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In vivo bioavailability, absorption, excretion and pharmacokinetics of [^{14}C] procyanidin B2 in male rats

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Running title: Bioavailability of ^{14}C-procyanidin B2

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List of Abbreviations: low-density-lipoprotein (LDL)

V_d: apparent volumes of distribution

AUC: area under the curve

Cl_p: total clearance

EDTA: ethylenediaminetetraacetic acid

SLC: liquid scintillation counting

C_{max}: maximum blood concentration

$t_{½}$: terminal half-lives

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Abstract. Procyanidins are important biologically-active compounds but the pathway and extent of absorption and metabolism is controversial. We conducted a mass balance study to evaluate the total radioactivity excreted in urine and feces after oral administration of [^{14}C]-procyanidin B2 to male rats (n = 5). Urine and feces were collected daily from 0 to 96 h. Absolute bioavailability of ^{14}C from [^{14}C]-procyanidin B2 was calculated as ~82 % using the values for total urinary ^{14}C (U_{∞}). A pharmacokinetic study measured total radioactivity in the blood (n = 9). Blood samples were collected at designated time intervals (0.5 to 24 h) post-administration. Three treatments were used: (I) intravenous, (II) oral higher dose (21 mg.Kg^{-1} of body weight), and (III) oral lower dose (10.5 mg.Kg^{-1}). Blood concentration of total [^{14}C] reached a maximum at ~6 h after ingestion of [^{14}C]-procyanidin B2 (groups II and III) and AUC was dependent on oral dose. After intravenous or oral administration, the terminal half-lives were similar, while 8-fold larger values were obtained after oral dosing for total clearance (Cl_p) and the apparent volumes of distribution (V_d). These pharmacokinetic differences explain the apparently lower ^{14}C bioavailability (8-11%) for [^{14}C]-procyanidin calculated from blood ($AUC_{(0\text{-}24)}$) values. Following oral administration of [^{14}C]-procyanidin B2, 63 % was excreted *via* urine within 4 d. The data suggest that much of the parent compound administered orally is degraded by the gut microflora prior to absorption and that these microbial metabolites have a different distribution from the compounds circulating after the intravenous dose.

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Introduction. In recent years, polyphenols have been the subject of intense investigation because of their wide distribution in plants and plant foods and their documented association with decreased risk of chronic diseases such as cardiovascular diseases and some forms of cancer (Williamson and Manach, 2005). Proanthocyanidins (*syn* condensed tannins), the oligomeric forms of flavan-3-ols, are amongst the most widespread polyphenols in plants (Ferreira and Slade, 2002) and also in the human diet (Gu et al., 2004). Procyanidins are the commonest type of proanthocyanidin with (+)-catechin and (–)-epicatechin their main constituent units (Ferreira and Slade, 2002; Gu et al., 2004). The chemical structure is complex and consists of several different linkages between the molecules and different arrangements of the two flavanol components. Procyanidin-rich beverages and foods include cocoa, grapes, apples, strawberries and red wine. The average dietary intake of proanthocyanidins in the USA has been estimated at 58 mg/day (Gu et al., 2004). The most ubiquitous proanthocyanidin dimers present in nature and in human diets are the B-type procyanidins where the flavan-3-ols are linked via an interflavan bond between the benzylic C-4 carbon of the heterocyclic ring of the upper unit and the C-8 carbon of the flavan-3-ol A-ring of the lower unit (4→8) (figure 1). Procyanidins with a 4→6 link have also been reported in nature but occur less frequently (figure 2) (Ferreira and Slade, 2002).

Previous studies have demonstrated the pharmacokinetics of the monomeric flavan-3-ols (–)-epicatechin and (+)-catechin *in vivo* in rats (Baba et al., 2001b; Baba et al., 2002; Catterall et al., 2003; Gott and Griffiths, 1987; Hackett and Griffiths, 1982) but very little is known about the bioavailability and pharmacokinetics of procyanidin dimers and/or other related higher molecular weight oligomers, which are consumed at high levels by

most of the population. The physiological and biological activities of such abundant compounds will be dependent on their absorption and metabolism after ingestion.

Early studies in the 1970s reported the presence of unconjugated procyanidin oligomers in urine and in different organs/tissues of rats and mice after oral administration of [^{14}C] flavan-3-ol oligomers (Harmand and Blanquet, 1978). Later studies in animals, using LC-ESI-MS analyses, reported the presence, following enzymatic hydrolysis, of unconjugated procyanidin dimers, including B1, B2, B3, B4 and B5, in various tissues including plasma and urine after feeding large doses of proanthocyanidin extracts or proanthocyanidin-rich seeds (Baba et al., 2002; Shoji et al., 2006; Tsang et al., 2005). Similarly, human studies have detected unconjugated procyanidin B1, B2 and B5 in plasma and serum within 30 min of consumption of the test material (Holt et al., 2002; Sano et al., 2003). Unconjugated procyanidin B2 concentration in the systemic circulation reaches a peak at approximately 2 h post administration in both animals (Baba et al., 2002; Shoji et al., 2006) and humans (Holt et al., 2002; Sano et al., 2003). However the levels of intact procyanidin B2 detected in human plasma (~ 10 to 40 nmol/L) after high doses of proanthocyanidins are lower, sometimes by several orders of magnitude, than the concentrations observed to be effective in various *in vitro* tests.

Other reports have provided evidence on the *in vitro* degradation of crude cocoa proanthocyanidins by human colonic bacteria into a complex mixture of smaller molecular weight phenolic acids (Deprez et al., 2000). It is known that these microbial metabolites enter the portal circulation and have been proposed to account, at least in part, for the biological activities of the parent compound. Such microbial metabolites have been detected in the urine of rats in the free form (Gonthier et al., 2003b; Gonthier

et al., 2003a) and/or as mammalian conjugates produced by a combination of *O*-methylation, glucuronidation and/or sulfation post-absorption (Baba et al., 2001a; Baba et al., 2002; Gonthier et al., 2003b). It has been demonstrated that some flavan-3-ol conjugates (e.g. the 5-β-D-glucuronides) but not all (e.g. 3`-*O*-methyl) retain their *in vitro* antioxidant activity (Harada et al., 1999). However, it has been suggested that their transient occurrence and low concentrations in plasma, and relatively high redox potential compared with tocopherols and ascorbate, make it unlikely that their antioxidant properties explain any possible benefit to health and that alternative mechanisms should be sought and evaluated (Clifford, 2004).

The objective of the current study was to investigate *in vivo* in male rats the bioavailability, pharmacokinetics and mass balance of purified [^{14}C] procyanidin B2 (figure 3), the major dietary proanthocyanidin.

Materials and Methods

Chemicals. [^{14}C] Procyanidin B2 was synthesized by Selcia Limited (Ongar, Essex, UK) according to our previously published procedure (Viton et al., 2008). It was received in a liquid form (water solution) in two batches with a specific activity 49.58 MBq mmol^{-1} and 78.08 MBq mmol^{-1}, for the first and second batch, respectively, and used immediately on the same day. Radiochemical purity was confirmed prior to use by radio-HPLC. Solvable^{TM} was obtained from Perkin Elmer LAS Ltd. (Beaconsfield, UK), hydrogen peroxide, Scintillation cocktail (Biochemika, for radiometry) and ethylenediaminetetraacetic acid (EDTA), BioUltra, ≥ 99.0 % (KT) were purchased from Sigma-Aldrich Chemical Co., UK.

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Animals. Wistar albino rats (WA), specific pathogen free (SPF), 200-250 g were purchased from B&K Universal Ltd (Hull, UK). The animals were kept in controlled conditions within the provisions of Home Office license, UK, and were adapted for one week (12 h dark-light cycle), constant temperature 22 ± 1^{o}C, and 50 % humidity and with free access to a standard rodent diet (B&K Universal) and water. The animals were weighed and were divided randomly into three groups (I–III). For the intravenous administration (I), NaCl was added to [^{14}C] procyanidin B2, to make a solution of 0.9 % saline and a single dose was applied *via* the caudal vein (~150 µL/animal) adjusted to provide a dose of 21 mg Kg^{-1} body weight for each animal. For the oral administration, a single dose was applied, approximately 500 µL/animal, adjusted to provide a dose of 21 mg Kg^{-1} (II) and 10.5 mg Kg^{-1} (III) of body weight for each rat. The weight of the gavage syringe used to deliver the dose was recorded before and after dosing and the exact amount administered to each rat was calculated by mass difference.

Mass balance and blood pharmacokinetics. After dosing rats were housed individually in metabolic cages (Arrowmight Biosciences, Hereford, UK) and urine and feces were collected daily for four days at time points of 0, 24, 48, 72 and 96 h after dosing. For the blood pharmacokinetics, approximately 2 mm was removed from the end of the tail with a scalpel blade and blood samples (0.1 mL) were collected at regular intervals covering a time scale between 0.5 and 24 h (sampling: 0.5, 1, 2, 3, 4, 5, 6, 7, 9, 12, 15, 18, 20, 22 and 24 h) into lithium-heparinized centrifuge microtubes.

Mass balance analysis. Duplicate aliquots of urine (250 µL) were mixed with 5 mL scintillation cocktail and total [^{14}C] was counted. Fecal homogenates (10 % w/v in water) were prepared and duplicated aliquots (250 µL) were placed into scintillation vials. Each

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sample was digested using Solvable™ (160 μL). Samples were placed in a shaking water bath (50 ºC for 1 h). They were allowed to cool to room temperature and then 30 μL EDTA (0.1 M) and 160 μL H_2O_2 (30 %, v/v) were added. The vials were incubated further (50 ºC for 1 h) and then allowed to cool to room temperature. Scintillation cocktail was added (5 mL) to each sample and radioactivity content was determined by liquid scintillation counting (SLC) (Wallac 1400 DSA, 2.50). Total radioactivity content was quantified by SLC and each analysis was carried out in triplicate. Total [^{14}C] in urine and feces was calculated as a percentage of the dose administered. Animal data were analyzed individually and are presented as mean ± SD for five animals.

Analysis of blood pharmacokinetics. Duplicate aliquots (40 μL) of blood were placed into scintillation vials. Each sample was digested and decolorized as described for the mass balance. Measurements for each sample were taken in triplicate. The concentration of radioactivity was calculated as micrograms of [^{14}C] procyanidin B2 equivalents per gram of blood and the pharmacokinetic parameters were calculated using the PK solutions™ software package (v.2.0, Summit Research Services, Montrose, CO, USA) and fitted to a non-compartmental model. Animal data were analyzed individually and are presented as mean ± SD for nine animals.

Results

Mass balance. Excretion of radioactivity from 0 to 96 h was higher in urine than in feces (table 1). During the first 24 h post-administration, ~69 % of the dose was excreted in urine for the intravenous group (I) while ~58 % was recovered for both oral groups (II and III). Total urinary excretion at the end of the collection period was calculated as ~76 % after intravenous dosing (group I) and 62–63 % after oral dosing (groups II and III)

respectively. Fecal excretion following intravenous administration was approximately 25 % within the first 24 h and increased to ~28 % at the end of the collection period. Following oral administration, almost the entire amount excreted in the feces (~ 41 %) at the end of the collection period was recorded within 24 h (~39-40 %) (table 1) for both groups II and III.

Following intravenous administration of [^{14}C] procyanidin B2, approximately 93 % of the total dose was excreted within the first 24 h. Following oral administration, total radioactivity excreted in urine and feces, from groups II and III, was calculated as ~98 and ~99 % respectively (≤ 24 h post-administration). Low levels of total radioactivity were excreted even 96 h after administration for all three groups of animals (I, II and III). The sum of total radioactivity collected from urine and feces in all three groups of animals suggests full recovery (103.2 ± 7.2%) within experimental error. Results are the mean ± SD for five animals.

Bioavailability (F) obtained from the ratios of the total amount of ^{14}C excreted in urine after oral dosing ($U_{\infty oral}$) to the total amount of the ^{14}C excreted in the urine after intravenous dosing ($U_{\infty iv}$) was calculated as 82–83 % (table 2).

Blood pharmacokinetics. Total [^{14}C] radioactivity in blood was plotted against time after intravenous and oral administration (figures 4 and 5 respectively). Following intravenous administration (group I), a prominent secondary peak shows a transient increase in total blood [^{14}C] radioactivity at approximately 7 to 8 h post administration (figure 4). The blood profiles of total [^{14}C] radioactivity for both oral doses (groups II and

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III respectively) are compared in figure 5 and show no evidence of a secondary peak. Results are the mean ± SD for three animals per time-point.

The pharmacokinetic parameters calculated from the non-compartmental analysis subsequent to intravenous or oral doses (groups I, II and III respectively) are shown in table 2. Following intravenous administration (group I), the terminal half-life was 6.67 ± 0.95 h and the rate constant for elimination (K_{el}) was 0.12 ± 0.02 h^{-1}. Total clearance ($Clp_{(iv)}$) and the apparent volume of distribution (V_d) were calculated as 0.56 ± 0.1 mL min^{-1} and 1.28 ± 0.21 L Kg^{-1} respectively.

After oral administration, the maximum blood concentration (C_{max}) for group II was twice that of group III and the T_{max} values were similar for both groups. Similar values were also calculated for total clearance ($Clp_{(oral)}$) and apparent volume of distribution (V_d) for both groups II and III, assuming bioavailability of 83%.

The $AUC_{(0-24)}$ value for group I was 149 ± 21 µg.h min^{-1}, but those for group II and group III were only 11% and 8% of this value respectively. Consequently use of these $AUC_{(0-24)}$ values gave much lower values for bioavailability than those obtained from the urinary data.

Discussion. The present study, in which pure radiolabelled [^{14}C] procyanidin B2 was administered to male rats, defines its bioavailability, absorption, excretion and pharmacokinetics. Note that in this report, data represent cumulative total radioactivity, i.e. the parent compound and/or its metabolites.

A summary of the mass balance study is illustrated in table 1. Following intravenous administration, ~76 % of the dose was excreted *via* urine by the end of the collection period (≤ 96 h) (table 1), reflecting extensive renal clearance. However, ~28 % of the dose was excreted in the feces indicating biliary excretion along with a prominent secondary peak (figure 4) suggesting enterohepatic recycling.

Studies with rats fed unlabelled procyanidins have reported the excretion of unchanged procyanidins in urine to be very low (< 0.5 %) (Baba et al., 2002; Shoji et al., 2006; Tsang et al., 2005) and while methylated forms have been observed (Shoji et al., 2006), there are no reports of glucuronidated or sulfated metabolites. This is in contrast to studies on the flavanol monomers ((+)-catechin and (–)-epicatechin) where substantial amounts of glucuronide and sulfate conjugates have been observed in urine within 24 h, accounting for 27 % and 36 % of the dose respectively (Tsang et al., 2005). Baba *et al* (Baba et al., 2001b) also reported that conjugated forms accounted for the majority (ca 70 to 90%) of the flavanol monomers excreted in urine but did not express this as a percentage of the dose. Baba *et al* (Baba et al., 2002) reported the excretion of trace amounts of epicatechin after feeding rats with unlabelled procyanidin B2, and again a substantial portion of this epicatechin was present as methylated and glucuronidated derivatives, but the exact percentage was not clearly expressed. There is also evidence for the absorption of phenolic acids produced from ingested procyanidins by the gut microflora and their excretion in urine. Gonthier *et al* (Gonthier et al., 2003b) reported that 14 phenolic acids together accounted on a molar basis for 6.5 % of the ingested procyanidin B3.

In this study, the first to use pure [^{14}C] procyanidin B2, ~58 % of the ingested procyanidin and/or its [^{14}C] mammalian and microbial metabolites were excreted in urine (≤ 24 h post-administration) following administration of higher or lower oral doses (groups II and III respectively) (table 1). Although excretion in urine in the current study was substantially higher than previously reported, none of the earlier studies were able to investigate all the forms excreted.

Maximum concentrations (C_{max}) of conjugated metabolites in the systemic circulation after administration of grape seed extract containing oligomeric and polymeric procyanidins (Shoji et al., 2006; Tsang et al., 2005) and after 3-palmitoyl-(+)-catechin administration (Hackett and Griffiths, 1982) in rats, have been reported to be between 3 and 8 h, in the range of the T_{max} of ^{14}C observed after oral dosing of [^{14}C] procyanidin in the present study. Such conjugates, because of their large molecular weight and relative hydrophilicity, would be expected to undergo biliary excretion in the rat and enterohepatic recirculation, but such recycling is not necessarily complete (Benet and Massoud, 1984), as indicated in this study where following administration of both higher or lower doses (groups II and III respectively), ~40 % of the total radioactivity of [^{14}C] procyanidin B2 was excreted in the feces (≤ 96 h post administration) (table 1). However, the ^{14}C detected in the feces could include unmetabolised procyanidin B2, its microbial breakdown products, procyanidin B2 metabolites excreted in bile, and procyanidin B2 metabolites effluxed from the enterocytes.

In the present study, approximately 60 % of the oral dose was recovered in the urine (≤ 96 h post administration) (table 1). Similar behavior with flavonoids in rats has been observed by others reporting total [^{14}C] excretion in urine to reach up to 60 or 67 % after

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a single administration of [^{14}C] (+)-catechin (40 mg Kg^{-1}) (Gott and Griffiths, 1987) or genistein (4 mg Kg^{-1}) respectively (Coldham and Sauer, 2000).

Almost the entire dose was excreted within the first 24 h (table 1) and such observations are in fair agreement with the terminal half-lives ($t_{½}$) for groups II and III respectively (table 2). Similarly, previous *in vivo* studies with rats have reported the total excretion of flavan-3-ols and/or their metabolites to occur within the first 24 h post-administration (Catterall et al., 2003; Gott and Griffiths, 1987; Hackett and Griffiths, 1982; Tsang et al., 2005). Extensive enterohepatic cycling of related flavonoids and their conjugated metabolites has been reported elsewhere in male rats (Coldham and Sauer, 2000; Gott and Griffiths, 1987; Silberberg et al., 2006) and has been reported to be proportional to the dose administered reaching a net transfer of biliary secreted conjugates of up to 50 % of the dose (Silberberg et al., 2006).

The plasma clearance values (Cl_p) are not the same after intravenous and oral dosing as shown in table 2. This suggests that the radioactivity counted after oral dosing was present predominantly in a form(s) other than the parent compound administered. Since the terminal half-lives were similar after intravenous and oral dosing, it is proposed that, predominantly, the difference in the clearance values is due to the 8-fold larger V_d after oral dosing compared with the intravenous dosing (table 2). This emphasizes that the measurement of [^{14}C] is monitoring different chemical entities after oral dosing compared with intravenous dosing. Additionally, the 8-fold larger V_d calculated from the total [^{14}C] after oral dosing (higher or lower), compared with the intravenous dosing, indicates that some of the compounds formed after the oral dosing are comparatively more hydrophobic than the parent compound. This observation is consistent with previous findings (Stoupi

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et al., 2009) that the gut flora metabolites produced in an *in vitro* model of procyanidin B2 catabolism have greater retention times (14.06 to 22.71 min) compared with the substrate (13.53 min) during reversed phase HPLC.

Bioavailability (F) from the U_∞ values (table 1) was calculated as ~ 82 % (table 2). The discrepancy between the F values obtained from the AUCs and U_∞ (table 2) is attributable to the difference in the clearance values between intravenous and oral dosing (table 2) reflecting the difference in the compounds being monitored. In the present study, a significant level of radioactivity was detected in blood even at 0.5 h after oral administration suggesting that some of the [^{14}C] procyanidin B2 was absorbed in the stomach or upper GI tract, probably in the intact form (figure 5). However, the maximal concentrations (C_{max}) for total [^{14}C] in blood were not attained until 5 to 6 h after oral administration suggesting that much of the radioactivity was absorbed from the distal part of the small intestine and/or the colon. Taken collectively, these data strongly suggest that much of the radioactivity detected in the blood after oral administration is derived from low-molecular weight phenolic acids and other compounds produced from procyanidin B2 by microorganisms in the gut prior to absorption as has been previously demonstrated for procyanidin B3 by rat cecal microflora (Gonthier et al., 2003b), crude proanthocyanidins by human colonic microflora (Deprez et al., 2000) and procyanidin B2 by human colonic microflora (Stoupi et al., 2009) *in vitro*.

Similar observations have been made previously, but have been explained by suggesting that the major bioavailable form of procyanidin dimers (B2 and B5) was (–)-epicatechin formed by depolymerization based on studies using an *in vitro* small intestinal model (Spencer et al., 2001). Subsequent studies in humans demonstrated that this

depolymerization did not occur *in vivo* (Rios et al., 2002). In this regard, it is interesting to note that following a single oral dose of (–)-[3-^{3}H]-epicatechin (4.5 mg Kg^{-1}), rats excreted 2.9 % and 90.3 % of the total radioactivity in urine and feces respectively and the bioavailability was 7.8 % calculated from the U_{∞} values (Catterall et al., 2003). This behavior is in marked contrast to that observed with [^{14}C] procyanidin B2 and also suggests that in the present study little if any of the procyanidin was metabolized to an intermediate monomer, consistent with previous reports (Stoupi et al., 2009). In the present study, C_{max} increased in proportion with the oral dose, the value for group II being twice that of group III (table 2), thus providing no evidence for saturation at least at these 2 doses and suggesting linear first order elimination at such doses.

To conclude, [^{14}C] procyanidin B2 is bioavailable in male rats (~82 %) as calculated from the [^{14}C]-U_{∞} values, and most of the radioactivity is absorbed from the distal part of the small intestine and/or the colon ($T_{max} \approx 6$ h). These data suggest that a greater fraction of the parent compound is transformed by the gut microflora and that it may be these metabolites that are responsible for any biological activities or health benefits that might be associated with proanthocyanidin consumption.

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Figure legends

Figure 1: Structure of C(4→8) procyanidins, modified from (De Bruyne et al., 1999).

Figure 2: The structure of C(4→6) procyanidins modified from (De Bruyne et al., 1999).

Figure 3: Procyanidin B2 radiolabelled at position 2 (*) of the heterocyclic C-ring of the upper unit.

Figure 4. Profiles of total radioactivity in blood sampled from male rats. The concentration of radioactivity is expressed as µg equivalents of procyanidin B2 mL^{-1} after intravenous administration of [^{14}C] procyanidin B2 for group I (21 mg Kg^{-1}). Results are mean ± SD for nine animals.

Figure 5: Profiles of total [^{14}C] radioactivity in blood sampled from male rats in group II (21 mg/kg, square symbols) and group III (10.5 mg/kg, round symbols). The concentration of radioactivity is expressed as µg equivalents of procyanidin B2 mL^{-1} after oral dosing. Results are mean ± SD for nine animals.

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TABLES

Table 1. Excretion of total radioactivity following single intravenous and oral administrations of [^{14}C] procyanidin B2 in male rats.

Time after administration (h)	Excretion in urine (% of dose)	Excretion in feces (% of dose)	Sum (% of dose)
Group I			
0-24	68.7	24.6	93.3
24-48	4.3	2.9	7.2
48-72	1.8	0.2	2
72-96	0.8	0.2	1
Total (% of dose)	75.6 ± 5.4	27.9 ± 5.6	103.5 ± 5.5
Group II			
0-24	58.3	39.6	97.9
24-48	2.7	0.1	2.8
48-72	1.4	0.1	1.5
72-96	0.5	0.05	0.55
Total (% of dose)	62.9 ± 5.8	39.9 ± 9.2	102.8 ± 7.5
Group III			
0-24	57.8	40.8	98.6
24-48	3	0.2	3.2
48-72	0.8	0.05	0.85
72-96	0.6	0.05	0.65
Total (% of dose)	62.2 ± 7.6	41.1 ± 9.4	103.3 ± 8.5

Note. [^{14}C] procyanidin B2 was administered to rats as either a single intravenous dose (group I received 21 mg Kg^{-1}) or oral dose (group II received 21 mg Kg^{-1}; group III received 10.5 mg Kg^{-1}). Data represent total [^{14}C] radioactivity, i.e. the parent compound and/or metabolites. Results are mean ± SD for nine animals.

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Table 2: Summary of blood pharmacokinetics

Intravenous administration		Oral administration		
Pharmacokinetic parameters	Mean ± SD Group I	Pharmacokinetic parameters	Mean ± SD Group II	Mean ± SD Group III
$t_{1/2}$ (h)	6.67 ± 0.95	C_{max} (µg mL^{-1})	2.60 ± 0.93	1.38 ± 0.28
Cl_p (mL min^{-1})	0.56 ± 0.1	t_{max} (h)	6.11 ± 0.43	5.56 ± 0.98
V_d (L Kg^{-1})	1.28 ± 0.21	$t_{1/2}$ (h)	7.3 ± 2.07	4.57 ± 1.46
k_{el} (h^{-1})	0.12 ± 0.02	* Cl_p (mL min^{-1})	4.02 ± 0.44	4.06 ± 0.47
$AUC_{(0-24)}$ (µg.h min^{-1})	149 ± 21	* V_d (L Kg^{-1})	11.4 ± 1.9	10.4 ± 2.67
		k_{el} (h^{-1})	0.11 ± 0.03	0.17 ± 0.05
		$AUC_{(0-24)}$ (µg.h min^{-1})	17.0 ± 2.7	5.18 ± 1.35
		Bioavailability (from $AUC_{(0-24)}$)	10.6 ± 1.7	7.71 ± 1.48
		Bioavailability (from U_∞)	83.2 ± 6.3	82.3 ± 7.01

Note. [^{14}C] procyanidin B2 was administered to rats as a single intravenous dose (group I received 21 mg Kg^{-1}) or oral dose (group II received 21 mg Kg^{-1}; group III received 10.5 mg Kg^{-1}). Data were analyzed using the PK Solutions 2.0 pharmacokinetic software and fitted to a non-compartmental model. Results are mean ± SD for nine animals.

$t_{1/2}$, blood half-life; Cl_p, total clearance; V_d, apparent volume of distribution; $AUC_{(0-24)}$, cumulative area under the curve for experimental time points only; C_{max}, maximum blood concentration; t_{max}, time to reach maximum blood concentration; k_{el}, rate constant for elimination. * Assuming bioavailability (from U_∞) at least 82 %.

R_1	R_2	Interflavan bond	
OH	OH		Procyanidin B1
OH	OH		Procyanidin B2
OH	OH		Procyanidin B3
OH	OH		Procyanidin B4

Figure 1

R_1	R_2	Interflavan bond	
OH	OH	▬	Procyanidin B5
OH	OH	·····	Procyanidin B6
OH	OH	·····	Procyanidin B8

Figure 2

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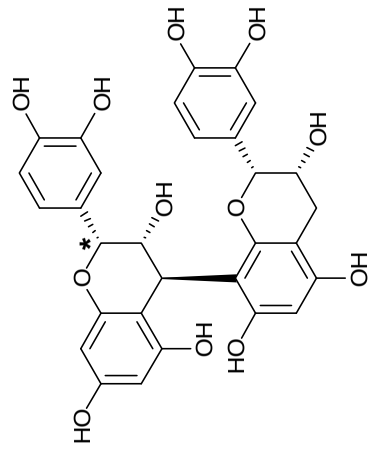

Figure 3

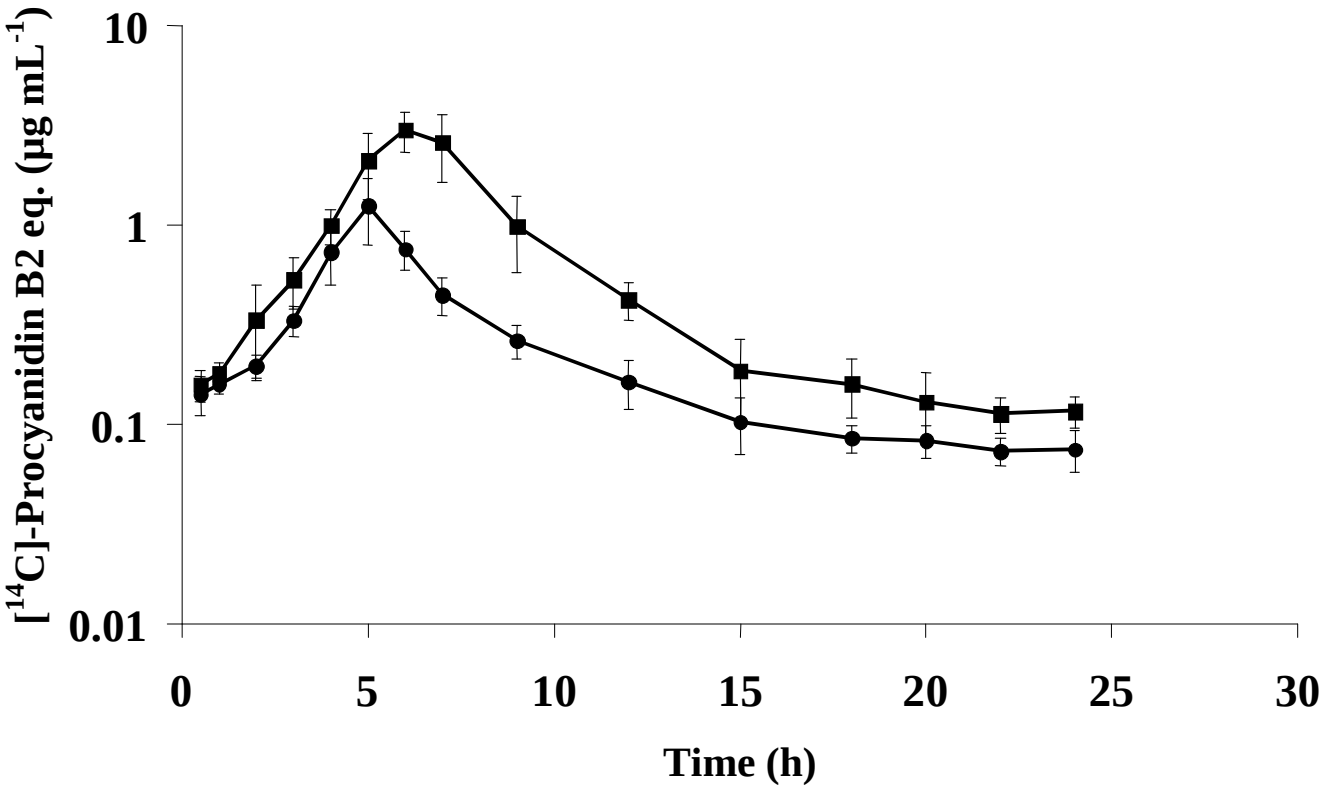

Figure 4

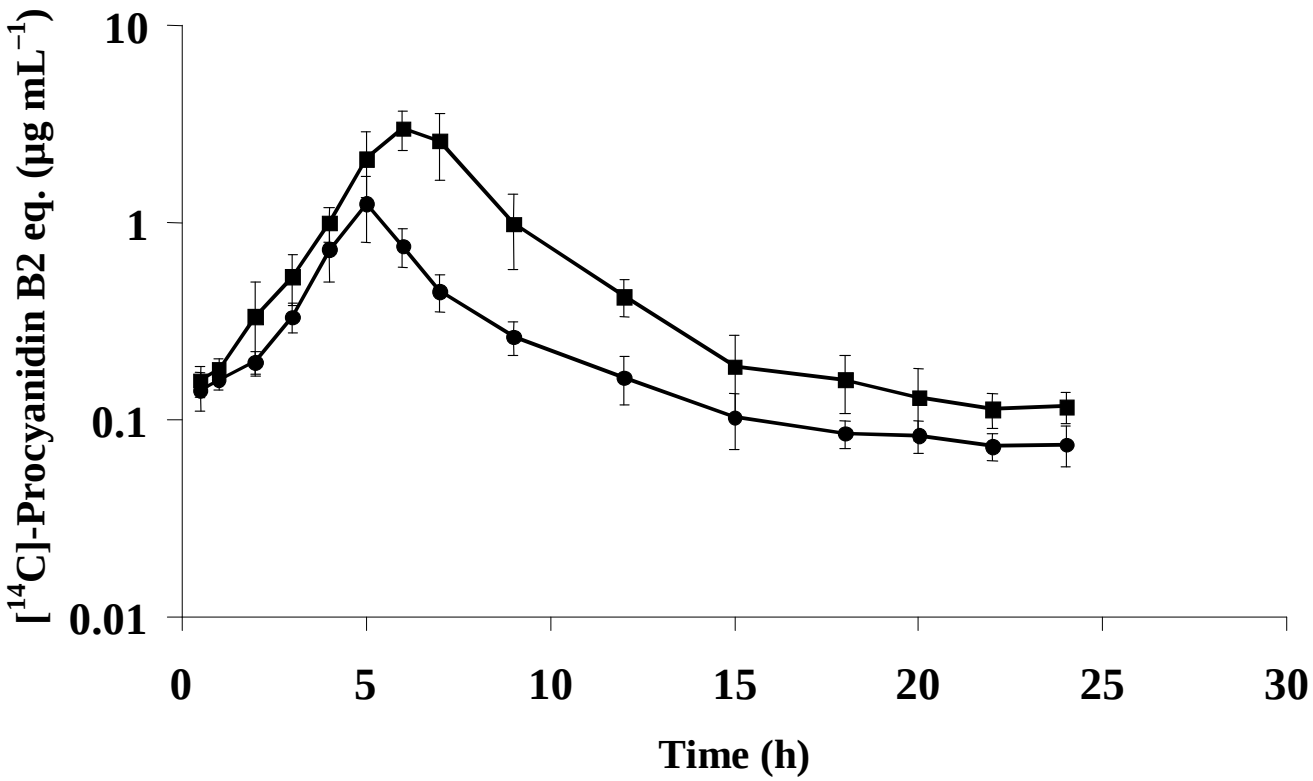

Figure 5