Cancer Chemother. Pharmacol. 3, 217–221 (1979)

Cancer Chemotherapy and Pharmacology

Original Articles

Comparative Pharmacology of Three New Nitrosourea Analogues: RFCNU, RPCNU, and Chlorozotocin

I. Oncostatic Effects in Mice

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Summary. *We have compared the LD_{50} of three new nitrosourea derivatives, RFCNU, RPCNU, and chlorozotocin (CZT), in L1210 leukemia-bearing mice: CZT is the most toxic and RFCNU the least.*

We studied the oncostatic effect of different doses, varying from 5 mg to 150 mg/kg, given three times on the survival of mice bearing L1210 leukemia, and found a range of maximally efficient doses for these three compounds, while BCNU, CCNU and MeCCNU present only one maximally efficient dose (MED). The range of MED is greater for RFCNU.

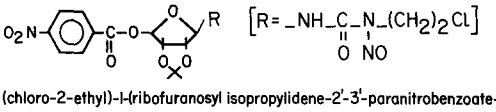
The oncostatic effects of these three nitrosourea derivatives on other tumors (E♂ G2 leukemia, C1498 myeloid leukemia, TM2 mammary tumor, Lewis Lung tumor, and B16 melanoma) were studied with the median dose of the range of the MED determined for L1210 leukemia: RFCNU is efficient in all tumors except TM2 and LLT; RPCNU is effective in all except E ♂ G2 and is lethally toxic for Lewis tumor-bearing mice; and CZT acts only on E ♂ G2 leukemia and not on any of the solid tumors tested.

No correlation was found between these effects and chemical parameters such as the alkylating activities of these three compounds. Further explanation will be given in subsequent papers devoted to hematopoietic and immunopharmacologic properties.

Introduction

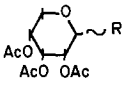
The available chloroethyl nitrosourea derivatives, which include BCNU, CCNU, and methyl CCNU, are an important family of oncostatic agents with a broad enough spectrum of activity [see 3, 4]. However, their contribution to cancer chemotherapy is limited. Firstly, they exhibit cumulative, hence long-term, bone marrow toxicity, demonstrated mostly by megacaryocyte cytostasis [4, 12], which considerably shortens the duration of their administration and/or reduces it to doses below the optimal oncostatic levels. Secondly, they exert a short-term immunosuppressive effect [6], which is now attracting more and more attention from chemotherapists, since at least some chemotherapies are less efficient in immunodepressed animals than in those with normal immune functions [8, 9]. This is also observed during the combined use of chemoimmunotherapy interspersion, where the effect of some oncostatics is decreased when an immunity adjuvant is applied before their administration [10].

RFCNU (Imbach-Montero) ICIG 1105



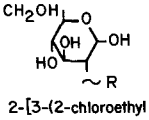
(chloro-2-ethyl)-1-(ribofuranosyl isopropylidene-2'-3'-paranitrobenzoate-5')-3nitrosourea

RPCNU (Imbach-Montero) ICIG 1163



(chloro-2-ethyl)-1-(ribopyranosyl triacetate-2'-3'-4')-3nitrosourea

CHLOROZOTOCIN (NSC 178248)



2-[3-(2-chloroethyl)3-nitrosoureido]D-glucopyranose

Fig. 1. Formulas of RFCNU, RPCNU, and chlorozotocin

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0344-5704/79/0003/0217/$ 01.00

Table 1. Alkylating activities of RFCNU, RPCNU, and chlorozotocin compared with that of CCNU

Name	Synthesis	Carbamoylating *activity: % carbamoylated 14C-lysine*	Alkylating *activity (% CNU)*	LD_{10} (μmol/kg)	$T_{1/2}$ (min)
CCNU	*Montgomery*	*90*	*10*	*171*	*117*
RFCNU	*Montero Imbach, 1974*	*38*	*40*	*210*	*25*
RPCNU	*Montero Imbach, 1974*	*45*	*111*	–	*12.5*
Chlorozotocin	*Montgomery, 1975*	*4*	*64*	*64*	*48*

These were the principal reasons for synthesizing new nitrosourea derivatives that would be less toxic to platelets and less immunosuppressive than the compounds currently available.

Taking as their main objective the synthesis of non-myelotoxic nitrosourea derivatives, Schein et al. [17] observed first that myelotoxicity in animals could be reduced by attachment of the cytotoxic group on the C-2 position of glucose. Then, to evaluate the influence of the glucose carrier on chloroethyl nitrosourea myelotoxicity, chlorozotocin tetracetate or 2-[3-(2-chloroethyl)-3-nitrosoureido]-2-deoxy-glucopyranose tetracetate was synthesized, and the same researchers [17] noted that this substance did not inhibit DNA synthesis in bone marrow. Subsequently they found the same property in a water-soluble form of this compound, chlorozotocin (CZT) or 2-[3-(2-chloroethyl)-3-nitrosoureido]-D-glucopyranose (Fig. 1) [1], which was synthesized by Montgomery's group [7].

Simultaneously, we used L1210 leukemia in mice to screen a series of derivatives synthesized by Imbach et al. [13, 14], in which the cyclohexyl group of CCNU was replaced by a sugar molecule [18].

When studying the correlation between the activity, expressed as the oncostatic index and the dose, we observed that the maximal effect was obtained over a range of doses for RFCNU [(chloro-2-ethyl)-1-(ribofuranosyl-isopropylidene-2′-3′paranitrobenzoate-5′)-3-nitrosourea] (Fig. 1) and RPCNU [(chloro-2-ethyl)-1-(ribopyranosyl triacetate-2′,3′,4′)-3-nitrosourea] (Fig. 1), while for the other nitrosourea derivatives, including BCNU, CCNU, and MeCCNU, this effect could only be obtained with a single dose [6].

All this led us to make a detailed comparison of the antitumor effects and toxicity of these two nitrosourea sugar derivatives, which are not hydrosoluble [6] and of CZT, which is hydrophilic [1, 16]. The alkylating activity, LD_{10} (μol/kg), and half time life ($T_{1/2}$) of each are given in Table 1 (P. S. Schein et al., personal communication; Gouyette, personal communication).

In a series of papers, we shall deal with the respective oncostatic actions of these compounds on several murine tumors, their immunopharmacology [5] and hematopharmacology [15], and, finally, their respective oncostatic and toxic effects in man, as observed in phase II clinical trials [2, 11].

Materials and Methods

1. L1210 Leukemia Test for Simultaneous Detection of the Oncostatic Action and the Evaluation of Acute LD_{50}

For each compound, 80 Fl ($DBA_2 \times C_{57}Bl_{10}$) mice were inoculated with 10^5 leukemia cells IP on day 0.

On day 1, 70 of them received 5, 10, 15, 20, 30, 40, 50, 60, 90, or 150 mg RFCNU, RPCNU, or CZT per kg body weight, each mouse being weighed individually. Compounds were injected IP as olive oil suspensions (RFCNU and RPCNU) or watery solutions (CZT).

On days 5 and 9, drug and placebo injections were repeated in mice with no macroscopic signs of toxicity. The mortality of mice was monitored daily and autopsies were performed to find out whether deaths were due to leukemia or to a toxic action of the drug. The acute LD_{50} for each compound was determined graphically, as indicated in Fig. 2.

The oncostatic effects of specified doses for each compound were expressed as I (oncostatic index) = T/C × 100 (T representing the median survival in the treated group of mice and C the median survival in the control group). When I > 125 and the difference between treated and control groups was statistically significant according to the nonparametric W test of Wilcoxon, the agent was considered active at the given dose.

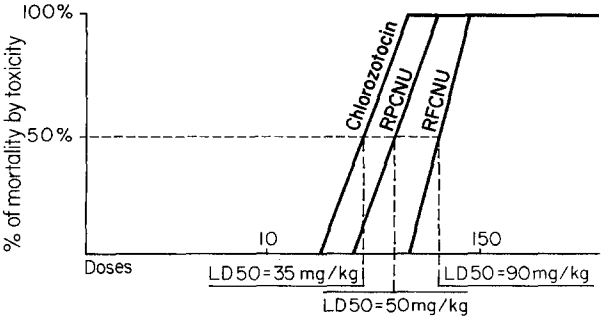

Fig. 2. Determination of the acute LD_{50} of the three nitrosoureas

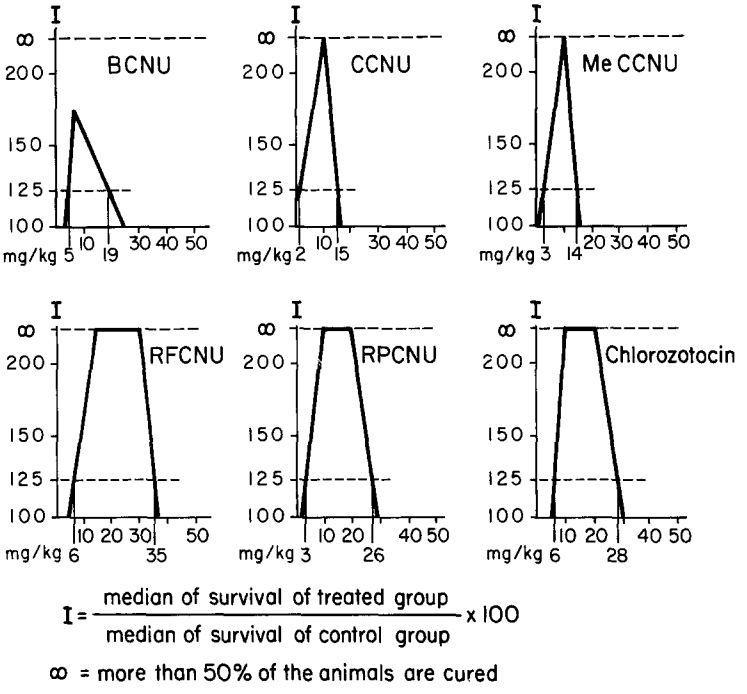

Fig. 3. The correlations of the oncostatic effects on L1210 leukemia of RFCNU, RPCNU, and chlorozotocin, and doses, compared with those of BCNU, CCNU, and MeCCNU

For each nitrosourea, a graph representing the correlation of the oncostatic effect and of the dose (mg/kg) was made. The value ∞ indicated that more than 50% of treated animals in the group concerned had been cured (Fig. 3).

2. Other Murine Tumors

Five other murine tumors were used: (a) E♂ G2 Gross virus-induced lymphoid leukemia: 5.10^5 cells were injected IP to $C_{57}Bl/10$ male mice; (b) C 1498 myeloid leukemia: 5.10^5 cells were inoculated SC to $C_{57}Bl/10$ mice; (c) Lewis lung carcinoma (LLT): 10^6 cells were injected IM to $C_{57}Bl/10$ mice; (d) TM2 mammary carcinoma: 10^6 cells were inoculated SC in C_3H/he male mice; (e) B16 melanotic melanoma: 0.2 ml tumor homogenate containing 50 mg tumor was implanted SC in $C_{57}Bl/10$ mice.

In each experiment, the tumor inoculum was injected on day 0 to 34 mice, which were divided at random into four groups: three groups received RFCNU, RPCNU, or CZT on days 1, 5, and 9. For each compound, the dose selected was the one that corresponded to the median of the MED range found in L1210 leukemia: 20 mg/kg for RFCNU and 15 mg/kg for RPCNU and for CZT. The drugs were administered i.p. as olive oil suspensions or watery solutions, and the control group received the placebo.

Mortality was noted daily.

Results were expressed as I (oncostatic index) = T/C × 100, and Wilcoxon's test was used for statistical evaluation.

Results

The acute LD_{50} dose for each of the three compounds is indicated in Fig. 2. As shown, it is 35 mg/kg for CZT, 50 mg/kg for RPCNU, and 90 mg/kg for RFCNU.

There is no real correlation between the alkylating activity and the oncostatic effect on L1210 leukemia, since the three new compounds have a higher alkylating activity than CCNU and all three of them possess a wide range of MED, while CCNU, MeCCNU, and BCNU each have only one MED.

The MED range of RFCNU was largest among the six compounds studied for activity on L1210 leukemia, as shown in Fig. 3.

The existence of a large range of MEDs offers an advantage, since it provides the possibility of modulating the doses to avoid the toxic effects without losing the maximal oncostatic effect.

To compare the oncostatic action of the three new nitrosoureas on other tumors, we used the medians of the intervals of the MED range, which seems a better parameter than a given dose, since it is oncobiologically determined. It gives each product its maximum chance of being active at a dose that is not toxic to mice bearing L1210 leukemia.

Table 2 shows that RFCNU is significantly oncostatic in all the tumors studied except TM2 mammary tumor and Lewis lung tumor, and that RPCNU also has a broad spectrum of activity, except on E♂ G2 leukemia and Lewis lung tumor: it is lethally toxic at the chosen dose for the mice bearing this tumor. This will be tentatively explained in one of the next papers, which will show that of these three nitrosoureas, only RPCNU is immunosuppressive [5].

CZT is only active on E♂ G2 leukemia and is not active in any of the solid tumors tested. This might be related to the preliminary clinical results we have already obtained [2].

Table 2. Oncostatic effects of RFCNU, RPCNU, and chlorozotocin on several leukemic and solid murine tumors

	RFCNU 20 mg/kg		RPCNU 15 mg/kg		Chlorozotocin 15 mg/kg	
	I[a]	Statistics[b]	I[a]	Statistics[b]	I[a]	Statistics[b]
L1210	∞	+++	∞	+++	∞	+++
E_{δ} G2	156	++		NS	156	++
C 1498 (myeloid)	135	+	153	+		NS
TM2 (mammary)		NS	134	+		NS
LLT (lung)		NS	Toxic			NS
B 16 (melanoma)	144	++	151	++		NS

[a] $I = \frac{\text{median survival of treated group}}{\text{median survival of control group}} \times 100$; ∞, over 50% of the animals in this group are cured

[b] Wilcoxon's nonparametric test: + $P = 0.05$; ++ $P = 0.01$; +++ $P \leqslant 0.001$; NS, not significant

Discussion

The comparative oncostatic toxicity on these tumors cannot be correlated, as in the case of L1210 leukemia, with the respective alkylating activities of the three compounds.

This observation is in accordance with that of Panasci et al. [16], who found no correlation between the alkylating activity of different nitrosourea derivatives and their oncostatic activities. Similarly, he found no correlation with the other chemical parameters.

It is worth noting that the least efficient compound, which is not active in any solid tumors, is the hydrosoluble CZT. Hence it will be of interest to study the pharmacokinetic characteristics of these three compounds, which might be very different for CZT than for the two others (which are not hydrosoluble).

In conclusion, one of the most frequently active compounds, RFCNU, has the lowest alkylating activity and is the least hydrosoluble.

Acknowledgement: This work has been conducted under the auspices of French-American Cooperation (NCI-INSERM).

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Received August 29, 1979