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Effect of polysaccharide peptide (PSP) on in vivo sulphation and glucuronidation of paracetamol in the rat

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SUMMARY

The effect of polysaccharide peptide (PSP), an immunomodulator isolated from ***Coriolus versicolor COV-1***, on the disposition of paracetamol was investigated in the rat. PSP (100 and 200 mg/kg, i.v.) was administered 30 min before a moderate dose (100 mg/kg, i.v.) of paracetamol was given. Plasma and bile concentrations of paracetamol, paracetamol glucuronide and paracetamol sulphate were measured by high performance liquid chromatography. The pharmacokinetics of paracetamol (100 mg/kg) alone was consistent with those reported previously, using a one-compartment model. PSP (200 mg/kg) significantly ($P < 0.05$) increased the clearance (controls, 19.06 ± 2.74 ml/min/kg: PSP treated, 26.22 ± 0.84 ml/min/kg)and volume of distribution (controls, 1.35 ± 0.11 l/kg: PSP treated, 1.61 ± 0.04 l/kg) of paracetamol by 37% and 21%, respectively. These changes were associated with concomitant increases in the glucuronide and sulphate metabolites in plasma, with significant increases in the C_{max} and T_{max} for both metabolites. The biliary excretion rate of paracetamol glucuronide and paracetamol sulphate were also measured. The C_{max} values of paracetamol sulphate were significantly ($P < 0.01$) increased by 2.4-fold from 907.8 ± 157.7 µg/ml (controls) to 3061 ± 331 µg/ml after PSP treatment. The lower dose of PSP (100 mg/kg) had no significant effect on the disposition of paracetamol in this study, which agreed with previous reports that a low dose of PSP (100–200 mg/kg, i.p.) was less effective in the protection against paracetamol-induced hepatotoxicity. The time course of the increase in paracetamol sulphate in plasma and bile in this study coincided with the transient perturbation of glutathione (GSH) turnover by a similar dose range of PSP previously described, such that more cysteine was available for oxidation to inorganic sulphate. This increase in sulphate conjugation by PSP would, in part, contribute to the increase in disposition of paracetamol and may be related to the ability of PSP to decrease the covalent binding of paracetamol to microsomal proteins previously reported. Further studies are necessary to understand the mechanism(s) involved in the PSP-induced increases in paracetamol glucuronide and paracetamol sulphate formation and biliary excretion.

INTRODUCTION

Polysaccharide peptide (PSP) is an immunomodulator isolated from *Coriolus versicolor COV-1* and characterised at the Shanghai Teachers University in China. PSP has been shown to be effective against a number of human tumour cells in vitro (1). Like PSK, another immunomodulator developed in Japan, PSP is indi-

Abbreviations : AUC, area under curve: CL, clearance: PSP, polysaccharide peptide: $T_{1/2}$, half life: Vd, volume of distribution.

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cated in anticancer therapy, and as an adjuvant to chemotherapy, radiotherapy and surgical operation (2). It has also been shown that PSP may be useful in the treatment of hepatitis and other symptoms associated with immunodeficiency (3). Preliminary studies have shown that PSP is effective in stimulating immunological responses as well as in protecting the liver from hepatotoxins in laboratory animals (4,5), although the full pharmacological and toxicological profiles remain to be fully understood.

The paracetamol model is a well studied and understood model of drug-induced toxicity and is particularly relevant to the proposed use of PSP as an adjuvant in cancer chemotherapy when multiple drugs are administered. The effect of PSP on the glutathione (GSH) status has been investigated recently when PSP increased the oxidation of GSH to its disulphide, GSSG, without causing any toxic effects (5). PSP also protected against paracetamol-induced hepatotoxicity by decreasing the covalent binding of [^{14}C]-paracetamol in vitro (5). In this study, the effect of PSP on paracetamol metabolism was investigated to determine whether or not the protective effect of PSP against paracetamol-induced toxicity has an underlying metabolic basis. A second objective of this study was to investigate the effect of PSP on sulphation and glucuronidation since these conjugation processes are the prime routes for the disposition of paracetamol at low to moderate doses (15–100 mg/kg).

MATERIALS AND METHODS

Reagents

Polysaccharide peptide (PSP), isolated from *Coriolus versicolor COV-1*, was kindly donated by Winsor Health Products Limited (Hong Kong). Paracetamol (4-acetyl-*p*-aminophenol), 3-acetyl-*p*-aminophenol, and other general reagents were obtained from Sigma Chemical Co. (St Louis, MO, USA). All solvents were redistilled before use. Authentic standards of paracetamol-glucuronide and paracetamol-sulphate conjugates were kindly donated by Sanofi Winthrop Production Division (UK).

Effect of polysaccharide peptide (PSP) on paracetamol metabolism

Male Sprague-Dawley rats (250–300 g), with free access to food and water, were anaesthetized with urethane (14% w/v in saline, 10 ml/kg, i.p.) and the trachea, carotid artery, jugular vein and the bile duct cannulated with polythene tubing of the appropriate sizes. Polysaccharide peptide (PSP, 100–200 mg/kg, i.v.) was administered 30 min before paracetamol (100 mg/kg) in saline was administered as a bolus dose over 1 min via the jugular vein. Blood samples (0.5 ml) were collected at 15, 30 45, 60, 90, 120, 150 and 180 min. after paracetamol administration. Plasma was obtained immediately by centrifugation (2000 *g*). The volume of blood taken was replaced by an equal volume of saline. Bile samples were also collected, at 30 min intervals, into pre-weighed vials. Paracetamol metabolites in plasma and in bile were stored at –20°C before quantitation by high performance liquid chromatography (HPLC).

Determination of paracetamol metabolites by HPLC

The metabolites of paracetamol were determined by HPLC using a Hewlett Packard (HP) 1050 series pumping system and multiple wavelength detector (wavelength at 254 nm), a Model 7125 syringe loading sample injector and a chart recorder. The eluates were analyzed by passing through a Hiber pre-column (50 mm × 4.6 mm I.D.) and a Waters μBondapak C_{18} analytical column (8 mm × 100 mm I.D.). The operation conditions for the HPLC system were: mobile phase, consisting of 8% organic phase (2 ml of ethyl acetate for every 150 ml methanol) and 92% acetate buffer (0.05 M, pH 3.6); flow rate at 1 ml/min; temperature, ambient (25 ± 1°C). 3-acetyl-*p*-aminophenol was used as an internal standard. The metabolites of paracetamol were identified by comparing the retention times of authentic standards. Peak height ratios of the metabolites were measured. Standard curves of paracetamol, the glucuronide and sulphate metabolites in plasma (10–160 μg/ml) and in bile (0.2–2 mg/ml) were linear ($r > 0.99$) and were prepared every time before an analysis was carried out. Under the experimental conditions described, the retention times of paracetamol glucuronide, paracetamol sulphate, paracetamol and 3-acetyl-*p*-aminophenol (internal standard) were 3.0, 4.9, 7.3 and 10.8 min, respectively.

Data analysis

The paracetamol concentration-time curve was analyzed using a one compartment open model and the nonlinear least squares regression program TOPFIT

(version 1.0) in conjunction with a model-specific subroutine and a weighting function of 1/(concentration). Although a multi-compartment model has been used by some researchers to describe the non-linearity in the pharmacokinetics of paracetamol caused by the saturation in the sulfo-conjugation pathway (6,7), the inadequate description of a distribution phase from the early time points in this study necessitate the use of a one-compartment open model as previously described by others (8,9). The area under curve (AUC) from zero to the last measured time point was calculated by the trapezoidal rule. Extrapolation of the AUC to infinity was made by adding the ratio between the last plasma concentration and the terminal elimination rate constant. The CL was estimated by the ratio of the paracetamol dose to the calculated AUC from zero to infinity. The maximum plasma concentrations (C_{max}) and the times taken to achieve those concentrations (T_{max}) for paracetamol, paracetamol glucuronide and paracetamol sulphate were directly obtained from the experimental observations.

Statistical analysis

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

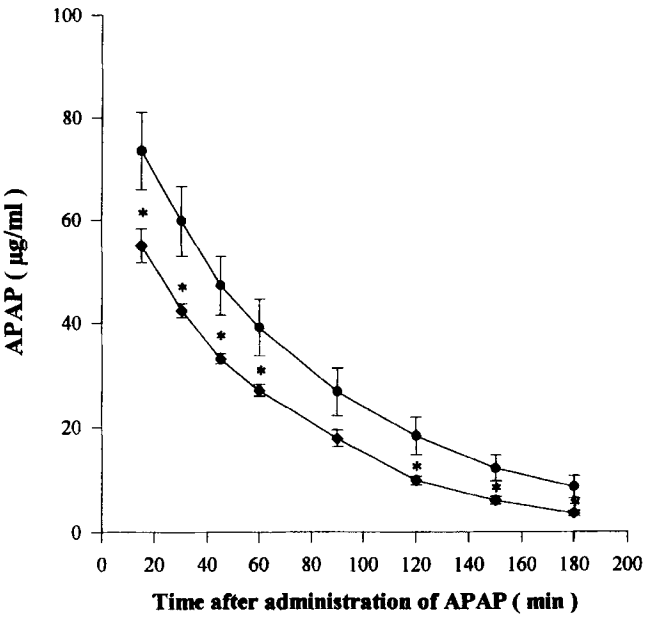

Fig. 1 : Concentration-time profiles of paracetamol in plasma after i.v. administration of paracetamol (100 mg/kg) alone (filled circles) and after pretreatment with 200 mg/kg polysaccharide peptide, PSP (filled diamonds). Results are mean ± SD of 8 animals. *$P < 0.05$ compared with controls using Student's t-test.

RESULTS

Effect of polysaccharide peptide (PSP) on paracetamol metabolism

The effect of polysaccharide peptide (PSP, 200 mg/kg) on paracetamol concentrations in plasma following an intravenous bolus dose of paracetamol (100 mg/kg) is shown in Figure 1. Paracetamol concentrations declined monoexponentially with time in both groups. The clearance (CL) and volume of distribution (Vd) of paracetamol were significantly ($P < 0.05$) increased by co-administration of PSP (Table I), such that the CL of paracetamol was increased by 37% (controls, 19.06 ± 2.74 ml/min/kg: PSP treated, 26.22 ± 0.84 ml/min/kg) and Vd was increased by 21% (1.35 ± 0.11 l/kg in the controls and 1.61 ± 0.04 in the PSP treated animals).

The concentration of paracetamol-glucuronide in plasma was increased by PSP co-administration over the time period studied (Fig. 2), with the increase

Table I : Pharmacokinetics of paracetamol in rats after a single i.v. dose of paracetamol (100 mg/kg) alone or in combination with polysaccharide peptide (PSP, 200 mg/kg, i.v.).

Parameter	*Paracetamol only*	*Paracetamol with PSP*
AUC (µg.h/ml)	87.5 ± 11.0	63.6 ± 7.5
$T_{1/2}$ (min)	47.3 ± 6.0	41.5 ± 1.3
Vd (ml/kg)	1352 ± 110	1610 ± 40*
CL (ml/min/kg)	19.1 ± 2.7	26.2 ± 0.8*

Data are mean ± SD of 8 animals. *$P < 0.05$ compared with control using Student's t-test.

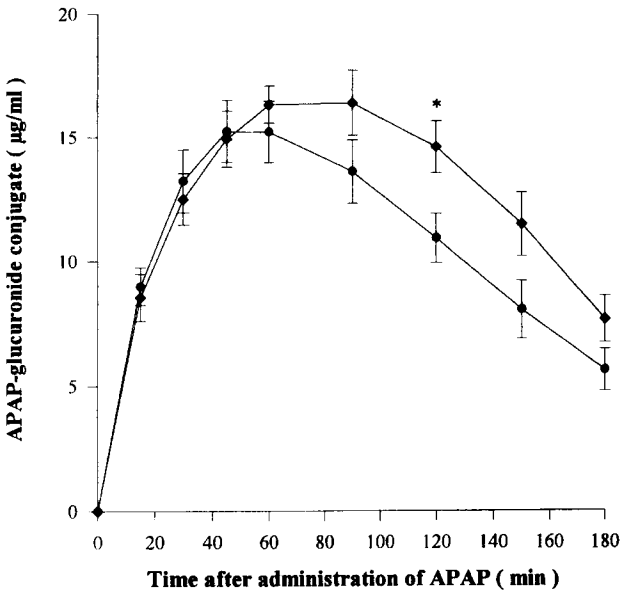

Fig. 2 : Concentration-time profiles of paracetamol glucuronide in plasma after i.v. administration of paracetamol (100 mg/kg) alone (filled circles) and after pretreatment with 200 mg/kg polysaccharide peptide (filled diamonds). Results are mean ± SD of 8 animals. *$P < 0.05$ compared to controls using Student's t-test.

more prominent after 60 min. The maximum concentration (C_{max} = 15.22 ± 1.26 µg/ml) of paracetamol glucuronide in plasma at 45 min (T_{max}) was increased to 16.34 ± 1.32 µg/ml (C_{max}) and 90 min (T_{max}) after PSP treatment. The paracetamol-sulphate concentrations in plasma was also increased by PSP (200 mg/kg) co-administration (Fig. 3). The C_{max} for paracetamol sulphate was 79.18 ± 4.5 µg/ml for the controls and 105 ± 4.8 µg/ml after PSP treatment, with the T_{max} remained unchanged at 60 min. The increase in plasma paracetamol sulphate was more than 40% above the controls.

The lower dose of PSP (100 mg/kg) did not significantly affect the disposition of paracetamol and its sulphate and glucuronide metabolites.

Effect of PSP on the biliary excretion of paracetamol and its conjugates

The bile flow during the experiment decreased in all the animals over the course of the study. The mean bile flow was 60 ± 18 µl/min/kg. These values were consistent with bile flow values previously described (9). Biliary excretion of paracetamol glucuronide and paracetamol sulphate conjugates are presented in Figures 4 and 5, respectively. The overall biliary excretion of paracetamol glucuronide appeared to be higher after PSP (200 mg/kg) treatment, but the data were not statistically significant ($P = 0.06$). However, the biliary excretion of paracetamol sulphate was significantly increased by PSP at each of the time period studied. The C_{max} was increased by 2.4-fold, from 907.8 ± 157.7 µg/ml of the controls to 3061 ± 331 µg/ml of the PSP treated animals. The maximum rate of biliary excretion of paracetamol sulphate occurred during the 30–60 min collection interval for both the control and PSP treated animals, with significant ($P < 0.01$) increases in the biliary excretion rate of paracetamol sulphate in the PSP treated animals throughout the period studied.

As in the case of the plasma data, the lower dose of PSP (100 mg/kg) did not affect the biliary excretion of paracetamol glucuronide and paracetamol sulphate.

DISCUSSION

Polysaccharide peptide (PSP) is a protein bound peptide containing more than 20 different amino acids and

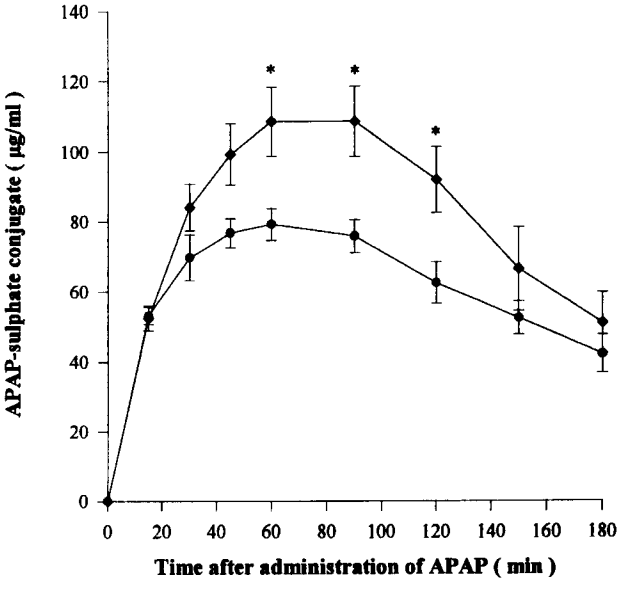

Fig. 3 : Concentration-time profiles of paracetamol sulphate in plasma after i.v. administration of paracetamol (100 mg/kg) alone (filled circles) and after pretreatment with 200 mg/kg polysaccharide peptide (filled diamonds). Results are mean ± SD of 8 animals. *$P < 0.05$ compared to controls using Student's t-test.

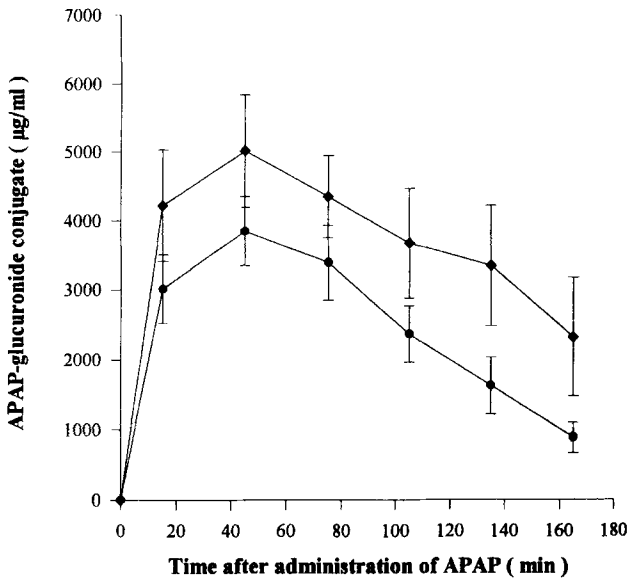

Fig. 4 : Concentration-time profiles of paracetamol glucuronide in bile after administration of paracetamol (100 mg/kg) alone (filled circles) and after pretreatment with 200 mg/kg polysaccharide peptide (filled diamonds). Results are mean ± SD of 8 animals.

5 different sugar residues. Metabolic studies have shown that PSP may be broken down to smaller peptides (3). Recently, it has been shown that PSP produced a dose-dependent transient depletion of hepatic glutathione (GSH) by increasing its oxidation to its disulphide (GSSG) (5). PSP also decreased dose-dependently the covalent binding of paracetamol which suggested that the protective effect of PSP on paracetamol-induced hepatotoxicity was either due to direct conjugation of PSP to the reactive metabolite or was a result of a decrease in the formation of the chemically reactive metabolite through the effect of PSP on paracetamol metabolism (5).

This study has examined the effect of polysaccharide peptide (PSP) on the disposition of paracetamol, paracetamol glucuronide and paracetamol sulphate in the rat. PSP treatment did not significantly alter the overall pharmacokinetics of unchanged paracetamol, but did affect its disposition by increasing the formation of paracetamol glucuronide and paracetamol sulphate. The biliary excretion of these conjugates was also increased by PSP. Paracetamol is metabolized by three pathways: sulphation; glucuronidation; and oxidation via the cytochrome P450 system (10,11). The major pathways of biotransformation following administration of paracetamol at low to moderate doses (15–100 mg/kg), as used in this study, are sulphation and glucuronidation. Sulphation and glucuronidation are important conjugation reactions for drugs such as paracetamol. When the dose of paracetamol is increased, saturation of these conjugation pathways has been reported for both sulphation (75–150 mg/kg) and glucuronidation (300 mg/kg) (12). Sulphation is catalysed by sulphotransferases which utilizes endogenous inorganic sulphate in the form of sulphate esters with 3′-phosphoadenosine 5′-phosphosulphate (PAPS) as the sulphate donor. Therefore, sulphation relies on the availability of PAPS (13) which in turn depends on ATP and inorganic sulphate whose store in the body is limited. It has been shown that an increased utilization of endogenous cysteine during an increase in glutathione (GSH) will compete with the inorganic sulphate. The time course of the increase in paracetamol sulphate in plasma and bile in this study coincided with the transient depletion of GSH through oxidation to GSSG in a previous study (5). Since cysteine is the common precursor for both inorganic sulphate and GSH, it follows that a decrease in GSH turnover may indirectly increase sulphate by increasing the amount of cysteine available for oxidation to inorganic sulphate. Inorganic sulphate concentrations,

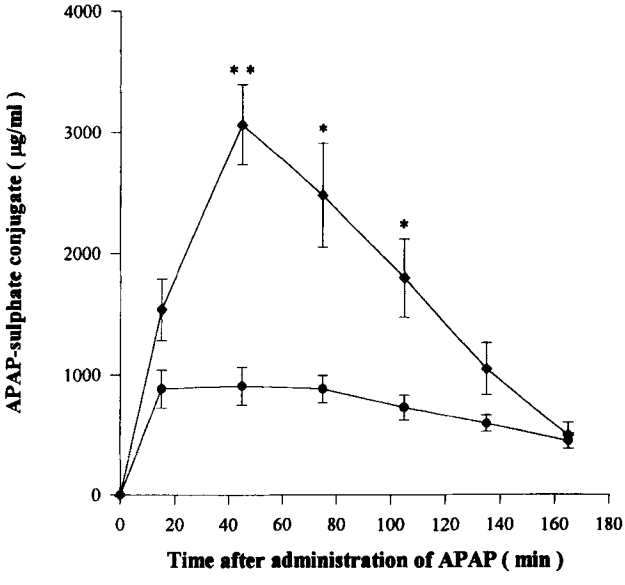

Fig. 5 : Concentration-time profiles of paracetamol sulphate in bile after administration of paracetamol (100 mg/kg) alone (filled circles) and after pretreatment with 200 mg/kg polysaccharide peptide (filled diamonds). Results are mean ± SD of 8 animals. *$P < 0.05$ compared to controls using Student's t-test.

in turn, correlate with 3′-phosphoadenosine 5′-phosphosulphate concentrations and paracetamol sulphation (11,14,15)

It is uncertain how PSP increased glucuronidation in this study since the rate of glucuronidation *in situ* is primarily influenced by the absolute amount of uridine diphosphate glucuronyl transferase and the cofactor, uridine diphosphate glucuronic acid. PSP may increase the activity of uridine diphosphate glucuronyl transferase as reported for phenobarbital (16) and oleanolic acid, another natural compound that has been used to treat hepatitis in China (17). PSP may also stimulate the carrier-mediated transport of paracetamol glucuronide and sulphate conjugates (18) in the liver cells.

In man, rat and mouse, it has been shown that cytochrome P450 2E1 and 1A2 were the isoforms responsible for the bioactivation of paracetamol (19). It would be of interest to further investigate the effect of PSP on the activities of these cytochrome P450 isoforms, given that PSP caused a dose-dependent decrease in the covalent binding of paracetamol (5). The effects of PSP on the activities of enzymes responsible for glucuronidation and sulphation also merits further investigation.

In conclusion, PSP increased the formation and biliary excretion of paracetamol glucuronide and, more significantly, paracetamol sulphate. It has been suggested that this increase in sulphate conjugation may be related to the previously reported effect of PSP on GSH turnover (5) and would, in part, contribute to the increase in disposition of paracetamol in moderate doses by PSP. We are at present further investigating the mechanism(s) of drug interactions of PSP with paracetamol.

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