Influence of Diet on Exposure to Acrylamide – Reflections on the Validity of a Questionnaire

Birgitta Kütting^a Thomas Schettgen^a Matthias W. Beckmann^b
Jürgen Angerer^a Hans Drexler^a

^aInstitute and Outpatient Clinic of Occupational, Social and Environmental Medicine and ^bGynaecological Hospital, Friederich Alexander University, Erlangen-Nuremberg, Germany

Key Words

Acrylamide · Acrylamide, food-related exposure ·
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Abstract

Aim: This pilot study attempts to assess how far the standardized questionnaires are a valid tool to detect the food-related burden of acrylamide. Acrylamide is a toxic substance classified by the International Agency of Research on Cancer, as well as the Deutsche Forschungsgemeinschaft, as a probable human carcinogen. **Methods:** A venous blood sample was taken in order to determine the smoking-specific acrylonitrile adduct N-cyanoethylvaline and the acrylamide adduct N-2-carbamoylvaline in a female study population expecting delivery soon. A standardized questionnaire was used to determine the consumption of acrylamide-contaminated food. The results of our questionnaire were transferred to a linear evaluation system. Finally, anamnestic data of the questionnaire were correlated to objective parameters such as blood levels of hemoglobin adducts of acrylamide and acrylonitrile. **Results:** A positive correlation between the acrylamide intake and the levels of hemoglobin adducts in our study population was not proven. **Conclusions:** Evaluation of food-related exposure to

acrylamide is difficult due to several reasons. Firstly, the validity of anamnestic data strongly depends on the patient's ability to remember precisely all consumed food (quality as well as quantity) over a 3-month period. In addition, the contamination of acrylamide in food varies from one product to another; even the contamination of the same product is variable. Therefore, the missing correlation between the questionnaire and hemoglobin adduct rates is rather due to restricted validity of anamnestic data.

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Introduction

Seven years ago the accepted doctrine had been that the main human exposure to acrylamide was of occupational origin, as used in the manufacture of plastics, industrial processes and in polyacrylamide gel electrophoresis in laboratories. For the general public a potential source of exposure had only been seen by drinking water that had been treated with polyacrylamide in a refining process [1]. In order to minimize the risk for the general population, a maximum tolerable level of 0.1 µg acrylamide/l water had been established within the European Union [2].

In 1997, Bergmark [3] determined the level of acrylamide adducts in blood samples from laboratory personnel working with polyacrylamide gels for electrophoresis. The levels of hemoglobin adducts in smokers were two times the level of those in the non-smoking study population. This high background of acrylamide in non-smoking subjects was unexpected and surprising at the time, and the reason for this phenomenon left unexplained for almost 3 years. Furthermore, increased levels of hemoglobin adduct rates in smokers could be repeatedly confirmed by others [4, 5].

Then, in 2000, Tareke et al. [6] found increased hemoglobin adduct levels in rats fed a fried animal standard diet and suggested that the high background levels might be food-related. In 2002, Tareke et al. [7] were also the first to show that acrylamide was formed by heating certain starch-based food, e.g. potato products such as crisps and french fries.

These results were supported by others, and since then a variety of food products containing acrylamide have been identified [8–10]. Based on these observations, certain food products were suspected of being a potential source of exposure to acrylamide. Recently the chemical mechanism governing this food-related acrylamide production has been described. All findings indicated that acrylamide was generated from food products as a result of the Maillard reaction, and could be released by the thermal treatment of certain amino acids (e.g. asparagine), particularly in combination with reducing sugars [11, 12]. The almost exclusive formation of acrylamide from asparagine could explain the selective occurrence of acrylamide in certain food products that are rich in concentration of the dominant free amino acid asparagine such as potatoes and cereals [11].

Reports on the presence of acrylamide in a range of fried and oven-cooked foods have caused a worldwide concern. Acrylamide is known to have neurotoxic, carcinogenic, genotoxic and toxic properties in laboratory animals. Up until now, only the neurotoxic properties with damage to the peripheral nervous system have been confirmed in human beings. Clinical trials proving the genotoxic and carcinogenic properties of acrylamide in human beings are still missing.

The probable intake of acrylamide with food led to a controversial debate about possible health risks for the general population. Two case-control studies were retrospectively analyzed to solve the question of a lifetime cancer risk related to nutritional acrylamide intake. A recently published population-based case-control study from Sweden [13] showed no association between the lev-

el of alimentary acrylamide intake and the risk of developing cancers of larger bowel, bladder or kidney. Pelucchi et al. [14] could not detect any statistically significant correlation between consumption of fried and baked potatoes and cancer risk. The cancer sites considered were the oral cavity and pharynx, esophagus, larger bowel, breast and ovary. However, the statistical evaluation of these two case-control studies was based on data of preexisting population-based studies. Therefore, both case-control studies had the enormous disadvantage that all information concerning consumption of acrylamide-containing food and the selection of study population were related to data raised to assess completely different questions. A cohort study [15] on a total of 8,854 workers at four plants (three plants were located in the USA and one in the Netherlands) with potential exposure to acrylamide could not detect any significant association between mortality of cancers at different sites such as the nervous system, thyroid gland, testis and respiratory system and exposure to acrylamide during the observation time of 1925–1983. A follow-up study over an 11-year period (1984–1994) on 8,508 workers of the three US plants confirmed the results of the former study [16] for nearly all cancer sites. Except for pancreatic cancer, Marsh et al. [16] found a significant 2.26-fold risk in mortality among workers within the highest category of cumulative exposure to acrylamide. Still, no consistent exposure-response relations were detected. The findings of Marsh et al. [16] and Collins et al. [15] were controversially discussed in the literature due to methodological deficiencies. Hogan and Scott [17] criticized the selected control group. Schulz et al. [18] detected a direct correlation between alimentary acrylamide consumption and mortality of pancreatic cancer by changing the cut-off points in re-analyzing the data of Marsh et al. [16]. Granath et al. [19] proposed a re-analysis of all data under consideration of potential confounders such as smoking status, the so-called ‘healthy worker effect’ and the duration of exposure.

Although the already performed epidemiological studies do not give convincing evidence for the carcinogenic potential of acrylamide in human beings, it would still be too early to give the all-clear sign. Reliability of epidemiological risk assessment strongly depends on the validity of acquiring all data related to exposure. Based on the mutagenic potential of acrylamide, one has to conclude that a grade of exposure without risk does not exist [20]. In order to answer the question how far questionnaires are a valid tool to assess the alimentary intake of acrylamide, we initiated a pilot study on 10 pregnant women expecting delivery soon.

Patients

Ten women admitted to the maternity unit of the University of Erlangen-Nuremberg for delivery were included in this pilot study. All patients gave their written consent. Smoking is usually abandoned or at least dramatically reduced during pregnancy, thus we did not expect smoking to be an important confounding factor in our selected study population. Due to legislation related to maternity protection, an occupational exposure to polyacrylamide or to other dangerous substances at the workplace does not play any role in this study population.

Material and Methods

A venous blood sample was taken promptly prior to or shortly after delivery in order to determine the smoking-specific acrylonitrile adduct N-cyanoethylvaline and the acrylamide adduct N-2-carbamoylvaline in our study population. This point in time for taking the blood samples was simply chosen for pragmatic reasons [21]. The analytical method has already been described in detail [4, 5, 21]. A standardized questionnaire was used to determine consumption of acrylamide-contaminated food over the last 3 months. The questionnaire comprised 40 items: 16 questions were related to general aspects of medical history including drug intake and the particular gynecological history, and 24 questions referred to potential sources of exposure to acrylamide either by consumption of acrylamide-contaminated food, smoking habits or circumstances of hair washing (frequency of hair washing, products used for shampooing, hair length). Four categories such as regular, sometimes, rare and never were chosen to assess the frequency of alimentary intake for different acrylamide-rich products such as potato chips, crackers, popcorn, french fries, roasted onions, baked potatoes, fried calamari, crisped bread, cereals, whole grain bread, toasted bread, coffee and hot chocolate. Quantitative evaluation was done by collecting data about daily intake of regularly consumed products in numeric categories such as number of slices or cups. Potentially confounding factors, such as occupational exposure and use of cosmetics, were taken into account. The results of our questionnaire were transferred to a linear evaluation system. The category regular received 10 points, the other categories were respectively given 5, 2 and 0 points. Analogously, the quantitative assessment of consumption was performed. After addition of all numeric values for one subject, the resulting data were correlated to the levels of N-2-carbamoylvaline in the taken samples of venous blood.

Results

The non-smokers ($n = 9$) were, as expected, predominant in our selected study population. Anamnestic data related to smoking status corresponded very well to the levels measured for smoker's specific acrylonitrile adduct N-cyanoethylvaline (level of detection: 4 pmol/g globin). The only smoker, smoking 10 cigarettes a day, showed a level of 185 pmol/g globin, whereas levels were below de-

tection (<4 pmol/g globin) in the 9 non-smokers. Passive smoking, even intensively, did not increase the level of N-cyanoethylvaline.

The non-smoking study population showed levels of N-2-carbamoylvaline (hemoglobin adduct of acrylamide) in the range of 18–34 pmol/g globin (median 20), whereas the only smoker had a much higher level of 104 pmol/g globin.

The so founded point score of the alimentary acrylamide intake of each single patient was correlated to the patient's corresponding result of adduct monitoring of N-2-carbamoylvaline performed in the venous blood sample. Based on our data, a positive correlation was missing.

Discussion

The biological half-time of free acrylamide is, within a range of approximately 4.7 h in human beings, quite short and therefore detectable plasmatic levels of acrylamide have rarely been found [22], even in workers who experienced high exposure levels.

In the human organism, acrylamide binds on the hemoglobins of red blood cells. These resulting adducts are supposed to represent valid biomarkers to assess the exposure with acrylamide. Due to erythrocytes' lifetime of 120 days, these adducts give a sensitive and valid possibility to assess the internal burden with acrylamide over a 3-month period [23]. These adducts are also considered to be a surrogate of toxicity, given that if acrylamide can react with hemoglobin it should also be able to react with other larger molecules such as functional and important proteins and DNA [24].

The validity and reproducibility of our measurement results are supported by the fact that the smoker-specific acrylonitrile adduct was increased with 185 pmol/g globin in the only smoking subject included in this study. In contrast, the non-smoking subjects had levels of cyanoethylvaline below detection level (<4 pmol/g globin). Recently, elevated levels of acrylonitrile adducts have been identified as characteristic for smoking [5] and the data of our pilot study are in line with this observation. Additionally, a significant positive correlation between maternal and fetal N-2-carbamoylvaline levels could be detected as well [21].

To acquire the reliable anamnestic data related to individual nutrition habits, including the kind of products consumed as well as their quantity over the past 3-month period, is considered extremely difficult or even impos-

sible. Every subject was asked to determine as exactly as possible their average frequency and quantity of consumption in relation to different food products supposed to contain acrylamide. A precise quantitative assessment of all consumed food over a 3-month period is in our opinion almost impossible. Validity of anamnestic data strongly depends on the patient's ability to remember precisely all consumed food products (quality as well as quantity) over such a long period.

Questions related to the size of the consumed portions of acrylamide-contaminated foods strongly depend on subjective estimation and the so founded data are of restricted use for comparison. Consequently, a missing positive correlation between anamnestic data and results of measurement is a result of the restricted validity of these anamnestic data and does not necessarily represent a real counterevidence for a missing correlation. The fact that the contamination of acrylamide varies from one product to another (even the contamination of the same product is variable) has a negative impact on the accuracy, e.g. data related to the acrylamide content of crisp bread may vary from non-detectable up to the range of 2,840 µg/kg [9]. Based on all these reflections on reliability and validity of anamnestic data we gave up the idea to assess the average daily intake of acrylamide for all our study subjects. A food product containing low levels of acrylamide has to be taken into account if it is frequently consumed and might also become an important origin of exposure. For all these reasons, anamnestic data often might lack the preciseness and exactness.

Recently, Madle et al. [20] estimated the mean internal burden resulting from acrylamide based on data of the national consumption study, which had already been performed at the end of the 1980s. Hereby they determined the daily average alimentary intake for subjects with average consumption of acrylamide, as well as for subjects with a high consumption of acrylamide at the age of 15 up to 18 years. However, we can assume that the food pattern has completely changed since then, which is the reason why all available data up to now are only imprecise estimations. At the moment, actual and representative data are not available.

For the general population there are sources of exposure to acrylamide other than food, for example the use of cosmetics. In recent years however, a reduction of content from a maximum of 100 to 0.1 mg/kg per product was reached for cosmetics, which cannot be rinsed off immediately, and a reduction to 0.5 mg/kg for all products, which can be rinsed off, by EU directives [25]. Hence, the use of cosmetics is negligible as a potential source of ex-

posure. Polyacrylamide-containing package of food is not supposed to play an important role for exposure of the general public. The migration of acrylamide from polyacrylamide-containing food packages is not allowed to be >10 µg/kg due to the German directive for articles of daily use [26]. The WHO concluded that the use of cosmetics, drinking of water and migration of polyacrylamide by food packages were negligible factors for human beings compared to the alimentary intake for the internal burden with acrylamide [27]. Smoking, however, has to be considered more seriously, since only 1 cigarette produces 1–2 µg of acrylamide and corresponding levels of acrylonitrile in the range of 3–15 µg. The mean value for the exposure to acrylamide is four times the level than in non-smokers [5]. Exposure evaluations suggest that the contribution of other sources except smoking is below the nutritional intake. Based on these reflections, it is hypothesized that for non-smokers, without occupational exposure, the nutritional intake remains the most important factor. The missing positive correlation between nutritional intake and corresponding hemoglobin adduct rate in our small study population reflects rather the restricted validity of a food-related questionnaire than it forms a real counterevidence for this association.

The exact cancer risk related to the nutritional intake of acrylamide still remains unclear. Mathematical models have been used in order to quantify the possible cancer risk. According to the different models, cancer risk was estimated in the range of 700–10,000 cases per 1 million inhabitants. These diverging results reflect the imprecision of these calculations [20]. For more exact risk evaluation, cohort studies, such as the one in progress in Bavaria (1,000 subjects), with a larger, representative cohort of the general population are needed to identify high-risk groups and to give further information on other possible sources. For this purpose, we have used a modified questionnaire assessing frequency of consumption and size of portions on a decimal scale in order to receive more exact data. Because a level with a negligible risk of carcinogenicity does not exist for acrylamide, there should be a requirement for the acrylamide content in food to be decreased as far as reasonably feasible.

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