WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 4, 78-84. Review Article ISSN 2277–7105

MIGHT TRACE AMOUNTS OF ACTINOMYCIN GIVEN OVER AN EXTENDED PERIOD OF TIME PROVE TO BE A POWERFUL ANTICANCER CHEMOTHERAPEUTIC REGIMEN?

Henry M. Sobell*

Departments of Chemistry and Molecular Biophysics University of Rochester, Rochester, New York 14642 USA.

Article Received on
27 Feb. 2017,

Revised on 02 Mar. 2017,
Accepted on 03 Mar. 2017

DOI: 10.20959/wjpr20174-8127

***Corresponding Author**
Henry M. Sobell
Departments of Chemistry and Molecular Biophysics University of Rochester, Rochester, New York 14642 USA.

ABSTRACT

This article presents our current understanding of how actinomycin D binds to DNA and exerts its mechanism of action. Actinomycin is proposed to bind to a premelted DNA-conformation (known as beta-DNA) present within the transcription complex. This acts to immobilize the complex, interfering with the elongation of growing RNA chains. Nucleolar (ribosomal) 5S RNA synthesis is particularly sensitive to the presence of actinomycin, and this probably accounts for its pharmacological activity as well as its extreme toxicity to mammalian cells. This paper asks – might trace amounts of actinomycin given over an extended period of time prove to be a powerful anticancer chemotherapeutic regimen?

KEY WORDS: Actinomycin toxicity and its anticancer activity, inhibition of the elongation of growing RNA chains in DNA transcription, actinomycin binds to a premelted beta-DNA conformation within the transcription complex preferentially inhibiting nucleolar (ribosomal) 5S RNA synthesis.

INTRODUCTION

Actinomycin D is a cyclic polypeptide containing antibiotic that binds to DNA and inhibits RNA synthesis (Figure 1)[1-4]. It does this by interfering with the elongation of growing RNA chains by the RNA polymerase enzyme[5]. Nucleolar (ribosomal) RNA synthesis is particularly sensitive to the presence of actinomycin, and this probably accounts for its pharmacological activity as well as its extreme toxicity to mammalian cells[6, 7].

Stereochemistry of actinomycin-DNA binding

A number of years ago, we determined the three-dimensional structure of an actinomycin-deoxyguanosine complex by x-ray crystallography[8-11]. This information suggested a model to understand the general features of how actinomycin binds to DNA. According to this model, the phenoxazone ring system on actinomycin intercalates between adjacent base-pairs, while pentapeptide chains lie in the narrow groove of the B- helix to form hydrogen bonds (in the case of d(pGpC) sequences) with guanine residues on opposite chains. Implicit in this model was the assumption that actinomycin binds to B-DNA, or a distorted form of B-DNA. The possibility that actinomycin might bind to some other discretely different DNA conformational state was not envisioned at that time.

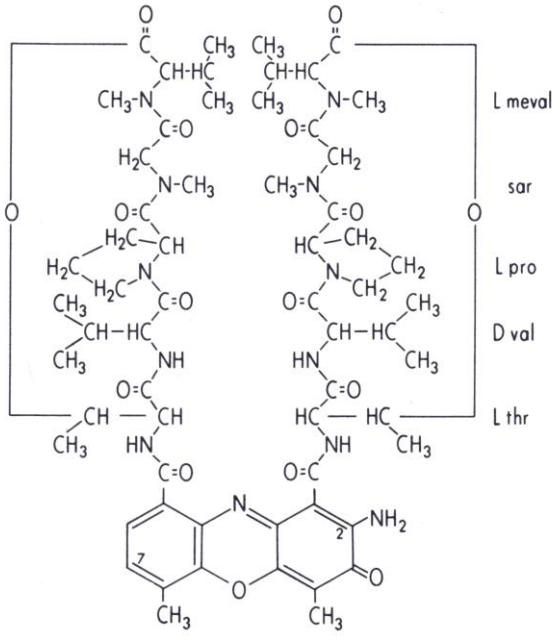

Figure 1. Chemical structure of actinomycin D. Abbreviations: L meval, L methyl valine; sar, sarcosine; L pro, L proline; D val, D valine; L thr, L threonine.

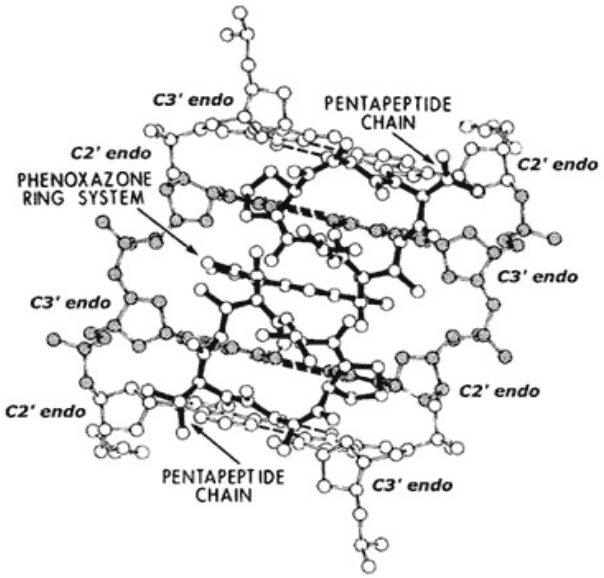

Figure 2: Actinomycin: beta-DNA binding model.

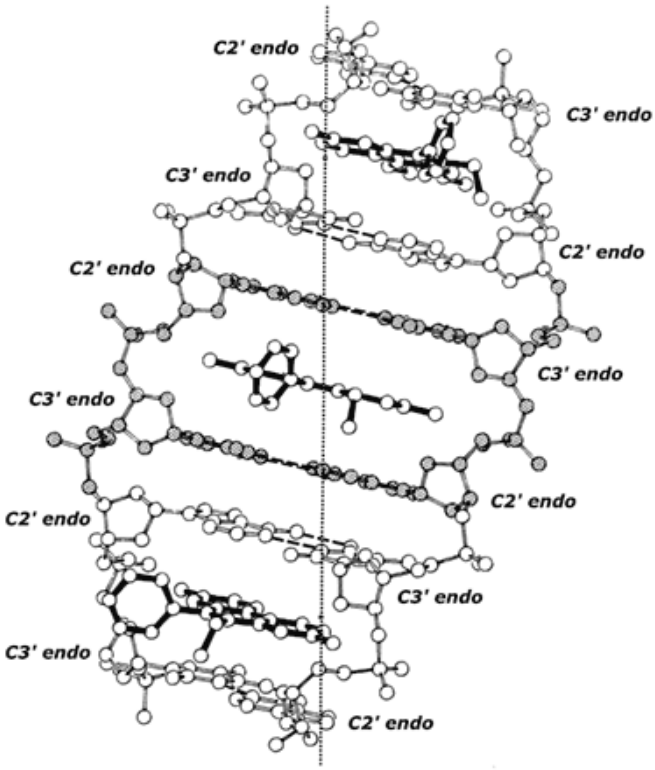

Figure 3: Ethidium: beta-DNA binding model.

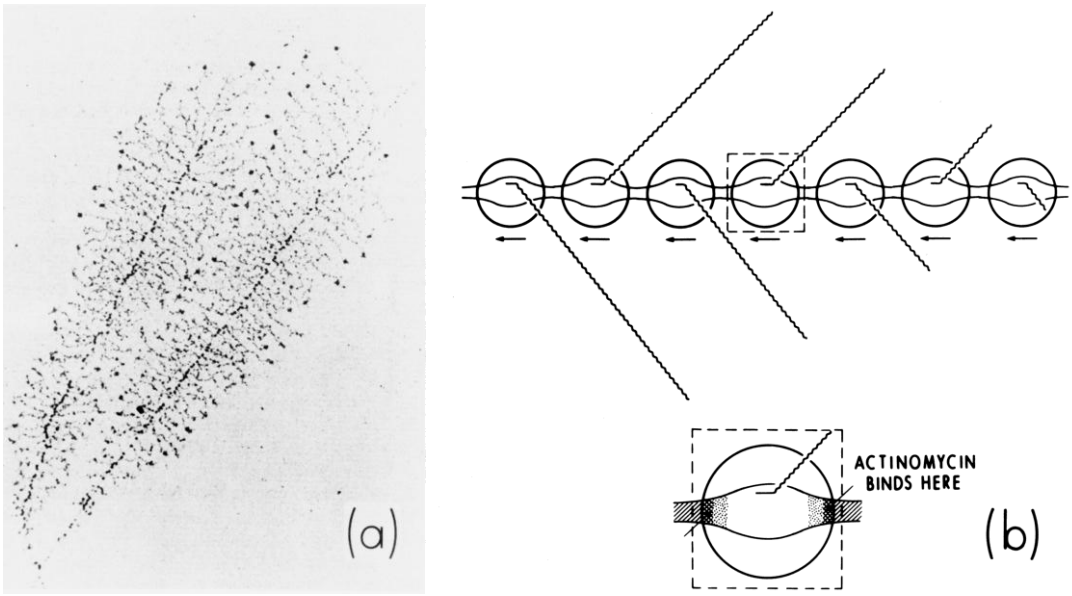

Figure 4: (a) Electron-micrograph of nucleolar genes undergoing transcription [18]. (b) Interpretation of the micrograph in (a) showing the mechanism of action of actinomycin. Actinomycin binds to beta-DNA, a conformational intermediate that exists within the boundaries connecting double-stranded B-DNA with single-stranded DNA in the transcription-complex. This immobilizes the complex, interfering with the elongation of growing RNA-chains.

A modification to this actinomycin-DNA binding model was subsequently proposed, which allows one to understand its mechanism of action (see Figure 2). This model is similar to the original one; however, it predicts that actinomycin binds to (what we have called) beta-DNA (i.e., *not* to B-DNA) – beta-DNA being a *metastable and hyperflexible* premelted form inferred from our wider crystallographic studies of planar drug molecules intercalated into a series of DNA-like and RNA-like self-complementary dinucleoside-monophosphates[12-14].

Figure 3 shows this same (extended) beta-DNA structure "pinned" by ethidium. The complex is an organized right-handed double helical structure in which the beta-structural element plus the intercalator form the asymmetric unit of the helix. This maximally elongated and unwound DNA duplex-structure, pinned by ethidium (and other planar intercalators) at saturating concentrations, readily explains the well-known observation of neighbor-exclusion intercalative drug-binding[15-17].

Mechanism of action of actinomycin D

I have next proposed beta-DNA to be an obligatory intermediate (i.e., a transition-state intermediate) in DNA-melting. This concept readily leads to understanding the mechanism of action of actinomycin D.

Figure 4 (a and b) shows an electron-micrograph of nucleolar genes undergoing very active transcription[18], and my interpretation of this process which indicates the mechanism of action of actinomycin D[19, 20].

Actinomycin intercalates into beta-DNA found within the boundaries connecting double-stranded B-DNA with single-stranded DNA in the transcription-complex. This immobilizes (i.e., "pins") the complex, interfering with the elongation of growing RNA-chains. In extremely active genes such as these, RNA polymerases lie in a close-packed arrangement along DNA. Interference with the movement of one polymerase by actinomycin is expected to inhibit the movement of other polymerases. This can explain why nucleolar RNA synthesis is so sensitive to the presence of actinomycin[21, 22].

Can the extreme toxicity of actinomycin D be exploited to preferentially kill malignant cells?

As described above, nucleoli within each nucleus in normal and malignant cells are known to contain large numbers of tandem repeats that contain 5S (ribosomal) genes undergoing transcription. Since rapidly dividing malignant cells require increased numbers of ribosomes to carry out protein synthesis, one might expect malignant cells to have many more 5S tandem repeats per nucleoli than normal cells (or alternately, it is possible that each nucleus within malignant cells contains many more nucleoli than normal cells – the number of 5S tandem repeats in each nucleolus remaining the same).

The presence of an underlying differential effect such as this, could allow actinomycin D to preferentially kill malignant cells. For this reason, trace amounts of actinomycin given over extended periods of time could be a powerful anticancer chemotherapeutic regimen. Of course, it is first necessary to carry out the appropriate experiments in mice or in other related mammals before attempting clinical use.

REFERENCES

1. Kirk, JM (1960) Biochim Biophys Acta 42: 167-169.
2. Goldberg, IH, Rabinowitz, M (1962) Science 136: 315-316.
3. Goldberg, I.H, Rabinowitz, M, Reich, E (1962) Proc Natl Acad Sci USA 48: 2094-2101.
4. Reich, E, Goldberg, IH (1964) Prog Nucleic Acid Res Mol Biol 3: 183-234.
5. Sentenac, A, Simon, FJ, Fromageot, P (1968) Biochim Biophys Acta 161: 299-308.
6. Perry, RP (1963) Exp Cell Res 29: 400-406.
7. Goldberg, IH (1975) Handb Exp Pharmacol. 38: 582-592.
8. Sobell, HM, Jain, SC, Sakore, TD, Nordman, CE (1971) Nature (London) 231: 200- 205.
9. Jain, SC, Sobell, HM (1972) J Mol Biol 68: 1-20.
10. Sobell, HM, Jain, SC (1972) J Mol Biol 68: 21-34.
11. Sobell, HM (1974) Scientific American 231: 82-91.
12. Sobell, HM (1985) Proc Natl Acad Sci USA 82: 5328-5331.
13. Sobell, HM (2009) Premeltons in DNA, Explanatory Publications, Lake Luzerne, NY ISBN 978-0-615-33828-6.
14. Sobell, HM (2013) Organization of DNA in Chromatin, Explanatory Publications, Lake Luzerne, NY ISBN 978-0-692-01974-0.
15. Crothers, DM (1968) Biopolymers 6: 575-583.
16. Wells, RD, Larson, JE (1970) J Mol Biol 49: 319-342.
17. Bond, PJ, Langridge, R, Jennette, KW, Lippard, SJ (1975) Proc Natl Acad Sci USA 72: 4825-4829.
18. Miller, OL, Beatty BR (1969) Science 164: 955-957.
19. Sobell, HM (2016) J Struct Funct Genomics 17: 17-31.
20. Sobell, HM (2016) How actinomycin binds to DNA and exerts its mechanism of action: http://atlasofscience.org/ (Henry M Sobell)
21. Leroy Liu and James Wang have provided a key insight into the nature of DNA supercoiling accompanying transcription that has shed additional light on this question. They have theorized that – in the presence of significant resistance to the rotational motion of the RNA polymerase and its nascent RNA chain around DNA during transcription – the advancing polymerase generates positive superhelicity in the DNA template ahead of it, and negative superhelicity behind it. In nucleolar genes, where there may as many as 200 RNA polymerases moving down the DNA template while synthesizing growing ribosomal RNA-chains, positive and negative superhelical DNA regions between them annihilate one-another, causing adjacent chains to bond-together to

form “trains” of transcription complexes, these now moving synchronously along DNA. *If this were the case, then the binding by one actinomycin molecule is sufficient to stop the entire “transcription-train” from moving along DNA.*

22. Liu, LF., Wang, JC (1987) Proc Natl Acad Sci USA 84: 7024-7027.