Glutathione Depletion by Buthionine Sulfoximine Induces Oxidative Damage to DNA in Organs of Rabbits *in vivo*[†]

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ABBREVIATIONS AND TEXTUAL FOOTNOTES

[1]Abbreviations: GSH, glutathione; γ-GCS, γ-glutamyl cystein synthetase; Tau, taurine (2-aminoethanesulfonic acid); BSO, buthionine sulfoximine; 8-OH-Gua, 8-hydroxyguanine; 8-OH-Ade, 8-hydroxyadenine; 8-OH-dG, 8-hydroxy-2′-deoxyguanosine; 8-OH-dA, 8-hydroxy-2′-deoxyadenosine; S-cdA, (5′S)-8,5′-cyclo-2′-deoxyadenosine; FapyGua, 2,6-diamino-4-hydroxy-5-formamidopyrimidine; GC/MS, gas chromatography/mass spectrometry; LC/MS, liquid chromatography/mass spectrometry; SIM, selected-ion monitoring; $^{\bullet}$OH, hydroxyl radical.

[2]Certain commercial equipment or materials are identified in this paper in order to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

ABSTRACT: Glutathione (GSH) exists in mammalian tissues *in vivo* at high concentrations and plays an important protective role against oxidatively induced damage to biological molecules including DNA. We investigated oxidatively induced damage to DNA by GSH depletion in different organs of rabbits *in vivo*. Rabbits were treated subcutaneously with buthionine sulfoximine (BSO), an effective GSH-depleting compound. GSH levels were measured in heart, brain, liver and kidney of animals. BSO treatment significantly reduced GSH levels in heart, brain and liver, but not in kidney. DNA was isolated from these tissues to determine whether GSH-depletion causes oxidatively induced DNA damage *in vivo*. Gas chromatography/mass spectrometry and liquid chromatography/mass spectrometry with isotope dilution were applied to measure typical products of oxidatively induced damage in isolated DNA samples. Several such products were identified and quantified in all organs. BSO treatment caused significant formation of 8-hydroxyguanine, 2,6-diamino-4-hydroxy-5-formamidopyrimidine, 8-hydroxyadenine and (5′S)-8,5′-cyclo-2′-deoxyadenosine in DNA of organs of rabbits. Animals were fed with the semi-essential amino acid 2-aminoethanesulfonic acid (taurine) during BSO-treatment. Taurine significantly inhibited GSH-depletion and also formation of DNA products. Depletion of GSH correlated well with formation of DNA products, unequivocally proving the role of GSH in preventing oxidatively induced DNA damage. Our findings might contribute to understanding of pathologies associated with DNA damage, oxidative stress and/or defective antioxidant responses.

Glutathione (L-γ-glutamyl-L-cysteinylglycine) (GSH)[1] is a pervasive tripeptide of glycine, glutamic acid and cysteine, and is known to play a crucial role in protecting tissues against the deleterious effects of oxidative damage to biological molecules. As a nucleophile, GSH also reacts with electrophilic and reactive intermediates derived from exogenous compounds such as drugs and xenobiotics (*1;2*). GSH is synthesized in the cytosol in two steps. In the first step, the enzyme γ-glutamyl cystein synthetase (γ-GCS) catalyzes the formation of L-γ-glutamyl-L-cysteine. In the second step, the glycine residue of the GSH tripeptide is added by glutathione synthetase. Cellular GSH exists at high concentrations in cells and predominantly in a reduced form, but small amounts of the oxidized disulfide form GSSG can also be detected. The GSH/GSSG ratio is generally >100/1 and considered an indicator of cellular redox status (*3;4*). Within the context of the biological importance of the role of GSH, diseases associated with GSH defiency have been an area of considerable interest (*4*). Inborn metabolic deficiencies have been described for several GSH-related enzymes such as γ-GCS, GSH synthetase and glutathione reductase. Furthermore, low GSH levels have been shown to be associated with the pathology of a number of diseases such as type II diabetes, acquired immune deficiency syndrome (AIDS), hepatitis C, ulcerative colitis, idiopathetic pulmonary fibrosis, adult respiratory distress syndrome (ARDS) and cataracts (*5-9*). GSH is also an antioxidant and plays an important role in protecting organisms against ionizing radiation and free radical-generating agents by repairing resulting radicals. Thus, it can react with peroxyl radicals, preventing them from propagating chain reactions, and with highly reactive hydroxyl radicals ($^{\bullet}$OH) and other reactive species *in vivo* (*4*).

Although samples from patients with genetic deficiencies of GSH biosynthesis are available and useful for some studies, the nature of these deficiencies have not been well

understood. Since GSH metabolism is dynamic, involving many tissues, *in vivo* studies of the effects of GSH deficiency are worth pursuing further. Treatment with buthionine sulfoximine (BSO), a selective inhibitor of γ-GCS, has been shown to lead to decreased cellular GSH levels, and its application can provide a useful experimental model of GSH deficiency (*10;11*). Since GSH is the most frequently found nonprotein intracellular thiol, its depletion by BSO leads to oxidative stress and to increase in radiation sensitivity (*4;12*). While damage caused by oxidative stress to any cellular constituent may be detrimental to the cell affected, damage to DNA is of particular concern, since it can lead to mutagenesis, carcinogenesis and aging among other biological effects. The mechanisms of formation, repair, measurement and biological effects of oxidatively induced DNA damage have been reviewed in detail (*13*). However, the effects of oxidative stress, experimentally induced by GSH deficiency, on cellular DNA have not been well understood.

The objective of the present study was to investigate oxidatively induced damage to DNA by GSH depletion in different organs of rabbits *in vivo*. Moreover, we hypothesized that 2-aminoethanesulfonic acid [taurine (Tau)], a semiessential amino acid, may protect the tissues from oxidatively induced DNA damage.

MATERIALS AND METHODS

Materials.[2] Nuclease P1 (from *Penicillium citrinum*) was purchased from United States Biological (Swampscott, MA). Buthionine sulfoximine (BSO), orthopthaldialdehyde and snake venom phosphodiesterase were obtained from Sigma (St. Louis, MO). Alkaline phosphatase was purchased from Roche Applied Science (Indianapolis, IN). Acetonitrile (HPLC grade) was from Burdick and Jackson (Muskegon, MI). Biomax5 ultrafiltration membranes (5 kDa molecular mass cutoff) from Millipore (Bedford, MA) were used to filter hydrolyzed DNA samples. Water (HPLC-grade) for analysis by liquid chromatography/mass spectrometry (LC/MS) was from J. T. Baker (Phillipsburg, NJ). Water purified through a Milli-Q system (Millipore, Bedford, MA) was used for all other applications. N,O-bis(trimethylsilyl)trifluororacetamide containing 1% trimethylcholorosilane was obtained from Pierce Chemicals (Rockford, IL).

Animal model of GSH deficiency. The animal experiments were carried out in accordance with guidelines described by the Ethics Committee of the Faculty of Pharmacy, Ege University. A total of 20 rabbits were used in this study. White rabbits of either sex (2.5-3 kg) were divided into four groups. The first group (n = 5, BSO group) received a single subcutaneous (s.c.) injection of BSO (75 mg kg^{-1} body weight day^{-1}). The second group (n = 5, control group) received only the vehicle (0.9% NaCl, 0.8 mL kg^{-1} body weight day^{-1}). The third group (n = 5, Tau group) received Tau in drinking water (1.0%, w/v). The fourth group (n = 5, BSO+Tau group) received the same dose of BSO plus Tau in drinking water. Throughout the 2-week treatment period, each rabbit was kept in a separate cage and allowed free access to rabbit chow and tap water. At the end of the treatment period, the rabbits (n = 20) were sacrificed by means of an overdose of sodium pentobarbitone. Heart, brain, liver and kidney were harvested for the

measurement of DNA damage and GSH levels. Tissue samples, after being frozen under liquid nitrogen, were stored −80 °C until the analyses were performed.

Measurement of tissue GSH levels. The levels of reduced GSH and total GSH after dithiothreitol reduction in metaphosphoric acid-denaturated samples were measured by pre-column derivatization with orthopthaldialdehyde by HPLC with fluorescence detection. Reversed-phase chromatographic condition included a Macherey/Nagel Nucleosil MN C18 column (250/4.6 mm, 5 μm particle size) (Germany), an isocratic separation with sodium acetate (50 mM)/acetonitrile (70:30) mixture at a flow rate of 0.7 mL/min, a column temprature at 30 °C, and a detector settings at Ex340/Em420. The level of reduced GSH was directly calculated from oxidized GSH graph and oxidized GSH from "total GSH−reduced GSH/2" equation (*14*), and was expressed as μmol/g wet tissue.

DNA isolation. Tissues from control and all treatment groups were suspended in 15 mL of polypropylene centrifugation tubes with 3 mL of nuclei lysis buffer (10 mM Tris HCl, 400 mM NaCl and 2 mM EDTA, pH 8.2) and incubated 60 min at 37 ºC. The cell lysates were digested overnight at 37 ºC with 0.2 mL of 10% SDS and 0.5 mL of protease K solution (1 mg protease K in 1% SDS and 2 mM EDTA). After digestion was complete, 1 mL of saturated NaCl (approximately 6 M) was added to each tube and then shaken vigorously for 15 sec until foam from protein appeared. The sample was then incubated for 10 min at 56 ºC followed by centrifugation at 5000g for 30 min at room temperature. The protein pellet was left at the bottom of the tube and the supernatant fraction containing the DNA was transferred to another 15 mL polypropylene tube. Two volumes of absolute ethanol kept at room temperature were added and the tubes were inverted several times until the DNA precipitated. The precipitated DNA strands were removed by spooling. Isolated DNA pellets were washed twice with 70% ethanol and

centrifuged. After the removal of ethanol, pellets were air-dried for 60 min at room temperature. The DNA was allowed to dissolve in water for 24 h at 4 ºC. The UV spectrum of each DNA sample was recorded by absorption spectrophotometry between wavelengths of 200 nm and 350 nm to ascertain the quality of DNA and an accurate quantification of the DNA concentration. The absorbance at 260 nm was used to measure the DNA concentration of each sample (absorbance of 1 = 50 μg of DNA/mL). Aliquots (50 μg) of DNA samples were dried in a SpeedVac under vacuum.

Analysis of DNA samples. Liquid chromatography/mass spectrometry (LC/MS) with isotope-dilution was used to identify and quantify (5′S)-8,5′cyclo-2′-deoxyadenosine (S-cdA), and 8-hydroxyguanine (8-OH-Gua) and 8-hydroxyadenine (8-OH-Ade) as their nucleoside forms 8-hydroxy-2′-deoxyguanosine (8-OH-dG) and 8-hydroxy-2′-deoxyadenosine (8-OH-dA), respectively (*15-17*). A stable isotope-labeled analog of 8-OH-dG, i.e., 8-OH-dG-$^{15}N_5$, was purchased from Cambridge Isotope Laboratories (Cambridge, MA) and used as an internal standard. Stable isotope-labeled analogues of and S-cdA and 8-OH-dA, i.e., S-cdA-$^{15}N_5$ and 8-OH-dA-$^{15}N_5$, respectively, were prepared as described (*18;19*) and used as internal standards. Aliquots (50 μg) of DNA samples were supplemented with aliquots of internal standards, hydrolyzed with nuclease P1, snake venom phosphodiesterase and alkaline phosphatase for 24 h, and then analyzed by LC/MS as described (*19*). A Synergi 4 μ Fusion-RP column (25 cm x 2 mm i.d., 4 μm particle size) (Phenomenex, Torrance, CA) with a guard column (1 cm x 2.1 mm i.d.) was used. Solvent A was acetonitrile plus water (98:2, v/v) and solvent B was 100% acetonitrile. A gradient of 1%/min of solvent B was used. The flow rate was 0.25 mL/min. The column was kept at 40 ºC. An aliquot of 25 μL of filtered samples was injected onto the LC column. For identification and quantification, selected-ion monitoring (SIM) was used to monitor the

characteristic ions of 8-OH-dG (m/z 168 and 306), 8-OH-dG-$^{15}N_5$ (m/z 173 and 311), 8-OH-dA (m/z 152 and 290), 8-OH-dA-$^{15}N_5$ (m/z 157 and 295), S-cdA (m/z 164 and 250) and S-cdA-$^{15}N_5$, (m/z 169 and 255) at the appropriate retention time periods during LC/MS analyses (*15-17*)**.**

4,6-Diamino-5-formamidopyrimidine (FapyAde), 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua) and 8-OH-Gua were identified and quantified using gas chromatography /mass spectrometry (GC/MS) with isotope-dilution, following hydrolysis of DNA samples with *E. coli* Fpg to release these lesions (*20*). Stable isotope-labeled analogues of FapyAde and FapyGua, i.e., FapyAde-$^{13}C,^{15}N_2$ and FapyGua-$^{13}C,^{15}N_2$, were purchased from Cambridge Isotope Laboratories (Cambridge, MA). The stable isotope-labeled analog of 8-OH-Gua, i.e., 8-OH-Gua-$^{13}C,^{15}N_3$ was obtained as described (*21*). Aliquots (50 μg) of DNA were supplemented with aliquots of FapyAde-$^{13}C,^{15}N_2$, FapyGua-$^{13}C,^{15}N_2$ and 8-OH-Gua-$^{13}C,^{15}N_3$, and hydrolyzed with 2 μg of *E. coli* Fpg (*22*)**.** After hydrolysis, precipitation of DNA by ethanol and subsequent centrifugation, supernatant fractions and DNA pellets were separated. Supernatant fractions were freed from ethanol in a SpeedVac under vacuum, frozen in liquid nitrogen and lyophilized for 18 h. The dried hydrolysates were trimethylsilylated and then analyzed by GC/MS as described (*20*). For identification and quantification, SIM was used to monitor the characteristic ions of the trimethylsilyl derivatives of FapyAde (m/z 354, 368 and 369), FapyAde-$^{13}C,^{15}N_2$ (m/z 357, 371 and 372), FapyGua (m/z 442 and 457), FapyGua-$^{13}C,^{15}N_2$ (m/z 445 and 460), 8-OH-Gua (m/z 440 and 455) and 8-OH-Gua-$^{13}C,^{15}N_3$ (m/z 444 and 459) (*20*). The quantification of the monitored compounds was achieved using integrated areas of the signals of their ions. The levels of 8-OH-Gua measured by GC/MS and by LC/MS as its nucleoside 8-OH-dG agreed well with each other.

Statistical analysis of the data. Statistical analysis was performed by using GarphPad Prism (Version 3.02, San Diego, CA, USA). The statistical comparisons between the groups were performed by Kruskall-Wallis one-way analysis of variance (ANOVA) followed by Mann-Whitney test or ANOVA followed by Tukey's multiple comparison test when appropriate. $P<0.05$ was considered statistically significant.

RESULTS

We hypothesized that the depletion of GSH *in vivo* causes oxidatively induced DNA damage and leads to its accumulation. To test this hypothesis, we investigated the formation of some typical oxidatively induced DNA lesions and GSH levels in organs of rabbits *in vivo* treated with the GSH-depleting compound BSO. Furthermore, we hypothesized that the non-essential amino acid Tau, which is abundantly present in most mammalian tissues including the brain, heart, skeletal muscles and nervous system (reviewed in refs (*23;24*), may reverse the action of BSO and inhibit DNA damage. We assayed four groups of rabbits, each of which had five animals, namely the control group and three other groups treated with BSO, BSO+Tau or Tau. The organs chosen for analysis were liver, kidney, brain and heart. Rabbits tolerated BSO and Tau treatments well. Tau did not appear to cause any visible side effects. However, corneal opasity was observed in 4 rabbits from the BSO group. The body weight of the rabbits from each group was not changed by the treatment (data not shown). The levels of GSH were measured in liver, kidney, brain and heart from all groups of rabbits. Treatment with BSO significantly reduced GSH levels in liver (Figure 1A). When animals were fed with Tau at the time of BSO treatment, no significant depletion of GSH was observed when compared to the control group, indicating the protection afforded by Tau against GSH depletion [also compare the BSO group and the BSO+Tau group]. Tau alone had no significant effect on GSH level when compared to the control group. In kidney, however, BSO treatment did not significantly affect GSH level (Figure 1B). Similar to liver, GSH levels in brain and heart significantly decreased in response to BSO treatment (Figures 1C and 1D, respectively). Tau significantly prevented GSH depletion by BSO. In heart, however, Tau did not reverse the action of BSO completely and GSH level was

significantly lower than those in the control group and in the Tau group. In both cases, Tau alone had no effect on GSH level when compared to the control group (Figures 1C and 1D).

We also investigated the formation of some typical oxidatively induced DNA lesions in liver, kidney, brain and heart of control and treated rabbits. The structure of the lesions identified and quantified are shown in Figure 2. Figures 3A and 3B illustrate the level of 8-OH-Gua in brain and kidney, respectively. No significant change in the level of 8-OH-Gua was observed in either organ in all three cases of treatment when compared to the control group. No results were obtained from brain of animals treated with Tau alone (Figure 3A). In liver and heart, however, treatment with BSO caused significant formation of 8-OH-Gua (Figures 3C and 3D, respectively). In both cases, feeding the animals with Tau at the time of BSO treatment significantly inhibited the formation of 8-OH-Gua (Figures 3C and 3D). Tau alone had no effect on the level of 8-OH-Gua observed in liver and heart of control animals.

In contrast to 8-OH-Gua, BSO treatment caused significant formation of FapyGua in brain when compared to the control group and Tau inhibited it (Figure 4A). In this case, FapyGua could not be detected in brain of animals fed with Tau alone. Similar results were observed in liver and heart. BSO treatment significantly increased the level of FapyGua and Tau inhibited the formation of this modified DNA base in these tissues (Figures 4B and 4C). As in the case of 8-OH-Gua, treatment with BSO, BSO+Tau or Tau had no significant affect on the level of FapyGua in kidney (Figure 4D). S-cdA and 8-OH-Ade were detected and quantified in liver only. Statistically significant formation of both S-cdA and 8-OH-dA was observed in response to BSO treatment (Figures 5A and 5B, respectively). Tau inhibited the formation of these lesions. 8-OH-Ade could not be detected in liver of Tau-treated animals (Figure 5B).

DISCUSSION

The model of continuous depletion of GSH used in this study, depends on the inhibition of the enzyme γ-GCS by BSO in mice (*5;25;26*), rats (*27-30*) and rabbits (*31*). This experimental model of GSH deficiency has also been used as a model of oxidative stress (*12*) and as an experimental model of hypertension (*27;32*). In the present study, we show for the first time that GSH depletion induced by BSO treatment results in formation of oxidatively induced DNA lesions in several organs of rabbits *in vivo*. Thus, our results unequivocally demonstrate the protection afforded by GSH *in vivo* against oxidatively induced DNA damage. Moreover, we show the protective effect of Tau against DNA damage induced by GSH depletion.

Consistent with the results of previous studies (*26;28*), subcutaneously administered BSO significantly decreased GSH levels in liver, heart and brain of animals. However, it exhibited no significant effect on GSH level in kidney, suggesting an organ-specific response *in vivo* to a GSH-depleting agent. Tau in drinking water of animals inhibited a decrease in GSH levels in liver, heart and brain. Tau is found at high concentrations in mammalian tissues (*33;34*). An important role in many physiological processes has been attributed to this non-essential amino acid, such as regulation of cardiovascular system and blood pressure, cell proliferation, and protection agaist oxidative stress, neurodegenerative diseases, atherosclerosis among other marked activities on various disorders (reviewed in refs (*23;24;35*)). The exact mechanism of Tau-induced protective effect against GSH depletion is not well known. Feeding animals with Tau during BSO treatment inhibited the formation of DNA lesions detected in this study. This effect is likely to result from the inhibition of GSH depletion by Tau rather than from antioxidant action of Tau., Tau is unlikely to have a biological role as an antioxidant because it is a poor antioxidant *in vitro* and because Tau chloramines resulting from its reaction with hypochlorous

acid can possibly oxidize biomolecules (*4;36*). In contrast, an effective antioxidant role has been attributed to hypotaurine *in vivo*, which is the precursor of Tau, as a scavenger of •OH (*4;34*). Hypotaurine is converted into Tau by certain reactive oxygen species including •OH and hypochlorous acid (*4*).

It is important to emphasize that, in this work, depletion of GSH correlated well with formation of DNA lesions, unequivocally proving the role of GSH in preventing oxidatively induced DNA damage. Simultanous inhibition of GSH depletion and DNA damage by Tau strongly supports this conclusion. Kidney was the only organ that did not exhibit GSH depletion by BSO and oxidatively induced DNA damage observed in the other organs. This result also supports the correlation of GSH depletion to DNA damage. With respect to oxidatively induced DNA damage in this model of GSH deficiency, liver was found to be the most affected organ with more DNA lesions detected than in other organs, indicating the importance of GSH for protection against oxidatively induced DNA damage in this organ. Overall, these results strongly suggest organ-specific DNA damage oxidatively induced by GSH-depletion *in vivo*.

The role of GSH in preventing DNA damage by oxidative stress is of great interest (*37-40*). In our study, we demonstrated the formation of FapyGua, 8-OH-Gua, 8-OH-Ade and S-cdA in DNA of several organs of rabbits by GSH depletion. These compounds are typical products of reactions of •OH with purine bases in DNA. Their mechanisms of formation have previously been discussed in detail (for reviews see refs (*13;41;42*). In spite of the blood-brain barrier, we observed a significant decrease in GSH level in the brain of BSO-treated animals. With regard to DNA damage in this organ, the background level of 8-OH-Gua was not significantly affected by BSO treatment. In contrast, FapyGua was significantly formed in conjunction with the decreased GHS level in brain. In heart and liver, however, the formation of both 8-OH-Gua and FapyGua

was observed and this corrolated with decreased levels of GSH. Tau treatment prevented the formation of these two lesions. Tau crosses the blood-brain barrier (*43;44*), and thus may play an important role in preventing DNA damage in brain as our results suggest. The difference in the formation of DNA lesions between different tissues is noteworthy. 8-OH-Gua and FapyGua result from the same parent OH-adduct radical of guanine by one-electron oxidation and by one-electron reduction, respectively (reviewed in refs (*13;42*)). Thus, the ratio of the yields of 8-OH-Gua and FapyGua may be affected by the redox status of cells and by the availability of transition metal ions (*4*). These factors may vary among different tissues, affecting the background levels and formation of these lesions. The formation of S-cdA and 8-OH-Ade was observed in liver only. Generally low *in vivo* background levels of these lesions may have prevented their detection in other tissues by the techniques used.

In conclusion, we demonstrated, for the first time, accumulation of some major products of oxidatively induced damage to DNA in several organs of rabbits due to GSH depletion *in vivo*, indicating the role of GSH in overall protection of cells against this type of DNA damage. Moreover, we show the protective effect of Tau against oxidatively induced DNA damage resulting from GSH depletion. These findings might have implications in pathologies associated with oxidative stress and/or defective antioxidant responses. In addition, an understanding of the regulatory mechanisms of GSH levels that impair oxidatively induced damage in different tissues is clearly of considerable importance and our results confirm that.

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FIGURE LEGENDS

FIGURE 1A. Level of GSH in liver of control animals and animals treated with BSO, BSO+Tau or Tau. Data are expressed as mean of 5 independent measurements using 5 animals in each group. Uncertainties are standard deviations. Statistical significance: a, $p < 0.05$ (compared to control group); b, $p < 0.05$ (compared to BSO group); c, $p < 0.05$ (compared to BSO group).

FIGURE 1B. Level of GSH in kidney of control animals and animals treated with BSO, BSO+Tau or Tau. Data are expressed as mean of 5 independent measurements using 5 animals in each group. Uncertainties are standard deviations.

FIGURE 1C. Level of GSH in brain of control animals and animals treated with BSO, BSO+Tau or Tau. Data are expressed as mean of 5 independent measurements using 5 animals in each group. Uncertainties are standard deviations. Statistical significance: a, $p < 0.05$ (compared to control group); b, $p < 0.05$ (compared to BSO group); c, $p < 0.05$ (compared to BSO group).

FIGURE 1D. Level of GSH in heart of control animals and animals treated with BSO, BSO+Tau or Tau. Data are expressed as mean of 5 independent measurements using 5 animals in each group. Uncertainties are standard deviations. Statistical significance: a, $p < 0.05$ (compared to control group); b, $p < 0.05$ (compared to BSO group); c, $p < 0.05$ (compared to BSO+Tau group); d, $p < 0.05$ (compared to BSO+Tau group); e, $p < 0.05$ (compared to control group).

FIGURE 2. Structure of the DNA lesions dealt with in this work.

FIGURE 3A. Level of 8-OH-Gua in brain of control animals and animals treated with BSO or BSO+Tau. Data are expressed as mean of 5 independent measurements using 5 animals in each group. Uncertainties are standard deviations.

FIGURE 3B. Level of 8-OH-Gua in kidney of control animals and animals treated with BSO, BSO+Tau or Tau. Data are expressed as mean of 5 independent measurements using 5 animals in each group. Uncertainties are standard deviations.

FIGURE 3C. Level of 8-OH-Gua in liver of control animals and animals treated with BSO, BSO+Tau or Tau. Data are expressed as mean of 5 independent measurements using 5 animals in each group. Uncertainties are standard deviations. Statistical significance: a, $p < 0.05$ (compared to control group).

FIGURE 3D. Level of 8-OH-Gua in heart of control animals and animals treated with BSO, BSO+Tau or Tau. Data are expressed as mean of 5 independent measurements using 5 animals in each group. Uncertainties are standard deviations. Statistical significance: a, $p < 0.001$ (compared to control group); b,c, $p < 0.001$ (compared to BSO group).

FIGURE 4A. Level of FapyGua in brain of control animals and animals treated with BSO or BSO+Tau. Data are expressed as mean of 5 independent measurements using 5 animals in each group. Uncertainties are standard deviations. Statistical significance: a, $p < 0.001$ (compared to control group); b, $p < 0.001$ (compared to BSO group).

FIGURE 4B. Level of FapyGua in liver of control animals and animals treated with BSO, BSO+Tau or Tau. Data are expressed as mean of 5 independent measurements using 5 animals in each group. Uncertainties are standard deviations. Statistical significance: a, $p < 0.001$ (compared to control group); b,c, $p < 0.001$ (compared to BSO group).

FIGURE 4C. Level of FapyGua in heart of control animals and animals treated with BSO, BSO+Tau or Tau. Data are expressed as mean of 5 independent measurements using 5 animals in each group. Uncertainties are standard deviations. Statistical significance: a, $p < 0.001$ (compared to control group); b,c, $p < 0.001$ (compared to BSO group).

FIGURE 4D. Level of FapyGua in kidney of control animals and animals treated with BSO, BSO+Tau or Tau. Data are expressed as mean of 5 independent measurements using 5 animals in each group. Uncertainties are standard deviations.

FIGURE 5A. Level of S-cdA in liver of control animals and animals treated with BSO, BSO+Tau or Tau. Data are expressed as mean of 5 independent measurements using 5 animals in

each group. Uncertainties are standard deviations. Statistical significance: a, $p < 0.05$ (compared to control group); b, $p < 0.01$ (compared to BSO group).

FIGURE 5B. Level of 8-OH-Ade in liver of control animals and animals treated with BSO or BSO+Tau. Data are expressed as mean of 5 independent measurements using 5 animals in each group. Uncertainties are standard deviations. Statistical significance: a, $p < 0.001$ (compared to control group); b, $p < 0.01$ (compared to BSO group).

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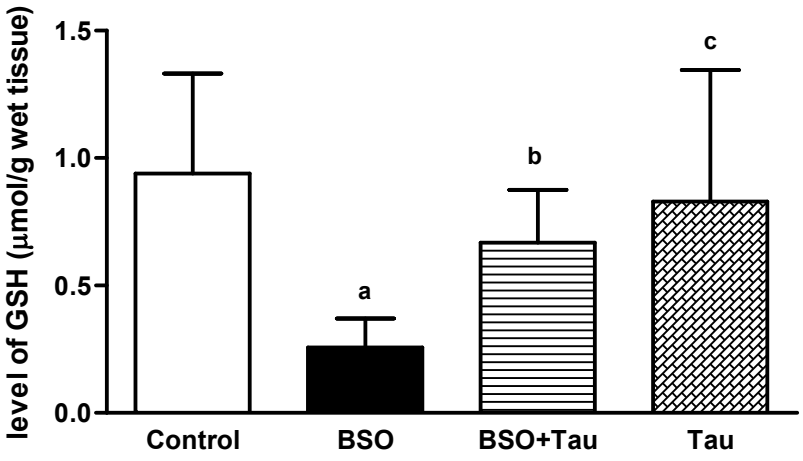
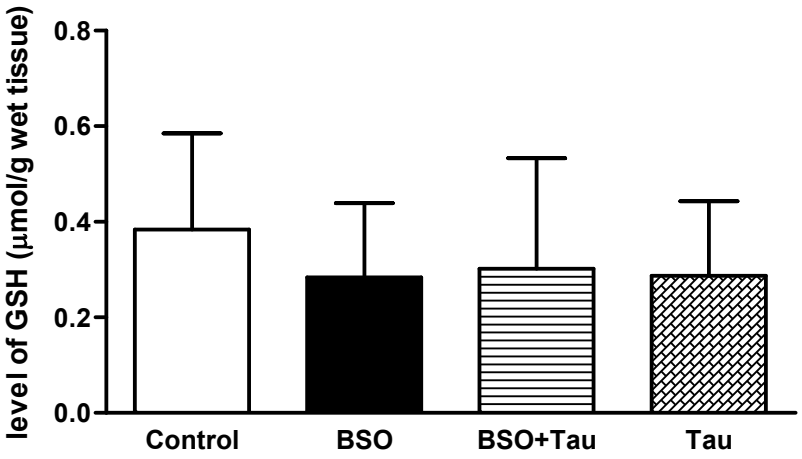


Figure 1A



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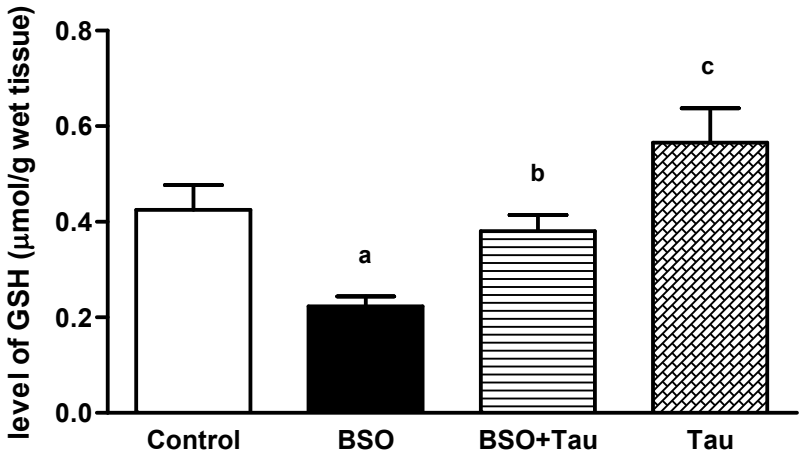

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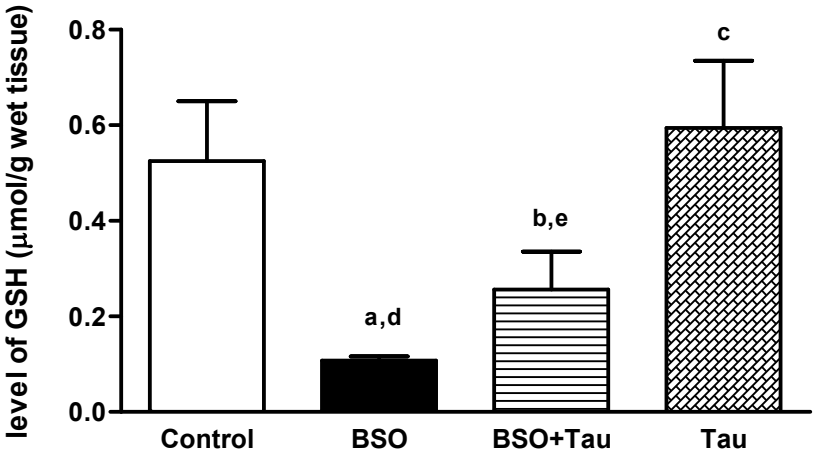

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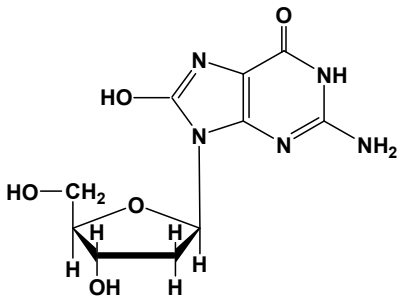

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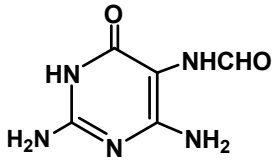

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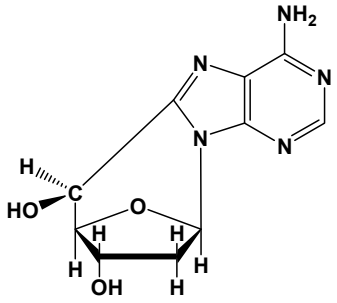

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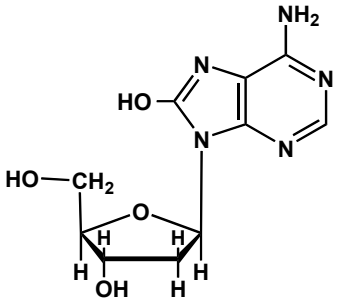
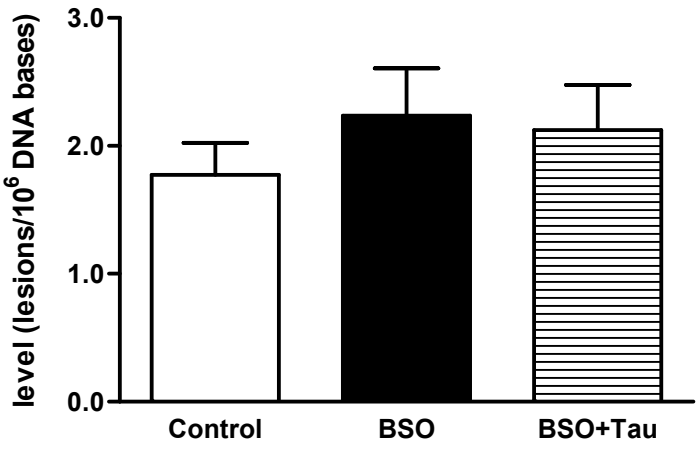


8-hydroxy-2′-deoxyadenosine

Figure 2



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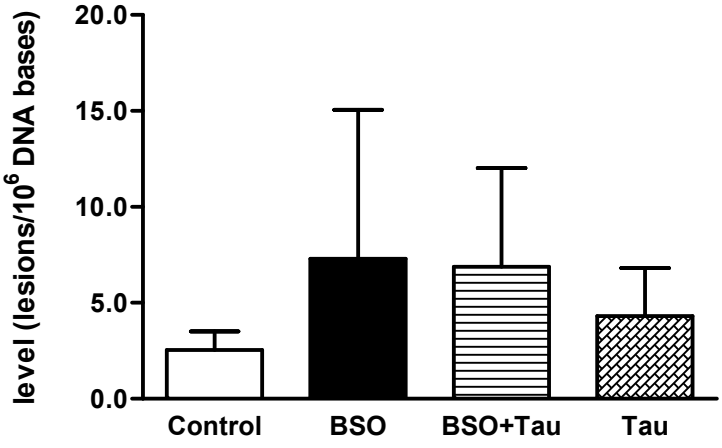

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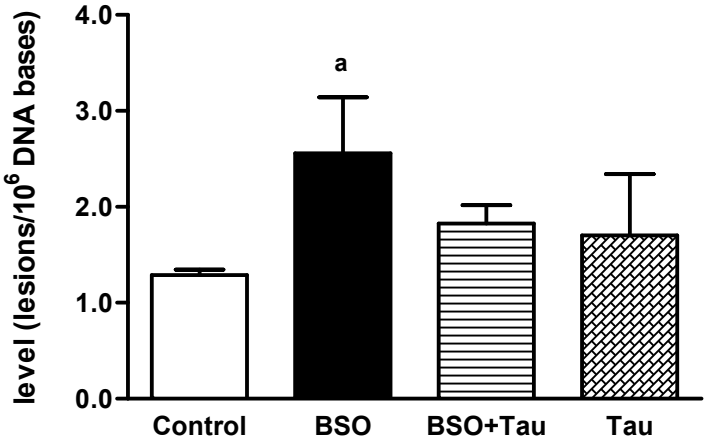

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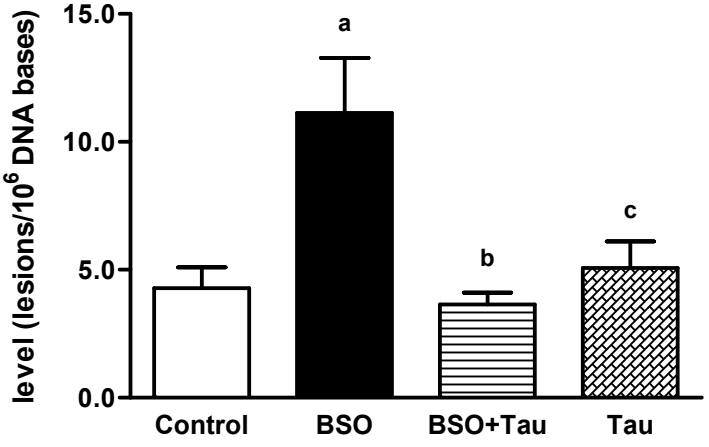

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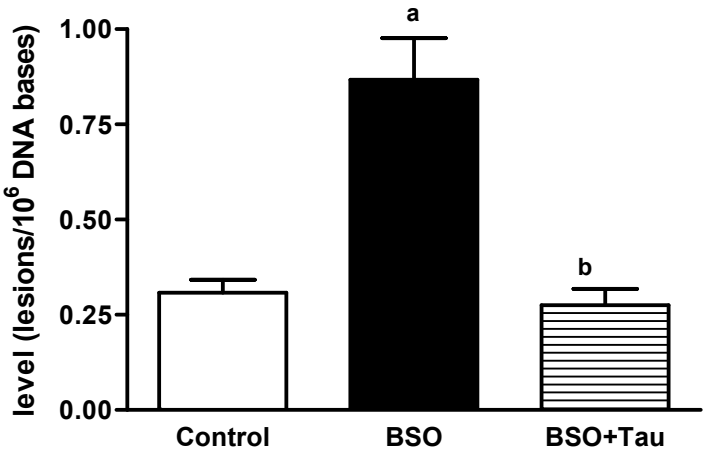

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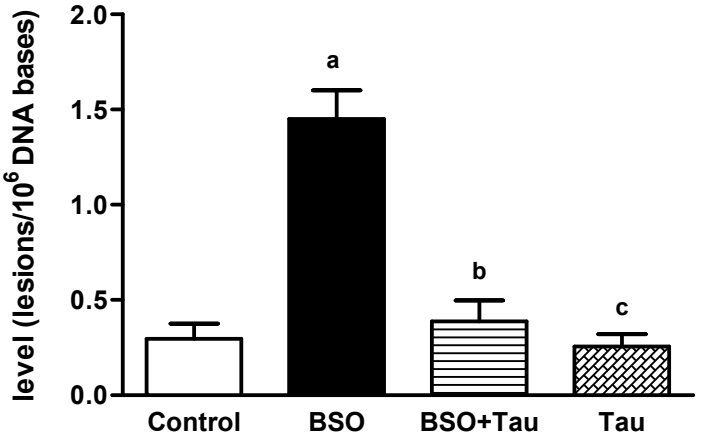

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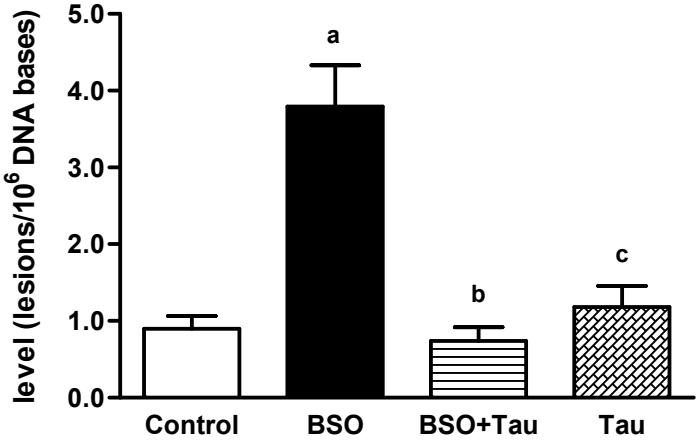

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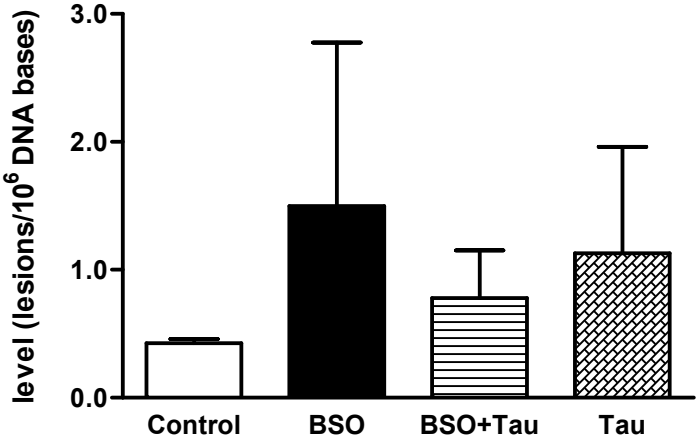

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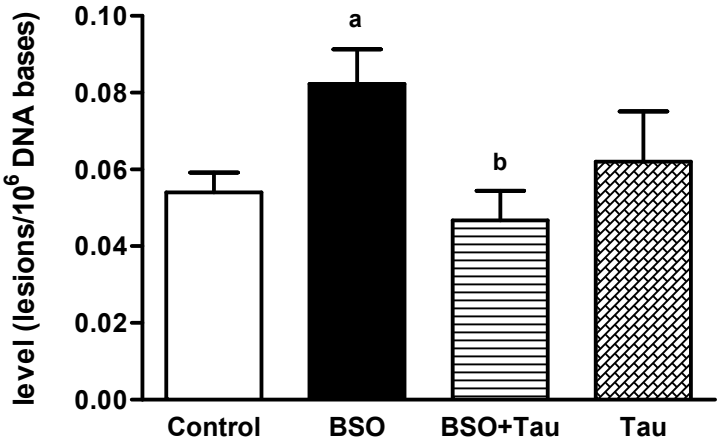

Figure 5A

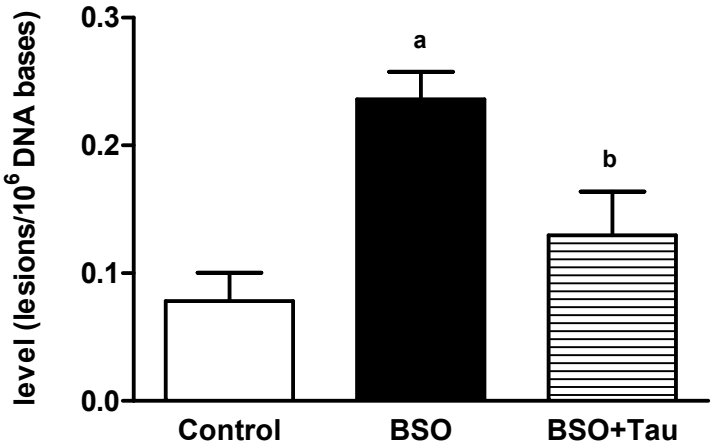

Figure 5B