Research Signpost
37/661 (2), Fort P.O., Trivandrum-695 023, Kerala, India

Cancer: Disease Progression and Chemoprevention, 2007: 185-202 ISBN: 81-308-0150-7
Editor: Takuji Tanaka

Chemopreventive effects of a plant lignan 7-hydroxymatairesinol on mammary and uterine cancer development in rat models

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Summary

7-Hydroxymatairesinol (HMR) is a plant lignan found in the heartwood and knots of Norway spruce (Picea abies). HMR is metabolized to enterolactone (ENL), which is a mammalian lignan produced by intestinal

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bacteria from plant lignans present in fiber-rich diets. Although serum ENL levels have been epidemiologically linked to a low risk of breast cancer, biological activity of ENL as anticarcinogenic agent is not fully established. This chapter is focused on chemopreventive actions of HMR in the experimental models of hormone responsive mammary and uterine cancers. Dietary HMR reduced the growth of DMBA-induced mammary cancer in female SD rats. No significant reduction in tumor multiplicity (number of tumors/rat) was observed. To examine influence of HMR on uterine carcinogenesis, the adult Donryu rats were initiated with N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG) and thereafter maintained on HMR containing diet. Significant reduction in the incidence of uterine adenocarinomas and a delay in the start of persistent estrus were observed in rats on HMR diet. Urinary analysis confirmed that HMR was metabolized mainly to ENL and 7-hydroxyenterolactone in both strains. It is not yet clear whether HMR or its metabolites or both exert chemopreventive effects in rat mammary and uterine carcinogenesis. The mechanisms of their acticarcinogenic effects remain unclear. Small quantities of HMR are found in human diets, and it is metabolized to ENL also in man. It is possible that diets enriched with plant-derived compounds such as HMR may be used as cancer reducing agents in the future.

Introduction

The hypothesis that low-fat and fiber-rich diets reduce the risk of breast and uterine cancers is supported by the epidemiological and experimental findings. The existence of various anticarcinogenic compounds such as lignans in e.g. vegetables, fruits, and whole grains has increased the interest in their use as a part of potentially cancer risk reducing diets.

Lignans are a large group of secondary plant metabolites consisting mainly of phenylpropanoid units. Nearly 500 different lignan structures have been identified from plant parts (roots, leaves, stem, seeds, and fruits). Plant lignans such as matairesinol (MR) and secoisolariciresinol (SECO) are associated with fiber and mainly occur as glycosidic conjugates in edible plants which complicate their isolation process. Lignans such as MR, SECO, arctigenin, lariciresinol, pinoresinol, syringaresinol and sesamin are converted by gut microbiota to mammalian lignans, enterolactone (ENL) and enterodiol (END), respectively [1-5].

Plant lignans are found in high quantities in coniferous trees. The far most abundant single component of lignans in Norway spruce (*Picea abies*) is 7-hydroxymtairesinol (HMR, Figure 1) [6, 7]. It constitutes about 60 percent of the total lignans. HMR is the most abundant in the heartwood of branches (5-10%) and especially in the inner knots in which the amount of HMR may be higher than ten per cent [7]. HMR mainly occurs in the unconjugated form

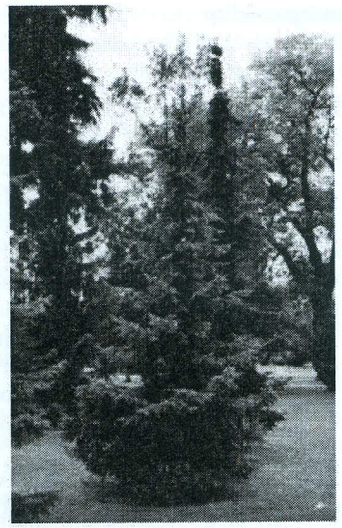

Figure 1. Norway spruce (*Picea abies*).

making extraction and purification of this plant lignan possible in large quantities. The availability of HMR from a novel source, spruce tree, made the preclinical studies with this plant lignan possible.

Objective of the HMR studies

Association of lignan-rich diets and high concentrations of ENL in serum and urine with reduced risks of breast cancer [8-10] and endometrial cancer [11] has increased interest in the diets enriched with lignans. However, because of conflicting epidemiological findings [12, 13] the question of the role of lignans in cancer prevention remains open. Experimental research focused on the cause and effect relationship between a high plant lignan or ENL concentration in serum and the risk of cancers is still limited. Several lignans have been isolated in quantities sufficient for *in vitro* testing but only a very limited number of compounds have been available in quantities sufficient for *in vivo* testing. This has been one of the major obstacles in testing the lignan effects in long-term *in vivo* models such as cancer models. Up till now, the effects of HMR on cancer development have been tested in the following tumor models: mammary, uterine, prostate, and liver cancers. Our own studies have focused on the effects of dietary HMR on hormone-dependent, 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced mammary cancer and

N-ethyl-*N'*-nitro-*N*-nitrosoguanidine (ENNG)-induced uterine cancer in rats. The findings for these two models will be discussed in detail in this chapter.

Plant lignans as precursors of mammalian lignans

Secoisolariciresinol diglucoside (SDG) and MR, were the first precursors found for mammalian lignans, END and ENL, respectively (Figure 2). Orally administrated SDG increased both END and ENL quantities in urine of the rat while MR increased ENL in urine [2, 14] also demonstrated a conversion of SECO, MR, and the alcoholic extracts from flaxseed to both END and ENL by human fecal bacteria *in vitro*. No conversion of ferulic acid to lignans by fecal bacteria was observed. An *in vitro* fermentation study with human fecal microbiota demonstrated that plant lignans, namely syringaresinol, arctigenin, and lariciresinol as well as pinoresinol also served as precursors for END and ENL production [3, 15]. The list of the precursors of ENL has increased further when sesame seed lignan, sesamin, was shown to be converted to END and ENL both in rats [4] and in humans [16, 17].

The conversion of HMR to mammalian lignan ENL was first demonstrated in rats [18]. In these studies, the administration of HMR increased the urinary ENL excretion dose-dependently. In addition to ENL production, HMR was

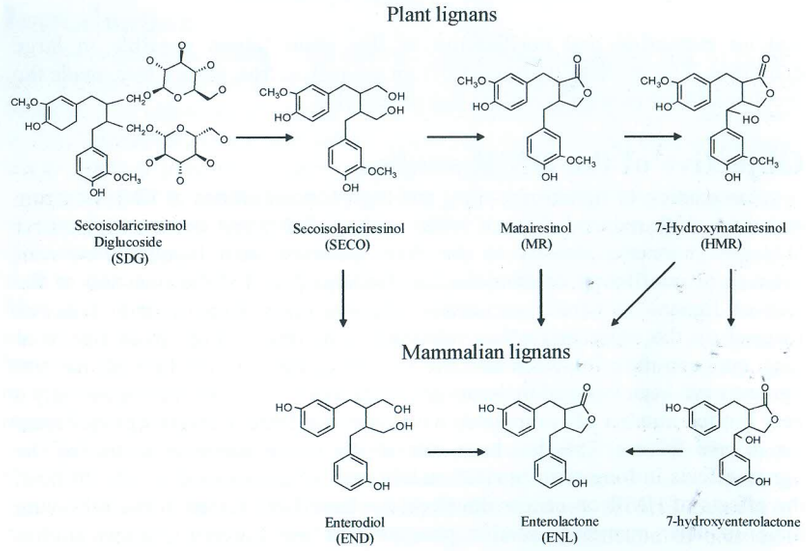

Figure 2. Metabolism of plant lignans to mammalian lignans by intestinal micobiota.

efficiently absorbed as such and part of it was metabolized to 7-hydroxyenterolactone. The absorption and metabolism of HMR to mammalian lignans were later confirmed also in humans [19] indicating that the rat is a suitable model for testing the conversion of purified plant lignans to mammalian lignans despite the obvious differences in gut microbiota.

In addition to plant lignans, degradation of lignin of plant fibers has been suggested as a possible source for mammalian lignan production. In a five-day feeding study in rats, lignans were estimated to account for 26-32% of the ENL production from cereal brans [20]. However, in humans the consumption of lignin-rich fiber (60 g per day for 5 days) did not significantly increase the excretion of lignans [21]. These studies indicate that there may be a fundamental difference between the rat and man in the capacity to produce ENL from lignans despite the similarities in metabolism of pure lignans. Therefore, the use of rats as a mammalian model for testing the putative biological significance of lignins or lignin-rich diets may require further investigation.

Mammalian lignans undergo enterohepatic circulation in rats and in humans. Axelson and Setchell [1] demonstrated in rats that mammalian lignans are absorbed from the intestine, transported to liver, and excreted into bile mainly in conjugated form. The majority of the mammalian lignans were found as glucuronides. Monosulphate and disulphate conjugates were also found in urine (≥74.9%) and in bile (≥97.5%) and low percentages of monosulphates and disulphates were also found in urine (≤6.3%) and traces in bile (≤ 1%). Similarly in humans, urinary lignans mainly occur as glucuronides [22, 23]. In urine, the major conjugate of END and ENL was the monoglucuronide (73-94%). Small quantities (≤ 14%) of monosulphates and diconjugates (glucuronides, sulphoglucuronides, and disulphates) were also found but hardly any free lignans (0.3 – 1%) [22]. Similarly in human plasma, END and ENL were present mainly as glucuronides but as much as 21-25 percent of END and ENL occurred in fraction containing free lignans and the sulphate conjugates [24]. Sulphate and glucuronide conjugates are generally considered as biologically inactive forms of compounds. However, several target tissues for lignan actions (e.g. mammary and endometrium) have sulphatase and/ or glucuronidase activities. This suggests that the free fraction of lignan in serum may poorly predict the amount of biologically active lignan available in target tissues.

The anticarcinogenic effects of HMR on DMBA-induced mammary cancer model in rats

The effects of orally administered HMR on mammary cancer were investigated on DMBA-induced rat mammary cancer at different stages of tumor development. HMR effects were tested using open-formula RM1 diet and semipurified C1000 diet as basal diets for the rats. RM1 contains whole

grains and soy as ingredients while C1000 is composed of purified sources of oil, protein, carbohydrates, and fiber, and is free of grain – and soy-derived bulk materials. In a study using RM1 basal diet, daily administration of HMR (15 mg/kg b.w.) starting at nine weeks after the DMBA -induction reduced tumor growth i.e. cumulative tumor volume when compared to controls (Figure 3A, [18]). In another experiment with HMR, a semipurified lignan and soy-free C1000 diet was used as a basal diet. In this experimental set up, dietary HMR showed also growth inhibition of the established tumors [25]. However, the effects of HMR were more significant when the administration was started prior to DMBA-induction (Figure 3B). Moreover, in both studies HMR administration resulted in decreased proportion of growing tumors (Table 1).

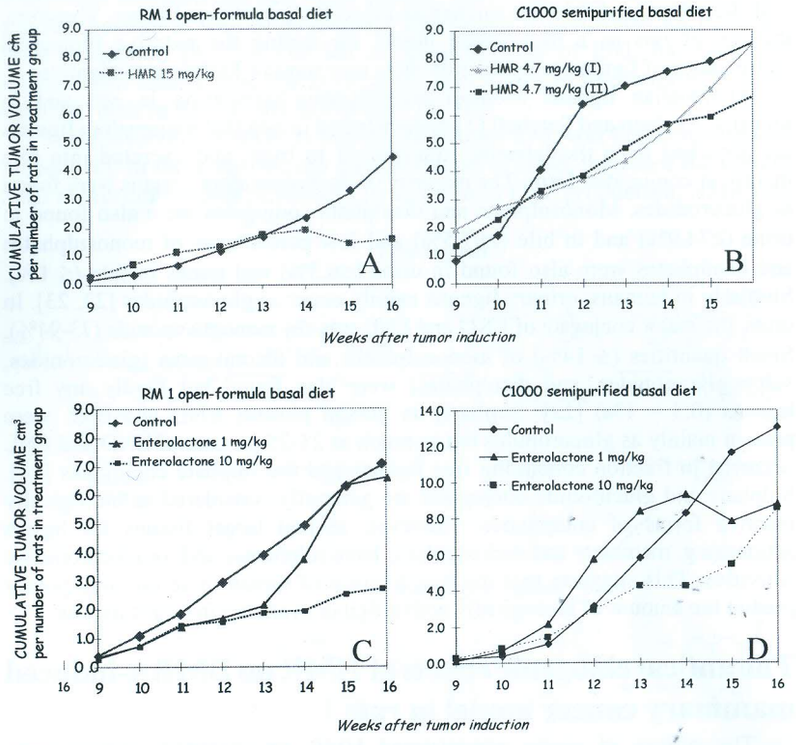

Figure 3. Cumulative mammary tumor volumes in DMBA-induced rats. The graphs A, B, and C are modified from Saarinen *et al.* 2000, 2002a and 2002b [18,25,27]. The graph D is unpublished.

Table 1. Number of rats and DMBA-induced tumors and the growth responses of tumors to lignan administration.

	HMR, experiment A RM1 open-formula chow diet		HMR, experiment B C1000 Semi-purified diet			Enterolactone, experiment C RM1 open-formula chow diet			Enterolactone, experiment D C1000 Semi-purified diet		
	Control	HMR 15 mg/kg b.w.	Control	HMR Group I 4.7 mg/kg b.w.	HMR Group II 4.7 mg/kg b.w.	Control	Enterolactone 1 mg/kg b.w.	Enterolactone 10 mg/kg b.w.	Control	Enterolactone 1 mg/kg b.w.	Enterolactone 10 mg/kg b.w.
Number of rats											
at start	8	7	13	13	13	13	13	13	10	8	10
at end	8	6	9	12	11	11	12	13	9	6	8
Total number of established tumors during the experiment	14	17	43	40	29	30	24	25	53	35	28
Multiplicity											
at start	0.8	1.6	1.2	1.3	1.0	0.9	0.9	1.0	2.2	2.5	1.9
at end	1.8	2.3	2.7	2.8	1.7	2.0	1.5	1.3	5.3	4	2.7
Total tumor volume per rat											
at start	0.16	0.27	0.80	1.93	1.37	0.40	0.34	0.28	0.21	0.09	0.26
at end	4.50	1.62	8.55	8.53	8.64	7.10	6.59	2.73	13.04	8.78	8.56
Growth pattern of the tumors[1]											
Growing (%)	86	59	68	70	43	56	55	30	76	54	64
Stabilized (%)	14	18	5	5	7	20	18	22	13	23	4
Regressing (%)	0	17	23	24	32	12	14	22	9	14	18
Disappeared (%)	0	8	5	0	18	12	14	26	2	9	14

The growth rate of the individual tumors was variable. The high mitotic activity was balanced by abundant spontaneous apoptosis also in untreated tumors which is a typical feature of the DMBA-induced mammary carcinomas [26]. Spontaneous apoptosis and regression of the tumors were observed in control animals. However, HMR increased the number of stable and regressing tumors when compared to controls (Table 1).

HMR is metabolized to ENL as we first demonstrated in rats [18] and which has later been confirmed to occur also in human subjects [19]. In DMBA-induced female rats on HMR-containing diet, ENL was the major lignan found in serum and urine [27]. ENL, a mammalian metabolite of selected plant lignans, has been suggested to mediate at least part of the anticancer effects of HMR and other plant lignans which are converted to ENL. This is supported by some epidemiological studies, indicating a link between high serum or urine ENL concentrations and reduced breast cancer risk in pre- and postmenopausal women [10, 28-30]. We investigated whether racemic ENL affects the growth of DMBA-induced mammary cancers in rats. Similarly to HMR, the effects of ENL were tested by using both open-formula RM1 chow diet and semipurified lignan and soy-free C1000 diet as basal diets. In both experiments, administration of ENL reduced the cumulative tumor volume during the seven-week treatment period (Figures 3C and D). The effect was more pronounced in rats fed with ENL in a dose of 10 mg/kg b.w. than in rats fed with a dose of 1 mg/kg b.w. In the study using RM1 basal diet (Figure 3C), a transient tumor growth attenuating effect of smaller ENL dose was observed during the first weeks of the experiment but at the end of the experiment the cumulative volume reached that of the control. In these studies we also demonstrated that the inhibition of tumor growth was more pronounced in tumors which developed into palpable size during the seven-week treatment period than in tumors which were palpable already at the start of the ENL regimen. This suggests that the early stages of the tumor development are more susceptible for ENL effects. However, the higher ENL dose inhibited also the growth of those tumors established prior to the start of the treatment [27]. Similar to HMR, administration of ENL in both basal diets resulted in a decreased proportion of growing tumors and increased proportions of stable and regressing tumors when compared to controls (Table 1).

The DMBA-induced rat mammary tumors rarely metastasize, but they behave like locally malignant rapidly proliferating neoplasms and fulfill all microscopic criteria for malignancy. The histology of the tumors is variable, which is a common feature of this tumor model [31]. In two of the studies (study A with HMR and study C with ENL) the histology of the DMBA-induced adenocarcinomas was further evaluated [18, 27]. The four tumor categories were: type A, poorly differentiated; type B, well differentiated; type

C, atrophic; type D, secretory carcinoma [32]. In both studies, the predominant tumor type was B, while the regressing tumors were of type C. Type D structure was seldomly present in these experiments (one tumor in HMR study and 5 tumors in ENL study). In these studies, the growth inhibition effect of HMR or ENL was not found to be restricted to any specified histological type of tumor.

Administration of HMR or its metabolite ENL had no major effects on the mammary tumor multiplicity (mean number of tumors per rat) or incidences (data not shown). In all four studies, the multiplicity tended to be smaller in rats treated with HMR or ENL than in control rats (Table 1) but the difference did not reach statistical significance. These findings indicate that anticarcinogenic effects of HMR in DMBA-induced mammary cancer model are rather targeted to attenuation of the growth of the tumors than development of new tumors. The growth inhibition may be due to the antiproliferative action of HMR or its mammalian metabolite ENL.

Lignans have some structural similarities with endogenous estrogens suggesting a possible estrogen-like activity. In DMBA-induced non-ovariectomized rats, however, no significant estrogen-like response was observed with HMR or ENL on mammary tumor growth (Figure 4). This is in line with the results from the immature rat uterine growth tests showing no uterine growth induction by HMR or ENL [18, 27]. ENL has also been associated with weak antiestrogenic effects. Waters and Knowler [33] demonstrated that ENL given in a subcutaneous dose of 1 mg/kg reduced the estradiol stimulated RNA synthesis in uterus, when given to rats 22 hours

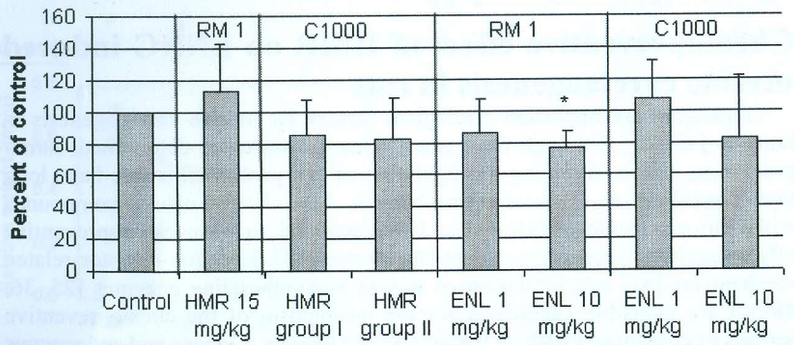

Figure 4. The relative uterine weights of DMBA-induced rats in studies A-D. The basal diets used were either open formula soy-containing RM1 or semipurified soy-free C1000. The control is set to 100%. The significant difference from the control is marked with an asterisk.

before estradiol. When ENL was administered at the same time or up to 12 hour before estradiol no significant effect was measured. The authors pointed to the structural resemblance between ENL and antiestrogens such as tamoxifen and suggested the competition with estradiol for the receptor binding as a possible mechanism of action. No significant direct binding of ENL to ERα or ERβ *in vitro* have been demonstrated [18, 34]. However, other estrogen receptor mediated mechanisms of lignans cannot be excluded.

The inhibition of aromatase enzyme activity by ENL resulting in reduced estrogen formation has been suggested to be a mechanism by which lignan-rich diets contributes to reduction of estrogen dependent diseases such as breast cancer [35, 36]. Aromatase is a cytochrome P450 enzyme converting testosterone and androstenedione to estradiol and estrone, respectively. Inhibition of aromatase has been demonstrated to inhibit mammary cancer growth in humans even more efficiently than antiestrogen tamoxifen [37, 38]. In the DMBA-induced mammary carcinoma model, aromatase inhibitors have also been effective [39-41]. In study C, a statistically significant decrease in uterine weight was measured among the DMBA-treated female rats after the long-term administration of ENL suggesting the possibility for aromatase inhibition or other antiestrogenic activity in rats. However, in short-term *in vivo* tests using androstenedione induced uterine growth assay, no significant effect of ENL administration was observed [27] leaving the hypothesis of ENL as an aromatase inhibitor or as antiestrogen *in vivo* without solid evidence. The anticarcinogenicity of HMR may, at least in part, be associated with the antioxidative capacity [18]. However, the specific mechanism of the lignans in mammary cancer still remains largely unresolved.

Chemopreventive effect of HMR on ENNG-induced uterine carcinogenesis in rats

Estrogens are important etiological factors for uterine carcinogenesis in humans [42-45]. Although their exact roles remain to be determined, tumor promoting effects involving up-regulation of cell proliferation rate have long been considered as a causative mechanism. Accordingly, natural compounds with anti-estrogenic activity have been assumed to have chemopreventive effects against estrogen-dependent carcinomas [46]. Estrogen-receptor-related mechanisms [47] and inhibition of steroid biosynthesizing enzymes [25, 36, 48-50] are plausible candidates for the mechanism of the chemopreventive actions of compounds such as flavonoids and lignans. Lignans and endogenous estrogens have some structural similarities, suggesting possible estrogen-like or anti-estrogen-like activity for lignans. In a case-control study, consumption of lignans and isoflavones were found to be associated with a low risk of endometrial cancer of pre- and postmenopausal women [11].

Direct effects of HMR on uterus were investigated in ovariectomized adult rats. Both 200 and 600 ppm HMR containing diets were fed for 4 weeks to rats starting at the age of 7 weeks. Neither uterotrophic effect nor estrous cycling of the vaginal smear was found in the dosing groups. Next, normal estrus cycling rats at the age of 8 weeks were fed HMR containing diet at 600 ppm for 2 weeks. No effects on estrus cycling, number of ovulation, weights of uterus, ovaries, and pituitary in the individual stages of the estrus cycles were found (Figure 5). These results show that low doses of HMR have no significant estrogenic effects on uterine tissue and normal estrous cycling in young adult rats (unpublished data), being in line with the previous data described above.

There is only limited evidence that environmental chemicals/hormones can induce or reduce endometrial adenocarcinomas in rodents [51]. We have documented that the Donryu rat is a high incidence strain for spontaneous development of endometrial adenocarcinomas, associated with a hormonal imbalance, characterized by an age-dependent increase in the estrogen-progesterone

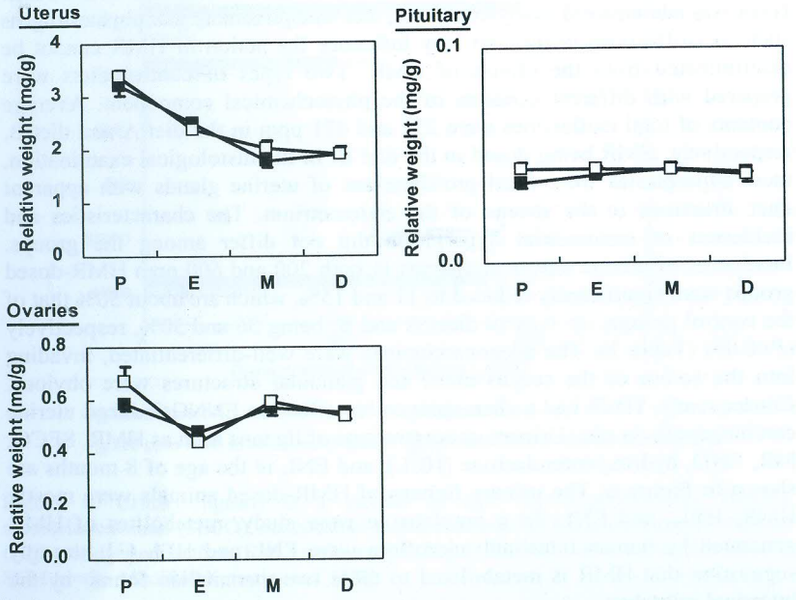

Figure 5. Relative weights of uterus, pituitary, and ovaries in the individual stages of the estrous cycle after dosing 600 ppm HMR to the rats for 2 weeks. Data are mean ± SEM. Open square (□), controls; black squares (■), 600 ppm HMR; P, proestrus; E, estrus; M, metestrus; D, diestrus.

(E2/P) ratio, which is due to early development of persistent estrus resulting from anovulation [52-54]. The incidence of spontaneous endometrial adenocarcinomas in this rat strain showed a tendency to decrease in animals having reproductive experience, compared to the nulliparous case, suppression being associated with changes in the hormonal milieu [55]. These results indicate that the Donryu rat may be a good animal model for endometrial adenocarcinoma linked to endogenous estrogens in humans. An elevated incidence of such tumors develops in this rat strain with a single intra-uterine administration of ENNG. A two-stage rat uterine carcinogenesis model has been established [56]. This animal model has advantages for clarification of tumor promotive effects of long-term exposure to estrogens and/or estrogenic compounds during adulthood [51].

Chemopreventive effect of HMR on uterine carcinogenesis was studied in the Donryu rat model [57]. Adult rats were treated with ENNG and transferred to diets with 200 and 600 ppm HMR at 11 weeks of age when they had regular estrus cycles. They were maintained on HMR diets until 15 months of age. HMR was administered in soy-containing diet. The possibility that phytoestrogens such as isoflavones in the diet may influence the action of HMR cannot be discriminated from the effects of HMR. Two types of control diets were prepared with different contents of the phytochemical component. Average contents of total isoflavones were 257 and 471 ppm in the diet A and diet B, respectively, HMR being dosed in the diet B. In the histological examination, most hyperplasias were focal proliferations of uterine glands with apparent duct structures in the stroma of the endometrium. The characteristics and incidences of endometrial hyperplasia did not differ among the groups. Incidences of uterine adenocarcinomas in both 200 and 600 ppm HMR-dosed groups were significantly reduced to 11 and 15%, which are about 50% that of the control groups, i.e. control diets A and B, being 36 and 30%, respectively ($P<0.05$) (Table 2). The adenocarcinomas were well-differentiated, invading into the serosa of the corpus uteri, and glandular structures were obvious. Consequently, HMR had a chemopreventive effect on ENNG-induced uterine carcinogenesis in rats. Urinary concentrations of lignans such as HMR, SECO, MR, END, hydroxyenterolactone (HEL), and ENL at the age of 8 months are shown in Figure 6. The urinary lignans of HMR-dosed animals were mostly HMR, HEL, and ENL. In a previous *in vitro* study, metabolites of HMR generated by human intestinal microflora were ENL and HEL [3], strongly suggesting that HMR is metabolized to these two mammalian forms by the intestinal microbes.

Persistent estrus associated with an increase of the E2/P ratio is thought to have exclusive relevance on pathogenesis of the development of uterine tumors [51]. Hormonal imbalance leads to anovulation, morphologically detectable as atrophic ovaries with polycystic atretic follicles and loss of corpus lutea, eventually

Table 2. Incidences (%) of uterine proliferative lesions at 15 months of age in the four experimental groups. Modified Katsuda *et al.* [57].

Group	None	Hyperplasia			Adenocarcinoma
		+	++	+++	
1: Control diet A	0	8	32	24	36
2: Control diet B	7	7	30	26	30
3: 200 ppm HMR in the control diet B	11	19	33	26	11*
4: 600 ppm HMR in the control diet B	4	15	46	19	15*

*Significant difference was detected in number of adenocarcinomas among the groups ($P<0.05$).

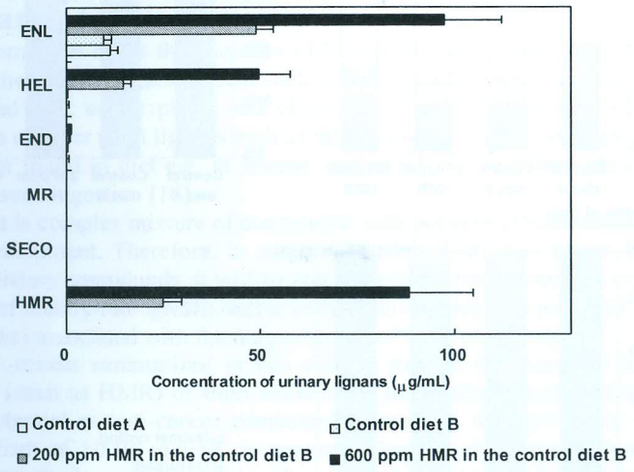

Figure 6. Urinary lignans at 8 months of age. Hydroxymatairesinol (HMR), secoisolariciresinol (SECO), mateiresinol (MR), enterodiol (END), hydroxyenterolactone (HEL), and enterolactone (ENL) were analyzed by HPLC-MS-MS. Values are mean ± SEM (n=6). Modified Katsuda *et al.* [57].

resulting in persistent estrus [58]. In the HMR study, delay in the start of persistent estrus due to HMR dosing was significant (Figure 7). Persistent estrus started at the age of 30.3 and 32.1 weeks in control groups. In HMR-dosed groups, the start of persistent estrus was significantly delayed by 3 to 5

weeks ($P<0.05$). Aromatase and/or hydroxysteroid dehydrogenase inhibitors, as well as anti-estrogenic pharmaceuticals, can prevent persistent estrus by reducing estrogen levels, followed by elevation of FSH, and growth of ovarian follicles. Several studies suggest, that ENL may act as weak inhibitor of enzymes (e.g. aromatase and/or hydroxysteroid dehydrogenase) involved in steroidogenesis [25, 36, 48, 50]. However, HMR did not have a strong impact on female reproductive systems of young and normal cycling rats. Therefore, the mechanisms underlying the delay of persistent estrus with HMR dosing remain to be confirmed.

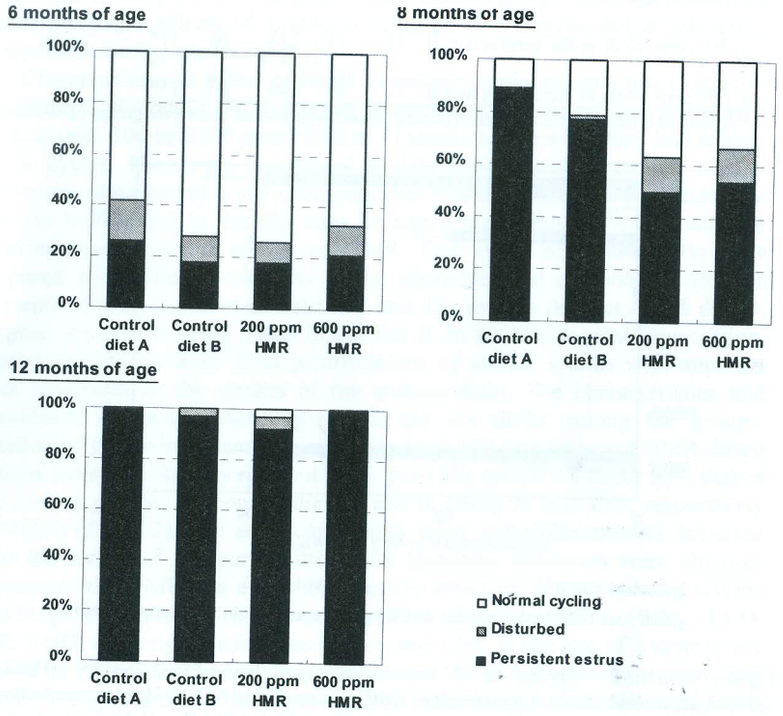

Figure 7. Incidence of persistent estrus among the 4 experimental groups. Two control groups were provided with control diets A and B. HMR was mixed in the control diet B doses of 200 and 600 ppm. At least, two repetitions of four-day precise estrous cycle were regarded as normal cycling. Disturbed estrous cycle was identified by contamination with large amounts of epithelial cells in vaginal smears at metestrus and/or diestrus stages. Persistent estrus was determined on the basis of continued estrus for at least 4 days.

Chemopreventive effects of HMR on carcinogenesis in other animal models

In addition to breat and uterine cancers, HMR has shown anticarcinogenic effects in experimental prostate cancer [59]. In athymic nude male mice bearing LNCaP androgen responsive human prostate cancers, dietary HMR inhibited the growth of the tumors when started at the early phase of the tumor development. The tumor growth inhibition was measured as smaller tumor volume, lower tumor take rate, increased proportion of non-growing tumors and higher tumor cell apoptotic index when compared to controls [59]. The recent studies on the effects of dietary HMR and its mammalian metabolite ENL on growth and metastasis of AH109A hepatomas in rats revealed that both lignans reduced the growth and metastasis of these tumors [60].

Future perspectives for research on lignans as anticarcinogenic compounds

Information about the presence of lignans in edible plants has increased during the past few years. Recent studies have demonstrated that in addition to SDG and MR, edible plants and plant-based foods contain also significant amounts of other plant lignans such as lariciresinol and pinoresinol [61]. HMR has been found in diet e.g. in sesame seeds [16] and in human plasma after sesame seed ingestion [16].

Diet is complex mixture of compounds with putative effects on cancer risk and development. Therefore, in addition to identification of the biologically active dietary compounds, it will be crucial to focus the research on combined effects of dietary biologically active compounds to elucidate potential benefits (and risks) associated with the long-term use of these compounds.

The results summarized in this chapter suggest that some of the plant lignans (such as HMR) or their mammalian metabolites (such as ENL) may have potential as anti-cancer compounds. However, it is not likely that the metabolism of HMR to ENL is necessary for the chemopreventive action. Moreover, the lowest observable effect level (LOEL) is not known for HMR, ENL or any other lignan either in the experimental cancer models or in human subjects. Therefore, the optimal daily dose of lignans associated with a reduced risk of cancer among human subjects thus remains to be established. In addition, the time of exposure (e.g. the stage of disease) when these compounds are used is likely to play a crucial role in cancer chemoprevention. Diet-derived components such as selected lignans may have potential as cancer risk reducing agents when time of exposure occurs at phases critical for cancer development, although the idea needs to be confirmed in experimental models as well as in clinical studies. It is possible that specific dietary compounds,

such as lignans, may have potential in chemoprevention of selected types of cancer in selected target groups.

Conclusion

Our results demonstrate that the dietary HMR has chemopreventive effects on the development of chemically induced mammary and uterine cancers. Whereas mechanisms of action of HMR in these tumor developments are not fully determined, and might be different in these animal models and not related to their antiestrogenicity, elevated serum level of ENL, a metabolite of HMR, may be an crucial mediator of these beneficial effects.

References

1. Axelson, M., Setchell, K.D.R. 1981, FEBS Lett., 123, 337.
2. Borriello, S.P., Setchell, K.D.R., Axelson, M., Lawson, A.M. 1985, J. Appl. Bacteriol., 58, 37.
3. Heinonen, S., Nurmi, T., Liukkonen, K., Poutanen, K., Wahala, K., Deyama, T., Nishibe, S., Adlercreutz, H. 2001, J. Agric. Food Chem., 49, 3178.
4. Liu, Z., Saarinen, N.M., Thompson, L.U. 2006, J. Nutr., 136, 906.
5. Rickard, S.E., Orcheson, L.J., Seidl, M.M., Luyengi, L., Fong, H.H.S., Thompson, L.U. 1996, J. Nutr., 126, 2012.
6. Ekman, R. 1979, Acta Academiae Aboensis, Ser. B, 39, 1.
7. Willför, S., Hemming, J., Reunanen, M., Eckerman, C., Holmbom, B. 2003, Holzforschung, 57, 27.
8. Adlercreutz, H., Fotsis, T., Heikkinen, R., Dwyer, J.T., Woods, M., Goldin, B.R. 1982, Lancet, 11, 1295.
9. Ingram, D., Sanders, K., Kolybaba, M., Lopez, D. 1997, Lancet, 350, 990.
10. Pietinen, P., Stumpf, K., Mannisto, S., Kataja, B., Uusitupa, M., Adlercreutz, H. 2001, Cancer Epidemiol. Biomarkers Prev., 10, 339.
11. Horn-Ross, P.L., John, E.M., Canchola, A.J., Stewart, S.L., Lee, M.M. 2003, J. Natl. Cancer Inst., 95, 1158.
12. den Tonkelaar, I., Keinan-Boker, L., Veer, P.V., Arts, C.J., Adlercreutz, H., Thijssen, J.H., Peeters, P.H. 2001, Cancer Epidemiol. Biomarkers Prev., 10, 223.
13. Zeleniuch-Jacquotte, A., Lundin, E., Micheli, A., Koenig, K.L., Lenner, P., Muti, P., Shore, R.E., Johansson, I., Krogh, V., Lukanova, A., Stattin, P., Afanasyeva, Y., Rinaldi, S., A.A., A., Kaaks, R., Berrino, F., Hallmans, G., Toniolo, P., Adlercreutz, H. 2006, Int. J. Cancer, 119, 2376.
14. Axelson, M., Sjövall, J., Gustafsson, B.E., Setchell, K.D.R. 1982, Nature, 298, 659.
15. Owen, R.W., Würtele, G., Haubner, R., Hull, W.E., Giacosa, A., Spiegelhalder, B., Bartsh, H. in International Symposium on Whole Grain and Human Health, Haikko Manor, Finland 2001.
16. Peñalvo, J.L., Heinonen, S.M., Aura, A.M., Adlercreutz, H. 2005, J. Nutr., 135, 1056.
17. Peñalvo, J.L., Heinonen, S.M., Aura, A.M., Adlercreutz, H. 2006, J. Nutr., 135, 1056.

18. Saarinen, N., Wärri, A., Mäkelä, S., Eckerman, C., Reunanen, M., Ahotupa, M., Salmi, S., Franke, A.A., Kangas, L., Santti, R. 2000, Nutr. Cancer, 36, 207.
19. Hormos Medical Corp., New dietary ingredient notification for 7-hydroxymatairesinol (HMR) potassium acetate complex. http://www.fda.gov/ohrms/dockets/dockets/95s0316/95s-0316- rpt0235-05- Contents-vol 167.pdf, 2004.
20. Begum, A.N., Nicolle, C., Mila, I., Lapierre, C., Nagano, K., Fukushima, K., Heinonen, S.M., Adlercreutz, H., Remesy, C., Scalbert, A. 2004, J. Nutr., 134, 120.
21. Setchell, K.D., Lawson, A.M., McLaughlin, L.M., Patel, S., Kirk, D.N., Axelson, M. 1983, Biomed. Mass Spectrom, 10, 227.
22. Adlercreutz, H., van der Wildt, J., Kinzel, J., Attalla, H., Wähälä, K., Mäkelä, T., Hase, T., Fotsis, T. 1995, J. Steroid Biochem. Mol. Biol., 52, 97.
23. Axelson, M., Setchell, K.D.R. 1980, FEBS Lett., 122, 49.
24. Adlercreutz, H., Fotsis, T., Lampe, J., Wähälä, K., Mäkelä, T., Brunow, G., Hase, T. 1993, Scand. J. Clin. Lab. Invest., 53 (Suppl. 215), 5.
25. Saarinen, N.M., Huovinen, R., Wärri, A., Mäkelä, S.I., Valentín-Blasini, L., Needham, L., Eckerman, C., Collan, Y.U., Santti, R. 2002a, Nutr. Cancer, 41, 82.
26. Huovinen, R., Wärri, A., Collan, Y. 1993, Int. J. Cancer, 55, 685.
27. Saarinen, N.M., Huovinen, R., Wärri, A., Mäkelä, S.I., Valentín-Blasini, L., Sjöholm, R., Ämmälä, J., Lehtilä, R., Collan, Y.U., Santti, R.S. 2002b, Mol. Cancer Ther., 1, 869.
28. Dai, Q., Franke, A.A., Jin, F., Shu, X.O., Hebert, J.R., Custer, L.J., Cheng, J., Gao, Y.T., Zheng, W. 2002, Cancer Epidemiol. Biomarkers Prev., 11, 815.
29. Keinan-Boker, L., van Der Schouw, Y.T., Grobbee, D.E., Peeters, P.H. 2004, Am. J. Clin. Nutr., 79, 282.
30. McCann, S.E., Muti, P., Vito, D., Edge, S.B., Trevisan, M., Freudenheim, J.L. 2004, Int. J. Cancer, 111, 440.
31. Huovinen, R., Collan, Y. 1994, Tumour Biol., 15, 345.
32. Archer, F.L., Orlando, R.A. 1968, Cancer Res., 28, 217.
33. Waters, A.P., Knowler, J.T. 1982, J. Reprod. Fert., 66, 379.
34. Mueller, S.O., Simon, S., Chae, K., Metzler, M., Korach, K.S. 2004, Toxicol. Sci., 80, 14.
35. Adlercreutz, H., Bannwart, C., Wähälä, K., Mäkelä, T., Brunow, G., Hase, T., Arosemena, P.J., Kellis, J.T., Vickery, L.E. 1993, J. Steroid Biochem. Mol. Biol., 44, 147.
36. Wang, C., Mäkelä, T., Hase, T., Adlercreutz, H., Kurzer, M.S. 1994, J. Steroid Biochem. Mol. Biol., 50, 205.
37. Bonneterre, J., Thurlimann, B., Robertson, J.F., Krzakowski, M., Mauriac, L., Koralewski, P., Vergote, I., Webster, A., Steinberg, M., von Euler, M. 2000, J. Clin. Oncol., 18, 3748.
38. Mouridsen, H., Gershanovich, M., Sun, Y., Perez-Carrion, R., Boni C, M., A., Apffelstaedt, J., Smith, R., Sleeboom, H.P., Janicke, F., Pluzanska, A., Dank, M., Becquart, D., Bapsy, P.P., Salminen, E., Snyder, R., Lassus, M., Verbeek, J.A., Staffler, B., Chaudri-Ross, H.A., Dugan, M. 2001, J. Clin. Oncol., 19, 2596.
39. Houjou, T., Wada, T., Yasutomi, M. 1993, Clin. Ther., 15, 137.
40. Iino, Y., Karakida, T., Sugamata, N., Andoh, T., Takei, H., Takahashi, M., Yaguchi, S., Matsuno, T., Takehara, M., Sakato, M., Kawashima, S., Morishita, Y. 1998, Anticancer Res., 18, 171.

41. Sugamata, N., Koibuchi, Y., Iino, Y., Morishita, Y. 1999, Int. J. Oncol., 14, 259.
42. Fox, H. 1984, Pathol. Res. Pract., 179, 13.
43. Hilliard, G.D., Norris, H.J. 1979, Recent Results Cancer Res., 66, 49.
44. Lingeman, C.H. 1979, Recent Results Cancer Res., 66, 1.
45. Zeil, H.K. 1982, Obstet. Gynecol., 60, 509.
46. Adlercreutz, H., Mazur, W. 1997, Ann. Med., 29, 95.
47. Wang, L.-Q. 2002, J. Chormatogr. B. Analyt. Technol. Biomed. Life Sci., 777, 289.
48. Brooks, J.D., Thompson, L.U. 2005, J. Steroid Biochem. Mol. Biol., 94, 461.
49. Lacey, M., Bohday, J., Fonseka, S.M., Ullah, A.I., Whitehead, S.A. 2005, J. Steroid Biochem. Mol. Biol., 96, 279.
50. Mäkelä, T.H., Wahala, K.T., Hase, T.A. 2000, Steroids, 65, 437.
51. Maekawa, A., Takahashi, M., Ando, J., Yoshida, M. 1999, J. Toxicol. Pathol., 12, 1.
52. Maekawa, A., Onodera, H., Tanigawa, H., Furuta, K., Matsuoka, C., Ogiu, T., Hayashi, Y. 1986, Jpn. J. Cancer Res. (Gann), 77, 882.
53. Nagaoka, T., Onodera, H., Matsushima, Y., Todate, A., Shibutani, M., Ogasawara, H., Maekawa, A. 1990, J. Cancer Res. Clin. Oncol., 116, 623.
54. Nagaoka, T., Takeuchi, M., Onodera, H., Matsushima, Y., Ando-Lu, J., Maekawa, A. 1994, Toxicol. Pathol., 22, 261.
55. Nagaoka, T., Takegawa, K., Takeuchi, M., Maekawa, A. 2000, Jpn. J. Cancer Res., 91, 375.
56. Ando-Lu, J., Takahashi, M., Imai, S., Ishihara, R., Kitamura, T., Iijima, T., Takano, S., Nishiyama, K., Suzuki, K., Maekawa, A. 1994, Jpn. J. Cancer Res., 85, 789.
57. Katsuda, S.-I., Yoshida, M., Saarinen, N., Smeds, A., Nakae, D., Santti, R., Maekawa, A. 2004, Exp. Biol. Med., 229, 417.
58. Yoshida, M. 2006, J. Toxicol. Pathol., 19, 57.
59. Bylund, A., Saarinen, N., Zhang, J.-X., Bergh, A., Widmark, A., Johansson, A., Lundin, E., Adlercreutz, H., Hallmans, G., Stattin, P., Mäkelä, S. 2005, Exp. Biol. Med., 230, 217.
60. Miura, D., Saarinen, N.M., Miura, Y., Santti, R., Yagasaki, K. 2006, Nutr. Cancer, in press.
61. Milder, I.E.J., Arts, I.C.W., van de Putte, B., Venema, D.P., Hollman, P.C.H. 2005, Br. J. Nutr., 93, 393.