Lymphocyte reactivity of workers exposed to carcinogenic and non-carcinogenic chemicals

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ABSTRACT Immunological studies have shown an increased lymphocyte reactivity in patients with early stage bladder cancer and individuals with pre-stage-T 1 exposed to bladder carcinogens (2-naphthylamine and industrial 1-naphthylamine containing 4-8% 2-naphthylamine) before 1952—that is, those at high risk of developing bladder cancer. Because of the close chemical similarity of Tobias acid (2-naphthylamine—1 sulphonic acid) to 2-naphthylamine, the lymphocytotoxicity of workers exposed to this chemical was investigated. The results show that the workers using Tobias acid unlike those exposed to 2-naphthylamine have lymphocytes with a normal range of reactivity towards bladder cancer cells, which is in keeping with the suggestion that Tobias acid is non-carcinogenic. The probable reason for its non-carcinogenicity is that the relatively very large size and hydrophilic properties of the sulphonic acid group prevents enzymic action on the amino group by steric hindrance. The present lymphocytotoxicity results strengthens our earlier conclusion that the observed increased lymphocyte reactivity in workers exposed to bladder carcinogens is specific.

Bladder cancer was first suspected as an occupational hazard in 1895 after Rehn¹ observed three cases from a single factory making magenta dye. At the time the cause was wrongly attributed to aniline. Over the years, as the dyestuffs industry expanded, an increasing number of cases of bladder tumours were reported among workers producing aromatic amines. Case *et al*² found that in one factory the mortality rate from bladder cancer was at least 30 times higher than in the normal male population of England and Wales. The work of Scott³ and Case *et al*² incriminated three chemicals, 2-naphthylamine (beta), 1-naphthylamine (alpha), and benzidine as potential bladder carcinogens. Commercial 1-naphthylamine contained up to 8% 2-naphthylamine and the latter was considered to be entirely responsible for its carcinogenic activity. Another chemical, 4-aminobiphenyl was also found to be a potent bladder carcinogen.⁴ The manufacture of these materials in Britain was prohibited by 1967. Tobias acid (2-naphthylamine 1-sulphonic acid), which chemically is similar to 2-naphthylamine, is being commercially used as an intermediate in the manufacture of a wide variety of dyestuffs.

Koss^{5, 6} used the standard exfoliative cytology

originally developed by Papanicolaou and identified the majority of bladder tumours to have resulted from exposure to 4-aminobiphenyl. Cytological evaluation of the urine sediment, however, proved to be of less value in diagnosing low grade papillary tumours. The limitations of Papanicolaou staining of urinary sediments in identifying cellular abnormalities among workers exposed to bladder carcinogens have been well recognised.⁷

Recent immunological studies have shown an increased lymphocytotoxicity to bladder cancer cells in patients with bladder cancer.⁸⁻¹² It has been further reported that specific lymphocytotoxicity is related to the stage of the disease, with a higher proportion of early-stage patients (T1 and T2) showing increases compared to late-stage (T3 and T4).¹⁰ Our own lymphocytotoxicity studies^{13, 14} have shown increased reactivity in lymphocytes, not only from patients with early-stage bladder cancer, but also from pre-stage-T 1 individuals (exposed before 1952 to 1- and 2-naphthylamine and benzidine)—that is, those considered to be at high risk of developing bladder cancer but who have no detectable evidence of the disease. Because of the chemical similarity of Tobias acid to 2-naphthylamine we have investigated the lymphocyte reactivity against bladder cancer cells of workers exposed to this substance,

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Table 1 Details of 70 workers exposed to naphthylamine and 19 workers exposed to Tobias acid

	Naphthylamine workers	Tobias acid workers
Mean age on assay (yr)	62.6 $\pm$ 5.6	44.0 $\pm$ 10.6
Mean age on first exposure (yr)	28.6 $\pm$ 7.7	35.2 $\pm$ 9.9
Mean duration of exposure (yr)	3.6 $\pm$ 6.2	6.5 $\pm$ 5.7

Materials and methods

INDIVIDUALS STUDIED

Tobias acid group—Nineteen male workers exposed to Tobias acid (table 1).

Naphthylamine group—Seventy male workers with no detectable bladder cancer exposed to either pure naphthylamine (2-NA) or 1-naphthylamine, or both, containing 4–8% 2-naphthylamine before 1952.

Bladder cancer group—Twenty-three untreated patients with transitional cell cancer of the bladder. There were 19 men and 4 women; 19 were in clinical stage I, two in stage II, and two in stage III. Six of the 19 male patients had been employed in industries in which there was potential exposure to known carcinogens.

Normal donors—Forty-two adult healthy male blood donors with no known exposure to bladder or other carcinogens.

Peripheral blood lymphocytes from the above four groups were separated following the procedure published recently.¹³

Lymphocytotoxicity test—The details of the test were reported recently.¹³ Briefly, the ability of separated peripheral blood lymphocytes to kill bladder cancer target cells was measured in a reproducible manner. The results are expressed as numbers of lymphocytes required to kill 50% of standard number of target cells. This number, LD₅₀, is thus high if the lymphocyte reactivity of an individual worker is low, conversely low if reactivity is high.¹⁴

Results

Application of the Kruskal Wallis test using all four groups indicated that the results of at least one of the groups differed significantly from the rest ($p < 0.001$). Analysis by the Mann-Whitney U test showed that there was no significant difference in lymphocyte reactivity between the naphthylamine workers and the patient with bladder cancer ($p > 0.05$), although they were significantly different to normal blood donors ($p < 0.001$). Tobias acid workers, however, were significantly different from naphthylamine workers ($p < 0.01$) and patients with bladder cancer ($p < 0.05$), but were not significantly different from normal blood donors ($p > 0.05$). These differences are reflected in the median values as shown in table 2. The interquartile range for Tobias acid workers is almost identical with that of normal blood donors, whereas the range for patients with bladder cancer and naphthylamine workers is much narrower and lower, indicating greater lymphocyte reactivity by these two groups.

In addition, our data were analysed to examine if differences in the mean ages of the four groups investigated could have influenced lymphocytotoxicity. The age had no effect whatsoever on lymphocytotoxicity (our unpublished data).

Discussion

Tobias acid workers, unlike those exposed to 2-NA, have lymphocytes with a normal range of reactivity towards bladder cancer cells. This is in keeping with the suggestion that Tobias acid is non-carcinogenic despite its similarity to 2-NA. Theoretically, two different mechanisms of biotransformation of Tobias acid into carcinogenic metabolite are possible. Firstly, it may be desulphonated to give 2-NA. Secondly, the amino group of Tobias acid may be oxidised to give hydroxylamine as in the accepted mechanism of carcinogenicity of 2-NA that entails oxidation of amino groups to give 2-naphthylhydroxylamine. So far as we are aware carcinogenicity bioassays have not been carried out in dogs

Table 2 Lymphocytotoxicity results using bladder tumour cells

	Normal donors	Patients with bladder cancer	Naphthylamine workers	Tobias acid workers
Median LD ₅₀	481	204	221	365
Interquartiles	246-5000	88-603	121-374	250-5000
Group size	42	23	70	19

Kruskal Wallis test: Null hypothesis that the four groups have similar data distribution is rejected $p < 0.001$.

Mann-Whitney U test: Normal donors v patients with bladder cancer, significant difference $p < 0.01$; normal donor v naphthylamine groups, significant difference $p < 0.001$; normal donor v Tobias acid workers, no difference $p > 0.05$; patients with bladder cancer v naphthylamine group, no difference $p > 0.05$; patients with bladder cancer v Tobias acid workers, significant difference $p < 0.05$; Tobias acid workers v naphthylamine group, significant difference $p < 0.01$.

(the only suitable model for human bladder cancer), but the metabolic studies of P L Batten (personal communication), who fed dogs with Tobias acid, have shown that the acid is readily absorbed and excreted by the dog. The 2-NA concentration in the urine of treated and control dogs were similar; furthermore an N-hydroxy metabolite was not detected in the urine of the test dogs. Batten suggested that the lack of carcinogenic activity of Tobias acid might be due to the relatively very large size and hydrophilic properties of the sulphonic acid group, which prevents any enzymic action on the amino group by steric hindrance.

The present results also strengthen our earlier conclusions that the observed increased lymphocyte reactivity in workers exposed to bladder carcinogens is specific as the workers exposed to chemically similar, but non-carcinogenic, substances do not show this increased lymphocyte reactivity towards bladder cancer cells.

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