Induction of P22 Prophage in *Salmonella typhimurium* by Hycanthone

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Received for publication 13 December 1973

Hycanthone was found to be an inducer of P22 prophage in *Salmonella typhimurium* LT-2(P22).

Hycanthone (HCT) is a highly effective chemotherapeutic agent for the treatment of schistosomiasis (10, 12). Despite this noted efficacy in clinical usage, studies at the bacterial and cellular level suggest a variety of other biological activities, some of which are possibly undesirable. These properties include mutagenicity (2-4), teratogenicity (13), carcinostatic activity (9, 16), and interaction with DNA (8, 17). Furthermore, in vitro cell culture studies (7) have revealed HCT to be capable of inducing transformation of rat embryo cells infected with Rauscher leukemia virus.

In view of the correlation often observed between the above-mentioned properties of certain chemical agents and their ability to induce prophage replication in lysogenic bacteria (5), an attempt was made to determine whether HCT also was capable of this induction phenomenon.

Salmonella typhimurium LT-2 and LT-2(P22) were used as the indicator and the lysogenic strain, respectively. The 50% end points for the antibacterial action of hycanthone methanesulfonate and mitomycin C (MC) against the lysogenic strain were determined by the rapid turbidimetric method (14). These values were found to be 3.2×10^{-4} M and 5.5×10^{-6} M, respectively. MC was used as a known inducer of P22 prophage (11). Lysogenic cells exponentially growing at 37 C in synthetic mineral salts-glucose medium (15) were treated with the 50% end point dose for 30 min, and then diluted 100-fold into drug-free 1% Bacto-Tryptone broth. Incubation was continued for an additional 80 min to allow maturation and release of free phages. Samples were withdrawn at intervals, appropriately diluted in chilled (4 C) T-2 buffer (6), and assayed for PFU by Adams' overlay technique (1). It should be pointed out that the PFU observed here really represent a mixture of free phages and infectious centers. These are contributed by sponta-

neously and chemically induced cells, and the liberated phage progeny from both kinds of cells.

The degree of induction, or induction index, has been defined as the ratio of the number of PFU in the test sample (T) to that in the control (C). By using *Escherichia coli* W1709 (λ), Price et al. (15) have obtained data that indicated that compounds yielding T/C values of 3.0 could be considered with some assurance to be inducers of the lytic cycle. The induction indices were determined for HCT and MC, and these are given in Table 1. It is clear from these data that HCT, with an induction index of 13.7, acts as an inducing agent in this *Salmonella* system. A more detailed investigation on the kinetics of prophage induction by HCT is currently under way in this laboratory.

TABLE 1. Effect of HCT and MC on phage production in the lysogenic LT-2(P22)

Drug ^a	PFU/ml ^b	Induction index
Mitomycin C	240	30.0
Hycanthone	110	13.7
Control	8	1.0

^a Concentrations used were the 50% end point dose.

^b These values ($\times 10^6$) were recorded 110 min after the beginning of drug treatment.

LITERATURE CITED

1. Adams, M. H. 1959. Bacteriophages. Wiley-Interscience, Inc., New York.
2. Cline, D., W. G. Flamm, and M. R. Machesko. 1972. Mutagenicity of hycanthone in mammalian cells. *Mutat. Res.* 14:262-264.
3. Green, S., F. M. Sauro, and M. S. Legator. 1973. Cytogenetic effect of hycanthone in the rat. *Mutat. Res.* 17:239-244.
4. Hartman, P. E., K. Levine, Z. Hartman, and H. Berger. 1971. Hycanthone: a frameshift mutagen. *Science* 172:1058-1060.
5. Heinemann, B. 1971. Prophage induction in lysogenic

- bacteria as a method of detecting potential mutagenic, carcinogenic, carcinostatic, and teratogenic agents, p. 235-266. In A. Hollaender (ed.), Chemical mutagens: principles and methods for their detection, vol. 1. Plenum Press, New York.
6. Hershey, A. D., and M. Chase. 1952. Independent functions of viral protein and nucleic acid in growth of bacteriophage. *J. Gen. Physiol.* **36**:39-56.
 7. Hetrick, F. M., and W. L. Kos. 1973. Transformation of Rauscher virus-infected cell cultures after treatment with hycanthone and lucanthone. *J. Pharmacol. Exp. Ther.* **186**:425-429.
 8. Hirschberg, E., and I. B. Weinstein. 1971. Comparative ability of hycanthone and Miracil D to interact with DNA. *Science* **174**:1147-1148.
 9. Hirschberg, E., I. B. Weinstein, R. Carchman, and S. Archer. 1968. Search for the carcinostatic metabolite of Miracil D. *Proc. Amer. Cancer Res.* **9**:30.
 10. Katz, N., J. Pellegrino, and C. A. Oliveira. 1969. Further clinical trials with hycanthone, a new antischistosomal agent. *Amer. J. Trop. Med. Hyg.* **18**:925-929.
 11. Levine, M. 1961. Effect of mitomycin C on interactions between temperate phages and bacteria. *Virology* **13**:493-499.
 12. Maritz, J. C. 1970. Hycanthone therapy in Bilharziasis. *S. Afr. Med. J.* **44**:126-128.
 13. Moore, J. A. 1972. Teratogenicity of hycanthone in mice. *Nature (London)* **239**:107-109.
 14. Muschel, L. H., and K. Schmoker. 1966. Activity of mitomycin C, other antibiotics, and serum against lysogenic bacteria. *J. Bacteriol.* **92**:967-971.
 15. Price, K. E., R. E. Buck, and J. Lein. 1964. System for detecting inducers of lysogenic *Escherichia coli* W1709 (λ) and its applicability as a screen for antineoplastic antibiotics. *Appl. Microbiol.* **12**:428-435.
 16. Sieber, S. M., J. Whang-Peng, D. G. Johns, and R. H. Adamson. 1973. Effects of hycanthone on rapidly proliferating cells. *Biochem. Pharmacol.* **22**:1253-1262.
 17. Waring, M. 1970. Variations of the supercoils in closed circular DNA by binding of antibiotics and drugs: evidence for molecular models involving intercalation. *J. Mol. Biol.* **54**:247-279.