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COMMENTARY

BIOLOGICALLY ACTIVE INTERMEDIATES GENERATED BY THE REDUCED GLUTATHIONE CONJUGATION PATHWAY

TOXICOLOGICAL IMPLICATIONS

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The most widely known biological role of reduced glutathione (GSH) is that of conjugation with a variety of xenobiotics or their chemically reactive metabolites arising from the activity of the poly-substrate mixed-function oxidase [1]. The major molecular requirement for conjugation with this nucleophilic thiol molecule is the possession of a sufficient electrophilic center. Because of the uniqueness of the GSH molecule [2], this role fulfills the dual function of enhancing excretion and detoxifying reactive chemical species. GSH conjugates are frequently less toxic than their parent compounds. They are excreted in the bile by a carrier-mediated transport system in the liver canalicular [3], or in the urine, after further biotransformation to *N*-acetyl-L-cysteine derivatives (generically called mercapturic acid) or thiomethyl metabolites (—SCH_3) [4]. Therefore, GSH serves to intercept highly electrophilic compounds before they can bind to tissue nucleophiles, possibly leading to toxic and/or mutagenic sequelae.

The interaction of foreign compounds with GSH may be spontaneous (non-enzymatic) or catalyzed by cytosolic, microsomal, mitochondrial or nuclear GSH *S*-transferases (GSTs) present as different isozymes in several mammalian and non-mammalian tissues [5, 6]. The primary substrate for GST is GSH; neither cysteine, *N*-acetyl-L-cysteine, pathotheine nor a range of simple exogenous thiols can substitute for it [7]. GSH is the predominant intracellular non-protein sulfhydryl (NPSH) in both procaryotic and eukaryotic cells, plant and animal [8]; GSTs constitute the major cytosolic protein in rat liver [9].

There are now recorded a number of instances where the classical GSH conjugation reactions serve to activate a xenobiotic, despite the considerable physicochemical changes brought about in the molecule. GSH/GST-mediated metabolism can be categorized into reversible and irreversible loss of GSH. Irreversible loss of GSH is a consequence of thioether formation, such as the mercapturic acid pathway. Resynthesis of GSH is required for the restoration of its cellular levels in response to irreversible loss. In contrast, reactions involving the formation of oxidized glutathione (GSSG), mixed, disulfides and thioesters result in reversible loss, since resynthesis of GSH from its constituent amino acids is not required for cellular GSH maintenance. This commentary addresses the toxicological sequelae of GSH-mediated metabolism (enzymatic and non-enzymatic) including its hydrolytic (or metabolic) reactive products after conjugation, i.e. L-cysteine and/or S-substituted *N*-acetyl-L-cysteine analogs.

Irreversible loss of GSH: Endogeneous compounds

Leukotrienes. Activation involving the first endogenous secondary substrate for GST is where the conjugates are biologically important molecules. Leukotrienes were originally detected as a factor present in perfusates from dog lungs that caused contraction of guinea pig intestines [10]. The factor was called "slow reacting substance" (SRS) [11] or "slow reaction substance of anaphylaxis" (SRS-A) [12] until recently when its structure was elucidated [13, 14]. Leukotriene C_4 (LTC_4) is a GSH conjugate of arachidonic acid [15]. After hydrolytic release from phospholipids of cell membrane, arachidonic acid is oxygenated by a lipoxygenase to 5-hydroperoxy-6,8,11,14-eicosatetraenoic acid (5-HPETE). This product is then converted to leukotrienes by elimination of 10-pro-R hydrogen and $OH^{\ominus}$ from the hydroperoxy group to give leukotriene A (arachidonic acid epoxide). Nucleophilic opening of the epoxide at C-6 by the sulfhydryl group of GSH gives LTC_4, which is metabolized to LTD_4 and LTE_4 by sequential elimination of glutatmic acid (catalyzed by γ-glutamyl transpeptidase) and glycine (catalyzed by a particulate dipeptidase from the kidney) (Fig. 1). In addition to arachidonic acid, other polyunsaturated fatty acids are transformed to analogous products [16]. Leukotrienes are under active investigation because of their possible role in diseases such as cystic fibrosis [17], asthma [18] and certain types of leukemia [19]. Compared to the previously known agonist histamine, which causes constriction of the airways and edema, the leukotrienes are 3–4 orders of magnitude more potent, and their effects

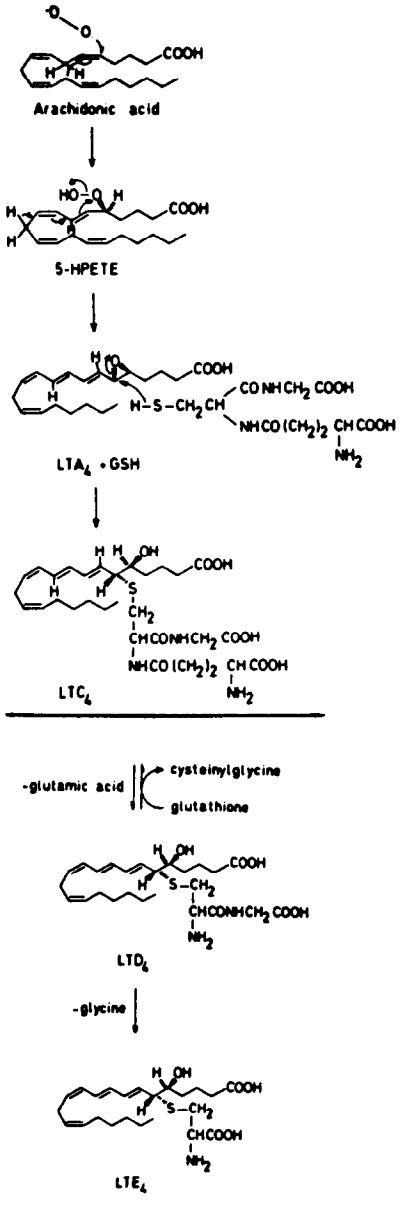

Fig. 1. Biosynthesis of leukotriene C_4 and its metabolism to leukotrienes D_4 and E_4.

are of longer duration [20, 21]. These properties further implicate the role of these agents in various pathological conditions.

Irreversible loss of GSH: Exogenous compounds

Alkylnitrosoguanidines. *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) and its ethyl analog (ENNG) are activated by GSH to products of enhanced alkylating potential [22, 23]. Thiolate anions of GSH (GS^-) react by nucleophilic attack at two reactive sites: attack on the imino carbon of MNNG gives rise to the thiazoline derivative while attack on the nitroso group accounts for the formation of *N*-methyl-*N'*-nitroguanidine which liberates free radicals [22]. Treatment of cultured mammalian cells with MNNG results in rapid methylation of nucleic acids, the extent being enhanced by the GSH content of the cells; proteins are less methylated. About 7% of the methylation of DNA occurs at the 0–6 position of the guanine residues with higher levels of methylation occurring at N-7 and N-3 positions of guanine and adenine respectively [23]. ENNG has a tendency to alkylate the 0–6 position of guanine [24].

Alkylation of the 0–6 atom of guanine is a critical promutagenic lesion produced in DNA by N-nitroso compounds and the only one clearly shown to correlate with cytotoxicity and carcinogenicity of such compounds [24, 25].

The involvement of GSH in enhancing the activation of alkylnitrosoguanidines has recently been further characterized in mutants of *Escherichia coli* and *Salmonella typhimurium* strains TA 1535 and TA 100 with lowered GSH levels (designated GSH^- mutants) but with normal GST activity [26–28]. The mutagenic activities of MNNG and ENNG, but not of *N*-ethylnitrosourea (ENU), are greatly reduced in the GSH^- mutants compared to the GSH^+ wild types. However, exogenous supply of GSH increases mutant yields considerably [26]. Thus, this finding unequivocally supports the view than MNNG and ENNG can be activated through reaction with GSH to species of higher reactivity and mutagenicity, and also corroborates the *in vitro* activation by mammalian GSH.

Dihalomethanes. Dihalomethanes such as dibromo-, diiodo- and dichloromethanes are activated via enzymatic conjugation with extracellular GSH to mutagens in a bacterial test system [29, 30]. These compounds are metabolized by microsomal cytochrome P-450 to carbon monoxide [31] and by the cytosolic GSH/GST to formaldehyde [32].

The mutagenic activities of these compounds were increased in *Salmonella* TA 100 with the addition of S-9 liver fraction with appropriate cofactors or S-100 liver fraction containing soluble GST metabolizing system [29]. This may be explained on the basis of conjugation with GSH (Fig. 2) and the production

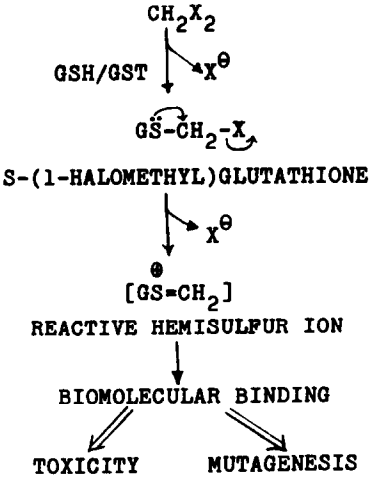

Fig. 2. Proposed reactive intermediate generated by the GSH/GST pathway for dihalomethanes in mammalian system (adapted from Ref. 29) (X = Cl, Br or I).

of highly reactive electrophilic intermediates, 1-halogenothioethers [33] which possess some structural similarity to the known mutagenic and carcinogenic 1-chloro ethers [34]. The rate of metabolism of the dihalo methanes by the GSH/GST pathway follows the carbon—halogen bond order of: C—I > C—Br > C—Cl. Diiodo methane has the shortest half-life in aqueous system and dichloromethane the longest. Thus, the chlorothioether of GSH may further be catabolized to the L-cysteine and/or *N*-acetyl-L-cysteine analogs which retain their mutagenicity.

Vicinal dihalogen compounds. Dihaloethanes may be metabolized by the microsomal mixed-function oxidase to yield 2-haloacetaldehyde [35, 36]. In contrast, activation also occurs via conjugation with GSH, resulting in the generation of highly reactive 2-halogenothioethers, sulfur half-mustards [36] (Fig. 3). Using dihalo-substituted cyclohexanes, the mutagenic activity of this group of compounds via the GSH/GST pathway was shown to be dependent upon stereochemical factors [37].

1,2-Dichloroethane [or ethylene dichloride (EDC)] is weakly mutagenic in the Ames assay, but addition of rat liver (100,000 *g*) supernatant fraction results in a marked enhancement of mutagenicity [38]. This activation is NADPH independent, non-microsomal, and caused by a factor in the soluble fraction. Increase in the number of revertants is duplicated by replacement of the liver supernatant fraction with GST isozymes A or C, and GSH, but not with GST isozyme B or denatured S-9 post-mitochondrial liver fraction and other thiols such as L-cysteine, *N*-acetyl-L-cysteine, or 2-mercaptoethanol [38]. The GST-catalyzed displacement (S_N2) reaction between EDC and GSH yields sulfur half-mustard, which may form the putative electrophilic thiiranium ion by the internal displacement of the second chlorine atom by the sulfur atom [39]. The EDC half-sulfur mustard, *S*-(2-chloroethyl)glutathione, a monofunctional alkylating agent, and its electrophilic thiiranium ion were postulated to be responsible for the mutagenic action of EDC in the bacterial test system. The *S*-(2-chloroethyl)glutathione and its L-cysteine analogs are not as reactive (i.e. easily hydrolyzable) as are the war gases, nitrogen and sulfur mustards [40]. The bile collected from EDC-treated rats [41], and diluted with buffer, still retains appreciable mutagenic activity. This implies that the half-sulfur mustard is translocatable from point of generation. These species are relatively stable in aqueous systems [42].

In addition, the synthetic conjugates, *S*-(2-chloroethyl)-L-cysteine and *N*-acetyl-*S*-(2-chloroethyl)-L-cysteine, but not *S*-(2-hydroxyethyl)-L-cysteine, produce mutagenic effects in the *Salmonella* bacterial system at molar concentrations in which no effects are seen with the parent EDC [38], thus showing that *in vivo* enzymatic catabolism of the GSH moiety by γ-glutamyl transferase, cysteinylglycinase and *N*-acetyl transferase to the L-cysteine and *N*-acetyl-L-cysteine derivatives does not abolish the mutagenic properties of the conjugate, whereas a substitution of the chlorine atom with a hydroxy group does. Recent evidence also indicates that a non-thiiranium ion alkylating species can be generated from *S*-(2-chloroethyl)-L-cysteine via the involvement of the α-amino group of the cysteine moiety [43].

S-(2-Chloroethyl)-L-cysteine and its bromine analog induce relaxation (possibly strand breaks at alkylated labile sites) in supercoiled plasmid pBR322

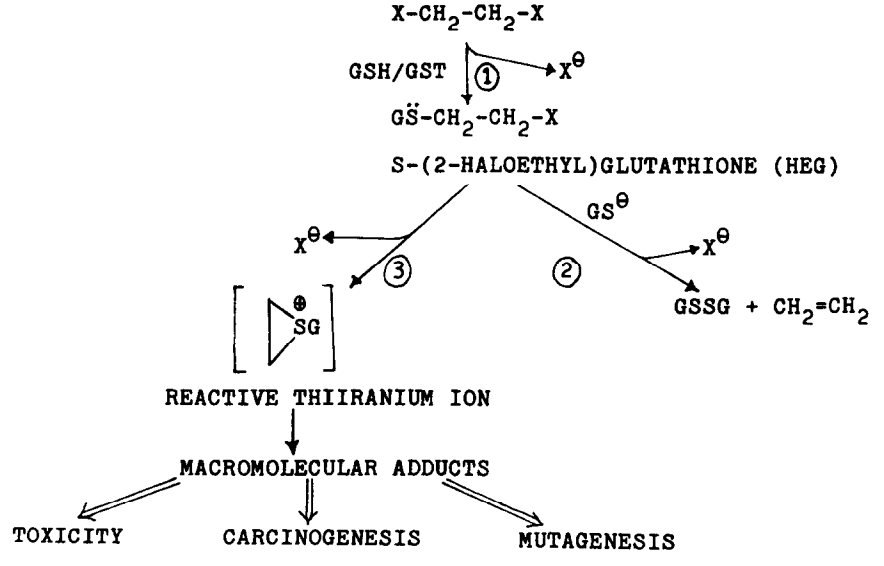

Fig. 3. Scheme (after Ref. 51) for the proposed formation of reactive intermediate(s) from the reaction of vic-dihaloalkanes with GSH (X = Cl, Br or I). (1) S_N2 nucleophilic attack on the stereospecific vic-dihaloalkanes by GSH in the presence of GST. (2) $GS^{\ominus}$ attack on the sulfur atom of HEG followed by anticlimination of the halogen ion yields ethylene and oxidized glutathione (GSSG). HEG may undergo further metabolism to its L-cysteine and *N*-acetyl-L-cysteine analogs before conversion to the thiiranium ion. (3) Intramolecular attack of the sulfur atom lone electron pair on the neighboring carbon atom leads to the expulsion of the halogen ion and the formation of the putative thiiranium ion.

DNA, as determined by gel electrophoresis and electron microscopy; *S*-(2-chloroethyl)-glutathione and *N*-acetyl-*S*-(2-chloroethyl)-L-cysteine have no effect [44]. This implies that the direct GSH adduct and its *N*-acetyl-L-cysteine analog do not interact with DNA as such and may not be responsible for the EDC mutagenic response through the GSH/GST metabolic pathway.

These findings are relevant to the observed increase in toxic manifestations in the rat liver and testes noted in an EDC/disulfiram (DSF) interaction study [45]. DSF inhibits cytochrome P-450, increases hepatic GSH level, and induces GST in both the liver and testes.* Of particular interest is the observed organotrophy of increased toxicity in the rat liver and testes exhibited by the EDC/DSF combination treatment which followed the quantitative organ distribution of GST activity [6]. DSF also modulates EDC binding to protein and DNA respectively. In the presence of DSF, the metabolites of [^{14}C]EDC bind to DNA; in the absence of DSF, they bind to proteins [46]. GSH/GST activation of EDC is also implicated in the *in vivo* hepatic DNA damage in mice, pretreated with piperonyl butoxide, a potent inhibitor of microsomal oxidative metabolism [47]. These findings may have relevance to the observed carcinogenicity of EDC after chronic exposure [48].

A similar activation process described for EDC was observed for 1,2-dibromoethane (ethylene dibromide or EDB), which is itself highly mutagenic and carcinogenic [38, 41, 49]. The contribution of the GSH/GST pathway in the metabolism of EDB was unequivocally demonstrated using primary deuterium isotope, tetradeutero derivative (EDB-d_4 or BrD_2C-CD_2Br) [50]. It was found that EDB-d_4 was more genotoxic than was EDB. This observation showed that the oxidative metabolism of EDB was not the source of genotoxic products and suggested that the GST pathway was responsible for the genotoxicity. This conclusion seemed reasonable because the oxidation of EDB was found to be its major route of elimination *in vivo* [50, 51]. Therefore, by slowing this route of elimination by deuterium substitution, a greater proportion of the administered dose of EDB-d_4 was available for metabolism by the GSH/GST pathway.

In addition, EDB is metabolized in rats to *S*-(2-hydroxyethyl)-L-cysteine in a series of reactions that, based upon *in vitro* studies with liver supernatant fraction plus GSH, presumably proceed via *S*-(2-bromoethyl)glutathione conjugate to its thiiranium ion [52]. This intermediate and its catabolic L-cysteine and, presumably, *N*-acetyl-L-cysteine products react with water or could conceivably alkylate tissue nucleophiles. Evidence exists for the formation of *S*-(2-(*N*^{7}-guanyl)ethyl]glutathione adduct in GSH/GST isozyme B mediated binding of EDB to calf-thymus DNA [53].

Two other highly toxic and carcinogenic vic-dibromoalkanes that are produced in large quantities are 1,2-dibromo-3-chloropropane and tris-(2,3-dibromopropyl)-phosphate. Since the administration of either compound to rats is accompanied by depletion of liver non-protein sulfhydryl groups (mainly GSH) [54, 55], similar GSH-mediated adducts formed with EDB may also be formed with them.

Glutathione conjugates and the thiomethyl shunt: Polyhalogenoalkanes and -alkenes. *S*-(1,2-Dichlorovinyl)-L-cysteine (DCVC) is a non-enzymatic reaction product, at high temperature, of trichloroethylene and soybean protein cysteinyl residues. It was first identified as a contaminant of cattle feed and was associated with causing aplastic anaemia in calves [56]. Conceivably, DCVC can be a product of the enzymatic (GST) reaction between GSH and trichloroethylene forming *S*-(1,2-dichloro vinyl)-glutathione which can further be catabolized to DCVC.

It was shown subsequently to be a potent nephrotoxin in cattle and laboratory animals [57]. DCVC is also implicated in the nephrotoxicity observed in rats treated with EDC [58]. The activation of DCVC to a toxic metabolite was later found to be catalyzed by β-lyase, a pyridoxal phosphate-dependent enzyme, to produce ammonia, pyruvate and a reactive sulfur-containing metabolite, possibly a thiol [59, 60]. The reactive fragment was shown to interact with protein and DNA [60] and to inhibit mitochondrial respiration [61]. The thiol metabolite, which can be methylated by thiol *S*-methyltransferase [62], may be responsible for the toxicity. β-Lyase has been isolated and purified from bovine kidney and liver, rat liver, and intestinal microflora [59, 63].

The action of β-lyase on compounds analogous to DCVC formed *in vivo* by conjugation with GSH would ultimately result in toxic thiol intermediates. GSH adducts of hexachlorobutadiene (HCBD) and chlorotrifluoroethylene (CTFE) have been isolated [64, 65]. Microsomal GSTs are more effective than cytosolic fraction in catalyzing the addition of GSH across the double bond of CTFE to give the haloalkane conjugate, *S*-(2-chloro-1,1,2-trifluoroethyl)glutathione (CTFEG) [66, 67] as well as the substitution/elimination reaction of HCBD to give the haloalkene conjugate *S*-(1,3-butadienyl-1,1,3,4-pentachloro)-glutathione [64]. The specific GST that results in the addition of GSH across a double bond is present in high concentrations in the kidney [68]. The organ specificity of the toxicity of these conjugates may be explained by the high activities of γ-glutamyl transpeptidase and cysteinylglycinase in the kidney compared to the liver [69]. Thus, the GSH adducts formed in the liver may be transported to the kidney for processing to the cysteine analogs.

The cysteine conjugates of HCBD, CTFE, tetrafluoroethylene (TFE), hexafluoropropene (HFP), trichloroethylene (TCE) and perchloroethylene (PCE) have been synthesized and a relationship determined between their structures and mutagenic potential *in vitro* [65, 70]. All conjugates are activated by β-lyase and cause decreased uptake of organic anion *p*-aminohippurate (PAH) and the cation tetraethylammonium bromide (TEA) into rat kidney slices or rabbit renal tubular suspensions. However, their mutagenicities differ markedly. The conjugates of HCBD, TCE and PCE are mutagenic in the Ames assay in the presence of kidney S-9

* O. J. Igwe, S. S. Que Hee and W. D. Wagner, presented at the *International Conference on Organic Solvent Toxicity*, Stockholm, Sweden, Oct. 15–17, 1984, Abstr. No. 166, p. 35.

fraction without metabolic cofactors, thus suggesting activation by β-lyase. However, the conjugates of TFE, CTFE and HFP are not mutagenic in the same test systems with and without metabolic cofactors [70]. The difference between these two groups of nephrotoxicants (predominantly chlorine- or fluorine-containing) was explained on the basis of the electrophilic nature of thiols derived from them by β-lyase activity, due to the electron withdrawing halogen substituents [70].

o-*Bromobenzene.* A disubstituted isomeric GSH conjugate of 2-bromohydroquinone, which is either 2-bromo-3,5-(diglutathion-*S*-yl)-hydroquinone or 2-bromo-3,6-(diglutathion-*S*-yl)-hydroquinone, has been implicated recently in the nephrotoxicity of *o*-bromobenzene and/or *o*-bromophenol in the rat [71]. Administration of the disubstituted GSH conjugate to rats causes high elevations in blood urea nitrogen (BUN) and histological alterations in the kidneys. These toxic manifestations are no different than those observed following the administration of *o*-bromobenzene, *o*-bromophenol or 2-bromohydroquinoline at equimolar doses to rats. However, the dose of the diglutathionyl conjugate required to produce toxicity is only a small fraction of that of the parent *o*-bromobenzene.

Miscellaneous compounds that produce thiol intermediates. Theoretically, all compounds shown to form thiomethyl metabolites [72] are candidates for the production of thiols through the β-lyase activity on the L-cysteine conjugate in an intermediary pathway. A common feature of thiols, even the simplest member of the group, hydrogen sulfide, is their extreme toxicities to mammalian cells [73]. Both methyl chloride (MC) and methyl iodide (MI) form glutathione conjugates [74, 75], and the glutathione conjugate of MC, *S*-methylglutathione, may be metabolized to methane thiol (CH_3SH), probably catalyzed by cysteine conjugate β-lyase [74]. The neurotoxicities of MC and MI [76, 77] are probably due to the formation of methane thiol [74].

Metals and GSH. Selenium is an essential nutrient required for the enzymatic activity of glutathione peroxidase [78]. Selenium causes a significant increase in hepatic non-protein sulfhydryls and oxidized glutathione [79]. Selenodiglutathione (GSSeSG) has been implicated in causing increased maternal deaths and fetal abortions and decreased body weight gains in surviving mothers of pregnant mice pretreated with GSH followed by sodium selenite (SS) [80]. Increased selenium content in maternal kidneys and spleen and in the fetus was also noted in the GSH/SS-treated group, when compared with the SS-treated group. GSSeSG is formed in mammalian plasma by a non-enzymatic reaction between SS and two molecules of GSH [81]. Its formation is the first step in the sequential reduction of SS from a +4 to a −2 oxidation state, utilizing GSH and NADPH as the source of reducing equivalents [82, 83]. GSSeSG toxicity may be due to its inhibition of protein synthesis as demonstrated in 3T3 fibroblast cells in culture; SS has no effect on protein synthesis in this system [84]. GSSeSG as GSSG possibly inactivates eucaryotic initiation factor 2 (eIF-2) by decreasing its phosphorylation mediated through a protein kinase [85], thus decreasing protein synthesis.

GSH plays an important role in the biliary secretion of several metals as GSH–metal complexes including mercury, copper, zinc, silver and chromium [86–88]. All metals secreted into the bile undergo some degree of reabsorption within the gastrointestinal tract. So, indirectly for GSH–metal complexes, this enterohepatic circulation significantly retards elimination, i.e. increases metal biological half-life, and is a major factor contributing to their hazardous properties.

A mercury–GSH complex is important in methylmercury ($MeHg^+$) uptake in the kidney, the target organ for mercury toxicity [89]. GSH in renal tissue binds to Hg^{2+} and serves as a sink for a renal accumulation. Treatment of rats with GSH depletors [diethylmaleate (DEM) which scavenges GSH, and buthionine sulfoximine (BSO) which inhibits γ-glutamylcysteine synthetase] decreases renal accumulation with no effect on liver accumulation [90]. Methyl mercury cations ($MeHg^+$) bind non-enzymatically with high affinity to thiolate anions (GS^-) [91]. The MeHg-SG adducts are possibly metabolized to their L-cysteine analogs in kidney, intestine and pancreatic tissue with high activities of γ-glutamyl transpeptidase (GGT) and the particulate dipeptidase cysteinylglycinase. A small but significant GGT activity is also present within the biliary tree [92]. GGT may have a role in the reabsorption from kidney tubular lumen of $MeHg^+$ as its L-cysteine analog. A low urinary activity of GGT in CFW Swiss mice has been associated with increased urinary excretion of $MeHg^+$ [93]. A stereospecificity of blood–brain permeability to MeHg-L-cysteine complex has been proposed [94], and this phenomenon may be important in the neurological deficits also associated with exposure to methyl mercury.

Reversible loss of GSH: Exogenous compounds

Neocarzinostatin. Neocarzinostatin (NCS) is reductively activated by GSH to a toxic and mutagenic species in Chinese hamster V-79 cell line [95, 96]. NCS is a polypeptide antibiotic with antitumor activity. The biologically active portion of the molecule, a non-protein chromophore [97], induces DNA single strand breaks [98] and alkali-labile breaks [99]. The cytotoxic and mutagenic manifestations of NCS to V-79 cell line are reduced by treating these cells with BSO and DEM, agents that deplete cellular GSH by different mechanisms [100]. This demonstrated that these GSH depletors do not inactivate NCS. However, L-2-oxothiazolidine-4-carboxylate, an agent that elevates cellular GSH [101], does not potentiate NCS cytotoxicity [96]. This is possibly because the high GSH concentration in undepleted cells is more than enough for the reduction of NCS to its bioactive moiety.

Azathioprine. This is a prodrug which is converted to active 6-thiopurine and a number of methylnitroimidazole metabolites. Thiolysis by GSH catalyzed by GST, with the formation of GSH-imidazole adduct, is the major route of azathioprine activation [102, 103]. The depletion of hepatic GSH and the formation of 6-thiopurine are observed after azathioprine administration.

Bleomycin. GSH is implicated in the activation of ferric iron–bleomycin complex in a catalytic oxidation–reduction cycle resulting in the formation of superoxide anion and other reactive oxygen intermediates [104]. Bleomycin is believed to act as an antineoplastic agent via an oxygen-dependent mechanism of DNA cleavage.

Conclusion, with suggestions for future research

A paucity of interest exists amongst pharmacologists and toxicologists in recognizing the significance of reactive intermediates generated by the GSH conjugation pathway. This may have arisen because of the frequency with which the phase I reactions give rise to products with interesting biological activity and the consequent concentration upon the detailed study of these processes.

The isolation and utilization of GSH-bacterial mutants that are isogenic to the wild type *Salmonella* and *E. coli* strains represent a useful and simple method to study and characterize GSH-interacting properties of xenobiotics in intact bacterial cells. Enormous potential exists for the development of such a mutant in a mammalian cell line possibly through partial mutation in the γ-glutatmylcysteine synthetase gene using the current knowledge in cell and molecular biology. Presently, BSO, which inhibits γ-glutamylcysteine synthetase, a product of the gene, is utilized in studying the effects of cellular GSH depletion *in vivo* and *in vitro* [100, 105, 106]. This is only marginally satisfactory, for not only does the BSO dose or concentration require careful titration because of its toxicity but it also exerts different effects on various cell lines. The proposed new cell line can be used (1) to characterize adducts formed by GSH–xenobiotic–macromolecule interactions and thus give insight into the toxicologic and/or mutagenic sequelae of these adducts, (2) to study the possible role of the GSH/GST pathway in enhancing DNA damage, or lack of it, caused by exposure to exogenous agents, e.g. vic-dihalogen compounds, and (3) to delineate GSH-interacting properties with chemotherapeutic agents, particularly regarding the development of pleoitropic primary and/or cross-resistance, a major drawback in chemotherapy.

The specific activities of GST isozymes in a particular organ may determine the rate of formation of reactive GSH conjugates, hence the extent of toxicity. This needs further study especially for vic-dihydrogen compounds in which target organs of toxicity seem to follow the quantitative distribution of GST activity [6]. Species resistance to the carcinogenic properties of vic-dihalogen compound may well correlate with the degree of GSH conjugate formation.

Because bacteria appear to possess β-lyase activity [107, 108], the role that the gut flora may play in the formation of sulfur-containing conjugates is of toxicological importance. Intermediary metabolites of the GSH-xenobiotic conjugation, which are secreted in the bile can become substrates for microfloral β-lyase, which appears to be chemically similar to L-cysteine conjugate β-lyases in the liver and kidney [109]. Degradation of the GSH-adduct and the mercapturates in the intestine would lead to the formation of new reabsorbable metabolites containing the reactive thiol group. Indeed, because of the intimate relationship between the bacterial mass and the intestinal microflora, the existence of a metabolic pathway that leads to the production of reactive groups in an organ such as the colon (with its high susceptibility to cancer) may be of significance. This needs more study.

With the foregoing discussion, it can be recognized that the traditional toxicological role of GSH must be expanded. GSH conjugation may have critical influences on the biological response of an organism to a foreign compound. The function of the GSH/GST pathway is therefore no longer limited to facilitating the excretion and occasionally the detoxification of xenobiotics. Biologically active GSH adducts may be formed with endogenous and exogenous substrates, and these may be further metabolized to sulfur-containing products with alkylating and mutagenic potential.

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