Apparent Antimutagenic Activity of Quinacrine Hydrochloride in Detroit-98 Human Sternal Marrow Cells Grown in Culture

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SUMMARY

The ability of a small proportion of the cells in cultures of Detroit-98 (D-98) human sternal marrow cells to attach to glass in the presence of 8-azaguanine has been previously shown to be caused by a mutation to resistance to this compound. This paper presents results of experiments dealing with the effect of the addition of quinacrine hydrochloride to the culture medium of D-98 cells on the frequency of this mutation. Such cells have been previously shown to be azaguanine-resistant mutants. In three independent experiments, cultures which were grown for several subcultivations in the presence of 0.06 μg/ml of quinacrine hydrochloride contained one-half to one-third the mean number of azaguanine-resistant mutants found in control cultures. On the other hand, the incorporation of quinacrine hydrochloride into the culture medium of D-98/AG cells, an azaguanine-resistant subline of the D-98 line, had no effect on its cloning efficiency in the presence or absence of added azaguanine. The results are discussed as a possible confirmation of the proposed antimutagenic action of quinacrine hydrochloride on mammalian cells.

INTRODUCTION

Szybalski (7) has reported that the development of resistance to Azg3 in D-98 cells in culture is a multistep mutational event in which each step reflects a discrete level of resistance and has a finite incidence. The frequency of each single-step event in the overall process can be readily measured by simply employing the appropriate Azg concentration in the assay for mutation frequency. However, not all of the biochemical events which are involved in these steps are understood. Thus, while the later events in the acquisition of resistance are explained by the deletion of the enzyme, nucleotide pyrophosphorylase, no change in the level of this enzyme was found in the first-step mutants. Furthermore, cross-resistance with a considerable number of other nucleosides of variable mode of action was seen. Two striking observations were made by Szybalski and Smith (8) in relation to this series of mutants: First, the frequency with which some of them occur is remarkably high, ranging to $10^{-3}$ per cell per division, and second, the frequencies were not affected when the cells were treated with or exposed to a number of compounds or conditions which are known to cause mutations in bacterial and viral systems.

Previous studies in our laboratory (2, 3) have sought to demonstrate that quinacrine hydrochloride as well as a number of other polyamines are active mutation suppressants (antimutagens) in bacterial and mammalian systems. It seemed that the first step in the development of resistance to Azg as described by Szybalski thus offered an interesting test model for examining this effect further. This paper reports the results which were obtained.

MATERIALS AND METHODS

Detroit-98 cells, derived from a human sternal puncture and an Azg3 derivative of the same, were obtained from the cell repository of the American Type Culture Collection. The cells were grown on basal medium (Eagle), supplemented with 8% fetal calf serum, 80 μg/ml neomycin sulfate, and 25 μg/ml Polymyxin B. They were harvested by trypsinization with 0.25% pancreatin in saline and were washed several times with complete medium to remove the trypsin. Cloned sublines were used throughout the experiments. Five to eight thousand cells applied to each 50-mm Falcon disposable Petri plate in 5 ml of medium were incubated in a vibration-free incubator at 37°C in an atmosphere of 95% air and 5% CO2. The medium in the plates was changed every three days. Azg, when present, was added at each medium change. At the end of 12 days’ incubation, the medium was aspirated off, and the cells were fixed briefly with 10% neutral formalin and then stained for approximately two hours with a 1:2 dilution of Giemsa tissue stain. After destaining, the number of colonies per plate were counted by projecting the plates on a screen with the aid of a plate reader. The use of plates having a rectilinear grid in their base facilitated the counting considerably. Fifteen replicate plates were used for each determination.

RESULTS

Preliminary results using the Azg concentration reported by Szybalski (6 μg/ml) resulted in “mutation frequencies” ap-
proaching 10% in the D-98 cells. For this reason the effect of
the Azg concentration on the plating efficiency of D-98 and
D-98/AG cells was examined.

The results are shown in Chart 1. There was a two-fold dif-
ference in the concentration of Azg causing a 50% inhibition
between these cell lines; a dose of 10 μg Azg/ml was chosen
for all subsequent experiments.

The effect of the addition of quinacrine hydrochloride on
the plating efficiency of the D-98 cells was examined and the
results are presented in Chart 2. When 10^6 cells were plated, a
level of 0.2 μg quinacrine per ml was without deleterious ef-
fects. However, when only a few thousand cells were plated,
the same concentration of quinacrine resulted in a complete
inhibition of attachment. This observation, as well as similar
observations with L1210 cells (in press), suggests that the
toxicity of quinacrine is more dependent on the ratio of
drug concentration to the cell concentration than it is on
the absolute drug concentration. This implies that cells may
concentrate the drug from the medium and, when a certain
critical number of binding sites are occupied, toxicity results.
A dose of 0.06 to 0.10 μg/ml was used in all further experi-
ments.

Table 1 summarizes the results of three separate experiments
in which the effect of adding quinacrine to cultures of D-98
cells on the number of Azg^+ cells resulting was examined. The
appropriate cultures had been maintained in the presence of
quinacrine for a period of up to four months prior to the
experiments. This was necessary since it was found (line 4 of
Table 1) that the addition of the drug at the time of plating
had no effect on the number of mutants which were found.
Culture in the presence of quinacrine did not affect the plating
efficiency of D-98/AG cells in the presence of Azg (last line of
Table 1).

**DISCUSSION**

The literature contains contradictory reports regarding the
mutagenic and/or antimutagenic properties of the acridines (1,
5, 6). Thus either event could be documented with sufficient
precedents to make it appear credible. One of the key differ-
ences among the various studies is the dose of the drug which
was used. In many cases the concentrations used were un-
doubtedly toxic, and under those conditions mutagenesis was
frequently observed. The studies reported here carefully em-
ployed nontoxic concentrations of quinacrine.

In interpreting the results obtained as effects on mutation
frequency, it must be borne in mind that the mutation nature
of the Azg^+ event has not been established critically. Thus,
while the cells which grow out of colonies which had attached
in the presence of Azg are Azg^+ in the sense that they inherit
an increased efficiency for plating in the presence of the drug,
neither Szybalski nor we have shown clearly that this is not, in
fact, due to a selection from among a randomly variable popu-
lation. The distinguishing test (4), based on a comparison of
the variance of the mutation frequency within a population to
the variance between populations, is almost impossible to
carry out because of the enormously large number of samples
which would be required. The mutational nature of the Azg^+ event is, therefore, tentatively based on the fact that it can be
demonstrated even when clones which are derived from single
D-98 cells are used as the inoculum for these experiments.

Before the “antimutagenic” effect of quinacrine can be ac-
cepted as arising from an effect on the chromosomal mutation
itself, several alternative explanations must be ruled out; the
results of the present study can only do this in small part.
D-98 cells are genetically unstable, and even after cloning, in-
dividual cells may vary in the number of copies of a given gene
Mutation Suppression in Detroit-98 Cells

Table 1

<table>
<thead>
<tr>
<th>Condition</th>
<th>Experiment No.</th>
<th>Normal cells</th>
<th>Quinacrine-grown cells</th>
<th>R$^b$</th>
<th>95% confidence range</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-98$^c$ cells grown with/without quinacrine</td>
<td>1</td>
<td>0.010</td>
<td>0.003</td>
<td>3.3 ± 0.3</td>
<td>1.86–4.85</td>
</tr>
<tr>
<td>D-98 cells grown with/without quinacrine</td>
<td>2</td>
<td>0.025</td>
<td>0.012</td>
<td>2.1 ± 0.2</td>
<td>1.47–2.88</td>
</tr>
<tr>
<td>D-98 cells grown with/without quinacrine</td>
<td>3</td>
<td>0.011</td>
<td>0.0034</td>
<td>3.3 ± 0.4</td>
<td>1.70–6.40</td>
</tr>
<tr>
<td>D-98 cells; quinacrine added at time of plating</td>
<td>4</td>
<td>0.025</td>
<td>0.025</td>
<td>1.0 ± 0.2</td>
<td>0.71–1.40</td>
</tr>
<tr>
<td>D-98/AG cells grown with/without quinacrine</td>
<td>5</td>
<td>0.041</td>
<td>0.067</td>
<td>0.61 ± 0.29</td>
<td>0.37–1.02</td>
</tr>
</tbody>
</table>

Summary of results testing the antimutagenic activity of quinacrine on the Azg$^f$ mutation in D-98 cells. Cloned cells were carried with/without 0.06 $\mu$g/ml quinacrine for up to four months. The number of colonies formed (plating efficiency) in the presence of 10 $\mu$g/ml Azg was compared to the plating efficiency without Azg. The results are the averages of 15 individual determinations, and the ratios of control/treated are followed by the coefficient of variation (C.V.). 95% confidence limits on the ratios are obtained as the range

\[
\frac{R}{(1 + C.V.)^2} \text{ to } R \times (1 + C.V.)^2.
\]

If 1.0 falls outside this range, it may be concluded that results in control cells are significantly different from those in treated cells.

$^a$Mutation frequency = Average no. of mutant colonies (on Azg medium) / Average total no. of colonies (on unsupplemented growth medium)

$^bR$ = Mutation frequency in control cells / Mutation frequency in quinacrine-grown cells

$^c$Azg, 8-azaguanine; Azg$^f$, cells resistant to 8-azaguanine; D-98, Detroit-98 human sternal marrow cells; D-98/AG, Azg-resistant subline of D-98.

(polyploidy) which they contain. Assuming that the chromosomal mutation resulting in Azg$^f$ cells is a dominant event and, further, that the probability of this event occurring is the same for each copy of the respective gene which may be present in a given cell, it follows that cells which are polyploid with respect to this gene have a greater chance of developing resistance to Azg than do diploid cells. If we now postulate that quinacrine reduces the frequency of polyploid cells in the culture, this in itself might explain the observed reduction in the frequency of Azg$^f$ mutants. Since there cannot be a selective advantage to polyploid cells on a D-98 culture under normal conditions [otherwise they would have taken over by now], there are two ways in which quinacrine might do this: (a) it could selectively inhibit the replication of preexisting polyploids, and (b) it could decrease the probability of developing new polyploids. The second possibility is, in a sense, an "antimutagenic" action in its own right, and we cannot distinguish it from the direct action on chromosomal mutations by the present study. The first possibility seems unlikely, however, from a consideration of the quantitative aspects involved. Since quinacrine was used at a nontoxic concentration (< 20% I), any population of cells which was selectively inhibited from growing must have been small, certainly no larger than 20% of the total population. If we now attempt to explain the 2- to 3-fold reduction in the frequency of Azg$^f$ (Table 1) by the inhibition of growth of no more than 20% of all the cells, it follows that the mutation frequency in those 20% would normally be 2–3 X 5 or 10–15 times that of the remaining, diploid population. Thus, if the mutation frequency per gene is constant, this would require the polyploid population to have 20–30 copies of the susceptible gene, a value which seems absurd.

In many ways this model of a mutation closely resembles the bacterial model which we have described in the past (2). Thus it had been observed early in the course of the present studies that the apparent resistance to Azg in D-98 cells merely reflected the ability of the cells to attach to the Petri plates with increased efficiency in the presence of the drug. Once attachment had occurred in the absence of the drug, the addition of Azg had no significant effect on the number of attached cells which subsequently developed into colonies. Thus the "mutants" preexist in the population at the time of plating, and the plating in the presence of Azg is the selection device for demonstrating their presence. The requirement for the presence of quinacrine during the growth of the cells (and, therefore, presumably during the period when the mutations arise)
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is analogous to our findings (2) that spermine had to be present during the growth of E. coli in order to permit the demonstration of an antimutagenic effect in that system. Thus the results of the present study support the conclusion that the addition of nontoxic concentrations of quinacrine hydrochloride to D-98 cells during growth can significantly reduce the incidence of the "mutation to resistance to Azg."

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REFERENCES

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