Toxicology 296 (2012) 67–72

Contents lists available at SciVerse ScienceDirect

Toxicology

journal homepage: www.elsevier.com/locate/toxicol

Pre-diagnostic acrylamide exposure and survival after breast cancer among postmenopausal Danish women

Anja Olsen[a,*], Jane Christensen[a], Malene Outzen[a], Pelle Thonning Olesen[b], Henrik Frandsen[b], Kim Overvad[c,d], Jytte Halkjær[a]

[a] Danish Cancer Society Research Center, Danish Cancer Society, Copenhagen, Denmark
[b] National Food Institute, Technical University of Denmark, Søborg, Denmark
[c] Department of Epidemiology, School of Public Health, Aarhus University, Aarhus, Denmark
[d] Department of Cardiology, Aalborg Hospital, Aarhus University Hospital, Aalborg, Denmark

ARTICLE INFO

Article history:
Received 12 January 2012
Received in revised form 28 February 2012
Accepted 10 March 2012
Available online 20 March 2012

Keywords:
Acrylamide
Glycidamide
Breast cancer survival
Hemoglobin adducts

ABSTRACT

Acrylamide is a probable human carcinogen, with industrial contact, tobacco smoking and foods processed at high temperatures as the main routes of exposure. In animal studies oral intake of acrylamide has been related to cancer development, with indications that the increased cancer occurrence especially regards endocrine related tumors. In human epidemiological studies, dietary exposure to acrylamide has also been suggested related to higher risk of endocrine related tumors, like estrogen sensitive breast cancer. The aim of the present study was to evaluate if pre-diagnostic acrylamide exposure, measured by acrylamide and glycidamide hemoglobin adducts (AA-Hb and GA-Hb), were associated to mortality in breast cancer cases. Among 24,697 postmenopausal women included into a Danish cohort between 1993 and 1997, 420 developed breast cancer before 2001 and 110 died before 2009. AA-Hb and GA-Hb concentrations measured in blood samples were related to mortality by Cox proportional hazard models. Estimates are given per 25 pmol/g globin higher levels.

Among non-smokers, higher concentrations of GA-Hb were associated to a higher hazard rate of breast cancer specific mortality (HR (95% CI): 1.63 (1.06–2.51)), the hazard rate among women diagnosed with estrogen receptor positive tumors was (HR (95% CI): 2.23 (1.38–3.61)). For AA-Hb the tendency was similar, but only statistically significant among those with estrogen receptor positive tumors (HR (95% CI): 1.31 (1.02–1.69)). In conclusion, the present study indicates that pre-diagnostic exposure to acrylamide may be related to mortality among breast cancer patients and that this may especially concern the most endocrine related type of breast cancer.

1. Introduction

Acrylamide is classified as a probable human carcinogen (class 2A) (IARC, 1994). Industrial contact with acrylamide was originally the main concern with regard to acrylamide exposure, but more recently it has been established that tobacco smoking (Urban et al., 2006) and processing of foods at high temperatures (Tareke et al., 2000) are probably more important routes of exposure for a large majority of the population.

The human metabolism of acrylamide follows two main pathways: conjugation with glutathione or epoxidation. Acrylamide is oxidized to the epoxide glycidamide. Cytochrome P450 2E1 is the primary enzyme responsible for the epoxidation. Glycidamide itself is further metabolized through conjugation with glutathion or hydrolysis to 2,3-dihydroxy-propionamide (Calleman et al., 1990; Doroshyenko et al., 2009; Ghanayem et al., 2005; Hartmann et al., 2010; Sumner et al., 1999). Both acrylamide and glycidamide are reactive compounds that form adducts with proteins, including hemoglobin. Contrary to acrylamide, glycidamide is mutagenic and is generally considered the causative genotoxic metabolite of acrylamide (Besaratinia and Pfeifer, 2007; Paulsson et al., 2001).

The concentration of acrylamide bound to the N-terminal amino acid in hemoglobin is strongly correlated to the exposure of acrylamide. The glycidamide analog correlates to glycidamide DNA adducts (Tareke et al., 2006) and is considered a biomarker for the genotoxic dose reflecting the individual ability to metabolically activate acrylamide. Adducts formed by acrylamide and glycidamide with hemoglobin (AA-Hb and GA-Hb) are considered good

* Corresponding author at: Danish Cancer Society Research Center, Danish Cancer Society, Strandboulevarden 49, DK-2100 Copenhagen O, Denmark.
Tel.: +45 35257606; fax: +45 35257731.
E-mail address: anja@cancer.dk (A. Olsen).

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doi:10.1016/j.tox.2012.03.004

measurements of an individual's average exposure to acrylamide and glycidamide within the 4 months life-time of an erythrocyte (Bergmark, 1997; Paulsson et al., 2003).

In animal studies, acrylamide has been reported carcinogenic following oral dosing. Looking at the cancer sites evaluated in the published rat studies, there are indications that especially the occurrence of endocrine related tumors are increased following acrylamide exposure (Friedman et al., 1995; Johnson et al., 1986).

In human epidemiological studies, the association between dietary exposure to acrylamide and risk of cancer development has been evaluated in several studies, recently reviewed by Hogervorst et al. (2010). Some of the published epidemiological studies seem to support that acrylamide primarily is associated with endocrine related cancer, as the strongest associations have been found for endometrial and ovarian cancer as well as estrogen and/or progesterone receptor positive breast cancer (Hogervorst et al., 2007; Olesen et al., 2008; Pedersen et al., 2010; Wilson et al., 2010). Other studies evaluating the same cancer sites have, however, not found associations with acrylamide exposure and consequently do not agree with the hormone hypothesis (Hogervorst et al., 2010). Further, the biological mechanisms behind the potential effect on endocrine related cancer are not elucidated, but it has been suggested that acrylamide may influence the hormonal balance through binding to the estrogen receptor (Hogervorst et al., 2010).

Circulating estrogen levels and the binding of estrogen to the estrogen receptor are known to be among the most important risk factors for breast cancer and influence on circulating estrogen levels is the key mechanism behind several of the factors known to be associated to breast cancer incidence and/or prognosis (Folkerd and Dowsett, 2010; Kendall et al., 2007). Special concern has been given to the impact of obesity in postmenopausal women, where estrogens produced through aromatization in adipose tissue result in significantly higher concentrations of circulating estrogens in obese women compared to lean women (Liedtke et al., 2012).

If acrylamide and/or glycidamide affect the hormonal environment in women it is possible, that exposure to high levels of acrylamide is also related to a poorer prognosis after breast cancer diagnosis.

The aim of the present study was to evaluate if pre-diagnostic acrylamide exposure, measured by AA-Hb and GA-Hb, was related to mortality among 420 postmenopausal women diagnosed with breast cancer. This hypothesis has, to our knowledge, not previously been studied in epidemiological studies.

2. Materials and methods

2.1. Cohort

The Diet, Cancer and Health study is a prospective cohort study, established with the primary aim of studying the etiological role of diet on cancer risk. Between 1993 and 1997, 79,729 Danish women aged 50–64 and without previous cancer diagnoses were invited to participate in the study. A total of 29,875, corresponding to 37% of those invited, were enrolled into the cohort. A detailed description of the cohort, including socioeconomic factors associated to non-response has been published previously (Tjonneland et al., 2007).

The Diet, Cancer and Health study and the present substudy were approved by the regional ethical committees on human studies in Copenhagen and Aarhus and by the Danish Data Protection Agency. All participants provided written informed consent.

All cohort members attained one of the two established study centers, and each participant filled in a food frequency questionnaire and a life-style questionnaire. The life-style questionnaire included questions about reproductive factors, health status, social factors, and life-style habits. From this questionnaire, we obtained information about use of hormone replacement therapy (HRT; use at baseline yes/no), smoking at baseline (yes/no), smoking duration (years) and tobacco use (g/day). Information about alcohol intake (g/day) was obtained from the food frequency questionnaire.

In the study centers, 30 mL of blood (nonfasting, collected in citrated and plain Venojects) were drawn from each participant. The samples were spun and divided into 1-mL tubes of plasma, serum, erythrocytes, and buffy coat. All samples were processed and frozen within 2 h at −20 °C. At the end of the day of collection, all samples were stored in liquid nitrogen vapor (maximum temperature, −150 °C).

Of the initial 29,875 women, we excluded 326 who later were reported to the Danish Cancer Registry with a cancer diagnosed before the visit to the study clinic. In addition, eight women were excluded from the study because they did not fill in the life-style questionnaire. Because the present analysis aimed at women who were postmenopausal at study entry, we further excluded 4844 women, including 4798 who were considered premenopausal because they had reported at least one menstruation <12 months before entry and no use of HRT, nine women who gave a lifetime history of no menstruation, and 37 women who did not answer the questions about current or previous use of HRT, leaving 24,697 postmenopausal women for study.

Cohort members were identified from their unique personal identification number, which is allocated to every Danish citizen by the Central Population Registry. All the postmenopausal cohort members were linked to the Central Population Registry to obtain information on vital status and emigration. Information on cancer occurrence among cohort members was obtained through record linkage to the Danish Cancer Registry, which collects information on all cases of cancer diagnosed in Denmark (Storm et al., 1997). Linkage was done by the use of personal identification number and follow-up was nearly complete (99.8%). Each cohort member was followed up for breast cancer occurrence from date at entry, that is, date of visit to the study center until the date of diagnosis of any cancer (except for non-melanoma skin cancer), date of death, date of emigration, or December 31, 2000, whichever came first. Incident breast cancer was diagnosed in 434 women during the follow-up period. Of these 14 were excluded due to lack of blood sample or failure in the adduct analysis, leaving 420 breast cancer cases for study. The 420 women diagnosed with breast cancer before 1/1 2001 were linked to the Danish Death Certificate Registry to obtain information about date and cause of death. All cases (100% follow-up) were followed from date of diagnosis (between baseline and end 2000) until death or December 31, 2008.

A clinical registry exclusively about breast cancer also exists in Denmark and information on estrogen receptor α (ERα) status was obtained by linkage with the Danish Breast Cancer Co-operative Group, which holds records on a range of details for approximately 90% of all breast cancers diagnosed in Denmark (Fischerman and Mouridsen, 1988). A standardized immunohistochemical method was used in all medical centers. The cutoff level used to define positive ERα status was ≥10% positive cells. Information on ERα status was registered for 388 (92%) of the breast cancer cases, of these 299 women were diagnosed with an ERα positive tumor and 89 with an ERα negative tumor.

2.2. Adduct analysis

The analysis of the blood samples was conducted according to the method described in (Bjellaas et al., 2007). In short, the globin was purified from the blood samples and stored at −20 °C until analysis. Globin (20 mg) was subjected to a modified Edman reaction where phenyl isothiocyanate reacts with the N-terminal amino acid (valine) in hemoglobin, undergoes cyclization and decouples from the hemoglobin molecule releasing a phenylthiohydantoin derivative of N-alkylated valine adducts. The released phenylthiohydantoins were purified by solid phase extraction and analyzed on a LC ion-trap MS using multiple reaction monitoring.

The limit of quantification (LOQ) of the analysis was determined from the standard deviation of blanks (LOQ = 10 × SD/slope of calibration curve) to 2.4 pmol/g globin and 6.8 pmol/g globin for the AA-Hb and GA-Hb, respectively. In total, 42 batches were run. All samples were injected into the LC/MS in triplicate. All values were above the LOQ for AA-Hb but 8 values were below LOQ for GA-Hb.

The association between AA-Hb and GA-Hb concentrations and incidence of breast cancer has previously been reported based on these 420 breast cancer cases and a corresponding number of controls (Olesen et al., 2008).

2.3. Statistical methods

Analyses of the relations between AA-Hb and GA-Hb and mortality were based on Cox proportional hazard models using follow-up (from date of diagnosis) as the time axis and stratifying by age at diagnosis in 5 year intervals. All analyses were stratified on smoking status at baseline (yes/no), as tobacco smoking is an important source of acrylamide exposure. Former smokers and never smokers were pooled, as AA-Hb and GA-Hb concentrations were identical in these two groups. Special concern was given to recent former smokers (within 6 months of study entry), but their adduct levels did not differ from never smokers. To further elucidate influence of smoking on AA-Hb and GA-Hb levels, analyses conducted on smokers were adjusted for smoking duration (years) and tobacco use (g/day), both included as continuous variables. Analyses were also stratified according to the estrogen receptor status (positive or negative) of the tumor at diagnosis. Only 30% of the breast cancer cases were diagnosed with an estrogen receptor negative tumor, and when this minor sub-group was also stratified on smoking status, the statistical strength became very limited. Results are consequently only presented for either all breast cancer cases or for those with estrogen receptor positive tumors.

Factors previously found related to mortality among breast cancer patients were evaluated as potential confounders (Barnett et al., 2008; Carlsen et al., 2008; Dal et al., 2008). The associations are presented with and without adjustment for time