Cancer Chemother Pharmacol (1989) 24: 311–313

Cancer Chemotherapy and Pharmacology

Absence of cross-resistance between two alkylating agents: BCNU vs bifunctional galactitol

Etel Institóris[1], Károly Szikla[1], László Ötvös[2], and Ferenc Gál[1]

[1] National Institute of Oncology, 1122 Budapest, Ráth Gy. u. 7–9, Hungary
[2] Central Research Institute for Chemistry, Hungarian Academy of Sciences, 1525 Budapest, Pusztaszeri-u. 57–59, Hungary

Summary. Dianhydrogalactitol (DAG) increased the life span of both BCNU-sensitive and -resistant L1210 tumor-bearing mice. However, the BCNU-resistant strain showed slightly lower sensitivity against DAG, which could be overcome by an increase in drug dose of ca. 20%. The somewhat lower sensitivity was proportional to a slightly reduced DNA cross-linking formation induced by DAG in BCNU-resistant cells. The amount of DNA cross-links was determined by measurement of the 1,6-di(guaninyl)-galactitol content of DNA. The slight reduction in cross-links is not attributable to DNA repair but rather to other factors that seem to prevent the formation of DNA-drug adducts. The absence of cross-resistance is explained by different kinds of DNA damage caused by the two alkylating agents and the presumably different defense mechanisms developed by cells against these lesions.

Introduction

In the past it has generally been assumed that cross-resistance occurs among alkylating agents. However, some recent observations have revealed the absence of cross-resistance among certain alkylators [6, 20]. We studied the possibility of cross-resistance and DNA cross-linking formation induced by dianhydrogalactitol (DAG) in BCNU-sensitive and -resistant strains of L1210 leukemia. DAG exhibits antitumor activity in a wide spectrum of experimental rodent tumors [18], and its usefulness in clinical practice has also been established [1, 4, 5, 9]. The tumor inhibitory effect was ascribed to its capability to cross-link DNA [11, 14] forming 1,6-di(guanin-7-yl)galactitol [10, 12]. The DNA cross-linking formation in both strains was followed by the measurement of diguaninyl moieties.

Materials and methods

Materials. 1,2:5,6-Dianhydro-[1-^{3}H]-galactitol (spec. act. 580 μCi/mg; 21.46 mβg/mg) and unlabelled dianhydrogalactitol (DAG) were supplied by Chinoin Pharmaceutical Work (Budapest, Hungary). The reference compounds used in the present study, namely, 7(1-deoxygalactit-1-yl)guanine, 7(1-deoxy-3,6-anhydrogalactit-1-yl)guanine, and 1,6-di(guanin-7-yl)-1,6-dideoxygalactitol were synthesized as previously described [12, 13]. For column chromatography, Sephadex G-10 (Pharmacia Uppsala, Sweden) and the H^+ form of Dowex AG (50 W X 4) (Bio-Rad Labs, Ltd; Richmond, Calif) were used.

Animal experiments. The BCNU-resistant strain of L1210 leukemia was originally obtained from Dr. A. E. Bogden, NCI, E. G. & G. Mason Research Institute (Worcester, Mass), and the standard L1210 leukemia was supplied by Dr. D. Gericke, Hoechst AG (Frankfurt am Main, FRG). Groups of BDF_1 mice, each comprising six animals, were inoculated i.p. with 10^6 cells of BCNU-sensitive or -resistant strains of L1210 leukemia. In the survival studies, mice bearing the two tumor strains were injected i.p. with single doses of BCNU or DAG on the 2nd day after transplantation. Sensitivity to drugs was evaluated on the basis of survival in days (T/C × 100%).

Assay of DAG-DNA adducts. The amounts of cross-linked DNA adducts and monoadducts were determined as previously reported [10, 13]. Mice bearing L1210 leukemia sensitive and resistant to BCNU were given single i.p. injections of [^{3}H]-DAG on the 3rd day following tumor inoculation. DNA was extracted by phenol [17] from the ascites cells and hydrolyzed at 100° C for 30 min at neutral pH to release 7-alkylated guanines and 3-alkyladenine. Nonradioactive marker compounds synthesized as previously described [12, 13] were added to the DNA hydrolysate; on a column of Sephadex G-10, the 7-alkylated guanines were separated from the partially apurinized DNA and 3-alkyladenine as previously reported [10, 13]. Fractions containing 7-alkylated guanines were rechromatographed on a column of Dowex (H^+ form) using 1–2 *M* HCl as an eluent. All radioactivity applied to the column distributed in three peaks, coinciding with the appropriate markers, namely, 7-galactitylguanine, 7-(anhydrogalactityl)guanine and 1,6-di(guaninyl)galactitol. The radioactivity of all evaporated fractions except those eluted with 2 *M* HCl was measured in aqueous Bray's solution. The latter were dissolved in NH_4OH and, after drying, burnt to tritiated water in a C306 Tri-Carb sample oxidizer (Packard) and measured in a liquid scintillation spectrometer.

Results

Animal experiments

DAG administration increased the life span of both BCNU-sensitive and -resistant L1210 tumor-bearing mice.

Offprint requests to: E. Institóris

Table 1. Effects of BCNU and DAG on BCNU-sensitive and -resistant L1210 leukemia

Agents	Doses (mg/kg i.p.)	L1210/S (T/C %)	L1210/R (T/C %)
BCNU	20	254	105
	35	401	98
DAG	6.0	175	156
	7.5	198	176
	9.0	231	210

T/C %, life span of the treated group (days)/life span of the control group (days) × 100

The maximum tolerable dose of DAG was 9 mg/kg, with higher doses resulting in occasional deaths due to toxicity. The sensitivity of the two strains to DAG was not completely equal (Table 1). To evoke the same response in both strains, it was necessary to increase the DAG dose by ca. 20% in the treatment of animals bearing BCNU-resistant L1210 leukemia.

Estimation of DNA-DAG adducts

Three guanine derivatives were identified in the DNA of sensitive and resistant cells: 7-galactityl-guanine(I) and 7-anhydrogalactityl-guanine(II) as monoadducts and 1,6-di(guanin-7-yl)galactitol(III) as cross-linked DNA adducts (Table 2). Equal doses of DAG produced somewhat lower guanine alkylation in the DNA of resistant cells than in that of sensitive cells (Table 2). The reduced alkylation level was proportional to the somewhat lower antitumor effect exerted by DAG on the BCNU-resistant strain.

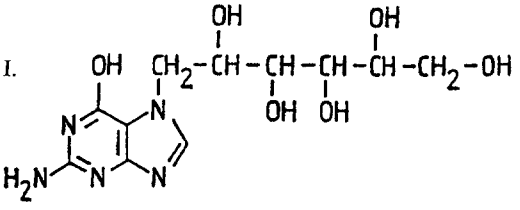
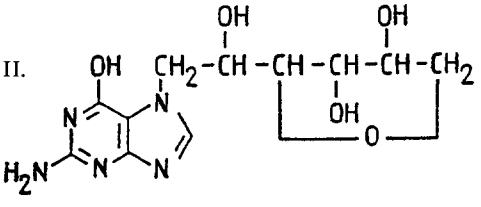
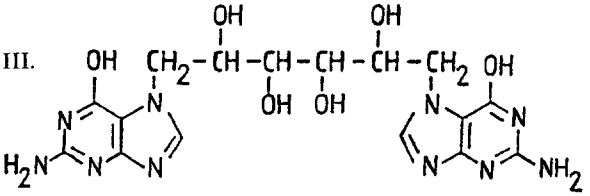
In the time-course study, the amount of diguaninyl moieties was related to the sum of the 7-monoalkylguanines as relatively stable alkylated products in DNA. The ratio of monoG:diG remained unchanged in both cell types, showing the lack of preferential removal of diguaninyl moieties from DNA (Table 2). The level of monoadducts in DNA during the observation period also proved to be constant, indicating the very similar degree of inhibition of DNA synthesis by DAG in both sensitive and resistant cells.

Discussion

The cross-resistance demonstrated among some alkylating agents [2, 3, 7, 8, 16, 21, 23] cannot be generalized since the mechanism of resistance developed by various alkylators can be qualitatively different. Resistance to BCNU involves a specific repair enzyme, dealkylating O6-alkylguanine [19], whereas resistance to DAG is ascribed to the "unhooking" DNA-repair mechanism Institóris et al., submitted for publication. The diversity of these mechanisms accounts for the lack of cross-resistance between the two agents studied.

The somewhat lower sensitivity of the BCNU-resistant strain to DAG was proportional to a slightly reduced cross-linking formation in DNA. The reduction in cross-

Table 2. Amounts of DNA-DAG adducts induced in BCNU-sensitive and -resistant L1210 leukemia cells by two different doses of [^{3}H]-DAG after i.p. injection

	Extent of alkylation (μmol alkylated guanine/10 mol DNA-P):					
	Sensitive			Resistant		
Doses	7.5 mg/kg	9 mg/kg		7.5 mg/kg	9 mg/kg	
Time after treatment (h)	6	6	24	6	6	24
I. 	1.66	2.1	2.0	1.56	1.84	1.75
II. 	1.34	1.6	1.48	1.09	1.33	1.40
III. 	0.68	0.82	0.79	0.59	0.70	0.71
$\frac{I + II}{III}$	4.41	4.51	4.40	4.49	4.53	4.43

links cannot be the consequence of an increased repair activity, since throughout the observation period the level of monoalkylguanines was slightly lower in resistant than in sensitive DNA. This phenomenon may be associated with some process in the resistant strain that slightly decreases the amount of drug capable of interacting with DNA.

Acknowledgements. This work was supported by grant 1012 from the National Scientific Research Fund in Hungary.

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Received 10 May 1988/Accepted 17 January 1989