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# EFFECTS OF CYCLOPHOSPHAMIDE AND ADRIAMYCIN ON RAT HEPATIC MICROSOMAL GLUCURONIDATION AND LIPID PEROXIDATION

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**Abstract**—The effect of cytotoxic drug administration, as a single dose i.p. to rats (six rats/treatment group), on hepatic microsomal UDP-glucuronosyltransferase (UGT) activity was investigated. Glucuronidation of morphine in microsomes from control rats apparently involved at least two enzymes. Administration of cyclophosphamide (CP; 200 mg/kg 7 days prior to killing) significantly increased the rate of morphine glucuronidation over the range 0.05–10 mM, and significantly increased the apparent $V_{max}$ for the high capacity isoenzyme from 1.25 ± 0.12 to 1.95 ± 0.39 nmol/mg/min. In contrast, the activity of 1-naphthol UGT was not significantly altered by administration of CP. Rats treated with the same dose of CP 1 day prior to killing showed a significant decrease in microsomal morphine-UGT activity at 0.05 and 2.5 mM morphine, but a significant increase in activity was observed following administration of CP or Adriamycin® (AD; 10 mg/kg) 4 days prior to killing. The extent of microsomal lipid peroxidation was significantly increased in microsomes obtained from rats treated with CP or AD 4 days prior to killing, and was positively correlated ($P < 0.001$) with the rate of glucuronidation of 0.05 and 2.5 mM morphine. Preincubation of microsomes in the presence of CP (5 mM) and AD (100 μM) significantly decreased the rate of glucuronidation of 2.5 mM morphine. *In vitro* NADPH-mediated lipid peroxidation significantly increased the activity of both the high and low affinity morphine-UGT isoenzymes. Administration of the cytotoxic drugs CP and AD may alter microsomal morphine-UGT activity via the process of lipid peroxidation, although other mechanisms cannot be excluded.

Peroxidation of unsaturated membrane lipids by free radicals is one process which contributes to the toxic side effects observed following administration of, or exposure to, a wide range of chemicals, including cytotoxic drugs [1–5]. Lipid peroxidation, by altering membrane structure and function, alters the activity of a number of microsomal enzymes, including the cytochromes P450 [6–13], nucleoside diphosphatase [14], glucose-6-phosphatase [6, 12, 14] and UDP-glucuronosyltransferase (UGT†) isoenzymes [6, 10, 14, 15]. The cytotoxic drugs CP [16] and AD [17–20] when administered *in vivo* or added *in vitro* increase the extent of rat hepatic microsomal lipid peroxidation. Lipid peroxidation induced by administration of these agents has been associated with a depression in microsomal mixed-function oxidation [17–20], a decrease in glucose-6-phosphatase activity [17] and no significant change in *p*-nitrophenol glucuronidation [17].

The membrane-bound UGT family of isoenzymes exhibits a degree of latency which may be altered by perturbation of the membrane phospholipid bilayer [21, 22]. The isoenzymes have been segregated into two main "clusters" according to substrate specificity [23]: group 1 (UGT1) isoenzymes glucuronidate mostly planar molecules including *p*-nitrophenol and 1-naphthol and are induced by 3-methyl cholanthrene; group 2 (UGT2) isoenzymes glucuronidate, in general, bulkier aglycones including morphine and are induced by phenobarbital. A more recent UGT nomenclature system has been proposed based on divergent evolution of the genes [24]; however, morphine-UGT has not as yet been cloned or sequenced.

Morphine is a narcotic analgesic which in man is metabolized predominantly via glucuronidation to form the metabolites M3G and morphine-6-glucuronide; rats do not produce morphine-6-glucuronide [25, 26]. Formation of M3G in man [26, 27] and in rats [25, 26, 28] involves at least two isoenzymes of morphine-UGT: a low capacity, high affinity form and a high capacity, low affinity form. Morphine is widely used for the treatment of severe chronic pain experienced by cancer patients and is commonly coadministered with a variety of drugs including cytotoxic agents, anxiolytics and antiemetics. Inhibition of morphine metabolism has been shown by benzodiazepines [29, 30], codeine [31] and chloramphenicol [27]. Little work has been conducted on the interaction of other classes of drugs with morphine, but in view of the increasing use of high and often escalating doses of morphine, there is a growing interest in drugs which potentially interfere with morphine metabolism. Given the toxicity of anticancer drugs such as CP and AD on cell membrane integrity [3–5, 16–20] and drug-metabolizing enzyme activity [17–19, 32–36], and that cytotoxic drugs are commonly coadministered with morphine, there is a possibility of an interaction between these two classes of drugs.

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† Abbreviations: UGT, UDP-glucuronosyltransferase; CP, cyclophosphamide; AD, Adriamycin®; M3G, morphine-3-glucuronide; UDPGA, UDP-glucuronic acid; MA, malonaldehyde.

In this study we have investigated the effect of administration of CP and AD to rats on the rate of hepatic microsomal morphine glucuronidation. The apparent Michaelis–Menten kinetic parameters for morphine glucuronidation to M3G for a two-enzyme system have been determined in control rats and rats treated with CP 7 days prior to killing. Consequently, a low and a high substrate concentration was used in an attempt to probe cytotoxic effects on both isoenzyme forms of morphine-UGT. Glucuronidation of 1-naphthol following CP administration has also been quantified in order to compare the effects of cytotoxic administration on the glucuronidation of a UGT1 substrate with that of a UGT2 substrate. The possible relationship between the cytotoxic drug-induced increase in extent of microsomal membrane lipid peroxidation and rate of morphine glucuronidation was investigated. In addition, control microsomes were pre-incubated with an NADPH-generating system in the presence and absence of CP or AD to determine the direct effects of the cytotoxic drugs and *in vitro* lipid peroxidation, respectively, on morphine-UGT activity.

## MATERIALS AND METHODS

*Chemicals and reagents.* Glucose-6-phosphate dehydrogenase, ADP, thiobarbituric acid, 1-naphthol β-D-glucuronide (sodium salt), UDPGA (sodium salt) and morphine-3-β-glucuronide were purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.). NADP and glucose-6-phosphate were purchased from Calbiochem (San Diego, CA, U.S.A.). Morphine HCl and 1-naphthol were from Macfarlan Smith Ltd (Edinburgh, U.K.) and BDH Chemicals (Poole, U.K.), respectively. CP was purchased from Farmitalia Carlo Erba (Hawthorn, Australia) who kindly provided AD as a gift. HPLC solvents were from Waters Associates (Lane Cove, Australia). All other reagents and solvents were of analytical reagent grade.

*Animal treatment and microsome preparation.* Adult male Hooded Wistar rats (180–200 g) were purchased from Gilles Plains Animal Resource Centre, Adelaide, Australia and acclimatized under standard conditions.

Two *in vivo* studies were conducted. In the first, which compared the effect of cytotoxic administration on kinetic parameters of morphine microsomal glucuronidation, and on rates of morphine and 1-naphthol glucuronidation, rats (N = 6) were injected i.p. with CP 200 mg/kg 7 days prior to killing; control animals (N = 6) were not treated. In the second study, rats (six/treatment group) were injected i.p. with CP (200 mg/kg) 1, 4 or 7 days prior to killing, or AD (10 mg/kg) 4 days prior to killing. Control animals were injected i.p. with the same volume of saline 7 days prior to killing. Animals were killed by cervical dislocation and exsanguination, and the livers were removed and immediately placed in ice-cold phosphate buffer (0.1 M, pH 7.4) with KCl (1.15%), prior to microsome preparation. All further steps were carried out at 4°. Microsomes were prepared by a method of differential centrifugation as described previously [28] and stored at −70° until used. Microsomes were thawed only once at the time of use.

*Pre-incubation of rat hepatic microsomes in the presence of a NADPH-generating system.* Pooled (N = 6) rat hepatic microsomes (5 mg/mL) from untreated animals were incubated at 37° for 45 min in the presence of 0.1 M Tris–HCl pH 7.4 and an NADPH-generating system (1 mM NADP, 4 mM glucose-6-phosphate, 1 IU/mL glucose-6-phosphate dehydrogenase and 8 mM $MgCl_2$), in a total volume of 1 mL. Reactions were started by the addition of NADP; control samples did not contain NADP. Reactions were stopped on ice.

Other incubations were performed as described (including NADP), except that either 5 mM CP or 100 μM AD were included in the incubation mixture. Control samples contained no CP or AD.

*Measurement and quantification of M3G production by rat liver microsomes.* Microsomal protein obtained from rats in the *in vivo* studies (1 mg/mL) or from pre-incubation studies (0.5 mg/mL) was incubated in the native state in the presence of morphine (using 16 concentrations over the range 0.05–10 mM morphine when determining kinetic parameters in microsomes obtained from rats in the first *in vivo* study, or using 0.05 and 2.5 mM morphine), 5 mM $MgCl_2$, 0.1 M Tris–HCl pH 7.4 and 5 mM UDPGA, in a total volume of 0.4 mL. Incubations were started by the addition of UDPGA and were performed at 37° for 30 min. Reactions were stopped by the addition of 1 mL acetonitrile containing 42 μg/mL phenytoin as internal standard and placing the tubes on ice. Samples were processed and the amount of M3G produced was quantified by a specific HPLC assay as described previously [28] or, in the case of the NADPH-generating system, the mobile phase was modified to contain 20% acetonitrile to improve chromatographic resolution.

*Fluorescence assay for the analysis of 1-naphthol glucuronide.* The fluorescence assay was based upon continuous monitoring of the formation of 1-naphthol glucuronide in a fluorescence cuvette, as originally described by Mackenzie and Hänninen [37]. The reaction mixture consisted of microsomal protein (0.1 mg/mL), 0.05 mM 1-naphthol [dissolved in 0.24% (v/v) dimethyl sulfoxide; final assay concentration being 0.048%], 4 mM $MgCl_2$ and 50 mM phosphate buffer pH 7.4 in a total volume of 2.5 mL. The mixture was pre-incubated at 30° and 2 mM UDPGA was added after the reaction baseline had stabilized (2 min). The formation of 1-naphthol glucuronide was monitored in a Perkin–Elmer MPF-3L Fluorescence Spectrophotometer at an excitation wavelength of 293 nm and an emission wavelength of 335 nm using a slit width of 5 nm. The output of the fluorescence spectrophotometer was connected to a chart recorder. Glucuronidation rate was assessed by measuring the slope of the linear portion of the reaction and reactions were shown to be linear up to a protein concentration of 0.2 mg/mL. Standards were prepared in the range 0–15 μM 1-naphthol glucuronide (using the same concentration of dimethyl sulfoxide as in the assay) such that the combined concentration of the glucuronide and

aglycone was 15 μM. Standards were measured at the assay temperature, 30°.

*Assessment of microsomal lipid peroxidation.* The extent of lipid peroxidation was assessed by measuring the amount of MA generated by an iron-dependent NADPH-generating system, based on methods described by Berrigan *et al.* [16] and Mimnaugh *et al.* [20]. Incubations to assess the *ex vivo* microsomal lipid peroxidation of control rats or those treated with cytotoxic drugs contained hepatic microsomal protein (1 mg/mL), 20 mM nicotinamide, 2 mM ADP, 0.12 mM $FeCl_3$, 150 mM KCl 50 mM Tris–HCl (pH 7.4) and an NADPH-generating system (1.9 mM NADP, 20 mM glucose-6-phosphate, 1.1 IU/mL glucose-6-phosphate dehydrogenase and 8.6 mM $MgCl_2$) in a total volume of 1 mL. Samples were incubated at 37° for 60 min. Reactions were stopped by the addition of ice-cold trichloroacetic acid [20% (w/v); 0.5 mL]. Zero time blanks were included as described by Mimnaugh *et al.* [20]. Thiobarbituric acid [0.67% (w/v), dissolved in 0.5 M KOH; 1 mL] was added to the incubations which were heated at 80° [38] for 30 min. Samples were cooled on ice, centrifuged at 12,000 *g* for 7 min, and the MA–thiobarbituric acid adduct measured using a Shimadzu UV-120-02 Spectrophotometer set at 530 nm. Results are expressed as nmol of MA produced/mg protein/60 min using a molar extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$ [39]. For microsomes assayed for lipid peroxide formation during pre-incubation in the presence of an NADPH-generating system, the assay procedure was essentially as outlined above, except that the protein concentration was 0.5 mg/mL and MA–thiobarbituric acid adduct formation was measured without any additional incubation period. Results are expressed as nmol of MA produced/mg protein/45 min.

*Other assays.* Protein was measured by the method of Lowry *et al.* [40] using bovine serum albumin as standard.

*Analysis of results.* All assays were performed in duplicate, unless otherwise stated. Group results are expressed as means ± SD. Michaelis–Menten parameters for a two-enzyme system were derived graphically from Eadie–Hofstee plots for individual rats and these values were used as initial estimates for a non-linear least-squares regression iterative computer program (Multifit 2.0®, Day Computing, Cambridge, U.K.). Data were weighted according to the reciprocal of the observations squared. Statistical comparisons between three or more groups were by ANOVA, otherwise data were analysed by unpaired Student's *t*-test. Spearman–Rank correlation analysis was performed on the rate of M3G formation versus the extent of microsomal lipid peroxidation. A P-value less than 0.05 (two tailed) was considered to be significant.

## RESULTS

### In vivo *effects of cytotoxic drug pre-treatment on rat hepatic microsomal UGT activity and kinetics, and lipid peroxidation*

The effect of CP treatment 7 days prior to killing on the rates of rat hepatic microsomal glucuronidation of a range of morphine concentrations was determined in the first study. The rate of morphine glucuronidation by CP-treated animals was significantly increased ($P < 0.05$) at all substrate concentrations, relative to the control group. Non-linear Eadie–Hofstee plots of these data (results not shown) illustrate the involvement of two or more isoenzymes in the glucuronidation of morphine. Mean apparent Michaelis–Menten parameters for morphine glucuronidation by the high and low affinity isoenzymes, determined by non-linear least-squares regression analysis, are shown for the control and CP-treated groups in Table 1. The mean maximal velocity for glucuronidation by the low affinity isoenzyme ($V_{max2}$) was significantly increased ($P < 0.01$) in microsomes obtained from CP-treated animals compared with those from control animals (1.95 ± 0.39 vs 1.25 ± 0.12 nmol/mg/min).

Table 1. Michaelis–Menten parameters for morphine glucuronidation in hepatic microsomes obtained from control rats and those treated with CP 7 days prior to killing

| Group | $K_{m1}$ (mM) | $K_{m2}$ (mM) | $V_{max1}$ (nmol/mg/min) | $V_{max2}$ (nmol/mg/min) |
|---|---|---|---|---|
| Control | | | | |
| Mean | 0.15 | 2.80 | 0.33 | 1.25 |
| SD | 0.11 | 1.94 | 0.32 | 0.12 |
| CP 7 day | | | | |
| Mean | 0.14 | 2.37 | 0.41 | 1.95* |
| SD | 0.13 | 1.94 | 0.29 | 0.39 |

The parameters were determined for individual rats by non-linear least-squares regression analysis (*$P < 0.01$ vs the corresponding control group).

Morphine-UGT activities at 0.05 and 2.5 mM morphine (largely representative of high affinity and low affinity isoenzymes, respectively), and 1-naphthol-UGT activity in rat hepatic microsomes from the first study are shown in Fig. 1a. Morphine glucuronidation at each substrate concentration was increased significantly ($P < 0.05$) by CP treatment 7 days prior to killing. In contrast, the activity of 1-naphthol-UGT was not significantly different between the CP 7-day and control groups ($P > 0.05$).

In the second study, the effect of administration of CP to rats 1, 4 or 7 days, or AD 4 days prior to killing on morphine-UGT activity was determined at morphine concentrations of 0.05 and 2.5 mM (Fig. 1b). At both substrate concentrations, morphine glucuronidation was significantly decreased ($P < 0.05$) following CP treatment 1 day prior to killing, but was significantly increased ($P < 0.05$) when CP had been administered 4 or 7 days earlier, and following AD administration. The extent of lipid peroxidation was measured in hepatic microsomes from the control rats ($N = 5$), and from rats treated with CP 1 ($N = 6$) or 4 ($N = 6$) days prior to killing, or AD ($N = 4$) (Fig. 1b). Insufficient quantities of microsomes from the CP 7-day group precluded a determination of their lipid peroxidation. Relative to the control group, lipid peroxidation was significantly increased ($P < 0.05$) for CP 4-day and

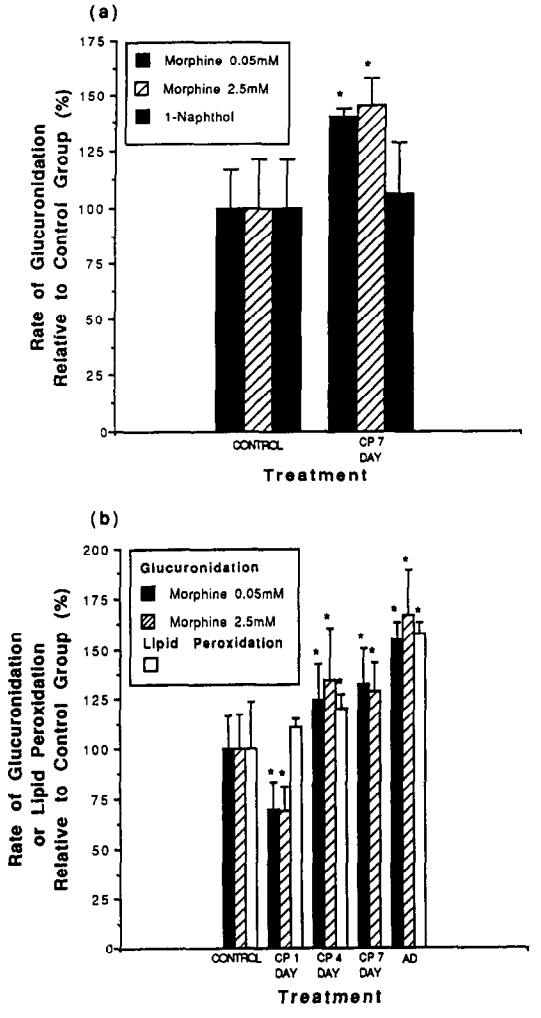


Fig. 1. The effect of cytotoxic drug administration on rat hepatic (a) glucuronidation of morphine and 1-naphthol in microsomes from the first study, and (b) glucuronidation of morphine and the extent of microsomal lipid peroxidation as measured by the amount of MA produced/mg of microsomal protein/hr in microsomes from the second study. (*$P < 0.05$ vs the corresponding control group). Control values were (a) $0.17 \pm 0.03$, $1.15 \pm 0.24$ and $0.62 \pm 0.13$ nmol/mg/min for the rate of glucuronidation of 0.05 and 2.5 mM morphine, and 1-naphthol, respectively; and (b) $0.20 \pm 0.03$ and $1.68 \pm 0.29$ nmol/mg/min, and $24.97 \pm 5.93$ nmol MA/mg/hr for the rate of glucuronidation of 0.05 and 2.5 mM morphine, and the extent of lipid peroxidation, respectively.

AD groups. Correlations between microsomal morphine-UGT activity at 0.05 and 2.5 mM morphine, and the extent of lipid peroxidation, were performed utilizing data from control, CP 1- and 4-day, and AD groups (N = 21). Spearman–Rank correlations were found to be significant ($P < 0.001$) at morphine concentrations of 0.05 mM ($r_s = 0.74$) and 2.5 mM ($r_s = 0.76$). The correlations remained significant ($P < 0.01$, N = 17) when the data from the AD-treated animals were omitted.

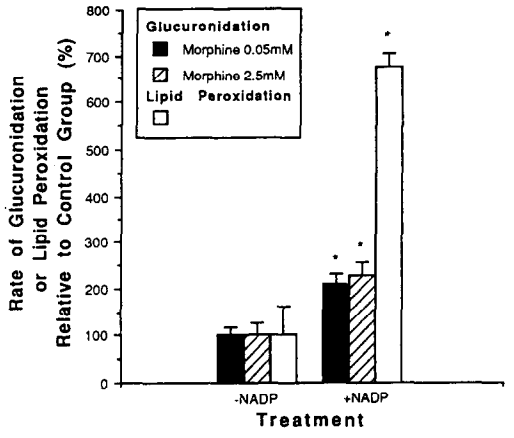


Fig. 2. The rate of morphine glucuronidation of 0.05 and 2.5 mM morphine, and the extent of lipid peroxidation as measured by the amount of MA produced/mg of microsomal protein/45 min, in rat hepatic microsomes following pre-incubation in the presence (+NADP) or absence (−NADP; control) of NADP (N = 16) (*$P < 0.0001$). Control values were $0.07 \pm 0.01$ and $0.76 \pm 0.20$ nmol/mg/min, and $5.74 \pm 3.44$ nmol MA/mg/45 min for the rate of glucuronidation of 0.05 and 2.5 mM morphine, and the extent of lipid peroxidation, respectively.

In vitro *effects of cytotoxic drug pre-treatment on rat hepatic microsomal morphine-UGT activity*

Morphine-UGT activities at 0.05 mM morphine following pre-incubation in the absence of cytotoxic drug (N = 12), in the presence of CP (N = 8) and in the presence of AD (N = 4) were $0.15 \pm 0.02$, $0.09 \pm 0.01$ and $0.14 \pm 0.01$ nmol/mg/min, respectively, and at 2.5 mM morphine were $1.78 \pm 0.34$, $1.06 \pm 0.07$ and $1.27 \pm 0.18$ nmol/mg/min, respectively. Pre-incubation of microsomes in the presence of CP significantly decreased ($P < 0.05$) the rate of morphine glucuronidation at 0.05 and 2.5 mM morphine, while AD significantly decreased the rate of glucuronidation of 2.5 mM morphine ($P < 0.05$).

*Effects of* in vitro *NADPH-dependent lipid peroxidation on rat hepatic microsomal morphine-UGT activity*

The effects of pre-incubation of rat hepatic microsomes, in the presence (N = 16) or absence (N = 16) of an NADPH-generating system, on the extent of lipid peroxidation and on the rate of morphine glucuronidation are shown in Fig. 2. The presence of NADP in an NADPH-generating system significantly increased the extent of microsomal lipid peroxidation and morphine-UGT activity at both substrate concentrations ($P < 0.0001$).

## DISCUSSION

Rats were dosed with CP and AD at 200 mg/kg (1300 mg/m$^2$) and 10 mg/kg (64 mg/m$^2$), respectively, following considerations of interspecies scaling based on body surface area [41]. In man, CP may be administered at a maximal dose of about 60 mg/

kg/day or 2300 mg/m$^2$/day, while AD may be administered at about 60 mg/m$^2$/day. Thus, the clinical doses of CP and AD used in man compare with the doses of the cytotoxic drugs administered to rats in this work.

Glucuronidation of morphine to M3G in hepatic microsomes obtained from control rats followed biphasic kinetics in agreement with previous reports [25, 26, 28]. We observed a mean apparent $K_m$ for the high affinity morphine-UGT form ($K_{m1}$) of 0.15 ± 0.11 mM, although this value should be viewed with a degree of caution as some individual predicted values were in the vicinity of the lowest concentration of morphine used in the study. The mean $K_{m2}$ value that we observed was 2.80 ± 1.94 mM. Miners *et al.* [27] reported a two-enzyme system for the glucuronidation of morphine in human hepatic microsomes described by a $K_{m1}$ and $K_{m2}$ of 2.5 ± 0.6 and 798 ± 123 μM, respectively. Hepatic microsomal glucuronidation of morphine in various animal species has been described by Michaelis–Menten kinetics for a single-enzyme system. In rats, $K_m$ values of 0.2 [31, 42] and 0.3 [43, 44] mM have been reported. In rabbits, a $K_m$ of 0.5 mM has been observed [45], while in monkeys the $K_m$ varied from 0.78 to 3.70 mM [46]. Our evidence supporting the existence of at least two morphine-UGT isoenzyme forms in the rat led us to use concentrations of 0.05 and 2.5 mM morphine in subsequent studies in order to probe the high and low affinity forms, respectively. It should be noted, however, that the high and low affinity isoenzymes will still contribute to activity measured at 2.5 and 0.05 mM, respectively.

The influence of i.p. administration of a single dose of CP 200 mg/kg on rat hepatic microsomal morphine glucuronidation was characterized by a temporal effect. Microsomal transferase activity was significantly decreased following administration of CP 1 day prior to killing, whereas a significant increase was observed at 4 and 7 days. When the cytotoxic drug was administered 7 days prior to killing, the activity of morphine-UGT was significantly increased over the range of substrate concentrations (0.05–10 mM) investigated, as was the mean apparent $V_{max}$ for the low affinity isoenzyme form. In contrast, the rate of glucuronidation of the UGT1 substrate, 1-naphthol, was not altered. This finding is in agreement with that of Gurtoo *et al.* [32] who, using the UGT1 substrate *p*-nitrophenol, observed that the rate of rat hepatic microsomal glucuronidation was not significantly altered by administration of a single dose of CP 180 mg/kg i.p. 7 days prior to killing. LeBlanc and Waxman [36] have recently reported a selective alteration in the activity of hepatic P450 enzymes over a 9-day period following administration of CP 130 mg/kg i.p. It was postulated [36] that the differential responses to CP was mediated through drug-induced perturbations of hormonal control of the enzymes. It is possible that isoenzymes of the UGT family may also be affected in a differential manner following exposure to CP.

AD 10 mg/kg was administered i.p. to rats 4 days prior to killing as this dosage regimen has been shown to produce a significant decrease in cytochrome P450 content [35]. We observed hepatic microsomal morphine glucuronidation to be significantly increased, whereas Mimnaugh *et al.* [20], using the UGT1 substrate *p*-nitrophenol, observed no change in glucuronidation rate in microsomes obtained from rats treated i.p. with AD either 15 mg/kg 1 day prior to killing or 5 mg/kg 7, 4 and 1 day prior to killing.

Consideration of possible mechanisms of the CP and AD modulation of UGT activity requires knowledge of CP and AD pharmacokinetics in rats. The apparent plasma elimination half-lives of CP and its metabolites following i.v. bolus administration of CP to rats have been reported to be between 29 and 58 min [47–50], although the half-life for acrolein, a toxic metabolite, has not been documented. Since the elimination of a compound is not complete until the passage of about five half-lives, CP and its metabolites might be expected to be present in the rat for up to approximately 5 hr following a single dose of CP. The elimination of CP is complicated, however, by binding of its metabolites to sulphydryl groups of tissue macromolecules and cellular proteins. Acrolein generated *in vitro* binds to and denatures the cytochromes P450 [33, 34], and may undergo further biotransformation to another protein-binding metabolite [34]. Studies of the plasma elimination half-life of AD have been rendered difficult due to its rapid and extensive distribution into tissues. AD exhibits significant protein and DNA binding [3, 5] so that the elimination half-life of the cytotoxic drug becomes dependent upon that of the macromolecule to which it is bound. There is an incomplete recovery of the radioactivity of labeled AD 96 hr after administration to rats [51].

The disruption of cell membrane integrity by the process of lipid peroxidation is thought to be one important event contributing to the toxicity of a variety of compounds [1, 2]. The extent of rat hepatic microsomal membrane lipid peroxidation has been shown to affect *p*-nitrophenol UGT activity. A low level of peroxidation increased the rate of *p*-nitrophenol glucuronidation, but the activity declined as the extent of lipid peroxidation was augmented [6, 15]. Others [10, 14] have observed an increase in *p*-nitrophenol glucuronidation induced by lipid peroxidation. We observed a significant increase in microsomal lipid peroxidation by the administration of CP 4 days prior to killing. Gurtoo *et al.* [32] reported that CP (100–600 mg/kg i.p.) administered to rats 2 hr prior to killing increased lipid peroxidation in a dose-dependent manner. AD treatment significantly increased hepatic microsomal lipid peroxidation, in agreement with others [17–20]. Wills [12] found that the effect of microsomal lipid peroxidation on some membrane-bound enzymes could be imitated by treating microsomes with detergents. It is common practice in many laboratories to increase the activity of microsomal UGT enzymes by including a membrane-perturbing agent, such as a detergent, thus favorably altering the conformation of the enzymes and/or allowing greater accessibility of the substrate or co-substrate, UDPGA. It has been observed, however, that addition of detergents to microsomal incubations can either increase, decrease, or have no effect on

the rate of glucuronidation, depending on the concentration of the detergent [28] or the substrate [52]. Cytotoxic drug-induced lipid peroxidation may contribute to alterations in microsomal morphine-UGT activity through a perturbation of the phospholipid membrane environment. We found a correlation between the rate of glucuronidation of morphine (0.05 and 2.5 mM) and the extent of microsomal lipid peroxidation induced by prior administration of CP or AD. That microsomal lipid peroxidation may be an important modulator of morphine-UGT activity was demonstrated by inducing lipid peroxidation *in vitro* with an NADPH-generating system [6, 11–13, 17, 20, 39] and observing a significant increase in morphine-UGT activity.

It is apparent that cytotoxic-induced lipid peroxidation may not be the only mechanism influencing UGT activity as suggested by a decrease in morphine-UGT activity following pre-incubation of microsomes in the presence of these cytotoxic drugs. The effects with CP observed *in vitro* are compatible with decreases in morphine-UGT activity observed 1 day after administration *in vivo*. CP and AD are known to exert toxicity via a number of effects, including binding to DNA [3, 34], altering transcription/translation [36], interacting with components of biomembranes [3–5], increasing membrane fluidity [4, 5], binding to proteins [33, 34, 36] and depleting antioxidant defense mechanisms [16, 18, 20, 33, 34]. The process of lipid peroxidation has been shown to selectively degrade content [11, 13] and activity [11] of individual forms of cytochrome P450. Changes in hormonal regulation of the UGTs [53] should also be considered. Any of these or more factors may have a role to play in the cytotoxic drug-induced alteration in enzyme activity.

In conclusion, glucuronidation of morphine to M3G involved biphasic kinetics in microsomes obtained from both control rats and rats treated with CP 7 days prior to killing. At this time, CP administration increased the apparent $V_{max}$ of the low affinity isoenzyme form and the rate of glucuronidation at all morphine concentrations investigated. Although the activity of morphine-UGT was increased by CP administration, the rate of 1-naphthol glucuronidation was not significantly different to that of the control group, indicating that CP has a differential effect on the UGTs. When CP was administered 1 day or 4 days prior to killing, morphine glucuronidation was decreased or increased, respectively, indicating a temporal effect. The administration of AD 4 days prior to killing caused a marked increase in morphine-UGT activity. Although evidence from the *in vivo* and *in vitro* studies suggests that cytotoxic-induced microsomal lipid peroxidation contributes to the alterations in morphine-UGT activity, possibly via a detergent-like effect, other mechanisms may also be involved, including direct effects of the cytotoxic drugs on the enzyme.

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