Effects of Dietary Constituents on the Metabolism of Chemical Carcinogens

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Summary

Dietary constituents of 2 types have been shown to affect the metabolism of chemical carcinogens by the microsomal mixed-function oxidase system. Naturally occurring inducers of increased activity of this system are present in plants. Cruciferous vegetables including Brussels sprouts, cabbage, and cauliflower are relatively potent in this regard. From these vegetables, three indoles with inducing activity have been identified. These are indole-3-acetonitrile, indole-3-carbinol, and 3,3'-diindolylmethane. A 2nd type of dietary constituent affecting the microsomal mixed-function oxidase system is added phenolic antioxidant, i.e., butylated hydroxyanisole (BHA) and butylated hydroxytoluene. Studies of the effect of BHA on metabolism of benzo(a)-pyrene by liver microsomes have been carried out. BHA feeding results in microsomal changes. The cytochrome P-450 shows altered spectral characteristics, and the aryl hydrocarbon hydroxylase system of these microsomes has an increased sensitivity to inhibition by α-naphthoflavone. In addition, there is a decrease in binding of metabolites of benzo(a)-pyrene to DNA upon incubation of these microsomes with benzo(a)-pyrene. A number of experimental studies of induction of increased mixed function oxidase activity have shown that increased levels of activity protect against administration of chemical carcinogens. BHA and butylated hydroxytoluene also have been found to exert a protective effect against chemical carcinogens. Thus the constituents of the diet could be of consequence in the neoplastic response to exposure to carcinogens in the environment.

Introduction

Geographical diversities in the incidence of substantial numbers of neoplasms are well established. Likewise, changes occur in the incidence of particular neoplasms in the same geographical area over a period of time. In both instances, the differences that are found may be due to either alterations in levels of exposure to carcinogenic agents or, alternately, to changes in factors protecting the host against the effect of the carcinogenic agent involved (44). This presentation deals with the latter possibility. In particular, the role of the microsomal mixed-function oxidase system will be considered along with dietary constituents that alter the properties of this system.

The microsomal mixed-function oxidase system is a complicated biochemical entity that metabolizes a wide variety of xenobiotic compounds including many chemical carcinogens (7). Three components of the system have been identified: a characteristic cytochrome designated cytochrome P-450, cytochrome P-450 reductase, and a lipid (20, 21). For activity, the system requires NADPH and oxygen. Mixed function oxidase activity can be increased by administration of various inducers (8). These can differ in the spectrum of substrates that they cause to be metabolized at an increased rate (6). The inducers may or may not bear a chemical relationship to the substrates for which activity is increased (7). The induction process entails new protein synthesis (11). With the very broad substrate specificity of the mixed-function oxidase system, the question arises as to how many specific enzyme species exist. Two distinctive cytochromes have been demonstrated in liver microsomes, the naturally occurring one, i.e., cytochrome P-450, and a closely related species designated cytochrome P-448 (38). Cytochrome P-448 occurs on induction with certain classes of inducers, in particular, aromatic polycyclic hydrocarbons. There are substantial differences in the metabolic characteristics brought about by the 2 cytochromes. The range of substrates metabolized differs as do their responses to inhibitors (21, 38). In addition, differences in metabolite patterns resulting from incubation with the same substrate have also been found (33). How many other related cytochromes exist has not been established. The occurrence of cytochromes with additional distinctive reaction properties could be of considerable importance. At the moment, the technology of identification of various cytochrome P-450's is poorly developed, so that only a paucity of information exists.

Induction of Increased Mixed Function Oxidase Activity by Dietary Constituents

Since induction of increased mixed function oxidase activity could alter the response to chemical carcinogens as well as other environmental hazards, there has been considerable interest in the characteristics of inducers (7). Substantial work has been reported on inducers of increased hydroxylation of aromatic polycyclic hydrocarbons by the microsomal mixed-function oxidase system. This particular
group of reactions has been termed AHH.\textsuperscript{a} Polycyclic hydrocarbons, phenothiazines, flavones, and 2-phenylbenzothiazoles have all been shown to induce increased AHH activity. Some data on the relationship of chemical structure to inducing activity have been obtained (9, 43, 54, 55).

In early studies of AHH activity, it had been assumed that normal levels of activity of substantial magnitude existed. This assumption is correct for liver but is incorrect for the small intestine and lung. Most if not all of the AHH activity in these 2 organs, which are major portals of entry, result from exposure to exogenous inducers present in crude diets such as Purina rat chow, Charles River rat diet, Rockland rat diet, or Cargill rabbit diet (45–47). The 1st evidence for this came from studies of starved female Sprague-Dawley rats. These animals showed almost total loss of AHH activity in the small intestine and lung. Subsequently, studies were carried out using a balanced purified diet, i.e., vitamin-free casein, 27%; starch, 59%; corn oil, 10%; salt mix, 4%, plus a complete vitamin supplement (Normal Protein Test Diet, Nutritional Laboratories, Cleveland, Ohio). Again there was almost a total loss of AHH activity in the small intestine and lung. Determination of the effects of starvation and of feeding a purified diet have also been carried out on 3-methyl-4-methylaminoazobenzene N-demethylase activity of the small intestine. Both of these regimens result in a profound decrease in the activity of this reaction as compared to the level of activity in animals fed Purina rat chow (3). Crude diets enhance microsomal metabolism of this substrate in the liver (4).

Efforts were begun to identify the inducers of increased AHH activity present in the crude diets using Purina rat chow as a prototype. Various constituents of the diet were tested. Considerable inducing activity was found in the vegetable component, which consists of alfalfa meal. Experiments with alfalfa meal showed that this material has inducing activity. Subsequently, the plant itself was obtained from a farm in which no chemical treatment of the soil or plant had been used. This alfalfa also had inducing activity when added to purified diets fed to rats (47).

Because of the results obtained with alfalfa, investigations of the inducing activity of other vegetables were carried out. Many vegetables have inducing activity, some do not. The most potent vegetables studied thus far are members of the Brassicaceae family, including Brussels sprouts, cabbages, turnips, broccoli, and cauliflower. Inducing activity has also been found in spinach, dill, and celery (47). Further studies with Brussels sprouts and cabbage showed that quite marked differences in inducing activity occur with different varieties of the vegetable, more so for cabbages than for Brussels sprouts. Age at the time of harvesting can markedly affect inducing activity in cabbages, as can soil conditions in which they are grown. Determinations of the distribution of inducing activity throughout the Brussels sprouts plant have been performed. The inducing activity is not uniformly distributed but is largely confined to the sprouts.

Work was initiated to characterize the naturally occurring inducers in Cruciferous plants using Brussels sprouts, cabbages, and cauliflower as starting materials. The inducing activity was found in a fraction containing indoles. From this fraction, 3 indoles having inducing capacity were identified. These are indole-3-acetonitrile, indole-3-carbinol, and 3,3′-diindolylmethane (19). These indoles result from the hydrolysis of a parent compound, indolylmethyl glucosinolate. The parent compound along with the enzyme myrosinase, which hydrolyzes the compound, occur in plants in their viable states. With cellular disruption, the enzyme and substrate are brought together and hydrolysis occurs. The relative amounts of the various hydrolysis products are dependent upon the conditions of the reaction (19).

Other compounds found in plants also have inducing activity. A number of flavones induce increased AHH activity (54). Flavone, which is not a naturally occurring compound, is an inducer. Polyhydroxylation of this compound results in loss of inducing activity. Since the vast majority of naturally occurring flavones are polyhydroxylated, they are ineffective as inducers. A few flavones such as 5,6,7,8,3′,4′-pentamethoxyflavone (tangeretin) and 4,5,7,8,3′,4′-hexamethoxyflavone (noateline) contain methoxy rather than hydroxy groups. These compounds, which occur in citrus fruits, do have inducing activity. Additional naturally occurring compounds reported to induce mixed-function oxidase activity include safrole, isosafrole, and β-ionone (17, 18, 31, 32). Oxidation of a variety of sterols including cholesterol results in their having inducing activity (4). A number of insecticides and polychlorinated biphenyls are inducers, so that contamination of the diet with these compounds could enhance mixed-function oxidase activity (2, 13, 36).

**Effects of Induction of Increased Mixed-Function Oxidase Activity on the Response to Chemical Carcinogens**

The microsomal mixed-function oxidase system has the capacity to both detoxify chemical carcinogens and to activate them to proximate or ultimate carcinogenic forms (27, 28). However, the in vivo studies that have been reported thus far have shown that induction of increased mixed-function oxidase activity protects against challenge by chemical carcinogens (Table 1). Because of the existence of activation reactions, the possibility that induction of increased mixed-function oxidase activity might increase the carcinogenic response to a chemical carcinogen exists. However, data clearly showing such an increased carcinogenic response in vivo have not been published. This may be due to the fact that rapid activation of carcinogens simply does not enhance carcinogenicity. Chemical carcinogenesis is a low-threshold (or perhaps even no-threshold) phenomenon. Thus, rapid activation could result in loss of activated carcinogen species due to nonavailability of critical binding sites or due to secondary reactions. In contrast, induction of increased activity of microsomal mixed-function oxidase

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\textsuperscript{a}The abbreviations used are: AHH, aryl hydrocarbon hydroxylase; BHA, butylated hydroxyanisole; BP, benzo(a)pyrene; ANF, α-naphthoflavone.
Inhibition of carcinogenesis by induction of increased microsomal enzyme activity

<table>
<thead>
<tr>
<th>Carcinogen</th>
<th>Inducer</th>
<th>Species</th>
<th>Organ</th>
<th>Refs</th>
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<tbody>
<tr>
<td>3'-Methyl-4-dimethylaminoazobenzene</td>
<td>Polycyclic hydrocarbons, α-benzene hexachloride, polychlorinated biphenyls</td>
<td>Rat</td>
<td>Liver</td>
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<td>2-Acetylaminofluorene</td>
<td>Polycyclic hydrocarbons, polychlorinated biphenyls</td>
<td>Rat</td>
<td>Liver, breast, small intestine</td>
<td>22, 26</td>
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<tr>
<td>Diethylnitrosamine</td>
<td>Polychlorinated biphenyls</td>
<td>Rat</td>
<td>Liver</td>
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<tr>
<td>4-Dimethylaminostilbene</td>
<td>Polycyclic hydrocarbons</td>
<td>Rat</td>
<td>Ear duct</td>
<td>40</td>
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<tr>
<td>Urethan</td>
<td>Pentobarbital</td>
<td>Mouse</td>
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<tr>
<td>Urethan</td>
<td>β-Naphthoflavone, chlordane, phenobarbital</td>
<td>Mouse</td>
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<td>BP</td>
<td>β-Naphthoflavone</td>
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<td>7,12-Dimethylbenz(a)anthracene</td>
<td>Polycyclic hydrocarbons</td>
<td>Rat</td>
<td>Breast</td>
<td>15, 56</td>
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<td>Phenothiazines</td>
<td>Rat</td>
<td>Breast</td>
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<td>β-Naphthoflavone</td>
<td>Rat</td>
<td>Breast</td>
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<td>Phenobarbital</td>
<td>Rat</td>
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<tr>
<td>Bracken fern carcinogen</td>
<td>Phenothiazine</td>
<td>Rat</td>
<td>Small intestine, bladder</td>
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</tbody>
</table>

Alterations of Microsomal Mixed-Function Oxidase Activity by Phenolic Antioxidants

At least 1 category of food additive, phenolic antioxidants, alters the microsomal mixed-function oxidase system. Studies of the effects of 2 of these compounds, i.e., BHA and butylated hydroxytoluene on aminopyrine demethylase, hexobarbitone oxidase, nitroanisole demethylase, and biphenyl 4-hydroxylase, have been reported (10, 12, 24). Butylated hydroxytoluene induces increased activity towards all of these substrates. BHA is inactive in this regard except for biphenyl 4-hydroxylase, and then induction occurs only when the antioxidant is used at a high dosage level.

Antioxidants have been found to inhibit chemical carcinogenesis (42, 48–50). A considerable number of studies of this nature have been done with BHA, which inhibits carcinogenesis resulting from administration of polycyclic hydrocarbons as well as a variety of other chemical carcinogens (48, 49). The mechanism or mechanisms by which BHA produces this inhibition is not known. Several possibilities exist that can be divided into 2 major categories. The 1st involves some type of direct interaction between BHA and reactive species of carcinogens. The 2nd possibility is that BHA is acting in an indirect manner. Of primary interest in this regard is alteration of enzyme reactions detoxifying carcinogens would have a protective effect. Thus, there is the expectation, which is in fact supported by the results of animal experimentation, that enhanced microsomal mixed-function oxidase activity would inhibit chemical carcinogenesis if it has any effect at all. This latter qualification is added since in many tissues mixed-function oxidase activity is low, so that inhibition will not occur if doses of carcinogen beyond the metabolic capacities of the system are used.

In the previous discussion, implications of in vivo alterations in mixed-function oxidase activity have been presented. Considerable in vitro work has been done. AHH activity is demonstrable in many cell lines grown in culture. Because of the nature of the enzyme system involved, there is a potential hazard in assuming that biological effects of alteration of microsomal mixed-function oxidase activity in cell cultures can be carried over to the intact organism. The mixed-function oxidase system is a highly integrated mechanism for rendering compounds more polar so that they can be further metabolized, frequently by conjugation, and excreted. In cell cultures, instead of being excreted, metabolites remain within the culture medium and may cause a variety of changes that would not have occurred had they been removed in comparable fashion to that existing in vivo. Thus, while increased transformation can be produced in cell cultures by induction of increased mixed-function oxidase activity, no such in vivo counterpart has been reported (23). In contrast, as has been previously discussed, a considerable number of experiments in which induction of increased mixed-function oxidase activity protects against chemical carcinogens do exist, as shown in Table 1.
activity. Both types of mechanisms are currently under investigation, but it is with the latter that the present discussion is concerned.

Determinations of the effects of BHA on the metabolism of polycyclic hydrocarbons by the microsomal mixed-function oxidase system had not been carried out in the earlier studies. Subsequently, work of this nature was initiated. In mice (female A/HeJ) fed BHA under conditions that inhibit BP carcinogenesis, alterations in the metabolism of BP by the microsomal mixed-function oxidase system were found (39). This was not apparent from determinations of AHH activity. The AHH activity of liver microsomes from BHA-fed mice was not significantly different from controls. However, the AHH determination measures petroleum ether-alkaline-extractable hydroxylated products as a group. Other metabolites are not measured, and distinctions between various hydroxylated metabolites of BP are not made. With different procedures, changes in the properties of the liver microsomes from the BHA-fed mice were demonstrable. Incubation of BP and DNA with microsomes from BHA-fed mice resulted in significantly less binding of BP metabolites to DNA than with control microsomes. There is approximately one-half of the binding of BP metabolites to DNA in the presence of microsomes from BHA-fed mice as compared to controls (39).

Since changes in the amount of BP metabolite binding to DNA could result from an altered cytochrome P-450, the ethyl isocyanide-binding spectra were measured to see if any other forms of cytochrome P-450, such as cytochrome P-448 might have been produced by BHA feeding. At pH 7.4, the peak at 430 nm was the same in control and treated animals. However, the peak at 455 nm was lower in the microsomes from BHA-fed mice than from controls. This resulted in a ratio of the maxima at 455 nm:430 nm that was significantly different in BHA-fed mice as compared to the controls. These spectral characteristics do not correspond to those found with cytochrome P-448. The spectral studies indicate the presence of an altered cytochrome P-450, but its nature remains to be determined (39).

A further difference in the microsomal metabolism of aromatic polycyclic hydrocarbons brought about by BHA feeding is the response to ANF. If ANF is added to the microsomal reaction mixture, considerably less of this flavone is required to produce inhibition of AHH with microsomes from BHA-fed mice than from control microsomes. ANF has been used as an inhibitor of AHH activity by Wiebel et al. (57). These investigators observed that induction with polycyclic hydrocarbons produces an increase in AHH activity in liver that is much more sensitive to inhibition by ANF than is enzyme from control animals. Further work indicated that cytochrome P-448 is responsible for this increased sensitivity. The liver microsomes from mice fed BHA are quite different, in that there is no increase in AHH activity and the ethyl isocyanide spectra do not indicate the presence of an increase in cytochrome P-448. In addition to the qualitative changes in the cytochrome P-450, it was found that BHA feeding causes an increase in the amount of P-450 per unit weight of microsomal protein and per unit weight of liver. The liver weight is also increased by BHA feeding, a finding reported by others previously in rats fed this compound (10).

Thus the work that has been carried out with BHA has shown that this compound causes altered properties of liver microsomes including a decreased binding of BP metabolites to DNA. The cause of the decrease in binding has not been determined. Considerable advances have been made in the study of the metabolism of BP (14, 16, 35). Epoxides, oxy radicals, and hydroxymethyl derivatives have been implicated as possible reactive species of importance in macromolecular binding (5, 16, 29). A next step in the study of BHA inhibition of binding of BP metabolites to DNA is the determination of the effects of BHA on the microsomal metabolite pattern of BP. From these data it might be possible to identify specifically the alterations in metabolism of BP associated with the decreased macromolecular binding.

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