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THE COMT-MEDIATED METABOLISM OF FLAVONOIDS AND ESTROGEN AND ITS RELEVANCE TO CANCER RISK

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Catechol-*O*-methyltransferase (COMT) is the Phase II enzyme able to efficiently catalyze detoxification of endo- and exogenous compounds containing catechol groups which otherwise may become toxic, mutagenic or even carcinogenic. Therefore, the enzyme activity itself and the factors which influence the kinetics of such an inactivation are extensively studied. The *COMT* gene contains a single-nucleotide polymorphism (SNP), which is associated with an amino acid shift val -> met. This gene is present in up to 75% of Caucasians. Such a mutation results in a significant decrease in enzyme activity. It was shown in several studies that individuals having the low activity allele ($COMT^L$) face greater risk for developing breast cancer. The development of carcinogenesis may originate from catechol metabolites of estrogen which are not sufficiently *O*-methylated into inactive compounds because of low COMT activity. Beside this genetic determinant, an expression of the enzyme in a given individual or in a population is likely to be affected by environmental and lifestyle factors. Particularly, exogenous compounds which compete for enzyme activity, like dietary polyphenol with catechol motif, the drug being a direct or indirect COMT inhibitor, or long-term exposure to exogenously administrated estrogen may contribute to carcinogenicity.

COMT transfers a methyl group from S-adenyl-L-methionine to the catechol substrate. Therefore, the differences in metabolism of folate may also affect the individual response to catechol inactivation and then the cancer risk. Relationships between the level of endogenous DNA modifications involved in risk cancer and polymorphism in *COMT* gene, dietary habits, particularly high-flavonoid diet as well as life style (estrogen level and drugs) are discussed in the paper.

INTRODUCTION

Polyphenolic compounds are among the most discussed dietary ingredients. They are a class of phytochemicals found in high concentrations in wine, tea, grapes and a wide variety of other plants and are associated with prevention of heart disease and cancer [Hertog, 1996; Hanninen, *et al.*, 1999; Hollman & Katan, 1999a; Riemersma *et al.*, 2001; Silalahi, 2002, Mojzisova & Kuchta, 2001]. One of the most nutritionally important polyphenols are flavonoids and isoflavones, commonly called "phytoestrogens" [Potter & Steinmetz, 1996; Reinli & Block, 1996]. Their antioxidant and estrogenic effect has led researchers to regard these compounds as potential anticarcinogens [Adlercreutz *et al.*, 1992; Zava & Duwe, 1997; Griffiths *et al.*, 1998].

While reviewing the literature of the subject it became clear that although high fruit and vegetable intake has been linked with a reduced risk of cancer, epidemiological data do not confirm that observation [Waladkhani & Clemens, 1998]. Several studies reported inverse relationship between intake of vegetables and fruits and cancer [Eastwood, 1999; Shih *et al.,* 2000; Birt *et al.*, 2001; Silalahi, 2002], while a non-significant inverse association of fruit, vegetable and flavonoid intake and cancer were announced by others [Arts *et al.*, 2001; Hertog, 1996]. What is the reason for such an inconsistence? One possible answer is the existence of inter-individual differences between metabolism of flavonoids due to the polymorphisms in catechol-*O*-methyltranferase, the dietary status, particularly the flavonoid and folate intake, and the exposure to estrogen. This hypothesis is confronted below with the results of our pilot studies.

FLAVONOID INTAKE AND ITS BIOACTIVE ROLE

The average, daily, mixed flavonoids, human intake is estimated to be in the range of 20 to 1 000 mg [Hertog *et al.*, 1993; Hollman & Katan, 1997, 1998, 1999b; Scalbert & Williamson, 2000; Aherne & O'Brien, 2002; Pierpoint, 1986]. These compounds are present in fruits, vegetables, grains, nuts, tea, and wine [Hertog *et al.*, 1992]. It was shown that after a quercetin-free diet followed by a single meal containing 200 mg of quercetin, human plasma levels reached >1 μM of quercetin alone as soon as 1.5-2 h after ingestion. Moreover, the half-life of quercetin was 25 h, implying that repeated dietary intake of quercetin can lead to much higher blood concentrations [Hollman, 1997; Hollman & Katan, 1997, 1998, 1999a; Hollman *et al.*, 1997a, b]. Thus, the biological effects of this compound should be studied within this concentration range for nutritional relevance. Our results showed that quercetin at the concentration as low as 1 μM has the protective effect on

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the oxidative DNA damage induced by hydrogen peroxide in human lymphocytes (Figure 1). This confirms earlier findings [Aherne & O'Brien, 1999; Anderson *et al.*, 2000; Collins & Dusinska, 2002] that some flavonoids are among the most potent anti-oxidants and because of that might protect against cancer through inhibition of oxidative damage, which is likely to be an important cause of mutation. Other proposed mechanisms include antiproliferation, estrogenic/anti--estrogenic activity, induction of cell-cycle arrest and apoptosis, modulation of activities of many enzymes, induction of detoxification enzymes, and changes in cellular signaling [Aherne & O'Brien, 1999, 2000; Anderson *et al.*, 1997, 1998, 2000, 2001; Kong *et al.*, 2001; Myhrstad *et al.*, 2002; Sato *et al.*, 2002; Walle & Walle, 2002].

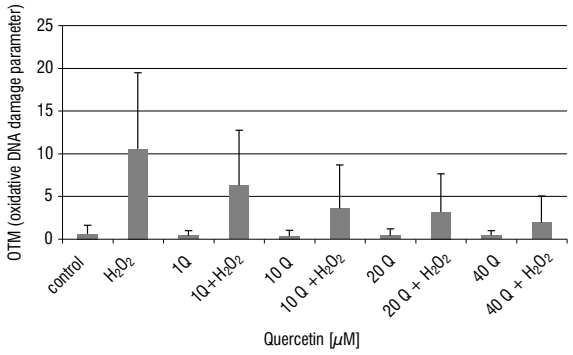

FIGURE 1. Effect of different doses of quercetin on oxidative DNA damage induced by hydrogen peroxide treatment. Lymphocytes were incubated with different concentration of quercetin for 1 h and next cells were exposed for 5 min to 25 μM hydrogen peroxide at 4°C. The oxidative DNA damage was assesed by Comet assay, modified by using the endonuclease III which recognized apurinic sites and oxidized pirimidine.

To estimate the amount of flavonoid intake among Polish young women, nineteen subjects recorded their food, beverages, herbs and spices consumption for two weekdays and one weekend throughout a three-week period in a winter month (9 records). As all individual questionnaires were computerized, the amount of dietary compounds was estimated according to a specifically developed program, linked to recently updated Polish food database. The intake of four subclasses of flavonoids containing catechol groups: flavonols, flavonons, flavanones and flavan-3-ols, as well as anthocyanidines, was

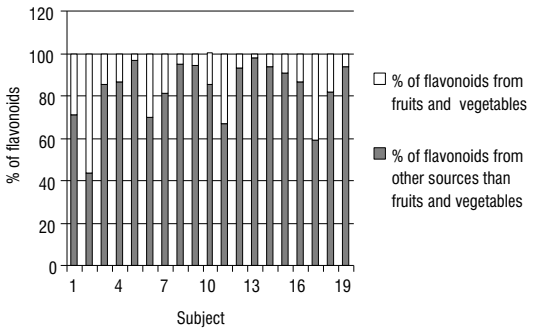

FIGURE 2. The percentage of the total daily flavonoid intake from different sources of common food for individual subject is calculated by using USDA database (for the flavonols, flavonons, flavanones, flavan-3-ols and anthocyanidines content of selected food in vegetables, fruits, beverages, herbs, spices, tea, coffee, chocolate *etc*. based on collected dietary diary).

calculated using published values for content (USDA database for the flavonoids content of selected food in vegetables, fruits, beverages, herbs, spices, tea, coffee, chocolate *etc.*). According to our results (Figure 2) the main dietary sources of flavonoids are not fruit and vegetables but tea, coffee, chocolate, wine and beer. These results confirm data published by Scalbert and Williamson [2000].

EFFECT OF FLAVONOIDS AND ESTROGENS ON OXIDATIVE DNA DAMAGE

The oxidative DNA damage is induced by oxygen radicals and other reactive species which are normally formed during cellular metabolic processes and are also raised by metabolism of environmentally available xenobiotics, subsequently inhibiting DNA replication and transcription [Stohs, 1995; Williams & Jeffrey, 2000; Fujikawa & Kasai 2001; Owuor & Kong, 2002]. The oxidatively damaged DNA can play a significant role in mutagenesis, cancer, aging, and other human pathologies. The level of oxidative DNA damage depends on the level and activities of anti-oxidative enzymes and can be decreased by supplementation with pure antioxidants or with food rich in antioxidants, namely vitamins C, A, E and beta carotenoids [Krishnaswamy & Raghuramulu, 1998; Collins *et al.*, 2003]. Furthermore, the antioxidant ability of flavonoids and other micronutrients may also account for the beneficial effects of fruits and vegetables in human health. Among the numerous oxidized DNA bases, most often occurs the 8-oxo-dG, 8-oxo-7,8-dihydro--2'deoxyguanosine (8-oxo-dG) and 2,4-diamino-5 formamidopyrimidine (Fapy-G). The elevated levels of 8-oxo-dG have been reported in tumor tissue; this can suggest that accumulation of oxidative DNA damage can play a significant role in carcinogenesis. Moreover, an enhanced level of such lesions may be used as a predictive assay of the risk cancer and as biomarkers [Cadet *et al.*, 1997]. Oxidative DNA damage occurs due to autoxidation of catechol estrogens and can be fixed as mutation due to proliferating activity of estradiol result in enhancement of the risk of cancer. Oxidized DNA bases can be directly measured by the high-performance liquid chromatography (HPLC). An alternative approach to the measurement of oxidized bases makes the use of repair endonucleases with the appropriate lesion specificities-endonuclease III, for oxidized pyrimidines and formamidopyrimidine glycosylase (Fapy-G for 8-OHgua) for oxidized pyrines [Cadet *et al.*, 2000; Lunec *et al.*, 2002]. These enzymes introduce breaks at sites of damage in DNA, which creates the condition for their detection by the comet assay (single cell gel electrophoresis) [Duthie & Hawdon, 1998; Angelis *et al.*, 1999, 2000; Collins & Dusinska, 2002]. This modified comet assay, like other enzyme-linked DNA breakage assays, gives value for endogenous oxidative base damage that is more than 10-fold lower than most estimated from HPLC. The method, which measures DNA strand breaks at the level of single cells, is very easily applied to human lymphocytes, and therefore lends itself to human biomonitoring studies.

Not only flavonoids but also some nutrients affect the production, metabolism and bioavailability of steroid hormones [Xu *et al.*, 1999]. It means that the level of estrogens

TABLE 1. The statistically significant correlation between the oxidative DNA lesion and uracil misincorporation into DNA (assessed by Comet assay single cell gel electrophoresis, respectively with endonuclease III or uracil DNA glycosylase) and flavonoid intake, serum folate concentration and COMT polymorphism, before or after 400 μg/day folic acid supplementation for four weeks.

DNA damage (comet assay parameter)	Σ Flavonoids	Folate in serum	COMT*H
Endogenous oxidative DNA damage	positive (after folate supplementation)	no	positive (before folate supplementation)
Uracil misincorporation into DNA	negative (before folate supplementation)	negative (after folate supplementation)	negative (after folate supplementation)

during the menstrual cycle phase or pregnancy must be considered while studying the influence of nutritional status on the endogenous DNA damage. Results of population-based and of *in vitro* and *in vivo* studies suggest that estrogens, under certain conditions, may be carcinogenic [Sasco, 2001].

The mechanism of estrogens contribution to the development of human cancer is still unknown. However, two possible hypotheses are subsequently advanced. The estrogen-induced tumor development is mediated by the estrogen-receptor-based proliferation of cells, accompanied by increased probability of mutation and the induction of DNA damage by the reactive oxygen species, such as superoxide [Lord *et al.*, 2002]. Moreover, the catechol estrogens may give rise to reactive quinones capable of forming adducts to DNA and enhance the single/double DNA strand breaks as well as oxidative modification of nitrogen purine and pirimidine bases [Li & Li, 1987; Zhu & Liehr, 1994]. That reaction can be blocked *via* several detoxication pathways, including *O*-methylation of catechol estrogens by catechol-*O*-methyl transferase (COMT). COMT is polymorphic in human population, which results in differences in enzyme activity and causes different detoxication of catechol estrogens [Millikan *et al.*, 1998; Lavigne *et al.*, 2001]. The increase of cancer risk was also observed after hormonal replacement therapy [Sitruk-Ware, 2002]. It means that the factors which affect the COMT activity can modify the risk of hormone--dependent cancers due to the accumulation of potentially carcinogenic metabolites of estradiol.

Our studies did not show any statistically significant correlation between phases of menstrual cycle, defined as follicular (9-17 days) or non-follicular, and the oxidative DNA damage. The amount of oxidized DNA, incised by endonuclease III digestion, was evaluated using the alkaline comet assay in blood samples taken during collection of nutritional data. In turn, the positive correlation was found between the oxidative DNA damage/modification and flavonoids intake (Table 1) but only after four weeks of folate supplementation (400 μg/day). It was reported, that the amount of oxidative DNA damage and DNA adduct in human lymphocytes was higher during the summer season [Giovannelli *et al.*, 2002, Li & Li, 1987; Milikan *et al.*, 1998; Mojzisova & Kuchta, 2001]. The impact of coffee consumption on the lymphocyte DNA oxidation is not consistent: in one case - a positive correlation was found [Giovannelli *et al.*, 2002], in another - a negative one [van Zeeland *et al.*, 1999]. Similarly to our findings, the positive tendency between the total consumption of vegetables and fruits and oxidative damage was found in peripheral lymphocytes in a Mediterranean population [Giovannelli *et al.*, 2002].

INFLUENCE OF THE HOST FACTORS (A GENETIC POLYMORPHISM – COMT GENE, FOLATE, FLAVONOIDS, B_{12}, B_6 INTAKE, AND ESTRADIOL LEVEL) ON BIOACTIVITY OF DIETARY FLAVONOIDS

The circulating levels of unmetabolized dietary phytochemicals, particularly quercetin, depend on the activity of catechol-*O*-methyltransferase (EC 2.1.1.6), an enzyme which rapidly converts quercetin to the 3'-*O*--methylquercetin, a non-bioactive form. The rate of the COMT-catalyzed methylation depends on several factors, such as *COMT* genotype, concentration of catechol estrogen, folates, S-adenosyladenine (SAM, vitamin B_{12} and B_6) [Goodman *et al.*, 2001a, b]. The catechol-*O*--methyltransferase catalyses the transfer of the methyl group to catecholamines and catechol estrogens. Inhibition of the COMT-mediated *O*-methylation of endogenous 2- and 4-hydroxylated estrogens by phytochemicals may lead to elevated tissue levels of pro-carcinogenic 4-hydroxyestradiol and to a decreased tissue level of the anti-carcinogenic 2-MeO-E_2 [Lavigne *et al.*, 2001]. It was shown, that chronic administration of dietary quercetin enhances the E_2-induced kidney tumor formation in male Syrian hamsters [Zhu & Liehr, 1996].

COMT exists in most tissues of human body. Its activity in some peripheral tissues was shown to have age- and sex--related differences [Mannisto, 1999] The distribution of COMT activity in Caucasian individuals was polymorphic with the trimodal distribution, with 25% of the subjects homozygous ($COMT^{LL}$) for the low activity thermo-labile COMT; 25% of the subjects homozygous ($COMT^{HH}$) for the high-activity thermostable enzyme; and 50% of the subjects heterozygous ($COMT^{LH}$) [Ishiguro *et al.*, 1999; Goodman *et al.*, 2001a, b]. The significantly lower COMT activity may suggest that under chronic estrogen treatment at high doses, and with the excess intake of dietary flavonoids, the concentration of quercetin may exceed the capacity of COMT to effectively catalyze their *O*-methylation into inactive metabolites. The resulting accumulation of catechol estrogens and quercetin may contribute to the carcinogenicity in such individuals [Zhu *et al.*, 1994; Zhu & Liehr, 1994]. Some studies suggested that $COMT^{L}$ allele was more frequently seen in patients with breast cancer than in healthy controls [Lavigne *et al.*, 1997, 2001; Huang *et al.*, 1999]

The postmenopausal women with the $COMT^{LL}$ genotype, which used estrogens for longer than 30 months, and women with the $COMT^{L}$ allele-containing genotypes which had menarche at <12 years of age had the significantly increased risk of cancer. Moreover, a recent study showed that the presence of the increased number of $COMT^{L}$ allele was significantly associated with higher breast cancer risk in women with the below median level of folate intake or the above median level of homocyteine. Both folate and homocyteine can indirectly affect the COMT-mediated *O*-methylation of catechol estrogens through modulating levels of S-adenosylhomocyteine (SAH), a strong non--competitive inhibitor of COMT. It was shown that folate deficiency significantly increases strand breakage and uracil misincorporation in cell [Duthie *et al.*, 2000]. These results demonstrate that folic acid modulates DNA repair, DNA strand breakage, and uracil misincorporation in immortalized human colonocytes, and that folate deficiency substantially decreases DNA stability in cell.

Our studies indicate that COMT *H polymorphism (higher enzyme activity) is positively correlated with the amount of oxidative DNA damage and negatively correlated with uracil misincorporation into DNA. Moreover, the uracil misincorporation into DNA is much lower after folate supplementation (Table 1), indicating that both COMT activity and folate level can influence genetic stability defined as amount of DNA damage/modification.

SUMMARY

This paper reviews most recent studies analyzing the association between consumption of fruits and vegetables, their flavonoid content and cancer risk. Reasons for the inconclusiveness of the epidemiological data on this matter are discussed in the light of factors, such as genetic polymorphism of the catechol-*O*-methyltransferase (COMT), an enzyme responsible for the inactivation of flavonoid and catechol estrogen, and the dietary status, particularly folic acid and flavonoid intake. The results of reviewed studies, as well as our own preliminary research, showed that health benefits of flavonoids, defined as a correlation between their intake and the amount of endogenous DNA damage/modification, depend on inherited variation amongst individuals in their genetically defined ability to metabolize flavonoids. In addition, our research suggests the importance of folate, as a methyl group supplier, in maintaining genetic stability (as reflected by a decreased amount of uracil misincorporation into DNA). These findings were discussed in relation to COMT polymorphism. It was concluded that the bioactivity of flavonoids and therefore their beneficial effect on health can be related to variation in polymorphic form of COMT, to dietary status and lifestyle (estrogen lifetime exposure).

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