N-acetyltransferase 2 Phenotype in Painters with Bladder Cancer and Controls

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Abstract

***Aim**: This study was designed to evaluate the impact of N-acetyltransferase 2 (NAT2, substrate: aromatic amines) in painters with bladder cancer and controls. **Background**: Until the beginning of the 1960s, painters in Germany have used, among others, azo dyes based on carcinogenic aromatic amines. **Materials and Methods**: Sixteen painters with bladder cancer and 26 healthy painters (controls) who were from the same areas in Germany and in the same age group (± 5 years) were recruited into the study. All subjects were phenotyped for NAT2 by the molar ratio of two caffeine metabolites in the urine which was determined by the high performance liquid chromatography (HPLC) method. The number of years working as a painter, age at first exposure to paints and the life-time smoking habits of subjects were noted. **Results**: Fourteen cases and 23 controls had been exposed to paints before 1960. Age at first exposure to paint was 15.5 years (SD 5.3) in cases and 16.3 (SD 4.9) years in controls. Cases had worked 31.1 years (SD 15.0) and controls had worked 44.8 years (SD 7.2) as painters. Four cases and 7 controls were non-smokers. In this study, 88% of cases and 65% of controls were of the "slow" acetylation phenotype. **Conclusions**: The results point to an impact of the "slow" acetylation status as an individual risk factor for bladder cancer in persons occupationally exposed to amounts of carcinogenic aromatic amines released from water-soluble azo dyes.*

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Key words: Aromatic amines, Enzyme polymorphism, Occupational disease, Susceptibility factor, Transitional cell carcinoma

Introduction

In 1989, the International Agency for Research on Cancer acknowledged "painting" as an occupation that definitely causes cancer.[1] Lung cancer was most strongly associated with painting, but an excess for bladder cancer was also noted. Since then, additional publications have pointed towards an elevated bladder cancer risk in painters,[2-4] but whether there was an elevated bladder cancer risk in painters remained controversial until two recent large studies.[5,6] Urothelial bladder cancer (syn: transitional cell carcinoma) may be caused by carcinogenic aromatic amines. Azo dyes, based on carcinogenic aromatic amines, had been used by painters in former decades. It has been shown that water-soluble, i.e., bioavailable azo dyes based on carcinogenic aromatic amines release carcinogenic aromatic amines into organisms.[7,8] The polymorphic enzyme N-acetyltransferase 2 (NAT2) metabolises aromatic amines. Persons with a low metabolic capacity of this enzyme are called "slow" acetylators. In this group, the oxidative metabolic pathway metabolises the aromatic amines to a larger extent, resulting in greater amounts of specific metabolites (arylnitrenium ions) reacting with the DNA of the urothelium.[9] Several studies have shown an over-representation of the "slow" acetylation status in bladder cancer cases occupationally exposed to aromatic amines.[10] Until the beginning of the 1960s, some azo dyes used by painters were based on carcinogenic aromatic amines, especially benzidine. Therefore, it was investigated whether there was an over-representation of the "slow" acetylation status in painters with bladder cancer who had been exposed only to presumably small amounts of aromatic amines compared to workers in the dyestuff production.

Materials and Methods

Participants

All cases were painters histologically diagnosed with urothelial bladder cancer. They were from different areas in Germany. Their medical histories, life-time smoking habits, drug intake, and occupational histories were recorded. Matched controls were drawn with the help of

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members of the local guilds of painters and varnishers, families of painters with bladder cancer and others. Matched controls were non-diseased painters of the same gender, comparable age (± 5 years) and living in the same area. All cases and controls were of German origin and gave informed consent to participate in the study.

NAT2 Phenotyping

The caffeine test method first described by Grant et al[11,12] was used for NAT2 phenotyping. Individuals were asked to empty their bladder and after that to drink 1 to 2 cups of coffee. Urine samples for NAT2-phenotyping were obtained 2 to 3 hours after caffeine intake. The urine samples were immediately adjusted to pH 3.5 by adding small quantities of 1 M HCl and were stored in 10 mL "monovettes" (plastic tubes) at -20°C until processing. The urine samples from the controls were collected at the workplace or at home by one of the authors in the same way, cooled and immediately transported to the laboratory. Individual storage times until analysis did not exceed 6 weeks.

Quantitation of the caffeine metabolites AFMU (5-acetylamino-6-formylamino-3-methyluracil; acetylated metabolite) and 1X (1-methylxanthine; non-acetylated metabolite) was done by high performance liquid chromatography (HPLC) analysis, using the standard addition procedure.[10] Standard 1X is a commercial product of Sigma (Deisenhofen, Germany) while AFMU was synthesized by Röhrkasten et al.[13] For details of the preparation of the standard, see Golka et al.[10] The ratio of the antimode used to distinguish between slow and rapid acetylators was 1.0. This value is laboratory-dependent and has been evaluated by both genotyping and phenotyping for NAT2 in two previous studies.[10,14]

Statistical Analysis

Odds ratios (OR) were calculated according to Brandt and Snedecor.[15]

Results

The slow acetylation status was over-represented in the diseased painters. Fourteen out of 16 diseased painters (88%) were slow acetylators. In contrast, in their non-diseased colleagues, only 17 out of 26 (65%) were slow acetylators. This is within the percentage range of slow acetylators in normal Caucasian populations. Fourteen diseased painters and 23 non-diseased colleagues had been exposed to colorants before 1960. Cases and controls showed comparable smoking habits. The relevant occupational and non-occupational risk factors for bladder cancer in cases and controls are given in Table I. The OR for bladder cancer of slow acetylators compared to rapid acetylators was 3.0 (95% CI, 0.64-14.04).

TABLE I: NON-OCCUPATIONAL AND OCCUPATIONAL RISK FACTORS FOR BLADDER CANCER IN PAINTERS

Possible risk factors	16 cases	26 controls
Year of birth	1934 (SD 9.5)	1933 (SD 9.1)
Smoking habits		
Smokers	6 (38%)	12 (46%)
Ex-smokers	5 (31%)	7 (27%)
Non-smokers	4 (25%)	7 (27%)
Unknown	1 (6%)	0 (0%)
Age at first exposure to colorants (y)	15.5 (SD 5.3)	16.3 (SD 4.9)
No. of years working as a painter (y)	31.1 (SD 15.0)	44.8 (SD 7.2)
No. of persons exposed to colorants before 1960	14	23

Discussion

Discussions of elevated bladder cancer risk in painters have been remained controversial until recently. In 1999, Steenland and Palu[6] published a large cohort mortality study on more than 42,170 American painters based on union records. In comparison to the US population, they observed a standardised mortality ratio for bladder cancer of 1.23 (95% CI, 1.05-1.43). In direct comparison to the control group of more than 14,000 organised non-painters, the bladder cancer risk increased to 1.77 (95% CI, 1.13-2.77). In 1998, Chen and Seaton[5] published a meta-analysis on cancer mortality in painters. They also reported an elevated risk for bladder cancer in painters; the standard mortality ratio was 1.3 based on a meta-analysis of 17 cohort studies. Therefore, these 2 studies showed a slight but definitely increased bladder cancer risk for painters; however, the prognosis of bladder cancer must also be considered.

Cheng et al[16] investigated 83 consecutive patients with non muscle-invasive bladder cancer (stage T1 according to TNM) with histologic confirmation of the diagnosis between 1987 and 1992. All patients underwent transurethral resection of the bladder. About 82% of these bladder cancer cases had a progression-free survival rate of 5 years, i.e., no development of muscle-invasive or more advanced stage carcinoma was observed.

Malmstrom et al[17] analysed survival rates in 29,055 bladder cancer cases diagnosed in Sweden from 1960 to 1986. Between 1960 and 1964, about 60% survived the first 5 years as compared to 71% in those diagnosed between 1980 and 1984. The Swedish authors concluded that patients with a history of bladder cancer for at least 15 years ran an almost negligible risk of dying from their disease. Therefore, an analysis of mortality from bladder cancer will clearly underestimate the incidence.

Smoking is the most important risk factor for transitional cell carcinoma of the bladder. Tobacco smoke contains numerous carcinogenic substances, including carcinogenic aromatic amines, mainly 4-aminobiphenyl. Therefore, it should be taken into account that the slow acetylation status might also be a risk factor for bladder cancer in smokers. Surprisingly, the percentage of slow acetylators in smokers suffering from bladder cancer is only slightly higher compared to non-smokers suffering from bladder cancer. This is in-line with the observation that the percentage of subjects with a slow acetylation status in bladder cancer study groups is similar to the percentage of subjects with a slow acetylation status in the general population in Germany in the 1990s.[10] In contrast, in diseased subgroups occupationally exposed to aromatic amines, the percentage of slow acetylators was higher.[10] Therefore, smoking habits are not likely to explain the over-representation of the slow acetylation status in the diseased painters investigated.

In Germany, an elevated bladder cancer risk for painters and varnishers was also seen in all 4 hospital-based case-control studies (Table II). These studies showed different risks for different types of painters. This points to a major problem in investigation of bladder cancer in painters—different exposures to possible carcinogens result from the different materials and techniques used.[22,23] This may explain why some studies did not show an increased bladder cancer risk. Based on our own studies[3,20,21] and on medical data of diseased painters and varnishers claiming compensation,[22] there are several characteristic findings:

1) the diseased persons began their occupational career as a painter early in their life;
2) relevant exposures dated before the 60s;
3) the painters were regularly exposed to dust from colour compounds as a result of preparing the colorants by themselves, handling powdery colouring matters and/or removing old paints; and
4) there was intensive skin contact with these materials.

In former decades, paints contained several carcinogenic compounds, e.g. water-soluble azo dyes based on carcinogenic aromatic amines, which may release aromatic amines into the organism (Fig. 1). A number of individual aromatic amines is classified as bladder carcinogens in humans.[24] In 1982, Cartwright et al[25] first reported an over-representation of the slow acetylation status in bladder cancer patients occupationally exposed to aromatic amines previously: 22 out of 23 bladder cancer patients were slow acetylators. The over-representation of the slow acetylation status in Caucasian bladder cancer patients occupationally exposed to aromatic amines was confirmed by several other studies, in contrast to a large study in China.[10]

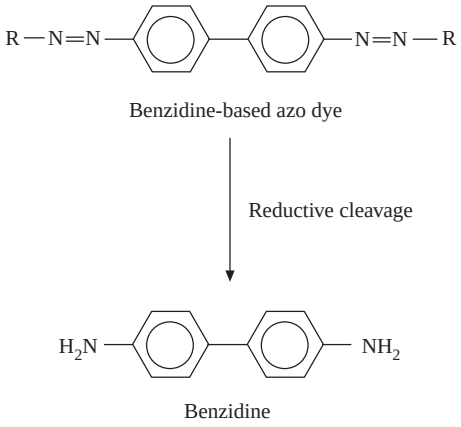

Fig. 1. Reductive metabolic cleavage of benzidine-based azo dyes to benzidine in the organism. R = coupling compound

TABLE II: BLADDER CANCER RISK REPORTED IN GERMAN PAINTERS AND VARNISHERS

Authors	Study size (Cases/Controls)	Painters and varnishers (Cases/Controls)	OR	95% CI
Claude et al[18] 1988	531/531	0^1-15^2-12^3 (3^1-49^2-17^3)[a]	1.25* (2.88)	0.59-2.67 (1.7-4.88)
Bolm-Audorff et al[19] 1993	219/219	10/5 (6/2)[b]	1.56 (2.34)	0.51-4.78 (0.46-11.83)
Golka et al[20] 1998	412/414	20/8	2.42	1.05-5.57
Golka et al[21] 1999	156/336	7/7	1.98	0.64-6.11

* not adjusted for smoking; OR: odds ratio

[a] spray painting, [b] house painters (included in painters and varnishers)

[1] concordant pairs, ever exposed

[2] discordant pairs, only cases ever exposed

[3] discordant pairs, only controls ever exposed

Therefore, the aim of the present study was to elucidate the impact of carcinogenic aromatic amines released from water-soluble azo dyes which had been used in former decades.

Concentrations of aromatic amines released from bioavailable azo dyes in painters are considered to be much lower than those exposed to workers in the dyestuff industry in the past. Vineis et al[26] have reported on the impact of the N-acetyltransferase polymorphism on low-level environmental exposure to carcinogens. In the non-diseased painters, the percentage of the slow acetylation status was similar to the general population in the present study. Therefore, an over-representation of the slow acetylation status also in non-diseased painters, resulting for example from a selection bias, can be ruled out. The present study shows a trend towards over-representation of the slow acetylation status in diseased painters. This finding points to an impact of carcinogenic aromatic amines also in the professional user of the colorants in former decades.

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