Phenobarbital, Drug Metabolism, and Human Cancer

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Abstract

To investigate the possible influence of anticonvulsant treatment on cancer risk, a nested case-control study of 104 lung cancers, 18 bladder cancers, and 322 cancer-free controls was conducted. The background for the study was previous observations among 8004 epileptics in Denmark with a significantly high risk for lung cancer and a significantly low risk for bladder cancer. Cigarette smoking appears to explain the lung cancer excess but not the low risk for bladder cancer, another tobacco-related disease. Information was abstracted on 94 and 95% of the cases and controls, respectively. Lung cancer was not associated with any anticonvulsant drug, but bladder cancer was inversely related to use of phenobarbital (PB). The apparent protective effect of PB was further evaluated in a study of rats given 4-aminobiphenyl (ABP), a bladder carcinogen. The levels of 4-aminobiphenyl adducts in hemoglobin and in bladder and liver DNA were significantly lower in rats given PB prior to 4-aminobiphenyl, compared to controls. These studies suggest that PB may induce drug-metabolizing enzymes of the liver that deactivate bladder carcinogens found in cigarette smoke and provide clues to the role of activation and detoxification of carcinogens in humans.

Introduction

Our survey of cancer incidence among 8004 epileptics in Denmark revealed a significant 1.4-fold excess of lung cancer and a significant 0.6-fold deficit of bladder cancer (1). An explanation for these divergent risks in terms of cigarette smoking seemed unlikely since tobacco is known to cause both cancers. PB,[2] a common barbiturate given to epileptics to control seizures, has been reported to increase the risk of human lung cancer (2), while decreasing the risk of bladder cancer (3). Anticonvulsant drugs are also known to increase liver enzymes that, under certain circumstances, may both activate and detoxify carcinogens such as those found in cigarette smoke (4, 5). To investigate the influence of cigarette smoking, anticonvulsant therapies, and drug metabolism on the risk of lung and bladder cancers, a nested case-control study and a laboratory experiment on rats were carried out.

Methods

Case-Control Study. An earlier cohort study of 8004 epileptics identified 111 lung cancers and 19 bladder cancers via record-linkage procedures with files at the Danish Cancer Registry (1). Patients had been treated at the Filadelfia epilepsy center between 1932 and 1962 and were followed for cancer incidence through 1984. The term "bladder cancer" in this study includes cases of bladder papillomas (6). Two controls were individually matched to each case on the basis of sex, year of birth (±1 year), and survival time. Eight cases (6.2%) and 13 controls (5.0%) were excluded because medical records were missing. An additional 14 controls, no longer matched to a case, were excluded, leaving 122 cases and 233 controls for study (Table 1).

Detailed drug use was abstracted from the medical records at the epilepsy center. Indication of exposure to Thorotrast, a radioactive contrast medium, was obtained by seeking information on cerebral angiography and through record-linkage with files from the Danish Thorotrast study (7, 8).

PB in daily doses of 100–300 mg was frequently prescribed at Filadelfia to prevent seizures (9). Phenytoin (5,5-diphenyl-hydantoin; Dilantin; 100–400 mg/day) became popular in the 1940s and primidone (500–1500 mg/day) became popular in the mid-1950s. Cumulative doses were computed by assuming that treatment continued daily at the prescribed dose after each hospital discharge until the date of cancer diagnosis (or equivalent date for matched control) or the end of 1964, whichever occurred first. After the mid-1960s many new anticonvulsants were released, and credible assumptions about continuation of previous treatments after discharge were not possible. The median cumulative dose was chosen to separate low from high drug exposures; specifically, it was 750 g for PB and phenytoin and 2000 g for primidone.

The effect of the anticonvulsants was assessed by means of conditional logistic regression for matched sets (10). Crude and adjusted risk estimates were calculated, where adjustment was undertaken for other anticonvulsant treatments. Estimates of relative risk and the corresponding 95% confidence intervals were calculated. Because of the link between Thorotrast exposure and lung cancer risk (5 exposed cases; 0 exposed controls), analyses excluding all patients given Thorotrast were also conducted.

Smoking Survey. Because tobacco use was not recorded in medical records during the years patients were hospitalized at Filadelfia, a mail survey was conducted to characterize smoking habits. Controls under the age of 80 years and alive in 1991 were sent a questionnaire. Practically all cases had died and thus were not included. Among the 142 controls

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[2] The abbreviations used are: PB, phenobarbitol; ABP, 4-aminobiphenyl.

Table 1 Relative risk of developing lung or bladder cancer following epilepsy, and numbers selected for case-control study

Cancer	Epilepsy cohort[a]		Case-control study	
	n	RR (95% CI)	Cases (*n*)	Controls (*n*)
Lung	111	1.45 (1.19–1.75)	104	200
Bladder	19	0.60 (0.36–0.94)	18	33

[a] Sources: Refs. 1 and 7. RR, relative risk; CI, confidence interval.

contacted, 44 did not respond, 3 refused, and 6 had recently died or were too ill to reply. Regular use of tobacco products, defined as smoking for 10 or more years, was reported by 61 and 69% of the men and women, respectively. The proportion of tobacco users in the general population, accounting for birth cohort differences, was 52 and 42% for men and women, respectively (11).

Animal Experiment. The effect of PB on the formation of ABP adducts, reactive metabolites which bind to DNA, was studied in Wistar rats (own breed). The PB group (12 rats) and controls (8 rats) were both given water and Altrumin 1314 laboratory chow (Christian Petersen A/S, Ringsted, Denmark) *ad libitum*. The exposed group was given 0.1% PB (Rigshospitalets Apotek, Copenhagen, Denmark) in the drinking water for 8 days (average dose, 101 mg/day/kg). On day 7, all rats in both groups were given 1 mg ^{14}C-ABP/kg (Chem-Syn Science Laboratories, Lenexa, KS) diluted to 7.3 Ci/mol with cold ABP (Sigma Chemical Co., St. Louis, MO) in 10 μl dimethylsulfoxide and 190 μl corn oil by i.p. injection. Twenty-four h later the rats were anesthetized with ether and killed by cardiac puncture. Blood, liver, and bladder epithelial cells were isolated (12, 13). Liver and bladder DNA and hemoglobin (14, 15) were prepared. The amount of ABP adducts in hemoglobin and in liver and bladder DNA was determined by liquid scintillation counting. Differences in ABP adduct levels among PB-treated and nontreated rats were tested by use of Student's *t* test.

Results

Case-Control Study. Phenobarbital was the most commonly prescribed antiseizure drug (70%), followed by phenytoin (54%) and primidone (23%) (Table 2). Approximately 25% of the epileptics had no record of taking any anticonvulsive drug. Thorotrast had been given during cerebral angiography to 5 lung cancer cases and 2 bladder cancer controls; all 7 were also treated with PB; 6 were treated with phenytoin and 3 were treated with primidone.

Lung cancer was not associated with any particular anticonvulsant drug (Table 3), but the risk following Thorotrast exposure was highly significant (lower 95% confidence limit 2.6). A reanalysis after exclusion of the five matched sets with Thorotrast-exposed cases did not change the results in any significant direction. However, PB use prior to the mid-1960s was linked to a significantly low risk of bladder cancer (relative risk, 0.3). Dose-response analyses revealed no consistent relationship between lung cancer and cumulative exposure to PB or any other barbiturate (Table 4). The risk associated with the highest exposure was 1.0. The risk for bladder cancer, however, declined significantly with increases in cumulative PB exposure (P trend < 0.01). For those in the highest exposure group, the risk was one-fifth lower than that seen among patients never given PB. Adjustment for treatment with other anticonvulsive drugs did not appreciably change these results.

Table 2 Anticonvulsant drug exposure among epileptic patients who develop cancers of the lung and bladder and their controls[a]

Type of drug	Lung cancer		Bladder cancer		All controls	
	n	(%)	*n*	(%)	*n*	(%)
Phenobarbital	76	(73)	6	(33)	162	(70)
Phenytoin	60	(58)	7	(39)	126	(54)
Primidone	29	(28)	5	(28)	53	(23)
Ospolate	4	(4)	0	(0)	8	(4)
Carbamazepine	6	(6)	0	(0)	7	(3)
Oxazolidine	4	(4)	0	(0)	5	(2)
Etosuximide	1	(1)	0	(0)	3	(1)
Other anticonvulsants	6	(6)	0	(0)	21	(9)
None	23	(22)	10	(56)	58	(25)
Total	104		18		233	

[a] Totals to more than 100% because some patients received more than one drug.

Table 3 Odds ratio and 95% confidence intervals for lung and bladder cancer associated with treatment with three major anticonvulsants[a]

Cancer	Anticonvulsant drug		
	Phenobarbital	Phenytoin	Primidone
Lung cancer			
Ever-exposed cases/controls	76/139	60/115	29/47
OR	1.2	1.0	1.3
95% CI	0.7–2.2	0.6–1.7	0.7–2.3
Bladder cancer			
Ever-exposed cases/controls	6/23	7/11	5/6
OR	0.3	1.1	1.6
95% CI	0.1–0.9	0.4–3.5	0.4–6.3

[a] OR, odds ratio; CI, confidence interval.

Table 4 Risk of lung cancer and bladder cancer by cumulative lifetime use of phenobarbital[a]

Cancer	Cumulative dose (g)		
	0	1–749	≥750
Lung cancer			
Cases/controls	28/61	46/68	30/71
OR	1	1.6	1.0
95% CI		0.8–3.0	0.5–1.8
Bladder cancer			
Cases/controls	12/10	4/8	2/15[b]
OR	1	0.6	0.2
95% CI		0.1–2.7	0.0–0.9

[a] OR, odds ratio; CI, confidence interval.
[b] Test for trend, $P < 0.01$.

Animal Experiment. Rats given PB in drinking water became drowsy during the first 3 days but then recovered and behaved normally. At preparation, their livers were larger (16.0 ± 0.3 g) than those of rats given PB-free water (11.1 ± 0.4 g) and were darker red. After injection of ABP, the liver and bladder DNA from PB-treated rats was found to contain significantly fewer ABP-DNA adducts than from control rats (Table 5). The reduction in DNA adducts was approximately 50%. ABP-blood hemoglobin adduct levels were also markedly reduced in the PB group compared to controls.

Table 5 Covalent binding of 4-aminobiphenyl to DNA (DNA-adducts) and hemoglobin in rats given phenobarbital (n = 12) and control rats not given phenobarbital (n = 8) for various tissue sites

Adduct site	4-aminobiphenyl adducts (pmol/mg)		P
	Phenobarbital group	Control group	
Bladder DNA	9.6 ± 2.5[a]	21.4 ± 5.7	0.048
Liver DNA	3.1 ± 0.3	5.6 ± 0.5	< 0.001
Hemoglobin	20.6 ± 6.4	40.7 ± 1.9	0.003

[a] Mean ± SE.

Discussion

Our epidemiological findings suggest that the significantly high risk of lung cancer previously seen among Danish epileptics (1) is due to cigarette smoking and not to long-term exposure to anticonvulsant drugs. On the other hand, the low risk of bladder cancer among epileptics appears related to the protective effect of high-dose PB, mediated perhaps through the induction of liver enzymes that metabolize or otherwise deactivate bladder carcinogens found in cigarette smoke and other exposures. This explanation is supported by our experimental study which demonstrated 50% fewer ABP adducts in hemoglobin and in liver and bladder cells of rats given PB prior to injection with ABP, a bladder carcinogen, than similarly treated rats not given PB.

While our study has several limitations, such as the small number of bladder cancers evaluated, there is little evidence of serious bias. Abstraction of medical records was done blindly. Assumptions regarding cumulative drug exposure seemed reasonable given the extensive records available during hospitalizations, although uncertainty exists on drug use after discharge. The most serious concern is the lack of detailed smoking data on most of the population and inferences had to be made from a small sample of elderly epileptics who survived until 1991. Although smoking cigarettes was discouraged at Filadelfia (9), it was not uncommon for epileptics to be given a daily pack of cigarettes during confinement in other hospitals. Our survey indicated a much higher proportion of regular cigarette smokers than expected based on national statistics. While the response rate was only 63%, the reasons for nonresponse appeared related to age and mental status, not to smoking history. Since smoking is associated with early mortality, a survey of survivors may have, we suspect, underestimated the true smoking prevalence of the entire cohort. Even so, the 10% higher proportion of smokers estimated from the survey would account for the 40–50% increased risk from lung cancer in the cohort compared to the general population.

Two other studies have reported a modest increase of lung cancer among epileptics (16, 17). Barbiturate exposure was an unlikely cause, since no dose response was evident and risk declined with time after initial treatment. A record-linkage study utilizing pharmacy records indicated a 1.7-fold risk for lung cancer associated with PB but, again, risk was not related to duration or intensity of use (2). We were unable to find an overall association with lung cancer risk or dose response for any anticonvulsant drug, including PB, based on estimates of cumulative drug exposure. Laboratory studies in rats and mice have also shown that PB treatment has no effect on the incidence of lung tumors induced by other agents (18, 19).

The association between lung cancer risk and Thorotrast is noteworthy, even though it is based on small numbers. Radon at sufficiently high doses is known to cause lung cancer in humans so that continuous exposure of the bronchial epithelium to exhaled radon, translocated from Thorotrast after radioactive decay, conceivably could increase risk (7). Dosimetry calculations suggest that cumulative radiation dose to the lung from Thorotrast could be as high as 370 mGy (37 rad) (20). However, recent results from the Danish Thorotrast study, while showing a lung cancer excess, indicate no dose response with administered volume of Thorotrast (8).

Accepting that cigarette smoking is related to the elevated risk of lung cancer in Danish epileptics, it was surprising to find a lowered bladder cancer risk associated with prolonged exposure to PB. Although previous studies of epileptics have not reported risks separately for bladder cancer (16, 17), a record-linkage survey of prescription drugs revealed an inverse relation between PB use and bladder cancer (3). Furthermore, our small experimental study showed that continuous exposure to PB significantly reduced the level of ABP adducts in hemoglobin, liver, and bladder cells. Since ABP is a potent bladder carcinogen found in cigarette smoke as well as some occupational settings, the reduced number of reactive metabolites binding to DNA suggests a possible mechanism for the protective effect of PB.

PB is known to interact with other chemical compounds by changing their efficiency, duration, and clearance. These effects are due primarily to altered levels of drug-metabolizing enzymes, particularly in liver cells. The induction by PB of certain cytochrome P450 enzymes has been well studied.

The human bladder carcinogen ABP may be the major bladder DNA damaging agent in tobacco smoke (21). It is thought to be activated in the liver by metabolism to an *N*-hydroxamino compound, which then circulates and eventually reaches the lumen of the bladder where it reacts with urothelial DNA. The metabolism in the liver of ABP to the hydroxyamino metabolite is catalyzed efficiently by the P450 1A2 enzyme. However, other P450 enzymes can oxidize aromatic amines to detoxified metabolites, thus reducing the level of reactive metabolites (22). Thus, it is possible that PB may contribute to a lower bladder cancer risk by inducing higher levels of P450 enzymes in liver cells which are capable of deactivating carcinogenic metabolites that are released in the blood and transported to the bladder mucosa.

Studies in laboratory animals indicate that the relation between PB and carcinogenesis is complex. Depending on the age, sex, and strain of the animal, PB may either inhibit or enhance the development of liver tumors. Furthermore, the sequence of exposure is critical (23). PB appears protective if given before exposure to a liver carcinogen, perhaps by increasing detoxifying enzymes and thus reducing DNA-binding metabolites. However, it may act as a promoter if administered subsequently to the carcinogen, perhaps by stimulating the division of cells already transformed. Although PB has been reported to promote the development of liver and thyroid tumors in laboratory animals under certain conditions, most published reports indicate no effect on the occurrence of bladder tumors. However, PB was reported to promote bladder carcinogenesis in one study (24) and to inhibit this process in another (18).

In conclusion, our findings suggest that epileptics smoke more than the general population, thus accounting for their elevated risk of lung cancer. However, it appears

that PB treatment of epilepsy lowers the risk of bladder cancer, perhaps by increasing the levels of detoxifying enzymes in the liver. This hypothesis could be evaluated by examining the metabolic fate of tobacco carcinogens among PB-exposed groups of smokers.

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