Biochemical Pharmacology, Vol. 39, No. 12, pp. 1869–1875, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00

CYTOTOXIC AND CYTOPROTECTIVE ACTIVITIES OF CURCUMIN

EFFECTS ON PARACETAMOL-INDUCED CYTOTOXICITY, LIPID PEROXIDATION AND GLUTATHIONE DEPLETION IN RAT HEPATOCYTES

IMONO ARGO DONATUS,* SARDJOKO and NICO P. E. VERMEULEN†
Department of Pharmacochemistry, Division of Molecular Toxicology, Free University, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands

(*Received* 17 *July* 1989; *accepted* 23 *November* 1989)

Abstract—The cytoprotective effect of curcumin, a natural constituent of *Curcuma longa*, on the cytotoxicity of paracetamol in rat hepatocytes was studied. Paracetamol was selected as a model-toxin, since it is known to be bioactivated by 3-methylcholanthrene inducible cytochromes P450 presumably to *N*-acetyl-*p*-benzoquinone imine (NAPQI), a reactive metabolite which upon overdosage causes protein- and non-protein thiol-depletion, lipid peroxidation and cytotoxicity measured as LDH-leakage. At low concentrations curcumin was found to protect significantly against paracetamol-induced lipid peroxidation, without protection against paracetamol-induced LDH-leakage and without protection against paracetamol-induced GSH-depletion. At a 100 times higher concentration of curcumin the observed protective effect on lipid peroxidation was accompanied with a tendency to increase cellular GSH-depletion and LDH-leakage. No time-dependency was found as to the curcumin-induced effects: treatment of the hepatocytes 1 hr before, concomitantly or 1 hr after the addition of paracetamol to the cells had similar effects. In contrast to what was expected on the basis of previous *in vivo* experiments, at higher concentrations curcumin itself was found to be slightly cytotoxic. Curcumin-induced LDH-leakage was accompanied by a significant depletion of GSH. It has been concluded that the observed cytoprotective and cytotoxic activities of curcumin may be explained by a strong anti-oxidant capacity of curcumin and the capability of curcumin to conjugate with GSH. Furthermore, it has been concluded that lipid peroxidation is not playing a causal role in cell-death induced by paracetamol or by curcumin.

Paracetamol (acetaminophen) is a widely used analgesic and antipyretic drug, which is known to cause severe hepatic necrosis at very high doses both in man and experimental animals [1–3]. As to the molecular mechanism of cell injury induced by high doses of paracetamol, so far several hypotheses have been put forward. Firstly, covalent binding of a reactive metabolite of paracetamol, presumably *N*-acetyl-*p*-benzoquinone imine (NAPQI) formed by cytochrome P450, to thiol groups of glutathione (GSH) and cellular proteins was thought to initiate the paracetamol-induced hepatic necrosis [4, 5]. More recently, however, the paracetamol-induced hepatic necrosis was also due to a cellular oxidative stress, resulting both in lipid peroxidation [6–8], protein and non-protein thiol oxidation [9–11] and in changes in the intracellular calcium homeostasis [9, 10, 12]. Moreover, recent evidence indicated that alkylation of membrane protein(s) by the reactive metabolite may also be a factor of importance in the onset of hepatic necrosis after paracetamol [12, 13]. Taking the various bio-activation and bio-inactivation mechanisms into consideration, much research has been directed to the prevention of paracetamol-induced hepatotoxicity. Thus the inhibition of cytochrome P450 with cobaltous chloride,

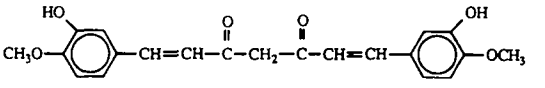

Fig. 1. Chemical structure of curcumin.

the stimulation of GSH-resynthesis by administration of *N*-acetyl-*l*-cysteine or methionine [1, 15, 16], the protection against oxidative stress by α-tocopherol or ascorbic acid [17, 18], as well as co-administration of acetylsalicylic acid [19] have been shown to protect against hepatotoxicity under experimental conditions in animals. Alternatively, however, prevention of paracetamol-induced hepatotoxicity has been accomplished by modification of the chemical structure of paracetamol. Thus, for example, *N*-methylation [20], 2,6-dimethylation [21] and 3,5-dialkylation [22–24] have been shown to be effective in the protection against the hepatotoxic potential, with or without negative effects on the analgesic activity of paracetamol [20, 25]. However, none of these approaches do further improve the risk-benefit ratio, as far as the anti-inflammatory activity of paracetamol is concerned.

Curcumin (diferuloyl methane, Fig. 1), which is an important constituent of *Curcuma longa* has been used widely and for a long time in the treatment of sprain and inflammation in indigenous medicine [26].

* Visiting fellow from the Laboratory of Pharmacology and Toxicology, Faculty of Pharmacy, Gajad Mada University, Yogyakarta, Indonesia.
† To whom correspondence should be addressed.

The anti-inflammatory activity of curcumin has been reported to be as potent as that of phenylbutazone in an acute model inflammation test, and half as potent as that of phenylbutazone and in a chronic inflammation test [27, 28]. In addition to that, relatively high concentrations of curcumin (10^{-4}–10^{-3} M) have been shown to inhibit lipid peroxidation induced by carragenin in liver homogenates of oedemic mice [29] and in rat brain homogenates [30]. These activities most likely can be due to strong antioxidant properties of curcumin [30]. Interestingly, curcumin has also been found to possess an anti-hepatotoxic activity [31]. Thus hepatotoxicity in rats induced by an oral overdose of paracetamol was reported to be prevented efficiently by oral pretreatment of the animals with relative small doses (2 mg/kg) of curcumin [32]. However, the mechanism of this hepatoprotective activity has not been elucidated yet.

The primary aim of the present study was to investigate possible mechanisms underlying the reported anti-hepatotoxic action of curcumin *in vitro*. For this purpose, paracetamol was used as a model-toxin, and the effects of curcumin on the paracetamol-induced GSH-depletion, lipid peroxidation, and LDH-leakage were studied in freshly isolated hepatocytes from 3-methylcholanthrene pretreated rats. So far, no studies on curcumin in animals have shown significant toxicological effects of the drug, even at very high doses [33–36]. Recently, however, using bacterial indicator-systems it has been shown that curcumin might possess some phototoxic activity [37]. Therefore, and because curcumin itself has structural elements, which, in principle, might lead to GSH-conjugation, the cytotoxic potential of curcumin was also investigated in rat hepatocytes.

MATERIALS AND METHODS

Materials. Paracetamol was obtained from Brocacef (Delft, The Netherlands). Curcumin (97% purity) was purchased from Merck (Darmstadt, F.R.G.). Because of light sensitivity curcumin was kept in the dark, Collagenase and pyruvate were obtained from Boeringer (Mannheim, F.R.G.). 3-Methylcholanthrene, bovine serum albumin and reduced GSH were purchased from the Sigma Chemical Co. (St Louis, MO).

Animals and isolation of hepatocytes. Male albino Wistar Rats, weighing 200–225 g (Harland-T.N.O., Zeist, The Netherlands) were used. Since hepatocytes from 3-methylcholanthrene-induced rats are more susceptible to paracetamol-induced toxicity [22], the rats were pretreated with 3-methylcholanthrene (40 mg/kg, dissolved in arachides oil), once injected intraperitoneally 48 hr before use. Rats were fasted overnight before isolation of the hepatocytes took place. Hepatocytes were isolated by collagenase perfusion according to the method of Seglen [38], with some modifications [22]. Cells isolated in this way usually contained more than 90% viable cells as judged from trypan blue exclusion measurements.

Incubation of hepatocytes. Freshly isolated hepatocytes ($1.5–2 \times 10^6$ cells/mL) were incubated in 6 mL volumes of Krebs–Henseleit buffer containing

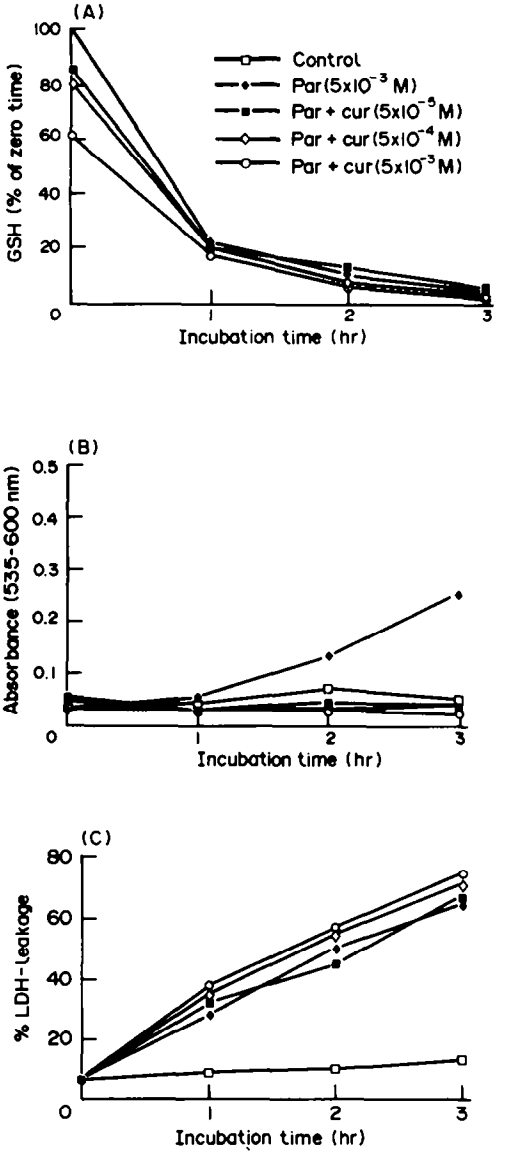

Fig. 2. Effects of curcumin pretreatment (1 hr before paracetamol incubation) on paracetamol-induced (A) GSH-depletion, (B) lipid peroxidation, and (C) LDH-leakage in isolated hepatocytes from 3-methylcholanthrene-pretreated rats. Results of one typical experiment out of three.

2% bovine serum albumin, 10 mM lactic acid and 1 mM pyruvate. The cells were equilibrated at 37° with 95% oxygen and 5% carbon dioxide for 15 min prior to the addition of curcumin and paracetamol, dissolved in incubation medium. During incubation aliquots of each time 0.5 mL were taken for the assay of LDH, GSH and LPO.

Assays. Cell-destruction was determined by measurement of cytosolic lactate dehydrogenase (LDH)-leakage from cells into the medium [38] and it was expressed as a percentage of the total LDH-activity present in cells. Lipid peroxidation (LPO) was monitored by measuring thiobarbituric acid

Table 1. Effects of curcumin on paracetamol-induced GSH-depletion, lipid peroxidation and LDH-leakage in hepatocytes of 3-methylcholanthrene pretreated rats

Treatment	GSH-depletion (%)		Absorbance 535–600 nm*		% LDH-leakage†	
	t = 0 hr	t = 3 hr	t = 0 hr	t = 3 hr	t = 0 hr	t = 3 hr
Control	100	81.4	0.030	0.072	8.8	15.0
Paracetamol (5×10^{-3} M)	100	5.0	0.030	0.250	8.8	61.1
+ Curcumin (5×10^{-5} M)	100	5.0	0.030	0.034	8.8	58.7
+ Curcumin (5×10^{-4} M)	100	7.1	0.030	0.037	8.8	64.3
+ Curcumin (5×10^{-3} M)	100	4.0	0.030	0.034	8.8	68.9
Curcumin (5×10^{-5} M)	100	77.8	0.030	0.034	8.8	17.1
Curcumin (5×10^{-4} M)	100	65.0	0.030	0.026	8.8	18.7
Curcumin (5×10^{-3} M)	100	41.1	0.030	0.025	8.8	28.1

Curcumin was added to the hepatocytes concomitantly with paracetamol. The effects of curcumin itself on these cytotoxicity parameters are also given. Results are expressed as means of two separate experiments.

* Corresponding to lipid peroxidation.

† Expressed as % of total activity in cells.

Table 2. Effects of curcumin on paracetamol-induced GSH-depletion, lipid peroxidation and LDH-leakage in hepatocytes of 3-methylcholanthrene pretreated rats

Treatment	GSH-depletion (%)		Absorbance 535–600 nm*		% LDH-leakage†	
	t = 0 hr	t = 3 hr	t = 0 hr	t = 3 hr	t = 0 hr	t = 3 hr
Control	100	84.3	0.048	0.070	8.25	13.4
Paracetamol (5×10^{13} M)	100	11.1	0.048	0.275	8.25	58.1
+ Curcumin (5×10^{-5} M)	100	9.9	0.048	0.075	8.25	56.2
+ Curcumin (5×10^{-4} M)	100	7.1	0.048	0.072	8.25	56.1
+ Curcumin (5×10^{-3} M)	100	4.3	0.048	0.050	8.25	60.0

Curcumin was added to the hepatocytes 1 hr after the addition of paracetamol. Results are means of two separate experiments.

* Corresponding to lipid peroxidation.

† Expressed as % of total activity in cells.

(TBA) reactive material [40]. Hepatocyte GSH-levels were determined with fluorescence detection after reaction of the supernatant of the trichloroacetic acid (TCA)-deproteinized hepatocyte solution with the reagent o-phthaladehyde at pH 8, according to the method of Hissin and Hilf [41]. Data shown are results of a representative experiment out of three.

RESULTS

Effect of curcumin on paracetamol-induced LDH-leakage

Using LDH-leakage as a parameter, paracetamol was found to be cytotoxic in hepatocytes freshly isolated from 3-methylcholanthrene pretreated rats. As shown in Fig. 2C, a concentration of 5×10^{-3} M of paracetamol strongly induced LDH-leakage after 3 hr of incubation, namely to about 65%. Pretreatment of the hepatocytes with curcumin at concentrations of 5×10^{-5}, 5×10^{-4}, or 5×10^{-3} M, 1 hr before the addition of paracetamol did not protect the cells against the paracetamol-induced LDH-leakage (Fig. 2C).

In order to clarify a possible time-dependent effect of curcumin-pretreatment on the paracetamol-induced LDH-leakage, we also measured the cytotoxicity after treating the cells with curcumin concomitantly, and 1 hr after the addition of paracetamol. Neither curcumin-treatment concomitantly with the addition of paracetamol nor 1 hr afterwards influenced the paracetamol-induced LDH-leakage from the hepatocytes, however (Tables 1 and 2, respectively).

Effect of curcumin on paracetamol-induced lipid peroxidation

As is shown in Fig. 2B, 5×10^{-3} M of paracetamol consistently and strongly induced lipid peroxidation, measured as the formation of TBA-reactive material, in the hepatocytes. The paracetamol-induced lipid peroxidation was completely prevented by pretreatment of the hepatocytes with all of the three curcumin concentrations created by adding curcumin 1 hr before the addition of paracetamol (Fig. 2B). Concomitantly added with paracetamol, or 1 hr afterwards, curcumin similarly protected against the

lipid peroxidation induced by paracetamol (Tables 1 and 2, respectively).

Effect of curcumin on paracetamol-induced GSH-depletion

Addition of paracetamol (5×10^{-3} M) to the isolated hepatocytes within 1 hr resulted in depletion of GSH-levels to approximately 20% of the control levels and subsequently within another 2 hr of incubation to less than 5% (Fig. 2A). Pretreatment of the hepatocytes with curcumin at concentrations of 5×10^{-5}, 5×10^{-4} and 5×10^{-3} M, 1 hr before the addition of paracetamol, did not protect the hepatocytes against the paracetamol-induced GSH-depletion (Fig. 2A). In contrast, curcumin itself was found to increase the depletion of GSH-levels to some extent. Treatment of the cells with curcumin concomitantly nor 1 hr after the addition of paracetamol influenced the paracetamol-induced GSH-depletion (Tables 1 and 2, respectively).

Cytotoxic effects of curcumin

Since curcumin has two α, β-unsaturated ketone functionalities, which in principle could enable curcumin to conjugate with GSH, the cytotoxic effects of curcumin itself were also studied in hepatocytes of 3-methylcholanthrene-pretreated rats. At concentrations of 5×10^{-5} and 5×10^{-4} M curcumin did not significantly increase the LDH-leakage normally observed in control incubations of the hepatocytes. However, a concentration of 5×10^{-3} M curcumin tended to increase LDH-leakage from the cells, i.e. from 15% to about 25% (Fig. 3C). In the case of curcumin alone, when added at concentrations of 5×10^{-5}, 5×10^{-4} or 5×10^{-3} M, no lipid peroxidation was observed. In contrast, curcumin tended to prevent the slight spontaneous lipid peroxidation normally observed in control incubations of the hepatocytes (Fig. 3B). As far as GSH-levels are concerned, curcumin was indeed found to deplete cellular GSH by about 10% at a curcumin concentration of 5×10^{-5} M (Fig. 3A). Increasing the concentration of curcumin up to 5×10^{-3} M even resulted in a depletion of GSH to about 40% of the control levels (Fig. 3A).

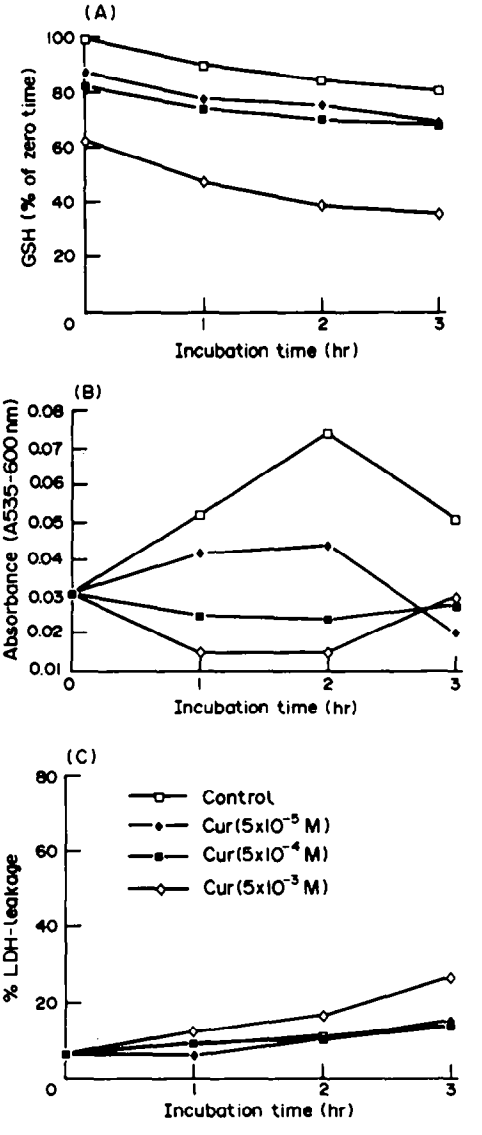

Fig. 3. Cytotoxic effects of curcumin in hepatocytes from 3-methylcholanthrene-pretreated rats. Effects of curcumin on (A) GSH-levels, (B) lipid peroxidation and (C) LDH-leakage. Results are of one typical experiment out of three.

DISCUSSION

The primary aim of the present study was to investigate effects of curcumin on the paracetamol-induced GSH-depletion and lipid peroxidation in rat hepatocytes, as possible mechanisms underlying the previously reported anti-hepatotoxic action of curcumin [32].

Curcumin is a yellow pigment, isolated from the rhizome of the perennial herb *Curcuma longa* (Turmeric). The compound or an alcoholic extract of curcuma rhizome has a reputation as colour agent in textile and pharmaceutical industry, as a food-additive and as an active principle in indigenous medicine [26, 28, 42]. Apart from strong anti-inflammatory and anti-oxidant activities [28, 30], curcumin was also shown to possess anti-hepatotoxic activities *in vivo* at relatively low doses [32], apparently without significant toxicological effects, even at rather high doses [33, 36].

Since the hepatotoxic action of paracetamol in animal and man, as well as the underlying molecular mechanisms *in vivo* [1, 4, 23] and *in vitro* [11, 22, 43, 44] are rather well documented, the cytotoxicity induced by this drug in freshly isolated hepatocytes of 3-methylcholanthrene pretreated rats was used as a model-system to study the hepatoprotective action of curcumin. The mechanism by which paracetamol causes hepatic necrosis upon over-dosages involves metabolic conversion of the drug into a reactive *N*-acetyl-*p*-benzo-quinone imine (NAPQI) by cytochrome P450 [4, 43], notably 3-methylcholanthrene-inducible isoenzymes of cytochrome P450 [15, 45]. This electrophilic species has been shown to be responsible for the irreversible depletion

of hepatocellular GSH stores [4, 9, 11, 24], for the covalent binding to cysteinyl thiol groups of proteins [4, 5, 46] and for the induction of an oxidative stress in hepatocytes manifesting itself in the form of lipid peroxidation [6, 9, 11, 24, 44], oxidative depletion of protein thiol-levels [9, 10, 11, 24] and disturbances of the cellular Ca^{2+}-homeostasis [9, 12]. Several studies with paracetamol, NAPQI [8, 18, 44, 48] or analogues of these compounds [11, 24, 47], have demonstrated that the processes of covalent binding to GSH and proteins, lipid peroxidation and oxidative protein thiol-depletion are more or less independent.

Unexpectedly, the present investigation demonstrated that curcumin in hepatocytes of 3-methylcholanthrene pretreated rats at a concentration of 5×10^{-5} M did not result in any significant protective effect on the cell death induced by paracetamol (Fig. 2C). This lack of protection was found to be independent from the moment of administration of curcumin to the hepatocytes, since the effect of administration of curcumin, 1 hr before, simultaneously and 1 hr after the addition of paracetamol was not different (Tables 1 and 2). Increasing curcumin concentrations up to 5×10^{-4} and 5×10^{-3} M did not protect the cells from the paracetamol-induced cytotoxicity either. In contrast the cells tended to become slightly more sensitive as judged from LDH-leakage values (Fig. 2C; Tables 1 and 2).

Hepatic reduced GSH plays an important role in the protection against chemically-induced cellular injury [49]. Depletion of GSH-levels, e.g. by covalent binding to diethylmaleate [4] or by inhibiting GSH-reductase [9, 11, 24] has been shown to enhance the susceptibility to cytotoxicity, whereas the reverse is true for agents which promote GSH-synthesis, such as *N*-acetyl-*l*-cysteine and methionine [15, 16]. In the present study curcumin was found not to protect the hepatocytes from a strong depletion of GSH induced by paracetamol, neither in a concentration (Fig. 2A), nor in a time-dependent manner (Tables 1 and 2). The observed lack of protection of curcumin against the paracetamol-induced cytotoxicity seems to corroborate with the lack of protection against GSH-depletion, since paracetamol-induced GSH-depletion is generally thought to precede covalent binding of paracetamol to proteins and, furthermore, to be inversely related to it [4, 24]. However, a number of *in vivo* and *in vitro* studies have also claimed a "dissociation" of the covalent binding process from cell-death. For instance, *N*-acetyl-*l*-cysteine, (+)-catechin and α-tocopherol protected against hepatotoxicity without significant effects on covalent binding of paracetamol to proteins [17, 18]. Furthermore sulfhydryl antidotes [1] and acetylsalicylic acid [19] prevented against paracetamol-induced hepatotoxicity, even if administered up to 10 hr after paracetamol administration, i.e. when covalent binding already has taken place [4]. Recent studies using immunochemical detection of covalent adducts of paracetamol to proteins tend to support the hypothesis of critical and less critical covalent adducts as mediators of the hepatotoxicity [13].

In contrast to the observed lack of protection of curcumin against GSH-depletion pre-treatment with the lowest concentration of curcumin already completely protected the hepatocytes from lipid peroxidation induced by paracetamol and measured as TBA-reactive material (Fig. 2B). Lipid peroxidation induced by paracetamol was also completely prevented by higher curcumin concentrations and by adding curcumin simultaneously or 1 hr after administration of paracetamol (Tables 1 and 2). This inhibition of lipid peroxidation is most likely caused by the fact that curcumin may act as a strong anti-oxidant due to its two phenolic groups. Methylation of these phenolic groups was demonstrated to depress this activity significantly [30]. However, based on the present experiments, inhibition of the cytochrome P450-mediated bioactivation of paracetamol [45] or the NAPQI-induced uncoupling of cytochrome P450 leading to oxygen radicals [14] cannot be excluded. The fact that curcumin in the hepatocytes was found to protect against lipid peroxidation at rather low concentrations without protection against paracetamol-induced LDH-leakage and GSH-depletion supports the hypothesis that lipid peroxidation is not playing a causal role in the development of cytotoxic effects of paracetamol. This hypothesis was also supported by previous studies in mice treated with deferrioxamine and paracetamol [8], in hepatocytes treated with paracetamol and dithiolthreitol [48] as well as in hepatocytes treated with 3-alkyl- [24] and with 3,5-dialkyl-substituted analogues of paracetamol [22, 24].

In contrast to what was expected on the basis of literature data [33–36], curcumin itself was found to be slightly cytotoxic at higher concentrations. Curcumin (5×10^{-3} M) tended to increase LDH-leakage from the hepatocytes, i.e. from 15% to about 25% (Fig. 3C and Table 1). This slight increase in cytotoxicity was accompanied by an increasing depletion of GSH, namely up to 40% of the control-levels. As yet no biotransformation data on curcumin are available. However, due to the presence of two α,β-unsaturated ketone functionalities, spontaneous or enzymatic conjugation to GSH and/or protein thiols can be anticipated. Most likely due to a strong intrinsic anti-oxidant activity of curcumin no lipid peroxidation was observed in the hepatocytes. At the higher curcumin concentrations the control production of TBA-reactive material was even decreased instead. Further studies are needed to delineate the importance of these mechanisms *in vivo*.

In conclusion, we have shown that low concentrations of curcumin can protect rat hepatocytes against paracetamol-induced lipid peroxidation at concentrations which are not protective against paracetamol-induced cytotoxicity. These effects were independent of the moment of curcumin (pre-) treatment: 1 hr before, concomitant and 1 hr after addition of paracetamol to the cells gave similar results. At higher concentrations, however, curcumin itself decreased cellular GSH-levels which were accompanied by a slight curcumin-induced LDH-leakage. The relevance of the latter two cytotoxic effects at higher concentrations of curcumin in relation to the former cytoprotective effect at lower concentrations remains to be further elucidated in *in vivo* situations.

REFERENCES

1. Prescott LF, Paracetamol overdosage. Pharmacological considerations and clinical management. *Drugs* **25**: 290–314, 1983.
2. Hinson JA, Biochemical toxicology of acetaminophen. In: *Reviews in Biochemical Toxicology* (Eds. Hodgson E, Bend JR and Philpot RH), Vol. 2, pp. 103–129. Elsevier, Amsterdam, 1980.
3. Black M, Acetaminophen hepatotoxicity. *Annu Rev Med* **35**: 577–593, 1984.
4. Mitchell JR, Jollow DJ, Potter WZ, Gillette JR and Brodie BB, Acetaminophen-induced hepatic necrosis I. Role of drug metabolism. *J Pharmacol Exp Ther* **187**: 185, 1973.
5. Corcoran JB, Racz WZ, Smith CV and Mitchell JR, Effects of *N*-acetylcysteine on acetaminophen covalent binding and hepatic necrosis in mice. *J Pharmacol Exp Ther* **232**: 864–872, 1985.
6. Wendel D and Feuerstein S, Drug induced lipid peroxidation in mice. I. Modulation by mono oxygenase activity, glutathione and selenium status. *Biochem Pharmacol* **30**: 2513–2520, 1981.
7. Albano E, Poli G, Chiarpotto E, Brasi F and Dianzini M, Paracetamol-stimulated lipid peroxidation in isolated rat and mouse hepatocytes. *Chem Biol Interact* **47**: 249–262, 1983.
8. Younes M, Cornelius S and Siegers CP, Ferrous iron supported *in vivo* lipid peroxidation induced by paracetamol. Its relation to hepatotoxicity. *Res Commun Chem Pathol Pharmacol* **51**: 89–99, 1986.
9. Albano E, Rundgren M, Harvison PJ, Nelson SD and Moldeus P, Mechanisms of *N*-acetyl-*p*-benzoquinone imine cytotoxicity. *Mol Pharmacol* **28**: 306–312, 1985.
10. Moore M, Thor H, Moore G, Nelson SD, Moldeus P and Orrenius S, The toxicity of acetaminophen and *N*-acetyl-*p*-benzoquinone imine in isolated hepatocytes is associated with thiol depletion and increased Ca^{2+}. *J Biol Chem* **260**: 13035–13040, 1985.
11. van de Straat R, de Vries J, Debets AJJ and Vermeulen NPE, The mechanism of paracetamol-induced hepatotoxicity by 3,5-dialkyl substitution: the roles of glutathione depletion and oxidative stress. *Biochem Pharmacol* **36**: 2065–2071, 1987.
12. Landon EJ, Neukam RJ and Rama Sastry BV, Effects of calcium channel blocking agents on calcium and centrilobular necrosis in the liver of rats treated with hepatotoxic agents. *Biochem Pharmacol* **33**: 697–705, 1986.
13. Bartolone JB, Sparks K, Cohen SD and Khairalla E, Immunochemical detection of acetaminophen-bound proteins. *Biochem Pharmacol* **369**: 1193–1196, 1987.
14. van de Straat R, de Vries J, Vromans RM, Bosman P and Vermeulen NPE, Cytochrome P450 mediated oxidation of substrate by electron-transfer: Role of oxygen radicals and of 1- and 2-electron oxidation of paracetamol. *Chem Biol Interact* **64**: 267–280, 1988.
15. Pratt S and Ioannides C, Mechanism of the protective action of *N*-acetyl-cysteine and methionine against paracetamol toxicity in the hamster. *Arch Toxicol* **57**: 173–177, 1985.
16. Corcoran GB and Wong BK, Role of glutathione in prevention of acetaminophen-induced hepatotoxicity by *N*-acetyl-L-cysteine *in vivo*: study with *N*-acetyl-D-cysteine in mice. *J Pharmacol Exp Ther* **238**: 54–61, 1986.
17. Walker BE, Keleker J, Dixon MF and Lasowsky MS, *Clin Sci Molec Med* **47**: 449, 1974.
18. Devalia JL, Ogilvie RL and McLean AEM, Dissociation of cell death from covalent binding of paracetamol by flavones in a hepatocyte system. *Biochem Pharmacol* **31**: 3745–3752, 1982.
19. de Vries J, de Jong J, Lock FM, van Bree L, Mullink H and Veldhuizen RW, Protection against paracetamol-induced hepatotoxicity by acetylsalicylic acid in rats. *Toxicology* **30**: 297–304, 1984.
20. Harvison PJ, Forte AJ and Nelson SD, Comparative toxicities and analgesic activities of three monomethylated analogues of acetaminophen. *J Med Chem* **29**: 1737–1743, 1986.
21. Fernando CR, Calder JC and Ham KN, Studies on the mechanism of toxicity of acetaminophen: synthesis and reactions of *N*-acctyl-3,5-dimethyl-*p*-benzoquinone imines. *J Med Chem* **23**: 1153–1185, 1980.
22. van de Straat R, de Vries J, Kulkens T, Debets AJJ and Vermeulen NPE, Paracetamol, 3-monoalkyl-and 3,5-dialkyl derivates. Comparison of their microsomal cytochrome P450 dependent oxidation and toxicity in freshly isolated hepatocytes. *Biochem Pharmacol* **35**: 3693, 1986.
23. van de Straat R, de Vries J, de Groot EJ, Zijl R and Vermeulen NPE, Paracetamol, 3-monoalkyl and 3,5-dialkyl derivates: comparison of their hepatotoxicity in mice. *Tox Appl Pharmacol* **89**: 183–189, 1987.
24. Porubek DJ, Rundgren M, Harvison PJ, Nelson SD and Moldéus P, Investigation of mechanisms of acetaminophen toxicity in isolated rat hepatocytes with acetaminophen analogues 3,5-dimethylacetaminophen and 2,6-dimethylacetaminophen. *Mol Pharmacol* **31**: 647–653, 1987.
25. Dearden JC, O'Hara JH and Townsend MS, A double-peaked quantitative structure activity relationship (QSAR) in a series of paracetamol derivatives. *J Pharm Pharmacol* **32**: 102, 1980.
26. Chopra RN, Chopra JC, Honda KL and Kapur LD, *Indigenous Drugs of India*, 2nd Edn, p. 325. U.N. Dhar and Sons pvt. Ltd., Calcutta, 1985.
27. Srimal RC and Dharvan BN, Pharmacology of Curcumin. A non-steroidal and anti-inflammatory agent. *J Pharm Pharmacol* **25**: 447–452, 1973.
28. Mukhopadhyay A, Basu N, Ghatak N and Gujzal PK, Anti-inflammatory and irritant activities of curcumin analogues in rats. *Agents Actions* **12**: 508–515, 1982.
29. Sharma SC, Muhktar H, Skarun SK and Murti CRK, Lipid peroxide formation in experimental inflammation. *Biochem Pharmacol* **21**: 1210–1214, 1972.
30. Sharma OP, Anti-oxidant activity of curcumin and related substances. *Biochem Pharmacol* **25**: 1811–1812, 1976.
31. Kiso J, Suzuki Y, Watanabe A, Oshima Y and Hikiho H, Anti-hepatotoxic principles of *Curcuma longa* rhizomes. *Plant Res Med* **49**: 184–187, 1983.
32. Banerjee H, Sengupta M, Das J, Sikdar S and Mitra VK, *Calcutta Med J* **73**: 119–125, 1976.
33. Abrakam S, Abrakam SK and Radhamony G, *Cytologia* **41**: 591, 1976.
34. Shankar TNB, Shanta NV, Ramesh HP, Murthey IAS and Murthey VS, Toxicity studies on Turmeric (*Curcuma longa*): acute toxicity studies in rats, Guinea pigs and monkeys. *Ind J Exp Biol* **18**: 73–75, 1980.
35. Hjayalaxmi, *Mutation Res* **79**: 125, 1980.
36. Yambajak K, Ratankumar S, Kamanna VS, Satyanarayana MN and Rao MVL, *J Food Sci-Technol* **19**: 187, 1982.
37. Tønnesen HN, de Vries H, Karlsen J and Beyersbergen van Henegouwen G, Studies on Curcumin and Curcuminoids. IX. Investigation of the photobiological activity using bacterial indicator systems. *J Pharm Sci* **76**: 371–373, 1987.
38. Seglen PO, Preparation of rat liver cells. III. Enzymatic requirements for tissue dispersion. *Exp Cell Res* **82**: 391–398, 1973.
39. Moldéus P, Högberg J and Orrenius S, Isolation and use of liver cells. *Methods Enzymol* **52**: 60–71, 1978.
40. Haenen GRMM and Bast A, Protection against lipid peroxidation by a microsomal glutathione-dependent

labile factor. *Fed Eur Biochem Soc Lett* **159**: 24–28, 1983.
41. Hissin PJ and Hilf R, A fluorometric method for the determination of oxidized and reduced glutathione in tissues. *Anal Biochem* **74**: 214–220, 1976.
42. Govindarajan VS, *CRC Crit Rev Fd Sci Nutr* **12**: 199, 1980.
43. Hinson JA, Phol LR, Monks TJ and Gillette JR, Minireview: acetaminophen-induced hepatoxicity. *Life Sci* **29**: 107–116, 1981.
44. Gerson RL, Cosini A, Dilfor D, Serroni A and Farber JL, Oxygen-mediated cell injury in the killing of cultured hepatocytes by acetaminophen. *Biochem Biophys Res Commun* **126**: 1129–1137, 1985.
45. van de Straat R, de Vries J, de Boer HJR, Vromans RM and Vermeulen NPE, Relationship between paracetamol binding to and its oxidation by two cytochrome P450 isozomes: a proton nuclear magnetic resonance and spectrophotometric study. *Xenobiotica* **17**: 1–9, 1987.
46. Streeter AG, Dahlin DC, Nelson JD and Baillie TA, The covalent binding of acetaminophen to protein. Evidence for cysteine residues as major sites of arylation *in vitro*. *Chem Biol Interact* **48**: 348–366, 1984.
47. van de Straat R, Bijloo GJ and Vermeulen NPE, Paracetamol, 3-monoalkyl- and 3,5-dialkyl substituted derivatives: antioxidant activity and relationship between lipid peroxidation and cytotoxicity. *Biochem Pharmacol* **37**: 3473–3476, 1988.
48. Tee LBG, Boobis AR, Huggett AC and Davies, DS, Reversal of acetaminophen toxicity in isolated hamster hepatocytes by dithiothreitol. *Tox Appl Pharmacol* **83**: 294–312, 1986.
49. Reed DJ, Brodie AE and Meredith HJ, In: *Functions of Glutathione: Biochemical, Physiological, Toxicological and Clinical Aspects* (Eds. Larson A, Holmgren A, Orrenius S and Mannervik B), p. 39. Raven Press, New York, 1983.