ANTITUMOR ACTIVITY OF CHRYSOMYCINS M AND V

JAMES A. MATSON, WILLIAM C. ROSE, JAMES A. BUSH, ROBERT MYLLYMAKI, WILLIAM T. BRADNER and TERRENCE W. DOYLE

Pharmaceutical Research and Development Division, Bristol-Myers Company, 5 Research Parkway, Wallingford, Connecticut 06492-7660, U.S.A.

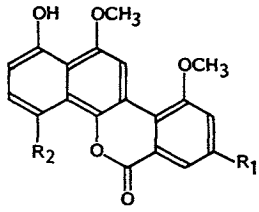
(Received for publication May 9, 1989)

While screening for new antitumor agents from cultured broths, the culture *Streptomyces albaduncus* strain C38291 (ATCC 14698) was selected for further evaluation. This investigation led to the discovery of the known antitumor antibiotics chrysomycins V (**1**) and M (**2**)[1]. These compounds are members of a family of compounds possessing the benzonaphthopyranone ring system (Fig. 1). Since there is considerable interest in this family of antitumor antibiotics not only as synthetic targets[2~4] but also as to their mechanism of action[5~15], we felt obligated to report our observations on the antitumor activity of chrysomycins V and M against various murine tumors.

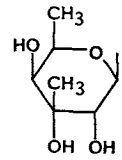
Commentary on Nomenclature

A variety of names have been used to identify

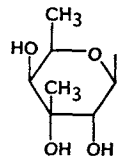
Fig. 1. Benzonaphthopyranone antitumor antibiotics.



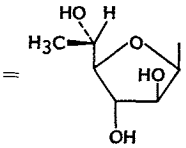
Chrysomycin V (**1**) (chrysomycin A, virenomycin V, albacarcin V) R_1=CH=CH$_2$ R_2=



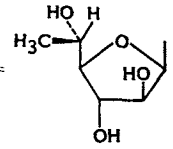
Chrysomycin M (**2**) (chrysomycin B, virenomycin M, albacarcin M) R_1=CH$_3$ R_2=



Gilvocarcin V (**3**) (toromycin, toromycin A, anandimycin A) R_1=CH=CH$_2$ R_2=



Gilvocarcin M (**4**) (toromycin B, anandimycin B) R_1=CH$_3$ R_2=



Ravidomycin (**5**) R_1=CH=CH$_2$ R_2=

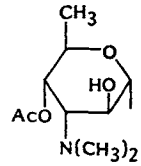


Table 1. Preclinical antitumor activities of chrysomycins M and V.

Tumor site[a]	Treatment		MST % T/C[b]	
	Schedule	Dose, ip (mg/kg/injection)	Chrysomycin M	Chrysomycin V
P388 (ip)	Day 1, 4, 7	256	200	100
		128	175	200
		64	150	194
		32	150	150
		16	119	131
		8	113	113
L1210 (ip)	Day 1	512	157	ND
		256	129	157
		128	107	129
		64	100	114
	Day 1, 4, 7	256	143	171
		128	136	157
		64	129	129
		32	100	121
	Day 1→5	256	143[c]	ND
		128	143	171
		64	136	143
		32	129	129
		16	114	114
B16 (ip)	Day 1, 5, 9	256	133	ND
		128	133	133
		64	114	133
		32	114	112
		16	102	110

[a] P388 and L1210 implants were 10^6 leukemic cell/mouse. For B16, each mouse received 0.5 ml of a 10% w/v tumor brei. Control median survival times were as follows: P388-9.0 days; L1210-7.0 days; B16-21.0 days. There were 6 CDFH1 mice in each treatment group in the P388 and L1210 experiments, and 9~10 BDF1 mice in each B16 experimental treatment group. Control groups in all experiments consisted of 10 mice.

[b] The median survival time (MST) of treated mice/MST of control mice, $\times 100 = \%$ T/C. Significant activity was considered to be a T/C of $\geq 125\%$ in each tumor model evaluated.

[c] Two of six mice had died by day-5 indicating excessive toxicity associated with this dosage.

ND: Not done.

subgroupings of this class: Chrysomycins A (**1**) and B (**2**)[1], chrysomycins V (**1**) and M (**2**)[2], virenomycins V (**1**) and M (**2**), albacarcins V (**1**) and M (**2**), toromycin (**3**), gilvocarcins V (**3**) and M (**4**), toromycins A (**3**) and B (**4**), anandimycins A (**3**) and B (**4**) and ravidomycin (**5**). We chose the root name chrysomycin for **1** and **2** because of the priority set by the paper of STRELITZ *et al.*[16] and the suffixes V and M because they reflect best the important variation in the aglycone.

Results

Table 1 shows the effectiveness of chrysomycins M and V in inhibiting three transplantable mouse tumors. Against P388 lymphatic leukemia chrysomycin V was capable of producing survival increases to an extent comparable to that seen with animals receiving about twice the dose level of chrysomycin M using an every day dose schedule. This evidence of a difference in potency in producing antitumor effects persisted in tests against L1210 lymphatic leukemia regardless of treatment schedule used, and in the marginal tumor inhibition observed with B16 melanoma.

Discussion

Against the three tumors investigated, both chrysomycins V and M were active. Chrysomycin V was consistently about twice as potent as chrysomycin M for a given treatment schedule. This observation is in marked contrast to that which is reported for ravidomycin and AY-

26,779 (ravidomycin E)[17]. The 8-ethyl analog was significantly more potent and toxic against P388 leukemia than the parent compound. It is also differs from that which is reported for gilvocarcins V and M. Gilvocarcin M was inactive[18].

There has been considerable speculation about the mechanism of action for these benzonaphthopyranone compounds[5~15]. The vast majority of the studies have focused on the 8-vinyl group and light activation in *in vitro* models. However, the activity observed for chrysomycin M and analogs of ravidomycin strongly suggest that other factors or modes of action must be involved in *in vivo* systems. Alternative hypotheses such as solubility[18], transport[17], inhibition of topoisomerase[19], induction of topoisomerase II-dependent DNA cleavage[19] and stronger or more stable binding to DNA[6,10,11,17,19] leading to cell death have been proposed. Clearly, the *in vivo* mechanism of action will require more careful evaluation to account for these observation.

References

1) WEISS, U.; K. YOSHIHIRA, R. J. HIGHET, R. J. WHITE & T. T. WEI: The chemistry of the antibiotics chrysomycin A and B. Antitumor activity of chrysomycin A. J. Antibiotics 35: 1194~1201, 1982
2) FINDLAY, J. A.; A. DALJEET, P. J. MURRAY & R. N. REJ: Total synthesis of the ravidomycin aglycone (defucogilvocarcin V). Can. J. Chem. 65: 427~431, 1987
3) MCGEE, L. R. & P. N. CONFALONE: On the photobiology of the gilvocarcins. Total synthesis of defuco-gilvocarcin V and a related photoactive vinyl phenol. J. Org. Chem. 53: 3695~3701, 1988
4) JUNG, M. E. & Y. H. JUNG: Total synthesis of the aglycone of the 8-methyl benzonaphthopyrone antibiotics, gilvocarcin M. virenomycin M, and albacarcin M. Tetrahedron Lett. 29: 2517~2520, 1988
5) WEI, T. T.; K. BYRNE, R. ELESPURU, M. GREENSTEIN, D. PICKLE, C. RICHARDSON, R. STROSHANE, U. WEISS & R. J. WHITE: Mode of action studies of the antitumor antibiotic toromycin and the related compound chrysomycin. Program and Abstracts of the 21st Intersci. Conf. on Antimicrob. Agents Chemother., No. 55, Chicago, Nov. 4~6, 1981
6) WEI, T. T.; K. M. BYRNE, D. WARNICK-PICKLE & M. GREENSTEIN: Studies on the mechanism of action of gilvocarcin V and chrysomycin A. J. Antibiotics 35: 545~548, 1982
7) TOMITA, F.; K. TAKAHASHI & T. TAMAOKI: Gilvocarcins, new antitumor antibiotics. 4. Mode of action. J. Antibiotics 35: 1038~1041, 1982
8) ELESPURU, R. K. & S. K. GONDA: Activation of antitumor agent gilvocarcins by visible light. Science 223: 69~71, 1984
9) ELESPURU, R. K. & V. M. HITCHINS: Wavelength dependence for the induction of bacteriophage lambda by antitumor agent gilvocarcin V. Photochem. Photobiol. 44: 607~612, 1986
10) SHISHIDO, K.; K. JOHO, M. URAMOTO, K. ISONO & T. JAIN: Studies on binding of toromycin, an antitumor antibiotic, to DNA. Biochem. Biophys. Res. Commun. 136: 885~890, 1986
11) TSE-DINH, Y. & L. R. MCGEE: Light-induced modification of DNA by gilvocarcin V and its aglycone. Biochem. Biophys. Res. Commun. 143: 808~812, 1987
12) SINGH, K.: Studies on the mechanism of action of ravidomycin (AY-25,545). J. Antibiotics 37: 71~73, 1984
13) GREENSTEIN, M.; T. MONJI, R. YEUNG, W. M. MAIESE & R. J. WHITE: Light-dependent activity of the antitumor antibiotics ravidomycin and desacetylravidomycin. Antimicrob. Agents Chemother. 29: 861~866, 1986
14) GASPARRO, F. P.; R. M. KNOBLER & R. L. EDELSON: The effects of gilvocarcin V and ultraviolet radiation on pBR322 DNA and lymphocytes. Chem. Biol. Interact. 67: 255~265, 1988
15) PEAK, M. J.; J. G. PEAK, C. M. BLAUMUELLER & R. K. ELESPURU: Photosensitized DNA breaks and DNA-to protein crosslinks induced in human cells by antitumor agent gilvocarcin V. Chem. Biol. Interact. 67: 267~274, 1988
16) STRELITZ, F.; H. FLON & I. N. ASHESHOV: Chrysomycin: A new antibiotic substance for bacterial viruses. J. Bacteriol. 69: 280~283, 1955
17) RAKHIT, S.; C. ENG, H. BAKER & K. SINGH: Chemical modification of ravidomycin and evaluation of biological activities of its derivatives. J. Antibiotics 36: 1490~1494, 1983
18) MORIMOTO, M.; S. OKUBO, F. TOMITA & H. MARUMO: Gilvocarcins, new antitumor antibiotics. 3. Antitumor activity. J. Antibiotics 34: 701~707, 1981
19) YAMASHITA, Y. & H. NAKANO: Difference between gilvocarcin V and gilvocarcin M on the light-dependent action on DNA *in vitro*. Nucleic Acids Symp. Ser. 20: 65~67, 1988