	T (2/2)[a]	T (1/2)[a]
	20	40	0	0	0	100 (5/8)	100 (5/8)	100 (5/8)
	10	74	106	473	1,000	89.2 (0/8)	81.4 (0/8)	80.2 (0/8)
TCNU	20	40	32	88	136	96.4 (3/8)	96.5 (3/8)	97.3 (2/8)
BCNU	40	76	221	446	608	75.4 (1/7)	82.5 (0/5)	88 (0/5)
Controls		63	900	2,548	5,054	0 (0/17)	0 (0/15)	0 (0/11)

Tumour fragments were implanted s.c. on day 0 in male BDF1 mice (18–23 g); drugs were given orally on days 9, 12 and 15. The median tumour weights and percentage of tumour inhibition (TI%) were evaluated on the days indicated
[a] Numbers in parentheses indicate the number of tumour-free mice/total number in the group on the day of evaluation

hibited tumour growth in four of eight mice on day 20 and, 1 week later, tumour regression was observed in another mouse. At lower doses, the therapeutic effectiveness was less pronounced. When injected i.v., 20 mg/kg Cy 233 given on days 8, 12 and 16 was lethal in three mice that died before day 20, whereas tumour regression was observed in the other five animals. At a dose of 15 mg/kg given i.v. on the same schedule, total tumour regression was observed in four of eight mice on day 20, and six of eight animals were tumour-free on day 27. TCNU given orally on the same schedule was very effective in inhibiting tumour growth at 20 mg/kg, but no tumour regression was observed between days 20 and 27. On the contrary, tumours started to re-grow in three of four mice. At lower doses the drug was less active.

In a final experiment in which Cy 233 was given orally, as were BCNU and TCNU on days 9, 12, and 15 post-implantation, the therapeutic effectiveness of the former was confirmed. Table 6 shows that 30 mg/kg Cy 233 was toxic, as six of eight mice died before day 20. However, no sign of toxicity was observed, and the therapeutic effectiveness at 20 mg/kg was very high, since the median tumour weight regressed from 40 mg on day 9 to 0 on day 20. Moreover, tumour regression lasted until day 35 (the last day of evaluation), when five of eight mice were tumour-free. TCNU showed slightly lower therapeutic effectiveness under the same conditions, and BCNU given at the maximal tolerated dose of 40 mg/kg was the least active; no tumour regression was observed.

Discussion

The present results show that Cy 233 has high antineoplastic activity in established murine tumours. Among these cancers, s.c. implanted B16 melanoma is a slow-growing tumour that metastasizes rapidly. Although this tumour model is considered to be refractory to chemotherapeutic agents [9], it was highly sensitive to Cy 233. There was a good correlation between the percentage of increase in life span (ILS = 102%) and tumour growth inhibition, which was observed in four of eight mice on day 20. In this tumour model, Cy 233 given i.v. showed high therapeutic activity on all schedules used. Although the q 6 h $\times 2$ schedule given for only 1 day induced the highest number of long-term survivors, we could not ascertain whether the effectiveness of the drug was schedule-dependent.

Another solid tumour known to be one of the most refractory to most compounds screened against it, including many nitrosoureas, colon 38 carcinoma [16, 19] was very sensitive to treatment with Cy 233. Chlorozotocin, which has a very similar chemical structure, could not exert a reproducible anti-tumour effect even after early treatment of Co 38, further evidence that two nitrosoureas with a similar structure may differ in their anti-tumour activity and pharmacological behaviour. An additional advantage is that Cy 233 exerts its anti-tumour activity against s.c. colon 38 carcinoma even when the treatment is delayed, regardless of the route of administration.

Staquet et al. have also reported that activity of a drug in the colon 38 model would increase the probability of its clinical activity [17]. This appears to be of practical relevance and is a further element warranting further studies involving the selection of drugs showing activity against naturally resistant and advanced solid tumours. Indeed, it would be of great interest to find out whether compounds producing high activity against a refractory solid tumour such as Co 38 would be more or less effective against human solid-tumour xenografts than agents selected on the sole basis of their activity against sensitive P388 leukaemia.

The investigation of the effectiveness of Cy 233 against human xenografts in nude mice, including a number of malignant melanomas and colon adenocarcinomas, is under way, as are toxicological studies to find out whether the chemical modification in this structure would reduce the side effects usually encountered with this group of agents. In conclusion, the high effectiveness of Cy 233 consistently observed in advanced tumours as well as its hydrosolubility and chemical stability in aqueous solutions appear to justify interest in this compound.

References

1. Aoshima M, Sakurai Y (1977) Comparative studies on the antitumor activity and the bone marrow toxicity of 1-(β-D-glucopyranosyl)-3-(2-chloroethyl)-3-nitrosourea and 2-[3-(2-chloroethyl)-3-nitrosoureido]-D-glucopyranose. Jpn J Cancer Res 68: 247
2. Atassi G, Briet P, Berthelon J, Collonges F (1985) Synthesis and antitumor activity of some 8-substituted 4-oxo-4H-1-benzopyrans. Eur J Med Chem-Clin Ther 20: 393
3. Idanami K, Komiyama K, Kuroda T, Umezawa I (1984) Antitumor activity of a nitrosourea derivative, CNUA, on murine tumors. Cancer Chemother Pharmacl 13: 22
4. Fox PA, Panasci LC, Schein PS (1977) Biological and chemical properties of 1-(2-chloroethyl)-3-(β-D-glucopyranosyl)-1-nitrosourea (NSC-254157), a nitrosourea with reduced bone marrow toxicity. Cancer Res 37: 783
5. Fujimoto S, Ogawa M (1982) A new nitrosourea derivative TA-077, 1-(-2-chloroethyl)-3-isobutyl-3-(β-maltosyl)-1-nitrosourea: I. Comparative study on anti-tumour activity. Cancer Chemother Pharmacol 9: 134
6. Geran GI, Greenberg NR, Macdonald MM, Schumacher AM, Abbott BJ (1972) Protocols for screening chemical agents and natural products against animal tumours and other biological systems. Cancer Chemother Rep 3: 11
7. Gorbacheva LB, Kukuschkina GV (1970) Some aspects of the mechanism of action of 1-propyl-1-nitrosourea. Biochem Pharmacol 19: 1561
8. Gosse C, El Abed I, Gouyette A, Atassi G, Ardouin P, Roger P (1988) Preliminary pharmacological properties and chemical stability of Cy 233 (NSC-609224). Abstract of the 8th International Symposium on Future Trends in Chemotherapy, Tirrenia, Italy, March 1988
9. Griswold DP Jr (1972) Consideration of the subcutaneously implanted B16 melanoma as a screening model for potential anticancer agents. Cancer Chemother Rep 3: 315
10. Komiyama K, Edanami K, Kuroda T, Umezawa I (1981) antitumor effect of 3-[3-(2-chloroethyl)-3-nitrosoureido]-3-deoxy-D-glucopyranose on murine tumors. Jpn J Cancer Res 72: 53
11. Molineux G, Schofield R, Testa NG (1987) Hematopoietic effects of TNCU in mice. Cancer Treat Rep 71: 837
12. Roger P, Monneret C, Fournier JP, Choay P, Gagnet R, Gosse C, Letourneux Y, Atassi G, Gouyette A (1989) Rationale for the synthesis and preliminary biological evaluation of highly active new antitumor nitrosoureido sugars. J Med Chem 32: 16
13. Sasaki K, Aisawa S, Satomi T, Akutsu H, Kawabata S, Momoki Y (1980) Synthesis and antitumor activity of *N*-nitrosoureido derivatives of kanosamine. J Antibiot 33: 517

14. Schabel FM Jr, Johnston TP, McCaleb GS (1963) Experimental evaluation of potential anticancer agents: VIII. Effect of certain nitrosoureas on intracerebral L1210 leukemia. Cancer Res 23: 725
15. Sedido S, Ninomeya K, Iwasaki M (1979) Biologic activity of MCNU: a new antitumor agent. Cancer Treat Rep 63: 961
16. Smyth J, Warington P, Cornbleet M, Leonard R (1985) Phase one trial of a novel nitrosourea, TCNU. Third European Conference on Clinical Oncology and Cancer Nursing, June 16–20 Stockholm, p 8
17. Staquet MJ, Byar DP, Green SB, Rozencweig M (1983) Clinical predictivity of transplantable tumor systems in the selection of new drugs for solid tumors. Rationale for a three-stage strategy. Cancer Treat Rep 67: 753
18. Sugiura K (1967) Effect of 1,3-bis(2-chloroethyl)-1-nitrosourea (NSC-409962) and two related compounds on a spectrum of tumors. Cancer Res 27: 179
19. Venditti JM, Wesley RA, Plowman J (1984) Current NCI preclinical antitumor screening in vivo: results of tumor panel screening 1976–1982 and future directions. In: Garattini S, Goldin A, Hawking F (eds) Advances in pharmacology and chemotherapy 20. Academic Press, New York, pp 1–20
20. Vibe-Petersen J, Bork E, Moller H, Hansen HH, Cornbleet M, Smyth J (1986) Early clinical experience with TCNU – a new water-soluble nitrosourea compound. Proc Am Soc Clin Oncol 5: 31

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