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Glutathione S-Transferases and Prevention of Cellular Free Radical Damage

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This paper describes the intervention of glutathione-dependent enzymes, in particular the glutathione S-transferases (GSTs), in both the detoxication of electrophilic decomposition products resulting from the attack of oxygen radicals on lipids and DNA; and the prevention of oxygen toxicity generated by redox cycling catecholamine derivatives. The continuing growth of our knowledge of the glutathione S-transferase polygene family is described in terms of the increase in members of known gene families, the discovery of new ones and our increasing knowledge of their activities towards endogenous substrates.

Keywords: Glutathione S-transferases, hydroxynonenal, base-propenals, catecholamines, oxygen radicals

INTRODUCTION

Herman Esterbauer made an invaluable contribution to our knowledge of the chemical effects of radical oxygen on lipids and nucleic acids and their biological consequences. In particular he had a leading role in the identification and chemical and biological characterization of the hydroxyalkenals, in particular 4-hydroxy-2,3-trans-nonenal (HNE).[1] He was kindly, unpretentious, intellectually rigorous, and attracted many distinguished collaborators. He was a scientist of the old tradition – a scholar and a gentleman. He is greatly missed.

His extensive work on toxic products of lipid peroxidation in particular HNE was ground breaking with respect to the importance of radical oxygen for the generation of endogenous toxins from cellular components.

This paper describes electrophiles resulting from oxygen radical attack on endogenous lipid[2] and DNA[3] and the generation of redox cycling metabolites of the catecholamine neurotransmitters and are believed to participate in the pathogenesis of Parkinson's disease and schizophrenia.[4,5] It also gives an account of our developing knowledge of the contribution of GSTs to both the detoxication of the electrophilic products of radical damage and the prevention of the generation of oxygen radicals by redox cycling. New gene families of glutathione S-transferases (GSTs) continue to be discovered

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and knowledge of their biologically significant substrates, rather than model substrates taken from the laboratory shelf, is beginning to accumulate. To date there are 9 gene families and 16 gene products which, together, contribute to the detoxication of a wide spectrum of electrophiles including both those resulting from the metabolism of xenobiotics and those from the attack of radical oxygen on endogenous molecules.[6]

Products of Radical Attack on Polyunsaturated Fatty Acyl Groups in Cellular Phospholipids

Hydroxyalkenals are end-products of the attack of radical oxygen on polyunsaturated fatty acyl groups in lipids. Hydroperoxy fatty acyl groups are formed initially and have several options. They may be detoxified directly by endoplasmic reticulum bound Se-dependent phospholipid hydroperoxide glutathione peroxidase (PHGPX).[7] Alternatively free hydroperoxy fatty acids may be released into the aqueous milieu by the lypolytic enzymes appropriate for either phospholipids or triglycerides, according to which undergoes peroxidation,[8] and then detoxified by both Se-dependent glutathione peroxidases (GPX) and appropriate glutathione S-transferases (GSTs).[9,10] The relative contributions of each of these different means of detoxifying lipid hydroperoxides depend on which enzymes are present in the tissue under attack and at what concentration.[7,9] Hydroperoxides that escape this initial detoxication, may either initiate further lipid peroxidation, or undergo oxidative decomposition to a wide range of compounds that include alkanes, alkenes and the electrophilic alkenals, hydroxyalkenals and hydroxydienals, from among which HNE is a major component.[1,2]

Esterbauer and his colleagues showed that the biochemical properties of HNE were many and varied, and that according to whether exposure was in μM or nM amounts the result could be either toxicological or patho-physiological. HNE may be inactivated by conjugation with GSH by the catalytic action of GSTs (see Table I) and, because of its normally high cellular concentration (~5 mM), GSH gives sustained protection against substantial quantities of HNE although persistent high concentrations bring about marked GSH depletion and toxicity ensues. For example cellular lipid etc. may become vulnerable to reactive oxygen species and macromolecular nucleophiles are exposed to electrophilic attack by HNE. In the case of proteins it reacts with the thiol of cysteine residues, the ϵ-amino group of lysine residues, and certain other residues including histidine, with the consequent potential to destroy or diminish the function of a number of enzymes and other proteins critical for cellular function.[2,11] One example is its ability to inactivate GSH reductase,[12] the function of which is to maintain suitable levels of reduced cellular GSH. As a result HNE, at appropriate concentrations, not only depletes GSH by HNE–GSH conjugate excretion, but also impairs its regeneration from glutathione disulphide.

Alkenals are also cytotoxic. For example acrolein, released by metabolism of the chemotherapeutic agent cyclophosphamide causes serious toxicity in the bladder epithelium, that can be mitigated by GSH analogues such as N-acetyl cysteine and the pharmaceutical agent sodium mercaptoethane sulphonate agent (Mesna). Cellular GSH and GST defences in the trilaminate bladder epithelium are apparently insufficient for the high level of intoxication they suffer during cyclophosphamide therapy.[13] The longer chain alkenals produced in lipid peroxidation, such as nonenal, are also important toxins.

Ethene, one of the alkenes produced during lipid peroxidation is a potential hazard. Though not itself toxic, it becomes so, if metabolized by cytochrome P450[14] to ethylene oxide, an electrophile and a substrate for GST.[15,16]

For examples of electrophilic products of lipid peroxidation including the metabolism of ethene to ethylene oxide, see Figure 1.

TABLE I Activities of human GSTs towards products of lipid peroxidation and DNA peroxidation

Substrate	hGSTA1-1	hGSTA4-4	hGSTM1-1	hGSTM2-2	hGSTP1-1	hGSTT1-1[39]	hGSTT2-2[45]	hGSTZ1-1
	(in $\mu mol\ min^{-1}\ mg^{-1}$)							
Ethylene oxide						+ve[14]		
Acrolein	0.9[38]	18[47]	7.1[38]		26.3[38]			
Crotonaldehyde	< 0.1[47]	7.5[47]	< 0.1[38]		1.6[38]			
Hydroxypentenal		7.4[47]						
Nonenal		205[47]						
Hydroxynonenal	5.0[45]	176[39]	3.2[38]		0.6[38]		0.12[45]	
	0.86[48]	189[47]						
		54[48]						
Hydroxydecenal		159[47]						
Hexa-2,4-dienal	0.09[48]	0.46[48]						
Cumene hydroperoxide	10[44]	0.7[47]	0.3[38]		< 0.1[38]	2.8[39]	6.9[45]	0.16[6]
9-Hydroperoxy linoleic acid	1.6[38]	0.4[45]	nil[38]		0.4[38]		9.7[46]*	
Dilinoleoyl phosphatidyl-choline hydroperoxide	nil	0.4[41]	nil		nil			
Adenine propenal	0.7[44]		3.7[44]		77[44]			
Cytosine propenal	< 0.1[44]		< 0.1[44]		1.0[44]			
Thymine propenal	< 0.1[44]		1.0[44]		8.7[44]			
Uracil propenal	< 0.1[44]		1.2[44]		15[44]			
Aminochrome	0.04[25]	< 0.01[25]	0.76[25]	148[25]	0.08[25]	nil[25]		
Dopachrome	0.02[25]	< 0.01[25]	1.2[25]	64[25]	0.04[25]	nil[25]		
Noradrenochrome	0.05[25]	< 0.01[25]	0.47[25]	75[25]	0.02[25]	nil[25]		
Adrenochrome	0.004[25]	< 0.01[25]	0.98[25]	0.63[25]	0.02[25]	nil[25]		

*The only available value for the activity of GSTT2-2 for hydroperoxylinoleic acid is from the rat. +ve indicates a result that is positive in a mutagenicity test, but has not been quantified. Spaces indicate that the substrate has not yet been tested.

Products of Radical Attack on DNA

The attack of radical oxygen on DNA is genotoxic involving both purine and pyrimidine bases and deoxyribose moieties. In the former case, one of the products is believed to be 5-hydroperoxymethyl uracil, which may be reduced by GPX and certain GSTs, to 5-hydroxymethyl uracil,[17] a recognized promutagenic lesion in DNA exposed to oxygen radicals. In the latter case, attack on deoxyribose moieties, results in promutagenic strand breakage and the release of cytotoxic and genotoxic base propenals, that are conjugated by GST.[3,18,19] For examples of these electrophilic products of oxygen radical attack on DNA see Figure 2.

HNE, a product of radical attack on lipids, is also a genotoxin. It is mutagenic in bacteria,[20,21] induces sister chromatid exchange, DNA fragmentation[22,23] and gives 1,N^2-etheno-deoxyguanosine adducts on reaction with DNA.[24]

Redox Cycling Ortho-Quinone Metabolites of Catecholamine Neurotransmitters

Oxidative metabolism of catecholamines e.g. dopa, adrenaline and noradrenaline gives rise to orthoquinone derivatives that undergo cyclization and further metabolism to aminochrome, dopachrome, adrenochrome and noradrenachrome. These quinones are susceptible to one-electron reduction by enzymes such as NADPH cytochrome P450 reductase and NAD(P)H quinone oxido-reductase to give

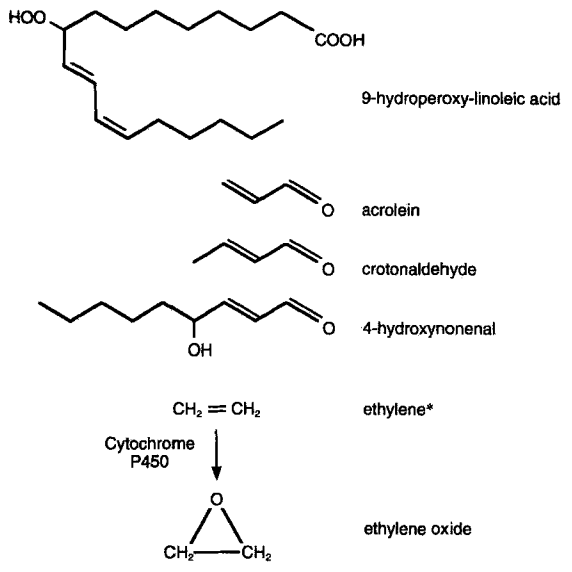

FIGURE 1 GST substrates derived from lipid peroxidation namely, the fatty acyl hydroperoxide 9-hydroperoxy-linoleic acid, the alkenals, acrolein and croton aldehyde the hydroxy-alkenal, 4-hydroxynonenal and the conversion of ethylene to ethylene oxide by the action of cytochrome P450.

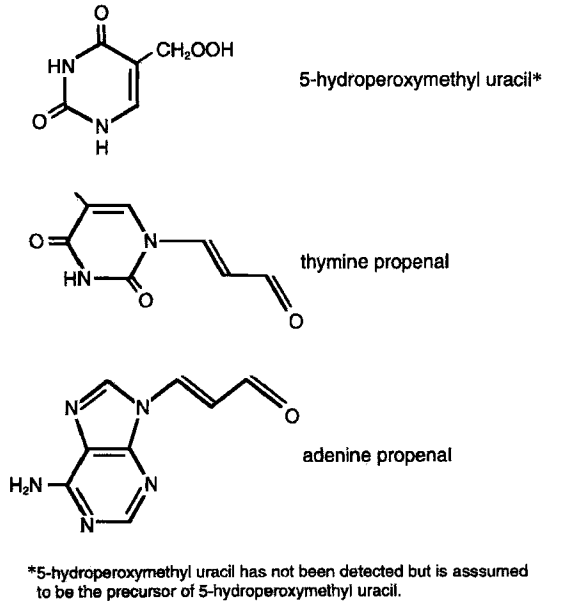

FIGURE 2 GST substrates derived from DNA peroxidation namely, 5-hydroperoxymethyl uracil the putative precursor of 5-hydroxymethyl uracil a stable product of DNA peroxidation and the base propenals, thymine propenal and adenine propenal.

semiquinone radicals that undergo rapid redox cycling, producing quantities of superoxide anion and the resultant toxic reactive oxygen species. Degeneration of the dopaminergic neurons in the substantia nigra as a consequence of the redox cycling of the dopamine derivative aminochrome, has been proposed to contribute to the etiology of Parkinson's Disease and it is suggested that a similar process in the mesolimbic system, may contribute to the development of schizophrenia. These orthoquinone derivatives are also substrates for GSTs which therefore compete with the redox cycling pathway preventing the formation of products of reactive oxygen. This reaction is shown in Figure 3.[25]

The Glutathione S-Transferases

From present evidence, GSTs occur in the vast majority of living forms that are aerobic.[26,27] They have similar overall structures, regardless of their phylogenetic origin. In man and many other organisms, they occur as polygene families, which co-operate to protect against a broad range of electrophilic toxins. These may be the products of either the metabolism (usually mixed function oxygenase dependent) of many of the xenobiotics

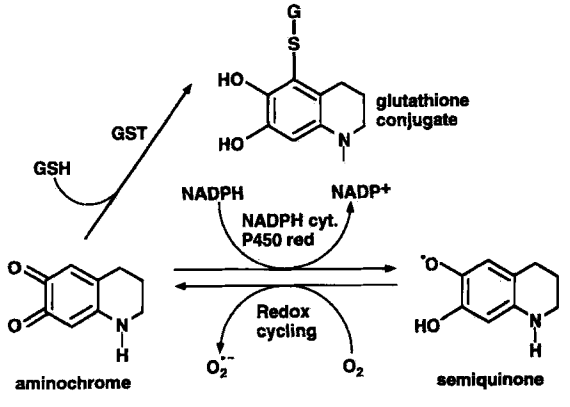

FIGURE 3 Competition by GST with the NADPH cytochrome P450 redox cycling pathway of aminochrome and other catecholamine derived ortho-quinones. The redox cycling pathway produces oxygen radicals in abundance, but GSTs compete with this pathway, produces unreactive GSH conjugates and are therefore potent anti-oxidants.

encountered in nature, or be produced endogenously as described above. Detoxication may be either by conjugation when the electrophilic centre is carbon or reduced when it is oxygen or nitrogen. In the former case stable conjugates result that are eliminated from the cell by a plasma membrane-bound GSH conjugate transporter[28,29] and are subsequently metabolized, mostly in the kidney, to mercapturic acids and excreted in the urine.[30]

The native enzymes are dimers. Each monomer has an active centre enclosed by two domains: one the G domain, is principally concerned with the binding and activation of GSH, while the other, the H domain, is concerned with the binding and orientation of the electrophilic substrate. Figure 4 shows ribbon diagrams of representatives of the alpha, mu, pi, theta and sigma families. In each of these the G-domain is on the right and the H-domain on the left of the active site which is shown occupied by GSH conjugates, or in one case GSH sulphonate, with their GSH moieties bound deep in the interior of the active site.[27]

At present six gene families are known in man: alpha, mu, pi, theta, kappa and zeta. A human sigma has yet to be reported, although one has been isolated from rat spleen.[31] The nomenclature of the known human gene loci according to the accepted nomenclature[32] are as follows. The alpha class comprises *hGST A1*, *A2*, *A3*, *A4* and *A5*; the mu class *hGST M1*A*, *M1*B*, *M1*O*, *M2*, *M3*A*, *M3*B*,[33] *M4* and *M5*; the theta class *hGSTT1*, *T1*O* and *T2*. Only one locus is known so far in each of the pi, kappa and zeta classes, namely, *hGST P1*A*, *P1*B*, *P1*C*,[34–36] *hGSTK1*[37]

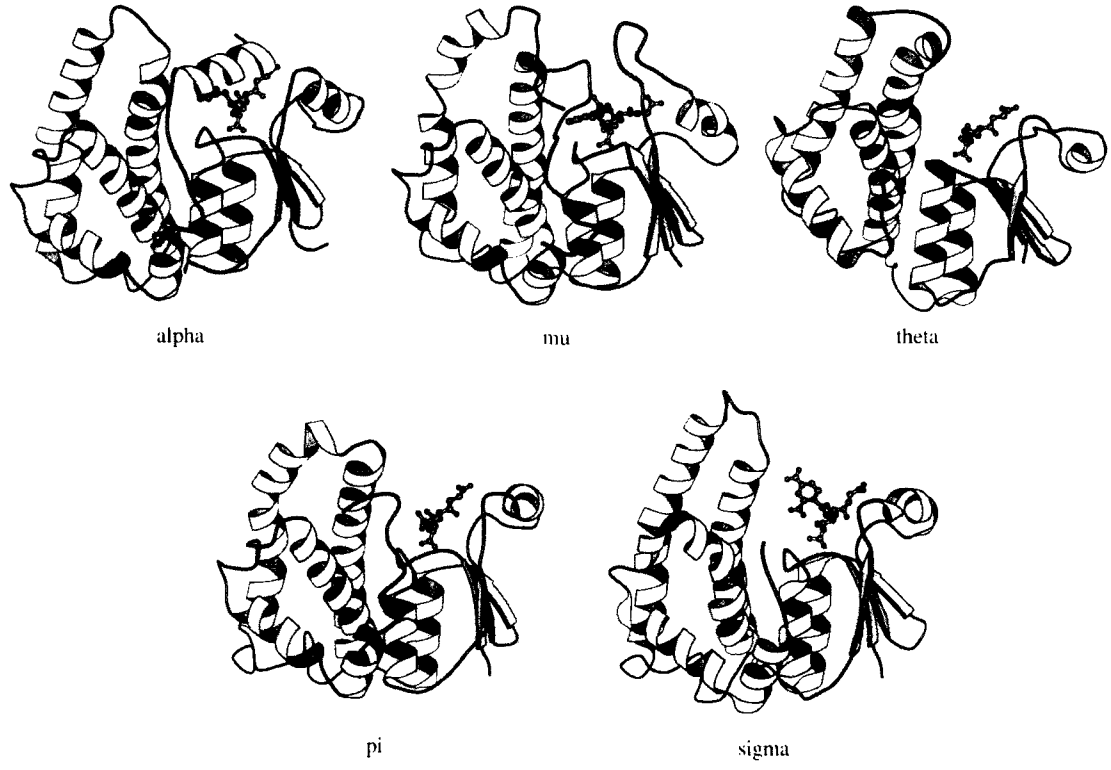

FIGURE 4 Ribbon diagrams of the three-dimensional structures of human(h) GSTA1, rat(r) GSTM1, hGSTP1, blowfly GSTT1 and squid GSTS1. The GSH domain is on the right hand side and the H domain on the left. The active sites of hGSTA1, rGSTM1, hGSTP1 and blowfly GSTT1 are occupied by S-benzylglutathione, (9S, 10S)-9-(S-glutathionyl)-10-hydroxy-9,10-dihydrophenanthrene, S-hexylglutathione, glutathione sulfonate and 1-(S-glutathionyl)-2,4-dinitrobenzene, respectively.[27]

and *hGSTZ1*.[6] It is seen GSTs *M1*, *M3*, *P1* and *T1* have allelic forms. The active dimeric forms of the enzymes are either homodimers, or heterodimers composed of closely related monomers from the same gene family. A similar nomenclature has been devised for mouse and rat GSTs.[38]

Knowledge of the spectrum of hGST isoenzyme expression in tissues is very important, but so far incomplete. More extensive information is often available for the more recently characterized isoenzymes, where information from quantitative immunochemistry is available. So far the general consensus is that the human liver expresses *hGSTs A1*, *A2*, *A4*, *M1*, *T1*, *T2*, *K1*, *Z1*; the lung *GSTs*, *A4*, *M1*, *M3*, *P1*, *T1* and *T2*; the erythrocytes *GSTs P1* and *T1*; the lymphocytes *GSTP1*and *M1*, but not *T1*; the colon and the kidney *GSTs A1*, *M1*, *P1* and *T1*; the skin *GSTs M1*, *M3*, and *P1*; the brain *GSTs M1*, *M3*, *P1*, *A4*, *T1* and *Z1* and the testis *GSTs M1*, *M3*, *P1*, *A1*, *A4*, and *T1*; the prostate *GSTs P1* and *T1*; the spleen, *GSTP1*; the small intestine, *GSTT1*; the pancreas *GSTT1*; and the muscle *GSTs M1*, *M2*, *P1*, *A4*, *T1* and *Z1*.[6,38–40] It is important to note that the recently characterised hGSTA4-A4, shown in Table I to have very high activity with HNE (and in addition activity with peroxidized phospholipid) is found in human liver, lung, brain, testis, muscle, small intestine, pancreas, bladder and in addition, retina, cornea, iris and ciliary body of the eye. It is very widely expressed.[41,42] The tissue distribution of GSTT2 in man is largely unknown, but studies in the rat record a broad distribution, which in order of tissue content is: liver, testis, adrenal, kidney, lung, brain, skeletal muscle, heart, small intestine and spleen.[43] Tissue GST distribution requires much more investigation and involve all tissues possible. Account should be taken of inter-individual variation by sampling numbers of donors and expressing results statistically and it will be seen below that racial variation should also be considered.

Tables I and II illustrate the broad overlapping specificities of the human GSTs towards some endogenous and exogenous substrates. Some of the exogenous substrates occur in Nature or are pollutants arising from modern industrial life, while others are laboratory chemicals.

In Tables I and II, the reactions that are catalyzed are classified as follows: CDNB, MS and CH_2Cl_2 are examples of nucleophilic displacement, BPDE, AFBO and ethylene oxide of attack of GSH on strained oxirane rings, EA, acrolein, crotonaldahyde, nonenal, HNE, hydroxydecenal, hydroxypentanal base propenals and aminochrome of Michael addition, Δ^5 AD, of GSH-dependent isomerism and CuOOH, linoleoyl hydroperoxide and dilinoleoyl phosphatidyl hydroperoxides of GSH dependent reduction. It is seen that the range in activities spans four orders of magnitude according to the substrate and that most substrates are utilized at different rates by more than one enzyme. For example, the best enzyme for CDNB is hGSTM2-2; for BPDE and AFBO, hGSTM1-1; for MS, hGST2-2; for CH_2Cl_2, hGSTT1-1; for EA, either hGSTA4-4 or hGSTP1-1; for Δ^5 AD, hGSTA1-1 and for CuOOH, hGSTA2-2 and hGSTT2-2. The best enzyme for many endogenous substrates derived from lipid peroxidation is hGSTA4-4; but there are exceptions: for example fatty acyl hydroperoxides are best detoxified by hGSTT2-2 and the limited information available so far indicates that GSTT1-1 is best for ethylene oxide. Present results from DNA peroxidation show hGSTP1-1 to be the most effective enzyme with base propenals. With respect to the redox cycling catecholamine derivatives, hGSTM2 is by far the best enzyme. However since neither substrates nor enzymes have been exhaustively tested, these data are incomplete.[32]

Genetic Polymorphism in the Glutathione Transferases

As seen above GSTs M1, M3, P1, and T1 exhibit genetic polymorphism. The substrate spectrum of hGSTM3 has been little studied, for example it

TABLE II Activities of human GST isoenzymes towards some exogenous substrates

Isoenzyme	CDNB	BPDE	*exo*-AFBO	*endo*-AFBO	MS	CH_2Cl_2	EA	Δ^5 AD	CuOOH
				(in $\mu mol\, mg^{-1}\, min^{-1}$)					
hGSTA1-1	82.0	0.038[49]	0.009[50]	< 0.01[50]			0.1	4.0	3.1
hGSTA2-2	80.0		0.01[50]	< 0.01[50]			0.1		10.4
hGSTA4-4	12.5						2.8		0.6
hGSTM1-1	190.0	0.57[49]	0.6[50]	1.4[50]			0.1	0.12	0.3
hGSTM2-2	276.0						0.2		0.1
hGSTM3-3	15.2						0.2		0.05
hGSTM4-4	1.4						0.1	nil	
hGSTP1-1	103.0	0.83[49]	0.06[50]	< 0.06[50]			1.22		0.03
hGSTT1-1	nil				nil	0.4[39]	nil		2.8[39]
hGSTT2-2	nil				0.3[45]		0.3[45]		6.9[45]
hGSTZ1-1	nil						0.05[6]		0.16[6]

CDNB, 1-chloro-2,4-dinitrobenzene; BPDE, benzo(*a*)pyrene-7,8-diol-9,10-oxide; AFBO, aflatoxin B_1-8,9-oxide; MS, 1-menaphthyl sulfate; CH_2Cl_2, methylene chloride; EA, ethacrynic acid; Δ^5 AD, Δ^5 androstene-3,17-dione; CuOOH, cumene hydroperoxide. Spaces indicate that the substrate has not been tested with the isoenzyme in question. All data are adapted from[38] except those otherwise marked.

is not known whether or not it utilizes products of reactive oxygen as substrates. *GSTM1* has three alleles, namely *M1*A*, *M1*B* and *M1*0*. Whereas *GSTM1*A* and *M1*B* differ by only one base in exon 7, without apparent effect on the activity of the enzyme phenotype, *GSTM1*0* represents a gene deletion and when homozygous, produces a null phenotype. *GSTP1* has four polymorphisms *hGSTP1*A*, *hGSTP1*B*, *hGSTP1*C* and *hGSTP1*D* as a result of variants at codons 104 and 113. At codon 104 *HGSTP1*A* codes for Ile and *hGSTP1*B* or Val. In the case of *HGSTP1*C* and *hGSTP1*D* both codons are involved.[36] These two codons are at the substrate binding site and are expected to affect activity. In the theta family *GSTT1*, like *GSTM1*, has a null allele that also results from a large deletion and gives a null phenotype when homozygous. Data relating these polymorphisms to susceptibility to disease are accumulating; but the causal connection between the substrates utilized by a particular gene product and the diseases associated with the polymorphism of this gene is not always clear. Perhaps one exception is the association of GSTM1 null with susceptibility to diseases such as smoking-dependent lung or bladder cancer. Benzo(*a*) pyrene is a major component of tobacco smoke and its carcinogenic metabolite, BPDE, is a good substrate for GSTM1 (see Table II).[51,52]

With reference to endogenous substrates, Table II shows that hGSTM1 is not a particularly powerful enzyme for any of the substrates resulting from radical attack on either lipid or DNA, and the effect of its polymorphism is probably small in this respect. It may however be important in diseases resulting from adrenochrome recycling. Adrenochrome, unlike other catecholamine derivatives is a better substrate for GSTM1 than GSTM2. The polymorphism of hGSTP1-1 on the other hand, might be important with respect to detoxication of the base propenals, particularly since GSTP1 is so widely distributed, but at present we know nothing about the relative activities of the three allelic forms for these substrates. In the case of GSTT1 null individuals, ethylene oxide would not be effectively detoxified and it has been proposed that this might explain the higher base-line sister chromatid exchange associated with the null phenotype.[53] The most remarkable enzyme with respect to lipid derived substrates is GSTA4-4 which may occur as several isoenzymes. The view at the moment is that these are not allelic forms.

The Frequency of GST Polymorphisms

The frequencies of polymorphisms vary with the GST and the racial type. Differences in

distribution of hGSTM1 null and hGSTT1 null phenotypes are often very significant. Thus in decreasing order the incidence of GSTM1 null is: Malays 64%; Chinese 63%; US Caucasians 50%; Indians 32% and African Americans 20%. In the case of GSTT1 null the incidence is: Chinese 64%; Koreans 60%; Malays 38%; U.S. Caucasians 24%; African Americans 22%; Indians 16% and Mexican Americans 9%.[51,54,55] The frequency of the polymorphic forms of hGSTP1 is not known.[36]

Induction and Chemoprotection

GSTs are inducible and their induction reduces susceptibility to reactive oxygen derived endogenous substrates and is therefore chemoprotective. Most of our information concerning induction and its potential for chemoprotection depends on work with experimental animals. In the rat the inducer 1,2-dithiole-3-thione and its derivative oltipraz induces hepatic alpha and mu enzymes, including rGSTA4-4, the orthologue of hGST4-4, and is also able strikingly to increase the expression of rGSTP1-1 and rGSTA5-5 from very low basal levels. Such induction has been recorded in several other tissues and is assumed to be a general effect.[56] In the case of aflatoxin B_1 (AFB)-induced hepatocarcinogenesis in the rat, oltipraz has been shown to be a very effective chemoprotector,[57] by virtue of its induction of rGSTA5-5, the most effective enzyme by far, for the detoxication of the carcinogenic metabolite AFB-*exo*-8,9-oxide.[50] In studies on both rats and mice, the plant product curcumin has also been shown to induce hepatic GSTs.[58,59] Relevant to substrates produced by oxidative stress is the observation that curcumin protects against HNE-induced cataract in the rat lens. In these experiments rGST4-4 in the lens is powerful in the detoxication of HNE and may also intervene at earlier stages of oxidative damage by detoxifying fatty acyl hydroperoxide-containing lipids.[59] This work showing that curcumin is anti-cataractogenic is comparable to the ground breaking experiments of Kensler *et al.*[57]

rGSTA4-4 is also selectively induced by iron-induced lipid peroxidation. It is suggested that the agent responsible for the induction is HNE, one of its principal substrates.[60]

Metabolism of HNE

There are three pathways of metabolism of HNE as follows: (1) as already, described, GST-dependent GSH conjugation; (2) oxidation of the aldehyde group by aldehyde dehydrogenase to give 4-hydroxy-2 nonenoic acid (HNA) and (3) reduction of the aldehyde by alcohol dehydrogenase to give 1,4-dihydroxy-2-nonene (DHN).[61–63] In studies on isolated rat hepatocytes, only one of these potential reactions appears to occur to each substrate molecule. That is, the products are either HNE GSH, HNA or DHN, HNE GSH being the major product and increasingly so with time, due to HNE induction of rGSTA4-4.[62] However HNE could be both GSH conjugated and, in addition, have its aldehyde group either oxidized or reduced and it is interesting that this does not happen in these isolated hepatocytes. However when whole animals were given HNE and the urinary mercapturic acids analysed, four were identified namely, hydroxynonenal mercapturic acid (HNE MA), HNA MA, HNA lactone MA, and DHN MA. It is shown that HNE MA (and presumably HNE–GSH) are not substrates for the dehydrogenases and cannot give rise to either HNA MA or DHN MA.[64] It would appear that *in vivo* in the rat, oxidation and reduction of the aldehyde group of HNE are essential initial reactions and that the products HNA and DNH are both substrates for GST. If this is true it is now necessary to determine the enzyme utilisation and kinetics for these new substrates, both the GSH conjugates and the mercapturic acid derivatives. It is clear that in the rat *in vivo* GSH conjugation is essential for excretion of HNE. These differences in behaviour between hepatocyte cultures and the whole animal are important and require evaluation.

CONCLUSION

This article, as explained in the Introduction, has arisen out of the work of Herman Esterbauer on lipid peroxidation and the identification of HNE, a major product of this process and a powerful electrophile. Its topic is principally the detoxication by GSTs of a range of endogenous substrates resulting, not only from the oxygen radical attack on polyunsaturated fatty acyl derivatives and DNA but, in addition, from redox cycling catecholamine derivatives. It is now established that the GST polygene family has indeed the potential to provide a large umbrella of protection against endogenous substrates resulting from oxygen toxicity, whereas in the past this was largely notional. There are enzymes that catalyse these detoxications with very high activities. For example the catalytic activity of hGSTA4-4 for alkenals and hydroxy alkenals may be as high as 205 and 189 μmol/min/mg respectively; of hGST P1-1 for base propenals, as high as 77 μg/min/mg; of hGSTM2 for aminochrome, 148 μg/min/mg and of hGSTT2 for 9-hydroperoxylinoleic acid, 10 μg/min/mg. Compared with carcinogenic electrophiles such as BPDE which hGSTs P1-1 and M1-1 detoxify at rates of 0.8 and 0.6 μg/mg/min respectively the above rates of detoxication of endogenous substrates are high indeed (Tables I and II). An important advance in our knowledge in this area would be the aquisition of data enabling quantitation of the tissue distribution of these enzymes, since this would enable the contribution of each tissue to the detoxication of a selected substrate to be computed.

In view of the presence of several enzymes with the capacity to detoxify HNE, it is interesting to learn that its basal level in plasma is about 0.3 μM[65] implying that low levels of reactive oxygen species are a normal feature of life. To confirm the power of the enzymes that detoxify HNE, it has been demonstrated that administration of up to 500-fold excess of HNE is necessary to affect the steady-state concentration in plasma.[66] The maintenance of these low levels of HNE seem to be important to provide a background against which small increments are able to carry out what appear to be physiological functions of HNE such as neutrophil chemotaxis[67] associated with activation of phosphoinositide-specific phospholipase C,[68] which has been studied in rats and humans: and also the stimulation of HeLa cell growth.[69] This area is naturally of considerable interest and could be pursued much further, but is not the subject of this review.

Perhaps one could conclude by mentioning the possible manipulation of hGSTA4-4 to advance clinical medicine. It has been pointed out that hGSTA4-4 has substantial activity towards many of the toxic products of lipid peroxidation (Table I). This enzyme is present in a large range of normal tissues, but probably not in tumour cells (such as the hamster ovary cells below). If it could be induced, it might provide normal tissue with useful protection against anti-cancer cytotoxic drugs, producing reactive oxygen species. This proposition has not yet been tested, but Chinese hamster ovary cells, apparently lacking GSTA4-4, have been transfected with mGST4-4 and challenged with adriamycin. Compared with controls the mGST4-4-transfected cells have a two-fold higher resistance to adriamycin, associated with 40% less membrane peroxidation,[70] which is not generally appreciated. This indicates that adriamycin toxicity is partially mediated by lipid peroxidation which is not generally appreciated. The induction of hGST4-4 in cancer patients about to undergo radiotherapy, where oxygen radicals are the principal toxins, might also be efficacious. This could be tested without delay since there is already a very powerful chemoprotective agent, namely oltipraz, that is licensed for clinical use.[56]

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