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SCIENCE DIRECT
Food and Chemical Toxicology 44 (2006) 1607–1612
www.elsevier.com/locate/foodchemtox

Modulation of antipyrine clearance by polysaccharide peptide (PSP) isolated from *Coriolus versicolor* in the rat

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Received 13 October 2005; accepted 21 March 2006

Abstract

Polysaccharide peptide (PSP), isolated from *Coriolus versicolor COV-1*, has been previously shown to have immuno-stimulatory, anti-tumour and analgesic effects in animal models. When used as an adjunct in cancer chemotherapy in clinical trials carried out in China, PSP improved the quality of life in the patients by improving appetite and alleviating symptoms associated with cancer chemotherapy. In this study, the effects of non-toxic doses of PSP on phase I metabolism was investigated in the rat, using the conventional probe antipyrine. Acute PSP (3–5 μmol/kg, i.p.) treatment did not produce significant changes in antipyrine clearance. Sub-chronic treatment with PSP (1–3 μmol/kg/day, i.p., 3 days) decreased the antipyrine clearance (30–35%), with an increase in the plasma half-life ($T_{1/2}$) by 55% and an increase in the area under concentration–time curve (AUC) by 61%. Total hepatic cytochrome P450 (P450) was dose-dependently decreased (32–54%) after sub-chronic, but not the acute treatment of PSP. Given that PSP can affect phase I metabolism and hepatic cytochrome P450 content, the concomitant use of PSP with other therapeutic agents that undergo phase I metabolism should be carefully monitored.

Keywords: Polysaccharide peptide (PSP); *Coriolus versicolor*; Pharmacokinetics; Antipyrine; Cytochrome P450

1. Introduction

Polysaccharide peptide (PSP) is a protein-bound polysaccharide isolated from a Chinese fungus, *Coriolus versicolor* strain *COV-1*. PSP consists of a polypeptide chain containing over 20 different amino acids and five different sugars including arabinose, glucose, galactose, mannose and xylose (Yang et al., 1993). The immuno-modulatory effect of PSP has been well established in human tumour cell lines in vitro (Wang et al., 1999) and in laboratory animals (Yang et al., 1993). It has been suggested that PSP may be useful as an adjunct to chemotherapy and radiotherapy. Phase III clinical trials completed in 14 hospitals throughout China suggested that PSP improved the quality of life of cancer patients by decreasing cancer treatment-related symptoms such as fatigue, loss of appetite, nausea and vomiting, and pain associated with cancer/cancer treatment (Liu et al., 1999). PSP improved symptoms like dryness of throat and mouth, night sweating, and vomiting in patients taking 5-fluorouracil for stomach cancer (Zhang et al., 1999); restored cyclophosphamide-induced immuno-suppression by increasing the natural killer cell functions in patients (Qian et al., 1999). In Hong Kong, PSP and similar polysaccharide peptide products are commonly used as health supplements to enhance "immuno-modulatory functions" and are freely available over-the-counter.

Despite the popular use of PSP as an adjuvant to cancer treatments in China, the metabolism of PSP has not been elucidated, although PSP is expected to be metabolized to its constituent polysaccharides and small peptides in vivo. Previous studies (Yeung et al., 1994) showed that PSP

Abbreviations: Area under concentration–time curve, (AUC); Cytochrome P450, (P450); Half-life, ($T_{1/2}$); Initial concentration, ($C_{initial}$); Polysaccharide peptide, (PSP); Volume of distribution, (V_d); Total clearance, (CL).

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doi:10.1016/j.fct.2006.03.008

protected paracetamol-induced hepatotoxicity by reducing the formation of the chemically reactive metabolite of paracetamol in the rat. Further studies showed that the phase II metabolites of paracetamol (glucuronide and sulphate) were significantly increased by PSP, which may reflect a decrease rate of metabolism through the phase I pathway (Yeung et al., 1995). More recently, PSP has been shown to delay the clearance of cyclophosphamide (Chan and Yeung, 2006), an anti-cancer agent commonly used with PSP in cancer chemotherapy (Qian et al., 1999). These results suggested that PSP may affect the pharmacokinetics of other therapeutic agents, possibly by altering the metabolism of these compounds. However, the effects of PSP on the activity of phase I and II drug metabolizing enzymes remained to be studied. In this study, the model compound antipyrine was used to investigate the potential of PSP in affecting phase I metabolism in vivo. Antipyrine clearance is a function of its metabolism in vivo since antipyrine is solely metabolized by hepatic cytochrome P450 (P450) (Tanaka et al., 1985). The study should provide information on how PSP may have contributed to the decrease in phase I metabolism-related generation of chemically reactive metabolite of paracetamol previously observed (Yeung et al., 1995) and more importantly, the effects of PSP on phase I metabolism per se after acute and chronic treatment, using antipyrine clearance as the in vivo indicator. In view of the popular use of PSP and similar polysaccharide peptide-based products, this study should provide additional insight on the natural product-drug interaction potential of PSP and similar compounds.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (250–300 g) were supplied by the Laboratory Animal Service Centre, The Chinese University of Hong Kong (CUHK). The animals were kept in animal holding room under standard conditions with 12-h light–dark cycle, with free access to rodent cubes (Glen Forrest Stockfeeders, Australia) and tap water. All the procedures had been approved by the Animal Experimentation Ethics Committee (CUHK) in accordance to the Department of Health (Hong Kong) guidelines in Care and Use of Animals.

2.2. Materials

Polysaccharide peptide (PSP), isolated from deep-layer cultivated mycelia of *C. versicolor*, was provided through Winsor Health Products Ltd. (Hong Kong) and authenticated by Professor Q.Y. Yang (Shanghai Teachers' University, China). The PSP sample was composed of 90% polysaccharides (74.6% glucose, 4.8% xylose, 2.7% galactose, 1.5% mannose, and 2.4% fucose) and 10% peptides (18 different amino acids, mostly aspartic acid and glutamic acid). The PSP sample used in this study has a molecular weight of 100 kD, analyzed by gel electrophoresis, according to the method by Zhou and Yang (1999) and was free from lipopolysaccharide. β-Nicotinamide adenine dinucleotide phosphate (NADP), D-glucose 6-phosphate, glucose 6-phosphate dehydrogenase, cimetidine, diclofenac sodium, dexamethasone-21-phosphate, heparin sodium, urethane, and phenacetin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Phenobarbitone sodium was purchased from Universal Pharmaceutical Lab. (Hong Kong). Carbon monoxide was supplied by Hong Kong Special Gas Company. All agents were dissolved in normal saline solution (0.9% NaCl) unless specified.

2.3. Effects of PSP treatments on the clearance of antipyrine

For the acute treatments, six animals per group were treated with PSP (3–5 μmol/kg, i.p.), cimetidine (258 μmol/kg, i.p.) or saline (control). Lower doses (2 μmol/kg) of PSP were not selected because PSP with lower doses did not affect the clearance of cyclophosphamide (Chan and Yeung, 2006). After the pre-treatments, the rats were anaesthetized with urethane (20% w/v, 6 ml/kg, i.p.). The carotid artery was cannulated for collecting blood sample, jugular vein for injection of antipyrine and replacing saline, and trachea for removing mucous. A single dose of antipyrine (398 μmol/kg, i.v.) was given via jugular vein 1 h after PSP treatment. Serial blood samples (0.3 ml) were collected via the carotid artery at 0, 10, 20, 40, 60, 90, 120, 150 and 180 min after antipyrine administration. Heparinized saline (100 units/ml) was given to prevent blood coagulation. Saline was replaced via jugular vein each time after a blood sample collection (Chow et al., 1992). Plasma was separated and stored at −20 °C prior to analysis by high performance liquid chromatography (HPLC).

For the sub-chronic treatments, six animals per group were pre-treated with PSP (1–3 μmol/kg/day, i.p.), phenobarbitone (102 μmol/kg/day, i.p.) or saline (control, i.p.) for 3 days. The dosages of PSP used were based on previous studies when PSP was well tolerated and showed no sign of toxicity to the laboratory animals (Yeung et al., 1994, 1995). During the pre-treatment period, the rats were kept in a 12-h light–dark cycle animal room with controlled temperature and humidity. Free access to laboratory rodent diet and tap water was allowed. One day after the final pre-treatment, the rats were anaesthetized and experiments were performed as described in previous section. In another set of experiment, animals were pre-treated with diclofenac (20 mg/kg/day, i.p.) or dexamethasone (1 mg/kg/day, i.p.) before PSP was administered, followed by study on antipyrine clearance.

2.4. Determination of plasma antipyrine by high performance liquid chromatography (HPLC)

Aliquot of each sample (100 μl) was mixed with 5 μl phenacetin (internal standard, 12.5 μg/ml final concentration) (Chow et al., 1992). Methanol (300 μl) was added for extraction to precipitate the plasma proteins. The mixture was vortex-mixed for 10 s, followed by centrifugation at 13,000 rpm for 10 min. The supernatant was passed through a 0.5 μm filter (Millex^{R}-LCR$_{13}$). The HPLC system included a Hewlett Packard 1050 series pumping system and multiple wavelength detector at 244 nm. Elutes (25 μl) were analyzed by reversed-phase C18 (Supelco Spherisorb S5/ODS2, 250 × 4.6 mm) column. The mobile phase was 30:70 acetonitrile–phosphate, 0.25 M, pH 6.4 with 0.1% triethylamine. The retention time of antipyrine and phenacetin were 6 min and 10 min, respectively. The limit of detection was 10 μg/ml. The relative standard deviation of the HPLC system for antipyrine was 3.7% at 50 μg/ml and 2.3% at 100 μg/ml. The accuracy of the standard curve is 96%, 98% and 99% at 25, 100 and 200 μg/ml antipyrine, respectively.

2.5. Effects of PSP on hepatic cytochrome P450 (P450) contents in vivo

Ten animals in each group were pre-treated with single dose PSP (4 μmol/kg, i.p.) or for 3 days (0.5–4 μmol/kg/day, i.p.), or saline. Another six animals were pre-treated with phenobarbitone (102 μmol/kg/day, i.p., 3 d). The animals were killed by cervical dislocation 1 h after acute treatment or 1 day after the final treatment for sub-chronic study. The procedures of collecting hepatic microsomal fraction were based on the method of Guengerich et al. (1982) at 4 °C. Briefly, the liver was removed and homogenized in Tris/KCl buffer (0.05 M, pH 7.4). The homogenate was centrifuged at 9100 rpm for 25 min. The supernatant was collected and centrifuged at 35,000 rpm for 1 h. The pellet was resuspended in Tris/KCl buffer, followed by centrifugation at 35,000 rpm for 1 h. Microsome