Lack of Effect of Smoking on the Excretion of Tryptophan Metabolites by Man

R. R. Brown, J. M. Price, S. W. Burney, and G. H. Friedell

Division of Clinical Oncology, University of Wisconsin Medical School, Madison, Wisconsin 53706 [R. R. B., J. M. P.], and the Cancer Research Institute, New England Deaconess Hospital, Boston, Massachusetts 02118 [S. W. B., G. H. F.]

SUMMARY

Urinary excretion of tryptophan and niacin metabolites was measured before and after oral loads of 2.0 g L-tryptophan in 12 normal smokers, in the same subjects after having stopped smoking for 3 weeks, and again after resumption of smoking. The metabolites measured were kynurenine, hydroxykynurenine, kynurenic acid, xanthurenic acid, acetylkynurenine, \( \alpha \)-aminohippuric acid, anthranilic acid glucuronide, \( \alpha \)-methylnicotinamide, and \( N \)'-methyl-2-pyridone-5-carboxamide. Also measured were 4-pyridoxic acid and creatinine as respective indices of nutrition and reliability of collections and nicotine as an index of smoking. Nicotine values indicated that the subjects had faithfully stopped smoking during the nonsmoking period. With the exception of an inconsistent change in acetylkynurenine excretion, no significant changes were observed in the excretion of any of the metabolites measured. Additional studies comparing 17 regular male smokers (average smoking history of 15 cigarettes/day for 9.5 years) with 13 male nonsmokers failed to show any differences in urinary excretion of these metabolites. The present data do not refute the statistical association between smoking and bladder cancer, but they do suggest that this association is not mediated by altered urinary excretion levels of tryptophan or niacin metabolites in smokers.

INTRODUCTION

Numerous studies, both retrospective and prospective (10, 16, 19), have indicated an association between cigarette smoking and an elevated incidence of bladder cancer. The association was stronger with cigarette smoking than with cigar or pipe smoking and was found in both sexes. Similarly, associations were found between cigarette sales by state and the incidence of bladder cancer observed (8).

The role of tryptophan metabolites in the urine as possible bladder carcinogens has been demonstrated by Allen et al. (1) and by Bryan et al. (4), who found that several normally occurring urinary metabolites of tryptophan produced bladder cancer in mice when implanted as a pellet into the lumen of the mouse bladder. The findings by Kerr et al. (9) of elevated levels of hydroxykynurenine and hydroxanthranilic acid and depressed \( N \)'-methylnicotinamide in the urine of smokers compared to nonsmokers suggested a possible relationship between smoking and bladder cancer through its effect on urinary excretion of tryptophan metabolites. Previous studies from this laboratory had shown that about 50% of patients with bladder cancer excreted elevated amounts of several tryptophan metabolites including some of those which are active in the mouse test system, but no information was available concerning smoking habits and the excretion of these metabolites (12).

This report presents our attempts to confirm and extend the observations of Kerr et al. (9) of the effects of smoking on the excretion of tryptophan metabolites.

MATERIALS AND METHODS

Fifteen healthy adults (ages 24 to 57, 4 women and 11 men) who smoked regularly were selected for tryptophan studies to be done while smoking, after having stopped for 1 and 3 weeks, and finally after resumption of smoking for 1 week. As an index of completeness of urine collections, creatinine was measured in each specimen (11) and as an index of abstention from smoking, urinary nicotine was assayed by slight modifications of the gas chromatographic method of Beckett and Triggs (2).

In each of the 4 investigational periods, tryptophan metabolites were assayed in basal urines and also after an oral load of 2.0 g L-tryptophan. The analytical methods used have been described in detail (13) and have been in use in these laboratories for a number of years. In view of the important role of vitamin \( B_6 \) in the metabolism of tryptophan, 4-pyridoxic acid, the chief urinary metabolite of this vitamin, was also measured as an index of any change in the intake of this vitamin (14). The tryptophan metabolites measured were kynurenine, hydroxykynurenine, acetylkynurenine, kynurenic acid, xanthurenic acid, \( \alpha \)-aminohippuric acid, anthranilic acid glucuronide, \( N \)'-methylnicotinamide, and \( N \)'-methyl-2-pyridone-5-carboxamide. Appropriate smoking and nonsmoking days were compared by means of a \( t \)-test.
for paired observations (18). This was felt to be the most sensitive means of detecting differences because each subject served as his own control, thus minimizing variations between subjects.

In addition to the above subjects, studies were done with 13 healthy control men who were known never to have smoked or who had stopped smoking completely for at least 1 year prior to the study of tryptophan metabolism. Only 2 of these subjects had ever smoked regularly and they had stopped approximately 1 and 2 years prior to their tryptophan studies. This group, having an average age of 27.4 years (range 19 to 38), was compared with another group of healthy control men with an average age of 29.7 (range 19 to 39) years who were regular smokers, averaging 15 cigarettes/day and with an average smoking history of 9.5 years (range 2 to 17). The urinary tryptophan metabolites listed above were measured in 24-hr urines from these subjects before and after a 2-g load of L-tryptophan. Differences between smokers and nonsmokers were compared in both basal and post-tryptophan days with Student's t-test (18).

RESULTS

Of the 15 smokers who agreed to stop smoking, 3 were dropped from the study, 2 because urinary nicotine values indicated that they had not stopped smoking completely and 1 because inconsistent urinary creatinine values indicated incomplete urine collections. The data from the remaining 12 subjects (3 women and 9 men, ages 24 to 57) were considered to be reliable.

Table 1 presents urinary nicotine, creatinine, and 4-pyridoxic acid levels in the 4 periods studied. Nicotine values were markedly decreased at both 1 and 3 weeks in the nonsmoking period and returned to their initial levels after resumption of smoking. Several assays on known nonsmokers demonstrated nicotine values of less than 0.05 mg/liter urine (less than 0.083 mg/day), whereas assays on the urine of 1 subject who never stopped smoking more than 2 to 4 cigarettes/day gave values of 0.363 mg/day. Hence it seemed possible to detect the use of as few as 2 or 3 cigarettes/day. The values found in the 12 subjects during the nonsmoking period indicate that they could not have been smoking more than 1 or 2 cigarettes/day, in agreement with their statements that they faithfully abstained during this period.

Creatinine values in Table 1 show that urine collections were comparable and complete throughout the study and also indicate that smoking had no significant effect on the excretion of creatinine in these subjects. Similarly, excretion of 4-pyridoxic acid was unchanged throughout the study, suggesting that nutritional intake of vitamin B6 was consistent and that dietary patterns

<table>
<thead>
<tr>
<th>Period</th>
<th>Nicotine (mg/day)</th>
<th>Creatinine (g/day)</th>
<th>4-Pyridoxic acid (μmoles/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Posttryptophan</td>
<td>Basal</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 wk</td>
<td>0.16 ± 0.027</td>
<td>0.098 ± 0.011</td>
<td>1.84 ± 0.18</td>
</tr>
<tr>
<td>3 wk</td>
<td>0.090 ± 0.019</td>
<td>0.119 ± 0.020</td>
<td>1.61 ± 0.15</td>
</tr>
<tr>
<td>Resumed smoking</td>
<td>1.02 ± 0.23</td>
<td>0.94 ± 0.18</td>
<td>1.95 ± 0.19</td>
</tr>
</tbody>
</table>

Table 2 presents urinary excretion of nicotine, creatinine, and 4-pyridoxic acid as affected by smoking.

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<table>
<thead>
<tr>
<th>Period</th>
<th>Kynurenine</th>
<th>3-Hydroxy-kynurenine</th>
<th>N'-Acetyl-kynurenine</th>
<th>Kynurenic acid</th>
<th>Xanthurenic acid</th>
<th>Anthranilic acid</th>
<th>o-Aminohippuric acid</th>
<th>o-Pyridoxic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Posttryptophan</td>
<td>Basal</td>
<td>Posttryptophan</td>
<td>Basal</td>
<td>Posttryptophan</td>
<td>Basal</td>
<td>Posttryptophan</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 wk</td>
<td>12.7 ± 2.1</td>
<td>40.1 ± 5.6</td>
<td>16.8 ± 3.5</td>
<td>37.7 ± 6.0</td>
<td>12.4 ± 1.5</td>
<td>18.8 ± 1.5</td>
<td>17.4 ± 7.1</td>
<td>70.4 ± 7.1</td>
</tr>
<tr>
<td>3 weeks</td>
<td>15.7 ± 1.5</td>
<td>40.4 ± 6.6</td>
<td>21.8 ± 2.9</td>
<td>41.7 ± 6.9</td>
<td>14.8 ± 1.5</td>
<td>20.7 ± 2.1</td>
<td>21.1 ± 4.6</td>
<td>65.1 ± 8.6</td>
</tr>
<tr>
<td>Resumed</td>
<td>11.8 ± 1.3</td>
<td>37.8 ± 5.0</td>
<td>14.4 ± 2.5</td>
<td>39.8 ± 7.5</td>
<td>10.2 ± 1.8</td>
<td>18.8 ± 1.9</td>
<td>25.3 ± 5.4</td>
<td>64.7 ± 7.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Period</th>
<th>β-Aminoisobutyric acid</th>
<th>N'-Methyl-2-pyridone-5-carboxamide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Posttryptophan</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 wk</td>
<td>12.1 ± 1.9</td>
<td>40.6 ± 6.9</td>
</tr>
<tr>
<td>3 weeks</td>
<td>8.9 ± 3.5</td>
<td>36.5 ± 7.4</td>
</tr>
<tr>
<td>Resumed</td>
<td>11.2 ± 1.1</td>
<td>43.8 ± 7.2</td>
</tr>
</tbody>
</table>

These subjects differ from each other significantly (p < 0.05).
were not markedly different in any of the experimental periods.

Excretion of tryptophan metabolites of the kynurenine pathway is shown in Table 2. With the single exception of basal acetyllykynurenine, none of the metabolites measured differed between the smoking and nonsmoking periods. In several instances, there was an indication that values may be higher during the nonsmoking periods but these differences were not statistically significant. Excretion of \( N^1 \)-methylnicotinamide and \( N \)-methyl-2-pyridone-5-carboxamide similarly were not apparently different in any of the study periods. Since no change was observed in the excretion of metabolites of either tryptophan or niacin, it appeared that smoking did not alter the efficiency of conversion of tryptophan to niacin and also that the dietary intake of niacin was relatively constant throughout the study.

Results of the comparison between male smokers and nonsmokers are given in Table 3. In none of the metabolites measured was there a significant difference between the smokers and nonsmokers, either in basal levels or in posttryptophan levels. Similarly, there was no appreciable difference in the excretion levels of these subjects and those reported in Tables 1 and 2. Thus, in both studies there is no evidence that smoking altered the urinary excretion of tryptophan metabolites in either basal urines or after a loading dose of \( L \)-tryptophan.

DISCUSSION

The studies reported here indicate no significant changes in urinary excretion of tryptophan or niacin metabolites in the same subjects while smoking or after having stopped smoking for 3 weeks. Further, no differences were found between nonsmoking subjects and those having smoked regularly for 9.5 years. Both basal and posttryptophan levels of metabolites were measured. These data are at variance with those of Kerr et al. (9), who found elevated levels of hydroxykynurenine and hydroxyanthranilic acid after tryptophan loading in 6 subjects after smoking for 5 weeks. They reported corresponding decreases in urinary \( N^1 \)-methylnicotinamide, suggesting that a block in the conversion of tryptophan to niacin occurred in smoking subjects. However, no statistical evaluation of their results was presented although they have been readily accepted and widely quoted. In the present study, no increases in urinary tryptophan metabolites were observed in smoking subjects, nor were there decreases in the niacin metabolites, \( N^1 \)-methylnicotinamide and \( N \)-methyl-2-pyridone-5-carboxamide.

No immediate explanation for these differences in results is available although somewhat different experimental conditions were used. The analytical methods used for some of the metabolites were presumably comparable since Kerr et al. used methods devised in our laboratories for several of the metabolites measured. We did not measure 3-hydroxyanthranilic acid in urine from our patients since an adequate method for this unstable metabolite is not available in our laboratory. One major difference between the 2 studies is that the loading dose of tryptophan used by Kerr et al. (50 mg/kg body weight) was larger than the 2.0-g load used in our study. There is some evidence that larger loads will produce somewhat different results than the smaller loads (3, 6, 7). The 2.0-g load used in our study was chosen because it is large enough to cause a consistent increase in urinary levels of metabolites in normal subjects. Since it is very unlikely that the usual dietary intake of tryptophan would ever exceed this loading dose, the smokers in our study would probably not excrete elevated levels of the measured tryptophan metabolites under any ordinary circumstances. Basal excretion levels of metabolites (without
tryptophan load) which were normal in the present studies were not reported by Kerr et al.

Although the data presented here in no way refutes the statistical data showing an association between smoking and bladder cancer, it does suggest that this association is not the result of the effect of smoking on excretion of tryptophan metabolites of the kynurenine pathway. It is entirely possible that systemic effects of smoking or urinary metabolites of absorbed smoke may act synergistically with substances in human urine (perhaps including normal levels of tryptophan metabolites) to produce the final bladder tumors. It has been shown by Bryan et al. (5) that at least one of the most carcinogenic of the tryptophan metabolites, the 8-methyl ether of xanthurenic acid, is an incomplete carcinogen for the mouse bladder and requires an additional factor or factors for activity.

Not studied in the present work or in that of Kerr et al. were the 5-hydroxyindole metabolites of tryptophan. It was reported by Sorrentino (17) that feeding bananas (which are high in 5-hydroxyindolic compounds) to guinea pigs resulted in a high incidence of bladder lesions. In this regard, Schievelbein et al. (15) reported that smoking causes an increased excretion of 5-hydroxyindole acetic acid by man. These data suggest the possible involvement of 5-hydroxyindoles in the bladder cancer process.

Previous studies failed to show altered tryptophan metabolism in workmen with industrial bladder cancer due to known exposure to 2-naphthylamine, benzidine, or 4-aminobiphenyl (12). Hence it was felt that elevated tryptophan metabolites were not directly involved in the carcinogenic process in the industrial cases. In view of the recognized activity of 2-naphthylamine as a human bladder carcinogen, it is of interest that cigarette smoke is reported to contain minute levels of this compound (20). These data suggest alternative explanations for the association of bladder cancer with smoking without involving altered tryptophan metabolism.

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REFERENCES

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