J. Pharm. Pharmacol. 1989, 41: 343–345
Communicated September 7, 1988

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Reduction by acetylsalicylic acid of paracetamol-induced hepatic glutathione depletion in rats treated with 4,4′-dichlorobiphenyl, phenobarbitone and pregnenolone-16-α-carbonitrile

L. VAN BREE, E. J. GROOT, J. DE VRIES, *Department of Medicinal Chemistry, Vrije Universiteit, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands*

Abstract—The role of enzyme induction in the reduction by acetylsalicylic acid (ASA) of paracetamol-induced hepatic glutathione (GSH) depletion has been studied in rats. Administration of an overdose of paracetamol to control rats resulted in an appreciable decrease of GSH concentration. Pretreatment with the enzyme inducers phenobarbitone, 3-methylcholanthrene (3-MC), pregnenolone-16-α-carbonitrile (PCN) and 4,4′-dichlorobiphenyl (4,4′-DCB) significantly potentiated the paracetamol-induced depletion of GSH. Simultaneous administration of an equimolar dose of ASA resulted in a reduction of the paracetamol-induced depletion of GSH in all instances except for those rats that were not pretreated and those given 3-MC. Benorylate, the ASA ester of paracetamol, depressed rat liver GSH to levels comparable to those produced by the combination of paracetamol and ASA. ASA itself caused only minor changes in liver GSH concentrations. The results demonstrate that ASA causes a diminution of paracetamol-induced GSH depletion in rats with phenobarbitone type of enzyme induction. Inhibition of the formation of the reactive metabolite of paracetamol or reduction of the absorption rate of paracetamol seem to be unlikely as mechanisms underlying the ASA-induced effect. An ASA-mediated effect via changes of the hepatic thiol status is proposed.

The urgent need to prevent the toxic effects of paracetamol has resulted in numerous reports concerning compounds which markedly reduce the paracetamol-induced necrosis and/or mortality in experimental animals (reviewed by Prescott & Critchley 1983).

Acetylsalicylic acid (ASA) pretreatment has been found to reduce the hepatotoxicity of paracetamol in the mouse (measured as decreased elevation of plasma transaminase) (Whitehouse et al 1976) and we have reported that ASA is effective in preventing paracetamol-induced hepatic GSH depletion in phenobarbitone-treated rats, but not effective in untreated animals (De Vries et al 1981). We also showed that paracetamol-induced hepatotoxicity in control rats (determined by plasma transaminase activities as well as histological examinations) is decreased by both simultaneous and delayed treatment with ASA (De Vries et al 1984).

We now report the effect of induction of cytochrome P-450 by 4,4′-dichlorobiphenyl (4,4′-DCB), phenobarbitone, 3-methylcholanthrene (3-MC) and pregnenolone-16-α-carbonitrile (PCN) on the action of ASA against the paracetamol-induced hepatic depletion of GSH in rats. We also examined the hepatic GSH levels after benorylate, an ester of ASA and paracetamol, which undergoes rapid hydrolysis by esterases in gut mucosal cells (Humphreys & Smy 1975) and the blood stream (Robertson et al 1972).

Materials and methods

Chemicals. 4,4′-DCB was purchased from Janssen Chimica (Beerse, Belgium) and was crystallized from heptane. Phenobarbitone was obtained from Brocades-Acf (Maarssen, The Netherlands). 3-MC and PCN were from ICN Pharmaceuticals (Plainview, NY: USA). 2,2′-Dinitro-5,5′-dithiobenzoic acid was purchased from Merck (Darmstadt, GFR). Paracetamol, ASA and benorylate came from the laboratory stock and were of pharmaceutical grade. All other chemicals were of analytical grade.

Animal treatment. Male albino Wistar rats (175–200 g) were housed in macrolon cages at 23°C with a 12 h normal light cycle and had free access to laboratory diet (RMH-10, Hope Farms, Woerden, The Netherlands) and tap water.

Rats were treated with enzyme inducers according to the following scheme: 4,4′-DCB (i.p., 44·6 mg kg^{-1} day^{-1} for 3 days) in arachis oil (2 mL kg^{-1}); phenobarbitone (s.c., 90 mg kg^{-1} day^{-1} for 2 days) in saline (5 mL kg^{-1}); 3-MC (i.p., 40 mg kg^{-1} day^{-1} for 1 day) in arachis oil (2 mL kg^{-1}); PCN (i.p., 33 mg kg^{-1} day^{-1} for 3 days) in saline containing 1% (w/v) Tween 80 (2 mL kg^{-1}). At 48 h after the first dose of phenobarbitone or 3-MC and at 24 and 96 h after the third dose of PCN and 4,4′-DCB respectively, overnight fasted animals were orally pretreated (10 mL kg^{-1}) with either vehicle (1% (w/v) amylum in 50 mM phosphate buffer, pH 7·4) or paracetamol, ASA, benorylate or a combination of equimolar amounts of paracetamol and ASA (6·62 mmol kg^{-1}) suspended in vehicle. Both analgesic-treated and vehicle-treated rats were decapitated 2 h later and the livers were rapidly prepared for GSH determination.

GSH determination. GSH levels were determined in the 12 000 *g* (20 min) supernatant prepared from a 33% (w/v) liver homogenate in 50 mM potassium phosphate buffer, pH 7·4, containing 155 mM sodium chloride, using the dithiobenzoic acid reagent by the method described by Kaplowitz (1977).

Values were analysed statistically using Student's *t*-test.

Results

The effect of pretreatment with various enzyme inducers on the action of ASA on the paracetamol-induced hepatic GSH depletion was investigated in rats. The results are shown in Fig. 1. Pretreatment with phenobarbitone, 3-MC, PCN and 4,4′-DCB did not significantly change the hepatic GSH content when compared with the control group. ASA itself caused only minor changes in GSH levels when given to untreated and pretreated animals. An overdose of paracetamol caused a statistically significant decrease of the GSH level. All pretreatments with enzyme inducers substantially potentiated the paracetamol-induced GSH depletion.

Simultaneous administration of ASA and paracetamol to control rats showed no significant change in GSH level when compared with paracetamol alone. Combined administration of the two drugs after pretreatment of rats with various enzyme inducers resulted, however, in a different response. After treatment with phenobarbitone, PCN or 4,4′-DCB, ASA afforded a significant reduction of the paracetamol-induced GSH depletion. However, ASA did not protect after 3-MC-treatment. The hepatic GSH depletion by benorylate resembled

Correspondence to: L. van Bree, Laboratory for Toxicology, National Institute of Public Health and Environmental Protection P.O. Box 1, 3720 BA Bilthoven, The Netherlands.

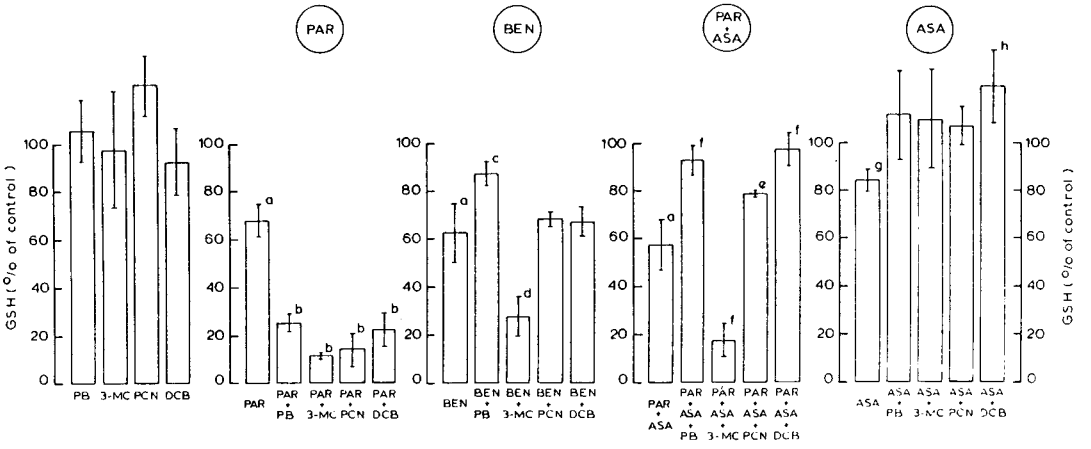

FIG. 1. Effect of enzyme induction on hepatic GSH depletion after administration of paracetamol (PAR), benorylate (BEN), acetylsalicylic acid (ASA) or a combination of equimolar amounts of PAR and ASA (PAR+ASA) to rats. Results show mean ± standard deviation for 3–11 animals. The average hepatic GSH level for 8 control rats was 131 ± 17 mg/100 g liver. [a] $P < 0.001$ when compared with control. [b] $P < 0.001$ when compared with PAR. [c] $P < 0.002$ and [d] $P < 0.001$ when compared with BEN. [e] $P < 0.01$ and [f] $P < 0.001$ when compared with (PAR+ASA). [g] $P < 0.02$ when compared with control. [h] $P < 0.01$ when compared with DCB.

that of the combined administration of ASA and paracetamol in untreated and pretreated rats.

Discussion

The protective action of ASA has not been clarified. In control rats it results in a slightly decreased liver GSH concentration, probably as a result of increased leakage (Kaplowitz et al 1980). Pretreated rats receiving ASA have hepatic GSH levels comparable to control values, suggesting that the ASA-induced protection is not associated with increased glutathione synthesis. The protection by ASA could involve inhibition of the biotransformation of paracetamol to reactive metabolite(s), thereby reducing the GSH depletion. However, ASA has a small effect on paracetamol metabolism by inhibiting its sulphate conjugation, resulting in an enhanced glucuronidation and mercapturate formation (Thomas et al 1974; Whitehouse et al 1975; Wong et al 1976). Furthermore, the covalent binding of paracetamol to liver proteins and the paracetamol-mercapturic acid excretion are preferentially stimulated by pretreatment with 3-MC or β-naphthoflavone and not with phenobarbitone (Jollow et al 1974; Gemborys & Mudge 1981; Ioannides et al 1983; Steele et al 1983). These data, therefore, strongly suggest that in our studies the ASA-induced reduction of GSH depletion in rats with a phenobarbitone type of enzyme induction is not due to a decrease in bioactivation of paracetamol. It has also been reported that ASA reduces the rate of paracetamol absorption from the gastrointestinal tract (Thomas et al 1974; Whitehouse et al 1976). Since the data in Table 1 indicate that ASA does not reduce the paracetamol-induced GSH depletion in untreated and 3-MC-treated rats, this mechanism will not have contributed to the protection by ASA observed in our studies.

From literature data, however, it might be hypothesized that ASA exerts its protective action by affecting the hepatic GSH homeostasis in animals possessing a phenobarbitone type of enzyme induction. The ASA-induced hyperglycaemia (Kawashima et al 1980) results in an enhanced glucose availability and glucose-6-phosphate (G6P) formation. An increased G6P concentration might lead to higher levels of NADPH by G6P dehydrogenase and consequently to a more extensive reduction of glutathione disulphide to GSH by the NADPH-dependent GSH reductase. Furthermore, G6P dehydrogenase is inducible by phenobarbitone but not by 3-MC (Kauffman et al 1980; Smith & Wills 1981; Conway et al 1983). Moreover, both the GSH-mediated reduction of the toxic paracetamol metabolite *N*-acetyl-p-benzoquinone imine to paracetamol (Huggett & Blair 1982), and the production of reactive oxygen species following the redox cycle of the paracetamol semiquinone radical (Powis et al 1984; Rosen et al 1984), ultimately result in increased glutathione disulphide formation.

It is not known whether our results also apply to man. Furthermore, those of our animals receiving overdoses of paracetamol or ASA suffered from severe liver or gastric injury, respectively. However, a previous study has demonstrated that both simultaneous and delayed treatment with ASA reduced the paracetamol-induced liver toxicity (De Vries et al 1984). Moreover, the protective action of paracetamol against the gastric damage provoked by ASA has been well documented (Seegers et al 1978). Therefore, the combined findings suggest that from a toxicological point of view, paracetamol-ASA combinations are preferable to drugs containing one of these compounds. Since paracetamol and ASA are equianalgesic (Cooper 1981), more attention should be paid to paracetamol-ASA combinations as well as benorylate in considering a safe analgesic therapy.

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J. Pharm. Pharmacol. 1989, 41: 345–346
Communicated April 27, 1988

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L-Ascorbic acid produces hypoglycaemia and hyperinsulinaemia in anaesthetized rats

J. T. CHENG, S. C. HSIEH-CHEN, C. L. TSAI, *Department of Pharmacology, College of Medicine, National Cheng Kung University, Tainan City, Taiwan 70101, Republic of China*

Abstract—L-Ascorbic acid (Vitamin C) produced a marked reduction in the blood glucose concentration following intravenous injection (20-100 mg kg^{-1}) to anaesthetized rats. This hypoglycaemic effect was accompanied by an increase in plasma insulin concentration. D-ascorbic acid produced a similar hypoglycaemic effect.

A relationship between scurvy and diabetes mellitus was proposed many years ago (Owens et al 1941). L-Ascorbic acid was found to lower the blood glucose concentration in animals (Losert et al 1980), in healthy human volunteers (Cheng & Yang 1983) and in diabetic patients (Dice & Daniel 1973). However, the mechanism of this hypoglycaemic effect remains obscure. The present study examined the effects of ascorbic acid on blood glucose and plasma insulin concentrations in the rat. To rule out the pharmacokinetic factors, the effect of L-ascorbic acid has been evaluated by intravenous administration.

Materials and Methods

Sprague-Dawley rats (200-280 g) of either sex were housed singly in suspended wire-mesh cages with free access to food and water in a colony room maintained at 23 ± 1°C under standard lighting conditions (05:00-19:00 h). After a 24 h fast, all the rats were anaesthetized with pentobarbitone (35 mg kg^{-1} i.p.). Before the experiment, their blood glucose content was measured every 30 min. Only the rats with a variation of blood glucose less than 5 mg dL^{-1} between two determinations were needed.

A solution of either L- or D- ascorbic acid, in distilled water was administered by intravenous injection into the femoral vein. Control animals received the distilled water, adjusted to the same pH.

Blood samples (2 mL) were collected from the carotid artery. Blood glucose was measured by the glucose oxidase method (Ames, USA) and plasma insulin was determined by radioimmunoassay (Amersham RIA-kits, UK) using rat insulin (Novo Research Institute, Copenhagen, Denmark) as standard. The insulin samples from the paired study were processed in the same assay, running in duplicate. The limit of detection was 0·8 ng mL^{-1} and the plasma dilution curves were parallel to the standard curves. Intra- and interassay coefficients of variation were 7·4 and 11·3%, respectively.

Addition of L-ascorbic acid (100 mg mL^{-1}) to the glucose standards or to fresh blood did not significantly modify the measured concentration of glucose ($P > 0·05$). Similarly, L-

Correspondence to: J. T. Cheng, Department of Pharmacology, College of Medicine, National Cheng Kung University, Tainan City, Taiwan 70101, Republic of China.