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CYP1A2 Activity as a Risk Factor for Bladder Cancer

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CYP1A2, CYP2D6 and N-acetyltransferase activities were estimated in 100 patients with bladder cancer and 84 control subjects from measurements of theophylline, metoprolol and isoniazid and their metabolites in urine, respectively. The frequency of occurrence of slow acetylators of isoniazid and poor metabolizers of metoprolol were 16.7% and 1.2% in the control group and 16.3% and 2.0% in the cancer patient group. These differences were not significant. The recovery ratio of 1-methyluric acid(1-MU) from theophylline was significantly higher in patients with bladder cancer than in control subjects(0.340 ± 0.016 versus 0.260 ± 0.020, $p<0.05$). The 1-MU recovery ratio was a significant, independent risk factor among the metabolic capacities tested as shown by logistic regression analysis, controlling for N-acetylation of isoniazid, hydroxylation of metoprolol, age, sex, and smoking. We concluded that the capacity for 3-demethylation of theophylline, as a reflection of CYP1A2 activity, is significantly associated with increased risk of nonoccupational urinary bladder cancer.

Key Words : *Theophylline demethylation, Isoniazid acetylation, Metoprolol hydroxylation, CYP1A2, CYP2D6, Bladder cancer.*

INTRODUCTION

Occupational exposure to arylamines has been linked to transitional-cell carcinoma of the human urinary bladder(Garner et al., 1984 ; Ward et al., 1988 ; Ward et al., 1991). Arylamines may also be significant in casusing nonoccupational (spontaneous, sporadic) bladder cancer, an association supported by the findings that cigarette smoking is proven to be a significant risk factor of bladder cancer(Mommsen and Agarrd, 1983) ; that aromatic amines such as 4-aminobiphenyl are present in nanogram quantities in cigarette smoke(Patrianakos and Hoffmann, 1979) ; and that smokers have higher levels of arylamine-hemoglobin adducts than nonsmokers(Bryant et al., 1987).

Aromatic amines are not carcinogenic in themselves but must undergo *in vivo* biotransformation to active carcinogens. This process involves the initial oxidation by cytochromes P450(Guengerich and Shimada, 1991) followed by conjugation mediated by acetyltransferase(Hein 1988 ; Wilson et al., 1989), UDP-glucuronosyltransferase(Kadlubar et al., 1977) and sulfotransferase(Chou et al., 1992). A wide variation in the activities of enzymes is regarded as an important determinant of individual susceptibility

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to chemical carcinogenesis(Conney 1982 ; Gelboin 1983). In this context, there is debate as to the relevance of polymorphic metabolism by CYP2D-6(Kasary et al., 1987 ; Hoari et al., 1989 ; Benitz et al., 1990), N-acetyltransferase(Cartwight et al., 1982 ; Evans et al., 1983 ; Hanssen et al., 1985 ; Kasary et al., 1987 ; Horai et al., 1989 ; Sinues et al., 199 2 ; Frederickson et al., 1992) and CYP2C(Kasary et al., 1987) with regard to the risk of bladder cancer.

CYP1A2 has recently been shown *in vitro* to affect the N-oxidation of primary amines, a step which is generally regarded as essential to carcinogenesis(Butler et al., 1988). However, no *in vivo* study has been done to investigate CYP1A2 activity as a risk factor for bladder cancer. Therefore, we have assessed the relationship between bladder cancer risk and CYP1A2 activity *in vivo*, measured using theophylline as a probe drug(Sarkar et al., 1991 ; Gu et al., 1992). In addition, N-acetyltransferase activity and CYP2D6 activity were estimated in the same Korean population of bladder cancer and control subjects.

MATERIALS AND METHODS

Subjects

The study was carried out on 100 patients with bladder cancer and 84 control subjects residing in the Seoul area. For matching of the groups by age and sex, age was constrained to over 20 years and female subjects were selected to represent at least 20 percent of both groups. To minimize any influence of habitat and socioeconomic status, all were selected from the same outpatient department of urology at Seoul National University Hospital. The patients were clinically stable for more than 3 months after surgical treatment without any evidence of metastatic cancer and were not receiving cancer chemotherapy. All of the bladder cancer patients had histological confirmation of transitional cell carcinoma and histopathologic grading(Ⅰ, Ⅱ and Ⅲ) at the time of the study. This classification was made independently before the study to remove bias. Control subjects were patients referred to the same outpatient clinic with definitive evidence that bladder cancers were not present as confirmed by radiology or endoscopy. The diagnoses of the control subjects included benign hyperplasia of the prostate, renal stone and sexual dysfunction. None of the cancer patients or control subjects had a history of industrial exposure to known bladder carcinogens. Subjects with renal, hepatic, or cardiac disease were excluded on the basis of measurements of serum creatinine, transaminases, and physical examination, as were patients receiving medications known to influence drug metabolism, Each subject gave informed consent to the study, which was approved by the Institutional Review Board fo Seoul National University Hospital.

Study design and determination of metabolic activity

All subjects abstained from any beverage containing caffeine for more than 3 days before the study. They were instructed to take 100 mg metoprolol tablet(Betaloc®, Yuhan-Astra Pharmaceutical Co., Seoul, Korea), 150 mg theophylline(Sigma, powder in gelatin capsule) and 400mg isoniazid(100mg×4 tablets, Yuhan Pharmaceutical Co., Anyang, Korea) as a cocktail before bedtime, and urine was collected over the subsequent 8h. The urine was stored at −20℃. Concentration of metoprolol and α-hydroxymetoprolol in urine were measured by high performance liquid chromatography(HPLC)(Horai et al., 1988). The interassay coefficients of variations of the assay for metoprolol and α-hydroxymetoprolol were less than 8.3 and 9.2% at concentrations of 0.5 to 10 μg/ml. The metabolic ratio(metoprolol/α-hydroxymetoprolol) (McGourty et al., 1985) and recovery ratio(α-hydroxymetoprolol/metoprolol plus α-hydroxymetoprolol) (Kasary et al., 1987) were used as indices of CYP2D6 activity. Isoniazid and acetylisoniazid were measured by a fluorophotometric method(Olson et al., 1977). The coefficients of variation of the assays were 3.3 and 2.4% for isoniazid and 6.4 and 4.5% for acetylisoniazid at 0.5 and 8 μg/ml. The recovery ratio(acetylisoniazid/isoniazid plus acetylisoniazid) was calculated(Kasary et al., 1987). Theophylline, its demethylated metabolites(3-methylxanthine ; 3-MX and 1-methyluric acid ; 1-MU) and a hydroxylated metabolite(1,3-dimethyluric acid ; 1,3-DMU) were measured simultaneously by HPLC(Muir et al., 1980). The coefficients of variation of the assays were 2.4% for theophylline at concentrations of 0.5 to 25 μg/ml and less than 6% for the metabolites. Theophylline oxidation activities were expressed as urinary recovery ratios of 1-MU, 3-MX and 1,3-DMU(urinary excreted amount of each meta-

bolite over that of metabolites plus parent drug).

Poor metabolizer phenotypes of CYP2D6(metoprolol) were defined by metabolic ratio values greater than 12.6(McGourty et al., 1985). Slow acetylator of isoniazid were defined as subjects whose urinary acetylisoniazid/isoniazid metabolic ratios were 0.99 or higher(Inaba and Arias, 1987 ; Shin et al., 1992).

Statistical method

Differences in the indices of metoprolol, theophylline and isoniazid metabolism between cancer patients and control subjects were compared by non-parametric methods(Wilcoxon rank sum test, median test or Kruskal-Wallis test). Differences in the distributions of the indices were illustrated by probit plots. Multivariate logistic regression analysis was used to test whether or not the individual indices of drug metabolizing activities were significant risk factors for bladder cancer ; the contribution of patient characteristics to the risk of bladder cancer such as age, sex and smoking were controlled simultaneously. The significance of each logistic regression coefficient was evaluated from its standard error and 95% confidence interval. The Statistical Analysis Package System(SAS) was used for all computations.

RESULTS

Of the 100 cancer patients, 68 were classified histopathologically as grade Ⅰ and Ⅱ(assigned as non-aggressive bladder cancer), 24 were grade Ⅲ and 8 were unclassified at the time of this study. Although we restricted the age of the subjects, the mean age and the proportion of smokers were significantly higher in the cancer patient group than in the control subjects(Table 1). This difference in age also resulted in a significant difference in renal functions calculated using the equation of Cockroft and Gault(Cockroft and Gault, 1976), in spite of restriction entry to subjects with serum creatinines less than 1.3 mg/dl. There were no differences in serum creatinine levels (cancer patients : 1.018±0.153 mg/dl versus control subjects : 1.009±0.152 mg/dl, $p=0.728$ by t test), but the estimated creatinine clearance was significantly different($p<0.001$ by t test) between control subjects(86.0±17.6ml/min) and cancer patients(69.3±14.9 ml/min).

The frequencies of slow acetylator were 16.7%(14/84) in control subjects and 16.3%(16/98) in the cancer patient group and were not different by chi-square test($p=0.89$ by Yate's corrected)(Table 1). The probit plot of urinary recovery ratio of acetylisoniazid indicated a non-normal distribution suggestive of bimodality, with overlapping for both groups of bladder cancer and control subjects(Fig. 1) The PM frequencies of CYP2D6 were 1.2%(1/84) in control subjects and 2.0%(2/100) in cancer patients, and were not significantly different($p=1.0$ by Fisher's exact test). The probit plot and histogram of metoprolol recovery ratio, in the cancer patient group was right-shifted(Fig. 2). The mean recovery ratio was significantly higher in the cancer patients than in control subjects by the Wilcoxon rank sum test($p<0.05$)(Table 2).

Among the recovery ratios of theophylline metabolites, only that of 1-methyluric acid was significantly different between the groups($p<0.02$)(Table 2). This difference was also noted in the probit plot as a right shift of the data for the cancer patient

Table 1. Demographic characteristics and frequencies of slow acetylators of isoniazid and poor metabolizers of metoprolol

	Control subjects (n=84)	Bladder cancer patients(n=100)	Aggressive cancer(n=24)	Non-aggressive cancer(n=68)
Age(years)	53.2±15.6	60.3±11.1*	65.4±9.9	58.6±11.0
Smoking(%)	57.1(48/84)	75.0(75/100)**	87.5(21/24)	76.5(52/68)
Male sex(%)	79.8(67/84)	79.0(79/100)	91.9(22/24)	80.9(55/68)
Slow acetylator of isoniazid(%)	16.7(14/84)	16.3(16/ 98)	12.5(3/24)	17.7(12/68)
Poor metabolizer of metoprolol(%)	1.2(1/84)	2.0(2/100)	0 (0/24)	2.9(2/68)

*$p<0.01$ by t-test

**$p<0.05$ by chi-square test

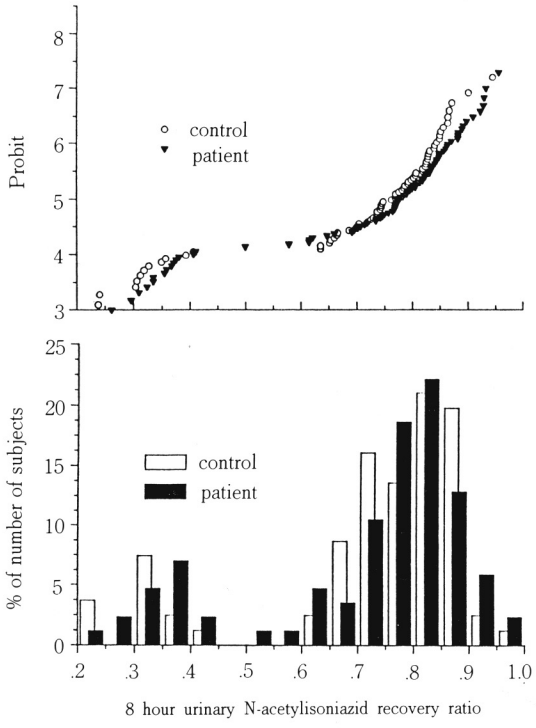

Fig. 1. Probit plot and histogram of 8 hour urinary recovery ratio of N-acetylisoniazid in 84 control subjects(open symbols) and 98 bladder cancer patients(closed symbols).

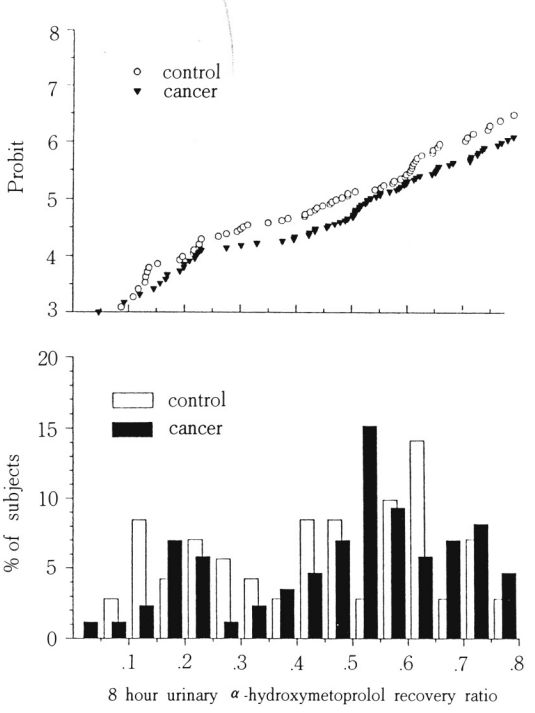

Fig. 2. Probit plot and histogram of 8 hour urinary recovery ratio of α-hydroxymetoprolol in 84 control subjects(open symbols) and 100 bladder cancer patients(closed symbols).

Table 2. Eight hour urinary recovery ratios of theophylline metabolites, α-hydroxymetoprolol and acetylisoniazid

	Control subjects (n=84)	Bladder cancer patients(n=100)	Aggressive cancer(n=24)	Non-aggressive cancer(n=64)
1-Methyluric acid*	0.260±0.020	0.340±0.016[a]	0.343±0.035[b]	0.333±0.019[c]
3-Methylxanthine	0.075±0.009	0.069±0.006	0.076±0.009	0.066±0.005
1,3-Dimethyuric acid	0.300±0.017	0.316±0.009	0.308±0.024	0.315±0.010
Theophylline excreted amount(mg)	19.343±2.833	11.444±1.076	14.160±3.952	11.050±0.707
α-hydroxymetoprolol	0.451±0.025	0.530±0.022[d]	0.548±0.039	0.515±0.028
Acetylisoniazid	0.711±0.021	0.720±0.019	0.715±0.037	0.740±0.113

Values are mean±standard error

*Kruskal-Wallis test comparing three means(control, aggressive and non-aggressive cancer patients) ; p=0.0168

Wilcoxon rank sum test between control subjects and cancer patients, p values ; [a]0.006, [b]0.041, [c]0.012, [d]0.027

group indicating more extensive theophylline 3-demethylating activity(Fig. 3). The recovery ratios of theophylline demethylation metabolites, when compared according to smoking status, were significantly defferent for the 1-MU ratio(smokers : 0.323±0.168, n=123, non-smokers : 0.251±0.150, n=61)(p=0.0102 by Wilcoxon rank sum test). The hydroxylated 1,3-dimethyluric acid recovery ratio was

Table 3. Multivariate logistic regression coefficients

	β (regression coefficient)	p value	Odds ratio (relative risk)	Confidence interval (95%)	Standardized coefficient*
1-MU recovery ratio	3.820	<0.002	45.60	4.16—499.77	0.35
α-hydroxymetoprolol recovery ratio	1.632	0.053	5.11	0.98— 26.74	0.20
Acetylisoniazid recovery ratio	0.386	0.70	0.68	0.21— 10.39	
Smoking	2.12	<0.001	8.37	2.40— 29.17	0.53
Age	0.054	<0.001	1.06	1.02— 1.09	0.40
Sex	2.063	<0.01	7.87	1.76— 35.21	0.42

1-MU ; 1-methyluric acid
*Standardized coefficient ; $\beta_i = \beta_i / \sqrt{var(x_i)}$

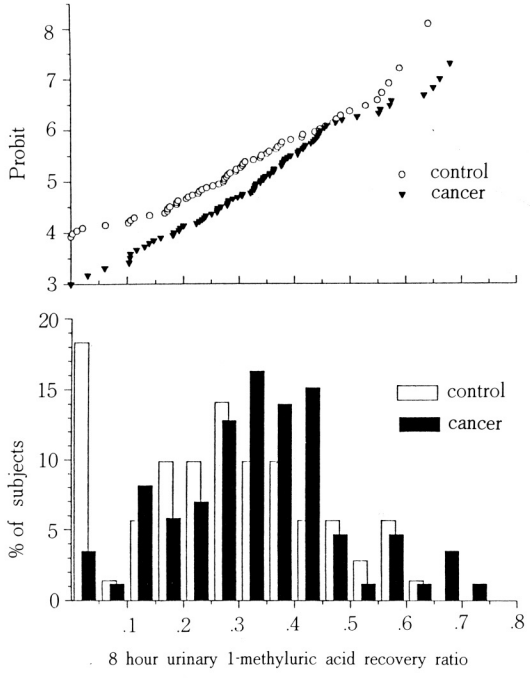

Fig. 3. Probit plot and histogram of 8 hour urinary recovery ratio of 1-methyluric acid in 84 control subjects(open symbols) and 100 bladder cancer patients(closed symbols).

significantly higher in the smoking cancer patients than in the non-smoking cancer patient group($p<0.05$).

The logistic regression results are summarized in Table 3. Age, smoking, and sex were independent and significant risk factors. The theophylline 3-demethylation index was the only one significant risk factor while N-acetylation and metoprolol hydroxylation indices were not. Although the α-hydroxymetoprolol recovery ratio was significantly different between groups by the Wilcoxon rank sum test(Table 2), the index was not a significant risk factor by logistic regression after adjustment for other indices of enzyme activity, age, smoking, and sex(Table 3). The theophylline 3-MX recovery ratio(=0.97) and the 1,3-DMU recovery ratio($p=0.70$) were not significant risk factors by logistic regression, neither were the urinary recoveries of unchanged theophylline and metoprolol. The p values and odds ratios were 0.62 and 1.000 for theophylline and 0.76 and 1.000 for metoprolol, respectively.

DISCUSSION

The control subjects in this study were younger and included fewer smokers than the cancer patients. As age and smoking status are important risk factors for bladder cancer and smoking is known to induce CYP1A2 activity(Pantuck et al., 1974 ; Sesadic et al., 1988), we controlled for age and smoking effects by logistic regression to reveal independent relationships between drug metabolizing activities and risk of bladder cancer.

In using the three probe drugs in a cocktail, we assume no interactions between the compounds. Metoprolol is reported to have no influence on the kinetics of theophylline(Upton, 1991) and theophylline does not alter the clearance and acetylation of isoniazid(Hoglund et al., 1987). Reports of the effects of isoniazid on the kinetics of theophylline

are contradictory with suggestions of increased-(Thompson et al., 1982) and decreased clearance-(Hoglund et al., 1987). Recent *in vivo* studies in rats(Zeruesenay et al., 1992 ; Bachman et al., 1993) indicated no interaction between isoniazid and theophylline. Therefore we assumed that any effect of isoniazid on the metabolism of other drugs would be minimal or if present, the effect would not distort comparisons between groups.

Although the *in vivo* determination method of CYP1A2 activity using theophylline has not previously been reported, the metabolic pathways of theophylline are less complex than those of caffeine(Kalow and Tang, 1992). We confirmed that 1-methylxanthine(1-MX ; a 3-demethylation product of theophylline) was not detectable(Tang-Liu et al., 1982). Therefore, variability in the 1-MU recovery ratio includes variability in the conversion of 1-MX to 1-MU by xanthine oxidase. However, xanthine oxidase shows only 4 fold variability compared to a greater than 30 fold interindividual difference in CYP1A2 activity(Vistisen et al., 1992). The recovery ratio of 1-MU was the only significant index of theophylline oxidation which related to bladder cancer in our study. Recently, Gu et al.(1992) showd *in vitro* that both the 3- and 1-demethylation pathways of theophylline are catalyzed by CYP1A2. Therefore, it is of relevance in our study that 3-MX recovery ratio was not different between control subjects and bladder cancer patients(Table 2) nor was it a significant variable by logistic regression($p=0.97$). Sarkar et al.(1991) showed *in vitro* that although both the pathways in human liver correlated with CYP1A2 content measured by immunoblot analysis, inhibition of the activities by a specific antibody were different. Therefore, they suggested that 1- and 3-demethylation pathways of theophylline might be mediated by closely related but different enzymes. At this moment further studies to differentiate the two pathways are needed before making any conclusion but those two pathways might be under different genetic or environmental regulation.

Slow acetylation has long been known to be a risk factor for occupational bladder cancer(Cartwright et al., 1982 ; Ladero et al., 1985 ; Inaba and Arias, 1987 ; Horai et al., 1989). However, in nonoccupational bladder cancer patients Kasary et al.(1987) and Horai et al.(1989) did not find this to be a significant factor. Furthermore, even in occupational bladder cancer the association is doubted(Hayes et al., 1993). This lack of association might be anticipated since the initial activation of arylamines is mediated by CYP1A2 and acetylation is a minor secondary pathway compared to N-hydroxylation. Furthermore N-acetylation can both activate and inactivate aromatic amines(Grant, 1993). Slow acetylators lack the detoxifying mechanism of competing N-oxidation by CYP1A2 but they produce less electrophiles by way of O-acetylation product of hydroxylamines. Another explanation for this lack of association is that, although acetylator phenotype is determined by NAT2 isoenzymes, arylamines are N-acetylated by both the NAT1 and NAT2(Grant, 1993). Although NAT may be a significant risk factor for occupational bladder cancer it is possible that in non-occupational bladder cancer unknown environmental carcinogens other than aromatic amines, which are activated by CYP1A2, are involved. Kasary et al.(1987) suggested CYP2D6 activity as a risk factor of aggressive bladder cancer, but this was not suggested by others(Cartwright et al., 1984 ; Horai et al., 1989 ; Benitz et al., 1990). In our study patients with aggressive cancer did not show any more relevance with CYP2D6 activity index. The odds ratios for metoprolol recovery ratio in aggressive and non-aggressive bladder cancer patients were 6.1($p=0.71$) and 4.1($p=0.73$), respectively.

The well known risk factors for bladder cancer, smoking, age, and sex were also found to be significant risk factors in this study(Table 3). The relative importance of each variable can be estimated from the standardized coefficient(coefficient divided by the square root of variance of the observed variable). On this basis, smoking was the most important risk factor associated with a 8.4 fold increased risk. The recovery ratio of 1-MU was a significant risk factor which was independent of smoking, age, sex and other enzyme activities. In the control population, subjects in the 25 and 75 percentiles of the distribution of the 1-MU recovery ratio had values of 0.14 and 0.38, respectively. Therefore, subjects in the 75 percentile would have a 2.48 fold increased relative risk compared to subjects in the 25 percentile.

In conclusion, ous observations are consistent with the hypothesis that activation of as yet unidentified environmental procarcinogens can be mediated by mixed function oxidases to form proximate carcinogens responsible for bladder cancer unrelated to occupational exposure. The variability in the activities of these drug metabolizing mixed function ox-

idases may be a significant risk factor determining interindividual difference in susceptibility to bladder cancer.

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