NUCLEAR GLUTATHIONE S-TRANSFERASE \(\kappa\) PREVENTS APOPTOSIS
BY REDUCING THE OXIDATIVE STRESS-INDUCED FORMATION
OF EXOCYCLIC DNA PRODUCTS

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Abstract — We previously found that nuclear glutathione S-transferase \(\kappa\) (GST\(\kappa\)) accumulates in cancer cells resistant to anticancer drugs, suggesting that it has a role in the acquisition of resistance to anticancer drugs. In the present study, the effect of oxidative stress on the nuclear translocation of GST\(\kappa\) and its role in the protection of DNA from damage were investigated. In human colonic cancer HCT8 cells, the hydrogen peroxide (H\(_2\)O\(_2\))-induced increase in nuclear condensation, the population of sub-G\(_1\) peak, and the number of TUNEL-positive cells were observed in cells pretreated with edible mushroom lectin, an inhibitor of the nuclear transport of GST\(\kappa\). The DNA damage and the formation of lipid peroxide were dependent on the dose of H\(_2\)O\(_2\) and the incubation time. Immunological analysis showed that H\(_2\)O\(_2\) induced the nuclear accumulation of GST\(\kappa\) but not of glutathione peroxidase. Formation of the 7-(2-oxo-heptyl)-substituted 1,\(N^2\)-etheno-2'-deoxyguanosine adduct by the reaction of 13-hydroperoxyoctadecadienoic acid (13-HPODE) with 2'-deoxyguanosine was inhibited by GST\(\kappa\) in the presence of glutathione. The conjugation product of 4-oxo-2-nonenal, a lipid aldehyde of 13-HPODE, with GSH in the presence of GST\(\kappa\), was identified by LS/MS. These results suggested that nuclear GST\(\kappa\) prevents H\(_2\)O\(_2\)-induced DNA damage by scavenging the formation of lipid-peroxide-modified DNA.

INTRODUCTION
The role of oxidative stress as a mediator of apoptosis has been extensively studied. In particular, hydrogen peroxide (H\(_2\)O\(_2\)), a by-product of oxidative stress and a major reactive oxygen species (ROS), has been implicated in triggering apoptosis in various cells. H\(_2\)O\(_2\) induces peroxidation of cellular components such as proteins, lipids, and nucleic acids [1]. H\(_2\)O\(_2\) also stimulates intracellular signal cascades, such as mitogen-activated protein kinases, and activates transcription factors, such as AP-1 and nuclear factor kappa-B [2].

Glutathione S-transferase (GST, EC 2.5.1.18) is mainly expressed in the cytoplasm and is ubiquitous in nature. GST functions in xenobiotic biotransformation [3], drug metabolism [4], protection against peroxidative stress of lipids and the nucleus [5–7], and isomerization of prostaglandins [8]. Human GST\(\kappa\) is one of a family of
GSTs; it has been reported to accumulate in various human cancer tissues or precancer tissues and is employed in cancer research as a tumor marker [9–13]. An increase in GST\(\pi\) was also found in cancer cell lines resistant to doxorubicin hydrochloride (DOX), cis-diaminedichloroplatinum(II) (cisplatin: CDDP) [14–16], and alkylating agents [17].

In addition to its main location in the cytoplasm, GST\(\pi\) has been found in the nucleus in uterine cancer cells [18] and glioma cells [19]. These findings suggest a negative correlation between the existence of GST\(\pi\) in the nucleus of cancer cells and the survival of the patient. However, there has been no report on the mechanisms responsible for the nuclear survival of GST\(\pi\) or on the physiological role of nuclear GST\(\pi\).

Edible mushroom lectin (Agaricus bisporus lectin; ABL) efficiently internalizes into the cytoplasm of cultured cells, localizes around the nucleus, and inhibits the nuclear transfer of proteins [20]. Previous reports presented evidence that ABL inhibits the nuclear transport of GST\(\pi\) and increases the sensitivity of cancer cells to anticancer drugs [21,22].

Endogenous lipid peroxidation products react with DNA and exocyclic DNA adducts to cause the covalent modification of nuclear bases [23,24]. During the lipid peroxidation process, lipid hydroperoxides are formed as the initial products, and the decomposition of the lipid hydroperoxides leads to the formation of aldehydes as the end products. Several aldehydes possess high reactivity against DNA bases, especially guanine [25–27]. Lipid-peroxide-induced DNA adduct formation and site-specific cleavage of double-stranded DNA have been reported [28,29]. Previously, Kawai et al. [30] studied the reaction of lipid hydroperoxides with DNA components and established a method to detect the formation of 7-(2-oxo-hepyl)-substituted 1,2'-etheno-2'-deoxynanosine adducts (oxo-heptyl-edG) by the reaction of 13-hydroperoxyoctadecadienoic acid (13-HPODE) with 2'-deoxyguanosine (dG).

Recently, it was reported that 4-hydroxy-2-nonenal (4-HNE) and 4-oxo-2-nonenal (4-ONE), the end products of lipid peroxides, are nonenzymatically transformed to conjugate with GSH [31]. Moreover, 4-ONE, a major end product of 13-HPODE, had a higher affinity for the nucleus than 4-HNE. Even though it has been found that GSTs catalyzes the formation of a conjugate of 4-HNE with GSH [32], its role in the formation of 4-ONE-GSH adducts was not known. In this study, we examined whether the nuclear GST\(\pi\) plays a role in the cellular sensitivity to oxidative stress caused by \(\text{H}_2\text{O}_2\) and found that GST\(\pi\) prevents DNA damage by scavenging the oxo-heptyl-edG formed from 13-HPODE and forming a conjugate of 4-ONE with GSH.

### Materials and Methods

**ABLE** was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). RPMI 1640 medium and fetal bovine serum (FBS) were obtained from Invitrogen Corp. (Carlsbad, CA). Sheep polyclonal antibodies against human glutathione peroxidase (GPX) were purchased from The Binding Site Ltd. (Birmingham, UK). Horseradish peroxidase (HRP)-labeled anti-rabbit IgG, HRP-labeled anti-mouse IgG, and HRP-labeled anti-sheep IgG were from DAKO A/S (Glostrup, Denmark). The Enhanced Chemiluminescence (ECL) kit was obtained from Amersham Biosciences (Buckinghamshire, UK). All other chemicals and reagents were purchased from Sigma Aldrich (St. Louis, MO).

**Preparation of cells**

We used the human cancer cell lines HCT8 (colonic carcinoma) kindly donated by Dr. K. J. Scanlon. HCT8 cells were supplemented with 10% FBS at 37°C in 5% CO\(_2\) with 100% humidity. Six hours before treatment with ABL, the cells were maintained in medium with 1% FBS. About \(2 \times 10^6\) cells were harvested with trypsin and washed with phosphate-buffered saline (0.137 M NaCl, 2.68 mM KCl, and 10 mM Na\(_2\)HPO\(_4\)/NaH\(_2\)PO\(_4\), pH 7.4: PBS) twice at 4°C. The pellets were stored at −80°C before use.

**TUNEL assay**

The terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay was performed using an Apop Tag Plus Fluorescein in situ Apoptosis Detection Kit (Intergen Co., Purchase, NY). Briefly, approximately \(2 \times 10^6\) cells were harvested, fixed in 70% ethanol, treated with terminal deoxynucleotidyl transferase for 1 h and then fluorescein isothiocyanate (FITC) conjugate anti-digoxigenin for 1 h at room temperature, washed with 0.1% Triton X-100/PBS, and resuspended in propidium iodide containing RNase A. Fluorescence intensity was estimated simultaneously using a FACScan flow cytometer (Becton–Dickinson, San Jose, CA).

**Nuclear condensation**

For the histochemical analysis, HCT8 cells were maintained with RPMI 1640 medium containing 10% FBS in a four-well Lab Tec Chamber (Nalge Nunc International, Naperville, IL). After treatment with \(\text{H}_2\text{O}_2\), cells were treated with 10 \(\mu\)M Hoechst 33342 for 30 min to estimate the extent of nuclear condensation. They were then washed again with PBS. Fluorescence intensity was examined using an Axioskop2 fluorescence microscope (Carl Zeiss, Jena, Germany), and the findings were
analyzed using a charge-coupled device camera (AxioCam) and AxioVision software.

**Analysis of double-stranded breaks of DNA**

DNA damage was determined by flow cytometry, based on the formation of sub-G1 peaks of DNA as described by Gong et al. [33]. HCT8 cells were washed with PBS, fixed with 70% ethanol for 12 h at -20°C, and then centrifuged and further incubated with citrate-phosphate buffer (1 v of 0.1 M citric acid and 24 v of 0.2 M Na2HPO4) for 15 min at 25°C. The DNA content per nucleus was evaluated in a FACScan flow cytometer after the nuclei were stained with propidium iodide.

**Preparation of proteins**

The cytoplasmic and nuclear proteins were prepared as described by Digram et al. [34]. Proteins in the whole cells were prepared as described previously [35].

**Preparation of antibodies**

GST\(\pi\) was purified from human placenta, and polyclonal antibody against human GST\(\pi\) was obtained by immunizing rabbits as described previously [21]. The monoclonal antibody to Oxo-heptyl-edG was prepared as described previously [30].

**Immunological estimation**

Immunological levels of GST\(\pi\) in the cytoplasm and nucleus were estimated by Western blotting. Lysate from the extract of cells was separated by SDS-polyacrylamide gel electrophoresis (SDS–PAGE) in a 12.5% gel, transferred to a nitrocellulose membrane, and immunologically stained using rabbit IgG against human GST\(\pi\) or sheep IgG against human GPX as the primary antibody and HRP-labeled anti-rabbit IgG or HRP-labeled anti-sheep IgG as the secondary antibody. Blots were developed with enhanced chemiluminescence using the ECL kit and the relative immunological activity was analyzed by NIH Image. The protein concentration was determined according to Redinbaugh and Turley [36], with bovine serum albumin as the standard.

**Estimation of lipid peroxide in the nucleus**

Nuclei extracts were prepared as described by Abmayr and Warkman [37]. Nuclear thiobarbituric acid reactive substance (TBARS) levels were determined according to the method of Ohkawa et al. [38] using tetramethoxypropane (Wako Pure Chemical Industries).

**Estimation of o xo-heptyl-edG**

Cells incubated in various conditions were harvested with trypsin and washed with PBS two times at 4°C. The cells were then suspended in 10 mM citrate buffer (pH 6.0) and incubated for 10 min at 95°C. After a wash with PBS two times, the cells were suspended in 2 M HCl for 30 min at room temperature and rewashed with PBS two times. The levels of o xo-heptyl-edG in the cells were estimated by flow cytometry using anti-oxo-heptyl-edG mouse monoclonal antibody (mAb6A3) and FITC-conjugated anti-mouse IgG antibody.

**Effect of GST\(\pi\) on the formation of o xo-heptyl-edG**

13-HPODE (20 mM) was mixed with 1 mM FeCl2 and stood for 12 h at 37°C. The solution (13-HPODE, 5 mM and FeCl2, 0.2 mM, as a final concentration) was incubated with or without GSH (1 or 5 mM) and GST\(\pi\) (0.2 U) in the presence of 5 μg of calf thymus DNA for 1 h at 37°C. Then 1 and 5 μg of DNA extracted from the solution by ethanol precipitation

![Image](image_url)

**Fig. 1. Nuclear condensation.** For the estimation of nuclear condensation, cells were incubated in a four-well Lab Tec Chamber. After treatment with various concentrations of \( \text{H}_2\text{O}_2 \) for 24 h, cells were treated with \( 10 \mu\text{M Hoechst 33342} \) for 30 min for the estimation of nuclear condensation (top). The observation of fluorescence intensity was done using an Axioskop fluorescence microscope, and the findings were analyzed using a charge-coupled device camera and AxioVision software. Cells were pretreated with ABL (40 μg/ml) for 10 h and then treated with \( \text{H}_2\text{O}_2 \) (bottom).
were spotted on a nitrocellulose membrane and immunologically stained using MAb6A3 as the primary antibody and HRP-conjugated anti-mouse IgG antibody as the secondary antibody. Blots were developed by enhanced chemiluminescence using the ECL kit and the relative immunological activity was analyzed by NIH Image.

**Liquid chromatography/mass spectrometry**

The chemical structure of the product of the incubation of 4-ONE and GSH in the presence of GST was characterized by liquid chromatography/mass spectrometry (LC/MS). The LC/MS was conducted using a Platform II (VG Biotech) in an electrospray ionization positive (ESP+) mode. The gradient condition (solvent A, 0.01% trifluoroacetate; solvent B, acetonitrile containing 0.01% trifluoroacetate) was as follows: 100% A (0 min), 50% B (20 min), 100% B (30 min), 100% B hold (30–35 min), 100% A (40 min).

**Statistical analysis**

Data are presented as the mean ± SD. Differences were examined using a Student t test. A value of \( p < 0.05 \) was considered significant.

**RESULTS**

**Nuclear condensation**

Nuclear condensation is a characteristic of apoptosis. The nuclear condensation caused by \( \text{H}_2\text{O}_2 \) was estimated morphologically using Hoechst 33342 (Fig. 1). Human colonic cancer HCT8 cells were incubated with various concentrations of \( \text{H}_2\text{O}_2 \) for 24 h. No DNA condensation was observed (100–400 \( \mu\text{M} \) \( \text{H}_2\text{O}_2 \)). ABL, a mushroom lectin, inhibits the nuclear transfer of GST \( \pi \) [1]. The cells were previously treated with 40 \( \mu\text{g/ml} \) of ABL for 10 h and further incubated with \( \text{H}_2\text{O}_2 \) for 24 h. Nuclear condensation was observed in

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Fig. 2. Flow cytometric analysis of the DNA damage. (A) Effect of \( \text{H}_2\text{O}_2 \) on the DNA damage was analyzed using a FACScan flow cytometer. The sub-G1 peak was estimated as a marker of the double-strand break of DNA. Treatment of cells with \( \text{H}_2\text{O}_2 \) or pretreatment with ABL was performed as described in Fig. 1 legend. (B) Effect of various concentrations of ABL (left) and \( \text{H}_2\text{O}_2 \) (right) on the formation of the sub-G1 peak (%). ○ 400 \( \mu\text{M} \) \( \text{H}_2\text{O}_2 \) ( ); ● \( \text{H}_2\text{O}_2 \) ( ); □ 40 \( \mu\text{g/ml} \) ABL ( ); ■ ABL ( ). * \( p < 0.05 \) compared with cells without \( \text{H}_2\text{O}_2 \) treatment. \( p < 0.05 \) compared with cells without ABL pretreatment. (C) Effect of incubation time on the formation of the sub-G1 peak (%). ○ 400 \( \mu\text{M} \) \( \text{H}_2\text{O}_2 \) ( ); ● control; □ 40 \( \mu\text{g/ml} \) ABL ( ); □ 400 \( \mu\text{M} \) \( \text{H}_2\text{O}_2 \) with ABL pretreatment. * \( p < 0.05 \) compared with \( \text{H}_2\text{O}_2 \)-treated cells. Data are the means of three independent analyses. Bars show the SD.
Fig. 3. TUNEL assay. The effect of ABL on the cytotoxicity of H₂O₂ was examined by TUNEL assay using an Apop Tag Plus Fluorescein In Situ Apoptosis Detection Kit as described under Materials and methods. (A) Cells (2 × 10⁶) treated with 400 μM H₂O₂ for 12 and 24 h (left) or pretreated with ABL (40 μg/ml) for 10 h (right). (B) Effect of incubation time on the TUNEL-positive cells (%). ○400 μM H₂O₂ (+); ●control; □40 μg/ml ABL (+); ◆400 μM H₂O₂ with ABL pretreatment. Data are the means of three independent analyses. Bars show the SD. *p < 0.05 compared with H₂O₂-treated cells.
a manner dependent on the dose of H$_2$O$_2$ when the cells were pretreated with ABL. These results suggest that inhibition of the nuclear transfer of GST by ABL enhanced the H$_2$O$_2$-induced DNA damage.

**Effect of H$_2$O$_2$ on DNA damage**

To understand the extent of the DNA damage by H$_2$O$_2$, the sub-G$_1$ peak was estimated flow cytometrically as a marker of the double-stranded breaks of DNA (Fig. 2A). The DNA damage induced by pretreatment with ABL was not apparent (Fig. 2B, left) and damage was slightly induced by H$_2$O$_2$ alone (Fig. 2B, right). ABL increased the population of the sub-G$_1$ peak induced by 400 μM H$_2$O$_2$ in a dose-dependent (10–40 μg/ml) manner (Fig. 2B, left). Pretreatment with 40 μg/ml of ABL for 10 h enhanced the population of the sub-G$_1$ peak induced by H$_2$O$_2$ (100–400 μM) for 24 h dose dependently (Fig. 2B, right). The effect of ABL on the H$_2$O$_2$-induced DNA damage was dependent on the incubation time with H$_2$O$_2$ (Fig. 2C). These results suggest that ABL increases the sensitivity of cells to H$_2$O$_2$, leading to DNA damage and apoptosis.

**Immunological estimation of nuclear GST**

The amount of GST by H$_2$O$_2$ was estimated. Fig. 4 shows results of Western blotting for GST. H$_2$O$_2$ increased the levels of cytoplasmic GST in a time-dependent manner (top). A concomitant increase in the level of GST was observed in the nucleus. The effect of ABL on the nuclear transfer of GST was studied (bottom). The H$_2$O$_2$-induced transfer of GST to the nucleus was inhibited by ABL. The results were consistent with previous data [21].

In the cytoplasm, H$_2$O$_2$ is detoxified to H$_2$O by GPX. Then, the immunological activity of GPX was estimated (Fig. 4). Treatment with H$_2$O$_2$ increased the levels of GPX in the cytoplasm. During the experiment, no GPX was transferred to the nucleus. Similarly, the immunological activity of glutathione reductase was not found in the nucleus in the presence or absence of H$_2$O$_2$ (data not shown).

**Estimation of lipid peroxide in the nucleus**

The formation of lipid peroxides was determined as the TBARS levels (Fig. 5). H$_2$O$_2$ increased the levels of nuclear TBARS when the cells were pretreated with ABL.

**Role of nuclear GST**

Kawai et al. [30] reported that DNA bases are modified with lipid peroxide of linoleic acids leading to DNA damage. 4-ONE is nonenzymatically formed from 13-HPODE, which reacts with dG to form oxo-heptyl-dG. Doorn and Petersen [31] reported that 4-ONE has a higher affinity for nucleotides than 4-HNE and, on the other hand, spontaneously reacts with GSH to form its GSH conjugate [31]. We then speculated that
human GST\(\pi\) catalyzed the formation of 4-ONE conjugated with GSH, which can then prevent the DNA from being modified with lipid peroxide. The immunological activity of lipid-peroxide-modified DNA was estimated flow cytometrically using anti-oxo-heptyl-\(\varepsilon\)-dG mouse monoclonal antibody (mAb6A3) and FITC-conjugated anti-mouse IgG antibody. The formation of oxo-heptyl-\(\varepsilon\)-dG was observed following treatment with 400 \(\mu\)M \(\text{H}_2\text{O}_2\) for 12 h (Fig. 6, panel 3) and increased on pretreatment with ABL (Fig. 6, panel 4). The possible role of GST\(\pi\) in preventing the formation of lipid peroxide-DNA was affirmed in vitro. A mixture of 13-HPODE and \(\text{FeCl}_2\) stood for 12 h at 37\(^\circ\)C. The mixture was incubated with calf thymus DNA for 1 h at 37\(^\circ\)C in the presence or absence of 5 mM GSH and 0.2 U of GST\(\pi\). The formation of oxo-heptyl-\(\varepsilon\)-dG was estimated from immuno blots (Fig. 7). The formation of oxo-heptyl-\(\varepsilon\)-dG was inhibited by 20\% in the presence of GSH (Fig. 7, lane 2) and by 60\% in the presence of GST\(\pi\) and GSH (Fig. 7, lane 4). The results suggest that GST\(\pi\) inhibits the formation of oxo-heptyl-\(\varepsilon\)-dG in the nucleus. Fig. 8 shows the results of LC/MS measurements of the adduct formation of 4-ONE and GSH in the presence or absence of GST\(\pi\). In the absence of GST\(\pi\), the LC/MS analysis of the product gave a pseudomolecular ion peak \([M + H]^+\) at \(m/z\) 462 (Fig. 8B). In the presence of GST\(\pi\), this value apparently increased (Fig. 8C). Since the possible molecular weight of the ONE-GSH adduct is 641.18 (Fig. 9), the data obtained by LC/MS support the idea that GST\(\pi\) catalyzes the formation of the product.

**DISCUSSION**

In this study, we found for the first time that nuclear GST\(\pi\) functions to scavenge lipid-peroxide-induced DNA damage. We showed that (1) hydrogen peroxide increased the modification of nuclear DNA induced by lipid peroxide to cause DNA damage followed by the induction of apoptosis, (2) the nuclear GST\(\pi\) prevented DNA damage from lipid peroxide by scavenging the oxo-heptyl-\(\varepsilon\)-dG formed by the reaction of 13-HPODE with \(\text{dG}\) (the product of the conjugation of 4-ONE, one of the major breakdown products of 13-HPODE, with GSH catalyzed by GST\(\pi\) was identified), and (3) ABL inhibited the nuclear transfer of GST\(\pi\) to increase the sensitivity of the nucleus to oxidative stress. These findings suggest that nuclear GST\(\pi\) prevents \(\text{H}_2\text{O}_2\)-induced DNA damage by scavenging lipid-peroxide-modified DNA.

**Fig. 6. Immunological estimation of oxo-heptyl-\(\varepsilon\)-dG.** Effects of ABL (panel 2), \(\text{H}_2\text{O}_2\) (panel 3), and \(\text{H}_2\text{O}_2\) with ABL pretreatment (panel 4) on the levels of oxo-heptyl-\(\varepsilon\)-dG in the cells were estimated by flow cytometer using anti-oxo-heptyl-\(\varepsilon\)-dG mouse monoclonal antibody (mAb6A3) and FITC-conjugated anti-mouse IgG antibody.

**Fig. 7. Effect of GST\(\pi\) on the formation of oxo-heptyl-\(\varepsilon\)-dG in vitro.** (A) 13-HPODE was mixed with \(\text{FeCl}_2\) and stood for 12 h at 37\(^\circ\)C. The solution was incubated with or without GSH and GST\(\pi\) in the presence of calf thymus DNA for 1 h at 37\(^\circ\)C. DNA extract was spotted on a nitrocellulose membrane and immunologically stained using MAb6A3 as the primary antibody and HRP-conjugated anti-mouse IgG antibody as the secondary antibody. Blots were developed by enhanced chemiluminescence using the ECL kit and the relative immunological activity was analyzed by NIH Image. (B) Relative intensity (%) of the levels of oxo-heptyl-\(\varepsilon\)-dG in each lane corresponds to A. Data are the means of three independent analyses. Bars show the SD. *\(p < 0.05\) compared with control cells; **\(p < 0.05\) compared with \(\text{H}_2\text{O}_2\)-treated cells.
Previously, we found that the nuclear GST\(\kappa\) is an important factor in the acquisition of drug resistance in cancer cells [21,22]. Cancer cells which expressed the nuclear GST\(\kappa\) in response to anticancer drugs such as DOX and CDDP showed resistance to these drugs, whereas the cells that did not express nuclear GST\(\kappa\) were more sensitive to the drugs. The conjugation of the drugs with GSH was found in the resistant cells and correlated with decreased drug-induced DNA damage. In the present study, \(\mathrm{H}_2\mathrm{O}_2\)-induced DNA damage was observed when the cells were previously treated with ABL, an inhibitor of the nuclear transfer of GST\(\pi\) (Figs. 1–3). Strikingly, HCT8 cells were not sensitive to \(\mathrm{H}_2\mathrm{O}_2\) (400 \(\mu\)M). Treatment of the cells with \(\mathrm{H}_2\mathrm{O}_2\) increased the nuclear transfer of GST\(\pi\) in a dose- (data not shown) and time-dependent manner (Fig. 4). The resistance of HCT8 cells to oxidative stress was abolished by pretreatment with ABL. The results strongly suggest an important role for the nuclear GST\(\pi\) in the sensitivity of the cells to oxidative stress.

There are many antioxidants in cells. Most of them are localized in the cytoplasm. In addition, each microorgan possesses its own defense system against oxidative stress. A nuclear superoxide dismutase, GPX, and GST\(\pi\) have been reported [21,39,40].

Adler et al. [41] reported that GSTp associates with Jun N-terminal kinase (JNK) to regulate its activity in mouse fibroblast NIH3T3 cells. Moreover, Yin et al. [42] demonstrated that GSTp coordinates the activation of extracellular signal-regulated kinases/p38 mitogen-activated protein kinase/inhibitor of \(\kappa\) kinase and suppression of JNK as part of the mechanism underlying its ability to elicit protection against \(\mathrm{H}_2\mathrm{O}_2\)-induced cell death. These findings indicate that GSTp plays an important role in the defense system against oxidative stress through its function as a regulator of stress kinases. It is interesting that GSTp has at least two different functions, to scavenge lipid peroxide and to regulate stress kinases as an antioxidant.

Lipid hydroperoxides are known to be relatively short lived. They are enzymatically and/or nonenzymatically metabolized to stable alcohols in vivo. They also react with metal to form reactive end products.
such as aldehydes. However, the importance of lipid hydroperoxides to the covalent modifications of biological components has not been thoroughly investigated. oxo-heptyl-ε-dG is formed by the reaction of 13-HPODE with dG. During this reaction, 4-ONE directly mediates the formation of oxo-heptyl-ε-dG, suggesting that the lipid-hydroperoxide-derived production of 4-ONE contributes to DNA damage. 4-ONE and 4-HNE also form adducts with proteins. These adductions of proteins and DNA are thought to be involved in the pathogenesis of several diseases such as atherosclerosis, diabetes mellitus, and carcinogenesis.

With regard to the reduction of lipid peroxide, reduction of linoleic acid hydroperoxide by GPX was reported. Lipid peroxide once formed is reduced to alcohol by GPX. With regard to the role of GST in the reduction of lipid peroxide, Cao et al. reported that GST and function in protecting against the cytotoxicity of 4-HNE in vascular smooth muscle cells. Depletion of GST by buthionine sulfoximine and inhibition of GST activity by sulfasalazine potentiated the 4-HNE-mediated cytotoxicity. The results suggested that GST functions to form a conjugate of 4-HNE with GSH.

Zimniak et al. reported that mouse GSTA4-4 belongs to the alpha subclass of GST and functions to form a conjugate of 4-HNE with GSH. Additionally, Singhal et al. reported that the human GST corresponding to mouse GSTA4-4 catalyzes the conjugation of 4-HNE with GSH. These reports indicate that GSTA4-4 plays an important role with GSH in the removal of 4-HNE. It is possible that GSTA4-4 functions to form a conjugate of 4-ONE with GSH. On the other hand, the colon cancer cell line employed in the present study possessed mainly GSTπ, which may detoxify 4-HNE and 4-ONE. It has been reported that aldose reductase prevents the formation of 4-HNE. However, there has been no report on the role of GST in the reduction of another lipid peroxidation product, 4-ONE. As shown in Fig. 9, this is the first report to show that GSTπ reduces the formation of DNA adducts with 13-HPODE, characterized as oxo-heptyl-ε-dG. GSTπ catalyzes the conjugation of 4-ONE, a lipid-peroxide-derived product, with GSH, the adduct of which is thought to contribute to age-related diseases or carcinogenesis.

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