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The influence of phytoestrogens on long-term prognosis of breast cancer and possible biological mechanisms

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Phytoestrogens are plant-derived hormone-like compounds, with lignans (i.e. oilseeds, whole grains, berries) and flavonoids, including isoflavones (i.e. soy beans), as the two main subgroups. There is growing evidence that phytoestrogen intake improves survival after postmenopausal breast cancer diagnosis, yet the biological mechanisms underlying this association still remain largely unknown. Besides widely accepted biological hormone-dependent effects of phytoestrogens through structural similarity (e.g., binding to the estrogen receptor), hormone-independent anti-carcinogenic effects, including anti-proliferative and anti-inflammatory properties, are less well studied in epidemiological study designs. In addition, studies with exposure assessments at multiple time points assessing both potential long-term effects of phytoestrogens and the influence of postdiagnosis changes in biomarker concentrations with respect to the subsequent breast cancer survival are still lacking.

To examine possible hormone-independent mechanisms underlying the protective effect of phytoestrogens on postmenopausal breast cancer survival, (1) inflammatory markers were investigated as potential mediators of the relationship of the major metabolites of lignans and isoflavones, enterolactone and genistein, with respect to breast cancer prognosis. (2) The association between enterolactone/genistein and the cell proliferation marker Ki-67 and HER2 receptor status in tumor tissue of breast cancer patients was examined. Moreover, this was the first study quantifying the extent to which both (3) eight phytoestrogen metabolites measured in long-term breast cancer survivors a median 6 years postdiagnosis and (4) changes in metabolite concentrations since the patients' recruitment influence the subsequent breast cancer prognosis.

Data from a German multi-center prospective cohort of 3,813 breast cancer patients aged 50-74 years at diagnosis of primary breast cancer were used to address these topics. At recruitment (2002-2005) and both follow-up studies, median 6 years (2009) and 11 years (2014/15) postdiagnosis, participants completed standardized interviews and were requested to provide a blood sample. Postdiagnosis enterolactone and genistein levels (n=2,270/1,021 patients) at recruitment were available and 8 phytoestrogen metabolites including enterolactone and genistein were measured in blood samples taken a median 6 years postdiagnosis (n=2,051). For standardized assessment of tumor prognostic markers, paraffin tissue blocks were collected from 1,505 patients. Vital status was assessed at follow-up via local population registries, and data on recurrences and secondary tumors were verified by clinical records or treating physicians. Multivariable linear, logistic and delayed-entry Cox proportional hazards regression models were performed. The product method was applied for mediation analysis.

(1) After a median follow-up time from recruitment to event or censoring of 5.3 years (min-max 0.1-7.4) until end-2009 (first follow-up), 180 (9.7%) of 1,743 patients had died, 121 (67.2%) due to breast cancer, and 171 (10.9%) recurrences occurred. Higher enterolactone levels were significantly associated with a reduced risk for all-cause mortality, breast cancer-specific mortality and distant-disease (per doubling of concentrations: multivariable Hazard Ratio (HR) 0.93, 95% confidence interval (0.87, 0.99); 0.91 (0.84, 0.99); 0.92 (0.87, 0.99)). No evidence for an association between genistein and any of the outcomes was observed. Higher enterolactone concentrations were strongly associated with lower levels of the pro-inflammatory marker C-reactive protein (CRP). Following mediation analysis, estimated 18%, 14% and 12% of the effects of enterolactone on all-cause mortality, breast cancer-specific mortality and distant-disease, respectively, were mediated through the inflammatory marker C-reactive protein. A mediational effect by C-reactive protein was apparent only in patients with low or moderate grade tumors (28%, 20% and 20%).

(2) Higher genistein concentrations were found to be associated with a significantly lower Ki-67 expression, when breast tumors are highly proliferative (Ki-67 ≥20% *versus* <20%, Odds ratio 0.93 (0.87, 0.99) per 10 nmol/L increment) after multivariable adjustment including hormonal factors. No association between enterolactone and Ki-67 expression or between enterolactone and genistein with tumor HER2 expression was observed.

(3) During a median follow-up time from blood draw at first follow-up to death or censoring by mid- 2015 of 5.8 years (0.3-6.1) (second follow-up), 142 (8.4%) of 1,686 women died, 73 (51.4%) due to breast cancer. No clear overall prognostic associations for enterolactone or for flavonoid metabolites, including genistein, daidzein, formononetin, naringenin, luteolin, resveratrol and kaempferol, measured median 6 years postdiagnosis, were observed with respect to the subsequent survival. (4) No clear evidence for an overall effect of changes in enterolactone and genistein concentrations between recruitment and the first follow-up with respect to the subsequent survival was detected.

This thesis provides first evidence that the beneficial effects of lignans, ascertained as the major metabolite enterolactone, on postmenopausal breast cancer survival may be partly mediated by the inflammatory marker C-reactive protein, which supports the potential anti-inflammatory effect of lignans and thus the involvement of hormone-independent mechanisms linking lignans to breast cancer prognosis. In addition, higher isoflavone exposure as indicated by genistein levels were related to lower tumor proliferation at high levels of Ki-67 expression. No clear overall prognostic associations for phytoestrogen metabolites measured in long-term breast cancer survivors as well as for changes in phytoestrogen exposure since recruitment were found with respect to the subsequent survival. Yet, potential misclassification of exposure status cannot be excluded as two different technologies were used to measure phytoestrogen metabolites at these two time points.

Large long-term longitudinal studies involving blood collection at multiple time points are required to confirm the present findings and to further elucidate long-term effects of phytoestrogens and potential biological hormone-mechanisms underlying the effects of phytoestrogens with respect to breast cancer prognosis. The long-term objective here should be to provide more specific dietary recommendations for a lignan-rich diet to breast cancer patients in clinical practice in order to further improve survival after breast cancer diagnosis.