ISSN: 0975-766X

Available Online through
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Research Article

PARACETAMOL DISPOSITION FOLLOWING ACUTE AND SUB-CHRONIC TREATMENT OF FRIED ONIONS

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Received on 27-10-2010 *Accepted on 15-11-2010*

ABSTRACT

Objective: To investigate the effect of fried onion pretreatment on disposition of paracetamol and its major metabolites in humans

Design: Before / after pretreatment non-blind investigation conducted in healthy male volunteers

Subjects: Twelve healthy men aged between 21 and 30 y submitted their written informed consent to participate in the study after thorough screening

Intervention: All the subjects received 1000 mg of paracetamol alone after overnight fasting, and urine was collected at 0-4 and 4-8 h intervals. Unchanged paracetamol and its metabolites were estimated using HPLC. After a washout period (10 half lives) 250 g of fried onions were ingested 3 h after oral administration of paracetamol 1000 mg, and paracetamol and its metabolites were again determined. In the second study 250 g of fried onions were ingested daily for 10 days and on the 11th day 250 g of fried onions were ingested prior to 1000 mg paracetamol administration. Urinary metabolites were estimated in the same way.

Results: Pretreatment with fried onions (one day treatment) decreased the urinary levels of paracetamol and its metabolites over 8 h, but statistically insignificant. 10 d pretreatment showed statistically significant reduction of urinary paracetamol and its metabolite levels. Unchanged paracetamol, sulfate conjugate, glucuronide conjugate,

cysteine conjugate and mercapturic acid conjugate levels were decreased by 56.99%, 27.20%, 60.70%, 67.47%, and 49.62% respectively.

Conclusions: Fried onion pretreatment decreased the urinary excretion levels of paracetamol and its metabolites indicate that fried onions might impairs the absorption and / or induce the drug transporters like P-glycoproteins.

Keywords: fried onions; paracetamol; glucuronide; sulfate; cysteine; mercapturate

INTRODUCTION

Flavonoids are a group of polyphenolic compounds, which are widely distributed throughout the plant kingdom, and consist of about 3000 varieties (5). They have a variety of biological effects on various mammalian cell systems, *in vitro* as well as *in vivo* and have been shown to inhibit the growth of various cancer cell lines and reduce tumor progression in experimental animals (9). Absorption distribution, metabolism and excretion of various flavonoid compounds in experimental animals and in humans have been studied using flavonoid rich diets like onions, apples and tea (10). Flavonoids appear to influence Cytochrome P450 mediated metabolism of some drugs and some of them have been reported to modulate P-glycoprotein activity, thus affecting the efflux of drugs and other xenobiotics from the cells (10). Dietary intake of flavonoids in Western Europe is estimated to be about 25 mg per day of flavonol (quercetin, kaempferol and myricetin) and flavone (luteolin and apigenin) aglycones. The main sources are tea, onions, apples and red wine; 70% - 90% of the total flavonol intake was quercetin(4,8) Major dietary sources of quercetin show considerable geographical and cultural variation; in Italy the main source is red wine, in china the main source is tea and in US and Northern Europe the main source is onions (4).

Dietary flavonols are absorbed and appear in plasma and urine as potential biomarkers in concentration related quantitatively to intake (8). There are five different glucuronides of quercetin estimated in human plasma samples by means of HPLC-UV-MS/MS following consumption of fried onions (14).

Paracetamol is an analgesic-antipyretic agent; available without prescription and recommended therapeutic dosage is usually well tolerated. However, acute overdose causes fatal hepatic damage and the number of self-poisoning and suicides with paracetamol have grown alarmingly in the recent years. The metabolism of paracetamol has been a

subject of interest in view of the discovery of metabolic activation of paracetamol by Cytochrome P-450 dependent mixed function oxidase to a reactive arylating intermediate, which is normally conjugated with glutathione and eventually excreted as cysteine and mercapturic acid conjugates. Upon over dosage of paracetamol, the reactive arylating intermediate becomes bound covalently to hepatocytes causing hepatic necrosis. There are individual differences in susceptibility to hepatotoxicity depending on the rate of metabolic activation and the extent of glutathione depletion. An important group of conjugation reactions are catalyzed by the uridine 5'-diphosphate (UDP) – glucuronosyltransferase (UGTs); to date at least 10 different UGT isoforms have been identified (1). Conjugation with sulfate is a major pathway for the biotransformation of phenolic drugs like paracetamol in humans and many animal species (7). The disposition of paracetamol could be described by a two-compartment model with simultaneous first-order and Michaelis-Menten type elimination kinetics for paracetamol sulfate conjugation (13). There were no reports available on pretreatment by flavonoids rich diets (fried onions) on reactive oxygen species produced compounds like paracetamol. Hence we studied the role of fried onions on the disposition of paracetamol by estimating the urinary levels of paracetamol metabolites by using HPLC in two investigations (acute and sub-chronic study).

METHODS

Study population:

Twelve healthy male volunteers aged between 21 to 30 y and weighing 50 to 75 kg participated in the study after undergoing thorough physical examination. The volunteers were briefed about the study and informed consent was obtained from them. The local ethical committee approved the study. Volunteers had no history of ill health during the preceding 6 mo and none had taken any medication at least a week prior to the administration of paracetamol tablets in the study. Only non-smokers, non-tobacco chewers, and non-alcoholics were included into the study. Volunteers who were excluded from the study were allergic to food and paracetamol.

Study protocol:

After an overnight fast the subjects were given orally 1000 mg (two 500mg tablets) of paracetamol (Calpol®, Burroughs Wellcome, Mumbai, India) with a glass of water. Entire urine that was voided during 0-4 and 4-8 h following drug administration was collected and volume measured. Aliquots were stored at -20°C until analysis. Unchanged paracetamol and its metabolites were estimated using HPLC. After a washout period (10 half lives) 250 g of fried onions were ingested, after 3 h paracetamol 1000 mg was given orally, paracetamol and its metabolites were estimated. In the second study 250 g of fried onions were ingested daily for 10 d and on the day 11^{th} 250 g of fried onions were ingested prior to administration of 1000 mg of paracetamol. Urinary metabolites were estimated in the same way.

HPLC analysis:

Paracetamol and its metabolites in urine samples were estimated by a reversed phase high performance liquid chromatography (HPLC) method (11). Chromatography was performed with a Shimadzu system including a LC-8A solvent delivery module and SPD-10A*VP* UV-Visible spectrophotometric detector. An octadecyl silane reverse phase C_{18} stainless column (Adsorbosphere C_{18}) of length 25 cm and 4.6mm internal diameter was used with 1% acetic acid: methanol: ethyl acetate (90:15: 0.1) as mobile phase. The flow rate was 1 ml/min and detector wavelength was set to 250 nm. 0.25 ml of urine sample was diluted with double distilled water and made up to 5 ml. After vortexing 20 µl was injected on to the HPLC column. The metabolites and unchanged paracetamol were eluted in the following sequence, sulfate conjugate, glucuronide conjugate, unchanged paracetamol, cysteine conjugate and mercapturic acid conjugate. The peak areas were measured for paracetamol and all the metabolites. A calibration graph of paracetamol was drawn in the concentration range of 0.5 to 50 µg/ml ($r^2 = 0.99$).

Data Analysis:

The proportions of unchanged paracetamol and its conjugates were expressed as percentage dose excreted during 0-4 and 4-8h. The mean percentage recovery of metabolites obtained was compared using Student's t-test. A difference was considered significant when the probability of chance explaining the results was less than 0.05.

RESULTS

Pretreatment with fried onions (one day treatment) decreased the urinary levels of paracetamol and its metabolites over 8 h, but the reduction was statistically insignificant. The mean ± SD values of unchanged paracetamol, sulfate conjugate, glucuronide conjugate, cysteine conjugate, and mercapturic acid conjugate levels were 30.92 ± 6.82, 136.86 ± 28.71, 425.64 ± 119.62, 24.61 ± 9.47 and 13.33 ± 4.57 respectively (Figure 1). A 10 day pretreatment (sub-chronic study) showed significant reduction of urinary paracetamol and its metabolite levels. The mean ± SD values of unchanged paracetamol, sulfate conjugate, glucuronide conjugate, cysteine conjugate, and mercapturic acid conjugate levels were 13.71 ± 6.11, 99.13 ± 25.19, 167.78 ± 31.08, 8.08 ± 3.15 and 6.76 ± 2.87 respectively (Figure 2). Unchanged paracetamol, sulfate, glucuronide, cysteinate, and mercapturate levels were decreased by 56.99%, 27.20%, 60.70%, 67.47%, and 49.62% respectively.

DISCUSSION

The glucuronide conjugate is the major urinary metabolite of paracetamol. This conjugation occurs in the liver by a family of UGTs. Gender and use of oral contraceptives affect glucuronidation. Glucuronidation of paracetamol is saturable in humans following over dosage and this would cause liver damage because a disproportionately large fraction of dose is then shunted towards the pathway of toxic metabolic activation (6). Sulfate conjugation of paracetamol is saturable and dose-dependent and a major parallel route of non-toxic elimination of paracetamol in many species and usually there is a reciprocal relationship with the extent of glucuronide conjugation. Sulfation of paracetamol in humans is dependent on the availability of inorganic sulfate and doses of paracetamol in the range 750-1500 mg causes in proportion of serum sulfate concentration (12). In our study there was increased levels of both glucuronoid and sulfate conjugates following paracetamol administration alone as suggested by earlier authors.

Quercetin glycosides from onions need to be liberated from the food marix before being absorbed. This may interfere in the absorption of paracetamol. Previous studies also suggest that quercetin aglycone affects the P-glycoprotein (P-gp) mediated transport of some drugs. Hence the components present in the fried onions may induce the P-gp efflux of paracetamol from the serosal to luminal side resulting in the decreased plasma levels of

paracetamol and also its metabolites. This may be one of the beneficial effects that the fried onions decrease the liver toxicity by generation of reactive oxygen free radicals produced by some phenolic drugs like paracetamol.

Quercetin can be detected in plasma and urine by measured the sum of free quercetin along with glucuronides and sulfates formed by conjugation in the liver or the small intestine (4). The interaction of components of fried onions in the process of glucuronidation and sulfation competitively with the paracetamol conjugation cannot be ruled out; hence paracetamol and its metabolite levels were decreased following ingestion of fried onions.

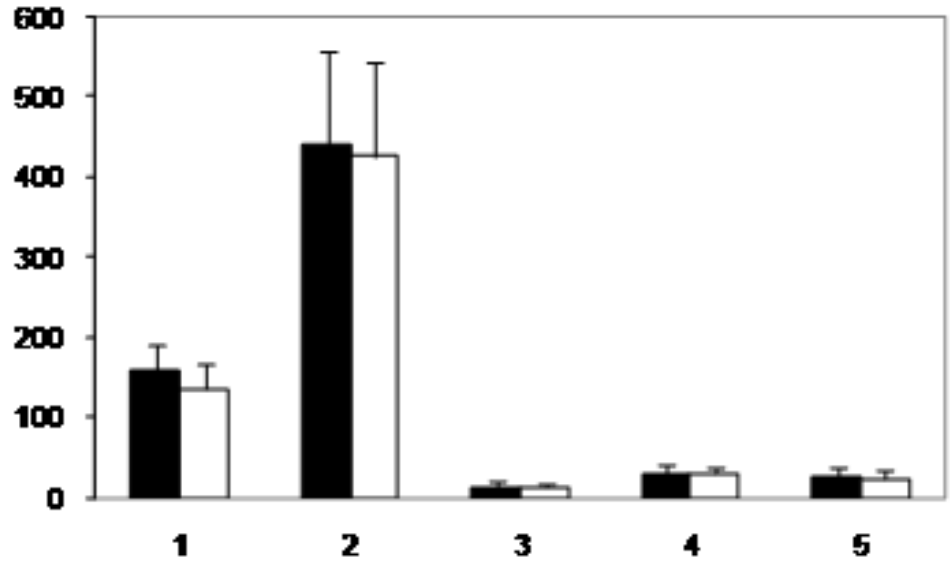
CONCLUSION

Fried onion pretreatment decreased the urinary excretion levels of paracetamol and its metabolites indicating that fried onions might impair the absorption of paracetamol and / or enhance it exorption into the intestines by inducing the drug transporters like P-glycoproteins.

ACKNOWLEDGEMENT

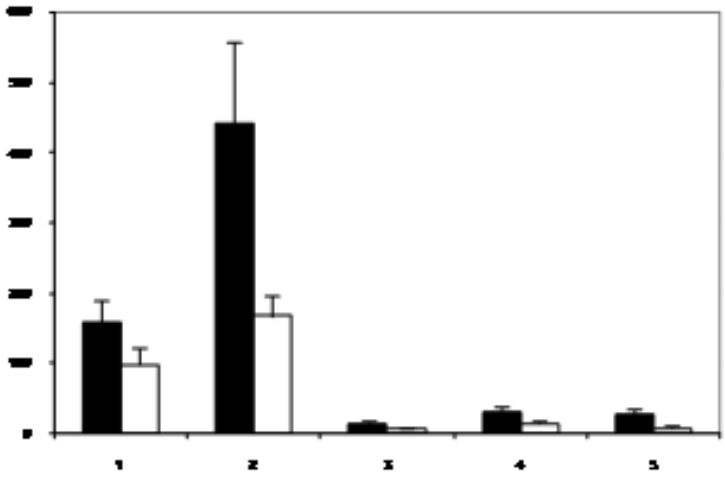
This work was funded by the university grants commission (ugc) and council of scientific and industrial research (csir), new delhi, india.

Fig-1:



Sulfate Glucuronide Mercapturate Paracetamol Cysteine

Fig-2:



Sulfate Glucuronide Mercapturate Paracetamol Cysteine

Legends to figures:

Paracetamol and its metabolite levels following ingestion of fried onion pretreatment.

(A) One day pretreatment of fried onions, (B) Ten days pretreatment of fried onions

Y-Axis: Urinary levels (mg/ml)

■ Paracetamol alone □ Paracetamol and Fried onions

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