Glutathione Dependence of Neocarzinostatin Cytotoxicity and Mutagenicity in Chinese Hamster V-79 Cells

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ABSTRACT

Neocarzinostatin (NCS) is mutagenic in bacteria, yeast, fungi, and mammalian cells. In cell-free systems, DNA strand breakage induced by NCS requires a reducing agent like 2-mercaptoethanol, unless very high (>100 μg/ml) concentrations of NCS are used. In this study, we have investigated the role of the sulfhydryl compound glutathione (GSH), which is usually the most common intracellular thiol, in the bioactivation of NCS to a toxic and mutagenic species. Chinese hamster V79 cells were pretreated with one of two GSH depleting agents, buthionine sulfoximine or diethyl maleate. These agents deplete GSH via different mechanisms, but both will lower GSH levels within the cell to <5% of control (untreated) values. GSH-depleted cells and control cells were then exposed to NCS concentrations of 0.5–2.5 μg/ml for 1 h, assayed for survival, and plated for expression of hypoxanthine-guanine phosphoribosyltransferase-negative (HGPRT−) mutants. After an expression period of 7 days, during which the cultures were subcultured twice, HGPRT− mutants were selected by plating in hypoxanthine-free medium containing 5 μg of 6-thioguanine per ml at a density of 2 × 10⁴ cells per 100 mm dish. NCS alone decreased the surviving fraction to about 1% at 2.5 μg/ml and produced dose-related increases in HGPRT− mutants that reached >10 times the spontaneous mutation frequency at 2.5 μg NCS per ml. In GSH-depleted cells, however, NCS was only mildly cytotoxic (60–80% surviving fraction) and did not produce dose-related increases in HGPRT− mutants over cells treated only with diethyl maleate or buthionine sulfoximine. Thus, GSH appears to be the main reducing agent for the bioactivation of NCS to a toxic and mutagenic species in Chinese hamster V79 cells.

INTRODUCTION

Neocarzinostatin is a polypeptide antibiotic that has shown some antitumor activity in clinical trials (1, 2) and which continues to be the subject of clinical investigation (3–5). The biologically active portion of the molecule is a nonprotein chromophore (6), the complete structure of which has recently been proposed (7). The chromophore induces single strand breaks in DNA by abstraction of a hydrogen from the C-5′ carbon of deoxyribose, which leads to the production of C-5′-aldehyde and a strand break (8). Chromophore-DNA adducts (9) and alkali-labile breaks (10, 11) are also induced by the NCS2 chromophore. In cell-free systems, DNA damage by NCS requires the presence of a reducing agent, such as a sulfhydryl group donor like 2-mercap-
Table 1

<table>
<thead>
<tr>
<th>Treatment condition</th>
<th>Relative surviving fraction</th>
<th>HGPRT(^+) mutants per (10^6) clonable cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>1.00</td>
<td>0.7</td>
</tr>
<tr>
<td>BSO (1 mM)(^a)</td>
<td>0.94</td>
<td>0.2</td>
</tr>
<tr>
<td>BSO (5 mM)</td>
<td>1.00</td>
<td>0.4</td>
</tr>
<tr>
<td>BSO (10 mM)</td>
<td>1.00</td>
<td>0.7</td>
</tr>
<tr>
<td>DEM (0.5 mM)</td>
<td>0.97</td>
<td>0.5</td>
</tr>
<tr>
<td>DEM (1.0 mM)</td>
<td>0.84</td>
<td>0.6</td>
</tr>
<tr>
<td>MNU (0.5 mM)</td>
<td>0.67</td>
<td>71.5</td>
</tr>
<tr>
<td>MNU (0.5 mM) plus</td>
<td>0.61</td>
<td>90.0</td>
</tr>
<tr>
<td>BSO (1 mM)</td>
<td>1.00</td>
<td>0.7</td>
</tr>
</tbody>
</table>

\(^a\) Exposure times were 8 h for 1 mM BSO, 4 h for 5 and 10 mM BSO, 2 h for DEM, and 1 h for MNU and MNU plus BSO.

Charts 1 and 2 show the results of NCS treatment on control and GSH-depleted cells. Both charts show the mean of two experiments, with the standard error when it is larger than the symbol. Both toxicity and mutagenicity are considerably reduced in cells that had been depleted of GSH at the time of NCS exposure. Mutation frequencies remained nearly the same when the expression period was extended to 9 days (data not shown). NCS did not induce any ouabain-resistant mutants in either of two experiments. The spontaneous mutation frequency in these experiments was 0.85 per \(10^6\) clonable cells, and the positive control (10 mM ethyl methane sulfonate) mutation frequency was 53 per \(10^6\) clonable cells.

DISCUSSION

These results indicate that GSH plays a major role in the activation of NCS to a toxic and mutagenic species in a mammalian cell line. The nearly identical results seen with BSO and DEM, which deplete GSH by different mechanisms, indicate that the reduction in mutagenicity and toxicity seen in GSH-depleted cells is a result of the GSH depletion and not simply inactivation of NCS by the depleting agent. In addition, we have shown that the same reduction in NCS toxicity is seen when BSO is rinsed off prior to NCS exposure (28).

NCS has been reported to be mutagenic in bacteria, yeast, thioguanine resistance assay. While BSO appears to give a dose-related increase in HGPRT\(^+\) mutants, all of the values are at or below the negative control value for that experiment. Likewise, DEM does not increase the mutation frequency over that seen in the control. It should be noted that the lowest concentrations of BSO and DEM shown in Table 1 were used in the experiments with NCS. The positive control, methylnitrosourea, was chosen because the literature indicates that, in bacteria at least, GSH does not play a role in the mutagenicity of methylnitrosourea (27). Data in Table 1 indicate that this is also true for V79 cells.
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fungi, and mammalian cells (13-17). In Salmonella, NCS reverts frameshift mutations and base pair substitutions (14) and is a potent mutagen in TA102, which has A-T base pairs at the site of mutation (29). In Saccharomyces, NCS induces reverse and possibly forward (supressor) mutations (16). Recently, Au et al. (17) have reported that NCS induces 6-thioguanine-resistant mutants and chromosome aberrations in Chinese hamster ovary cells. In this study, NCS was assayed for its ability to induce 6-thioguanine-resistant mutants and ouabain-resistant mutants. NCS did not produce detectable numbers of ouabain-resistant mutants. Since the ouabain resistance assay detects primarily base pair substitutions (30), the lack of activity in this assay indicates that NCS does not induce appreciable numbers of base pair substitutions in mammalian cells, while in bacteria it induces both base pair substitutions and frameshifts at frequencies high enough to be easily detected. In mammalian cells, NCS acts much like ionizing radiation, which does not induce ouabain-resistant mutants but does induce 6-thioguanine-resistant mutants (31).

NCS causes a unique type of single strand break in DNA that is the result of the oxidation of the 5'-carbon of deoxyribose (8). Thus, it falls into the class of compounds known as “oxidative mutagens.” Its mechanism of action appears to involve free radicals (19). In general, GSH helps protect cells from damage by free radicals, but in the case of NCS, GSH has the opposite role, being essential for the toxic and mutagenic activity of the drug. Another possible nonprotein thiol that could function in the reductive activation of NCS is cysteine. Both glutathione (32) and cysteine (33) have been used to activate NCS in a cell-free assay for DNA strand breakage. We have shown (28), however, that in V79 cells normal intracellular levels of cysteine are extremely low (less than 0.2 μM), making it unlikely that cysteine has any role in the bioactivation of NCS. The nearly complete absence of NCS activity in thiol-depleted cells, and the cysteine data, indicate that GSH is the only reducing agent functioning in the activation of NCS. Using BSO, it may be possible to deplete GSH at a faster rate from a normal human lung fibroblast than from a human lung tumor line, A549, judging from work that is in progress in our laboratory. If this were also true in vivo, non-tumor cells might be protected from the toxic and mutagenic activity of NCS, while tumor cells would still be sensitive to NCS.

In addition to making initial treatment with NCS more effective, this could also reduce the risk of secondary tumors being induced by NCS.

REFERENCES


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