Altered Aromatic Amine Metabolism in Epileptic Patients Treated with Phenobarbital

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Abstract
The fate of carcinogens differs among individuals who have different activities of drug-metabolizing enzymes that are important in activating and detoxifying carcinogens. A drug that profoundly alters the metabolism of drugs and carcinogens is the anticonvulsive agent phenobarbital. To investigate why epileptic patients appear to have a low risk of cancer of the urinary bladder, and on the basis of the observation that levels of aromatic amine-hemoglobin adducts are strongly associated with various risk factors for cancer at that site, we determined aromatic amine-hemoglobin adducts in 62 epileptic patients as a surrogate measure of the reaction of carcinogenic metabolites with DNA in target tissue. Although adducts were detected in all subjects, the levels were proportional to daily tobacco consumption. When the subjects were stratified into groups smoking 20 g tobacco/day or more, smoking <20 g/day, and not smoking, an effect of medication was detected. Epileptic patients treated chronically with phenobarbital or primidone, which is effectively metabolized to phenobarbital, were found to have lower levels of 4-aminobiphenyl adducts than patients on the other treatment (P = 0.02; ANOVA). In nonsmokers, no effect of medication could be demonstrated above background variation; however, an increasing effect was seen with tobacco consumption with only one-half the increase in adducts per g tobacco smoked as epileptic patients on other treatment. The difference in the increases (slopes of regression lines) was highly significant statistically. This reduction in the level of hemoglobin-aromatic amine adducts is probably due to induction of detoxification enzymes in the patients treated with phenobarbital.

Introduction
A recent survey of cancer incidence among 8004 epileptic patients in Denmark revealed a 1.4-fold excess of lung cancer but a statistically significant deficit (relative risk, 0.6) of urinary bladder cancer (1). A case-control study carried out within the cohort showed that the excess risk for lung cancer could be attributed to excessive smoking by the patients, and the risk for bladder cancer was inversely related to the cumulative dose of phenobarbital (2). It was suggested that the apparent protective effect of phenobarbital on the development of bladder cancer was a result of the induction by phenobarbital of drug-metabolizing enzymes that deactivate bladder carcinogens found in cigarette smoke (2). This hypothesis was supported by the results of a study of rats exposed to the bladder carcinogen 4-aminobiphenyl after treatment with phenobarbital; rats given phenobarbital for 1 week before exposure to the carcinogen had lower levels of 4-aminobiphenyl adducts in liver and bladder DNA than did rats not given phenobarbital (2). It is possible to detect aromatic amine-hemoglobin adducts in most human individuals, and there is a strong association between the levels of adducts and tobacco smoking and various other risk factors for bladder cancer (3). The present study was undertaken to determine if reductions of carcinogen adduct levels occur in epileptics treated with phenobarbital.

Materials and Methods
Phenobarbital is usually used only when other anticonvulsants fail to prevent seizure effectively because it may cause severe drowsiness. In a survey of 620 patients admitted to the Filadelfia Treatment Centre and associated centers in Denmark, we identified 39 patients who were being treated chronically with phenobarbital or primidone, which is metabolized mainly to phenobarbital. Of those patients, 32 (82%) agreed to participate in the study; 28 were treated with phenobarbital at 50-200 mg daily, and four were treated with primidone at 375-1000 mg daily. Thirty-nine control patients who were being treated permanently with anticonvulsants other than phenobarbital or primidone were selected from the same institutions matching them as closely as possible by smoking habits, sex, and age with the treated group; 30 control patients (77%) accepted the invitation (Table 1).

The patients were interviewed about their smoking habits during the previous 3 months by use of a structured questionnaire, which included questions on tobacco products used, daily consumption, and whether the smoke was inhaled. The daily tobacco consumption was estimated by assuming that a cigarette contains 1 g tobacco, a small cigar, 3 g, and a regular cigar, 5 g. Medication taken by the patients during the 3 months preceding the interview was registered by the nurse in charge of the department. At the time of the interview, 10 ml of blood was collected. Hemoglobin was isolated and analyzed for adducts with 3-aminobiphenyl, 4-aminobiphenyl, and 2-naphthylamine (4).

ANOVA (5) was used to test whether patients treated with...
phenobarbital or primidone (+Ph) had different levels of hemoglobin adducts with 3-aminobiphenyl, 4-aminobiphenyl, and 2-naphthylamine from patients treated with other anticonvulsants (−Ph). The subjects’ smoking habits were stratified as none, <20, or ≥20 g tobacco/day. Multivariate linear regression models (5) were used to evaluate the effects of phenobarbital treatment and the amount of tobacco smoked on adduct levels. First, independent variables were tested individually for a relationship with the three-outcome measures. Independent variables not showing association with outcome were excluded from additional analysis. Finally, all two-way interactions were tested in the final model.

**Results**

The levels of 4-aminobiphenyl adducts in hemoglobin from the 15 nonsmoking +Ph patients (mean = 25.5 pg/g) and 10 −Ph patients (mean = 22.9 pg/g) were almost identical (Fig. 1). The level of 4-aminobiphenyl adducts was lower in +Ph patients than in −Ph patients when the amount of tobacco smoked did not influence the results in the presence of +Ph treatment (P < 0.001). Even when smokers were distributed into groups of nonsmokers, those smoking <20 g tobacco/day, and those smoking ≥20 g tobacco/day (P = 0.02; ANOVA; Fig. 1). This effect of medication increased with the amount of tobacco smoked. We, therefore, analyzed the effect of medication and smoking on adduct levels in +Ph and −Ph patients in linear regression models. The slope of the regression line for the level of 4-aminobiphenyl hemoglobin adducts (pg/g) divided by the amount of tobacco smoked (g/day; Fig. 2) was significantly smaller for the patients being treated chronically with phenobarbital (β = 2.53) than it was for patients on other treatment (β = 6.96; P < 0.001 in test for interaction, noninhaling excluded).

The variation in the levels of protein adducts with 3-aminobiphenyl and 2-naphthylamine by tobacco consumption and the types of anticonvulsative treatment paralleled closely with that of 4-aminobiphenyl. For all three aromatic amines, the amount of protein adducts with 3-aminobiphenyl, 4-aminobiphenyl, and 2-naphthylamine increased with the amount of tobacco smoked. We, therefore, analyzed the effect of medication and smoking on adduct levels in +Ph and −Ph patients in linear regression models. The slope of the regression line for the level of 4-aminobiphenyl hemoglobin adducts (pg/g) divided by the amount of tobacco smoked (g/day; Fig. 2) was significantly smaller for the patients being treated chronically with phenobarbital (β = 2.53) than it was for patients on other treatment (β = 6.96; P < 0.001 in test for interaction, noninhaling excluded).

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**Table 1** Distribution of study subjects by smoking habits and type of anticonvulsant treatment

<table>
<thead>
<tr>
<th>Smoking habits</th>
<th>Anticonvulsant</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-smokers</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>Smokers</td>
<td>17</td>
<td>20</td>
</tr>
<tr>
<td>Non-inhalers</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>Inhalers</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td>All patients</td>
<td>32</td>
<td>30</td>
</tr>
<tr>
<td>Female</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Age (mean ± SD)</td>
<td>56 ± 13</td>
<td>55 ± 11</td>
</tr>
</tbody>
</table>

*Includes four patients treated with primidone, a precursor to phenobarbital.

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**Table 2** Increase of hemoglobin-aromatic amine adducts/g tobacco smoked (β) in epileptic patients treated with phenobarbital or primidone (+Ph) or other drugs (−Ph), (β ± SE)

<table>
<thead>
<tr>
<th>Aromatic amine</th>
<th>Patients</th>
<th>−Ph</th>
<th>+Ph</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-Aminobiphenyl</td>
<td>0.81 ± 0.10</td>
<td>0.42 ± 0.03</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>4-Aminobiphenyl</td>
<td>6.96 ± 0.84</td>
<td>2.53 ± 0.22</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>2-Naphthylamine</td>
<td>0.75 ± 0.11</td>
<td>0.23 ± 0.05</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

*Only nonsmokers and smokers who inhaled the tobacco smoke were included. Values given as pg adduct/g hemoglobin/g tobacco/day.

Statistical significance of difference of β (slopes of regression lines) for +Ph and −Ph epileptic patients (test for interaction).
Discussion

This study was undertaken to test the hypothesis that aromatic amine-hemoglobin adducts would be lower in patients undergoing treatment with phenobarbital than in individuals treated with other anticonvulsant drugs. This hypothesis was based on the observations that phenobarbital treatment is associated with a lower risk of bladder cancer and that there is a high degree of association between aromatic amine hemoglobin adducts and various bladder cancer risk factors (3, 6). It was also hypothesized that the mechanism by which phenobarbital would modulate hemoglobin adducts was through the induction of enzymes that detoxify aromatic amines because previous studies with experimental animals had shown that phenobarbital treatment before administration of a specific aromatic amine, 4-aminobiphenyl, reduced the levels of adducts formed not only with hemoglobin, but also with DNA in bladder and liver (2).

Treatment with phenobarbital or primidone resulted in lower levels of hemoglobin adducts with 4-aminobiphenyl in smokers who inhaled, after adjustment for amount of tobacco consumed; no effect of treatment was observed in nonsmokers. The absence of an effect in nonsmokers may simply indicate that the effect was small compared to the variance of the measurements; the SD of the measurements (22 ± 13 and 26 ± 11 pg/g) and the sample sizes (n = 10 and 15, respectively) required that the difference between the two means be at least 8.3 pg/g to reach statistically significance in a one-tailed test and 10.0 pg/g to be statistically significant in a two-tailed test. These values represent a 30–45% difference in the means, which is larger than the difference observed among smokers without adjustment for tobacco consumption. Some of the adducts observed in nonsmokers undoubtedly arose from exposure to environmental tobacco smoke, whereas the remainder may have other sources. Without being able to adjust for these exposures, it is not surprising that we did not observe an effect of phenobarbital treatment in nonsmokers.

Our results support the hypothesis that phenobarbital induces drug-metabolizing enzymes that detoxify aromatic amines. The precise nature of the enzyme induction remains open to conjecture. Although the absence of an observable effect of phenobarbital in nonsmokers may reflect nothing more than the low statistical power of this study, it might also reflect a different role of aromatic amines in bladder cancer risk in smokers and nonsmokers. Epidemiological studies of bladder cancer and phenobarbital have not examined smoking as an independent factor so this possibility remains speculative. If there is a differential effect, it may arise from interactions between cigarette smoke and phenobarbital. Additional studies will be required to confirm or reject these possible explanations.

References

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