NOTES

BOHEMIC ACID COMPLEX. BIOLOGICAL CHARACTERIZATION OF THE ANTIBIOTICS, MUSETTAMYCIN AND MARCELLOMYCIN

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This paper describes some of the biological activities of musettamycin and marcellomycin, the two pyrromycinone glycosides isolated from the anthracycline mixture called bohemic acid complex[1].

In Vitro Biological Effects

Antimicrobial Activity

The minimum inhibitory concentration (MIC) of musettamycin and marcellomycin for a variety of organisms including bacteria, yeasts and fungi, was determined by using the standard 2-fold tube dilution procedure[2]. The results are shown in Table 1 along with tests of a number of other anthracycline products. In general marcellomycin and musettamycin possess the typical antimicrobial spectra of this class of antibiotics since they inhibit gram-positive bacteria and have little or no effect on gram-negative bacteria, yeasts and fungi. Results with streptococcal species suggest the following rank order of potency: marcellomycin>musettamycin> pyrromycin (free base). Marcellomycin is 8-fold more potent than the closely related cinerubin A against these organisms.

Induction of Lysogenic Bacteria

Marcellomycin and musettamycin have been tested for their ability to induce bacteriophage production in the lysogenic strain of *Escherichia coli* W1709 (λ) using the methods of PRICE, *et al.*[3] There was no evidence of induction at 12.5 μg/ml and toxicity to the lysogenic cells was observed at 50 μg/ml.

Tissue Culture Cytotoxicity

Tube dilution protein tests to determine cytotoxic effects of bohemic acid products on HeLa cells in tissue culture were performed as previously described[2], the 50% end points (ED_{50}) in μg/ml were: marcellomycin 0.033, musettamycin 0.041 (av. 2 tests), pyrromycin 0.037 and pyrromycinone 0.25, thus the 3 glycosides appear to have comparable activity and the aglycone somewhat less.

In Vivo Biological Effects

Acute Toxicity

The acute intraperitoneal LD_{50} of musettamycin, marcellomyin and pyrromycin was determined in BDF_1 male mice. The median day of death was based on all mice dying below an LD_{100} dose. During the 60-day observation interval, no mice died after Day 9. (Table 2)

Antitumor Effects

Tests for inhibition of L-1210 leukemia in mice were performed using procedures similar to those previously reported.[4] Dose-response titrations were run with pure musettamycin and marcellomycin using both a single dose Day 1 treatment and daily dosing for 5 days. (Table 3) There was little evidence of schedule dependence in achieving tumor inhibition. On the basis of comparing both the optimum doses and the minimum effective doses with the single injection schedule, marcellomycin appears to be 4 times as potent as musettamycin. In another experiment (Table 4) pyrromycin was compared with musettamycin and found to be at least 10 times less potent and very weak in terms of survival increase (T/C value). Tests on other tumor systems are continuing in this laboratory and in other laboratories of the National Cancer Institute as additional quantities of the antibiotics are prepared.

Discussion

Bohemic acid complex consists of a number of anthracycline antibiotics based on pyrromycinone. The antimicrobial spectra of marcellomycin and musettamycin are typical of anthracyclines. The lack of inducing effects in lysogenic bacteria is consistent with results found for other pyrromycinone antibiotics[5] and suggests possible interaction with RNA metabolism rather than with DNA metabolism alone. Marcellomycin and musettamycin are cytotoxic to KB

Table 1. Minimum inhibitory concentration (μg/ml)

Organisms		Aclacinomycin A	Adriamycin	Carminomycin I	Cinerubin A	Cinerubin B	Marcellomycin	Musettamycin	Pyrromycin	Pyrromycin HCl	ε Pyrromycinone
Streptococcus pneumoniae	A-9585	0.13	0.06	0.13	0.25	0.13	0.03	0.06	0.13	0.06	16
〃 *pyogenes*	A-9604	0.13	0.06	0.13	0.25	0.5	0.03	0.06	0.13	0.06	32
Staphylococcus aureus	A-9497	2	2	0.5	1	0.25	1	0.5	1	1	>125
〃 〃	A-9537	2	1	0.25	2	0.25	1	1	1	1	>125
Escherichia coli	A15119	125	63	63	>125	>125	125	>125	125	125	>125
〃 〃	A21780	32	4	0.25	16	>125	32	32	8	8	>125
Klebsiella pneumoniae	A-9977	63	63	8	125	>125	125	>125	63	125	>125
Proteus mirabilis	A-9900	125	125	63	>125	>125	>125	>125	63	125	>125
Candida albicans	A-9540	16	125	63	2	4	125	125	63	125	>125
〃 *tropicalis*	A15051	16	63	63	4	4	125	63	32	63	>125
〃 *krusei*	A15052	63	125	32	32	32	125	125	63	125	>125
Cryptococcus neoformans	A15053	63	63	32	63	63	125	63	32	63	>125
Trichophyton mentagrophytes	A-9870	>125	>125	>125	16	32	>125	>125	125	125	>125
Microsporum canis	A-9872	>125	>125	>125	32	63	125	>125	125	125	>125

Table 2.

Material	LD_{50} mg/kg	Days of death	
		Median	Last
Marcellomycin	10.56	Day 5	Day 8
Musettamycin	21.12	Day 6	Day 9
Pyrromycin	35.64	Day 5	Day 7

Material: Free base, initially dissolved in DMSO then diluted in saline
Dose: 0.5 ml i. p., 6 mice/dose
Evaluation: WEIL[8]

Table 3. Effect of musettamycin and marcellomycin on L-1210 leukemia

Compound	Dose mg/kg/day	Day of treatment	Total injs.	MST Days	Effect MST % T/C	Average weight change, g	Survivors Day 5
Musettamycin	12.8	1	1	10.5	150	−0.6	6/6
	6.4	1	1	10.0	143	+0.5	6/6
	3.2	1	1	9.0	129	+0.3	6/6
	1.6	1	1	9.0	129	+0.3	6/6
	0.8	1	1	8.5	121	+1.2	6/6
	0.4	1	1	8.0	114	+1.3	6/6
Musettamycin	6.4	1→5	5	6.0	86	−1.5	5/6
	3.2	1→5	5	11.0	157	−1.3	6/6
	1.6	1→5	5	10.0	143	+0.3	6/6
	0.8	1→5	5	9.5	136	+0.4	6/6
	0.4	1→5	5	9.0	129	+0.6	6/6
	0.2	1→5	5	9.0	129	+1.8	6/6
Marcellomycin	12.8	1	1	Tox	Tox	Tox	3/6
	6.4	1	1	11.0	157	−0.7	6/6
	3.2	1	1	11.0	157	−0.7	6/6
	1.6	1	1	10.0	143	+0.1	6/6
	0.8	1	1	10.5	150	0	6/6
	0.4	1	1	9.0	129	−0.8	6/6
Marcellomycin	6.4	1→5	4	Tox	Tox	Tox	0/6
	3.2	1→5	5	Tox	Tox	Tox	3/6
	1.6	1→5	4	6.0	86	−0.1	5/6
	0.8	1→5	5	10.0	143	−0.2	6/6
	0.4	1→5	5	9.0	129	0	6/6
	0.2	1→5	5	9.0	129	+1.1	6/6
Control	Saline	1→5	5	7.0	—	+3.0	10/10

Inoculum: 10^6 ascitic cells, i.p. into BDF_1 female mice
Treatment: i. p. in 0.5 ml volume
Evaluation: MST-median survival time in days; % T/C-MST treated/MST control ×100
Toxicity: <4/6 survivors, Day 5
Criteria: T/C≥125 considered significant tumor inhibition (prolongation of host survival)

Table 4. Effect of pyrromycin on L-1210 leukemia

Compound	Dose mg/kg/day	MST Days	Effect MST T/C	Average weight change, g	Survivors Day 5
Pyrromycin	32	9.0	129	+0.3	6/6
	16	8.0	114	+1.1	6/6
	8	9.0	129	+1.3	6/6
	4	8.0	114	+2.1	6/6
	2	8.0	107	+3.9	6/6
Musettamycin	3.2	10.0	142	+0.7	6/6
	1.6	9.5	136	+0.9	6/6
	0.8	9.0	129	+1.9	6/6
	0.4	8.0	114	+1.9	6/6
	0.2	8.0	114	+1.7	6/6
Control	Saline		—	+2.0	10/10

Treatment: i. p. once daily Days 1→5 for 5 injections. Other conditions as shown in Table 3.

cells in tissue culture and have moderate inhibitory effects on the transplanted mouse leukemia L-1210. Of particular interest is the gradation of biological effects associated with the number of sugar residues. In terms of tumor inhibitory effects and acute toxicity as well as a number of *in vitro* tests the potency decreases as follows:

marcellomycin > musettamycin > pyrromycin

though there is not a quantitative relationship between biological systems in relative response to the agents. Increased potency in one or more biological tests have also been reported for glycosides of carminomycinone[6,7] and of aklavinone with more than one sugar.[9]

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