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ORIGINAL ARTICLE

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A pilot study on the safety of combining chrysin, a non-absorbable inducer of UGT1A1, and irinotecan (CPT-11) to treat metastatic colorectal cancer

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Abstract *Purpose*: Recently, it was shown that chrysin causes upregulation of UGT1A1 in Caco-2 intestinal cells. Therefore, we proposed that oral chrysin may reduce irinotecan (CPT-11) induced diarrhoea by shifting the SN-38G/SN-38 equilibrium towards the inactive SN-38G in the gastrointestinal mucosa. The purpose of this study was to examine the safety of combining single agent CPT-11 with chrysin. *Patients and methods*: Twenty patients with previously treated advanced colorectal cancer were administered chrysin twice daily for 1 week preceding and succeeding treatment with single agent CPT-11 (350 mg/m^2 over 90 min every 3 weeks). Loperamide usage and bowel frequency/consistency were recorded by patients into a study diary and blood samples were collected for CPT-11 pharmacokinetic analysis. *Results*: There were no observable toxicities that could be attributed to chrysin use. The grades and frequency of delayed diarrhoea were mild, with only 10% of patients experiencing grade 3 toxicity. Loperamide usage was also modest with a median of 1–5 tablets per cycle (range: 0–22). Pharmacokinetic results revealed a mass ratio of plasma SN-38G/SN-38, which was very similar to historical controls (7.15 ± 5.67, $n = 18$). *Conclusions*: These findings, combined with the observation of clinical activity and grade 3/4 neutropenia in 25% of patients, suggest that combining chrysin with CPT-11 may be a safe and potentially useful means of preventing diarrhoea, although this needs to be further investigated in the setting of a randomised trial.

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Introduction

Irinotecan (CPT-11) is widely used in the treatment of patients with metastatic colorectal cancer and increasingly in the management of patients with advanced lung cancer. It is considered to be a pro-drug, given that it requires activation by carboxylesterases to produce the cytotoxic metabolite SN-38 [37].

SN-38 is a potent topoisomerase I poison [26] and is metabolised by UGT1A1 [25] to the inactive moiety SN-38 glucuronide (SN-38G). The most debilitating toxicity complicating the clinical use of CPT-11 is delayed diarrhoea which usually occurs 4–7 days post-therapy [4]. Although its exact etiology is unknown, this syndrome has been attributed to intestinal cytotoxicity mediated by reactivation of SN-38 from SN-38 glucuronide (SN-38G) in the gut. It has been demonstrated that SN-38G is almost completely hydrolysed to SN-38 by enteric bacterial β-glucuronidase [38, 39]. Furthermore, rats that were given inhibitors of β-glucuronidase or antibiotics in the form of neomycin experienced less delayed diarrhoea than control animals following treatment with CPT-11 [38].

There is no specific antidote to delayed diarrhoea from CPT-11 in cancer patients and heavy reliance has been made on supportive therapy. The use of high dose loperamide (4 mg qid) is the current standard of management, although it is not effective in all patients [29, 44]. In resistant cases, dehydration may occur, which when severe may lead to renal failure and thromboembolic complications. In addition, the combination of diarrhoea and severe neutropenia, another frequent complication of treatment with CPT-11, may also promote the occurrence of gram-negative sepsis. The combination of these complications has contributed to the relatively high incidence of mortality within 60 days of

commencement of treatment that has complicated recent randomised trials involving CPT-11 [35].

Selectively increasing the glucuronoconjugation of SN-38 in the gastrointestinal tract could potentially reduce local mucosal damage and subsequent diarrhoea. It has been recently shown that the flavonoid chrysin upregulates UGT1A1 in Caco-2 cells [20]. Furthermore, orally administered chrysin has very poor bioavailability [41], suggesting that it could selectively induce UGT1A1 in the gastrointestinal mucosa. This would favourably shift the SN-38G/SN-38 equilibrium in the gut towards SN-38G, thereby reducing delayed diarrhoea, without necessarily impacting on systemic or intra-tumoural levels of SN-38.

The current trial was designed to assess the safety of single agent CPT-11 when given in conjunction with chrysin (250 mg twice daily) for advanced colorectal cancer. Previously, normal volunteers have received oral doses of chrysin ranging from 300 to 625 mg without any reported toxicity [5, 6, 41]. Furthermore, there is anecdotal evidence of bodybuilders taking 2–3 g of chrysin/day without any associated side effects. Therefore, we believed that the chrysin dose chosen was likely to be well tolerated. To determine whether chrysin altered the disposition of CPT-11, pharmacokinetic analyses of CPT-11 and its metabolites SN-38, SN-38G and APC were conducted.

Delayed diarrhoea also appears to be increased in patients with a reduced ability to deactivate SN-38. One genetic polymorphism of UGT1A1 causing reduced SN-38 glucurono-conjugation is UGT1A1*28 [3] in which the gene contains an additional TA repeat in the promoter region. A higher incidence of gastrointestinal toxicity has been reported following treatment with CPT-11, in patients heterozygous (6/7) or homozygous for this allele (7/7) [2]. UGT1A1 genotype has also been shown to be predictive of risk of severe neutropenia following CPT-11 therapy [23]. Therefore, patients participating in the trial were also genotyped with respect to UGT1A1.

Patients and methods

Clinical study design and patient selection

The study was designed as an open-label pilot study of oral chrysin in combination with CPT-11 in patients with advanced or metastatic colorectal cancer receiving single agent CPT-11 after previously failing a 5-FU containing regimen. To be included in the trial, patients were required to have bidimensionally measurable, histologically confirmed colorectal cancer, be at least 18 years old with an ECOG performance status of < 3 and have an estimated life expectancy of at least 12 weeks. Pre-treatment biochemical analysis was required to demonstrate adequate renal function as documented by a serum creatinine ≤ 110 μmol/l (1.24 mg/dl) and adequate hepatic function as indicated by a serum bilirubin of ≤ 18 μmol/l (1.05 mg/dl), irrespective of the presence of hepatic metastases. Hepatic transaminases (SGOT/SGPT, AST/ALT) were required to be $\leq 3\times$ institutional upper limit of normal ($\leq 5\times$ institutional upper limit of normal in the presence of hepatic metastases).

Patients were excluded from the trial if they had previously received CPT-11, if they have active or uncontrolled infection, pre-existing intestinal disease causing diarrhoea, inflammatory bowel disease, bowel obstruction or faecal incontinence. Patients with Gilbert's syndrome, diagnosed based on elevation of bilirubin in the absence of clinical evidence of other liver abnormalities or haemolysis, were excluded as they may experience excessive CPT-11-induced toxicity. Patients with a psychiatric disorder that could interfere with consent or follow-up, a history of symptomatic ischemic heart disease, epilepsy requiring treatment, or any other intercurrent illness, which in the judgement of the investigator would make study treatment inappropriate, were excluded. Patients were also excluded if they had exhibited prior intolerance to loperamide, or were pregnant or breastfeeding.

All patients gave a written informed consent to participate, and the study was performed in accordance with the ethical standards established by the Helsinki Declaration. The experimental protocol was approved by the Central Sydney Area Health Service Ethics Review Committee (Sydney, Australia).

Treatment and assessment

Patients received 250 mg of oral chrysin (Prolab, USA) twice daily for 1 week preceding and succeeding treatment with CPT-11 administered intravenously at a dose of 350 mg/m^2 every 3 weeks. HPLC certificates of chrysin purity were provided by Prolab (USA).

Disease assessment by clinical and CT evaluation was undertaken within 3 weeks of commencement of treatment and repeated every 6 weeks and response graded according to standard UICC criteria. Treatment was discontinued in the presence of symptomatic or radiological progression, unacceptable toxicity not responding to dose modification, withdrawal of patient consent, or death. Adverse events were graded according to the National Cancer Institute Common Toxicity Criteria (NCI CTC). Survival curves were estimated by the Kaplan-Meier method [20].

Study end points

The primary end-point of the study was to evaluate the safety of CPT-11 when given in conjunction with chrysin. Secondary end-points included tumour response rate and an assessment of the pharmacokinetics

of CPT-11 and its major metabolites in the presence of chrysin compared to historical controls.

Plasma pharmacokinetics

For the first administration of CPT-11, blood was collected prior to and at the end of infusion of CPT-11 and 0.5, 1, 2, 4 and 24 h post infusion. Plasma was separated by centrifugation at 4,000*g* (10 min, 4°C) and removed by aspiration.

Plasma concentrations of total CPT-11 and metabolites were determined using a slight variation of a validated method [34]. Standards were prepared in a similar manner to the plasma samples by being mixed with 100-μl ice-cold acetonitrile:methanol (50:50, v/v) containing 5 ng CPT. These were centrifuged (8,000*g*, 10 min) and the supernatant was acidified with 2.5 μl of 2N HCl.

For SN-38G quantification, 50 μl of plasma was incubated at 37°C with 25 U of glucuronidase for 1 h. The incubated plasma was then treated as described above. Separation was performed at ambient temperature using a Waters Nova-Pak Radial-Pak C18 reversed-phase column (5×250 mm, 4 μm), with 7.5 mM ammonium formate buffer (pH 4.0):acetonitrile (78:22) as the mobile phase at a flow rate of 1.2 ml/min. Fluorescence detection (RF-10AXL; Shimadzu, Sydney, Australia) was optimised for the detection of SN-38 with excitation and emission wavelengths set at 380 and 530 nm, respectively. Aliquots (10 μl) were injected onto the chromatograph.

Pharmacokinetic analysis was performed using non-compartmental methods. Specifically, the $AUC_{0\text{-infinity}}$ was determined by the trapezoidal method for each of the compounds of interest. The clearance *(CL)* of CPT-11 was estimated from the dose divided by the AUC. The Kruskal-Wallis test was used to assess possible relationships between pharmacokinetic parameters and pharmacodynamics, with a P value < 0.05 considered significant.

UGT1A1 genotyping

DNA was extracted from the buffy coat following cell lysis, RNAse treatment to remove RNA, removal of protein, and DNA precipitation using the Qiagen DNAeasy kit. Approximately, 120 ng of DNA was subject to amplification by polymerase chain reaction. The amplification primers were as described previously [31], where the sequence of the forward primer was 5′-GTCACGTGACACAGTCAAAC-3′ and that of the reverse primer 5′-TTTGCTCCTGCCAGAGGTT-3′. These primers flank the polymorphic TA locus in the promoter region of the UGT1A1 gene and amplify a 98-bp fragment when a $(TA)_6TAA$ allele is present and a 100-bp fragment when a $(TA)_7TAA$ allele is present. The forward primer was 5′-end labeled with a fluorescent dye (FAM) to permit visualization of the amplification product. The amplification reactions were performed in a 15 μl volume mixture consisting of 1.5 mM of $MgCl_2$, 250 μM of dNTPs, 0.1 μM of each primer, and 1.5 U of MBI Taq polymerase (Fermentas, supplied by Progen, Australia). DNA was amplified in an Eppendorf Mastercycler for 34 cycles at 94°C for 30 s, 57°C for 30 s, and 72°C for 30 s, followed by a final extension at 72°C for 45 min. Control DNA from individuals known to have a 6/6, 6/7 and 7/7 (confirmed by sequencing) were included in the PCR analysis as controls.

PCR fragments were subjected to capillary electrophoresis using an ABI 310 genetic analyser (PE Applied Biosystems). The samples were electro-injected at 13.0 kV for 3 s to a 42-cm-long diameter, 50 μm capillary (PE Applied Biosystems) filled with GeneScan polymer diluted to an appropriate concentration with 1× TBE buffer. Electrophoresis was performed at 15 kV at 60°C for 13 min. The results were collected using the DataCollection program and analysed using GeneScan software. All electrophoregrams were calibrated by fixing the positions of peaks produced by the DNA length standard TAMRA 500 (PE Applied Biosystems). Positions of the analysed peaks were determined by the Local Southern method (GeneScan software, PE Applied Biosystems). Genotypes were assigned as 6/6, 6/7 and 7/7 for patients homozygous for allele 6, heterozygous for allele 7, and homozygous for allele 7, respectively.

Effect of chrysin exposure on SN-38 glucuronidation in Caco-2 cells

To examine whether chrysin exposure could affect SN-38 glucuronidation in the intestine, human intestinal Caco-2 cells were exposed to chrysin (25, 50 μM) or vehicle for 3 and 4 days. Chrysin exposure and preparation of cell homogenates and microsomes was done essentially as described previously [20]. Briefly, just prior to confluency Caco-2 cells were incubated for 3 or 4 days with chrysin (25, 50 μM) or an equivalent volume of vehicle (80:20 ethanol:DMSO, v/v). The final concentration of vehicle was $< 0.5\%$. The medium was changed every 24 h. Then cells were washed twice with phosphate-buffered saline (2.7 mM KCl, 137 mM NaCl, pH 7.4) and scraped off. The cells were centrifuged at 1,500*g* for 5 min at 4°C and the cell pellet resuspended in 0.15 M KCl in 10-mM sodium phosphate buffer (pH 7.4) with protease inhibitors (2 mM PMSF, 50 μg/ml leupeptin and 1 μg/ml pepstatin). The cells were then disrupted by sonication on ice (5×5 s). Part of the cell homogenate was set aside for enzyme activity studies and the rest was centrifuged at 9,000*g* for 20 min at 4°C to obtain the supernatant fraction. The supernatant was centrifuged at 100,000*g* for 60 min at 4°C and the microsomal pellet resuspended in 400 μl of homogenisation buffer. Samples were stored at −70°C until analysis.

SN-38 glucuronidation

SN-38 glucuronidation was measured based on the method of Haaz et al. [22]. Microsome or cell homogenate (1 mg/ml) was pre-incubated for 5 min at 37°C in 0.1 M Tris buffer (pH 7.4), containing 10 mM MgCl2, 4-mM saccharolactone and 5-μM SN-38 lactone (final volume = 360 μl). SN-38 lactone, provided by Rhone-Poulenc Rorer laboratories (Neuilly, France), was used to study SN-38 glucuronidation as it is the predominant form in which SN-38 exists in vivo [34], and in steady-state conditions there is no significant difference in the rate that the two molecular forms, the lactone and carboxylate, are glucuronidated [22]. The reaction was initiated by the addition of 4-mM UDP-glucuronic acid (UDPGA) and incubated for 1 h at 37°C. The reaction was stopped by the addition of ice-cold 100-μl acetonitrile:methanol containing 10-ng camptothecin as an internal standard. The samples were vortexed and centrifuged at 8,000*g* for 5 min at 4°C. The supernatant (100 μl) was acidified with 2.5 μl 2N HCl to convert all of the SN-38G into the lactone form.

HPLC analysis of SN-38G

The formation of SN-38G total (lactone + carboxylate) was monitored by HPLC with fluorescence detection. Chromatographic separation was performed at ambient temperature using a Waters Nova-Pak Radial-Pak C18 reversed-phase column (5×250 mm, 4 μm) with 75-mM ammonium formate buffer (pH 6.0):acetonitrile (78:22) containing 5 mM Pic A (tetrabutylammonium phosphate) as the mobile phase at a flow rate of 1.5 ml/min. Fluorescence detection (RF-10AXL; Shimadzu, Sydney, Australia) was optimised for the detection of SN-38G with excitation and emission wavelengths set at 355 and 515 nm, respectively. The retention times of SN-38G, camptothecin and SN-38 were 2.7, 9.2 and 11 min, respectively. SN-38G standards ranging from 3.13–2500 ng/mL were prepared in a similar manner to the samples and were linear with respect to concentration ($r^2 > 0.99$). The lower limit of quantitation was 3.13 ng/ml. An aliquot (75 μl) of the supernatant was injected onto the chromatograph.

Results

Twenty-one patients were enrolled in the clinical study between 10/2/02 and 18/4/03. Only 20 patients received CPT-11 because of withdrawal of consent of one patient. The patient characteristics are listed in Table 1. The median age was 64 years (range 56–79) and there was a slight predominance of males. Six patients were aged ≥70 years and five patients had received prior pelvic radiotherapy, and both of these factors increase the risk of severe diarrhoea [12, 36]. The median performance status was 1. All patients had received prior fluoropyrimidine-based chemotherapy, while 13 had also received prior oxaliplatin as part of their initial treatment regimen. The median overall survival was 9.7 months and the one-year survival was approximately 42%. One partial response was seen, providing a response rate of 5%. However, stable disease including two minor responses occurred in 11 patients.

Twenty patients who were treated received a median of three cycles of CPT-11 (range 1–8) and the median duration of treatment was 2.2 months (range 0.7–5.7 months). All were evaluable for assessment of toxicity and response. There were no toxicities that could be attributed to the administration of chrysin. Dose delay occurred in three patients (two due to ongoing neutropenia and one due to persisting fatigue). Dose reduction occurred in 6 patients (4 due to grade 4 neutropenia, 1 due to grade 3 fatigue and 1 due to grade 3 nausea/vomiting). The toxicities following treatment with CPT-11 are listed in Table 2. Grade 3 or 4 neutropenia occurred in 5 patients (25%). The principal non-haematological toxicities were alopecia, fatigue and nausea and vomiting.

Only 2 patients (10%) experienced grade 3 diarrhoea. They were the nineteenth and twentieth patient to be enrolled. The first was an obese 74-year-old woman who had received prior pelvic radiotherapy for pelvic recurrence of malignancy. The second patient was a 73-year-old man who was possibly non-compliant with the chrysin according to his diary card recordings. The experience of one other patient is worth noting. He experienced mild diarrhoea during the study and achieved a protracted response. Upon symptomatic relapse, some months later, he was re-challenged with CPT-11. He experienced severe diarrhoea with his first cycle that settled on reintroduction of the chrysin prior to subsequent cycles. No dose reduction was required.

The average loperamide usage recorded by patients after receiving CPT-11 is summarised in Table 3. Thirteen patients (65%) took < 5 loperamide tablets per cycle and in 4 others the diary recordings were incomplete. Three patients took more than ten loperamide tablets per cycle; however, in one patient this occurred in the absence of any diarrhoea. The other two patients were those who experienced grade 3 diarrhoea.

The pharmacokinetics of CPT-11 in this chrysin-treated population and in comparison to historical controls are summarised in Table 4. Considerable inter-patient variability in the pharmacokinetics of CPT-11 and its major metabolites APC, SN-38 and SN-38G was observed. The mean AUC of CPT-11 was 29.3 ± 11.6 μg/ml h and the mean clearance of CPT-11 was 13.4 ± 6.2 l/h m^2 . There was approximately 5, 10 and 4-fold variability in the AUC values for CPT-11, SN-38 and SN-38G, respectively, and approximately 23-fold variability in the AUC for APC. No associations were observed between pharmacokinetic parameters and clinical outcomes.

Table 1 Patient characteristics

Characteristic	No.
Gender	
Male	11
Female	9
Age	
Median (range)	64 (56–79)
< 70 years	14
≥70 years	6
ECOG performance status	
0	6
1	13
2	1
Stage at initial diagnosis	
2	2
3	7
4	11
Prior pelvic radiotherapy	5
Prior therapy	
5-FU/FA	1
Xeloda	6
FOLFOX	4
XELOX + T	1
2 regimens	6
> 2 regimens	2
Number of cycles of CPT-11	
0	1
1	4
2	5
3	4
4	1
> 4	5

Table 2 Frequency of toxicities attributable to treatment

Toxicity	0	1	2	3	4
Neutrophils	12	0	3	1	4[a]
Hemoglobin	5	9	5	1	0
Platelets	12	7	0	1	0
White cells	7	4	4	3	2
Diarrhoea	6	9	3	2	0
Fatigue	NE	NE	NE	2	0
Nausea	NE	NE	NE	2	0
Vomiting	NE	NE	NE	1	0

Toxicity was graded according to the NCI CTC*NE* Not evaluated
[a] One patient had febrile neutropenia

Table 3 Average loperamide use by patients per cycle

No. of tablets	No. of patients
0	6
1–5	7
6–10	0
11–15	2
> 16	1
Unknown	4

The median number of tablets taken per cycle was 1–5 (range 0–22)

Only three patients in the current study were heterozygous for the UGT1A1*28 polymorphism. All other patients were homozygous for the normal allele (6/6) and this cohort was in apparent Hardy-Weinberg equilibrium. No relationship was observed between severity of diarrhoea experienced and UGT1A1 genotype, plasma SN-38G:SN-38 mass ratio or total plasma bilirubin levels ($P > 0.05$ in each instance).

In both microsomal and cell homogenate preparations of Caco-2 cells, there were significantly higher rates of SN-38 glucuronidation following exposure to chrysin compared to control preparations, and this increase was concentration dependent (Table 5).

Discussion

There was a low frequency of diarrhoea in this study, which is consistent with the hypothesis that chrysin may be effective in reducing the severity of delayed diarrhoea associated with CPT-11 therapy. Only two patients experienced grade 3 diarrhoea (10%), despite all patients receiving 350 mg/m^2 CPT-11 and having received substantial prior therapy. This compares well with previous studies in which grade 3/4 diarrhoea occurred in 19% of patients treated with 350 mg/m^2 CPT-11 every 3 weeks [17]. Furthermore, one patient who experienced grade 3 diarrhoea had received prior pelvic radiotherapy and was 74 years of age. The other patient with severe diarrhoea may not have been compliant with the chrysin and was 73 years old. Of course, before conclusions can be drawn on the efficacy of this intervention strategy, it will need to be tested in a larger patient population in a randomised cross-over or placebo-controlled trial.

The low incidence of diarrhoea in this cohort may be partly the result of the low frequency of the UGT1A1*28 polymorphism (7.1%). This is lower than the incidence of this polymorphism previously reported [24]. However, the allelic frequency of UGT1A1*28 is race dependent [28], and the sample size in the present study is smaller than other studies which have examined genotype frequencies. Also, patients with elevated baseline bilirubin were specifically excluded and Gilbert UGT1A1*28 individuals should have also been excluded.

Earlier studies have reported conflicting findings regarding the association between plasma SN-38:SN-38G ratios and diarrhoea [14, 21]. Although the association between SN-38:SN-38G mass ratio and incidence of severe diarrhoea was not found to be statistically significant in the current study ($P = 0.054$), this is perhaps not surprising considering the small number of patients that experienced delayed diarrhoea.

Previously, loperamide use for relief from gastrointestinal toxicity has only been documented in patients receiving high doses of CPT-11 or weekly CPT-11. In a study in which patients received 400 to 600 mg/m^2 CPT-11, the median number of loperamide tablets taken was 21 (range 5–72) [1]. In another study, 54% of patients receiving CPT-11 took more than 10 loperamide tablets [30]. However, those patients were receiving 125 mg/m^2 CPT-11 weekly and the incidence of severe diarrhoea is higher with weekly schedules [17]. In the present study,

Table 4 Pharmacokinetics of CPT-11 and its major metabolites (mean ± SD)

Variable	AUC (μg/ml h)			
	APC	SN-38	SN-38G	CPT-11
Published data[a]				
Mean	6.38	0.451	3.24	29.3
SD	3.42	0.185	3.03	11.6
Mean	4.27[b]	0.559	2.28	25.8
SD		0.490	1.74	7.02

Variable	Metabolic ratio			CPT-11 *CL* (L/h/m^2)
	APC: CPT-11	SN-38: CPT-11	SN-38G: SN-38	
Published data[a]				
Mean	0.22	0.017	7.15	13.4
SD	0.12	0.008	5.67	6.21
Mean		0.0282[c]	6.46	15.20
SD		0.427	5.61	4.30

Pharmacokinetic data was available for 18 patients. Metabolic ratios are expressed as a ratio of AUC metabolite to AUC CPT-11
[a]All data taken from Canal et al. [10] unless otherwise stated
[b] Dodds et al. [15]
[c] de Jonge et al. [13]

use of loperamide was modest, with a median value of five tablets per cycle (range 0–22). The relatively low use of loperamide in the present study is encouraging, although by no means definitive that chrysin is effective in reducing diarrhoea. Interestingly, no association was observed between loperamide usage and worst diarrhoea experienced by patients ($P > 0.05$).

There were no observable toxicities that could be attributed to chrysin use. Oral administration of compounds is often associated with gastrointestinal disturbances or allergic reactions. However, neither of these side effects was observed in the first week of chrysin administration, prior to receiving chemotherapy. Previously, normal volunteers have received oral doses of chrysin ranging from 300 mg to 625 mg without any reported toxicity [5, 6, 41].

Chrysin is widely used as a sports supplement, with athletes taking doses of up to 2–3 g per day. This usage is based on reports of its in vitro activity as an aromatase inhibitor [9, 27], and it is therefore used to enhance androgen supplementation by inhibiting the conversion of testosterone to oestrogen. However, the extremely poor bioavailability of chrysin suggests that it is unlikely to have substantial systemic effects. No clinical evidence of an anabolic effect was observed in the present study. In healthy male volunteers, chrysin supplementation was ineffective at inhibiting aromatase or increasing serum testosterone levels [7, 8].

The pharmacokinetic parameters observed in the current cohort were comparable to historical controls. CPT-11 AUC and clearance were similar to values obtained in previous studies [10, 11, 33]. In a Phase II study in which patients with metastatic colorectal cancer received 350 mg/m^2 every 3 weeks, the CPT-11 clearance was 15.2 ± 4.10 l/h/m^2 with CPT-11 AUC of 24.8 ± 7.02 μg/ml h, [10] as compared to a clearance of 13.4 ± 6.2 l/h/m^2 and AUC of 29.3 ± 11.6μg/ml h observed in the present study.

The metabolic ratios observed are also similar to those previously reported. The SN-38:CPT-11 ratio observed (0.017 ± 0.008) was comparable to that reported in a previous study in which 350 mg/m^2 of CPT-11 was administered (0.0282 ± 0.427) [13]. This suggests that chrysin does not affect carboxylesterase activity (CPT-11 → SN-38); however, this will need to be confirmed in randomised trials.

The SN-38G:SN-38 ratio has been shown to be relatively constant over time [33] and has been used as an indirect measure of glucuronidating activity [33]. The mass ratio of SN-38G:SN-38 observed (7.15 ± 5.67) was similar to that reported in a previous study in which 350 mg/m^2 CPT-11 was administered every 3 weeks (6.46 ± 5.61) [10]. The SN-38 and SN-38G AUC values were also comparable to those observed in previous studies. This is indirect evidence to support the proposal that oral chrysin is unlikely to affect systemic UGT1A1 activity, and is consistent with previous studies that have demonstrated that chrysin has quite poor bioavailability [41]. Chrysin is highly protein-bound (> 99%) and the resulting free concentrations are negligible [41]. However, the possibility remains that chrysin may have an indirect effect on systemic SN-38 glucuronide levels by altering SN-38 metabolism in the colonic mucosa, as SN-38 is known to undergo enterohepatic recirculation.

The disposition of APC was also comparable to previous studies. Dodds et al. [15] observed a mean AUC of APC of 4.27 μg/ml h in patients treated also with 350 mg/m^2 CPT-11. This compares well with the mean AUC of APC of 6.34 ± 3.42 μg/ml h observed in the present study. However, in both the present and

Table 5 Effect of chrysin exposure on SN-38 glucuronidation. SN-38 glucuronidating activity in Caco-2 cells exposed to chrysin was compared to activity in cells exposed to vehicle to determine the fold-increase in activity. Values are expressed as mean ± SD

Condition	Activity (pmol/min/mg)	Fold-increase in activity
Microsome		
Control, 3-day exposure	0.17 ± 0.008	
25 μM chrysin, 3-day exposure	0.60 ± 0.04*	3.2
Control, 4-day exposure	0.17 ± 0.01	
25 μM chrysin, 4-day exposure	0.72 ± 0.12*	4.26
50 μM chrysin, 4-day exposure	1.03 ± 0.15*	6.09
Cell homogenate		
Control, 4-day exposure	0.16 ± 0.05	
25 μM chrysin, 4-day exposure	0.46 ± 0.06*	2.79
50 μM chrysin, 4-day exposure	1.14 ± 0.11*	6.93

*Significantly different to control ($P < 0.05$)

previous study extensive inter-patient variability in AUC of APC was observed. This large inter-patient variability makes it difficult to ascertain whether chrysin affects the systemic disposition of APC.

Clinical evidence of cytotoxic activity was observed. Out of 20 patients evaluable for tumour response assessment, stable disease including two minor responses was observed in 11 patients and one partial response was observed. Also grades 3 and 4 neutropenia were observed in 25% of patients, which is indicative of CPT-11 cytotoxicity in other tissues. This incidence of neutropenia is comparable to that observed in a study in which 34% of patients receiving three-weekly CPT-11 experienced Grade 3/4 neutropenia [17]. Survival in this group of cancer patients was similar to that observed in previous studies. In another trial of colorectal cancer patients receiving second-line administration of 350 mg/m^2 CPT-11 three-weekly, a median overall survival of 9.9 months and a one-year survival of 41% was observed [17], which is comparable to the survival data from the present study. Together, this data suggests that when combined with chrysin, CPT-11 retains anti-tumour activity.

Chrysin exposure resulted in a threefold to sevenfold induction of SN-38 glucuronidation in Caco-2 cells. Previously, chrysin has been shown to induce UGT1A1 protein and mRNA expression in Caco-2 cells [19]. In the human hepatoma cell line Hep G2, chrysin exposure (25 μM) also caused isoform-specific induction of UGT1A1 protein and mRNA as well as induction of ethinylestradiol and bilirubin conjugation [42]; substrates are glucurono-conjugated almost exclusively by UGT1A1 [16, 32]. As oral administration of chrysin is known to produce very poor bioavailability [41], it is plausible that chrysin exposure will selectively increase the glucurono-conjugation of SN-38 in the gastrointestinal tract, thereby reducing local mucosal damage and subsequent diarrhoea, without affecting the systemic activity of CPT-11.

Maximal induction of UGT1A1 by chrysin in Caco-2 cells occurs after 3–4 days and is linear with chrysin concentration [19, 20]. The concentrations of chrysin required to produce significant induction (10–50 μM) are likely to be obtained in the intestine after normal dietary exposure [40]. On the basis of a gastric fluid volume of 100–500 mL, and nearly complete absorption of chrysin in humans (based on rapid passive uptake across membranes [18, 43], the resulting chrysin concentration following oral administration of 250 mg chrysin would be in the range of 9.8–49 mM. Thus, it seems plausible that oral administration of 250 mg chrysin could result in sufficient concentrations in the gut to induce UGT1A1.

In conclusion, oral chrysin appears to be safe and worthy of further investigation as an intervention strategy to reduce delayed diarrhoea associated with CPT-11 therapy in advanced colorectal cancer patients. We are currently conducting a randomised phase II multi-centre, double-blinded, placebo-controlled study of chrysin in combination with CPT-11 to examine the efficacy of chrysin in reducing delayed diarrhoea.

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