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Structural determinants of plant lignans for the formation of enterolactone in vivo

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Abstract

The quantity of mammalian lignans enterolactone (ENL) and enterodiol (END) and of plant lignans secoisolariciresinol (SECO) and 7-hydroxymatairesinol (HMR) excreted in a 24-h rat urine sample was measured after a single p.o. dose of an equivalent quantity of secoisolariciresinol diglycoside (SDG), secoisolariciresinol (SECO), matairesinol (MR), 7-hydroxymatairesinol (HMR) and ENL. Plant lignans (SECO and HMR) were partially absorbed as such. The aglycone form of SECO was more efficiently converted into mammalian lignans END and ENL than the glycosylated form, SDG. Of plant lignans, MR produced the highest quantities of ENL: the quantity was over twofold compared with HMR or SDG. The majority of the animals, which had been given SECO, excreted higher quantities of END than ENL into urine, but ENL was the main lignan metabolite after SDG. The highest quantities of ENL in urine were measured after the administration of ENL as such. The (−)SECO isolated from *Araucaria angustifolia* was converted into (−)ENL only. The administration of (−)SDG, which was shown to produce (+)SECO, resulted in excretion of (+)ENL only and (−)HMR was converted into (−)ENL only. This confirmed that the absolute configurations at C8 and C8′ are not changed during the microbial metabolism. Whether the biological effects are enantiomer-specific, remains to be resolved.

Keywords: Enterolactone; Plant lignans

1. Introduction

Lignans are defined as a class of phenolic compounds, many of which possess a 2,3-dibenzylbutane skeleton. They are optically active compounds and may exist as two enantiomers, i.e. the right- and left-handed forms. Lignans are widely distributed in higher plants and the type of lignan varies in different species. Most dietary sources such as flax and rye contain lignans as glycosidic conjugates associated with fiber components. The presence of lignans as unconjugated forms in foods like flaxseed, has never been verified. In contrast to these dietary sources, large quantities of lignans are found as unconjugated forms in coniferous trees. As an example, the most abundant lignan in flax is seco-

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isolariciresinol diglycoside (SDG) while (−)7-hydroxymatairesinol (HMR) is the main lignan component in heartwood of spruce (*Picea abies*) [1]. Plant lignans such as matairesinol (MR) and SDG are converted by the intestinal microflora to mammalian lignans, enterolactone (ENL) and enterodiol (END), respectively (Fig. 1) [2].

Epidemiological studies [3–5] have shown an inverse relationship between serum and urine ENL concentrations and breast cancer risk and raised considerable interest in a potential chemopreventive action of ENL. Thompson and co-workers demonstrated chemopreventive properties of SDG in DMBA-induced mammary tumors in rats [6,7]. The daily administration of SDG significantly decreased the number of tumors per tumor-bearing rat and per number of rats in the group. SDG also inhibited the growth of the established tumors and the number of those tumors appearing during the late stage of carcinogenesis [7]. In our recent studies, orally administered HMR was shown to be metabolised to ENL and to inhibit the growth of DMBA-induced mammary tumors in rats [8]. HMR in a dose of 15 mg/kg of body weight starting at 9 weeks after DMBA-induction increased the proportion of stabilized and regressing tumors [8]. When the administration via diet was started 1 week prior to DMBA induction, HMR reduced both tumor volume and tumor growth but no significant reduction in tumor multiplicity was observed [9]. In another study, ENL

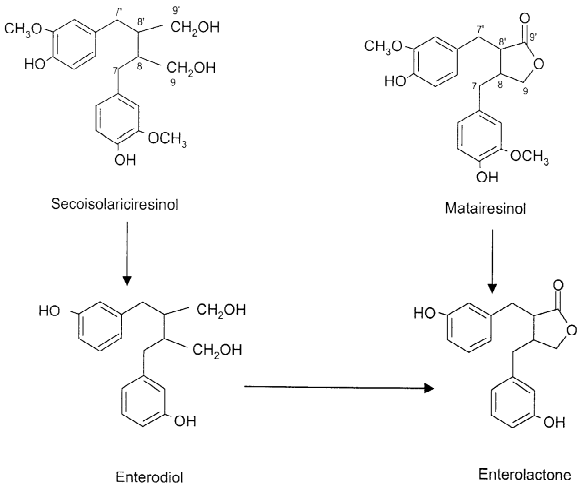

Fig. 1. Metabolism of plant lignans to mammalian lignans.

dose of 10 mg/kg body weight inhibited tumor growth in the DMBA model for mammary cancer [10]. Inhibition was more pronounced in tumors which developed during the 7-week treatment period but ENL also inhibited growth of tumors established prior to the start of the lignan administration. These findings do suggest that the growth inhibition by lignans of the DMBA-induced mammary carcinomas may be at least in part mediated by ENL. ENL synthesised for the DMBA-study was a racemic mixture of enantiomers containing both the right- and left-handed forms.

The lowest observable effect level (LOEL) has not been found for ENL or any other lignan in animals or in humans. In a population study [5], a significant difference was observed in serum ENL concentrations between breast cancer patients and their healthy controls (20 vs. 26 n*M*, respectively) while a wide individual variation was found in the ENL concentration. In the lowest quintile, the mean serum ENL concentration was 3 n*M* with the highest concentration being 54 n*M*. This implies that concentrations of tens of nanomolars would be significant in the chemoprevention of human breast cancer. In the serum of HMR exposed rats, however, the ENL concentrations were considerably higher or about 10-fold compared with those found in the serum of omnivores [9].

In addition to LOEL, critical structural properties determining the mechanisms of ENL formation need to be clarified. This becomes vital in a case in which the concentrations required for the chemoprevention will exceed those generally found in a population. Minor changes in the chemical structure of a lignan may result in major changes in the ENL formation. Theoretically, some lignans such as secoisolariciresinol (SECO), MR, and HMR could be important precursors of ENL in reducing breast cancer risk. Other lignans such as (−)nortrachelogenin, although very similar by structure, may not be metabolized to ENL and may not have the same potency in breast cancer chemoprevention (according to our unpublished observations). Therefore, it would be important to evaluate whether or not the compounds will be sufficiently converted to ENL, when assessing the role of the dietary lignans in breast cancer chemoprevention.

The objective of the present study was to compare

the ENL quantities in 24-h rat urine following a single p.o. dose of an equivalent quantity of SDG, SECO, MR, HMR, or ENL. Stereochemistry may be involved in a conversion of a precursor plant lignan to mammalian lignans, as well as in absorption, conjugation, and urinary excretion. Therefore, the enantiomers of ENL found in urine were analysed after the administration of different precursors.

2. Experimental

2.1. Chemicals

Secoisolariciresinol (SECO) was extracted from *Araucaria angustifolia* [11] and 7-hydroxymatairesinol (HMR) *Picea abies* according to the method described by Ekman [1,9]. HMR was a mixture of two stereoisomers, (−)-allo-HMR (HMR1) and (−)HMR (HMR2), the latter representing the majority. The purity analysed with gas chromatography was 95% for extracted HMR and 97% for SECO. Matairesinol (MR) was synthesised by a method used by Freudenberg and Knof [12]. We developed the method further and have described it previously when we described the synthesis of ENL [10]. The (−)ENL was synthesised in Dr Sjöholm's laboratory (Åbo Akademi University, Turku, Finland). The synthesis of (−)ENL will be reported in detail later. END was obtained from Fluka (Buchs, Switzerland). SDG was extracted from flaxseed using a method developed by Pihlava et al. [13]. Briefly, the method contains supercritical fluid extraction (SFE)-extraction, solvent extraction, base hydrolysis and clean-up of the lignan by C_{18}–flash chromatography. Purity of the isolated SDG was at least 90% according to analysis by HPLC equipped with a diode array detection system [13]. The majority of the impurities consisted of unidentified phenolic components. Water used in the HPLC eluent was purified using a water purification system (ELGA), and further purified by elution through a glass column packed with approx. 40 g of RP-18 material (Varian Bondesil 40 μm) in order to remove residuals of lignans occurring in the purified water. The RP-18 material had to be washed with approx. 250 ml methanol after each 1 liter elution of water, otherwise leaking of lignans from the material caused a higher background in the LC–MS–MS-MRM (multiple reaction monitoring) analyses.

Ascorbic acid and *Helix pomatia*, an enzyme mixture of glucuronidase and sulfatase, were purchased from Biosepra (Villenouve la garenne Cedex, France). Anhydrous sodium acetate was obtained from J.T. Baker (Deventer, Netherlands) and flavone was from Sigma (St Louis, MO, USA). Sodium azide and Lichrosolv-grade methanol were obtained from Merck (Darmstadt, Germany). Polyethylene glycol (M_r 3400) was from ICN Biomedicals Inc. (Aurora, OH, USA).

2.2. Animals

Thirty male Sprague–Dawley rats, which were 7 weeks old at the time of shipment, were obtained from Harlan (Horst, Netherlands). These rats were housed (two animals/cage) under a 12-h light, 12-h dark cycle at 21 °C with 50% humidity with free access to water and standard open-formula chow RM1 diet (SDS, Special Diet Services, Whitham, Essex, UK). The animals were acclimatised to standardised housing conditions for 2 weeks prior to the metabolism experiment.

2.3. Lignan feedings and urine collections

At the age of 9 weeks, the rats were randomised into four groups with six rats in each. Prior to baseline urine collections rats were administered p.o. with vehicle as placebo treatment. The vehicle used for the lignan administrations was 50% polyethylene glycol in water containing 10% ethanol and the administered volume was 200 μl per 100 g of body weight. For comparison of lignan metabolism, rats were administered p.o. with vehicle containing MR, SECO, SDG, or ENL in a dose of 25 mg per kg of body weight. In the case of SDG, the dose was 47.2 mg/kg which corresponds to 25 mg/kg of SECO in the aglycone form. Individual 24-h urine samples were collected. The collection jars of metabolic cages contained 120 μl of 0.56 *M* ascorbic acid and 120 μl of 0.15 *M* sodium azide as preservatives. The centrifuged urine volumes were measured and samples stored at −20 °C.

2.4. Sample preparation

A volume of 0.5 ml of thawed urine samples was mixed with 1.0 ml of 0.15 *M* sodium acetate buffer (pH 4.0) and 15 μl of *Helix pomatia* enzyme mixture. For hydrolysis of lignan conjugates, the samples were incubated at +37 °C overnight. The hydrolysed samples were extracted using Sep-Pak tC_{18} columns (Waters, Milford, USA). The columns were conditioned with 2.0 ml of methanol and equilibrated with 2.0 ml of 0.15 *M* sodium acetate buffer. The urine samples to which 2.5 μg of the internal standard flavone had been added were loaded into columns, washed with 0.15 *M* sodium acetate buffer and the polyphenolic fraction was eluted with 2.0 ml of methanol. The samples were gently evaporated to dryness under nitrogen flow in a water bath at +45 °C. The samples were then dissolved in 5.0 ml of methanol and an aliquot of 0.1 ml was diluted with 0.9 ml of 0.1% acetic acid (HAc). The final flavone concentration was 50 ng/ml.

2.5. Analysis of lignan concentrations in rat urine using HPLC–MS–MS

2.5.1. HPLC–MS–MS analyses

The samples were analysed by HPLC–MS–MS using a PE Sciex API3000 triple quadrupole mass spectrometer, which had been equipped with a Turbo ion spray ionisation source (electrospray ionisation). Air was used as the nebuliser gas and nitrogen as curtain gas. The mass spectrometer was interfaced to a Waters Symmetry C_{18} column (3.5-μm particle size, 2.1 mm I.D., 100 mm length) equipped with a Waters Sentry guard column. An Agilent 1100 series HPLC delivered vacuum degassed eluents, gradient eluent flow, thermostatically controlled warming of the column, and autosampler injection. The entire system from sample injection to data acquisition was computer controlled using PE Sciex Analyst software version 1.1.

The eluents used were methanol/0.1% HAc 90:10 (A) and 0.1% HAc (B). Both A and B contained isopropanol, 0.1% (A) and 1% (B). The gradient was from 22 to 73% A within 16 min (corresponding to 20–66% methanol), then to 95% A (85% methanol) within 1 min, which composition was held for 2 min. The initial composition (22% A) was then reached after 1 min and held for 5 min. The total analysis time was 24 min. The flow-rate was 0.20 ml/min, the injection volume 10 μl, and the column oven was kept at 30 °C. The retention times of the analytes HMR1, HMR2, SECO, END, and ENL were 10.3, 9.8, 12.4, 15.3, and 15.8 min, respectively. The retention time of the internal standard flavone was 20.4 min. The mass spectrometric analyses were carried out in the MRM (multiple reaction monitoring) mode. Negative ionisation mode was used for the analytes, whereas flavone was analysed in the positive mode. The duration of the negative MRM scan (period 1) was from 0 to 18 min and the positive MRM scan (period 2) from 18 to 25 min. The temperature of the ionisation source was maintained at 400 °C and the flow of Turbo ion spray gas at 8 l/min. The parameters in the negative mode included nebuliser gas (NEB) (13), curtain gas (CUR) (9), and collision activation decomposition gas (CAD) (7), ion spray (IS) voltage −3300 V, and entrance potential (EP) 10 V. The Q1 ion energy (IE) was −1.0 V, Q3 IE −2.0 V, deflector (DF) 150 V, and continuous electron multiplier (CEM) 2200 V. In the positive mode, NEB was 10, CUR 14, CAD 6, IS 5500 V, and EP −10 V. The Q1 and Q3 IEs, DF, and CEM values were the same as in negative mode but with opposite polarities. The resolution was set to unit resolution both in Q1 (quadrupole 1, the first mass analyser) and in Q3 (quadrupole 3, the second mass analyser). Unit resolution was defined as 0.7±0.1 a.m.u. full width at half height of the polypropylene glycol peaks chosen during the calibration.

The MRM method was optimised by infusion of pure compounds using the quantitative optimisation function with the molecular ion as precursor ion. The four most intense peaks were auto-selected, and of these the most intense product ion was chosen for the MRM method. Parent and daughter ion combinations and individual potentials used are presented in Table 1. The dwell time of each compound was 400 ms.

2.5.2. Quantitation

Standard samples for the calibration curve were prepared from aliquots of a stock standard solution containing HMR, SECO, END, and ENL at concentrations of 700, 500, 620, and 810 ng/ml (in

Table 1
Parent and daughter ion combinations and individual potentials used in LC–MS–MS (MRM) detection of analysed lignans

Compound	Q1 mass (parent ion)	Q3 mass (daughter ion)	DP	FP	CE	CXP
HMR	373.00	355.15	−61	−210	−24	−23
SECO	361.12	164.97	−61	−250	−36	−9
END	301.02	252.87	−51	−190	−32	−17
ENL	297.06	252.96	−46	−160	−28	−15
Flavone	223.24	76.86	61	220	61	14

DP, declustering potential; FP, focusing potential; CE, collision energy; CXP, collision cell exit potential.

methanol), respectively. Four dilutions of the stock solution were made: 1:2, 1:10, 1:20, and 1:40. All of the standard solutions contained 50 ng/ml flavone, i.e. the same quantity as in the study samples. The standard solutions were diluted with pooled blank urine collected from adult male rats fed at least 4 weeks with semipurified lignan-free C1000 diet (Altromin, Lage, Germany), and solid-phase extracted as described above. They were not hydrolysed. When the HPLC–MS–MS analyses were completed, the peaks were reviewed and the automatic integration of peak areas was corrected when necessary. HMR was determined as the sum of the two isomers. The Analyst program automatically calculated the concentration of unknowns on the basis of the standard curve.

2.6. Optical activity of plant lignans and analysis of ENL enantiomers

ENL enantiomers were analysed in the same samples as above using the HPLC–MS–MS (MRM) method in the negative mode optimised for ENL described above. The HPLC column used was a Chiralcel OD-R column (Daicel Chemical Industries, Ltd.) with the dimensions of 0.46 cm (I.D.)×25 cm and equipped with a OD-RH guard column (0.4 cm I.D.×1 cm). The eluents were the same, but an isocratic flow containing 78% of eluent A (corresponding to 70% methanol) with a flow-rate of 0.50 ml/min was used. The injection volume was 50 μl, the temperature of the ionisation source was maintained at 450 °C, and the dwell time was 1000 ms. The retention time of (−)ENL was 22.2 min and that of (+)ENL was 23.7 min. The identity of the two enantiomers was confirmed by analysis of a solution containing pure synthesised (−)ENL. The optical rotations for plant lignans HMR 1, HMR 2, SECO and SDG were measured with a Perkin-Elmer 241 digital polarimeter using a 1-dm cell.

2.7. Statistical analysis

Statistica Software for Windows (version 5.1) was used for the statistical analysis (Stat Soft, Tulsa, OK, USA). Normally distributed data of urine lignan concentrations and animal weights were analysed using one-way analysis of variance followed by Tukey's least significance test. The acceptable level of significance was $P \leq 0.05$.

3. Results

3.1. Plant lignans and their metabolites in urine

After the feeding of vehicle (50% PEG in water containing 10% EtOH), the average baseline excretions of ENL, END and SECO were 11.0±5.80, 0.089±0.095 and 0.142±0.109 μg per 24 h, respectively. No statistically significant differences were measured between the different treatment groups in baseline ENL or END excretion ($P > 0.05$). The rat body weights did not significantly differ from each other within the feeding groups ($P > 0.05$).

As expected, all of the tested compounds were metabolised to END and/or ENL. In the case of SECO and HMR, plant lignans as such were also excreted into urine. The aglycone forms of plant lignans were not excreted in equal quantities, i.e. when compared with SECO, unconverted HMR was detected in urine in higher quantities (Table 2).

The proportion of END and ENL excreted into urine was different after the administration of differ-

Table 2
Excretion of lignans into urine after a single oral dose of 25 mg/kg (aglycone) of lignans

Administered lignan	Lignans in urine (μg/24 h)			
	HMR	SECO	END	ENL
MR	nd	nd	1.03±0.80[a]	54.7±22.4[a]
Baseline	nd	0.082±0.023	nq	8.97±5.39
HMR	62.7±27.5	nd	0.46±0.30[a]	18.2±8.9[b,c]
Baseline	nd	0.067±0.034	0.089±0.093	9.68±4.44
SECO	nd	6.31±3.43[a]	46.2±34.9[b]	40.9±18.4[a,c]
Baseline	nd	0.13±0.064	0.076±0.10	10.05±4.44
SDG	nd	1.65±1.67[b]	1.32±2.49[a]	24.5±6.2[b,c]
Baseline	nd	0.10±0.038	0.15±0.038	7.04±2.40
ENL	nd	nd	1.93±0.54[a]	311±155[d]
Baseline	nd	0.33±0.09[#]	0.13±0.13	19.2±3.81

The number of individual urine samples for analysis in lignan groups was six, except for the MR administered group and baseline samples in which the number of samples available for analysis was five; nd, compounds not detected; nq, compounds not quantified (concentrations below quantitation limit). As for lignans, MR is used for matairesinol, HMR for 7-hydroxymatairesinol, SECO for secoisolariciresinol, SDG for secoisolariciresinol diglycoside, END for enterodiol and ENL for enterolactone.

[#] Statistically significant differences in baseline concentrations of measured compounds ($P \leq 0.05$).

[a,b,c] Statistically significant differences in concentrations of measured compounds between the lignan administered groups ($P \leq 0.05$).

ent plant lignans. ENL was a major metabolite after the administration of HMR, MR, and SDG. After the administration of SECO, however, four rats in six excreted more END than ENL to urine while two rats excreted more ENL than END. After the administration of SECO, three of the urine samples contained SECO and after the administration of SDG, four of the urine samples contained SECO in detectable quantities.

The highest ENL quantities in urine were measured after the administration of synthetic ENL. The excreted quantities of ENL were fivefold compared with the measured quantities after the administration of MR, the best precursor of ENL among the plant lignans tested in the present study.

3.2. Optical rotation of the plant lignans and stereochemistry of urinary ENL

The optical rotations of plant lignans were the following: HMR1 $[\alpha]_D^{22}$ −5.8° (c 4 in THF), HMR2 $[\alpha]_D^{22}$ −11.5° (c 4 in THF), SECO $[\alpha]_D^{22}$ −29.8° (c 1 in acetone), and SDG $[\alpha]_D^{22}$ −4.2° (c 1 in methanol). The optical rotation of MR, $[\alpha]_D^{24}$ −36.9° (c 3.33 in THF), has been determined previously [10]. The enantiomeric purities of HMR1, HMR2, and SECO were confirmed by chiral HPLC–MS–MS analyses. Enzymatically hydrolysed (−)SDG was shown to produce (+)SECO only by chiral HPLC–MS–MS analysis.

The proportions of (–) and (+) forms of ENL in urine samples expressed in percentages were measured prior to the lignan administration and following a single oral dose of different plant lignans again in the urine samples collected for 24 h. Due to the plant fiber material in open formula chow used as basal diet for rats, ENL was also found in baseline urine samples. In these baseline samples, the majority of ENL was in the (+) form (Table 3). The average proportion of (−)ENL was 29±5.4% in urine samples collected prior to the lignan feedings

Table 3
The proportion of (−)ENL in the total quantity of ENL expressed as percentages after a single oral dose of 25 mg/kg (aglycone) of lignans

Administered plant lignan	(−)ENL (%)	
	Baseline	24 h[a]
MR	26±2.5	76±2.8
HMR	28±4.6	59±8.8
SECO	28±3.3	58±7.4
SDG	35±6.4	17±4.7

[a] The urine samples were collected post lignan administration for 24 h.

($n=20$). The administration of SECO, HMR, and MR increased the proportion of (−)ENL. Only SDG, isolated from flaxseed, increased the proportion of (+)ENL in urine, i.e. decreased the proportion of (−)ENL.

4. Discussion

The results of our single-dose study with the various lignans are in accordance with the proposed general concept for metabolism of the lignans in the mammalian organism. First, lignans are converted to aglycones by intestinal glycosidases, and thereafter, they will be transformed through sequential demethylation and dehydroxylation reactions catalysed by intestinal bacteria (Fig. 1) [14]. Herewith, the *meta*-methoxy–*para*-hydroxy substitution pattern of the plant lignans is converted to a single-*meta*-hydroxy substitution typical for mammalian lignans. The free *para*-hydroxy-group seems to be necessary for *meta*-demethylation, i.e. the *meta*-demethylation occurs prior to *para*-dehydroxylation [14]. Finally, the lactone ring may be formed but not opened (Fig. 1).

Several experimental findings justify the use of the 24-h urinary excretion quantities as parameters for the total conversion of plant lignans in the intestinal tract of the rat to mammalian lignans, particularly ENL. Firstly, a dose-dependent increase in urinary lignans was measured after the administration of SDG [15] and HMR [8]. In the case of HMR, no saturation of absorption or excretion capacities were found within the tested concentration range (3–50 mg/kg of body weight) [8]. Secondly, the majority of urinary lignans are excreted after a single dose within 24 h (unpublished observations). Thirdly, the measurement of lignan excretion into urine as micrograms per 24 h takes into account the variations in the urine volumes, i.e. hourly variations in lignan concentrations measured as μg/ml.

Compared to the administration of SDG, the administration of its aglycone SECO produced more mammalian lignans into urine. The total quantity of plant and mammalian lignans END and ENL in urine was threefold increased after the administration of SECO as an aglycone compared with urinary excretion after the administration of its diglycoside form, SDG. Similarly, the total quantity of urinary lignans was higher after the administration of HMR or MR when compared to SDG. The hydrolysis of *O*-glycosides into aglycones is obviously one of the rate-limiting steps in the conversion of plant lignans to mammalian lignans. In addition to the differences in the total quantities, the glycosylation appears to alter the relative quantities of metabolites. Considerably more END was excreted in urine after the administration of aglycone (SECO) in comparison with the glycoside (SDG) while no significant difference was seen in the quantities of ENL. There is no obvious explanation for this.

Our results clearly indicated that SECO and especially HMR may be absorbed as such (Table 2), which raises a question about the putative effects of plant lignans. After the feeding of SDG, four of the urine samples contained a detectable quantity of SECO. This is in accordance with earlier findings by Rickard and co-workers [15] stating minor quantities of SECO in comparison with mammalian lignans (ENL and END) after the administration of SDG. SECO has also been found in human urine [16]. In rats, the single p.o. dose level of HMR increased considerably more in the urinary quantity of HMR than what was seen in the quantity of SECO after the administration of SECO. The presence of HMR has also been demonstrated in the serum of HMR-exposed rats [9]. The intestinal microflora is capable of eliminating the aliphatic 7-hydroxy group. However, the presence of the 7-hydroxy group in the lignan molecule (HMR) seems to slow down the formation of ENL (HMR vs. MR). This may result in a higher concentration of HMR in the intestinum and an increased absorption of HMR. Since the MR quantities were not measured in urine, no information is available on the effects of the lactone ring (SECO vs. MR) or the 7-hydroxy group (HMR vs. MR) on the absorption of plant lignans.

There is some controversy regarding the capability of the rat to convert END to ENL [14]. No conversion of END to ENL was observed after the incubations with a rat faecal suspension in vitro [14]. However, in previous studies [2,15] the conversion of SDG to mammalian lignans END and ENL has been shown in rats. The excretion of END and ENL following the administration of SDG or SECO was

confirmed in the present study. The majority of those animals, which were given SECO, demonstrated higher quantities of END than ENL, but after a single dose of SDG, ENL was the main lignan metabolite measured in urine. Further, ENL quantities were lower in SECO and SDG groups than those seen after the MR administration. A lignan structure with the lactone ring and the presence of *O*-glycoside in SDG are the most plausible explanations for the different metabolite patterns after the administration of SDG, SECO, and MR.

The quantity of ENL in urine after ENL feeding was over fivefold higher than that after MR, the plant lignan giving the highest ENL yield. ENL may be passively absorbed along the passage through the intestine resulting in higher urinary ENL excretion. Plant lignans (like HMR, MR, SECO) even though they maybe readily absorbed, must first be converted by microbes to ENL in the small intestine and/or colon. This allows the absorption of ENL to occur only in a limited part of the gastrointestinal tract. Therefore the ENL concentrations in the lumen would be lower and ENL would have less time for absorption.

Molecular chirality may be involved in the action of lignans, since the intestinal metabolism and absorption, as well as excretion, may require a specific stereochemical structure. No inversion of enantiomers by intestinal bacteria was observed in this study, indicating that the stereochemical structure of the plant lignan determines the chirality of the metabolites. ENL was detected as a (−)enantiomer after the administration of SECO, HMR, and MR. The increased proportion of urinary (−)ENL after the HMR or MR (synthesised from HMR) administration suggests that both HMR and MR have the same configuration at the asymmetric carbons of the lactone ring as (−)ENL. Furthermore, SDG having mirror image configurations of the carbons C-8 and C-8′ increased the proportion of urinary (+)ENL showing that the absolute configuration of these carbons is not changed during the microbial metabolism.

Synthetic ENL tested for the chemopreventive action in the DMBA-induced mammary cancer model was a mixture of two enantiomers [10]. Therefore, this experiment did not provide any answer to the requirements on the stereochemical structure in regard to the biological activity. (−)HMR showing chemopreventive potency in DMBA-induced rat mammary cancer, was converted to (−)ENL, suggesting that this ENL enantiomer is biologically active. HMR itself, however, was found in high concentrations both in serum and urine of DMBA-induced rats after chronic exposure to an HMR enriched diet, as well as in urine after HMR feeding in the current study. Thus, we cannot exclude the possibility that HMR itself has also anticarcinogenic activity. On the other hand, administration of SDG, also showing antitumorigenic activity in DMBA model, resulted in excretion of (+)ENL, suggesting that this enantiomer is also active [6,7]. Concentrations of SECO were not measured/reported in these studies, but on the basis of our current results, SECO is also absorbed as such, and may have contributed to the chemopreventive action. Both enantiomers of ENL may thus be biologically active.

The binding sites of receptors and enzymes have specific stereochemical requirements. When no molecular chirality is involved, the antitumor activity of ENL or other lignans may be based on other mechanisms of action. Plant lignan aglycones SECO, MR, and HMR have been shown to be potent antioxidants in vitro [8]. The potential antioxidant activity of lignans could represent a mechanism associated with the preventive action of lignans in the development of hormone-related diseases. HMR was a more effective antioxidant than any of the flavonoids or synthetic antioxidants [17]. No strict stereochemical structures would be required from the lignans to act as antioxidants.

Hepatic microsomes are known to oxidize END and ENL and similar metabolites are excreted in human urine [18]. These derivatives account for less than 5% of the total urinary lignans, and no evidence has been provided for their biological significance in mammals. No attempts were made to identify or quantify these minor urinary components found in this short-term, single-dose study. Finally, we regard our single-dose tests as preliminary and further studies with various dietary contents of lignans and different exposure times will be necessary to confirm our findings.

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