Increased Production of Mutagenic Metabolites of Carcinogens by Tissues from Senescent Rodents

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ABSTRACT

Activation of the procarcinogens benzo(a)pyrene and 2-fluorenamine by liver homogenates (S9) prepared from senescent male CFN rats and C57BL/6J mice resulted in an enhanced production of mutagenic metabolites when compared to young rodents, as indicated by an enhancement of the induced reversal frequency in a Salmonella typhimurium bioassay. Similar results were observed when carcinogen activation was mediated by purified hepatic microsomes, indicating that the age-related differences in carcinogen activation did not result from aging changes in carcinogen metabolism involving non-microsomal mechanisms.

The metabolites of many procarcinogens are thought to be the ultimate carcinogens in mammals. Therefore, the present findings are consistent with the hypothesis that some fraction of the markedly increased incidence of neoplasia observed in senescent mammals is a result of age-related alterations in the metabolism of chemical carcinogens.

INTRODUCTION

The incidence of cancer markedly increases with advancing age. The question then arises whether this relationship is a result of as yet unidentified factors intrinsic to the process of senescence or more simply a result of an increased length of exposure to ubiquitous carcinogenic factors in aged populations. Another possibility is that the development of adult forms of the markedly increased incidence of neoplasia observed in senescent mammals is a result of age-related alterations in the metabolism of chemical carcinogens.

EXPERIMENTS

Animals. Male Wistar-derived rats (CFN) and male C57BL/6J mice were maintained in our aging colony prior to use. Rats were housed in wire-bottomed cages, 2 animals per cage, and fed rodent chow (Purina laboratory chow) once daily. Mice were maintained in plastic boxes, 5 or 6 animals/box, and fed rodent chow ad libitum. All animals were presented with water ad libitum and were maintained at 23 ± 0.5°, 50% relative humidity, under a 12-hr-12-hr light-dark regimen. Under these environmental conditions, rats and mice have a mean life span of 26 to 29 months. Age of the rodents at time of sacrifice is given in the appropriate table heading or chart legends.

Chemicals. 2-FA (98% pure) and TCPO were obtained from Aldrich Chemical Co., Milwaukee, Wis. BP (98% purity) and DMBA (98% purity) were purchased from Eastman Kodak, Rochester, N. Y. Arochol 1254 was generously provided by Monsanto Chemical Co., St. Louis, Mo. Chemicals were not further purified prior to use.

The results of the present study support this hypothesis by demonstrating an increased production of mutagenic, therefore possibly carcinogenic, metabolites of the polynuclear aromatic hydrocarbon BP and the potent carcinogen 2-FA in a Salmonella bioassay system when those metabolites are generated by homogenates or microsomes from the livers of old rodents.
poured onto supporting agar in sterile Petri dishes. The molten top agar was rapidly spread over the surface of the supporting agar and allowed to harden. Plates were incubated in the dark, at 37°C for 48 hr. Revertants (his\textsuperscript{*}) were scored in strains TA98, which carries a frame-shift mutation, and TA100, which carries a base substitution mutation. Data are the average of triplicate plate counts and are expressed as normalized reversion frequency, which is defined as follows: number of revertants per number of cells per nmol of cytochrome P-450 per mg of protein. All experiments were performed at least 3 times using different tissue preparations. Care was taken to ensure that experiments were run in a range such that the reversion frequency was linear with increasing concentration of enzyme (fixed mutagen) or mutagen (fixed enzyme).

Protein content of all S9 or microsomal samples was measured according to the method of Lowry et al. (24). Determination of cytochrome P-450 content of S9 and microsomes was by the procedure of Omura and Sato (30). Cytochrome P-450 content was also determined according to an alternative method (25), by which we found that the error in cytochrome P-450 measurement in the homogenates due to hemoglobin contamination was less than 5%. All S9 and microsomal preparations were found to be free from microbial contamination by plating suitable aliquots of the tissue preparations onto nutrient agar.

RESULTS

Homogenates (S9) prepared from the livers of senescent male CFN rats were more effective in producing his\textsuperscript{*} revertants than were similar homogenates prepared from their younger counterparts (Table 1). This was observed both for BP and 2-FA and was most evident in the frame shift mutant TA98. There was a slight enhancement of BP-induced mutagenesis in the presence of TCPO, a competitive inhibitor of microsomal epoxide hydrase (28, 29), in agreement with observations made by Oesch (27). Trichloropropene, 2,3-oxide was found to be mutagenic in strain TA100 (Table 1) but not in strain TA98.

Similar results were obtained with homogenates prepared from the livers of male C57BL/6J mice. Activation of both BP (Chart 1) and 2-FA (Chart 2) to metabolites mutagenic in strain TA98 was linearly related to carcinogen concentration for young and old animals over the substrate concentration tested. There was a greater number of revertants when the S9 was derived from senescent mice (Charts 1 and 2).

BP is metabolized in rodent microsomes by aryl hydrocarbon hydroxylase, one of a family of microsomal mixed-function oxidases (12, 18). Since nonmicrosomal mechanisms exist in hepatic tissues which further metabolize the intermediate epoxide (11, 19), we examined the mutagenicity of metabolites generated from BP by liver microsomes prepared from rats of different ages. There is a markedly greater production of mutagenic metabolites of BP by microsomes prepared from old rats when tested either in strain TA98 (Chart 3) or TA100 (Chart 4). In addition, BP mutagenesis was enhanced in both bacterial strains by the addition of TCPO. The metabolites of BP were more effective mutagens in the frame-shift tester strain (Chart 3). In every instance, a greater number of his\textsuperscript{*} revertants was recorded when mutagen and bacteria were incubated in the presence of microsomes prepared from livers of old rats.

We confirmed that the observed age-related differences are enzymatic in nature by demonstrating that they were eliminated by heating the microsomal preparation (60°C for 15 min) or by incubating the plates in the absence of the cofactor NADPH. There were no significant age-related differences in either microsomal NADPH-cytochrome c reductase or microsomal P-450 content following induction with Arochlor 1254 (9). We also found that 2-FA-induced mutagenesis of strain TA98, following activation by hepatic S9 or microsomes prepared from rats or mice which had not been pretreated with Arochlor.

### Table 1

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Bacterial strain</th>
<th>None</th>
<th>TCPO</th>
<th>BP</th>
<th>TCPO + BP</th>
<th>2-FA</th>
</tr>
</thead>
<tbody>
<tr>
<td>300</td>
<td>TA98</td>
<td>19</td>
<td>19</td>
<td>262</td>
<td>302</td>
<td>285</td>
</tr>
<tr>
<td></td>
<td>TA100</td>
<td>45</td>
<td>81</td>
<td>245</td>
<td>325</td>
<td>439</td>
</tr>
<tr>
<td>700+</td>
<td>TA98</td>
<td>20</td>
<td>22</td>
<td>508</td>
<td>502</td>
<td>995</td>
</tr>
<tr>
<td></td>
<td>TA100</td>
<td>50</td>
<td>74</td>
<td>302</td>
<td>425</td>
<td>820</td>
</tr>
</tbody>
</table>

[Chart 1. Normalized reversion frequency in strain TA98 of S. typhimurium incubated with varying concentrations of BP and 100 µl hepatic S9 prepared from male C57BL/6J mice of different ages. •, 100 days of age; ○, 800+ days of age.]

[Chart 2. Normalized reversion frequency in strain TA98 of S. typhimurium incubated with varying concentrations of 2-FA and 5 µl hepatic S9 prepared from male C57BL/6J mice of different ages. •, 100 days of age; ○, 800+ days of age.]
The nature of the relationship between cancer and aging is presently unknown. Peto et al. (31) have suggested that tumor induction on mouse skin by direct application of BP is independent of the chronological age of the rodents but appears to depend upon the duration of exposure to the carcinogen, a conclusion tentatively reached earlier for most human cancers based on epidemiological studies (14). Burton (13) has also recently concluded that the probability of tumor initiation may be independent of age.

However, the results of other experimental studies suggest that a relationship exists between tumor induction and chronological age. Van Duuren et al. (32) have shown that the promotion phase of 2-stage skin carcinogenesis is strongly affected by senescence, and Ebesson (15, 16) demonstrated that the skin of old mice is more susceptible to tumor induction by DMBA than that of younger mice. Furthermore, heterochronic skin transplants followed by carcinogen treatment showed that skin from old animals retained a greater propensity for tumor induction than did that from young animals, indicating that the susceptibility of skin to tumor induction was intrinsic to the skin and not a result of extrinsic factors such as the functional state of the immune system (15-17). These findings suggest that old rodents are more susceptible to chemical carcinogens than are their younger counterparts, and there is no question that there is an increased incidence of cancer with advancing age. However, there is experimental evidence to the contrary that there is no age-associated increase in susceptibility of mouse skin to BP-induced carcinogenesis (31). Whether there is an age-associated intrinsic difference in response to chemical carcinogens (or to other carcinogenic agents) at organ sites other than skin remains to be determined. Thus, the role of aging in determining the incidence of cancer remains unresolved.

It has been shown previously that there are marked age-related differences in rodent hepatic mixed-function oxidase activity (5-9, 23), including aryl hydrocarbon hydroxylase activity (4). Furthermore, alterations in other microsomal and nonmicrosomal enzyme systems which further metabolize chemical carcinogens become manifest in senescent rodents (10). Thus, we suggest that these alterations may be of some significance in producing the increased incidence of cancer with advancing age. This suggestion becomes even more tenable in light of the now widely held idea that most cancer results from interaction of living organisms with environmental factors (20, 21, 33).

The results of the present study offer support for the hypothesis that age-associated alterations in carcinogen metabolism are one factor in determining the age-related increase in cancer incidence. Homogenates or purified microsomes prepared from the livers of old rodents produce metabolites of both BP and DMBA which are qualitatively or quantitatively distinct from those generated by tissue samples from their young counterparts, as evidenced by increased mutagenicity in a bacterial bioassay system. Other studies from this laboratory indicate that there is an increased extent of in vitro covalent binding of metabolites of BP and DMBA to calf thymus DNA following activation by tissue preparations from the liver of old rodents (10). If similar age-related alterations in carcinogen metabolism become manifest in the skin of senescent rodents, these results offer a reasonable alternative explanation for the observation of Ebesson (15-17) that those factors which render old mouse skin more susceptible to the carcinogenic action of DMBA are intrinsic to the skin.
Previous studies have shown that microsomes prepared from
the livers of old rats have less aryl hydrocarbon hydroxylase
activity than do liver microsomes from young animals (4). Since
measurable aryl hydrocarbon hydroxylase activity is lower, but
mutagenic activity is enhanced in a bioassay system, we sug-
gest that one consequence of senescence is that the metabo-

lism of procarcinogens, such as 2-FA and BP, is altered such
that a greater proportion of the metabolites of these compounds
are mutagenically active. Thus, the metabolites of chemical
carcinogens which are generated in old tissues are either
qualitatively distinct from those generated in tissues of young
organisms or there exist quantitative differences in the propor-
tion of the many metabolic forms of the carcinogens.

The extent of the usefulness of the Salmonella bioassay
system in partially bridging the obvious gap between bacterial
mutagenicity and carcinogenicity in mammals remains to be
determined and is at best only semiquantitative. However, our
results indicate that old animals exposed to some potential
carcinogens may be at greater risk than are their younger
counterparts. We suggest that these fundamental age-related
differences in the metabolism of carcinogens account for some
undetermined fraction of the increased frequency of neoplasia
observed in senescent animals.

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