Depletion of Cellular Glutathione by Exogenous Spermine in V79 Cells: Implications for Spermine-induced Hyperthermic Sensitization

Angelo Russo,1 James B. Mitchell, William DeGraff, Norman Friedman, and Janet Gamson
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ABSTRACT

The relationship between spermine-induced thermosensitization and modulation in the cellular redox state as measured by glutathione levels was studied using Chinese hamster V79 cells. Marked cellular glutathione depletion was observed for cells treated with exogenous 1 mM spermine at 37°C or 43°C. Glutathione depletion and thermal sensitization by spermine were found to be cell density dependent with maximum depletion and sensitization observed at low cell densities. These findings are discussed in the context that treatment of cells with exogenous polyamines such as spermine can result in cellular oxidative stress which may in part contribute to spermine-induced thermal sensitization.

INTRODUCTION

The cytotoxicity of hyperthermia is well documented (1, 2). However, the mechanism of hyperthermic cell death remains obscure. Initial perturbation of the cellular membrane with secondary biochemical ramifications is considered the most likely primary effect (2). Over the last decade effects of polyamines on thermosensitization has been extensively studied (3–13). These studies strongly suggest that exogenously applied positively charged diamino-organic compounds have a profound thermosensitization effect. How polyamines function in thermosensitization is not known. It is particularly difficult to define a definite mechanism by which polyamines sensitize to heat since the mechanism of hyperthermic cell death remains obscure. Initial perturbation of the cellular membrane with secondary biochemical ramifications is considered the most likely primary effect (2). Over the last decade effects of polyamines on thermosensitization has been extensively studied (3–13). These studies strongly suggest that exogenously applied positively charged diamino-organic compounds have a profound thermosensitization effect. How polyamines function in thermosensitization is not known. It is particularly difficult to define a definite mechanism by which polyamines sensitize to heat since the mechanism of hyperthermic cell death remains obscure.

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Catabolism of polyamines depends in part on amine oxidases (14, 15). These amine oxidases are enzymes that are oxygen dependent and are known to produce hydrogen peroxide (14). Previously we have shown that modulation of the cellular redox state by applying diethylmaleate, a compound that binds sulfhydryl compounds has a profound thermosensitization effect. How polyamines function in thermosensitization is not known. It is particularly difficult to define a definite mechanism by which polyamines sensitize to heat since the exact role that polyamines have in a normally functioning cell is not established. What is known about polyamines is that they are ubiquitous, their levels fluctuate as a function of cell cycle, and they are associated with the anionic biomolecule DNA (14, 15). Moreover exogenously applied polyamines cause fluctuation in the concentrations of naturally occurring intracellular polyamines (14, 15). These fluctuations in polyamine levels are thought to result from inhibition of S-adenosylmethionine decarboxylase and ornithine decarboxylase activity, two enzymes involved in polyamine biosynthesis (14, 15).

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SPERMINE-INDUCED GSH DEPLETION

RESULTS

The effects of exogenous spermine exposure on GSH levels for cells maintained at 37°C and 43°C are shown in Chart 1. Hyperthermic exposure alone resulted in a rapid elevation in GSH to a near plateau level of 160–180% of control. Similar heat-induced GSH elevation has been observed previously (19–21). When cells were exposed to 1 mM spermine at either 37°C or 43°C there was a steady decline in cellular GSH values. By 2–3 h of exposure at either temperature GSH values were <5% of controls and thus lower than the detection limits of the assay. Similar results were found using 1 mM spermidine (data not shown). It was concluded from these experiments that exogenous spermine was exerting marked effects on GSH levels even at 37°C, and in addition the elevation in GSH levels associated with 43°C heating alone was not only totally inhibited but was markedly reduced when spermine was present.

A series of experiments was next conducted to identify possible approaches to stop or modify the spermine-induced GSH depletion. For these studies only exposure of cells to spermine at 37°C was used since the GSH depletion was similar for 37°C or 43°C. A 4-h exposure time was selected which completely depletes cellular GSH (see Chart 1). The data from these experiments are shown in Table 1. Addition of catalase, a well-known scavenger of extracellular H₂O₂, or calcium chloride failed to prevent spermine-induced GSH depletion. Calcium chloride was added because it was observed that the spermine treatment often resulted in cells becoming detached from the flask surface.

![Graph showing GSH levels over time](https://via.placeholder.com/150)

Chart 1. Cellular GSH levels (expressed as a percentage of control values) for cells exposed to 43°C (●, A), 37°C + 1 mM spermine (□, □□), 43°C + 1 mM spermine (●, #) as a function of time. Different symbols represent replicate experiments.

A possible reason for this might have been due to spermine cationic chelation for ions important in cell adherence. The calcium chloride addition, however, did not prevent either the cell detachment or GSH depletion. The GSH synthetic cycle was stimulated and thus the GSH levels were increased by pretreatment of cells for 2 h with OTZ (23). Despite GSH levels being 200% of control, when spermine was added, GSH depletion was still observed. Providing additional substrate energy for GSH synthesis in the form of glutamine also failed to prevent GSH depletion. There were only two parameters that had any appreciable affect toward partially preventing the spermine-induced GSH depletion. Increasing the concentration of AG, a diamine oxidase inhibitor (24), to 10⁻³ M and increasing the cell density both resulted in less GSH depletion, to about 40% of control rather than the 5% level seen normally with spermine treatment. No agent studied completely prevented the spermine-induced GSH depletion. If GSH levels were an indicator of spermine-induced thermal sensitization, perhaps increasing AG concentrations or increasing cell density might reduce spermine sensitization. Survival of cells treated with 1 mM spermine at 43°C with two AG concentrations is shown in Chart 2. Thermal sensitization was observed for spermine addition, and the extent of sensitization was the same for both 10⁻⁵ and 10⁻³ M AG additions. GSH levels at 43°C were the same for both 10⁻³ and 10⁻⁵ M AG additions, 45 and 49% of control, respectively. Therefore increasing the AG concentration for heated spermine-treated cells afforded no protection from thermal sensitization or GSH depletion. In contrast variation of the cell density at the time of hyperthermic and spermine exposure had pronounced effects on cell survival and GSH levels as shown in Chart 3. Chart 3A shows the survival of cells exposed to 43°C for 90 min with or without 1 mM spermine. For higher cell densities there was less spermine-induced thermal sensitization. In like manner for higher cell densities the GSH depletion by spermine and hyperthermia was less as shown in Chart 3B. Therefore there appeared to be a relationship between the extent of spermine-induced thermal sensitization and GSH depletion levels as the cell number was varied.

DISCUSSION

We have shown previously that intracellular levels of glutathione are elevated by exposing cells to hyperthermic stress (19–

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**Table 1**

<table>
<thead>
<tr>
<th>Condition</th>
<th>GSH (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-h exposure, 1 mM spermine + 10⁻⁶ M AG</td>
<td>&lt;5</td>
</tr>
<tr>
<td>4-h exposure, 1 mM spermine + 10⁻⁴ M AG + catalase, 200 μg/ml (scavenger of extracellular H₂O₂)</td>
<td>&lt;5</td>
</tr>
<tr>
<td>4-h exposure, 1 mM spermine + 10⁻³ M AG + glutamine, 10⁻³ M and 10⁻⁴ M (provides substrate energy for GSH synthesis)</td>
<td>12, 6</td>
</tr>
<tr>
<td>4-h exposure, 1 mM spermine + 10⁻³ M AG + oxothiazolidine-4-carboxylate (stimulates GSH synthesis to 200% of control prior to addition of spermine)</td>
<td>&lt;5</td>
</tr>
<tr>
<td>4-h exposure, 1 mM spermine + 10⁻³ M AG + cell density at 1.3 x 10⁶ cells/cm², 6.7 x 10⁶ cells/cm², and 1.3 x 10⁷ cells/cm²</td>
<td>37, 18, 2</td>
</tr>
</tbody>
</table>

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SPERMINE-INDUCED GSH DEPLETION

Additionally we have shown that interference with this rise in GSH by either diethylmaleate or BSO results in thermal sensitization for temperatures ≤43°C (19–21). Recently Freeman et al. (26) demonstrated that acid pH conditions that sensitize cells to heat also results in reduction in cellular GSH levels. Therefore agents or conditions that have shown potential to thermosensitize are of interest to us. Since polyamines are exquisite thermal sensitizing agents (3–13), we reasoned that polyamines because they are thermal sensitizer may have some interaction with intracellular GSH. Furthermore this interaction with the cellular redox state may in part explain or offer an alternative mechanism for thermal sensitization by polyamines. Our data clearly show that exogenously applied spermine is a potent GSH depleter both at 37°C and 43°C. This observation is particularly attractive from a biochemical point of view because the catabolism of polyamines results in the generation of hydrogen peroxide, aldehydes, and the α-β unsaturated carbonyl acrolein as shown in the proposed catabolic pathway in Chart 4. GSH can detoxify hydrogen peroxide and acrolein in conjunction with glutathione peroxidase (27) and glutathione-S-transferase (28), respectively. The mechanism of GSH depletion could result from interaction of GSH enzymatic catalysis of and subsequent export of either GSSG or the acrolein-GSH adduct from the cell. There are several enzymes capable of such catalysis including diamine oxidase, found in fetal calf serum (14, 29) and various spermine oxidases (15). Aminoguanidine, a diamine oxidase inhibitor (24) failed to prevent spermine-induced sensitization when concentrations were increased (see Chart 2). There is precedent for GSSG export after detoxification of benzyl amine in in vivo systems (30). Moreover the adduct of acrolein and GSH would be exported from the cell. Hence, given that the catabolic pathways are operative, it is apparently straightforward to account for GSH depletion by polyamines. Yet it is not clear that this is the only means by which GSH might be depleted. If the polyamine catabolic products were to interact with GSH anabolic enzymes GSH depletion would result. This is the case for BSO, and BSO results in thermal sensitization (19–21). We attempted to increase the available intermediates for GSH synthesis by supplying cysteine via the intracellular cysteine delivery system OTZ (23, 31) or glutamine. Neither of these potential substrates for GSH synthesis could sustain intracellular levels of GSH in the presence of spermine. We have not ruled out the possibility that depletion of intracellular GSH by spermine is more complex than...
simple detoxification of catabolic polyamine products. Previously it has been shown in bacteria (32) that GSH can combine through an amide bond with polyamines. The assay system that we used in this study (25) is specific for GSH and GSSG; hence we would not have discerned this particular metabolite had it been formed and cannot rule out this possibility. Catalase also failed to prevent GSH depletion and thermosensitization (data not shown) for 1 mM spermine. This result is not surprising since exogenously applied catalase does not enter into the intracellular space and therefore would not be expected to protect the intracellular system from intracellularly generated hydrogen peroxide. Recently it has been shown that the cytotoxicity of 0.01 mM spermine can be reversed by catalase addition.\(^3\) The discrepancy in these findings may be related to cell density and/or concentration differences in the spermine used.

Increasing the cell numbers that are exposed to spermine results in less thermosensitization and greater levels of intracellular GSH (Chart 3). There seems to be a correlation between the cell density, the level of GSH, and the thermosensitization induced by spermine. This observation is consistent with that of Ben-Hur et al. (13) who showed less spermine-induced thermosensitization for cells treated as multicellular spheroids versus monolayer cultures. When a greater number of cells are exposed to spermine there is a larger quantity of metabolic machinery to detoxify spermine without resulting in drastic reductions in GSH. If GSH levels are an index of the amount of cellular stress that is being imposed, it is not surprising that there is greater relative survival associated with the use of larger numbers of cells.

This study does support in part the premise that oxidative stress as reflected in perturbation of intracellular levels of GSH may be important in thermal sensitization by spermine. It also shows for the first time that there is an interaction of exogenously applied polyamine and intracellular sulfhydryl groups. The exact implications of such biochemical interactions are not known at this time. We are currently undertaking studies to understand these interactions better and to determine the role that endogenous polyamines impose on the intracellular redox state.

REFERENCES

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