THE MODIFICATION BY ACRIDINE ANALOGS OF THE SURVIVAL OF ULTRAVIOLET IRRADIATED *Escherichia coli B**

John C. Lankford and K. Lemone Yielding**

The Laboratory of Molecular Biology
The University of Alabama in Birmingham

Received May 24, 1971

Summary: A comparison was made of the effects of mutagenic and non-mutagenic acridines on the survival of E. coli B following UV irradiation. Organisms were plated after various levels of UV exposure on both minimal and Brain Heart Infusion (Difco) agar either with no added drug or with 10^{-6} M 9-aminoacridinium chloride, acridine orange, or 10-methylacridine hydrochloride. The non-mutagenic acridine (10-methylacridinium chloride) caused no modification of UV survival. The most potent mutagen, 9-aminoacridine hydrochloride was a strong inhibitor; while acridine orange, which is less mutagenic was less effective in decreasing post-irradiation survival. Thus, with these 3 acridine analogs, mutagenic effects could be correlated with the ability to inhibit post UV survival.

Introduction

Since the initial studies on the mutagenicity of acridines by Orgel and Brenner (1961) and the investigation of acridine-DNA interactions by Lerman (1961), much effort has been aimed toward understanding better the mode of action of these drugs. It would seem there is not a simple relationship between the extent of drug binding and mutagenicity, although this has often been presumed to be the case.

It was shown by Lerman (1964) that methylation of the ring nitrogen of acridine could render the compound non-mutagenic. This information prompted Riva (1966) to study the strong and weak

*Supported by a grant from The American Cancer Society
**RCD Awardee, USPHS (GM22,698)

binding of several acridine analogs to DNA. Two of these acridine analogs were methylated on the ring nitrogen, and two other analogs were not. Riva's findings showed nearly identical binding to DNA when mutagenic acridines were compared with non-mutagens.

There has been much recent interest in the enzyme processes concerned with repair of exogenous mutagenic damage to DNA and to the regulation of these processes by drugs. Witkin (1963) demonstrated that acriflavin decreased the survival of a tyrosine requiring auxotroph of E. coli B/r following UV irradiation. The present investigations were aimed at seeking a correlation between the mutagenicity of various acridines and their ability to interfere with so called mutation repair.

Materials and Methods

Cultures of Eschrichia coli B were grown to late log phase in Brain Heart Infusion broth (Difco) at 37°C in a shaking water bath, and diluted to a density of about 10^5/ml with 0.9% NaCl. The bacteria were then irradiated in an open petri dish using a filtered GE G8T5 lamp such that essentially all emitted radiation was at 254 mμ. The ultraviolet dose was measured with a Blak-Ray short wave UV meter. Immediately after irradiation, 0.1 ml samples of the appropriate dilutions, were spread on 2 sets of 1.5% agar plates containing either minimal salts plus glucose or 3.7% Brain Heart Infusion. The acridine analogs were incorporated into the media at concentrations of 1×10^{-6} M. Care was taken to keep the organisms in the dark after irradiation, and incubation was conducted in the dark at 37°C. Bacteria on minimal-agar plates were incubated for about 40 hours while those on Brain Heart Infusion were incubated overnight. The surviving colonies were counted on a New Brunswick counter.

Results

The effects of the different acridines were demonstrated equally well in minimal media and Brain Heart Infusion agar. At 1×10^{-6} M, none of the acridines decreased survival of the unirradiated controls. This concentration is well below the optimum mutagenic concentration for acridines. The effects of irradiation on survival of the untreated E. coli B plated subsequently on minimal or nutrient media are illustrated in Figure 1 (curve A) and Figure 2 (Curve A) respectively. In each instance there was a characteristic decline in viability as a function of dose. It is also apparent that survival was enhanced on minimal over that on enriched media.

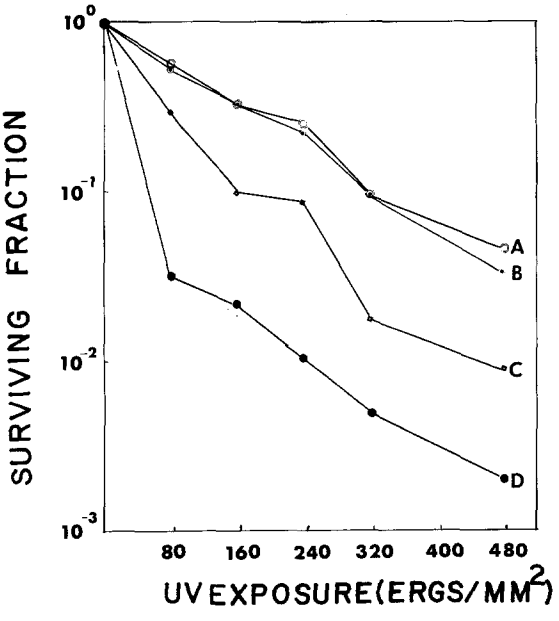

Figure 1. Survival of E. coli B plated on minimal agar with and without added drugs at 10^{-6} M after UV treatment: Control (Curve A); 10 methylacridinium chloride (Curve B); Acridine orange (Curve C); and 9-aminoacridine hydrochloride (Curve D).

The effects of 9-aminoacridine hydrochloride, acridine orange, and 10-methylacridinium chloride on post irradiation survival

were quite different although they have been reported to bind to DNA to approximately the same extent. (Riva, 1966)

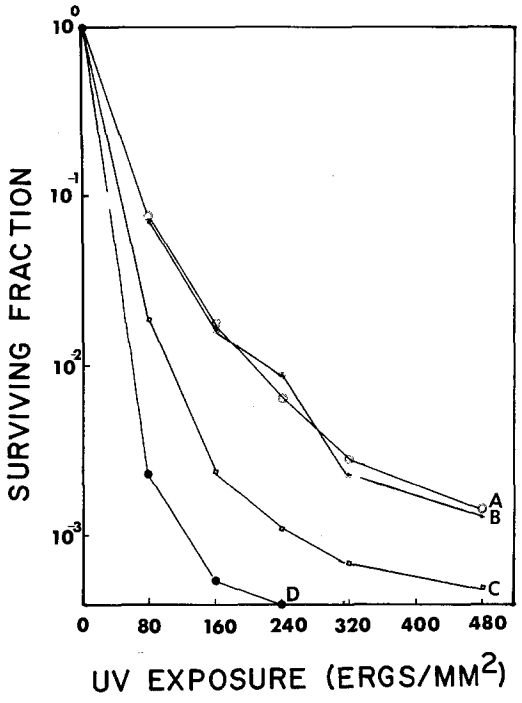

Figure 2. Survival of E. coli B plated on Brain Heart Infusion agar with and without added drugs at 10^{-6} M after UV treatment: Curves as in Figure 1.

The strongest mutagen, 9-aminoacridine hydrochloride caused a large decrease in survival (Curve D). For example, after only five seconds of irradiation at 16 ergs/mm^2/sec, survival on minimal media containing 9-aminoacridine hydrochloride decreased to 3% as compared to approximately 60% in the absence of the drug. In contrast, 10-methylacridinium chloride, which has been reported not to be mutagenic (Lerman, 1964), caused no decrease in post irradiation survival (Curve B).

Acridine orange, which is less mutagenic than 9-aminoacridine hydrochloride was also less inhibitory toward survival following UV irradiation (Curve C). Thus, in this limited number of acridines, mutagenicity appeared to be correlated with inhibition of the repair of UV damage.

It has been presumed that the mutagenic effects of the acridines depend on a direct interaction with DNA, although, as pointed out by Riva (1966), there is not a simple correlation with the extent of binding to DNA in vitro. The present experiments suggest another parameter which must be evaluated in the final interpretation of the biological effects of these drugs. It may be that the enzymes responsible for UV repair serve as sensitive detectors for subtle differences in binding between mutagenic and non-mutagenic drugs. Additionally, some of the 'acridine induced' mutations may, in fact, result from interference with a role of some of the "repair" steps in assuring genetic stability. Studies of additional drugs of this series in relation to mutagenicity and post-irradiation survival are obviously needed.

REFERENCES

1. Orgel, A. and Brenner, S., J. Mol. Biol., 3, 762, (1961).
2. Lerman, L. S., J. Mol. Biol., 3, 18 (1961).
3. Witkin, E. M., Proc. Nat. Acad. Sci. US, 50, 425, (1963).
4. Lerman, L. W., J. Cell. Comp. Physiol., 64 suppl. 1, 1 (1964).
5. Riva, S. C., Biochem. Biophys. Res. Com., 23, 606, (1966).