Short Communication

Consumption of Watercress Fails to Alter Coumarin Metabolism in Humans

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ABSTRACT:

Watercress is an excellent source of phenethyl isothiocyanate (PEITC), an effective inhibitor of nitrosamine carcinogenesis in rodents. The mechanism of inhibition is believed to be due in part to inhibition of cytochrome P450 (P450) enzymes. P450 2A6 is a catalyst for the metabolic activation of several nitrosamines. In this study, we investigated the effect of watercress consumption on coumarin 7-hydroxylation, a P450 2A6-specific reaction, in a group of 15 nonsmoking, healthy volunteers. The urinary excretion of 7-hydroxycoumarin (7OHC) was determined. For 6 of the 15 subjects, watercress consumption decreased the amount of 7OHC excreted in the first 2 h following coumarin administration. However, the mean 0- to 2-h excretion of 7OHC for all 15 subjects was not significantly lowered by the consumption of watercress, 2.8 ± 0.78 versus 3.1 ± 0.53 mg (±S.D.). The mean 7OHC excreted from 2 to 4 h (1.1 ± 0.50 mg) was significantly higher (P = 0.027) during watercress consumption than before (0.77 ± 0.22 mg), suggesting a delay in coumarin metabolism. Total excretion of 7OHC was unaffected by watercress consumption. Therefore, under the conditions of our study, PEITC and other constituents of watercress had at most a marginal inhibitory effect on P450 2A6-catalyzed coumarin 7-hydroxylation.
Materials and Methods

Study Design. The protocol was approved by the University of Minnesota Research Subjects’ Protection Programs Institutional Review Board Human Subjects Committee. Subjects were healthy nonsmokers ranging in age from 19 to 30 (mean, 24.7 ± 3.8). There were eight males and seven females. Coumarin was provided by Schaper and Brummer (Salzgitter, Germany) and 5-mg capsules were prepared by the Investigational Drug Pharmacy at the University of Minnesota.

The study was carried out over 2 weeks. During both weeks of the study, all subjects ate their usual diet, except that cruciferous vegetables, mustard, and salad dressing were excluded. Week 1 was the baseline period. On visit 1, subjects were given an orientation, and they signed consent forms. On visits 2 to 4, at 8:00 AM subjects ate a bagel with cream cheese. Fifteen minutes after finishing the bagel, they were administered a capsule containing 5 mg of coumarin with 200 ml of water. One and 3 h after taking coumarin, they drank 400 ml of water. Urine samples were collected 2 and 4 h after taking the coumarin. Week 2 was the watercress period. On visit 5, 4 days after visit 4, at 8:00 AM subjects ate a bagel with cream cheese and 2 oz (56.8 g) of watercress, obtained from Dehn’s (Andover, MN). They were given two more 2-oz portions of watercress in sealed plastic bags and were told to eat these uncooked at lunch and dinner. On visit 6, subjects reported at 8:00 AM and ate a bagel with cream cheese and 2 oz of watercress. Fifteen minutes after finishing the bagel and watercress, they were given a capsule containing 5 mg of coumarin, which they took with a glass of 200 ml of water. They then followed procedures exactly as in visits 2 to 4. They were given watercress to eat with lunch and dinner, as in visit 5. On visit 7, they followed the same procedure as in visit 6 but did not eat watercress at lunch or dinner. Coumarin was administered only on visits 6 and 7 because we were uncertain that a single watercress meal (e.g., breakfast on visit 5) would affect coumarin metabolism. All urine samples were stored at −20°C until analysis for 7OHC and/or N-acetyl-S-(N-phenethylthiocarbamoyl)-cysteine (PEITC-NAC).

Analysis of Urine. Analysis of urine for 7OHC was carried out by high-performance liquid chromatography essentially as described by Egan and O’Kennedy (1992) except detection was by fluorescence (excitation wavelength, 350 nm and emission wavelength, 453 nm). Analyses of PEITC-NAC in urine and gluconasturtiin in watercress were performed by high-performance liquid chromatography as described previously (Chung et al., 1992; Preestera et al., 1996). Data from baseline and watercress periods were compared using Student’s t test.

Results and Discussion

Each 2-oz portion of watercress used in this study contained 112 mg (0.26 mmol) of gluconasturtiin. Therefore, the maximum amount of PEITC that could be released was 43 mg. The urine of our subjects was analyzed for PEITC-NAC, a major metabolite of PEITC (Chung et al., 1992). An average of 12.6 ± 3.6 mg (mean ± S.D., n = 15) of PEITC-NAC, corresponding to 6.3 mg of PEITC, was excreted from 0 to 4 h following consumption of the watercress breakfast. Therefore, the minimum mean uptake of PEITC was 6.3 mg per subject.

Sixty to 75% of orally administered coumarin is converted to the glucuronide of 7OHC (Pelkonen et al., 1997). The glucuronide was hydrolyzed and the released 7OHC quantified. In the baseline period, the mean amount of 7OHC excreted in 4 h was 3.9 ± 0.6 mg, or 70% of the administered dose (Table 1, column 8) and the majority, 80%, was excreted in the first 2 h (Table 1, column 2). The amount of coumarin excreted as 7OHC was determined on baseline visits 2 to 4 for each subject. The mean standard deviation among analyses for all 15 subjects at baseline was 15 ± 0.9%. There was no significant variation in 7OHC excretion on days 2 to 4, and there was no evidence that administration of coumarin affected coumarin metabolism on subsequent days. The effect of watercress consumption on the level of urinary 7OHC was determined on visits 6 and 7 (Table 1). In six subjects, mean levels of 7OHC in urine collected 2 h after coumarin dosing were lower in the watercress period than in the baseline period, while in the other nine subjects there was no change (Table 1, columns 2–4). Overall, the amount of 7OHC in these urine samples at baseline (3.1 ± 0.53 mg) was not significantly different from the amount after watercress consumption (2.8 ± 0.78 mg).

In the urine samples collected in the 2 to 4 h period after dosing with coumarin, levels of 7OHC were higher in five samples after watercress consumption than at baseline, while no change was observed in the other 10 (Table 1, columns 5–7). Three of the increases...
were seen in samples from individuals (subjects 2, 12, and 15) whose levels of 7OHC were lower in the watercress consumption than in the baseline period, for the 0- to 2-h urine collection. Overall, levels of 7OHC after watercress consumption (1.1 ± 0.50) were significantly higher (P = 0.027) than at baseline (0.77 ± 0.22) in the 2- to 4-h urine samples. Total excretion of 7OHC, 0 to 4 h after taking coumarin, was similar to previous studies (Pelkonen et al., 1997) and was unaffected by watercress consumption (3.9 ± 0.6 versus 3.9 ± 0.84 mg).

In subjects 6 and 15, the excretion of 7OHC was markedly different on the two days of watercress consumption. Subject 6 showed delayed metabolism to 7OHC on the 1st day of watercress consumption (1.2 mg, 0–2 h; 2.3 mg, 2–4 h), but not on the 2nd (2.6 mg, 0–2 h; 0.11 mg, 2–4 h), while subject 15 showed the opposite effect (2.7 mg, 0–2 h; 2.2 mg, 2–4 h on day 1; 0.5 mg, 0–2 h; 3.1 mg, 2–4 h on day 2). This was not due to differing uptake of PEITC on the 2 days (data not shown), nor was it due to analytical variation. Subjects 6 and 15 excreted consistent amounts of 7OHC on each of the 3 baseline days. When the data for subjects 6 and 15 on the watercress consumption days were omitted, the means were 3.0 ± 0.70 (n = 13, 0–2 h period) and 1.0 ± 0.42 (n = 13, 2–4 h period). The results were essentially the same as those obtained when subjects 6 and 15 were included.

Measurement of urinary 7OHC, 0 to 2 and 2 to 4 h after dosing with coumarin, is an effective way to detect changes in coumarin metabolism due to changes in P450 2A6 (Pelkonen et al., 1997). A study that used a protocol similar to ours reported a 25% decrease in 7OHC excretion in 0 to 2 h when subjects were administered grapefruit juice 30 min before coumarin. Therefore, if watercress consumption had inhibited P450 2A6 activity, we would have expected to see a decrease in urinary 7OHC 0 to 2 h after coumarin administration. However, our results indicate that, under the conditions of our study, PEITC and other constituents of watercress had at most a marginal inhibitory effect on P450 2A6 activity.

These results are consistent with those of a recent study in which we analyzed nicotine metabolites in the urine of smokers before, during, and after a period of watercress consumption (Hecht et al., 1999). P450 2A6 appears to play a major role in the metabolism of nicotine to cotinine, as well as in the further metabolism of cotinine to trans-3′'-hydroxycotinine and other metabolites (Nakajima et al., 1996a; Yamazaki et al., 1999). We found no effect of watercress consumption on these reactions. However, watercress consumption did result in increased glucuronidation of nicotine, cotinine, and trans-3′'-hydroxycotinine (Hecht et al., 1999).

In humans, P450s 1A2, 3A4, and 2A6 appear to play a role in NNK metabolic activation (Hecht, 1998). P450 2A6 appears to play a major role in the metabolism of nicotine to cotinine, as well as in the further metabolism of cotinine to trans-3′'-hydroxycotinine and other metabolites (Nakajima et al., 1996a; Yamazaki et al., 1999). We found no effect of watercress consumption in these reactions. However, watercress consumption did result in increased glucuronidation of nicotine, cotinine, and trans-3′'-hydroxycotinine (Hecht et al., 1999).

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References


