Biochemical Pharmacology, Vol. 35, No. 23, pp. 4337–4339, 1986.
Printed in Great Britain.

0006–2952/86 $3.00 + 0.00
Pergamon Journals Ltd.

SHORT COMMUNICATIONS

Potentiation by reduced glutathione of Adriamycin-stimulated lipid peroxidation in kidney microsomes

(*Received* 17 *December* 1985; *accepted* 19 *May* 1986)

Considerable evidence suggests that the toxic properties of the antitumor agent Adriamycin result from its enzymatic reductive activation to a semiquinone free radical intermediate, which rapidly autoxidizes and generates toxic oxyradicals [1–3]. Adriamycin-mediated oxyradical production markedly increases the peroxidation of membrane polyunsaturated phospholipids in microsomes [4] and mitochondria [5], and Adriamycin-induced lipid peroxidation *in vivo* may be involved in the cardiotoxicity [6] and nephrotoxicity [7] of this important drug.

We have reported previously that the addition of glutathione (GSH) diminishes Adriamycin-enhanced lipid peroxidation in liver microsomes [4] and liver mitochondria [5]. Here, in contrast, we report an anomalous potentiation by GSH of NAD(P)H-dependent lipid peroxidation in kidney microsomes, by what appears to be an enzymatic process.

Materials and methods

Adult, male CDF_1 mice (Flow Laboratories, Dublin, VA), fed Purina laboratory rodent chow and water *ad lib*, were killed by cervical fracture, and the livers and kidneys were removed, rinsed in 150 mM KCl–50 mM Tris–HCl buffer and gently homogenized (10%, w/v) on ice in KCl–Tris. Kidney and liver microsomes were isolated from tissue homogenates as previously described [8]. The microsomes were incubated at 1 mg protein/ml in oxygenated KCl–Tris buffer, under a 100% oxygen atmosphere in the dark for 60 min, and Adriamycin, NADPH and NADH were added to the incubation mixtures just prior to initiating the reactions by adding GSH (neutralized solution). Microsomal lipid peroxidation was quantitated by adding 2-thiobarbituric acid to aliquots of trichloroacetic acid (TCA)-acidified samples and measuring the absorption at 533 nm as described in detail [4, 9]. Results are expressed as nmoles of malonaldehyde equivalents/mg microsomal protein/60 min.

Microsomal protein [10], GSH [11] and γ-glutamyltranspeptidase activity [12] were measured as described. All biochemicals were from the Sigma Chemical Co. (St. Louis, MO), and other laboratory chemicals were of high purity. Adriamycin (NSC-123127) was provided by the Drug Development Branch, NCI, NIH (Bethesda, MD). The water used for reagents was first deionized and then distilled in glass.

The data were analyzed statistically by Student's *t*-test [13]. Differences between mean values at $P < 0.05$ were considered significant.

Results and discussion

When GSH was added to liver microsomes, it inhibited both endogenous and Adriamycin-stimulated NADPH-dependent lipid peroxidation (>75%) in a concentration-dependent manner (1–10 mM). Under identical conditions, we found that GSH greatly enhanced kidney microsomal lipid peroxidation (Fig. 1). Endogenous kidney peroxidation was increased nearly 10-fold, and Adriamycin-stimulated peroxidation was more than doubled by 10 mM GSH. The stimulating effect of GSH on kidney microsomal lipid peroxidation was also observed with NADH as the source of reducing equivalents (Table 1). GSH in combination with either NADPH or NADH resulted in a powerful synergistic effect on kidney microsomal lipid peroxidation which was prevented by boiling the microsomes. Prior heat-inactivation of kidney microsomal enzymes essentially abolished peroxidation supported by GSH, GSH plus NADPH or GSH plus NADH, suggesting that the process was enzyme-mediated (Table 1). In the absence of NAD(P)H, peroxidation supported by GSH (5 mM) alone was inhibited >80% by Adriamycin (results not shown). The mechanism of this effect is unclear.

It has been well documented that GSH inhibits NADPH-dependent peroxidation of liver microsomal lipids [4, 14, 15]. Liver microsomes apparently contain a membrane-bound, GSH-utilizing enzyme, which is not glutathione peroxidase, but which is capable of controlling lipid peroxidation [16]. Although the mechanism of this enzymatic, GSH-dependent protection against lipid peroxidation has not been fully elucidated, it has been suggested that scavenging of oxygen and lipid radicals might be involved [17]. The contrasting, unexpected stimulatory effect of GSH on kidney microsomal lipid peroxidation could likewise be a consequence of an enzyme-mediated reaction, this one catalyzing the oxidation of GSH to reactive intermediates. The oxidation of thiols yields multiple, potentially reactive, and toxic chemical species which include: thiyl free radical, peroxysulfenyl radical, disulfide radical anion and sulfinic and sulfonic acids [18]. Moreover, concomitant with GSH oxidation, several partially reduced molecular oxygen species can be generated: superoxide anion radical, the perhydroxyl radical, hydrogen peroxide and hydroxyl free radical [19, 20]. The generation and reactions of these oxyradicals in biological systems have been shown to greatly potentiate diverse cytotoxic phenomena, including membrane lipid peroxidation [21].

Ames and Elvehjem [22] reported an oxygen-dependent conversion of GSH to GSSG which was catalyzed by mouse kidney homogenates. Both renal γ-glutamyltranspeptidase [23] and a relative nonspecific, membrane-bound thiol oxidase [24, 25] have subsequently been implicated in enzyme-catalyzed GSH oxidation. Griffith and Tate [26] concluded that in kidney the mechanism of GSH oxidation by γ-glutamyltranspeptidase is mediated by cysteinylglycine, which is converted to cystinyl-bis-glycine and undergoes metal ion-catalyzed disulfide-thiol exchange reactions with GSH. On the other hand, others claim that a distinct renal sulfhydryl oxidase activity could be resolved from γ-glutamyltranspeptidase activity and that this enzyme could catalyze the oxidation of a variety of thiols [27]. The thiol oxidase, however, was found to be localized predominately in renal cortical plasma membrane, with much lower activity in the kidney microsomal fraction [25]. It should be noted that kidney microsomes may contain brush-border and basal membrane-derived plasma membrane vesicles. We found appreciable γ-glutamyltranspeptidase activity in kidney microsomes (2.8 ± 0.5 μmoles *p*-nitroaniline formed/mg protein/min), and kidney microsomes catalyzed glutathione oxidation (9.7 ± 3.1 nmoles GSH oxidized/mg protein/min; corrected for the autoxidation of GSH, 1.6 ± 1.1 nmoles/min with GSH at 5 mM). The

BP 35:23-O

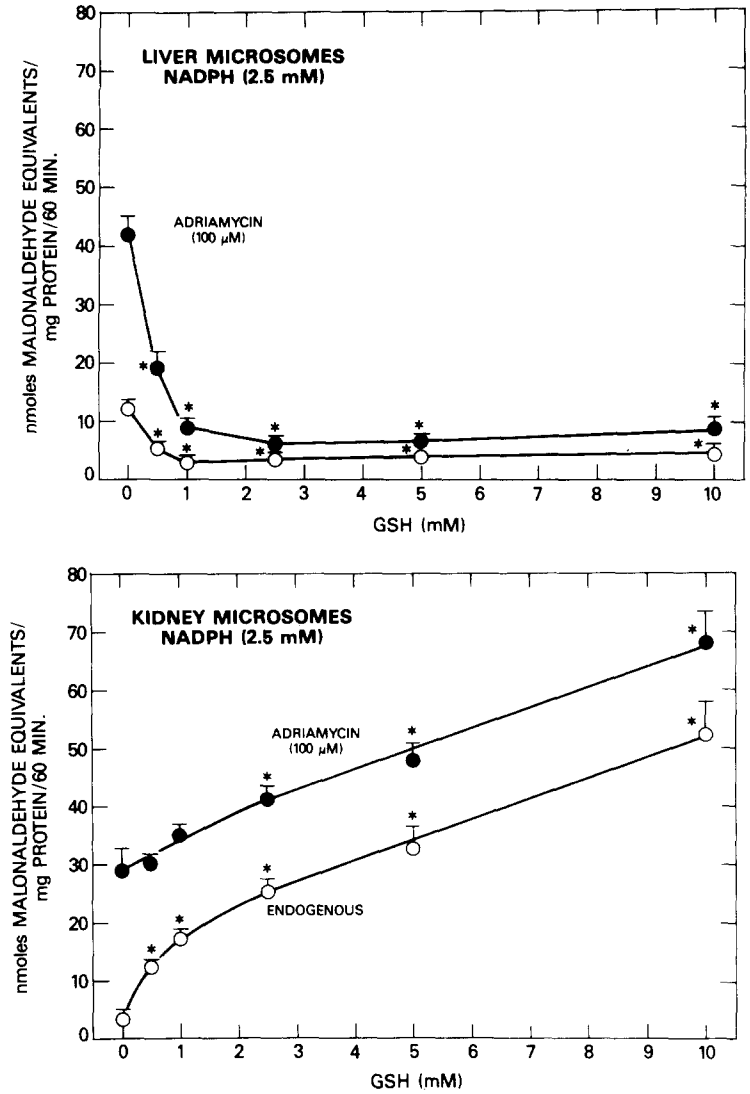

Fig. 1. Comparison of the divergent effects of GSH on liver and kidney NADPH-dependent microsomal lipid peroxidation in the absence and presence of Adriamycin (100 μM). Values statistically different from those in the absence of GSH ($P < 0.05$, $N = 3$) are shown by an asterisk.

Table 1. Effects of GSH on NADPH- and NADH-dependent lipid peroxidation in intact and heat-inactivated kidney microsomes

	Lipid peroxidation (nmoles malonaldehyde/mg protein/60 min)	
Reducing agent (concn)	Control microsomes	Heat-inactivated microsomes*
NADPH (2.5 mM)	2.9 ± 0.6	1.2 ± 0.2†
NADH (2.5 mM)	3.5 ± 0.3	0.5 ± 0.4†
GSH (5 mM)	3.3 ± 0.6	0.6 ± 0.3†
GSH (5 mM) plus NADPH (2.5 mM)	27 ± 1	1.9 ± 0.4†
GSH (5 mM) plus NADH (2.5 mM)	21 ± 2	1.0 ± 0.6†

* Microsomes were immersed in boiling water for 10 min. Heat-inactivation also prevented Adriamycin-stimulated lipid peroxidation, but had no significant effect on ascorbate-promoted peroxidation.

† Significantly different from values (mean ± SD) measured in control microsomes ($P < 0.05$, $N = 3$).

rate of oxidation of GSH in the presence of microsomes was enhanced slightly by NADPH, but GSH had no effect on the rate of kidney microsome-catalyzed NADPH oxidation (data not presented). Prior to this report, neither γ-glutamyltranspeptidase nor thiol oxidase has been implicated as participating in the process of thiol-promoted peroxidation, although it has been reported that certain thiols had a proxidant effect on lipid peroxidation in a reconstituted unsaturated lipid model membrane system containing relatively high concentrations of exogenous metal ions [28, 29]. Additionally, it has been suggested that the stimulation of microsomal peroxidation by cysteine results from nonenzymatic reactions between iron and the cysteine sulfhydryl group [30].

In conclusion, GSH greatly enhanced both endogenous and Adriamycin-stimulated NAD(P)H-dependent kidney microsomal lipid peroxidation, although, in contrast, liver microsomal peroxidation was inhibited potently by GSH. The enhancement of kidney lipid peroxidation by GSH was concentration dependent, significant within physiological concentrations, synergistic with either NADPH or NADH, and appeared to require enzymatic activity in that peroxidation was prevented by prior heat-inactivation of the kidney microsomes. The effect of GSH may involve GSH oxidation products resulting from renal γ-glutamyltranspeptidase or thiol oxidase activity or the interaction of GSH with NADPH–cytochrome P-450 reductase-generated oxyradicals. These results suggest that GSH-enhanced peroxidation may have toxicological significance in kidney if a similar process occurs *in vivo*; thus, it may play a significant role in the nephrotoxicity of Adriamycin and other nephrotoxicants.

Laboratory of Experimental Therapeutics and Metabolism
National Cancer Institute, NIH
Bethesda, MD 20892, U.S.A.

EDWARD G. MIMNAUGH*

REFERENCES

1. K. Handa and S. Sato, *Gann* **68**, 603 (1977).
2. B. Kalyanaraman, E. Perez-Reyes and R. P. Mason, *Biochim. biophys. Acta* **630**, 119 (1980).
3. J. H. Doroshow, *Cancer Res.* **43**, 460 (1983).
4. E. G. Mimnaugh, M. A. Trush and T. E. Gram, *Biochem. Pharmac.* **30**, 2797 (1981).
5. E. G. Mimnaugh, M. A. Trush, M. Bhatnagar and T. E. Gram, *Biochem. Pharmac.* **34**, 847 (1985).
6. C. E. Myers, W. P. McGuire, R. H. Liss, I. Ifrim, K. Grotzinger and R. O. Young, *Science* **197**, 165 (1977).
7. L. F. Fajardo, J. R. Eltringham, J. R. Stewart and M. R. Klauber, *Lab. Invest.* **43**, 242 (1980).
8. T. E. Gram, C. L. Litterst and E. G. Mimnaugh, *Drug Metab. Dispos.* **2**, 254 (1974).
9. F. Bernheim, M. L. Bernheim and K. M. Wilbur, *J. biol. Chem.* **174**, 257 (1948).
10. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
11. G. L. Ellman, *Archs Biochem. Biophys.* **82**, 70 (1959).
12. G. Szasz, *Clin. Chem.* **15**, 124 (1969).
13. G. W. Snedecor, *Statistical Methods.* The Iowa State University Press, Ames, IA (1956).
14. K. E. Hill and R. F. Burk, *Biochem. Pharmac.* **33**, 1065 (1984).
15. G. R. M. M. Haenen and A. Bast, *Fedn Eur. Biochem. Soc. Lett.* **159**, 24 (1983).
16. R. F. Burk, *Biochim. biophys. Acta* **757**, 21 (1983).
17. R. F. Burk, *Biochem. Pharmac.* **31**, 601 (1982).
18. L. S. Harman, C. Mottley and R. P. Mason, *J. biol. Chem.* **259**, 5606 (1984).
19. H. P. Misra, *J. biol. Chem.* **249**, 2151 (1974).
20. G. Saez, P. J. Thornalley, H. A. O. Hill, R. Hems and J. V. Bannister, *Biochim. biophys. Acta* **719**, 24 (1982).
21. I. Fridovich, *Science* **201**, 875 (1978).
22. S. R. Ames and C. A. Elvehjem, *J. biol. Chem.* **159**, 549 (1945).
23. A. Meister and M. E. Anderson, *A. Rev. Biochem.* **52**, 711 (1983).
24. L. H. Lash, D. P. Jones and S. Orrenius, *Biochim. biophys. Acta* **779**, 191 (1984).
25. K. Ormstad, P. Moldeus and S. Orrenius, *Biochem. biophys. Res. Commun.* **89**, 497 (1979).
26. O. W. Griffith and S. S. Tate, *J. biol. Chem.* **255**, 5011 (1980).
27. S. Ashkar, F. Binkley and D. P. Jones, *Fedn Eur. Biochem. Soc. Lett.* **124**, 166 (1981).
28. M. Tien, J. R. Bucher and S. D. Aust, *Biochem. biophys. Res. Commun.* **107**, 279 (1982).
29. J. R. Bucher, M. Tien, L. A. Morehouse and S. D. Aust, *Fund. appl. Toxic.* **3**, 222 (1983).
30. A. J. F. Searle and R. L. Willson, *Biochem. J.* **212**, 549 (1983).

* Address all correspondence to: Mr. Edward G. Mimnaugh, Biochemical Pharmacology Section, Clinical Pharmacology Branch, National Cancer Institute, NIH, Bldg. 10, Rm. 6N119, Bethesda, MD 20892.

Biochemical Pharmacology, Vol. 35, No. 23, pp. 4339–4340, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00

Allopurinol as an inhibitor of the *in vivo* formation of acyclovir from desciclovir

(*Received* 12 *May* 1986; *accepted* 12 *June* 1986)

Desciclovir (2-[(2-amino-9*H*-purin-9-yl)methoxy]ethanol), currently in clinical trials, is a prodrug of the antitherpetic agent acyclovir (9-[(2-hydroxyethoxy)methyl]guanine, ZOVIRAX) [1, 2]. The advantage of this prodrug over acyclovir is its greater bioavailability by oral administration. After absorption, desciclovir is oxidized to acyclovir, presumably by xanthine oxidase (EC 1.2.3.2). The basis for this proposed route of enzymatic activation is the ability of xanthine oxidase purified from bovine milk [1] and human liver [3] to catalyze the conversion of desciclovir to acyclovir. However, the possibility exists that, *in vivo*, other enzymes might be involved. Consequently, this study was undertaken to elucidate the effects of the xanthine oxidase inhibitor allopurinol (4-hydroxypyrazolo[3,4-*d*]pyrimidine, ZYLOPRIM) [4] on the renal elimination of desciclovir and acyclovir in the rat.

Materials and methods

Animals. Male Long–Evans rats were obtained from Blue Spruce Farms, Inc. Animals were housed in nalgene metabolism cages that separated urine from feces; all animals had free access to food and water. Each cage contained two rats. Urine from each group of two rats was collected after 8 hr and 24 hr.

Dosing. A single dose of desciclovir (20 mg/kg) was administered by stomach tube to all rats. After a 48-hr