Subcellular compartmentalization of glutathione: Correlations with parameters of oxidative stress related to genotoxicity

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Glutathione (GSH) is a major component of the antioxidant defence system of mammalian cells and is found in subcellular pools within the cytoplasm, nucleus and mitochondria. To evaluate the relationships between these pools and parameters of oxidative stress related to genotoxicity, wild type (WT) and 8-oxo-2'-deoxyguanosine glycosylase 1 (OGG1)-null (mOGG1^-/-) mouse embryonic fibroblasts (MEF) were treated with buthionine sulphoximine (BSO; 0–1000 μM, 24 h), an inhibitor of GSH biosynthesis. BSO treatment resulted in a concentration-dependent depletion of GSH from the cytoplasm, but depletion of mitochondrial and nuclear GSH occurred only at concentrations ≥100 μM. GSH levels were correlated with reactive oxygen species (ROS), lipid peroxidation (measured as the increase in the genotoxic end-product malondialdehyde (MDA)) and oxidative DNA modifications, measured as both frank DNA strand-breaks (FSB) and oxidized purine lesions (OxP) using the alkaline comet assay with formamidopyrimidine DNA glycosylase (FPG) modification; this system allowed for the identification of BSO-induced DNA modifications as primarily mutagenic 8-oxo-2'-deoxyguanosine lesions. A number of significant correlations were observed. First, negative linear correlations were observed between mitochondrial GSH and ROS (r = -0.985 and r = -0.961 for WT and mOGG1^-/- MEF, respectively), and mitochondrial GSH and MDA (r = -0.967 and r = -0.963 for WT and mOGG1^-/- MEF, respectively). Second, positive linear correlations were observed between ROS and MDA (r = 0.996 and r = 0.935 for WT and mOGG1^-/- MEF, respectively), and ROS and OxP (r = 0.938 and r = 0.981 for WT and mOGG1^-/- MEF, respectively). Finally, oxidative DNA modifications displayed a negative linear correlation with nuclear GSH (r = -0.963 and -0.951 between nuclear GSH and FSB and OxP, respectively, for WT MEF and r = -0.960 between nuclear GSH and OxP in mOGG1^-/- MEF), thus, demonstrating the genotoxic potential of compounds that deplete GSH. The findings highlight the critical roles of the mitochondrial and nuclear GSH pools in protecting cellular components, particularly DNA, from oxidative modification.

Introduction

Reduced glutathione (GSH; γ-glutamylcysteinylglycine) is involved in many important cellular functions including the detoxification of xenobiotics, by the formation of less-toxic GSH–xenobiotic conjugates often requiring the catalytic activity of GSH S-transferases [reviewed in Ref. (1)] and the deactivation of reactive oxygen species (ROS), either via direct GSH–ROS interaction or via the activity of GSH peroxidases (2). Thus GSH is involved in both chemical detoxification and antioxidant defence. Owing to its critical importance, GSH is maintained at high (mM) concentrations in mammalian cells by constitutive de novo biosynthesis via a two enzyme biosynthetic pathway (3). The first enzyme, glutamate-cysteine ligase [GCL; formally known as γ-glutamylcysteine synthetase (γ-GCS)], is rate-limiting and is efficiently inhibited by the xenobiotic buthionine sulfoximine (BSO) (4). The second enzyme is glutathione synthetase (GS). Although GSH is primarily, if not exclusively, synthesized in the cytoplasmic compartment of mammalian cells its sites of usage are extensive, with subcellular pools of GSH being found in the cytoplasmic, nuclear and mitochondrial compartments of mammalian cells. The cytoplasmic pool is the largest of the three, generally containing >85% of the total cellular GSH while the nuclear and mitochondrial pools have been shown to contain as much as 10 and 30% of the total cellular GSH, respectively (5–11). The toxicological implications of discrete nuclear and mitochondrial pools of GSH have been reviewed previously (12). However, their origins are poorly understood and contradictory reports have been published. For example, Ho and Guenthner (13), and Lash (14) reported the presence of both enzymes of de novo GSH biosynthesis (GCL and GS) in nuclei and mitochondria, respectively, while other groups reported no evidence of intra-organelle synthesis (5,15). Previous studies have also reported that GSH synthesized in the cytoplasm is actively transported into the mitochondrial matrix via the dicarboxylate and 2-oxoglutarate transporters (16,17). No active transport mechanism has been discovered for nuclear import, although it is assumed that GSH is capable of entering and exiting the nucleus via nuclear pores. In addition to the GSH-enriched cytoplasmic, nuclear and mitochondrial pools, discrete glutathione pools are also found in the endoplasmic reticulum and extracellular compartment, although these pools contain predominantly the oxidized form of glutathione (GSSG) (18,19). Depletion of GSH has been shown previously to result in increases in parameters of oxidative stress [defined as an imbalance of pro-oxidants (e.g. ROS) and antioxidants (e.g. GSH) (20)] evidenced by, for example, lipid peroxidation (21) and oxidative DNA modifications, particularly 8-oxo-2'-deoxyguanosine (8-oxo-dG) lesions (21–23). 8-Oxo-dG lesions are highly mutagenic and as such mammalian cells have...
evolved efficient 8-oxo-dG repair mechanisms, with the majority being repaired via the short-patch base excision repair (BER) pathway initiated by the DNA glycosylase 8-oxo-2′-deoxyguanosine glycosylase 1 (OGG1) (24). These increases in parameters of oxidative stress are due to reduced clearance of endogenously produced ROS, of which there are many sources including the electron transport chain of mitochondria, NADPH oxidase and the cytochrome P450 family of flavin mono-oxygenases [reviewed in Refs. (25–27)]. These endogenous sources primarily produce the relatively unreactive superoxide anion (O₂⁻) via the one electron reduction of molecular oxygen (O₂); however, O₂⁻ is readily converted into hydrogen peroxide (H₂O₂), which in turn can react with reduced metal ions to yield the extremely reactive hydroxyl radical (HO·) (28,29), which will only diffuse 1–2 molecular diameters before reacting with a cellular component (30).

An important reason for gaining information on the relationships between GSH and oxidative DNA damage is in relation to chemical safety assessment. For compounds that are classified as genotoxic, a non-threshold mechanism is adopted (31). It is essential, therefore, that apparent genotoxicity that may arise with chemicals, which can deplete GSH at high concentrations, can be recognized so as not to classify these incorrectly. This study represents a comprehensive analysis, being the first to examine the relationships between parameters of oxidative stress and cytoplasmic, nuclear and mitochondrial GSH simultaneously. Additionally, this study reports the novel observation that oxidative DNA modifications display negative linear correlation with nuclear GSH specifically.

Materials and methods

Chemicals and cell culture materials

Unless stated otherwise, all chemicals were of the highest available purity from Sigma-Aldrich Chemical Company (UK) and all cell culture materials were obtained from Gibco (UK).

Cells and cell culture

Wild type (WT) and 8-oxo-2′-deoxyguanosine glycosylase 1-null (mOGG1−/−) mouse embryonic fibroblasts (MEF) were a generous gift from Dr Thomas Lindahl (Cancer Research, UK). MEF were maintained at 37°C in 25 cm² flasks and passaged twice weekly using a standard DMEM supplemented with 10% foetal bovine serum, 2 mM L-glutamine, syringe filtration through a 0.2 μm filter. The stock solution was then diluted to 50 mM stock in supplemented DMEM and sterilized by autoclaving at 121°C (32). 100 mM NaF, 0.02% sodium deoxycholate, 2 mM Na3VO4; 0.02% mammalian protease inhibitor cocktail, 1 mM dithiothreitol, 100 mM Tris, adjusted to pH 7.6 and incubated on ice for 10 min. Western blots for β-actin, lamin A/C and cytochrome c were then performed using a standard protocol with 20 μg of protein per fraction separated by electrophoresis at 20 V/cm for 90 min on a 12.5% SDS–polyacrylamide gel. Primary antibodies used were: mouse anti-β-actin (Sigma-Aldrich; dilution: 1:10 000), rabbit anti-mouse lamin A/C (Santa Cruz, UK; dilution: 1:500) and rabbit anti-mouse cytochrome c (Abcam, UK; dilution: 1:500). Horseradish peroxidase coupled secondary antibodies were obtained from DakoCytomation (Denmark) and were used at dilutions of 1:10 000 for the detection of β-actin and 1:500 for the detection of lamin A/C and cytochrome c. All antibody incubations were for 1 h at room temperature in the dark, fluorescence was measured using a Perkin Elmer fluorescence cuvettes containing 1.8 ml of assay buffer (5 mM EDTA, 100 mM NaH2PO4, adjusted to pH 8.0). A standard curve (0–6.5 nmol) was also used using β-actin using a freshly made GSH stock solution (0.1 mg/ml in ice-cold assay buffer) by adding the appropriate volume (0–20 μl) to a cuvette containing 1.8 ml of assay buffer and 100 μl of 5% TCA. Next, 100 μl of o-phthalaldehyde solution (1 mg/ml in 100% methanol) was added to each cuvette before thorough mixing by agitation. Following a 15 min incubation at room temperature in the dark, fluorescence was measured using a Perkin Elmer fluorescence cuvettes with an excitation wavelength of 340 nm and an emission wavelength of 420 nm (slit width 4.0 nm). Total GSH (nmol) was determined from the standard curve and normalized to protein mass.

Assessment of intracellular ROS

Intracellular ROS were measured using the ROS sensitive dye 7′-dichlorodihydrofluorescein diacetate (H2DCF-DA) as described previously (37), except that MEF were labelled with H2DCF-DA before BSO treatment. Fluorescence units (F) at 520 nm were normalized to protein mass.

Assessment of lipid peroxidation

Lipid peroxidation was measured as the increase in the genotoxic end-product malondialdehyde (MDA) using the Calbiochem Lipid Peroxidation Assay Kit (Calbiochem, Germany) according to manufacturer’s instructions using the protocol for the detection of MDA only. Total MDA (nmol) was normalized to protein mass.

Alkaline comet assay

The alkaline comet assay, originally described by Singh et al. (38), was performed with Escherichia coli formamidopyrimidine DNA glycosylase (FPG) digestion for the detection of oxidized purine lesions (OxP) (39). Briefly, following treatment, MEF were harvested by gentle scraping into 1 ml of cold phenol red-free-DMEM and the cell pellet collected by centrifugation at 3000g for 10 min at 4°C. Cell pellets were then resuspended in 150 μl of phenol red-free-DMEM and an aliquot (30 μl) removed and mixed with 300 μl of 0.5% low melting point agarose. Two separate 150 μl aliquots of the resulting cell
Results

**MEF viability and apoptosis following BSO treatment**

Treatment with BSO (0–1000 μM, 24 h) had no statistically significant effect upon WT or mOGG1−/− MEF viability, as determined by the trypan blue exclusion and MTT assays (data not shown), nor did it significantly increase levels of apoptotic cells (data not shown).

**Subcellular fractionation of MEF**

The cytoplasmic, nuclear and mitochondrial fractions prepared using the method described were determined to be of high purity as qualitatively assessed via western blotting for ‘marker’ proteins: β-actin (cytoplasmic marker), lamin A/C (nuclear marker) and cytochrome c (mitochondrial marker) (Figure 1).

**BSO treatment results in the differential depletion of cytoplasmic, nuclear and mitochondrial GSH**

Treatment with BSO (0–1000 μM, 24 h) resulted in a statistically significant concentration-dependent depletion of GSH from the cytoplasmic compartments of both WT (Figure 2) and mOGG1−/− (Figure 3) MEF, with <20% of cytoplasmic GSH remaining following treatment with 1000 μM BSO. The nuclear and mitochondrial GSH pools, however, were more resistant to depletion in both cell types, with >70% and >40%, respectively, remaining following treatment with 1000 μM BSO (Figures 2 and 3). No statistically significant differences were observed between control levels of GSH in the cytoplasmic and nuclear compartments of WT and mOGG1−/− MEF however, mitochondrial GSH was significantly lower in mOGG1−/− MEF (P < 0.01, two-tailed Student’s t-test).

**BSO treatment results in the formation of ROS and MDA**

Treatment with BSO (0–1000 μM, 24 h) resulted in statistically significant increases in intracellular ROS (Figure 4) and MDA (Figure 5) only at concentrations ≥100 μM.

**BSO treatment results in the formation of oxidative DNA modifications**

Treatment with BSO resulted in the statistically significant concentration-dependent increase in frank DNA strand-breaks (FSB) in WT MEF (Figure 6), whereas there was no effect with mOGG1−/− MEF (Figure 6). However, incubation with FPG prior to electrophoresis to reveal OxP showed that mOGG1−/− accumulated OxP to a statistically significantly greater extent than WT MEF following treatment with BSO (Figure 7), while

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**Fig. 1.** Western blots for β-actin, lamin A/C and cytochrome c.

**Fig. 2.** GSH remaining in the cytoplasmic, nuclear and mitochondrial compartments of WT MEF following BSO treatment. Graph represents mean ± SEM from three independent experiments. #: Significantly different from untreated cytoplasmic control (19.7 ± 0.13 nmol GSH/mg cytoplasmic protein) with P < 0.05. #: ** and ††: Significantly different from untreated cytoplasmic, nuclear (8.2 ± 0.27 nmol GSH/mg nuclear protein) and mitochondrial (14.1 ± 0.38 nmol GSH/mg mitochondrial protein) controls, respectively with P < 0.01. P-values determined by two-tailed Student’s t-test.

**Fig. 3.** GSH remaining in the cytoplasmic, nuclear and mitochondrial compartments of mOGG1−/− MEF following BSO treatment. Graph represents mean ± SEM from three independent experiments. #: Significantly different from untreated cytoplasmic control (19.0 ± 0.30 nmol GSH/mg cytoplasmic protein) with P < 0.05. #: ** and ††: Significantly different from untreated cytoplasmic, nuclear (6.2 ± 0.36 nmol GSH/mg nuclear protein) and mitochondrial (8.7 ± 0.16 nmol GSH/mg mitochondrial protein) controls, respectively with P < 0.01. P-values determined by two-tailed Student’s t-test.
the total level of oxidative DNA modifications remained the same (Figure 8). These findings are in accord with the finding that a range of pro-oxidant compounds induced OxP in both WT and mOGG1−/− MEF while inducing FSB in WT MEF only (41). Additionally, significant depletion of GSH from the nuclear compartment had a marked effect upon oxidative DNA modifications, with high levels of FSB and OxP induced in WT and mOGG1−/− MEF, respectively, following treatment with 1000 μM BSO (Figures 6 and 7).

Intracellular ROS display negative linear correlation with mitochondrial and nuclear GSH

Linear correlation analysis was used to determine the relationship(s) between intracellular ROS and the cytoplasmic, nuclear, and mitochondrial pools of GSH (Table I). A statistically significant negative linear correlation was observed between intracellular ROS and both nuclear and mitochondrial GSH for both WT and mOGG1−/− MEF.

MDA and OxP display positive linear correlation with intracellular ROS

Linear correlation analysis revealed a statistically significant positive linear correlation between levels of intracellular ROS and MDA (Table II), as well as between intracellular ROS and levels of OxP (Table III) for both WT and mOGG1−/− MEF. No significant correlations were observed between intracellular ROS and FSB (Table III).
The relationship(s) between ROS and cytoplasmic, nuclear and mitochondrial GSH were assessed by correlation analysis (Table IV). Statistically significant negative linear correlation was observed between MDA and mitochondrial GSH for both WT and mOGG1−/− MEF. Additionally, statistically significant negative linear correlation was observed between MDA and nuclear GSH in WT MEF (Table IV).

**Oxidative DNA modifications display negative linear correlation with nuclear GSH**

Linear correlation analysis revealed a statistically significant negative linear correlation between OxP and nuclear GSH for both WT and mOGG1−/− MEF (Table V). Additionally, statistically significant negative linear correlation was observed between FSB and nuclear GSH and between OxP and cytoplasmic GSH in WT MEF (Table V).

**Discussion**

In the current study, treatment of WT and mOGG1−/− MEF with non-cytotoxic concentrations of the de novo GSH biosynthesis inhibitor BSO resulted in significant depletion of GSH from the cytoplasmic compartment at all concentrations tested (1–1000 μM) but in depletion of mitochondrial and nuclear GSH only at concentrations ≥100 μM. Similar observations regarding less efficient depletion of GSH from the nuclear (42,43) and mitochondrial (44,45) compartments by BSO treatment have been reported previously.

The levels of nuclear GSH (8.2 ± 0.27 and 8.2 ± 0.36 nmol/mg nuclear protein in WT and mOGG1−/− MEF, respectively) and mitochondrial GSH (14.1 ± 0.38 and 8.76 ± 0.16 nmol/mg mitochondrial protein in WT and mOGG1−/− MEF, respectively) in untreated cells reported here are consistent with those reported previously by groups employing subcellular fractionation techniques (5,7–9,11). In addition, fluorescence microscopy and cytometry-based approaches have been used to study GSH compartmentalization in intact cells. In agreement with this study, Thomas et al. (46) have reported previously that the mean nuclear GSH/cytoplasmic GSH ratio in tumour cells was 0.57 ± 0.05 and that nuclear GSH was more resistant to depletion by BSO treatment. Söderdahl et al. (47) also reported that in A549 cells the nuclear GSH concentration was below that of the cytoplasm, but that nuclear GSH did not appear to be resistant to depletion by BSO treatment, whereas the mitochondria retained significant levels of GSH following near complete depletion of both nuclear and cytoplasmic GSH. Therefore, the measurement of nuclear GSH and its depletion by BSO is complex and may depend on the experimental technique(s) employed and the cell type(s) analysed.

It was observed that the formation of significant amounts of intracellular ROS and the induction of significant levels of lipid peroxidation, measured as the increase in MDA, occurred only following significant depletion of GSH specifically from the mitochondrial and/or nuclear compartments. In addition, linear correlation analysis revealed a statistically significant negative linear relationship between intracellular ROS and both mitochondrial and nuclear GSH. The correlation between ROS and mitochondrial GSH is easy to rationalize as mitochondria are a major source of endogenous ROS, primarily as a result of inefficient electron transfers by complexes I and III during oxidative phosphorylation (48,49); thus, it is attractive to hypothesize that mitochondrial GSH plays a key role in the efficient deactivation of mitochondrially generated ROS. A similar relationship between mitochondrial GSH and ROS has been reported recently by Luéis et al. (11), who observed that selective depletion of mitochondrial GSH from HepG2 cells and primary rat hepatocytes by treatment with (R,S)-3-hydroxy-4-pentanoate resulted in enhanced ROS generation by the mitochondria, particularly under hypoxic conditions. The same study also suggested that a threshold of ROS generation was required to inflict cell death and that this threshold may relate to the threshold for mitochondrial GSH depletion to stimulate ROS generation. Indeed, a previous study (50) utilizing 1-chloro-2,4-dinitrobenzene to deplete mitochondrial GSH observed that depletion of <40% of GSH from isolated rat heart mitochondria had no effect upon ROS production, whereas depletion of >50% resulted in a linear increase in H2O2 production. Finally, a third study (51) observed that depletion of cellular GSH from lymphocytes by treatment with BSO resulted in an increase in mitochondrially generated ROS. The relationship between ROS and nuclear
GSH, however, is more difficult to explain as it is unlikely that nuclei generate large amounts of ROS, even following depletion of nuclear GSH. Linear correlation analysis also revealed significant relationships between MDA and both intracellular ROS and mitochondrial GSH. Mitochondrial GSH may play a key role in the protection of mitochondrial membranes from lipid peroxidation via either an ascorbic acid and α-tocopherol-dependent mechanism (52), a GSH peroxidase-dependent mechanism (53) or both. This hypothesis is supported by previous observations that treatment of HepG2 cells with BSO and pyruvate resulted in a significant increase in levels of MDA in the mitochondrial fraction but not in other subcellular fractions (e.g., microsomal) (54). While lipid peroxidation per se may have deleterious consequences for the cell, the formation of MDA from HepG2 cells resulted in increases in both 8-oxo-dG and total levels of oxidative DNA modifications, specifically 8-oxo-dG lesions, and total cellular GSH (22,23) and between OxP and total cellular GSH in the brains and livers of rats exposed to paint thinner (21). Previous work in our laboratory has shown that depletion of GSH from HepG2 cells resulted in increases in both 8-oxo-dG lesions and FSB (56). Also, Jevtic–Todorovic and Guenther (57) observed that the depletion of nuclear GSH corresponded with greatly enhanced cytotoxicity of the DNA alkylation agent melphalan. Interestingly, the combination of melphalan and BSO has shown encouraging results for the treatment of patients with cancers that had exhausted the standard therapeutic options for their disease during phase 1 clinical trials and is currently being assessed for its usefulness in treating ovarian cancer and melanoma as part of phase 2 clinical trials (58).

Although not assessed in the present study, a number of groups have reported a negative relationship between GSH and oxidative modifications of mitochondrial DNA. Hollins et al. (51) reported that depletion of total cellular GSH from human peripheral blood lymphocytes enhanced the susceptibility of mitochondrial DNA to oxidative modification by the pro-oxidant tert-butyl-hydroperoxide and that mitochondrial DNA appeared to be more prone to oxidative modification than nuclear DNA. Moreover, Garcia de la Asuncion et al. (59) demonstrated that the level of 8-oxo-dG lesions in mitochondrial DNA was positively correlated with the ratio of GSSG/GSH (i.e. negatively correlated with GSH) in mitochondria isolated from the livers of both mice and rats.

Finally, the observation of marked DNA oxidation associated with depletion of nuclear GSH has important implications for the interpretation of apparent genotoxicity when observed in in vitro screening tests, such as the mouse lymphoma assay (60). This effect may occur at relatively high

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### Table IV. Linear correlation analyses: MDA and GSH

<table>
<thead>
<tr>
<th></th>
<th>WT cytoplasmic GSH</th>
<th>WT nuclear GSH</th>
<th>WT mitochondrial GSH</th>
<th>mOGG1&lt;sup&gt;1−/−&lt;/sup&gt; cytoplasmic GSH</th>
<th>mOGG1&lt;sup&gt;1−/−&lt;/sup&gt; nuclear GSH</th>
<th>mOGG1&lt;sup&gt;1−/−&lt;/sup&gt; mitochondrial GSH</th>
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</thead>
<tbody>
<tr>
<td>MDA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$r$</td>
<td>$-0.810$;</td>
<td>$-0.982$;</td>
<td>$-0.967$;</td>
<td>$-0.817$;</td>
<td>$-0.905$;</td>
<td>$-0.963$;</td>
</tr>
<tr>
<td>$P$</td>
<td>0.290</td>
<td>0.009</td>
<td>0.021</td>
<td>0.275</td>
<td>0.105</td>
<td>0.026</td>
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</tbody>
</table>

$r$ = product moment correlation coefficient (Pearson’s). $P$-values determined by two-tailed Student’s $t$-test with Bonferroni’s correction ($k = 3$). Significant values of $r$ and $P$ are underlined.

### Table V. Linear correlation analyses: oxidative DNA modifications (FSB and OxP) and GSH

<table>
<thead>
<tr>
<th></th>
<th>WT cytoplasmic GSH</th>
<th>WT nuclear GSH</th>
<th>WT mitochondrial GSH</th>
<th>mOGG1&lt;sup&gt;1−/−&lt;/sup&gt; cytoplasmic GSH</th>
<th>mOGG1&lt;sup&gt;1−/−&lt;/sup&gt; nuclear GSH</th>
<th>mOGG1&lt;sup&gt;1−/−&lt;/sup&gt; mitochondrial GSH</th>
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<td>FSB</td>
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<td>$r = -0.963$;</td>
<td>$r = -0.856$;</td>
<td>$r = -0.732$;</td>
<td>$r = -0.823$;</td>
<td>$r = -0.619$;</td>
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<tr>
<td></td>
<td>$P = 0.185$</td>
<td>$P = 0.026$</td>
<td>$P = 0.193$</td>
<td>$P = 0.479$</td>
<td>$P = 0.260$</td>
<td>$P = 0.797$</td>
</tr>
<tr>
<td>OxP</td>
<td>$r = -0.948$;</td>
<td>$r = -0.986$;</td>
<td>$r = -0.920$;</td>
<td>$r = -0.906$;</td>
<td>$r = -0.960$;</td>
<td>$r = -0.860$;</td>
</tr>
<tr>
<td></td>
<td>$P = 0.042$</td>
<td>$P = 0.038$</td>
<td>$P = 0.080$</td>
<td>$P = 0.103$</td>
<td>$P = 0.029$</td>
<td>$P = 0.185$</td>
</tr>
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</table>

$r$ = product moment correlation coefficient (Pearson’s). $P$-values determined by two-tailed Student’s $t$-test with Bonferroni’s correction ($k = 3$). Significant values of $r$ and $P$ are underlined.
concentrations of certain chemicals that can deplete GSH and should not be interpreted as inherent genotoxicity as it is a secondary effect not relevant to low doses (55).

In summary, the current study represents a comprehensive identification of relationships between different subcellular pools of glutathione and parameters of oxidative stress, providing further evidence for the critical importance of mitochondrial GSH for the deactivation of endogenously produced ROS and presenting the novel observation that oxidative DNA modifications are negatively correlated with nuclear GSH.

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References


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