Disposition and metabolism of the flavonoid chrysin in normal volunteers

T. Walle, Y. Otake, J. A. Brubaker, U. K. Walle & P. V. Halushka

Department of Cell and Molecular Pharmacology and Experimental Therapeutics, Division of Clinical Pharmacology, Medical University of South Carolina, Charleston, SC 29425, USA

Aims To describe the oral disposition of the dietary flavonoid chrysin in healthy volunteers.

Methods Oral 400 mg doses of chrysin were administered to seven subjects. Chrysin and metabolites were assayed in plasma, urine and faeces by h.p.l.c.

Results Peak plasma chrysin concentrations were only 3–16 ng ml^{-1} with AUCs of 5–193 ng ml^{-1} h. Plasma chrysin sulphate concentrations were 30-fold higher (AUC 450–4220 ng ml^{-1} h). In urine, chrysin and chrysin glucuronide accounted for 0.2–3.1 mg and 2–26 mg, respectively. Most of the dose appeared in faeces as chrysin. Parallel experiments in rats showed high bile concentrations of chrysin conjugates.

Conclusions These findings, together with previous data using Caco-2 cells, suggest that chrysin has low oral bioavailability, mainly due to extensive metabolism and efflux of metabolites back into the intestine for hydrolysis and faecal elimination.

Keywords: bioavailability, chrysin metabolism, chrysin, flavonoids, healthy volunteers

Introduction

Flavonoids are dietary polyphenols derived from fruits and vegetables [1, 2]. Epidemiological observations strongly suggest flavonoids to be preventive in coronary heart disease [3, 4], stroke [5] and certain cancers [6]. Chrysin, 5,7-dihydroxyflavone, also is a potent inhibitor of the enzyme aromatase, which converts androgens to oestrogens [7]. As such, it is commonly used in high doses to boost testosterone concentrations.

However, very little is known about the oral bioavailability of flavonoids. Thus, whether biological activities observed *in vitro* can be extended to human subjects is questionable. We have used the human intestinal epithelial cell line Caco-2 as an *in vitro* model to study the absorption and bioavailability of these agents [8–10]. For chrysin, cell membrane penetration was not a limiting factor. However, extensive metabolism by these cells suggested strongly that the oral bioavailability of chrysin in humans may be low [9, 10].

Correspondence: Professor Thomas Walle, Department of Pharmacology, Medical University of South Carolina, 173 Ashley Avenue, P.O.Box 250505, Charleston, SC 29425, USA. Tel.: +1 (843) 792–2471; Fax: +1 (843) 792–2475; E-mail: wallet@musc.edu

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In the present study we tested this hypothesis by determining the disposition and metabolism of an oral dose of chrysin in seven human volunteers using plasma, urine and stool measurements. As an aid to the interpretation of these data, we also conducted experiments evaluating chrysin disposition in rats, including biliary elimination.

Methods

Study design

Seven healthy subjects (22–38 years of age; 56–89 kg) participated in the study. Two subjects were female; one was Black, one was Asian and five were Caucasian. One subject was a smoker. Written informed consents were obtained. The study was approved by the Institutional Review Board for Human Research.

All subjects were studied in a Clinical Research Unit. The diet during and for 4 days prior to the study was low in flavonoids. Two 200 mg capsules of chrysin (with 100 mg lysophosphatidyl choline; Price's Power Products, Newport News, VA) were administered orally in the morning after an overnight fast. Serial blood samples drawn at 0–48 h after the dose were centrifuged to separate plasma. Four consecutive 12 h urine samples were

collected with thiomersal and sodium bisulphite as preservatives. Stools were collected for 48 h from four subjects. All samples were stored at −20°C.

Analyses

Plasma and urine samples (2 ml) were subjected to solid phase extraction [10]. The methanol extracts were taken to dryness and reconstituted in mobile phase (55% methanol, 0.3% trifluoroacetic acid). Faecal homogenate samples (2 g) were freeze-dried and extracted three times with methanol. The extracts were taken to dryness and reconstituted in mobile phase. All samples were analysed for chrysin and its glucuronide and sulphate conjugates by h.p.l.c., using a Symmetry C18 column (Waters) with photodiode array detection (268 nm). Quantitative data were obtained from standard curves obtained from spiked predose samples. Chrysin glucuronide and chrysin sulphate were isolated as standard reference compounds from cellular incubates with chrysin [10]. The retention times for chrysin, chrysin glucuronide and chrysin sulphate were 19.8, 3.7 and 6.7 min. The coefficient of variation for chrysin analysis (at 5–25 ng ml^{-1} plasma) was 14%. Minimum detectable concentrations (three times background noise) were 1 ng ml^{-1}. AUCs were calculated by the trapezoidal rule and extrapolated to infinity based on the elimination rate constant obtained from least squares linear regression.

Identification of chrysin and metabolites

Chrysin and its glucuronide and sulphate conjugates were identified in plasma, urine and faecal samples by their characteristic h.p.l.c. retention times and u.v. spectra as compared with reference compounds [10]. Chrysin glucuronide in urine and chrysin sulphate in plasma were quantitatively hydrolysed by β-glucuronidase and aryl sulphatase, respectively [10]. Chrysin and metabolites were absent in predose samples.

Plasma binding of chrysin

Plasma protein binding of chrysin was determined by ultracentrifugation, as previously described for quercetin [11]. Plasma containing 20 μM chrysin was centrifuged for 20 h at 250 000 g. The protein-free layer immediately under the lipoprotein layer was assayed for chrysin.

Rat studies

Male Sprague-Dawley rats (150 g) were given single oral chrysin doses of 5 mg kg^{-1} in DMSO : Tween 20 : water. Urine and faeces were collected at 24 h intervals and assayed by h.p.l.c. as above. Other rats were given a 1–5 mg kg^{-1} i.v. or i.p. injection of chrysin in DMSO : Tween 20 : saline. The rats were anaesthetized and the bile duct was cannulated. Bile was collected for 3 h.

Results

The mean plasma concentrations of chrysin after a 400 mg oral dose in the seven subjects are shown in Figure 1a. The peak concentration, reached at about 1 h, was very low, 3–16 ng ml^{-1}, with large interindividual variability in AUC values (5–193 ng ml^{-1} h) (Table 1). The average apparent $t_{½}$ value for the 1–12 h time points was 4.6 h. The mean plasma concentrations of chrysin sulphate in the seven subjects exceeded those of chrysin by approximately 30-fold (Figure 1b), with AUC values of 450–4220 ng ml^{-1} h. Although a glucuronic acid conjugate of chrysin appeared to be present in some patient plasma

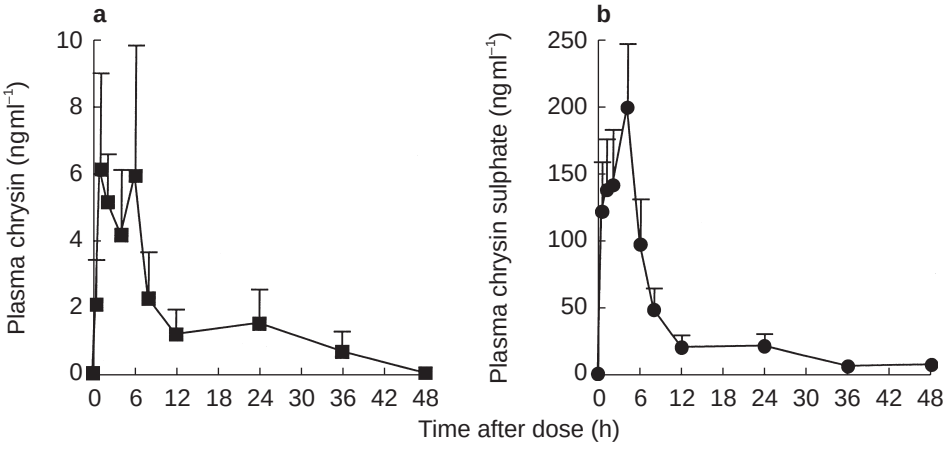

Figure 1 Plasma concentration-time curves for a) chrysin and b) chrysin sulphate in seven subjects receiving a single 400 mg oral dose of chrysin. The data shown are mean values ± s.e.mean.

Table 1 Areas under the plasma concentration-time curves (AUCs) and urinary recoveries of chrysin and metabolites after a single oral 400 mg dose of chrysin

	Plasma AUC (ng ml^{-1} h)		*Urinary recovery (mg)*	
Subject	*Chrysin*	*Chrysin sulphate*	*Chrysin*	*Chrysin glucuronide*
1	6	780	0.2	4.5
2	186	4220	3.1	17.5
3	38	1360	0.3	9.3
4	10	1860	0.8	9.8
5	12	803	1.7	25.8
6	5	450	0.2	2.2
7	193	990	1.0	10.2
Mean ± (s.e. mean)	64 (33)	1490 (485)	1.0 (0.4)	11.3 (3.0)

samples, the concentrations were too low to be measured accurately. As in previous cellular studies [10], there was no evidence of oxidative metabolism of chrysin.

The amount of unchanged chrysin excreted in urine was 0.2–3.1 mg, i.e. 0.05–0.8% of the dose. Interestingly, only trace amounts of chrysin sulphate were found in urine, whereas 2–26 mg of chrysin glucuronide was found. The overall recovery of the administered chrysin dose in urine was still low, only 1-7% of the dose.

As excretion via faeces may be the main route of elimination of chrysin and in particular its metabolites, faecal samples were collected in four subjects. The amounts of chrysin in the faeces were about 40, 160, 180 and 390 mg. The low value may be due to incomplete collection. The high value corresponds to 98% of the ingested dose.

To facilitate interpretation of the human data, several experiments were conducted in the rat *in vivo*. After single oral chrysin doses (5 mg kg^{-1}), the findings were very similar as in the humans, i.e. small amounts of chrysin glucuronide were found in urine and only unchanged chrysin in faeces. After i.v. and i.p. chrysin doses (1–5 mg kg^{-1}) no unchanged chrysin but high concentrations of chrysin metabolites appeared in the bile with chrysin glucuronide being excreted in 10-fold larger amounts than chrysin sulphate.

Discussion

The plasma concentrations of unchanged chrysin following a single 400 mg oral dose of this flavonoid were low. The plasma binding of chrysin was estimated to be > 99%, which is very similar to that of the flavonoid quercetin [11]. The volume of distribution for quercetin is low (2–18 l) [12], most likely due to its extensive plasma binding. Using this value of volume of distribution the oral bioavailability of chrysin was estimated to be 0.003–0.02%. The maximum concentrations of chrysin in plasma of 12–64 nM, with even lower unbound concentrations, should be compared with the K_i value of 2.6 μM for inhibition by chrysin of aromatase *in vitro* [7]. Thus the ability of chrysin to influence androgen and oestrogen concentrations in peripheral human target tissues by inhibiting this enzyme is questionable.

As in the human intestinal Caco-2 and hepatic Hep G2 cells [9, 10], the only metabolites observed were conjugates. However, the amounts of chrysin glucuronide and sulphonate in plasma and urine were small. Based on our previous findings [9], elimination of metabolites may depend on efflux by the MRP2 transporter. Experiments in rats strongly supported these findings, including the appearance of high concentrations of chrysin glucuronide and sulphate in the bile. After efflux into the intestine these conjugates would be expected to be hydrolysed by sulphatases and glucuronidases to chrysin, as observed in the stool samples. Although the appearance of large amounts of unchanged chrysin in the stool samples could be interpreted as poor absorption, our previous transport study in the Caco-2 cells does not support that possibility [9].

Even though the systemic availability of chrysin appears to be low, this does not exclude the occurrence of local biological effects of the flavonoid, particularly in the intestine. In summary, this study supports the view that the bioavailability of chrysin, and possibly other flavonoids, in humans is very low, due to extensive presystemic intestinal as well as hepatic glucuronidation and sulphation.

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