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A Bacterial Mutagenicity Study of Rivanol, an Acridine Derivative Used as an Abortifacient

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We have used the forward mutation to resistance to 6 azauracil to test the mutagenicity of rivanol (6, 9 diamino 2-ethoxy acridine) on *Escherichia coli*. Rivanol has been used to induce therapeutic abortions in midpregnancy and is considered safe and effective for this purpose. The findings reported here that rivanol, like other acridines, is a mutagen, at least in procaryotes, suggests that such use of rivanol be reconsidered in light of its possible genetic toxicity.

INTRODUCTION

Rivanol is a substituted diaminoacridine compound (6, 9 diamino 2-ethoxy acridine, Fig. 1) used in Asia and Sweden as an abortifacient for midtrimester abortion by extra-amniotic instillation [1,2,3]. Some reports suggest that rivanol is safer than saline for such abortions because of the absence of potential salt toxicity and because of the intrinsic antibacterial activity of rivanol and consequently fewer infections.

The apparent safety of rivanol has been documented in several large series of patients reported by Manabe and Manabe [2] and Ingemanson [3]. However, these reports considered only the short-term effects of this agent. Since rivanol is an acridine, a class of compounds known to bind strongly to DNA and to produce mutations [4,5,6], it is appropriate to consider the potential of rivanol to produce genetic damage, cancer, or other late sequelae. In this report we present evidence that in one bacterial mutagenicity test system, rivanol has significant mutagenicity. In light of this finding, it would seem reasonable that the need for rivanol abortions be especially carefully considered and that rivanol be evaluated in other systems to study its genetic toxicology.

MATERIALS AND METHODS

Bacteria: *Escherichia coli* SYMW is a derivative of *E. coli* K12 which has the genetic markers supF, supE, gal, and hsdR. The relevant phenotype for the present study is that the strain is wild-type at the *upp* locus (uracil phosphoribosyl transferase, called *ura*P by Miller [7]). Forward mutations from upp^+ to upp^- were scored by the ability of the mutant but not the wild-type bacteria to grow on minimal plates with glucose in the presence of 30 μg/ml of 6-azauracil [7]. *E. coli* SYMW was grown on Luria broth (10 gm tryptone, 5 gm yeast extract, 0.5 gm NaCl per liter) at 37°C.

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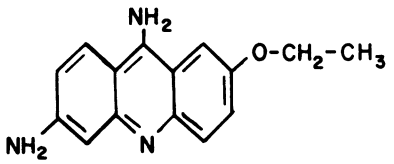

RIVANOL
(6,9-diamino-2-ethoxy-acridine)

FIG. 1. Structure of 6.9 diamino-2-ethoxy acridine. This compound is also known by the trivial names rivanol, ethodin, and ethacridine.

Chemicals: Rivanol (ethodin) was purchased from Sigma and was used without further purification. Acridine orange was purchased from Allied Chemical Company.

RESULTS

In order to test for the mutagenicity of rivanol and a related, known mutagen, acridine orange, we grew *E. coli* cells for many generations in the presence of these agents at various initial concentrations and then determined the frequencies of the cells with the mutant phenotype among the viable cells in the population. Specifically, we inoculated Luria broth cultures which had rivanol or acridine orange present at different concentrations and allowed growth to proceed at 37°C to stationary phase. A uniform inoculum, about 10^7 cells per ml, was used in each experiment. After 12–14 hours growth at 37°C with aeration, two determinations were made: the concentration of viable cells was determined by plating dilutions of each culture on Luria broth agar; the concentration of *upp* mutants in the population was determined by plating dilutions of each culture on minimal glucose containing agar which also

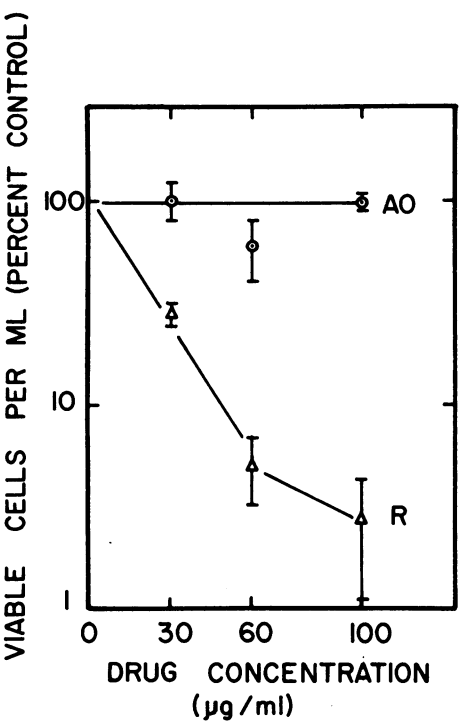

FIG. 2. Viable cell counts in cultures grown in the presence of various initial concentrations of rivanol or acridine orange. Four independent experiments were carried out and the data averaged. The error bars indicate the standard error of the mean for each drug concentration.

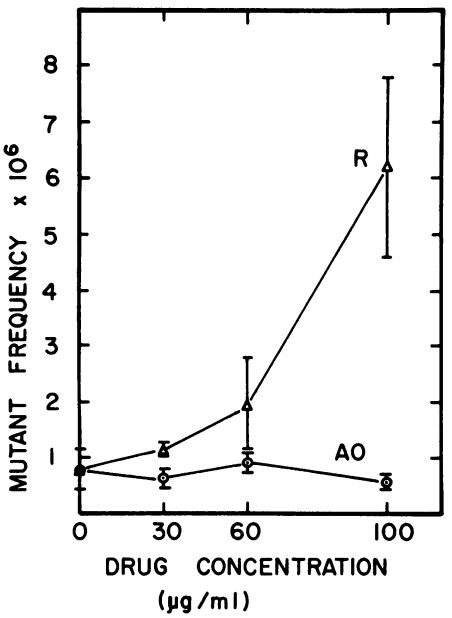

FIG. 3. Frequency of *upp* mutations in *E. coli* cultures grown in the presence of the indicated initial concentrations of either rivanol or acridine orange. Four independent experiments were carried out and the data averaged. The error bars represent the standard error of the mean for each drug concentration. These determinations were carried out on the same cultures analyzed in Fig. 2.

contained 30 μg/ml of 6-azauracil. The ratio of the latter to the former number gave the frequencies of *upp* mutants in each population.

Figure 2 shows the concentration of viable cells as a function of increasing concentrations of rivanol or acridine orange. In all experiments the rivanol-treated samples had fewer viable cells per ml than the untreated controls. Such toxicity by acridine orange was not observed at the concentrations tested.

The frequency of mutants in the treated and control cultures is shown in Fig. 3. Acridine orange at concentrations less than or equal to 100 μg/ml did not increase the frequency of *upp* mutants. However, rivanol exhibited a dose-dependent increase in mutant frequency up to eightfold at 100 μg/ml.

DISCUSSION

Rivanol has a structure quite similar to other acridines which are known bacterial mutagens [4,8]. In general, the acridines and proflavines have been found to be rather weak carcinogens when tested in mammalian systems [9,10]. Rivanol, however, binds two to five times more strongly to DNA than the commonly studied compounds acridine orange and proflavine [5,6]. This fact may account for the selective bacterial toxicity we observed when comparing rivanol and acridine orange.

Our failure to detect significant mutagenicity with acridine orange is not surprising since we used a concentration of drug insufficient to cause any inhibition of growth. Mutagenicity depends on achieving a significant intracellular concentration of the drug, a concentration that frequently results in killing of a certain fraction of the organisms. Also the relative permeability of a given bacterial strain to a specific agent has been related to its mutagenicity in the case of proflavine [11]. The bacteria grown in rivanol showed a dose-related increase in mutation frequency which was significantly different from the untreated controls at 100 μg/ml ($0.01 < p < 0.025$).

The mutational test system employed in this study is relatively general; that is, it should detect mutagens regardless of their mutational specificity or mechanism.

This is because the system scores forward mutations or loss of a gene function. Any type of mutation that results in a non-functional uracil phosphoribosyl transferase enzyme confers 6-azauracil resistance on the cell. Thus, frame-shift mutations, point mutations, and genomic rearrangements are all detected by this system. Although this system does not have the specificity of some of the reverse mutation test systems devised by Ames and his colleagues [8], it might be increased in sensitivity by addition of mutations which increase permeability to acridines and which block certain DNA repair pathways [11].

The use of rivanol for extra-amniotic instillation to induce midtrimenster abortion is widespread in Asia [1] and has been employed in Sweden as well [3]. Its safety in terms of immediate side effects seems to compare favorably with other agents. The genetic toxicity of this agent remains to be evaluated, however. It may be that rivanol is not absorbed into the maternal circulation, but it would be surprising if some of the extra-amniotic rivanol solution could not find its way through the decidua into the maternal blood and lymphatic drainages. Few physiologic studies have been published to help settle this issue. Lewis et al. [12] measured the rivanol concentration in plasma of women receiving this drug for therapeutic abortion. They found peak levels of 0.02 μg/ml. Rising et al. [13] found that orally administered rivanol is poorly absorbed by humans and that most of the administered drug is excreted in the feces. Still, they found that about one percent of the ingested rivanol was absorbed and partially metabolized. These low blood levels that seem to occur after oral or extra-amniotic application may be below the concentration which can cause significant genetic damage or induce malignancies. On the other hand, mammalian cells may be more permeable to these compounds than are bacterial cells, so low concentrations may pose significant risks. It is clear that such considerations need further investigation and that the group of patients treated with rivanol ought to be followed for a sufficient period of time to evaluate the possible long-term effects of this agent.

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