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Increased Salicylate Concentrations in Urine of Human Volunteers after Consumption of Cranberry Juice

GARRY G. DUTHIE,*,† JANET A. M. KYLE,† ALISON MCE. JENKINSON,† SUSAN J. DUTHIE,† GWEN J. BAXTER,§ AND JOHN R. PATERSON§

Rowett Research Institute, Aberdeen AB21 9SB, Scotland, U.K., and Dumfries and Galloway Royal Infirmary, Dumfries DG1 4AP, Scotland, U.K.

The aim of this study was to assess whether regular consumption of cranberry juice results in elevations in urinary salicylate concentrations in persons not taking salicylate drugs. Two groups of healthy female subjects (11/group) matched for age, weight, and height consumed 250 mL of either cranberry juice or a placebo solution three times a day (i.e., 750 mL/day) for 2 weeks. At weekly intervals, salicylic acid and salicyluric acid (the major urinary metabolite of salicylic acid) concentrations were determined in urine by HPLC with electrochemical detection. Concentrations of salicylic acid in plasma were also determined. Consumption of cranberry juice was associated with a marked increase ($p < 0.001$) of salicyluric and salicylic acids in urine within 1 week of the intervention. After 2 weeks, there was also a small but significant ($p < 0.05$) increase in salicylic acid in plasma. The regular consumption of cranberry juice results in the increased absorption of salicylic acid, an anti-inflammatory compound that may benefit health.

KEYWORDS: Salicylic acid; salicyluric acid; cranberry juice; salicylates

INTRODUCTION

Acetylsalicylic acid (aspirin, 2-acetoxybenzoic acid) (**Figure 1**) has been used for more than 100 years to provide pain relief and to treat inflammatory conditions and fevers. More recently, regular intake has been associated with decreased incidence of cancers (*1*−*3*), particularly colon cancer (*4*, *5*), and three double-blind, randomized, placebo-controlled trials of aspirin have demonstrated an unequivocal reduction in colorectal adenomas, a precancerous lesion (*6*−*8*). After absorption from the stomach and small intestine, aspirin is very rapidly hydrolyzed to salicylic acid (2-hydroxybenzoic acid) (**Figure 1**) in the liver and blood, where it is tightly bound to plasma proteins and widely distributed to all tissues in the body. The mechanism(s) of the anti-inflammatory action of aspirin and salicylic acid is (are) unknown, but recently it has been shown to suppress the transcription of cyclo-oxygenase 2 (COX-2), a key enzyme in inflammatory processes and tumorigenesis (*9*, *10*).

Salicylic acid is present in plants, where it functions as a hormonal mediator of the systemic acquired resistance response to pathogen attack and environmental stress (*11*), and it is present in a wide range of fruits, vegetables, herbs, and spices of dietary relevance (*12*−*17*). This has led to the suggestion that the recognized effects of consuming fruits and vegetables on lowering risk of colon cancer may be due in part to salicylates in plant-based foods (*18*). However, estimated daily intakes of

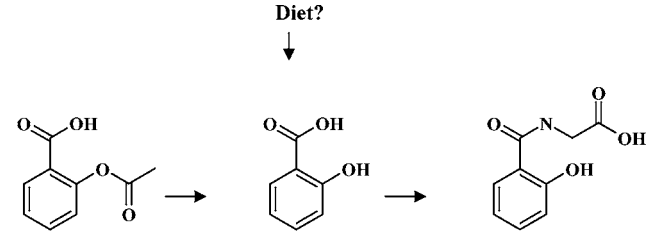

Figure 1. Structures of acetylsalicylic acid (aspirin), salicylic acid, and salicyluric acid.

natural salicylates range widely from 0 to 200 mg/day (*13*−*17*). Some authors (*17*) have concluded that the amounts of salicylic acid in fruits and vegetables may be too low and insufficiently bioavailable to have marked therapeutic significance, in addition to not being in the form of aspirin. However, a dietary source of salicylic acid is suggested by the detection of the compound and associated metabolites in the plasma and urine of individuals not taking aspirin or related preparations (*19*−*22*). Moreover, serum and urinary concentrations of salicylates in vegetarians overlap with those of individuals taking low-dose aspirin (*20*, *22*), suggesting significant absorption from ingested fruits and vegetables.

However, intervention studies with human volunteers to assess whether salicylic acid is absorbed from the diet are lacking. A study on a single volunteer that consumed 1800 mL of cranberry juice containing 5.6 mg of salicylic acid (*23*, *24*)

* Author to whom correspondence should be addressed (telephone 01224 716623; fax 01224716629; e-mail ggd@rri.sari.ac.uk).
† Rowett Research Institute.
§ Dumfries and Galloway Royal Infirmary.

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Table 1. Anthropometric Characteristics of the Volunteers[a]

parameter	intervention group: placebo drink, $n = 9$	intervention group: cranberry juice, $n = 11$
age (years)	28 ± 2	27 ± 2
height (m)	1.69 ± 0.02	1.66 ± 0.04
weight (kg)	64 ± 3	63 ± 3
body mass index (kg/m^2)	22 ± 1	23 ± 1

[a] Values are given as mean ± standard error.

detected salicylic acid by GC-MS analysis in two plasma samples collected at 45 and 270 min after the juice had been drunk. Consequently, we wished to study the absorption of salicylic acid from cranberry juice in more depth by accurately determining the salicylate concentration in a commercially available cranberry juice and measuring the effects of its consumption, over a period of 2 weeks, on the plasma and urinary concentrations of salicylic acid and salicyluric acid (**Figure 1**, the major metabolite of salicylic acid in urine) in a group of healthy human volunteers.

SUBJECTS AND METHODS

The fruit juice (Ocean Spray Cranberry Select Premium) was obtained from a local U.K. retailer. All other reagents were obtained from Sigma-Aldrich (Poole, U.K.) or Merck (Poole, U.K.) unless specified.

Healthy female subjects ($n = 22$) were allocated to two groups closely matched for age, weight, and height (**Table 1**). All were nonsmokers, normotensive, and free from clinical disorders and had not taken aspirin, vitamin/mineral supplements, or medication for at least 3 weeks before the start of the study. One subject was subsequently excluded from the study due to being prescribed antibiotics by her doctor, and another subject withdrew for reasons not related to the treatment. For 2 weeks the volunteers consumed 250 mL of either cranberry juice or a placebo solution having a sugar content similar to that of the fruit juice [9% (w/v) sucrose in water] three times a day (i.e., 750 mL/day). Early-morning urine samples were collected 1 week and immediately prior to the interventions and at weekly intervals thereafter. In addition, blood was collected from each subject into vacutainers containing EDTA as anticoagulant (Evacuette, Greiner Labortechnik, Kremsmuenster, Austria). All subjects were asked to fast from 10:00 p.m. the previous evening. The trial was approved by the joint ethical committee of the Grampian Health Board and the University of Aberdeen.

All samples were kept on ice, and blood was centrifuged (4 °C, 1500*g*, 15 min) within 60 min to obtain plasma. The plasma and urine were aliquoted, snap frozen in liquid nitrogen, and stored at −80 °C. Concentrations of salicylates in juice, plasma, and urine were measured by HPLC with electrochemical detection using 4-methylsalicylic acid as an internal standard. The cranberry juice was analyzed for salicylic acid following hydrolysis, 0.5 mL being added to sodium hydroxide (2.0 mL, 2.5 mol/L), mixed, and kept at room temperature for 24 h. These samples and the plasma and urine (0.5 mL) were then analyzed as previously described (*20*−*22*). In brief, following adjustment of the pH to 2.0 with hydrochloric acid (1 mol/L), the organic material was extracted twice with ethyl acetate (2 mL), evaporated to dryness at 70 °C under nitrogen, and reconstituted in 0.5 mL of the mobile phase containing 30 mmol/L citrate (pH 3.8) in 5% methanol containing 5.5 μL of EDTA (10 mM). Reconstituted extracts (50 μL) were eluted with a ternary gradient program to give maximum peak resolution (*20*, *21*), substances being detected electrochemically at an oxidation potential of +1.1 V. The limits of detection for plasma salicylic acid and urinary salicyluric and salicylic acids were 5 and <10 nmol/L, respectively. In the original work (*20*, *21*) the identities of the compounds being measured were confirmed by gas chromatography−mass spectrometry. Plasma and urinary salicylate measurements were made blind to the intervention status. Results are presented as mean ± SEM. Changes in parameters across time were assessed by repeated measures analysis of variance, and testing for differences at equivalent time points was carried out using Student's *t* tests.

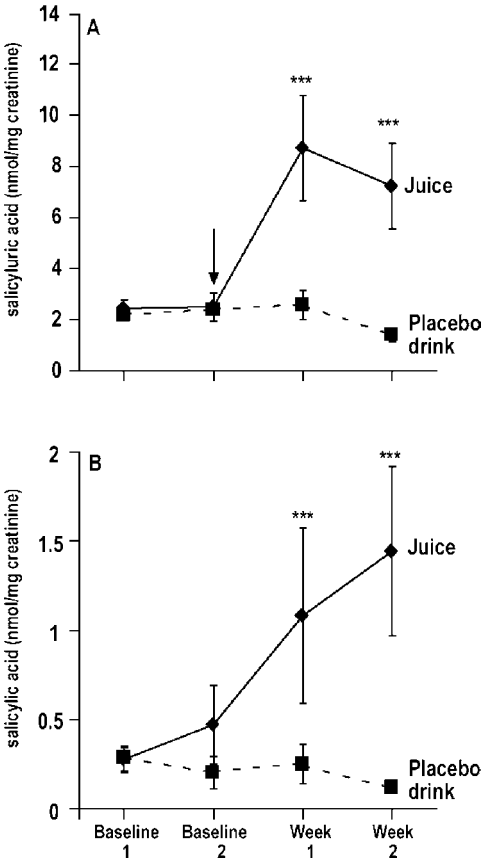

Figure 2. Effects of consumption of cranberry juice or a placebo drink on concentrations of salicyluric acid (**A**) and salicylic acid (**B**) in urine of human volunteers. Arrow denotes beginning of intervention. ***, $p < 0.001$.

RESULTS AND DISCUSSION

The total salicylate content of the cranberry juice determined in quadruplicate was 7.04 ± 0.02 mg/L. No salicylic acid was detected in the placebo solution.

Within 1 week of the intervention, daily consumption of the juice by volunteers was associated with marked increases ($p < 0.001$) in urinary salicylic acid and salicyluric acid, compared with those consuming the placebo solution (**Figure 2**). Concentrations of the urinary metabolite were an order of magnitude greater than salicylic acid. This observed increase in salicyluric acid in urine in response to consumption of the juice reflects known first-order kinetic elimination of salicylic acid from blood and its hepatic and renal conversion to the water-soluble metabolite prior to renal clearance (*25*, *26*). However, changes in plasma concentration of salicylic acid were less marked, a small but statistically significant increase ($p < 0.05$) being observed only after 2 weeks of juice consumption (**Table 2**). As the half-life of salicylic acid in blood is 2−3 h (*26*), the less marked change in plasma concentrations may be due to the blood samples having been taken after an overnight fast. This suggests that plasma concentrations may be poor indicators of dietary salicylate intake. In a study involving a single individual who consumed 1800 mL of cranberry juice (*24*), plasma salicylic acid concentrations of 7.1 and 3.04 mmol/L were found 45 and 270 min, respectively, following consumption. In contrast to the present study, in which we detected salicylic acid in all plasma samples analyzed, as have others

Table 2. Concentrations of Salicylic Acid (Micromoles per Liter) in the Plasma of Volunteers after 2 Weeks of Consumption of Cranberry Juice or Placebo

	preintervention		postintervention	
intervention	−1 week	0 week	+1 week	+2 weeks
cranberry, $n = 11$	0.11 ± 0.02	0.11 ± 0.01	0.10 ± 0.01	0.34 ± 0.20*
placebo, $n = 9$	0.09 ± 0.01	0.16 ± 0.02	0.17 ± 0.06	0.10 ± 0.02

[a] Values are mean ± standard error. *, $p < 0.05$, cranberry vs placebo groups.

(*20*), in this study (*24*) salicylic acid was not detected in the plasma sample taken prior to the consumption of the cranberry juice. This may reflect differences in sensitivities between the methods of detection.

In its acetylated form as aspirin, salicylic acid is widely prescribed to treat and prevent myocardial infarction and stroke, its therapeutic efficacy generally being ascribed to the acetyl group irreversibly binding to the active site of cyclo-oxygenase 1 (COX-1), thus preventing platelet aggregation and thrombus formation (*23*). Aspirin is a prodrug of salicylic acid, a compound with known anti-inflammatory activity, which is thought to account for the anti-inflammatory of aspirin in vivo. How salicylic acid reduces inflammation is still unknown. It has, however, been shown to reduce COX-2 transcription at nanomoles per liter concentrations, thereby decreasing the synthesis of proinflammatory and potentially neoplastic prostaglandins (*9*, *24*). Whether sufficient salicylic acid can be obtained from the diet to exert such potentially beneficial effects is unclear. Although salicylic acid is likely to be present in most foods of plant origin, Janssen et al. (*17*) concluded that the amounts of bioavailable salicylates in a "normal" diet (0−6 mg/day) are too low to affect risk for coronary heart disease or colon cancer. There is, however, great variability in the reported salicylate content of foodstuffs (*12*−*17*). Janssen et al. (*17*) calculated the estimated intake of dietary salicylates for four individuals consuming different diets using the published values of salicylate contents of food from four different studies (*12*−*14*, *16*) and compared the estimated dietary salicylate intakes against the amounts of total salicylates excreted in 24 h for the same individuals. The results show that the estimated intakes of dietary salicylates were significantly lower than the total salicylates excreted in urine, for three of the four published studies of dietary salicylate values. The discrepancies between estimated dietary salicylate intake and total salicylates excreted are not easily explained. However, serum and urinary concentrations of salicylic and salicyluric acids, respectively, are greater in vegetarians than in nonvegetarians and overlap with those of individuals consuming up to 150 mg of aspirin/day (*20*−*22*). This suggests that potentially therapeutic levels of salicylic acid could be achieved by eating salicylic acid-rich foods.

Intervention studies show that the regular intake of low-dose aspirin (81−325 mg/day) decreases the risk of developing colorectal adenomas, a precursor of carcinoma, in patients with a previous history of bowel adenomas or carcinoma (*6*−*8*). Such anticancer effects may be due to the action of salicylic acid suppressing the expression of COX-2 as induction of this enzyme is a key event in inflammation and tumorigenesis. Whether such a potentially beneficial effect could be obtained by daily drinking of cranberry juice containing lesser amounts of salicylic acid is not known.

Fruit and vegetable consumption and aspirin intake are both strongly and inversely associated with colon cancer risk (*4*, *5*, *27*, *28*). The increased concentrations in urine of salicyluric acid in response to regular intakes of cranberry juice may indicate that natural salicylates in plant-based foods can contribute to the recognized benefits of a healthy diet. It is possible that the bioavailabilty of salicylates from fruit juices is greater than that obtained from solid foodstuffs. However, it remains to be established which types of fruit and vegetables are the richest source of salicylates and whether intakes are sufficient to modify the key cellular events associated with the pathogenesis of colon cancer. Nevertheless, this is the first controlled intervention study to show that salicylates of dietary origin are bioavailable.

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