GLUTATHIONE DEPLETION OR RADIATION TREATMENT ALTERS RESPIRATION AND INDUCES APOPTOSIS IN R3230AC MAMMARY CARCINOMA

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Abstract: Glutathione depletion by L-buthionine sulfoximine inhibits the growth of Ehrlich mouse mammary carcinoma, R3230Ac rat mammary carcinoma and the PC3 human prostrate carcinoma cells, in vitro. Inhibition of growth occurs within the first 24 hours after exposure to the drug. The cell density does not increase over the initial cell density over 7 days. A549 human lung carcinoma and the DU145 human prostrate carcinoma cells show no inhibition of growth under the same treatment conditions. A comparative study of the R3230Ac and A549 cells demonstrated a marked increase in apoptosis following L-BSO treatment in R3230Ac, which was dependent on L-BSO concentration and incubation time. L-BSO did not induce apoptosis in A549 cells at any of the concentrations tested. The incidence of apoptosis for R3230Ac cells following exposure to 0.1 mM L-BSO was similar to the incidence of radiation-induced apoptosis observed after exposure to 10 Gy. Treatment with L-BSO or radiation alone inhibited O₂ utilization in of R3230Ac, while no effect on O₂ utilization was observed in A549 cells. LBSO altered the bioreductive capacity of both the R3230Ac and A549 cells. These results suggest that the ability of L-BSO to block mitochondrial O₂ utilization may be involved in the apoptotic response in R3230Ac cells.

Key words: apoptosis, bioreduction, carcinoma cells, glutathione, oxygen uptake, radiation
1. INTRODUCTION

Previously we demonstrated a role for glutathione (GSH) in the cellular radiation response [1,2]. Glutathione protects against the oxidative damage induced by ionizing radiation by three distinct mechanisms: First, GSH reacts chemically with reactive oxygen species (ROS) [1,2]. Second, GSH is a substrate for selenium-linked GSH peroxidases [1-3], which protect cells against radiation toxicity by reducing peroxide and hydroperoxides [1-3]. Glutathione disulfide (GSSG) produced in both of the above reactions is reduced via GSSG reductase with NADPH donated by the oxidative limb of the pentose phosphate cycle [4]. Finally, GSH-S-transferases conjugate GSH with the breakdown products of lipid peroxidation namely, malondialdehyde and 4-hydroxynonenal [5].

GSH has been shown to protect cells against radiation-induced apoptosis [6,7]. Moreover, the loss of cellular and mitochondrial GSH content is an early event in the generalized cellular apoptotic response [7-10]. While inhibiting the loss of GSH has been shown to block apoptosis [11-14], the direct inhibition of GSH synthesis by L-BSO can induce apoptosis in a number of cell lines [15-18]. It has been suggested that depletion of mitochondrial GSH precedes the loss of cytochrome c in the apoptotic response [13,14]. Loss of cellular GSH, during apoptosis, may increase oxidative damage via inhibition of antioxidant defenses [19]. While the loss of cytochrome c would inhibit mitochondrial energy production and cellular O$_2$ consumption [20].

Today there is continued interest in the role of glutathione (GSH) in apoptosis [cf. 6-20]. The exact mechanism for its involvement in the apoptotic response is not known. Cellular GSH is an important biochemical involved in the growth of many cells [14-18,21]. Some cells like the human A549 lung carcinoma cell and GSH' mutants are exceptions and can grow without GSH [1-3,22]. For the cells undergoing apoptosis GSH depletion inhibits protein, and DNA synthesis. as well as alters protein redox status. Glutathione is a substrate for γ-glutamyl-transpeptidase catalyzed amino acid transport and subsequent protein synthesis. Deoxyribonucleotide and DNA synthesis also in part involves GSH as coenzyme in the thioltransferase catalyzed reduction of ribonucleotide reductase [21]. GSH maintains thioltransferase in a reduced state necessary for reduction of nucleotide precursors to deoxyribonucleotides [23]. Thioltransferase's enzyme activity is also necessary for maintaining intracellular proteins in their correct oxidation status [1-3,23].