Apiaceous Vegetables and Cruciferous Phytochemicals Reduced PhIP-DNA Adducts in Prostate but Not in Pancreas of Wistar Rats

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ABSTRACT We previously showed rats fed with apiaceous vegetables, but not with their putative chemopreventive phytochemicals, reduced colonic DNA adducts formed by 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), a dietary procarcinogen. We report here the effects of feeding apiaceous and cruciferous vegetables versus their purified predominant phytochemicals, either alone or combined, on prostate and pancreatic PhIP-DNA adduct formation. In experiment I, male Wistar rats received three supplemented diets: CRU (cruciferous vegetables), API (apiaceous vegetables), and CRU+API (both types of vegetables). In experiment II, rats received three diets supplemented with phytochemicals matched to their levels in the vegetables from experiment I: P+I (phenethyl isothiocyanate and indole-3-carbinol), FC (furanocoumarins; 5-methoxypsoralen, 8-methoxypsoralen, and isopimpinellin), and COMBO (P+I and FC combined). After 6 days of feeding, PhIP was injected (10 mg/kg body weight) and animals were killed on day 7. PhIP-DNA adducts were analyzed by LC-MS/MS. In prostate, PhIP-DNA adducts were reduced by API (33%, $P < .05$), P+I (45%, $P < .001$), and COMBO (30%, $P < .01$). There were no effects observed in pancreas. Our results suggest that fresh vegetables and purified phytochemicals lower PhIP-DNA adducts and may influence cancer risk.

KEYWORDS: • 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine • apiaceous vegetables • cruciferous vegetables • DNA adducts • heterocyclic aromatic amines

HETEROCYCLIC AROMATIC AMINES (HAAs) are a group of environmental carcinogens and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) is one of the most mass abundant HAAs.^{1,2} Human exposure to PhIP is primarily through consumption of overcooked meats,³ and epidemiologic studies indicate a positive correlation between PhIP exposure and prostate and pancreatic cancer risk.^{4,5}

We recently showed that apiaceous vegetables, but not their putative chemopreventive phytochemicals, decreased colonic PhIP-DNA adducts.⁶ We also demonstrated that purified phytochemicals from cruciferous vegetables decreased PhIP-DNA adducts in rat colon, but with less effect from their whole vegetable source.⁷ However, there is no study that examined the effects of combined intake of two different vegetable families or their phytochemicals on prostate and pancreas PhIP-DNA adducts. Hence, we exam-

ined here whether feeding the aforementioned would result in similar effects in rat prostate and pancreas as seen in colon.

Male Wistar rats were acclimated for the first 5 days, had free access to water and diet, and were handled per protocols approved (0807A40602) by the University of Minnesota Animal Care and Use Committee. For experiment I, diets were supplemented at 21% w/w with three different vegetable combinations: cruciferous only (CRU; $n = 10$), apiaceous only (API; $n = 10$), and combination of both cruciferous and apiaceous (CRU+API; $n = 10$).⁶ CRU included fresh watercress, broccoli, and cabbage (7% per each vegetable), whereas API consisted of fresh celery and parsnips (10.5% per each vegetable); CRU+API included all of the aforementioned vegetables in half the amounts to maintain 21% w/w. In experiment II, the supplemented diets contained one of three different phytochemical combinations at levels matching their whole vegetable counterpart fed in experiment I: glucosinolate metabolites (PEITC and I3C; P+I, 183 mg of each compound/kg diet; $n = 10$), furanocoumarins (5-methoxypsoralen [5-MOP], 8-methoxypsoralen [8-MOP], and isopimpinellin; FC, 0.606, 0.299, and 1.24 mg/kg diet, respectively; $n = 10$) and the combination of the phytochemicals (COMBO; $n = 10$) as previously described and discussed.⁷ AIN-93G purified diet was used for control groups ($n = 10$ –12). After acclimation, rats were divided into five experimental feeding groups, including negative and

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positive control groups. After 6 days of feeding, all animals (except the negative control groups) were injected intraperitoneally (10 mg of PhIP/kg body weight). On day 7, animals were anesthetized and killed by exsanguination and then tissues were collected for further analyses.

Prostate and pancreatic tissues were harvested from the same animals we previously utilized.^{6,7} PhIP-DNA adducts in prostate and pancreas were measured using an online column-switching LC-MS/MS method in which the ratio between unlabeled PhIP-DNA adducts and isotopically labeled internal standard was used to quantify the adduct levels.⁶

Results are expressed as mean $\pm$ standard error of the mean. All analyses were conducted using the SAS package (SAS 9.2; Cary, NC, USA). $P < .05$ was considered statistically significant. Data were initially analyzed using ANOVA followed by the least square means test.

In both experiments, overall, animals had similar weight gain and growth patterns and no statistically significant differences among diet groups within each experiment were observed. In experiment I, supplementation of fresh apiaceous vegetables reduced PhIP-DNA adducts by 33% ($P < .05$) in the prostate compared with the positive control group (Fig. 1A). In experiment II, PhIP-DNA adducts were reduced in the P+I group by 45% ($P = .001$) compared with positive control and reduced by 30% ($P = .01$) in the COMBO group (Fig. 2A). We saw no reduction in PhIP-DNA adducts in the pancreas for either experiment (Figs. 1B and 2B).

To our knowledge, this is the first attempt to investigate the effects of combined intake of two different vegetable families, in their fresh vegetable form, and their isolated, matched phytochemical compounds, on a biomarker of carcinogenesis in the prostate and pancreas. We demonstrated that CRU did not decrease PhIP-DNA adduct levels in the prostate, but the P+I did. This lack of effect in the fresh CRU group could be because PEITC and I3C levels fed in the phytochemical diet were matched to the total glucosinolate content in our fresh cruciferous feeding due to the variety of glucosinolates in the vegetables with potential bioactivity, yet lack commercially available standards for individual measurement as previously discussed.⁷ Thus, these two specific components were higher than in our fresh cruciferous feeding, which would have contained a number of different glucosinolates in addition to the parent compounds of PEITC and I3C. Possibly, other factors might have influenced this difference, such as bioavailability or incomplete hydrolysis of parent glucosinolates.⁷

Similar to our prior observations in rat colon,^{6,7} API reduced PhIP-DNA adducts in the prostate, whereas no effect was seen with supplementation of the furanocoumarins, which have been suggested to be the responsible agents for the potential chemopreventive effects of apiaceous vegetables. These consistent results suggest that 8-MOP, 5-MOP, or isopimpinellin may not be the primary compounds responsible for the chemoprotective effect seen with the addition of fresh apiaceous vegetables to the diet and that, therefore, constituents other than furanocoumarins within the apiaceous vegetable family might reduce PhIP-DNA adduct levels in the prostate. CRU+API had no effect on

FIG. 1. PhIP-DNA adducts in prostate (A) and pancreas (B) after a 7-day diet intervention in Wistar rats. All results are expressed as least squares mean $\pm$ SEM ($n = 10$ –11 per group). * $P < .05$ compared with the positive control group. PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine; CRU, cruciferous vegetable supplemented; API, apiaceous vegetable supplemented; CRU+API, combined cruciferous and apiaceous vegetable supplemented. SEM, standard error of the mean.

adduct levels in the prostate. Given that apiaceous vegetables in API were effective as opposed to cruciferous, this lack of effect by CRU+API may be due to the lower dose of apiaceous vegetables provided in this diet (half of what was provided in API) to maintain 21% w/w of total vegetables across diets and thus tolerability of the diet.

The similarity in effect of the vegetables and phytochemicals on PhIP-DNA adducts in prostate and colon suggests a systemic modulation of PhIP metabolism and distribution, as opposed to tissue-specific metabolism. This is further supported by the dramatic effect of P+I on PhIP metabolizing liver enzymes; the P+I diet increased cytochrome P450 (CYP) 1A1 and CYP1A2 activities by 10.1- and 3.6-fold, respectively.⁷ In contrast, the effects of CRU on hepatic biotransformation enzymes were modest, and the CRU diet has failed to recapitulate the reduction of prostate and colonic PhIP-DNA adducts seen with P+I.⁶ In addition, the API diet increased urinary methylated PhIP metabolites,⁶ produced by another phase II biotransformation enzyme (e.g., *N*-methyl transferase). In both studies, methylated PhIP metabolites

FIG. 2. PhIP-DNA adducts in prostate (A) and pancreas (B) after a 7-day diet intervention in Wistar rats. All results are expressed as least squares mean $\pm$ SEM ($n=10-11$ per group). ** $P<.01$, and *** $P<.001$ compared with the positive control group. P+I, PEITC, and I3C supplemented; FC, furanocoumarin supplemented; COMBO, combination of P+I and FC supplemented.

were negatively correlated with colonic PhIP-DNA adducts,^{6,7} indicating a protective role of *N*-methyl transferases against PhIP-DNA adduct formation. Altogether, it seems that modulation of biotransformation enzymes may play a role in suppressing PhIP-DNA adduct formation in both prostate and colon, although the responsible enzymes may differ depending upon vegetable types.

We assessed PhIP-DNA adducts as a surrogate marker of carcinogenicity. However, it should be noted that induction of DNA lesions by chemical carcinogens is an early step of carcinogenesis. Also, there are different tissue-specific mechanisms involved in prostate carcinogenesis compared with those of colon. Specifically, PhIP causes genetic mutations in β -catenin and *APC*, thereby disrupting the Wnt/ β -catenin signaling pathway and eventually inducing oncogenes involved in colon cancer.⁸ In prostate, PhIP elevates p-AKT expression and induces the loss of a tumor suppressor gene, *PTEN*, to dysregulate the PTEN/PI3K/Akt signaling pathway,⁹ which is frequently disrupted in human prostate cancer.¹⁰ However, few studies have examined the

molecular mechanisms by which vegetable families and their phytonutrients may influence prostate cancer development and the PI3K/Akt signaling pathway. Traka *et al.* demonstrated that sulforaphane, a major isothiocyanate in broccoli, inhibited proliferation of human prostate cells and suppressed p-AKT-mediated gene expression in a murine prostate cancer model.¹¹ In addition, I3C also decreased phosphorylation of Akt and induced apoptosis in a prostate cancer cell line.¹² To our knowledge, no study has investigated the effects of furanocoumarins or apiaceous vegetables on the PI3K/Akt signaling pathway in relation to prostate cancer prevention. Further investigation of the effects of cruciferous and apiaceous vegetables (and bioactive constituents) on prostate cancer markers seems warranted.

There was no reduction in PhIP-DNA adduct levels in the pancreas in either experiment I or experiment II. Rat pancreatic tissue comprises multiple layers, including both lobular and connective tissues.¹³ This variability in tissue histology may have introduced inconsistency to our DNA extraction process, which led to the high intragroup variability in PhIP-DNA adduct levels. The high deviation in pancreatic PhIP-DNA adducts is similar to that of other studies in which the approximate standard deviation ranged from 40% to 75%¹⁴⁻¹⁶ with limited exception.¹⁷ This suggests a need to improve methods for analysis of pancreatic DNA adducts. Interestingly, the pattern of effect by each diet in the pancreas (lowest adducts in API group and P+I group) follows the same pattern seen in the prostate and the colon from these animals.^{6,7}

A key strength of the study is the feeding of two classes of vegetables, both alone and combined, at physiologically relevant doses that closely mimic the typical varied human diet.^{18,19} In addition, our investigation of the effects of apiaceous vegetables or their putative bioactive constituents against PhIP-DNA adducts in prostate and pancreas appears to be novel. In this, API reduced PhIP-DNA adducts in prostate, whereas furanocoumarins failed to reproduce the chemoprevention. Furthermore, we utilized prostate and pancreas tissues from the same rats previously examined for effects of vegetables on colon cancer risk; by doing so, the data suggest potential chemoprevention by the vegetables and phytochemicals primarily through systemic mechanisms such as modulation of PhIP metabolism and distribution, rather than tissue-specific metabolism. Our choice of PhIP-DNA adducts as the endpoint of our study has both advantages and disadvantages. Although DNA adduct formation is considered a necessary requirement for tumor induction,²⁰ Tang *et al.* found no statistically significant association between PhIP-DNA adduct levels and prostate cancer risk.²¹ Therefore, interpretation should be done with caution, particularly in the context of carcinogenesis, considering that such DNA lesions can be removed by repair mechanisms.²²

Overall, we conclude that fresh apiaceous vegetables, along with the cruciferous vegetable glucosinolate metabolites (P+I), play a role in modulation of DNA adduct formation in prostate, and thus may reduce cancer risk. Although a direct comparison between intact whole vegetable and pure

compound feeding was not feasible due to differences in PhIP-DNA adducts between control groups of their respective studies, our findings point to possible differences in action between whole foods and their bioactive constituents, suggesting that studies using isolated compounds may not replicate the effect found with whole foods.

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AUTHORS' CONTRIBUTIONS

S.P.T. and D.D.G. designed the research; M.A.M., J.K.K., C.M.G., D.D.G., and S.P.T. conducted the research; M.A.M., J.K.K., and D.D.G. analyzed the data; M.A.M., J.K.K., and S.P.T. drafted the article; M.A.M., J.K.K., D.D.G., and S.P.T. had primary responsibility for the final content. All authors read and approved the final article.

DISCLAIMER

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Cancer Institute or the National Institutes of Health.

AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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