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Biological Activities of Rubidazone

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Summary. *Rubidazone (benzoylhydrazone daunorubicin) is a semisynthetic compound with a good experimental antitumor activity. Its main interest is its lower toxicity and cardiotoxicity. In human chemotherapy, it is considered a valuable drug.*

Introduction

Among the derivatives of the parent compound daunorubicin (DNR), rubidazone (benzoylhydrazone DNR: RBZ) [13] is one of the more interesting derivatives if we consider the chemotherapeutic index, the tumor spectrum, the lower cardiotoxicity, and the lower immunodepressive potency. Therefore we have compared the different biological activities of RBZ with those of DNR and of 14-hydroxydaunorubicin (doxorubicin: DXR or adriamycin) (Fig. 1).

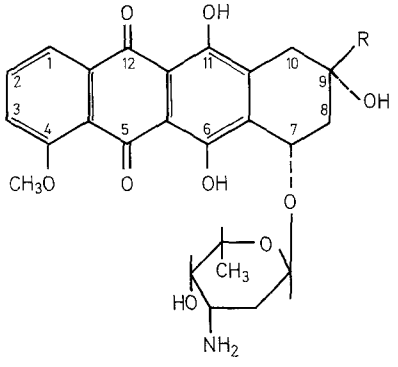

Fig. 1

Compound	–R	Molecular weight
Rubidazone N.S.C. 164 011	$-C(CH_3)=N-NH-C(=O)-C_6H_5$	645.671
Daunorubicin N.S.C. 82 151	$-CO-CH_3$	527.530
Doxorubicin (Adriamycin) N.S.C. 123 127	$-CO-CH_2OH$	543.510

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1. Cytotoxicity

A. As with the other anthracycline compounds, RBZ is a *cytostatic agent,* active at phases G_1 and S. As others [2] we have observed by pulse cytophotometry that RBZ induces a G_2 block (Fig. 2). Using KB cells in cul-

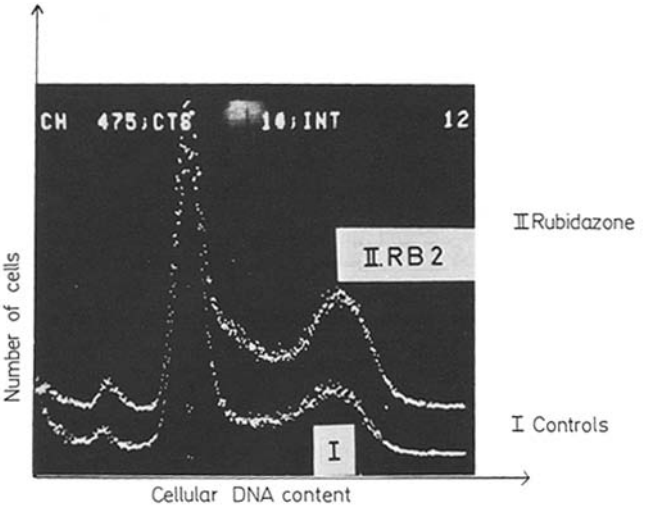

Fig. 2. Pulse cytofluorometry. Ehrlich ascitic tumor cell culture (30 h) cellular DNA content using propidium iodide (100,000 cells counted)

	$G_1 + O$	S	$G_2 + M$
I. Controls	60%	18%	22%
II. Rubidazone (1 nmol/ml)	30%	25%	45%

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ture, it is three to four times less toxic than the other compounds as it is shown in Table 1 (inhibiting concentration 50%: IC_{50}).

B. At the IC_{50}, the activity of the three compounds on *cell nucleic acid synthesis* was studied with KB cell culture according to the following protocol:
KB cell culture on day 0;
compound added on day 2;
cell harvest at different times;
cell pulsed with labeled precursor (1 μCi/ml [methyl-^{3}H] thymidine, 25 Ci/mmol or [5-^{3}H] uridine 24 Ci/mmol) 1 h before cell harvest.

The three compounds were found to inhibit DNA synthesis, RBZ being the *slowest* inhibiting agent (Table 2). The inhibiting activity on RNA synthesis was less than that for DNA synthesis. Neither RBZ nor the other compounds had an effect on cell protein synthesis.

Table 1. Cytotoxic activities (KB cells)

Compound		IC_{100}	IC_{50}
Rubidazone	μg/ml	4.0	0.5–1.0
	nmol/ml	5.8	0.7–1.4
Daunorubicin	μg/ml	0.5–1.0	0.3
	nmol/ml	0.9–1.8	0.5
Doxorubicin	μg/ml	0.5	0.15
	nmol/ml	0.9	0.3

Table 2. Inhibition of DNA and RNA synthesis in KB cells (%)

Time (h)	Rubidazone (IC_{50})		Daunorubicin (IC_{50})		Doxorubicin (IC_{50})	
	DNA	RNA	DNA	RNA	DNA	RNA
1.30	14	20	30	43	24	20
5.00	41	44	50	49	40	35
24.00	58	26	59	27	56	21
48.00	84	16	78	19	72	10

Table 3. Mutagenic activity (strain S. typhimurium TA 98) revertants/Petri dish

nmol/dish	Rubidazone	Daunorubicin	Doxorubicin
50.0	237	bactericide	bactericide
20.0	133	182	163
10.0	57	141	70
1.0	4	9	5
0.1	2	2	3

C. Mutagenic Activity. The mutagenic activity of compounds of the anthracycline family is well known [14, 17]. Using the Ames test [11], S. typhimurium strain TA 98 (frame-shift mutation) was the more susceptible (direct test without S-9). In this system and on a molar basis, RBZ was less mutagenic than the other drugs (Table 3).

2. Toxicity

A. *Subacute Toxicity in Mice.* CD-1 mice, 6 weeks old, were treated by subcutaneous (SC), intraperitoneal (IP), or intravenous (IV) route for five consecutive days; the animals were observed for a further period of 16 days. Table 4 shows the LD_{50} values and the maximal tolerated doses (MTD: the highest dose without lethality or weight loss). RBZ was the least toxic of the three compounds.

B. *Cardiac Toxicity.* Cardiac toxicity is the main problem with these drugs. Two major types of toxicity are observed: electrocardiographic disturbances and, more important, congestive heart failure.

Using the Zbinden and Brandle technique [16], A. Caillard (personal communication) of the Nicolas Grillet Research Center has examined electrocardiographic changes found with these drugs in rats (lengthening of the QRS interval); he has determined the 50% cumulative cardiotoxic limits ($CtC_{50}D$)< the cumulative dose

Table 4. Subacute toxicity in mice (treatments: 5 consecutive days) LD_{50} (mg/kg) – maximal tolerated dose (MTD)

Route of treatment	Rubidazone		Daunorubicin		Doxorubicin	
	LD_{50}	MTD	LD_{50}	MTD	LD_{50}	MTD
SC	15.00	5.0	11.1	2.50	5.5	2.50
IP	4.50	3.0	2.0	0.75	3.0	0.75
IV	8.50	5.0	8.2	2.50	5.0	1.25

Table 5. Cardiac toxicity (rat) Zbinden technique – $CtC_{50}D$

Compound	Daily dose mg/kg IP	$CtC_{50}D$ mg/kg IP
Rubidazone	4	76
	8	88
Daunorubicin	2	16
	4	16
Doxorubicin	4	16

that induces in 50% of the treated animals a QRS interval increase of 15% or more in comparison with the controls. The results (Table 5) show that RBZ is four to five times luss cardiotoxic than daunorubicin or doxorubicin ($CtC_{50}D$ IP: 76 to 88 μg/kg for RBZ and 16 mg/kg for DNR or DXR).

With respect to congestive heart failure, Jaenke [7] has found that RBZ is less cardiotoxic (at least three times less toxic) than the two other compounds in rabbits.

3. Antitumor Activity

The antitumor activity was studied on different types of grafted tumors in mice:
sarcomas (sarcoma 180, sarcoma induced by benzopyrene);
Ehrlich ascitic tumor;
leukemias: L 1210, P 388, AKR (grafted and 'spontaneous')
leucosarcomatosis $C_{57}Bl$;
carcinomas: mammary R III, uterine, stomach and lung carcinoma in $C_{57}Bl$ mice, Lewis lung carcinoma, melanocarcinoma B 16.

As usual, the activities were evaluated on the basis of tumor weight or survival time. The activities depend not only on the nature of the tumor, but also on the route and timing of the treatments. With leukemia L 1210, grafted IP on day 0, five treatments IP on days 0, 1, 2, 3, and 4, the chemotherapeutic indexes (LD_{50}/50% active dose) were, respectively: RBZ = 10–12, DNR = 6–10, DXR = 12. When the treatments (1/2 MTD) were delayed (on days 4, 5, 8, 9, and 10), RBZ was clearly more active than DNR.

Table 6 shows some of the results obtained with other tumors (leukemia P 388, leukemia AKR, Lewis lung carcinoma). At equivalent dosage, RBZ was as active as or more active than DXR or DNR. With the so-called 'S'-AKR leukemia ('spontaneous' AKR leukemia grafted IP for the first time in AKR mice), the activity of RBZ was definitively better than that of DNR (Table 7).

Using Ehrlich ascitic tumor, sensitive and resistant strains (resistance was induced in vivo by suboptimal treatments with DNR), we have observed a clear cross-resistance between the compounds.

Table 6. Antitumor activity. Long survival (40–50 days)

Tumor	Graft	Route of treatment	MTD	Rubidazone			Daunorubicin			Doxorubicin		
				L.S.[a]	I.L.S. (%)[b]	ΔP[c]	L.S.[a]	I.L.S. (%)[b]	ΔP[c]	L.S.[a]	I.L.S. (%)[b]	ΔP[c]
Leukemia P 388	IP	IP	1	7/10	190	+ 0.5	4/10	171	+ 3.1	8/10	191	+ 2.7
			1/5	1/10	152	+ 1.9	0/10	123	+ 3.9	1/10	157	+ 2.1
Leukemia AKR	IP	IV	1	6/10	181	+ 2.2	1/10	146	+ 0.8	4/10	165	+ 0.8
			1/2	1/10	112	+ 3.4	0/10	106	+ 2.7	0/10	113	+ 3.2
Lewis lung carcinoma	SC	IV	1	3/10	143	+ 1.2	1/10	128	+ 1.4	0/10	121	+ 2.6
			1/2	2/10	132	+ 2.3	0/10	122	+ 3.9	0/10	128	+ 2.4

[a] L.S. = Long survival treated/total mice
[b] I.L.S. (%): Percentage increase of the life-span (mean survival time: treated/controls × 100)
[c] ΔP: Weight variations (g)

Table 7. S-AKR leukemia. Graft IP on day 0 (10^3, 10^4, or 10^5 cells). Five treatments IP (1 MTD), on days 0, 1, 2, 3, and 4. End of assay: 4 × mean survival time of controls

No. of cells grafted	Rubidazone		Daunorubicin	
	I.L.S. (%)[a]	No. mice survivals/total	I.L.S. (%)[a]	No. mice survivals/total
10^3	348	12/20	139	0/20
10^4	311	12/19	148	0/20
10^5	231	6/19	142	0/20

[a] I.L.S. (%): Percentage increase of the life-span (mean survival time: treated/controls × 100)

4. Immunodepressive Activity

The immunodepressive activity was studied in the following systems:
localized hemolysis in gel (Jerne plaque assay) [8] in which the three drugs were depressant with no significant difference between them;
carbon clearance [5] in which no significant depressive activity was observed;
graft-versus-host reaction [15] in which immunodepressive activity for the three drugs (IP treatments) was similar;
antitumoral immunity (Ehrlich ascitic tumor system) [9] in which RBZ was less immunodepressive than the other drugs (IV treatments).

With cell culture of mouse peritoneal macrophages, we compared the toxicity of the drugs using two techniques:

1st Technique (cytotoxicity). We counted the macrophages in the culture flasks and considered the ratio R = number of macrophages per flask treated/control. Table 8 shows the results which were obtained at different culture times (days 4, 12, and 18). RBZ was clearly less toxic than DXR, and DXR was less toxic than DNR. These results confirm those previously reported by Mantovani [12] with DXR and DNR.

2nd Technique (functional toxicity). Day 0: the compound was added (various concentrations) in the culture medium. Day 1: Indian ink was added (1/2000). Day 4: cultured adherent macrophages were washed and destroyed (NaOH). Measure of absorbance (nm 660) of the cell lysate. The graph (concentrations of the compound on a log scale, absorbance on an arithmetic scale) was a straight line and the concentration which inhibits 50% of the phagocytic activity (IC_{50}) was determined. Table 9 shows that RBZ was less depressive than DNR and DXR with this experimental system.

Table 8. Mouse macrophage cytotoxicity. $R = n$ macrophages per flask treated/control. IC_{50} = Rubidazone: 0.6 μg/ml, Daunorubicin: 0.3 μg/ml, Doxorubicin: 0.15 μg/ml

Day	Compound	R		
		IC_{50}	$IC_{50}/2$	$IC_{50}/4$
4	Rubidazone	0.67	0.77	0.80
	Daunorubicin	0.12	0.57	0.77
	Doxorubicin	0.64	0.61	0.74
12	Rubidazone	0.38	0.54	0.79
	Daunorubicin	0.00	0.24	0.34
	Doxorubicin	0.47	0.50	0.46
18	Rubidazone	0.26	0.59	0.72
	Daunorubicin	0.00	0.19	0.39
	Doxorubicin	0.28	0.32	0.34

Table 9. Concentration which inhibits 50% activity of macrophages (mouse peritoneal macrophages)

Compound	IC_{50}	
	μg/ml	nmol
Rubidazone	0.120	0.18
Daunorubicin	0.025	0.05
Doxorubicin	0.020	0.04

Discussion and Conclusion

RBZ is an active semisynthetic derivative of daunorubicin. Its principal interests are its low toxicity and cardiotoxicity, low mutagenic activity, and low immunodepressive activity. In the spleen colony assay system, Alberts and van Daalen Wetters [1] found the same toxicity ratio for RBZ and DXR. The particular metabolism (Baurain et al., 1979) may explain the good antitumor activity of RBZ. Bachur [10] has suggested that the activity differences between DNR and DXR may be explained by the greater activity of the aldo-ketoreductase for DNR than for DXR. This hypothesis may be used also for RBZ which perhaps inhibits aldo-ketoreductase. In the human chemotherapy, RBZ (at twice the dosage of DNR) is at least as effective as the parent compound [6] but is less toxic (experimentally three to four times less cardiotoxic); it is highly active in the treatment of patients with acute leukemia [3, 4]. We consider that RBZ deserves more clinical studies.

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