Chain Length of Dietary Alkylresorcinols Affects Their In Vivo Elimination Kinetics in Rats

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Abstract

Two phenolic acids, 3,5-dihydroxybenzoic acid (DHBA) and 3-(3,5-dihydroxyphenyl)-propanoic acid (DHPPA), are the major metabolites of cereal alkylresorcinols (ARs). Like their precursors, AR metabolites have been suggested as biomarkers for intake of whole-grain wheat and rye and as such could aid the understanding of diet-disease associations. This study estimated and compared pharmacokinetic parameters of ARs and their metabolites in rats and investigated differences in metabolite formation after ingestion of different AR homologs. Rats were i.v. infused for 30 min with 2, 12, or 23 μmol/kg DHBA or DHPPA or orally given the same amounts of the AR homologs, C17:0 and C25:0. Repeated plasma samples, obtained from rats for 6 h (i.v.) or 36 h (oral), were simultaneously analyzed for ARs and their metabolites by GC-mass spectrometry. Pharmacokinetic parameters were estimated by population-based compartmental modeling and non-compartmental calculation. A 1-compartment model best described C25:0 pharmacokinetics, whereas C17:0 and AR metabolites best fitted 2-compartment models. Combined models for simultaneous prediction of AR and metabolite concentration were more complex, with less reliable estimates of pharmacokinetic parameters. Although the AUC of C17:0 was lower than that of C25:0 (P < 0.05), the total amount and composition of AR metabolites did not differ between rats given C17:0 or C25:0. The elimination half-life of ARs and their metabolites increased with length of the side chain (P-trend < 0.001) and ranged from 1.2 h (DHBA) to 8.8 h (C25:0). The formation of AR metabolites was slower than their elimination, indicating that the rate of AR metabolism and not excretion of DHBA and DHPPA determines their plasma concentrations in rats.

Introduction

Alkylresorcinols (ARs) are phenolic lipids present as several homologs in the outer layers of wheat and rye kernels. These cereals contain 5 major homologs with saturated alkyl chains of different lengths, ranging from 17 to 25 carbon atoms (Fig. 1A). Cereal ARs have been suggested and evaluated as biomarkers for intake of whole-grain (WG) and bran products of these cereals and may be used in intervention studies to assess compliance (2) or in observational studies as a surrogate measurement of intake (3). The major AR metabolites found in plasma and urine, 3,5-dihydroxybenzoic acid (DHBA) and 3-(3,5-dihydroxyphenyl)-1-propanoic acid (DHPPA), may also be used in a similar manner to estimate intake of foods containing ARs (Fig. 1B,C).

About 60% of ingested cereal ARs is absorbed in the small intestine (4). The main route of absorption has been hypothesized to be via the lymphatic pathway to the systemic circulation (5). However, alternative absorption processes might exist, e.g., direct HDL efflux to the portal vein, as has been observed in vivo for tocopherols (6). The metabolism of ARs is thought to include α-oxidation, β-oxidation, and conjugation reactions to form easily excreted hydrophilic metabolites (7,8). The 2 phenolic acids, DHBA and DHPPA, are the major metabolites of ARs, and they are present in plasma and urine as such or as conjugates of sulfonic and glucuronic acids (7,9–11). Apart from urinary excretion, AR metabolites may be excreted in bile, especially as conjugates of sulfonic and glucuronic acids, and in biliary excretion (7,11,12).
when ARs are consumed in high doses (12), and possibly undergo enterohepatic circulation, as indicated by the plasma concentration profile after ingestion of a single AR dose (13).

In human studies, elimination of AR metabolites has been suggested to be slower (13,14) than for their precursors (15) and therefore it has been hypothesized that plasma AR metabolites are good potential biomarkers for intake of WG wheat and rye. However, the half-life values reported for AR metabolites are probably overestimates, because they were determined after ingestion of rye bread and are therefore likely to reflect both formation and elimination of AR metabolites. Metabolite formation may be a rate-limiting process and thus determine the metabolite half-life. To our knowledge, homolog-specific differences in metabolite formation have not been investigated to date, as previous studies on AR elimination have been conducted in individuals consuming WG or bran products of wheat and rye containing substantial amounts of several AR homologs.

The objective of the present study was to determine and compare pharmacokinetic parameters of ARs and their metabolites in rats by compartmental pharmacokinetic modeling and noncompartmental parameter calculation. To better understand AR metabolite pharmacokinetics, parameters were estimated in rats after i.v. infusion of AR metabolites and oral administration of intact AR. An additional objective was to compare metabolism after separate ingestion of 2 different AR homologs, C17:0 and C25:0, to evaluate homolog-dependent differences in AR elimination.

Materials and Methods

Experimental animals and diets. Groups of male Sprague Dawley rats (Taconic), 7–8 wk old (190–200 g) upon arrival, were routinely acclimatized with free access to water and feed before their diet was changed to an AR-free basal diet (Supplemental Table 1). This was made freely available to the rats for 2 wk prior to the experiment start and throughout the study period. After the last blood sample was drawn, the rats were killed with pentobarbital (Allfataal vet., Apotek Produktion och Laboratorium) by vein catheter injection for i.v.-treated rats and by heart injection under anesthesia for orally treated rats. The study was approved by the Ethical Committee for Animal Experiments in the Uppsala region (application no. C329/10).

Administration and sample collection. One group of rats (n = 24) was acclimatized for 17 d after arrival before the feed was changed to the AR-free basal diet (Supplemental Table 1). After 2 wk of the basal diet, the rats were divided into 6 groups and were orally administered a single dose (2.3, 11.6, or 23.2 μmol/kg) of AR homologs C17:0 or C25:0 (in ~1 mL corn oil) by gavage. Cannulas were used to collect venous blood from the distal back leg at 0, 3, 6, 12, 24, and 36 h after administration. A second group of rats (n = 24) was acclimatized for 10 d after arrival before the feed was changed to the AR-free diet (Supplemental Table 1). Rats were divided into 6 groups and were given either DHBA or DHPPA (dissolved in saline) at 1 of 3 doses (2.32, 11.6, or 23.2 μmol/kg) by 30-min i.v. infusion and blood samples were drawn at 0, 15, 28, 60, 90, 210, and 390 min after infusion start. The infusion was administered through a femoral vein catheter and blood samples were taken through a femoral artery catheter. These catheters were surgically attached under anesthesia 1 day prior to infusion.

To simplify the dosing of intact ARs and AR metabolites, a blocked randomized design was utilized, where 2 rats were paired (based on their body weight) and given the same substance and dose amount on the same occasion. Plasma was immediately separated from blood after sampling and stored at −80°C until analysis.

Quantification of ARs and their metabolites in rat plasma. Three rats per treatment (36 rats total) were randomly selected for analysis of ARs and their metabolites in plasma. All plasma samples of the selected rats were individually analyzed as single samples and a modified GC-MS method (16) was used to simultaneously quantify ARs and their metabolites. In detail, plasma samples were thawed at room temperature and 50 μL plasma was added to 15 mmol syringic acid in 15 μL methanol (internal standard for AR metabolites) and 200 μL 0.1 mol/L sodium acetate buffer (pH 5.0) containing 7 μL and ± 13 μL β-glucuronidase and sulfatase activity, respectively. After gentle shaking by hand, the samples were incubated overnight at 37°C. Incubation was followed by acidification of the samples with 100 μL concentrated acetic acid, and 3 mL diethyl ether was added. Due to the immiscibility with the aqueous deconjugation solution, 15 ng of AR homolog C20:0 in 15 μL methanol (internal standard for ARs) was added to the samples after the addition of diethyl ether. Extractions and silylation were performed as previously described (16), though the silylation volume was reduced to 50 μL. Silylated samples were analyzed by GC-MS as previously described (16) but with a modified temperature program: 100°C (0.0 min), 180°C (9.3 min), 185°C (12.6 min), 260°C (14.5 min), 280°C (17.4 min), and 300°C (19.4–24.4 min). Quantifications of ARs and their metabolites were performed as previously described (11,16), with an exception of a reduced dwell time of each ion to 100 ms. A pooled sample of fasting human plasma was analyzed in quadruple replicates within each batch. All samples from each rat were analyzed within the same batch.

Estimation of pharmacokinetic parameters. Plasma concentration data were log-transformed before estimation of pharmacokinetic parameters. Population pharmacokinetic models were individually built for C17:0, C25:0, DHBA, and DHPPA using nonlinear mixed-effects modeling (17,18). Models were evaluated as previously described (12) and for AR metabolites, 3-compartment models were tested in addition to 1- and 2-compartment models. NONMEM 7.2 (Icon) was used for model building and model diagnostics were performed with Xpose 4 (19). Median predictions and 5th and 95th percentile predictions for each model were based on 200 simulated datasets per observation. Model selection was based on objective function values (OFVs), ratio of model OFVs, and the goodness-of-fit plots. The OFV indicates how much an individual parameter contributes to model performance, and a parameter should be added to the model only if it causes a substantially lower OFV than when it is excluded from the model (12). Parameters estimated from modeling were utilized to calculate hybrid distribution constants, hybrid elimination constants, and elimination half-lives (t1/2) as previously described (20).

In addition to parameter estimation by modeling, pharmacokinetic parameters were noncompartmentally calculated for individual rats as previously described (20) using Excel 2007 (Microsoft). In these calculations, the elimination constants were determined as the slopes of the linear regression between the natural logarithm of the plasma concentration and time in the intervals 360–2160 min (ARs) and 90–390 min (AR metabolites). AUCs were estimated by the trapezoid formula (20).

To create combined models describing the pharmacokinetics of ARs and their metabolites as well as the enzymatic transformation of parent ARs to DHPPA and DHBA for the individual AR homologs, metabolite
models were joined to AR models. Pharmacokinetic parameters of AR metabolites were re-estimated after combining metabolite models with the AR models.

**Statistics.** Differences in noncompartmental estimates between ARs and their metabolites were evaluated in mixed linear models with substance and dose as fixed factors. Least-square means of noncompartmental estimates were calculated for substances. For maximum concentration and AUC, least-square means were also calculated for dose, whereas other parameters did not differ between dose amounts. A general linear model was used to calculate the P value of a linear trend for \( t_{1/2} \), with carbon atoms in the side chain as independent variable and \( t_{1/2} \) as dependent variable. All statistical analyses were performed with SAS 9.1 (SAS Institute) and differences at \( P < 0.05 \) were considered important.

**Results**

Differences (\( P < 0.01 \)) between C17:0 and C25:0 were found for apparent clearance and apparent volume of distribution (Table 1). Compared with C17:0, C25:0 had a greater maximum concentration, total AUC, and dose-corrected total AUC (\( P < 0.01 \)). Times to reach maximum concentration were 3.4 ± 1.2 h and 6.0 ± 1.2 h for C17:0 and C25:0, respectively, and the difference was of borderline significance (\( P < 0.06 \)). No differences between AR metabolites were observed for clearance or total AUC (Table 2). The dose-corrected total AUC did not differ between dose amounts. No differences were observed in total AUCs of metabolites (DHBA, DHPPA, and their sum) between rats administered C17:0 and C25:0, but the metabolite AUC during the first 6 h was higher in rats given C17:0 than in those given C25:0 (\( P < 0.05 \)). Only minute amounts of DHBA were formed after administration of DHPPA. The AR metabolites DHBA and DHPPA had a lower (\( P < 0.001 \)) \( t_{1/2} \) (1.2 and 1.5 h, respectively) than intact ARs and \( t_{1/2} \) also differed (\( P < 0.05 \)) between C17:0 (5.4 h) and C25:0 (8.8 h) (Tables 1 and 2). The \( t_{1/2} \) of ARs and their metabolites increased linearly with increasing length of the side chain (\( P \)-trend < 0.001) (Fig. 2).

The AUC ratio between DHPPA and DHBA did not differ after oral administration of different doses or AR homologs (C17:0 or C25:0) and in rats given AR orally, DHPPA represented 87 ± 5% of the metabolite total AUC. The times of maximum AR metabolite concentration did not differ and were 5.0 ± 4.2 h after intake of C17:0 and 8.0 ± 4.0 h after intake of C25:0. Among evaluated models, 2-compartment models best described the plasma concentrations of C17:0, DHBA, and DHPPA, whereas a 1-compartment model best described C25:0 (Figs. 3 and 4). Models indicated a faster absorption of C17:0 compared with C25:0 and pharmacokinetic parameter estimates derived from modeling were in general similar to parameters estimated by noncompartmental estimation (Tables 1 and 2). The best combined models (describing both AR and metabolite concentrations after oral administration of C17:0 or C25:0) were structured as combinations of the best individual AR and AR metabolite models (Fig. 3C). The structures of the combined models included 3 elimination routes for ARs, where fractions of plasma ARs were transformed and returned to plasma as DHPPA or DHBA. For these models, re-estimation of parameters from the individual models did not improve the model fit, i.e., the OFV did not change substantially. Metabolite formation rates estimated in the model of C17:0 (DHBA, 0.019 h\(^{-1}\); DHPPA, 0.19 h\(^{-1}\)) were comparable with those estimated in the model of C25:0 (DHBA, 0.016 h\(^{-1}\); DHPPA, 0.17 h\(^{-1}\)), though the actual rate of DHPPA formation is determined by the elimination rates of the parental ARs (Table 1).

**Discussion**

The pharmacokinetics of ARs and their metabolites have been investigated in both humans and animals (12–15,21,22). However, no previous study has evaluated the pharmacokinetic parameters of AR metabolites after i.v. administration of pure DHBA and DHPPA, which is necessary to estimate the volume of distribution, clearance, and true \( t_{1/2} \). In the present study,

**TABLE 1** Pharmacokinetic parameters of orally administered ARs in rats estimated by population pharmacokinetic compartment models and by noncompartmental calculations

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Model(^5)</th>
<th>Calculation(^4)</th>
<th>Model(^5)</th>
<th>Calculation(^4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorption rate constant, ( h^{-1}\ (%)</td>
<td>60 (0.10)</td>
<td>(-)</td>
<td>72 (2.1)</td>
<td>(-)</td>
</tr>
<tr>
<td>Apparent volume of distribution, ( L ) (%)</td>
<td>(-5)</td>
<td>67 (22)</td>
<td>3.7 (19)</td>
<td>2.8 (15)</td>
</tr>
<tr>
<td>Central compartment, ( L ) (%)</td>
<td>3.2 (15)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>Peripheral compartment, ( L ) (%)</td>
<td>37 (41)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>Apparent intercompartmental clearance, ( L/h ) (%)</td>
<td>3.5 (48)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>Apparent clearance, ( L/h ) (%)</td>
<td>13 (8.2)</td>
<td>8.5 (18)</td>
<td>0.24 (16)</td>
<td>0.29 (19)</td>
</tr>
<tr>
<td>Elimination rate constant, ( h^{-1}\ (%)</td>
<td>(-5)</td>
<td>0.15 (12)</td>
<td>0.065 (25)</td>
<td>0.13 (27)</td>
</tr>
<tr>
<td>Hybrid distribution rate constant, ( h^{-1}\ (%)</td>
<td>3.4 (7)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>Hybrid elimination rate constant, ( h^{-1}\ (%)</td>
<td>0.083 (7)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>( t_{1/2} ), h (%)</td>
<td>11(^{11})</td>
<td>5.4 (14)</td>
<td>11 (25)</td>
<td>8.8 (23)</td>
</tr>
<tr>
<td>Proportional residual error, ( %)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>Additive residual error, ( \ln(\text{nmol/L}) ) (%)</td>
<td>2.6 (13)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
</tbody>
</table>

\(^1\) Values are expressed as estimates (RSEs), \( n = 9 \). \(^*\)Different from C17:0, \( P < 0.05 \); \(^**\) different from C17:0, \( P < 0.001 \). AR, alkylresorcinol; RSE, relative SE; \( t_{1/2} \), elimination half-life.

\(^2\) Parameter estimated by population pharmacokinetic compartment models built individually for the different homologs.

\(^3\) Estimates were noncompartmentally calculated for each rat (\( n = 9 \)) and are expressed as means (RSEs).

\(^4\) Parameters not estimated by noncompartmental calculation.

\(^5\) Not applicable in 2-compartment model.

\(^6\) Not applicable in 1-compartment model.

\(^7\) No SE calculated.

\(^{11}\) Hybrid \( t_{1/2} \).

\(^{12}\) Not included in the model.
pharmacokinetic parameters of ARs and their metabolites in rats were estimated by noncompartmental calculations and estimates were confirmed by population-based compartmental modeling. By administering pure compounds to rats, differences in parameter estimates between the 2 AR homologs and their 2 metabolites could be evaluated. In addition, the formation of AR metabolites was monitored following oral administration of 2 AR homologs to evaluate differences in relative bioavailability and elimination.

The lack of double peaks in the plasma concentration profile of rats orally administered ARs is in line with previous animal studies (21,22) but in contrast with a human study (15). Apart from interspecies differences in absorption, the differences may have been due to matrix effects, because rats in the present study were administered pure compounds in oil, whereas the human participants ingested large amounts of rye bran. The time to reach maximum AR and metabolite concentration in the present study was comparable with the time for radioactivity to peak (7–12 h) in plasma of rats fed radiolabeled AR homolog C21:0 (21).

Although the dose-corrected total AUC was higher for C25:0, it appears that the uptake of intact ARs from the gut does not differ between homologs, because the total AUC of metabolites formed (DHBA, DHPPA, and their sum) did not differ after ingestion of C17:0 or C25:0. It can be speculated that the faster absorption and greater metabolite formation in the earlier stages (<6 h) of C17:0 compared with C25:0 could reflect the existence of an alternative absorption pathway of ARs that complements lymphatic absorption. The concentration of ARs is higher in HDL than in LDL (5), which might indicate HDL-mediated absorption comparable with that of vitamin E (6). Like fatty acids, it is possible that AR homologs with different chain length possess diverse susceptibility for the different absorption pathways and thereby avoid first-pass metabolism to varying degrees (23). However, because ARs were not analyzed in portal blood or liver and thereby avoid first-pass metabolism to varying degrees (23).

The observed differences in plasma maximum concentration, the AUC, apparent clearance, and apparent volume of distribution between the 2 homologs could partly be due to faster elimination of C17:0. Unlike the findings in the present study, the elimination half-life (t_{1/2}) does not substantially differ between AR homologs in humans (15). However, the human participants consumed rye bran containing a mixture of AR homologs, which could affect elimination of individual homologs. Furthermore, the relative composition of plasma ARs in the human study changed over time; short homologs dominated early but declined compared with longer homologs over time. A possible explanation of different elimination rates between C17:0 and C25:0 might be diverse preferences for different AR homologs of enzymes responsible for the initial ω-hydroxylation, as seen for different forms of vitamin E (24). Further, C17:0 was the major homolog in the minute amounts of conjugated ARs detected in human urine after ingestion of wheat bran (7). In line with those findings, it is possible that rats in the present study were able to excrete conjugated C17:0 in urine. The similarities in qualitative metabolite formation (proportion of total metabolites present as DHPPA) after oral administration of C17:0 and C25:0 suggest a shared catabolic pathway of different AR homologs.

![FIGURE 2](https://i.imgur.com/3.jpg)  
**FIGURE 2** Estimated t_{1/2} of ARs and their metabolites in rat plasma. Noncompartmental estimates of t_{1/2} are plotted against number of carbon atoms in the side chain (P-trend < 0.001). Slope, intercept, and R^2 of linear regression are indicated in the diagram. Error bars represent SEEs. AR, alkylresorcinol; DHBA, 3,5-dihydroxybenzoic acid; DHPPA, 3-(3,5-dihydroxyphenyl)-propionic acid; t_{1/2}, elimination half-life.
The very small amounts of DHBA quantified after administration of DHPPA indicate that plasma DHPPA is primarily eliminated by excretion and not through further phase I metabolism. The rapid elimination of AR metabolites compared with their formation observed in the present study indicates that the slow decline in human plasma metabolite concentration observed after AR consumption (13) reflects the formation of DHBA and DHPPA rather than their elimination. Thus, the concentration of AR metabolite in plasma is not only dependent on the amount and time of AR consumption but also the rate of AR metabolism. As a consequence, plasma concentration of AR metabolites is likely to be affected by more factors than plasma ARs. This might be an important disadvantage of plasma AR metabolites as biomarkers. However, due to the cumulative nature of urine collections, urinary AR metabolites in 24-h urine collections would be less affected by variations in the rate of AR metabolism and thus more successful in reflecting WG wheat and rye intake.

Unlike C25:0, C17:0 was best described by a 2-compartment model. However, as shown by the parameter estimates (Table 1), the rate constant of the transfer from the peripheral to the central compartment ($Q/V_2$) was merely a fraction of the rate constant in the opposite direction ($Q/V_1$). Hence, the distribution of C17:0 from central to peripheral compartments could in practice be considered an additional elimination route. Parameter precision and overall predictive power of the pharmacometric models were higher for the metabolites than for the AR homologs. The i.v. infusion of AR metabolites led to less residual variability in the resulting datasets compared with the AR data, which were acquired after oral administration.

The combined models had higher complexity than the individual models, which could make it more difficult to get stable parameter estimates when estimating every parameter for all substances simultaneously due to the large number of available df needed. The estimated parameters for the metabolite models in the separate metabolite experiments can also be seen as more descriptive of the metabolite pharmacokinetics, because the measured concentrations of metabolite are not influenced by absorption and metabolism of ARs. In the combined models, ARs were eliminated via 3 routes. In 2 of those, eliminated ARs reappeared in plasma as DHPPA or DHBA. It can be speculated that the third route might represent biliary excretion of ARs metabolites and possibly also renal elimination of ARs.

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The results presented here can be used when designing future studies for evaluation of potential bioactivities of ARs and their metabolites in rats. Furthermore, the models established here can be used as the basis for creating similar models for humans. A combined human pharmacokinetic model for ARs and their metabolites could prove useful, e.g., in evaluating the utility of ARs and their metabolites (individual or total) in nonfasting plasma samples to reflect intake of WG rye and wheat.

In conclusion, the present study demonstrated substantially faster elimination of AR metabolites compared with their intact precursors. As a consequence, plasma concentrations of DHBA and DHPPA are determined by their formation and depend on the temporal relation between intake and measurement. The $t_{1/2}$ of ARs and their metabolites increased linearly with length of the side chain ($P$-trend $< 0.001$). There were no differences between the AR homologs in total formation of metabolites or proportions of metabolites. However, the longer AR homolog had a greater AUC than the shorter homolog. These findings may be useful in the design of future studies of ARs and their metabolites in rats, and the models presented could provide guidance for the development of similar models in humans.

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Literature Cited