EFFECT OF SULPHYDRYL DRUGS ON PARACETAMOL-INDUCED HEPATOTOXICITY IN MICE

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CONTENTS

SUMMARY

It has been shown that the major *in vivo* biotransformation of thiol-containing drugs such as captopril (CP) and penicillamine (PA) involve mixed disulphide formation with endogenous thiols derived from cysteine. At high doses, both drugs produced a dose-dependent depletion of glutathione (GSH) and may perturb GSH and related GSH-enzymes. In this study the possible interactions of these drugs with paracetamol, which produce hepatotoxicity after GSH depletion, were investigated. Following co-administration of CP (50-250 mg/kg) or PA (43-257 mg/kg) with paracetamol (300 mg/kg), the hepatotoxic effect produced by paracetamol was diminished. The protective effect was comparable to that produced by N-acetylcysteine (500 mg/kg) and L-cysteine (500 mg/kg). However, pre-treatment with buthionine sulfoximine (BSO), a specific inhibitor of GSH synthesis, abolished the protective effects of CP, N-acetylcysteine and L-cysteine while the protective effect of PA was unaffected. This suggests that, although both CP and PA may act as alternative sulphydryl nucleophiles to GSH to prevent arylation of essential cellular macromolecules by the reactive metabolite of paracetamol, the underlying mechanisms of these drug interactions may be distinctly different.

I. INTRODUCTION

Captopril (CP) is an angiotensin converting enzyme inhibitor used in the treatment of hypertension. Like D-penicillamine (PA), which is used in the treatment of Wilson's disease and diseases such as cystinuria, rheumatoid arthritis and active chronic hepatitis, CP is an amino acid derivative incorporating a free sulphydryl group. Although the properties and clinical uses of these drugs are distinctly different, previous studies have shown that the major *in vivo* biotransformation of these drugs involve mixed disulphide formation with endogenous thiols derived from cysteine /1,2/. At high doses, both drugs produced a similar dose-dependent depletion of hepatic glutathione (GSH), with CP producing an increase in serum transaminase activity and liver toxicity in the mouse /3,4/.

Hepatic GSH has many functions, among them conjugation with and detoxification of electrophilic species /5-7/. Depletion of GSH

by sulphydryl drugs such as CP and PA may affect the biotransformation and toxicity of drugs such as paracetamol, which produce hepatotoxicity after depleting hepatic GSH /8,9/. To study such a possible interaction, the effect of CP and PA on paracetamol-induced hepatotoxicity was investigated along with N-acetylcysteine and L-cysteine, which are used in treating paracetamol poisoning.

II. METHODS

2.1 Effect of sulphydryl drugs and paracetamol on hepatic GSH and SGPT activity in the mouse.

Male ICR mice (25-30 g) were divided into groups and allowed free access to food and water. A single oral dose of paracetamol (300 mg/kg) was given either alone or followed by i.p. administration of captopril (50-250 mg/kg), D-penicillamine (43-257 mg/kg), Nacetylcysteine (500 mg/kg) or L-cysteine (500 mg/kg). The choice of doses of paracetamol and the sulphydryl drugs were based on previous studies /3/. Paracetamol (300 mg/kg) would cause a hepato-toxic effect while the doses of N-acetylcysteine and L-cysteine would give a protective effect from paracetamol-induced liver toxicity. Control mice were given N-saline (0.9%) in place of paracetamol or other drugs.

Based on a previous time course study for GSH depletion /3/, the mice were killed after 6 hr and the livers taken for determination of hepatic GSH concentration. Serum was obtained for determination of serum glutamic-pyruric transaminase (SGPT) activity which best represented the type of liver toxicity induced by paracetamol as described previously /3/. All GSH concentrations were determined on the day of experiment.

2.2 Determination of hepatic GSH

The glyoxalase I method /10/ was used to determine the concentration of GSH in this study because CP and PA both interfere with the colorimetric assay with Ellman's reagent /11/.

2.3 Determination of SGPT activity

Serum was obtained from clotted whole blood centrifuged at 2000 xg for 15 min. The SGPT activity was determined by the method of Reitman and Frankel /12/, using a commercially available diagnostic kit.

2.4 Statistical analysis

All results are reported as the mean ± s.d. Differences between means were determined using the Student's t-test.

III. RESULTS AND DISCUSSION

The key functional group in CP and PA, both in terms of their metabolism and covalent hinding to plasma proteins, is the free sulphydryl group. The major biotransformation pathways of these drugs involve mixed disulphide formation with endogenous thiols (protein and non-protein) derived from cysteine /1,2,13/ and it would appear that none of the other functional groups of these drugs undergo significant biotransformation *in vivo*.

In this study, both CP and PA diminished the increase in SGPT activity produced by a toxic dose of paracetamol (Table 1). The protective effects of these drugs were comparable to that of L-cysteine and N-acetylcysteine, which is used clinically for the treatment of paracetamol overdose. This suggest that both CP and PA may act as alternative sulphydryl nucleophiles to GSH and thus prevent arylation of essential cellular macromolecules by the iminoquinone metabolite of paracetamol.

To explore the possible mechanism(s) of the interaction of CP and PA with paracetamol-induced toxicity, a specific inhibitor of GSH synthesis was used. The protective effects of CP, N-acetylcysteine and L-cysteine were abolished by pretreatment with BSO (Table 2), while the protective effect of PA was unaffected. Therefore, the underlying mechanism(s) of the protective effects of the sulphydryl drugs CP and PA would appear to be distinctly different. The protective effect of CP may be related to facilitation of GSH synthesis, although other mechanisms such as chemical reduction of the paracetamol reactive metabolite, direct conjugation of CP with

TABLE 1

Effect of sulphydryl drugs on paracetamol-induced depletion of hepatic glutathione (GSH) and hepatotoxicity in mice at 6 hr.

Treatment	Hepatic GSH (μmole/g)	SGPT Activity (U/L)
Control	5.27 ± 0.62	15.60 ± 5.83
Paracetamol 300 mg/kg	3.01 ± 0.59[2]	89.80 ± 10.33[2]
Paracetamol 300 mg/kg + N-acetylcysteine 500 mg/kg	4.50 ± 0.70[1,3]	11.50 ± 1.50[4]
Paracetamol 300 mg/kg + L-cysteine 500 mg/kg	5.09 ± 1.88[3]	16.80 ± 2.20[4]
Paracetamol 300 mg/kg + captopril 250 mg/kg	3.28 ± 0.65[2]	29.92 ± 6.18[4]
Paracetamol 300 mg/kg + penicillamine 210 mg/±g	2.23 ± 0.62[2]	23.21 ± 6.00[4]

Results are mean ± S.D. of 10 animals
1 $P < 0.05$
2 $P < 0.001$ compared to saline control only
3 $P < 0.005$
4 $P < 0.001$ compared to paracetamol only

the reactive metabolite or interaction of CP with the metabolism of paracetemol may be possible. Like N-acetylcysteine, CP may also be involved in promoting cystine uptake for utilization for cellular GSH synthesis /14/. By contrast, the protective effect of PA against paracetamol toxicity in this study is not dependent on GSH synthesis. Because of its low redox potential /15/, PA may interact with mechanisms by which the paracetamol reactive metabolite is formed or simply reduce the amount of reactive metabolite by reduction or direct conjugation. Although other sulphydryl compounds such as N-acetylcysteine, cysteamine and methionine have all been found useful in paracetamol overdose, a lot of controversies still remain with respect to the mechanism of protective effect of various sulphydryl nucleophiles /16,17/. We are presently further investigating the mechanisms of these drug-interactions.

TABLE 2

Effect of sulphydryl drugs on paracetamol–induced pertubation of hepatic glutathione (GSH) and serum glutamic-pyruvic transaminase (SGPT) activity in mice (6 hr) pretreated with buthionine sulfoximine (BSO).

Treatment	Hepatic GSH (μmole/g)	SGPT Activity (U/L)
Control + BSO	1.68 ± 0.65	18.54 ± 5.41
Paracetamol (300 mg/kg)	3.01 ± 0.59	89.80 ± 10.33[2]
Paracetamol (100 mg/kg) + BSO	0.66 ± 0.11[1]	85.88 ± 16.23[1]
Paracetamol (100 mg/kg) + captopril (250 mg/kg) + BSO	1.32 ± 0.97	74.29 ± 31.83[1]
Paracetamol (100 mg/kg) + penicillamine (210 mg/kg) + BSO	1.11 ± 0.37	27.80 ± 5.85
Paracetamol (100 mg/kg) + N-acetylcysteine (500 mg/kg) + BSO	0.24 ± 0.09[2,3]	95.22 ± 3.49[2]

Results are mean ± S.D. of 12 animals
1 $P < 0.01$
2 $P < 0.005$ compared to control + BSO
3 $P < 0.001$ compared to paracetamol + BSO

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