STUDIES ON THE MECHANISM OF ACTION OF GILVOCARCIN V AND CHRYSOMYCIN A*

Sir:

While evaluating a new antitumor prescreen, we isolated a species of *Streptomyces arenae* which produces an antibiotic complex containing a new antitumor compound, 2064A. This same antibiotic was also discovered recently by two separate groups of researchers and was designated toromycin by one[1], gilvocarcin V by the other[2]. Gilvocarcin V was reported to be active against a number of experimental tumors[3]. Our 2064 complex was detected using a biochemical version of a prophage induction assay (BIA)[4], a test for agents interacting with DNA. We wish to report on the mechanism of action of gilvocarcin V (2064A) and a closely related BIA-active compound chrysomycin A, which differs from the former only in its sugar moiety[5].

Fermentation of *S. arenae* and purification of 2064A were performed as previously described[6]. Our chrysomycin sample, obtained from an original stock[7], was assayed by HPLC to be 95% chrysomycin A.

The viability of *Bacillus subtilis* ATCC 6633 log-phase cells was monitored during treatment with these compounds. Both drugs demonstrated a minimum inhibitory concentration (MIC) of 0.5 μg/ml with this organism. Bactericidal activity was likewise exhibited by both drugs; at least a 1000-fold decrease in viability of *B. subtilis* occurred within 5 minutes of exposure to each drug at twice the MIC.

The effects of gilvocarcin V and chrysomycin A upon macromolecular synthesis were likewise studied in *B. subtilis*. Log-phase cells were grown in the presence of labeled thymidine, uridine, or valine to monitor DNA, RNA, or protein synthesis, respectively. Gilvocarcin V inhibited DNA synthesis earlier and to a greater extent than RNA synthesis (Fig. 1). Protein synthesis was the least inhibited under these conditions. Chrysomycin A at twice the concentration of gilvocarcin V demonstrated similar patterns of inhibition.

Activity in the BIA test is normally associated with a compound's ability to initiate DNA damage[4]. Intracellular DNA degradation due to drug exposure was assessed. The DNA in growing *B. subtilis* cells was labeled with [^{3}H]-thymidine (0.5 μCi/ml), the cells were resuspended in fresh drug-containing medium without isotope, and the label remaining in DNA was monitored. There was no detectable effect (Table 1) upon cellular DNA for either gilvocarcin V or chrysomycin A at concentrations two-

Table 1. Effect of gilvocarcin V and chrysomycin A on *B. subtilis* DNA *in vivo*.

Addition (μg/ml)		% Degradation†*			
		30 minutes	60 minutes	90 minutes	120 minutes
None		3.7	2.5	8.4	3.8
Streptomycin	50	0	2.4	0	0
Nalidixic acid	50	2.1	19.3	23.0	30.8
Gilvocarcin V	1	1.8	3.6	8.7	3.7
	7	12.5	19.1	26.6	26.6
	50	4.5	28.9	31.5	44.6
Chrysomycin A	1	0	8.3	6.5	0
	7	2.9	11.5	9.6	0
	50	0	5.2	6.8	0
DMSO, 2%**		0.5	2.8	1.8	1.3

* Data are a composite of two experiments in which controls contained 32,700 and 44,400 TCA-insoluble CPM at time zero.

† $\% \text{ Degradation} = \frac{(\text{TCA-insoluble CPM at time zero}) - (\text{TCA-insoluble CPM at time x})}{\text{TCA-insoluble CPM at time zero}} \times 100$

** A final concentration of 2% DMSO was used to solubilize the 50 μg/ml solutions of gilvocarcin V and chrysomycin A.

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Fig. 1. Effect of gilvocarcin V on macromolecular biosynthesis in *B. subtilis*.

Cells were grown to early logarithmic phase in minimal medium supplemented with D-glucose and casein at 4.0 and 8.0 g/liter, respectively. Additions of 12 μCi of [^{14}C]uridine, 13 μCi of [^{3}H]thymidine or 25 μCi of [^{3}H]valine were made to 60 ml of medium. Gilvocarcin V was added to each flask 12 minutes after isotope addition giving a final concentration of 0.5 μg/ml (△) or 1.0 μg/ml (○). A separate flask without drug was used as control (□). At appropriate times, a 1.0 ml sample was withdrawn and mixed with 1.0 ml of 10% ice-cold trichloroacetic acid (TCA). Acid insoluble materials were collected on glass fiber filters (Whatman GF/A), washed twice with cold TCA, once with 95% ethanol, and dried. Radioactivity was determined in PCS scintillation fluid (Amersham).

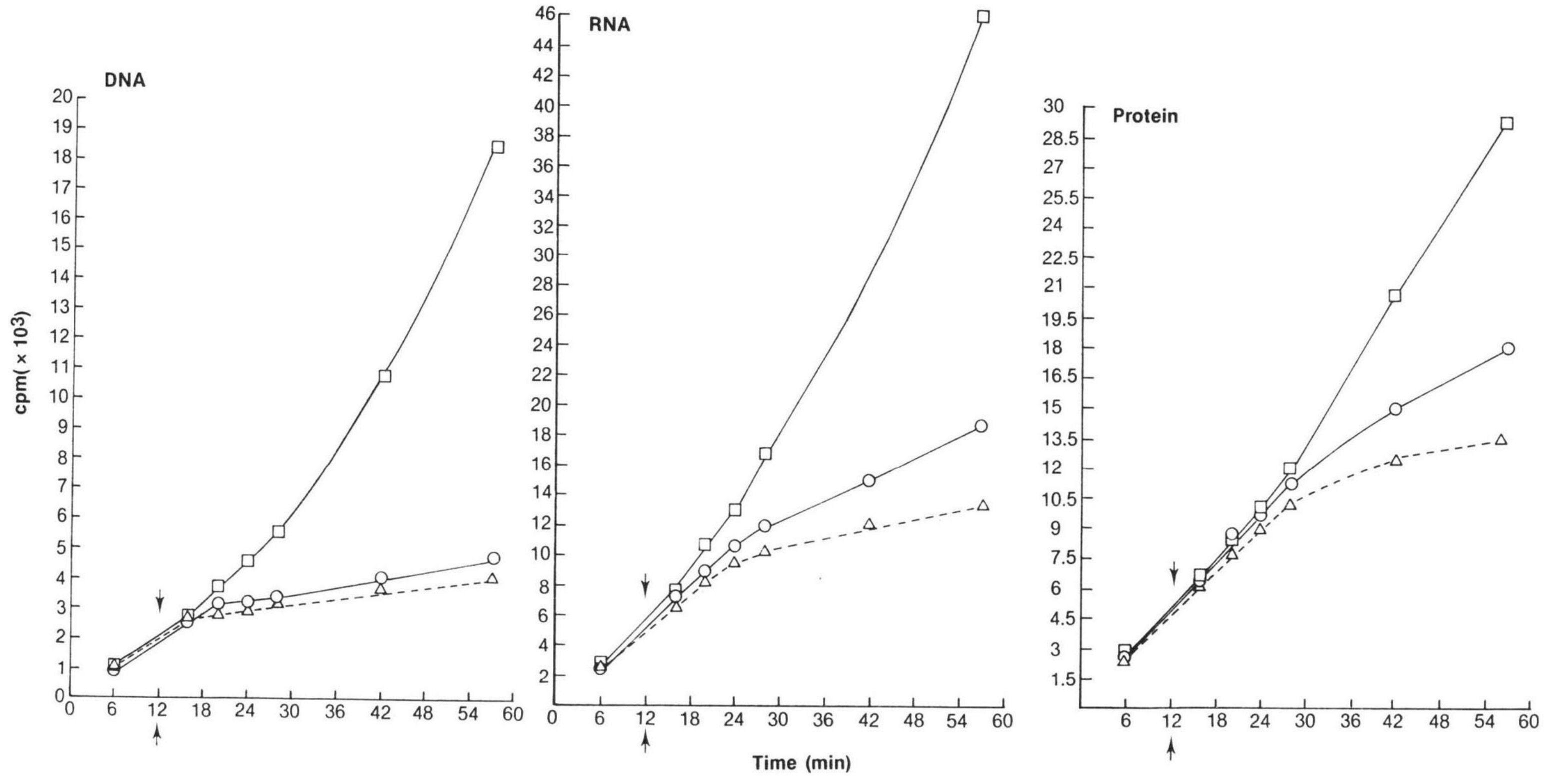
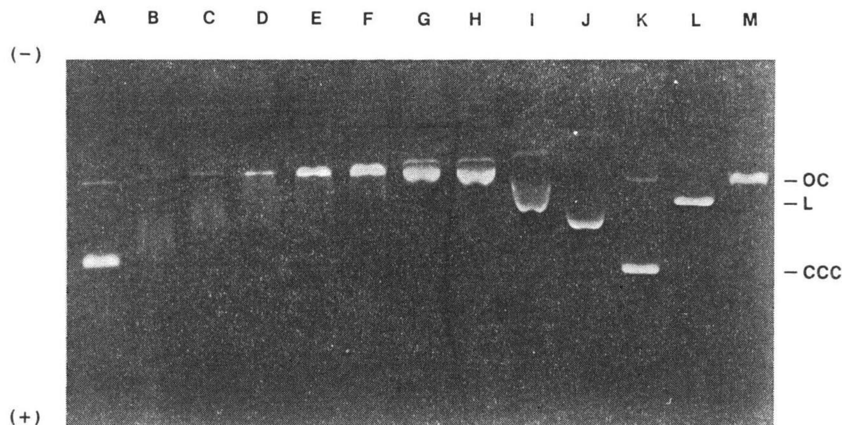


Fig. 2. Agarose gel electrophoresis of CCC-ϕX174 RFI DNA titrated with gilvocarcin V.

Electrophoresis in tris-acetate (pH 7.8) buffer was through a 1% gel at 48 mA for 18 hours. One μg of DNA was mixed with increasing concentrations of gilvocarcin V. A~J: 1, 2, 3, 4, 4.5, 5, 5.5, 6, 10, 30 μg/ml, respectively. K: Drug free control. L: *Pst* I-digested ϕX174 RFI DNA (indicates the position of linear duplex DNA). M: Same as L+4 μg/ml gilvocarcin V.



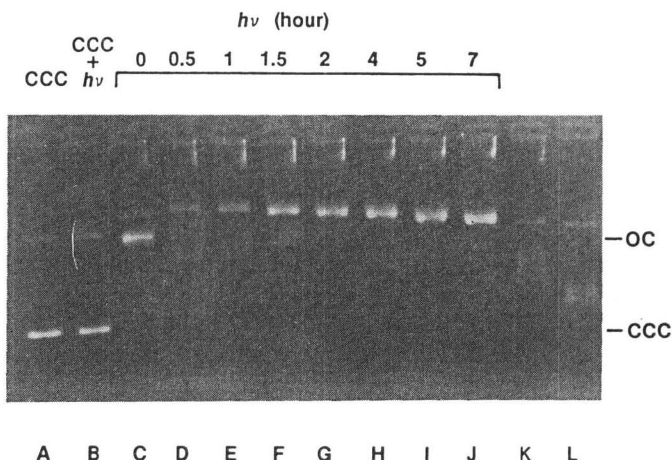
fold higher (1.0 μg/ml) than the MIC. Extensive DNA degradation did occur after 30~60 minutes when the concentration of gilvocarcin V was increased to 7 and 50 μg/ml. No measurable DNA degradation was elicited by chrysomycin A even at concentrations as high as 50 μg/ml. One cannot exclude the possible initiation of occasional interruptions in the DNA molecule by chrysomycin A which would not be detected under the conditions of this experiment. Both antibiotics, however, demonstrated a rapid bactericidal effect and strong BIA activity[6] at significantly lower concentrations. This suggests that the *in vivo* DNA degradation observed exclusively for gilvocarcin V must be unrelated to these other activities of the drug.

The *in vitro* interaction of gilvocarcin V and chrysomycin A with duplex covalently closed circular DNA (CCC-ϕX174 RFI) was evaluated by agarose gel electrophoresis (Fig. 2). Under our conditions, the order of anodal migration of the various conformational forms of DNA was: 1) CCC-DNA, 2) linear duplex (L) DNA, and 3) nicked circular (OC) DNA. As the gilvocarcin V concentration was increased, a diffuse DNA band with reduced mobility appeared while the CCC-DNA band decreased in its intensity, suggesting intercalative binding of the drug to DNA and a reduction in CCC-DNA superhelicity. At concentrations of 4.5~5 μg/ml, CCC-DNA formed a sharp band with a mobility almost equal to that of OC-DNA, suggesting that all negative superhelical turns had been removed. A diffuse band migrating between OC- and CCC-DNA again appeared at even higher drug concentrations, indicating supercoiling in the opposite direction. Chrysomycin A behaved similarly except that a totally relaxed conformation and winding of the CCC-DNA in the opposite direction were not observed, even at very high concentrations. This may suggest that the specific steric properties of chrysomycin A limit further binding to CCC-DNA when a particular drug concentration is exceeded. No DNA damage of CCC-DNA was apparent with either drug.

Since these compounds are readily altered chemically upon exposure to light[4,6,7], their capacity to elicit photoactivated DNA damage was investigated. A mixture of gilvocarcin V, at a concentration above that required for complete superhelical relaxation, and CCC-DNA was irradiated with incandescent light. With increasing exposure to light (Fig. 3), the OC-DNA band intensified as the diffuse DNA band decreased (channels C~J). Similar results were obtained with chrysomycin A (data not shown). When drug was irradiated prior to mixing with CCC-DNA (channels K and L) a decrease in relaxation of superhelical twists was observed exclusively. It is likely that the OC-DNA band contained primarily nicked circular DNA since material from this band did not return to a negative

Fig. 3. Agarose gel electrophoresis of gilvocarcin V-CCC-ϕX174 DNA complex under conditions of photoactivation.

Electrophoretic conditions were the same as given in Fig. 2. One μg of DNA was incubated with 8 μg/ml gilvocarcin for 30 minutes and then exposed for various times to a 25 watt incandescent light source 25.4 cm above the test solutions. A: drug free control. B: drug free+7-hour light. C: complete (DNA+drug) without light. D~J: C+0.5, 1.0, 1.5, 2.0, 4.0, 5.0, 7.0 hours of light. K: drug+5-hour light, followed by incubation with DNA for additional 30 minutes. L: same as K, except 7-hour light.



superhelical state after sustained exposure to light (channels H~J). This capacity to initiate DNA damage *in vitro* under special conditions may be related to the action of these drugs in living cells.

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References

1) HORII, S.; H. FUKASE, E. MIZUTA, K. HATANO & K. MIZUNO: Chemistry of toromycin. Chem. Pharm. Bull. 28: 3601~3611, 1981
2) TAKAHASHI, F.; M. YOSHIDA, F. TOMITA & K. SHIRAHATA: Gilvocarcins, new antitumor antibiotics. 2. Structural elucidation. J. Antibiotics 34: 271~275, 1981
3) MORIMOTO, M.; S. OKUBO, F. TOMITA & H. MARUMO: Gilvocarcins, new antitumor antibiotics. 3. Antitumor activity. J. Antibiotics 34: 701~707, 1981
4) ELESPURU, R. K. & M. B. YARMOLINSKY: A colorimetric assay of lysogenic induction designed for screening potential carcinogenic and carcinostatic agents. Environ. Mutagen. 1: 65~78, 1979
5) 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 (to be submitted)
6) WEI, T. T.; J. A. CHAN, P. P. ROLLER, U. WEISS, R. M. STROSHANE, R. J. WHITE & K. M. BYRNE: Detection of gilvocarcin antitumor complex by a biochemical induction assay (BIA). J. Antibiotics 35: 529~532, 1982
7) STRELITZ, F.; H. FLON & I. H. ASHESHOV: Chrysomycin: A new antibiotic substance for bacterial viruses. J. Bacteriol. 69: 280~283, 1955