Induction of Hepatic Aryl Hydrocarbon Hydroxylase in C57BL Mice by Ionizing Radiation

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SUMMARY

Inbred strains of C57BL mice were exposed to either 100, 500, or 1000 rads of whole-body irradiation. Another group of mice were given injections of 3-methylcholanthrene only, and a control group had no treatment. Forty-eight hr after treatment, the animals were killed and hepatic aryl hydrocarbon hydroxylase (AHH) activity was measured by spectrophotofluorometer. A comparison of hepatic AHH activity in treated and nontreated groups of mice showed that each treated group had a significantly increased AHH induction compared with the control group. Although radiation appeared to have a dose-related effect on AHH induction, the increase with dose level was not statistically significant.

INTRODUCTION

AHH has been extensively studied in various strains of mice (4, 17, 18, 23, 24). The activity of this enzyme is induced by some chemicals, for example, MC. Depending upon whether the hepatic AHH is inducible by MC, strains of mice are grouped into inducible strains and noninducible strains (3, 9, 16). The C57BL mice are inducible strains while DBA are noninducible strains. The enzyme AHH metabolizes polycyclic hydrocarbon and is cytochrome P-450 mediated (20). There are reports that AHH induction in human cultured lymphocytes and pulmonary alveolar macrophages is associated with cigarette smoking, and that individuals with higher AHH inducibility are more susceptible to lung cancer (1, 8, 14). On the other hand, some reports suggest that the relationship between smoking and lung cancer is related to α-radiation emitted from 32P deposited on the tobacco leaves (21). Epidemiological studies have shown that individuals working in uranium mines and exposed to 222Rn run a greater risk of developing lung cancer than the normal population (13). In fact, lung cancer has been induced in hamsters by exposing them to α-irradiation (10, 19). Because these reports indicate an association of radiation with lung cancer, we became interested in examining the relationship between radiation and AHH activity. Our purpose, therefore, was to observe the effect of various doses of radiation on the AHH activity of liver and lung tissues in inbred strains of C57BL mice. Here we present data that indicate that radiation does induce the hepatic AHH activity in C57BL mice. The data on the effect of radiation on lung tissue, which also show an increase in AHH activity, will be published elsewhere.

MATERIALS AND METHODS

C57BL mice were purchased from the L. C. Strong Research Foundation Inc., San Diego, Calif. At the time of experiments, animals were approximately 4 months old. Although for many of the experiments only male mice were chosen, female animals were also used in the study. The experiment was repeated 6 to 7 times at each dosage. The data represent the combined results on AHH activity in control animals (no treatment), 20 to 30 animals for each radiation dose, and 28 animals treated with MC, approximating about 350 AHH assays in total.

In a typical experiment involving treatment with 1 dose of radiation, 9 animals were used, 3 serving as controls, 3 receiving injections of MC in corn oil (100 mg/kg), and 3 animals receiving either 1000, 500, or 100 rads of X-ray whole-body irradiation. Whole-body X-ray exposures were achieved with an orthovoltage unit (200 kVp, 10 ma, and 0.25 mm of copper plus 1 mm of aluminum added filtration; mice target distance 75 cm). The dose rate was determined prior to each series of exposures and was 14.6 rads/min. The irradiations were carried out by placing the animals on a rotating platform.

Forty-eight hr after MC or radiation treatment, the animals were killed by cervical dislocation, and liver tissues were dissected. The tissue from each animal was then homogenized separately in cold homogenizing buffer (0.15 M KCl and 0.25 M K2HPO4, pH 8.5) prepared in the proportion of 6 ml of buffer per g tissue weight. The homogenate was then centrifuged at 10,000 × g for 30 min at 4°. The crude tissue extract was then obtained by collecting the supernatant. Aliquots of tissue extract from each sample were taken for protein analysis that was done according to the method of Lowry et al. (11).

The AHH assay was performed in triplicate according to methods described previously (7). The incubation buffer solution (0.9 ml of 0.05 M Tris and 3 mM MgCl2, pH 8.5) contained NADPH and NADH at a concentration of 7.5 mg/ml each and 0.1 ml of tissue extract (containing 3 to 5 mg of cellular protein per ml). The reaction was started by the addition of 0.1 μM benzo(a)pyrene in 50 μl of acetone at...
AHH inducibility in mice after whole-body irradiation

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>No. of animals</th>
<th>Median fluorescence</th>
<th>Median estimate</th>
<th>Significance (p)</th>
<th>95% confidence limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>38</td>
<td>13.7</td>
<td></td>
<td></td>
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<tr>
<td>MC</td>
<td>28</td>
<td>41.0</td>
<td>2.72</td>
<td>&lt;0.001</td>
<td>2.05 - 3.71</td>
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<tr>
<td>1000 rads</td>
<td>25</td>
<td>23.0</td>
<td>1.90</td>
<td>&lt;0.001</td>
<td>1.35 - 2.56</td>
</tr>
<tr>
<td>500 rads</td>
<td>23</td>
<td>21.0</td>
<td>1.40</td>
<td>&lt;0.05</td>
<td>1.01 - 1.86</td>
</tr>
<tr>
<td>100 rads</td>
<td>21</td>
<td>19.0</td>
<td>1.39</td>
<td>&lt;0.02</td>
<td>1.06 - 1.79</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

Chart 1 shows the variation in liver AHH activities in individual inbred mice. As shown in Chart 1, there were a few extreme values that would have distorted the mean; therefore, we have used median estimates throughout to compare the values obtained from different treatments. Table 1 illustrates the fluorescent readings and inducibility comparisons among our treatments. Significance of comparisons with controls was obtained with the Wilcoxon rank sum test. The tests, median estimates, and 95% confidence limits for AHH inducibility were obtained with non-parametric procedures (6). The data were transformed to logs, inferences and estimates were obtained based on differences, and these estimates were retransformed to represent the appropriate ratios. Statistically, each of the treated groups had significant AHH induction in comparison to the control group. It appears that the group receiving 1000 rads of radiation has greater AHH induction than the groups receiving either 500 or 100 rads. The possibility of a dose-response relationship was exhibited, but was not statistically significant.

There are reports that a sublethal dose of radiation inhibits the activity of other hepatic microsomal enzymes (2, 5). It is suggested that the radiation decreases the content of cytochrome P-450, which in turn results in the inhibition of enzyme activity (26). Nair et al. (15) postulate that the inhibitory effect of X-irradiation on the hepatic microsomal enzyme system is mediated through an action on the hormonal regulation of enzyme activity. Our investigation shows that X-irradiation induces AHH activity. Why the other hepatic microsomal enzymes are inhibited by radiation while AHH activity is enhanced cannot be explained at this time. It is possible that the enhancement of AHH by radiation might not be a universal effect on all species. We have used an inducible mouse strain (C57BL) for our study whereas the other studies of radiation effects utilized rats or rabbits. It is also possible that some enzyme systems are inhibited by radiation whereas others are activated. The more likely explanation of increased AHH activity by radiation may be the presence of multiple forms of cytochrome P-450 (25). The treatment of animals with MC has been reported to result in the appearance of cytochrome P-448 (12). Cytochrome P-450 and cytochrome P-448 differ in their substrate specificity (12, 22) and CO-binding capacity. It is probable that radiation may have a mode of action similar to that of MC, and the enhanced

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AHH activity by radiation may be due to the appearance of cytochrome P-448 or other forms of cytochrome P-450. Published reports show that radiation is associated with bronchogenic carcinoma, which in turn is associated with increased levels of AHH inducibility. Our results show that radiation is associated with an induced level of AHH activity. However, it is not clear from the data whether this level of AHH induction by radiation would be associated with lung cancer. The study of the AHH level in animals with lung cancer induced by radiation will necessarily be the next step in evaluating the level of AHH induction by radiation, and whether the inducibility actually is related either to lung cancer or susceptibility to lung cancer.

Statistically insignificant differences in AHH inducibility by treatment with various doses of radiation indicate that AHH induction may not be dose dependent. However, another study (N. Prasad and R. Prasad, unpublished observation) suggests that the radiation effect may be time dependent.

In conclusion, radiation is associated with AHH induction. Although production of some other microsomal enzymes are inhibited by radiation, the activity of hepatic AHH in C57BL mice is enhanced by radiation.

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REFERENCES

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