Reduction in the Frequency of Mutation to Resistance to Cytarabine in L1210 Murine Leukemic Cells by Treatment with Quinacrine Hydrochloride

Michael K. Bach
The Upjohn Company, Research Division, Kalamazoo, Michigan 49001

SUMMARY

The cloning of single cells in agar was employed to study the effect of the addition of quinacrine hydrochloride to cultures of L1210 cells on the frequency of the mutation to resistance to cytarabine (ara-C). In three independent experiments this frequency was reduced 5- to 16-fold after prolonged culture in the presence of quinacrine. Attempts to demonstrate a concomitant increase in the number of L1210-bearing leukemic mice which are “cured” by combining ara-C treatments with treatments with quinacrine under idealized experimental conditions yielded marginally significant results in the predicted direction. The results can be interpreted as supporting an antimutagenic effect of quinacrine on mammalian cells similar to the antimutagenic effect of this compound in bacterial cells which we had demonstrated previously.

INTRODUCTION

In a previous paper (2) we described the characteristics of a relatively frequent mutation to resistance to 1-β-D-arabinofuranosylcytosine (cytarabine) (ara-C) in the line of L1210 murine leukemia used in our laboratory. In view of our previous interest in finding methods for reducing the mutation frequency in bacterial systems (6-8), it seemed desirable to examine the effect of one of the more active antimutagens on the mutation frequency in this mammalian system. This paper describes the results obtained with L1210 cells in vitro and with L1210 leukemia in vivo.

MATERIALS AND METHODS

The culture, single cell cloning, determination of mutation frequency, and in vivo passage of L1210 cells have been described previously (2). Cultures which were treated with quinacrine hydrochloride were established from inocula of 100 cells and were maintained independently from one another and in the presence of 0.2 μg/ml of quinacrine hydrochloride for periods of several months. Control replicate cultures were established in the same manner but were maintained without the addition of quinacrine. For the in vivo experiments, groups of 50 to 75 female BDF₁ mice (16-18 gm) were injected intraperitoneally with 500 pooled L1210 cells (grown in vitro) in prewarmed complete medium RPMI 1634 and were treated as indicated in Table 2. All other injections were in 0.2 ml of physiologic saline and were made subcutaneously in the midback. Deaths were scored daily, and the experiments were terminated at least one week after the last deaths had occurred. The duration of the experiments after the last treatment with ara-C was longer than the period required for a single surviving L1210 cell to kill a mouse.

RESULTS

Since quinacrine, and acridines in general, are known to cause mutations when they are used at toxic concentrations in a variety of systems (1, 3, 9, 12), we have maintained the drug concentration in our experiment at a nontoxic level. Chart 1 describes the effect of increasing concentrations of quinacrine hydrochloride on the growth of L1210 cells. It should be stressed that our results with several different mammalian cell lines indicate that the critical factor in the toxicity of this drug is not the absolute concentration, but rather the ratio of drug concentration to the number of cells added in the inoculum, since the drug is known to be concentrated by the cells against a concentration gradient. A concentration of 0.2 μg/ml was used to maintain the cultures which were grown in the presence of quinacrine. Since the cell inoculum used under maintenance conditions was higher than the 2 × 10⁴ cells we used in Chart 1, no toxicity was encountered at this concentration.

Table 1 reports the results of several experiments in which the frequency of the cells resistant to ara-C (ara-C₁) mutation in cultures, which were maintained with and without quinacrine, was compared. Each number is the average frequency obtained in 10 independent cultures which had been maintained independent of one another for several months of semiweekly transfers. The differences in frequency are significant at P = 0.005 when the variation of the ratio of the frequency in the treated to the control groups from experi-
Table 1

<table>
<thead>
<tr>
<th>Independent culture No.</th>
<th>Experiment No. 1&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Experiment No. 2&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Experiment No. 3&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Quinacrine-grown</td>
<td>Control</td>
</tr>
<tr>
<td>1</td>
<td>3.7</td>
<td>&lt;0.2</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>1.7</td>
<td>&lt;0.2</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>1.0</td>
<td>&lt;0.2</td>
<td>13.5</td>
</tr>
<tr>
<td>4</td>
<td>&lt;0.2</td>
<td>&lt;0.2</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>1.0</td>
<td>&lt;0.2</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>1.4</td>
<td>0.5</td>
<td>4.5</td>
</tr>
<tr>
<td>7</td>
<td>1.7</td>
<td>0.2</td>
<td>18</td>
</tr>
<tr>
<td>8</td>
<td>1.0</td>
<td>0.2</td>
<td>15</td>
</tr>
<tr>
<td>9</td>
<td>1.7</td>
<td>0.8</td>
<td>14</td>
</tr>
<tr>
<td>10</td>
<td>3.1</td>
<td>0.5</td>
<td>16</td>
</tr>
</tbody>
</table>

Average 1.65 ± 0.34 0.32 ± 0.21 10 ± 1.6 0.58 ± 0.16 9.4 ± 2.3 0.57 ± 0.44

Mutation rate to resistance to 1-β-D arabinofuranosylcytosine (cytarabine) hydrochloride (ara-C) in L1210 cells <i>in vitro</i> when cells are grown in the presence or absence of quinacrine hydrochloride. Three and 6 × 10⁴ L1210 cells were inoculated with 5 ml of complete medium RPMI 1643 plus 0.1% Ionagar 5Î£ (Oxo, Ltd., London) containing 0.1 µM aria-C and incubated at 37°C for 12 days. The number of colonies in each tube were counted (Experiments 2 and 3), or the number of tubes having no colonies were counted (Experiment 1). Results are reported as the rate per cell per division times 10⁵, and each average value is based on 10 replicate tubes. For purposes of computing averages, the frequencies which were less than 0.2 (i.e., no mutant colonies found in any of the 10 replicate tubes) are taken as equal to 0.2.

<sup>a</sup>Computed by the null method, \( m = \frac{(-2.303 \log P_0)}{N} \) (14).

<sup>b</sup>Computed by the equation, \( r = \frac{mN}{\ln 2} \) (14).

where \( r \) is the average number of colonies, \( N \) is the number of cells added per tube, \( C \) is the number of replicate tubes, \( P_0 \) is the ratio of tubes without colonies to the total number of tubes used, and \( m \) is the mutation rate.
are shown in Table 2. As expected, the mean survival time of those animals which ultimately died was prolonged, even when ara-C treatments were administered relatively late in the course of the disease. On the other hand, there was little if any effect on the number of animals surviving ("cures") when ara-C was administered alone, except at the earliest time points. This is consistent with previous observations (11) that "cures" with ara-C can only be effected before there are $10^6$ L1210 cells in a mouse. Treatment with quinacrine had no effect on the median survival time of those mice which died in any of the groups (comparing Groups 1 and 2, 3 and 4, etc.). On the other hand, this treatment had a pronounced effect on the number of survivors in the group receiving ara-C on Days 6 to 10. In the first experiment, this difference is significant at the 1% level and there is a 95% confidence that it is real, using a one-tailed test for significance (4).

While the same trend exists in the second experiment, the results in that case were not significant. The interpretation of the results is encumbered by the unexpected increase in the number of survivors in the control group which received quinacrine in the first experiment. This difference, too, is statistically significant, although in numerous previous experiments no "cures" due to quinacrine had been seen. If the results from the two experiments are pooled, recognizing the pitfalls in such a procedure, the results in the third column of Table 2 are obtained. It is seen that only the difference between the groups receiving ara-C on Days 6 and 10 remains significant (5% level, 95% confidence) under this treatment.

![Chart 2. Theoretical plot of the expected survival time of mice inoculated with L1210 cells vs the dose of the inoculum. The plot is adapted from Skipper et al. (11). The horizontal lines intersecting this plot represent the range of cell concentrations which are likely to contain at least one cell resistant to 1-ß-D-arabinofuranosylcytosine (ara-C) (solid lines, control; dashed lines, grown with quinacrine) based on the mutation rates in vitro.](chart.png)
DISCUSSION

In designing experiments to test for the antimutagenic activity of a compound, it is essential that the inoculum be sufficiently small to assure that no mutant cells are introduced with the inoculum. Furthermore, the antimutagenic compound must not offer a selective advantage to the nonmutants. In the experiments described here, the inoculum for the culture experiments was at least 10 times less than the mutation frequency for the ara-C<sup>r</sup> mutation (14). By keeping the cultures separate from one another for several months, the cells present at the time of assay had undergone a large number of divisions in the presence or absence of the drug. The length of time in culture, based on the number of cell divisions which occurred, is very similar to that of a bacterial culture which divides twice an hour for one or two days. Thus, the ten cultures per treatment which were used can truly be considered to have been independent of one another. With regard to the second point, the addition of 0.2 µg/ml quinacrine to the cloning medium had no effect on the cloning efficiency of either normal or ara-C<sup>r</sup> cells.

While the results obtained do support an antimutagenic effect for quinacrine in these cells, and the analogy to the antimutagenic activity of this drug in E. coli makes this interpretation even more plausible, other interpretations cannot be ruled out completely. One possibility, which is particularly bothersome, concerns the appearance of the colonies in the two sets of cultures. Whereas the few ara-C<sup>r</sup> colonies, which appeared in the cultures that had been grown in the presence of quinacrine, were fairly large and discrete, there was a tendency to have small, relatively loose colonies in the cultures which had been grown in the absence of the drug. Possibly, the larger number of relatively small colonies might have arisen from the break-up of more loosely bound large colonies of these cells. The known ability of quinacrine to bind to membranes and to increase their stability (13) could bring about such a difference in cell-to-cell adherence and, therefore, in colony formation. Obviously, this objection does not apply when the null method (14) is used for the estimation of the mutation frequency. Fortunately, this was possible in one of the experiments, and the results obtained are essentially the same.

With regard to the in vivo results, it should be recalled that, in general, death from L1210 leukemia, following even massive therapy with ara-C, has not been correlated with the prevalence of ara-C<sup>r</sup> cells in the animal (10). Thus, several attempts to demonstrate antimutagenic effects of quinacrine by increasing the median survival time in animals receiving ara-C therapy were uniformly unsuccessful, as were a few efforts to show an increased frequency of ara-C<sup>r</sup> cells in ascitic fluid from animals taken at the median survival time. Despite this, it was hoped that some added cures might be obtained by combination therapy when treatment was initiated at a critical stage—after the time when ara-C alone can effect massive cures, but before the time when the disease is so far advanced that death will no longer be a reflection of the presence of resistant cells, but rather of cells which were inaccessible to the drug (11). On the surface, the results obtained (Table 2) do support this hypothesis, but the inconsistencies already mentioned (the apparent “curative” effect of quinacrine by itself in the first experiment and the lack of significance to the results in the second experiment) require that the interpretations be viewed with much caution and reservation. It is possible that more pronounced differences could be obtained by changes in the dosage regimen for ara-C, by introducing a third set of injections with ara-C at Day 14, by changing the dosage of quinacrine, or by other means. Unfortunately, the massive effort which is required to conduct these experiments prevents their execution at this time.

Since relapses in the treatment of leukemia are frequently associated with resistance and failure to reinroduce or maintain remission with the previously used drug, the development or selection of resistant cells remains a major problem in the therapy of these diseases. It is not possible to assess beforehand how large an antimutagenic effect would be useful in these situations or, indeed, whether the resistance which develops is due to selection of preexisting resistant cells or to mutations during therapy. The selection of preexisting mutants seems unlikely in the face of the three- and four-drug combinations which are now commonly used to induce remission. The known capacity of a single surviving leukemic cell to kill the host would justify the use of even the best antimutagen only under conditions when the primary drug combination being used can be expected to reduce the cell population below the expected frequency of the mutation to resistance. Under such conditions, it may be possible to discriminate between the direct mutation and the selection of preexisting mutants by the application of antimutagens of established potency, in combination with the best-known remission-inducing therapy. Clearly much remains to be done to develop such a situation.

ACKNOWLEDGMENTS

I wish to thank Mrs. Lois Smith for her devotion and patience in carrying out many of these experiments. I am indebted to Mr. Jack I. Northam for the statistical evaluation of the results.

REFERENCES


Reduction in the Frequency of Mutation to Resistance to Cytarabine in L1210 Murine Leukemic Cells by Treatment with Quinacrine Hydrochloride

Michael K. Bach


Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/29/10/1881

Sign up to receive free email-alerts related to this article or journal.

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.