INTERNATIONAL JOURNAL OF FOOD SCIENCES AND NUTRITION
2021, VOL. 72, NO. 4, 511–517
https://doi.org/10.1080/09637486.2020.1850650

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IN VITRO AND ANIMAL STUDIES

In vitro catabolism of 3′,4′-dihydroxycinnamic acid by human colonic microbiota

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ABSTRACT

3′,4′-Dihydroxycinnamic acid (aka caffeic acid) is a common dietary component found in a variety of plant-derived food products either in a free form or esterified as in chlorogenic acids such as 5-*O*-caffeoylquinic acid. The dihydroxycinnamate is produced principally by hydrolysis in the colon of 5-*O*-caffeoylquinic acid and other caffeoylquinic acid esters, and is catabolised by the resident microbiota prior to absorption. In the present study 3′,4′-dihydroxycinnamic acid was incubated *in vitro*, with or without glucose, under anaerobic conditions with faecal slurries obtained from five volunteers. The main resultant catabolites to accumulate were 3-(3′,4′-dihydroxyphenyl)propanoic acid (aka dihydrocaffeic acid), 3-(3′-hydroxyphenyl)propanoic acid and phenylacetic acid. Both the rate of degradation of the hydroxycinnamate substrate and the catabolite profile varied between the faecal samples from the individual volunteers. Overall there was no clear cut effect when glucose was added to incubation medium.

ARTICLE HISTORY
Received 20 October 2020
Revised 9 November 2020
Accepted 9 November 2020

KEYWORDS
3′,4′-Dihydroxycinnamic acid; faecal slurries; colonic catabolism; GC-MS analysis

Introduction

3′,4′-Dihydroxycinnamic acid (**1**)[1] (aka caffeic acid) represents >75% of the total hydroxycinnamate content of a diversity of plant-derived products. It occurs principally as the acyl-quinic acids 3-*O*-caffeoylquinic acid (**2**), 4-*O*-caffeoylquinic acid (**3**) and 5-*O*-caffeoylquinic acid (**4**) (5-CQA), which are found in especially high concentrations in coffee (Crozier et al. 2006; Clifford et al. 2020), and are reported to have a diversity of beneficial effects on health (Williamson 2012).

Structures 1-4

The bioavailability of 5-CQA has been investigated using a diversity of human and animal models (Olthof et al. 2001; Gonthier et al. 2003; Crozier et al. 2010; Stalmach et al. 2012; Williamson et al. 2018). There is evidence of absorption of intact 5-CQA in the stomach of rats (Lafay et al. 2006), although it appears to be relatively poorly absorbed in the upper GIT of humans (Stalmach et al. 2009). Some hydrolysis of the acyl-quinic acid, releasing 3′,4′-dihydroxycinnamic acid occurs in the small intestine mucosa but most 5-CQA progresses along the GIT entering the large intestine where it is broken down by the action of the resident microbiota with substantial amounts of the resultant catabolites, including 3′,4′-dihydroxycinnamic acid and it subsequent degradation products, being absorbed into the circulatory system, some after undergoing phase II metabolism (Stalmach et al. 2012).

This paper reports on a study of the *in vitro* catabolism of 3′,4′-dihydroxycinnamic acid when incubated with faecal slurries, from five volunteers, under anaerobic conditions in the presence and absence of glucose. The spectra of catabolites that were produced was analysed by GC-MS after derivatisation to produce trimethylsilyl derivatives.

Materials and methods

Chemicals

4′-Hydroxycinnamic acid (aka coumaric acid), 3′,4′-dihydroxycinnamic acid, 4′-hydroxy-3′-methoxycinnamic

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[1]The names used for the various phenolic compounds in this paper are based on recommendations for standardising nomenclature of catabolites of dietary (poly)phenols proposed by Kay et al. (2020).

acid (aka ferulic acid), 3′-hydroxy-4′-methoxycinnamic acid (aka isoferulic acid), 3-(phenyl)propanoic acid, 3-(3′,4′-dihydroxyphenyl)propionic acid (aka dihydrocaffeic acid), 3-(3-hydroxyphenyl)propionic acid, 2′,4′,5′-trimethoxycinnamic acid, phenylacetic acids and benzoic acid were purchased from AASC Ltd (Southampton, UK). Analytical grade chemicals were used in preparing the buffer, macromineral, micromineral and reducing solutions. Manganese chloride, cysteine hydrochloride, sodium sulphate, resazurin were purchased from Sigma-Aldrich (Dorset, UK). Ammonium bicarbonate and calcium chloride were obtained from Fisher Scientific (UK), while cobalt chloride and mangnesium sulphate were supplied by VWR International (UK). HPLC solvents were acquired from Rathburn Chemicals (Walkerburn, Peebleshire, UK).

Fermentation medium

The fermentation medium was prepared by mixing 2.5 g of tryptone in 450 mL of distilled water and 112.5 µL of micromineral solution (consisting of 13.2 g of $CaCl_2$. $2H_2O$, 10 g of $MnCl.4H_2O$, 1 g of $CoCl_2.6H_2O$, 8 g of $FeCl_3.6H_2O$ and distilled water up to 100 mL). This mixture was shaken to dissolve the chemicals and then 225 mL of buffer solution (consisting of 2 g NH_4HCO_3, 17.5 g $NaHCO_3$ and distilled water up to 500 mL), 225 mL of macromineral solution (consisting of 2.85 g of Na_2HPO_4, 0.3 g $MgSO_4.7H_2O$ and distilled water up to 500 mL) and 1125 µL of 0.1% (w/v) resazurin solution (redox indicator) were added. This fermentation medium was adjusted to pH 7 using 6 M HLC and then sterilised by boiling for a few minutes before allowed to cool under oxygen free nitrogen (OFN) for 30 min to remove oxygen indicated with the medium changing colour from blue to pink. For each 10 mL of the medium, 0.5 mL reducing solution (consisting of 312.5 mg of cysteine hydrochloride, 2 mL of 1 M NaOH, 312.5 mg $Na_2S.9H_2O$ and make up to 50 mL with distilled water) was added.

Subjects

Five Asian subjects, 3 female and 2 males, designated as volunteers V1-V5, were recruited for the study. They were all non-smokers, aged between 25-40 years, had no history of gastrointestinal problems and had not taken antibiotics for at least three months prior to the study. The volunteers were asked to follow a low (poly)phenol diet for 48 h prior to faecal collection. All collected faecal samples were processed within 30 min of passage. The project was approved by the Ethics Committee of the University of Glasgow College of Medicine, Veterinary and Life Sciences (Ref: 2011023).

In vitro fermentation

The *in vitro* fermentation was performed as described previously by Jaganath et al. (2009). For each donor, 6.4 g of freshly voided faecal samples was homogenised with 20 mL of sodium 0.07 M phosphate buffer (pH 7.0) to obtain 32% faecal slurry. Five mL of the slurry was added to 44 mL pre-reduced fermentation medium and 1 mL of the substrate in 100 mL fermentation bottles. The substrate was 55 µmole of 3′,4′-dihydroxycinnamic acid with and without 0.5 g of glucose. After the addition of the substrate, the fermentation bottles were purged with OFN and sealed airtight. Samples were placed horizontally in a shaking water bath at 60 strokes/min and incubated at 37 °C for 8 h, aiming to simulate the conditions in the colonic lumen. Aliquots of the fermented faecal samples (3-5 mL) were collected after 0, 1, 2, 4, 6, and 8 h and stored immediately at −80 °C. To distinguish between enzymatic and chemical degradation, substrates were also incubated in the presence of heat-inactivated faeces (100 °C, 1 h) obtained from one of the donors.

Extraction of faecal incubates

Phenolic acid extraction, derivatisation, and analysis of faecal slurries by GC-MS were based on the procedures of Roowi et al. (2010). A 60 µg aliquot of 2,4,5-trimethoxycinnamic acid was added to 1 mL of faecal slurry. Samples were acidified by adding 300 µL of 1 M HCL and partitioned twice against 1.5 mL volumes of ethyl acetate. The upper organic phases were combined, transferred to an amber glass vial and dried at 40 °C under a stream of nitrogen. Samples were further dried by the addition and evaporation of 200 µL of dichloromethane. Three hundred µL of a *N*-methyl-*N*-(trimethylsily)trifluoroacetamide and pyridine mixture (1:4, v:v) was added to each vial, and the head space flushed with a gentle flow of nitrogen before sealing. Samples were then heated at 80 °C for 20 min for complete silylation. A set of standard calibrations solutions of phenolic acids ranging from 54-216 µg/mL was prepared and treated in a similar manner to the extracted samples.

GC-MS analysis of phenolic catabolites

Silylated samples were analysed with GC-MS (Agilent 6890 Series) equipped with a split/splitless injector

and an Agilent 7683 autosampler. Separations were carried out on a ZB-5MS (30 m x 0.25 id x 0.25 µm) capillary column (Phenomenex, Macclesfield, UK) with helium carrier gas (1.2 mL/min). Derivatised samples (1 µL) were injected into the GC injection port in split mode with a 1:25 ratio. The GC-MS conditions were as follows: The inlet temperature was maintained at 220 °C. The oven was programmed from 40 °C (held 0.1 min) to 160 °C at 20 °C/min, to 200 °C at 1.5 °C/min, to 250 °C at 10 °C/min, and to a final temperature of 300 °C at 40 °C/min held for 5 min. The transfer line was maintained at 310 °C. Data analysis and acquisition were performed using MSD Chemstation software. Phenolic compounds were identified based on their retention time, mass spectra of authentic standards and NIST library screening.

Statistical analysis

All samples were analysed in triplicate for GC-MS. Data are presented as mean values ± standard error ($n = 3$). When appropriate, data were subjected to statistical analysis using (ANOVA) to determine the significance of glucose supplement. Statistical analyses were performed using PSPP software.

Results

Catabolism of 3′,4′-dihydroxycinnamic acid incubated with faecal slurries

GC-MS analysis of the faecal slurries from 5 volunteers incubated for 0-8 h with 55 µmole of 3′,4′-dihydroxycinnamic acid (**1**) in the presence and absence of glucose revealed degradation of the hydroxycinnamate by the colonic microflora. Concomitant with the disappearance of the dihydroxycinnamate was the appearance of three main catabolites which were identified as 3-(3′,4′-dihydroxyphenyl)propanoic acid (**5**, aka dihydrocaffeic acid), 3-(3′-hydroxyphenyl)propanoic acid (**6**) and phenylacetic acid (**7**). The levels of these metabolites in 0-8 h incubates, as well as that of the substrate, in the presence and absence of glucose are presented in Table 1, and illustrated in Figure 1. Trace levels of 3-(phenyl)propanoic acid (**8**) were detected in some faecal samples prior to the addition of the substrate and did not accumulate to any extent during incubation arguably as a result of its conversion via β–oxidation to phenylacetic acid. Very low levels of benzoic acid (**9**) were detected in a small number of incubates. In the absence of glucose the dihydroxycinnamate was completely metabolised within 6 h in samples from all the volunteers, with the exception of V-5 where an 8 h period was required. In the case of V-2 complete degradation occurred within 4 h. The effect of glucose supplementation varied. It significantly enhanced breakdown in faecal samples in V-5, but had a little impact in V-1, V-2, V-3 and V-4 (Figure 1).

Structures 5–9

3-(3′,4′-Dihydroxyphenyl)propanoic acid was the main catabolite to be detected (Figure 1). In samples from all five volunteers the disappearance of 3′,4′-dihydroxycinnamic acid was associated with a gradually increase in 3-(3′,4′-dihydroxyphenyl)propanoic acid, which was first detected at 1 h. It reached a peak between 4-6 h in faecal samples from all volunteers with the highest amount recorded being 42 ± 1.7 µmoles in V-1 in the presence of glucose (Table 1).

3-(3′-Hydroxyphenyl)propionic acid was detected in incubates from all volunteers. The highest amount of this catabolite was detected at 8 h, with the main producers being V-1 and V-2 (32 µmoles). Phenylacetic acid was also detected in incubates from all volunteers. In some instances, low amounts of this catabolite were detected in early incubates and levels increased to varying degrees over the time (Table 1). The highest accumulation of this catabolite was between 6-8 h with the highest amount detected in V-2 samples (21 ± 0.5 µmoles) after an 8 h incubation in the absence of glucose.

The effect of glucose supplementation varied between individuals and also in terms of catabolite formation (Table 1 and Figure 1). In the absence of glucose, the formation of phenylacetic acid was highest in V-1, V-2 and V-3, whereas in V-4 and V-5 the amount of phenylacetic acid was highest in the presence of glucose. In the case of production of 3-(3′,4′-dihydroxyphenyl)propanoic acid by the faecal incubates, glucose increased accumulation with V-1- and V-2 and had relatively little impact with V-3, V-4 and V-5.

It is noteworthy that the overall recovery of phenolics from the faecal incubations with 55 µmole of 3′,4′-dihydroxycinnamic acid was high, ranging from 41 to with 53 µmole indicating that there was little degradation to carbon dioxide.

Discussion

An earlier *in vitro* study with 5-CQA and 3′,4′-dihydroxycinnamic acid degradation by human faecal

Table 1. Degradation of 3′,4′-dihydroxycinnamic acid (55 μmoles) and the formation of catabolites in human faecal slurries from five volunteers in the presence (+) and absence (−) of glucose.

Subject	Metabolite	Glucose	0 h	1 h	2 h	4 h	6 h	8 h
V-1	3′,4′-Dihydroxycinnamic acid	−	49 ± 5.9	50 ± 0.8	44 ± 1.7	22 ± 0.7	nd	nd
		+	45 ± 1.2	47 ± 1.3	30 ± 2.5	nd	nd	nd
	3-(3′,4′-Dihydroxyphenyl)propanoic acid	−	nd	nd	4.4 ± 0.1	20 ± 0.7	30 ± 1.1	4.7 ± 0.1
		+	nd	nd	10 ± 5.1	35 ± 1.5	37 ± 1.6	42 ± 1.7
	3-(3′-Hydroxyphenyl)propanoic acid	−	nd	nd	nd	2.8 ± 0.1	13 ± 0.7	32 ± 1.0
		+	nd	nd	0.5 ± 0.0	4.0 ± 0.2	6.1 ± 0.1	8.8 ± 0.3
	Phenylacetic acid	−	nd	nd	1.0 ± 0.2	2.1 ± 0.1	4.9 ± 0.2	7.1 ± 0.1
		+	0.7 ± 0.1	1.4 ± 0.1	2.1 ± 0.3	2.4 ± 0.2	2.9 ± 0.1	2.9 ± 0.1
	Total	−	49 ± 2.6	50 ± 0.4	50 ± 0.7	47 ± 0.2	48 ± 0.4	44 ± 0.4
		+	46 ± 0.5	48 ± 0.5	43 ± 2.0	42 ± 0.6	46 ± 0.7	53 ± 0.7
V-2	3′,4′-Dihydroxycinnamic acid	−	46 ± 3.0	44 ± 2.5	35 ± 1.8	nd	nd	nd
		+	48 ± 3.3	48 ± 0.5	32 ± 2.5	nd	nd	nd
	3-(3′,4′-Dihydroxyphenyl)propanoic acid	−	nd	0.3 ± 0.0	3.9 ± 0.2	25 ± 8.2	8.6 ± 0.1	nd
		+	nd	2.7 ± 0.0	13 ± 0.3	45 ± 7.5	37 ± 2.9	32 ± 0.7
	3-(3′-Hydroxyphenyl)propanoic acid	−	nd	nd	nd	3.0 ± 0.1	20 ± 3.6	32 ± 6.7
		+	0.2 ± 0.2	0.3 ± 0.3	0.6 ± 0.5	1.6 ± 1.2	2.0 ± 1.7	2.6 ± 2.1
	Phenylacetic acid	−	0.4 ± 0.0	0.8 ± 0.0	1.5 ± 0.1	14 ± 5.6	18 ± 0.9	21 ± 0.5
		+	0.5 ± 0.0	1.0 ± 0.0	1.5 ± 0.1	3.9 ± 0.3	5.4 ± 0.2	6.8 ± 0.4
	Total	−	46 ± 1.3	45 ± 1.1	41 ± 0.7	41 ± 3.6	46 ± 0.5	53 ± 1.2
		+	50 ± 1.4	52 ± 2.1	47 ± 0.2	51 ± 3.1	44 ± 1.2	41 ± 0.8
V-3	3′,4′-Dihydroxycinnamic acid	−	52 ± 5.5	50 ± 8.9	47 ± 5.7	25 ± 4.8	0.8 ± 0.1	nd
		+	48 ± 0.9	42 ± 7.8	32 ± 0.7	7.9 ± 1.1	1.4 ± 0.1	nd
	3-(3′,4′-Dihydroxyphenyl)propanoic acid	−	nd	nd	nd	21 ± 5.8	26 ± 3.4	24 ± 1.8
		+	nd	0.3 ± 0.0	6.9 ± 0.3	29 ± 1.1	31 ± 0.6	30 ± 1.4
	3-(3′-Hydroxyphenyl)propanoic acid	−	nd	nd	nd	0.6 ± 0.0	13 ± 5.0	15 ± 5.0
		+	nd	nd	2.4 ± 0.8	5.2 ± 0.9	9.8 ± 1.6	7.0 ± 0.5
	Phenylacetic acid	−	nd	1.0 ± 0.2	1.6 ± 0.1	4.1 ± 0.6	6.7 ± 1.7	13 ± 0.9
		+	0.4 ± 0.0	0.8 ± 0.0	1.5 ± 0.1	1.1 ± 0.9	4.1 ± 0.2	5.4 ± 0.5
	Total	−	52 ± 2.4	51 ± 3.8	50 ± 2.5	51 ± 2.5	46 ± 1.8	52 ± 1.9
		+	47 ± 0.4	43 ± 3.4	43 ± 0.3	43 ± 0.1	46 ± 0.6	42 ± 0.5
V-4	3′,4′-Dihydroxycinnamic acid	−	47 ± 1.8	44 ± 1.5	23 ± 0.7	9.9 ± 0	nd	nd
		+	49 ± 2.1	38 ± 0.3	34 ± 1.5	nd	nd	nd
	3-(3′,4′-Dihydroxyphenyl)propanoic acid	−	nd	nd	17 ± 1.2	30 ± 0.7	33 ± 2.0	24 ± 2.6
		+	nd	0.4 ± 0.0	13 ± 1.0	44 ± 2.7	35 ± 1.6	27 ± 4.3
	3-(3′-Hydroxyphenyl)propionic acid	−	nd	nd	0.1 ± 0.0	0.1 ± 0.0	2.6 ± 0.2	14 ± 0.8
		+	nd	nd	1.2 ± 1.5	2.1 ± 1.5	1.3 ± 1.0	1.8 ± 2.0
	Phenylacetic acid	–	nd	5.9 ± 0	2.5 ± 0.3	1.5 ± 0.0	5.1 ± 0.3	11 ± 0.9
		+	0.4 ± 0.0	0.8 ± 0	2.8 ± 1.8	6.7 ± 5.5	14 ± 1.0	14 ± 0.3
	Total	−	47 ± 0.8	50 ± 0.6	43 ± 0.4	41 ± 0.3	40 ± 0.8	49 ± 0.9
		+	50 ± 0.9	39 ± 0.1	51 ± 0.2	52 ± 1.7	49 ± 0.6	43 ± 1.7
V-5	3′,4′-Dihydroxycinnamic acid	−	43 ± 2.1	43 ± 1.8	24 ± 5.9	9.4 ± 0.5	5.7 ± 1.1	nd
		+	51 ± 0.7	43 ± 3.2	40 ± 0.4	9.0 ± 0.5	1.2 ± 0.4	nd
	3-(3′,4′-Dihydroxyphenyl)propanoic acid	−	nd	nd	23 ± 11	32 ± 6.0	33 ± 12	37 ± 11
		+	nd	nd	6.5 ± 0.7	27 ± 1.3	37 ± 4.7	31 ± 4.2
	3-(3′-Hydroxyphenyl)propionic acid	−	nd	nd	nd	1.4 ± 0.5	2.8 ± 0.8	9.0 ± 0.8
		+	nd	nd	nd	nd	2.8 ± 0.3	6.6 ± 1.1
	Phenylacetic acid	−	nd	0.2 ± 0.1	0.4 ± 0.2	1.2 ± 0.1	1.0 ± 0.1	1.3 ± 0.4
		+	1.1 ± 0.3	1.8 ± 0.1	0.9 ± 0.1	4.6 ± 1.1	4.2 ± 2.9	3.1 ± 2.1
	Total	−	43 ± 0.9	43 ± 0.8	48 ± 4.6	44 ± 2.6	42 ± 5.0	47 ± 4.6
		+	52 ± 0.3	45 ± 1.4	48 ± 0.3	41 ± 0.5	46 ± 1.8	42 ± 1.5

Trace levels of 3-(phenyl)propionic acid and benzoic acid were detected in a small number of incubates.
Data are expressed in μmoles ± standard error ($n = 3$).

samples resulted in the appearance of 3-(3′-hydroxyphenyl)propionic and benzoic acid as the major catabolites (Gonthier et al. 2006). In the present study, catabolism of 3′,4′-dihydroxycinnamic acid in faecal slurries associated with the appearance of 3-(3′,4′-dihydroxyphenyl)propanoic acid, 3-(3′-hydroxyphenyl)propanoic acid and to a lesser extent phenylacetic acid with very minor production of 3-(phenyl)propionic acid and benzoic acid in a very limited number of the incubates. Colonic bacteria potentially involved in such catabolism include *Escherichia coli, Bifidobacterium lactis*, and *Lactobacillus gasseri* (Couteau et al. 2001). *In vivo* 3-(3′,4′-dihydroxyphenyl)propanoic acid and 3-(3′-hydroxyphenyl)propanoic acid, are absorbed via the large intestine and converted via a series of dehydroxylation, β-oxidation and glycination steps to 3-hydroxyhippuric acid and hippuric acid, prior to excretion in urine (Stalmach et al. 2009).

Free 3-(3′,4′-dihydroxyphenyl)propanoic acid appears in plasma after consumption of coffee, a rich source of caffeoylquinic acids (Stalmach et al. 2009). 3-(3′,4′-Dihydroxyphenyl)propanoic acid can enter erythrocytes that assist the lowering of the trans-

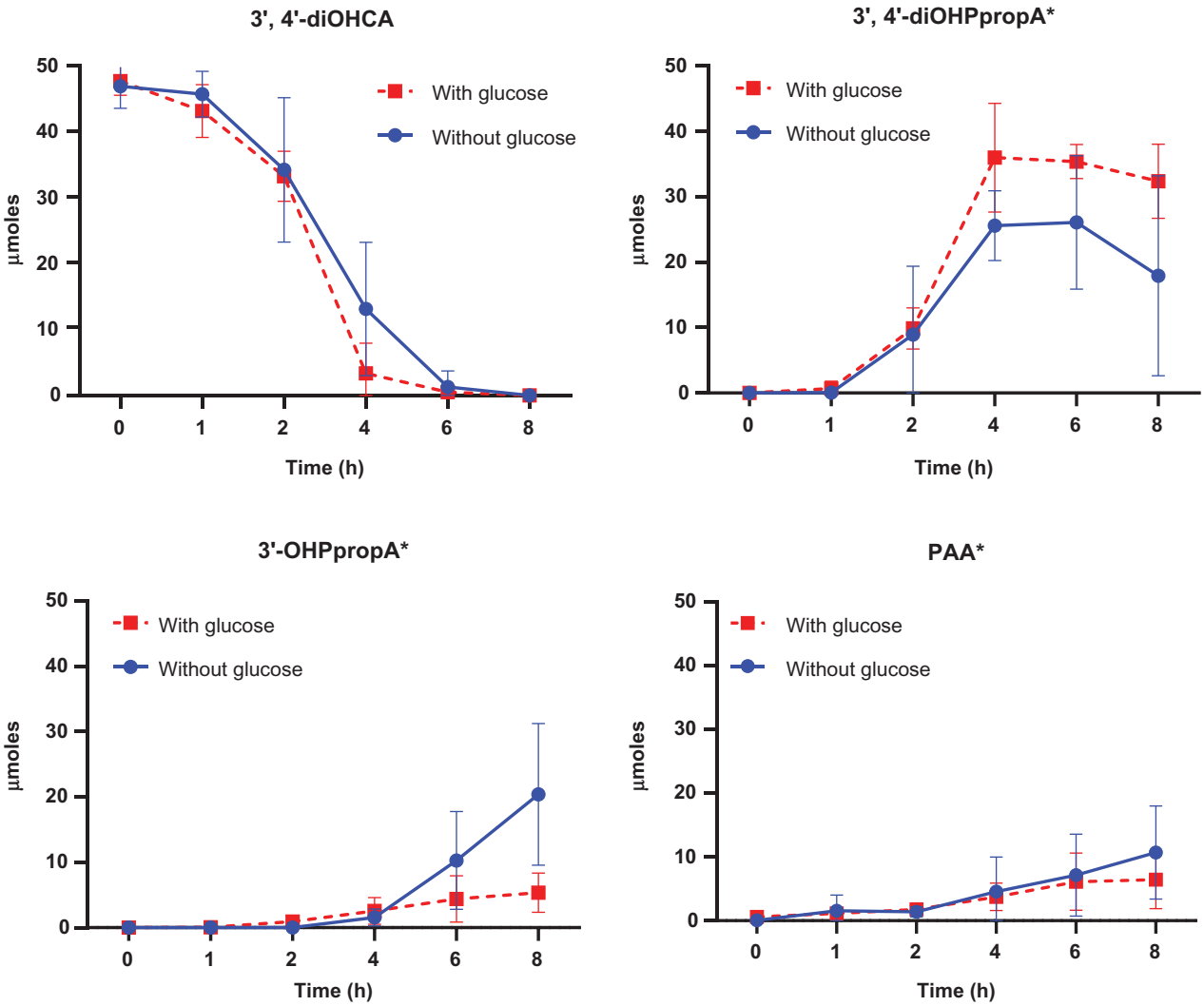

Figure 1. Substrate degradation and catabolite formation [3-(3′,4′-dihydroxyphenyl)propanoic acid (3′,4′- diOHPropA), 3-(3′-hydroxyphenyl)propanoic acid (3′-OHPropA) and phenylacetic acid (PAA) in human faecal slurries from five volunteers (V1-V5) incubated with 50 µmole of 3′,4′-dihydroxycinnamic acid (3′,4′-DiOHCA) in the presence and absence of glucose. Data expressed in µmole. Error bars indicate standard error ($n = 5$). *Profiles for with and without glucose significantly different ($p < 0.05$).

membrane oxidant stress generated by extracellular ferricyanide (Poquet et al. 2008). Phase II metabolites of 3-(3′,4′-dihydroxyphenyl)propanoic acid that have been detected in plasma and/or urine after coffee intake include 3-(4′-hydroxyphenyl)propanoic acid-3′-sulphate and 3-(4′-hydroxyphenyl)propanoic acid-3′-glucuronide (Stalmach et al. 2009). These conjugates were not detected in the faecal incubations indicating that while the colonic bacteria produce 3-(3′,4′-dihydroxyphenyl)propanoic acid from 3′,4′-dihydroxycinnamic acid, further metabolism producing glucuronide and sulphate conjugates requires mammalian enzymes probably located in colonocytes and/or hepatocytes .

Urinary excretion of radiolabeled 4′-hydroxycinnamic acid-3′-glucuronide, 3′-methoxycinnamic acid-4′-glucuronide (aka ferulic acid-4′-glucuronide), 3′-hydroxycinnamic acid-4′-sulphate, 4′-hydroxycinnamic acid-3′-sulphate, 3′-methoxycinnamic acid acid-4′-sulphate and 4′-methoxycinnamic acid-3′-sulphate (isoferulic acid-3′-sulphate) occurs after ingestion of [3-^{14}C]3′,4′-dihydroxycinnamic acid by rats (Omar et al. 2012). None of these compounds were produced *in vitro* by the human faecal samples in the current study further indicating the role of the liver and/or wall of the colon site as sites of phase II sulphation and glucuronidation as well as methylation.

Dehydroxylation of 3-(3′-hydroxyphenyl)propanoic acid will produce 3-(phenyl)propionic acid which is subject to β-oxidation with the resultant phenylacetic acid undergoing further side chain shortening and being converted to benzoic acid (Peppercorn and Goldman 1971; Scheline 1991). *In vivo*, benzoic acid is conjugated with glycine in the liver and excreted as hippuric acid in urine (Gonthier et al. 2003; Crozier

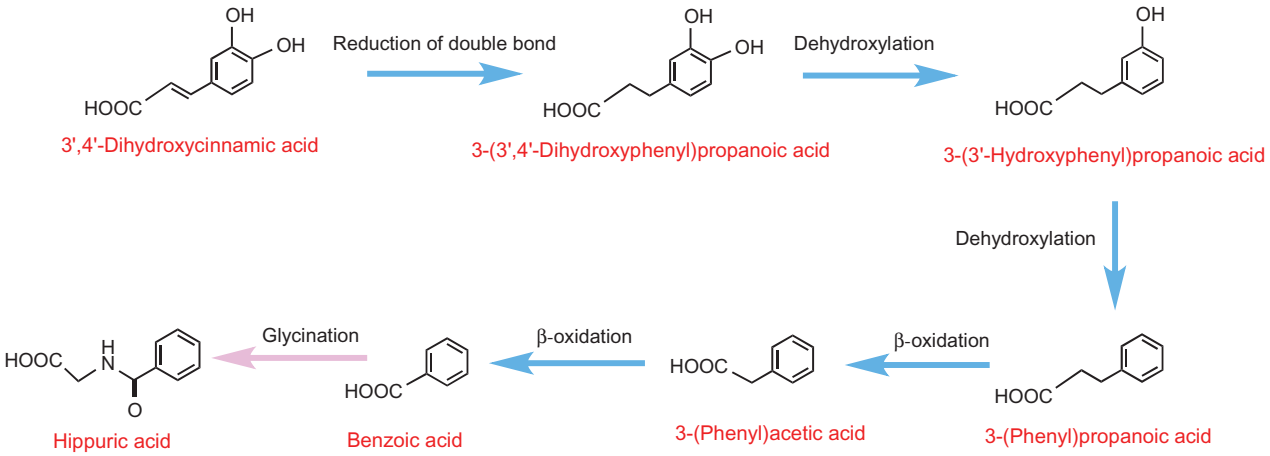

Figure 2. Proposed degradation pathway of 3′,4′-dihydroxycinnamic acid in humans and hepatic conversion of benzoic acid to hippuric acid. All conversions are microbiota-mediated steps except the glycination which is catalysed by a mammalian-enzyme.

et al. 2010). In the present study there was only traces amount of 3-(phenyl)propanoic acid were present although phenylacetic acid did accumulate (Figure 1) but was present in control faecal samples, indicating that this is not solely a product of 3′,4′-dihydroxycinnamic acid catabolism. Traces of benzoic acid were detected in the faecal samples of some volunteers. The results obtained thus support the operation of the catabolic pathway illustrated in the Figure 2 in which hepatic glycination of benzoic acid is also illustrated.

The rate of 3′,4′-dihydroxycinnamic acid degradation and catabolites produced varied among individuals, and thus may reflect the individual variations in intestinal bacterial colonisation (Meijer-Severs and van Santen 1986). It has been proposed there is a high degree of complexity of bacteria species among human individuals that is determined by the diet, which supplies nutrients not only to the host but also to the intestinal bacteria (Blaut and Clavel 2007). In the present study all volunteers were of Asian origin, which was thought would reduce variations in catabolism but in practice had little impact with volunteer-volunteer variations being similar to those observed in other studies in which (poly)phenols were incubated with faecal material (Roowi et al. 2010; González-Barrio et al. 2011; McDougall et al. 2014; Pereira-Caro et al. 2015).

Conclusions

In summary, the rate and extent of *in vitro* 3′,4′-dihydroxycinnamic acid degradation varied in the faecal samples provided by five Asian volunteers. 3-(3′,4′-Dihydroxyphenyl)propanoic acid was the main catabolite especially in incubates to which glucose was added. The data obtained was in keeping with dehydroxylation of 3-(3′,4′-dihydroxyphenyl)propanoic acid yielding 3-(3′-hydroxyphenyl)propanoic acid which was further dehydroxylated and subject to side chain shortening producing phenylacetic acid, trace amounts of which were, arguably, converted to benzoic acid which *in vivo* would undergo hepatic glycination yielding hippuric acid.

Acknowledgements

M.H.O. thanks the Director for the Institute for Medical Research (IMR), Setia Alam, Selangor and the Director General of Health Malaysia for the permission to publish this paper. The authors would also like to thank the volunteers who participated in the study, Ms June Lee Chelyn for her assistance with the Chem Draw.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

T.M.A. and A.C. are grateful for support from the Researchers Supporting Project of King Saud University, Riyadh, Saudi Arabia [grant number RSP-2020/273].

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