DISCUSSION FORUM

The role of peroxidase-catalyzed activation of aromatic amines in breast cancer

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Aromatic amines are mammary carcinogens in rodents and exposure to aromatic amines may be associated with increased risk of breast cancer in women. Peroxidases present in milk can oxidize aromatic amines to reactive electrophiles which bind to DNA and induce mutations. Hydrogen peroxide, required for peroxidase-dependent oxidations, is supplied by milk xanthine oxidase and by the respiratory burst of neutrophils, cells which are present in milk and activated by exposure to it. In this paper, I propose that lactoperoxidase and myeloperoxidase activate aromatic amines, within the breast ducts, and that these enzymes play a crucial role in the chemical induction of breast cancer.

Aromatic amines

Aromatic amines are synthetic and naturally occurring carcinogens. Many aromatic amines induce mammary tumours in female rodents; these include 3,3’-dimethoxybenzidine (Morgan et al., 1994), 4-aminobiphenyl, 2-aminofluorene (Garner et al., 1984), and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), the most abundant of the heterocyclic aromatic amines found in food (Ito et al., 1991). A recent review summarizes all of the rodent mammary carcinogens identified by studies under the aegis of the National Toxicology Program, USA (Dunnick et al., 1995). Of 34 chemicals causing mammary neoplasms in rats or mice, 16 were aromatic amines or nitro compounds.

This paper presents a biochemical-physiological paradigm for explaining the induction of mammary cancer by aromatic amines. Its crux is the hypothesis that, within the fluid present in the breast ducts, peroxidases activate aromatic amines to mutagenic products. The development of these ideas was stimulated by recent epidemiological evidence that aromatic amines may induce breast cancer in humans.

Breast cancer epidemiology

Breast cancer is second only to lung cancer as a cause of cancer mortality in women. A recent review of breast cancer research (Marshall, 1993) stated that: ‘epidemiologists have made little headway in explaining the long, relentless increase in the incidence of this disease over the past 50 years. . . . But epidemiologists have shown beyond doubt that something other than inherited vulnerability—something in the environment—is driving [breast] cancer rates upward.’ Aromatic amines are mammary carcinogens in rodents, and humans are exposed to aromatic amines via several routes. Are these compounds human breast carcinogens?

Smoking and breast cancer

The Canadian Cancer Society warns women that predisposition to breast cancer may be higher in smokers. Some epidemiological studies have found an association between cigarette smoking and breast cancer (Palmer et al., 1991; Calle et al., 1994). A very recent Danish study concluded that ‘women who have smoked for more than 30 years have a significantly increased risk of breast cancer, with a relative risk of 1.6’ (Bennick et al., 1995). Biggs et al. (1993) reviewed the distribution of p53 mutations in several types of human cancer, and concluded that ‘the similarity between the mutational spectra in lung cancer and breast cancer invites consideration of tobacco as a [breast] carcinogen’. However, the relative risks of breast cancer associated with smoking are generally reported to be low (<2), and other studies have found no such association (Field et al., 1992).

Smoking and breast cancer: the role of N-acetyltransferase

N-Acetylation, catalyzed by hepatic acetyl CoA:arylamine N-acetyltransferase (NAT), is a major route for the metabolism of aromatic amines and hydrazines, including natural products, such as caffeine metabolites; drugs, such as isoniazid, sulfamethazine and procainamide; and carcinogens, such as benzidine, β-naphthylamine, and the food pyrolysis product heterocyclic amines. The distribution of acetylation rates is bimodal: the human population is divided into ‘slow’ and ‘fast’ acetylators. Slow acetylation is inherited as a recessive autosomal Mendelian trait. The molecular basis of this genetic polymorphism has been elucidated (Grant, 1993; Meyer, 1994). Two distinct human NAT enzymes exist, NAT1 and NAT2. NAT2 is the locus of the ‘classical’ NAT polymorphism. The ‘slow’ alleles (bearing point mutations in the gene for NAT2) encode poorly translated mRNAs or less stable protein products. The slow acetylation phenotype is observed in ~50% of North American Caucasians, but is much less common in Asian populations. Slow NAT2 phenotype predisposes individuals to some drug-induced idiosyncratic toxicities (e.g. isoniazid-induced neurotoxicity; hydralazine- and procainamide-induced lupus). These drugs are excreted as acetylated metabolites in the urine; pharmacological half-lives are usually longer in slow-acetylator individuals, and the parent drug accumulates to toxic levels (Weber and Hein, 1985).

Recently, Ambrosone et al. (1995a) conducted a case-control study of the influences of both NAT2 genotype and smoking history on the risk of breast cancer among post-menopausal Caucasian women (159 cases and 203 controls). They observed that slow-acetylator-genotype women (about half the Caucasian population) showed a very significant risk of smoking-related breast cancer. Among slow acetylators, breast cancer risk increased with amount smoked. Smoking, 20 years prior to interview, increased breast cancer incidence with an odds ratio of 7.65 (highest quartile smokers versus never-smokers; 95% confidence interval, 2.22–26.32; Ambrosone et al., 1995b).
Among the fast-acetylator-genotype women, however, no association of smoking with breast cancer was observed. Since aromatic amines (and hydrazines) are the only known substrates for NAT2, the straightforward interpretation is that slow acetylaters are at greater risk because they metabolize and excrete aromatic amines in cigarette smoke more slowly and, therefore, suffer more chronic exposure to their toxic effects. Indeed, cigarette smoke contains aromatic amines, as noted by Ambrosone et al. (1995b); these include naphthylamines, aminobiphenyls, and other carcinogens (Pieraccini et al., 1992). 4-Aminobiphenyl is metabolized to an intermediate which reacts with hemoglobin cysteine residues; the levels of this adduct in human blood are consistently found to be higher in smokers (Skipper and Tannenbaum, 1994). N-acetylation detoxifies aromatic amines: the product amides are excreted more readily than the amines and are also very poor substrates for peroxidative metabolic activation (see below).

Passive smoking also results in exposure to aromatic amines; levels of these compounds are higher in side-stream smoke than in main-stream smoke (Pieraccini et al., 1992). Passive smoking may be a risk factor for breast cancer (Horton, 1992).

**Occupational and dietary exposure to aromatic amines**

One of the most-studied examples of occupationally induced human cancer is the epidemic of bladder cancer among (male) workers exposed to benzidine and other aromatic amines in the dyestuff industry (Vineis, 1994). A recent study of workers in Moscow shows that aromatic amines also induce bladder cancer in females; the numbers of cases were too small for evaluation of a possible increased risk of breast cancer (Bulbulyan et al., 1995). Another occupational study, in Oregon, identified 'chemical and gas handlers' as a group at significantly elevated incidence risk (Morton, 1995).

Grilled foods contain potently mutagenic heterocyclic aromatic amines (Layton et al., 1995), some of which induce mammary cancer in rodents, as mentioned earlier. When such compounds were administered per os to nursing rat dams, they were found at high levels in the breast tissue, excreted in the milk and absorbed by the suckling pups (Ghoshal and Snyderwine, 1993). The involvement of dietary exposure to heterocyclic aromatic amines in breast cancer etiology has been proposed (Snyderwine, 1994).

Petrakis and colleagues tested for the presence of mutagenic activity in nipple aspirates of breast fluid (Petrakis et al., 1980). Of 456 women tested, 31 gave positive results. The nature of the mutagens was not determined, but activity was detected with Salmonella typhimurium strain TA1538, which is sensitive to aromatic amines. The possible presence of aromatic amines in human milk and nipple aspirate fluid warrants examination.

**Metabolic activation**

Aromatic amines require metabolic activation to chemically reactive mutagenic species. Normal human mammary epithelial cells in vitro can activate genotoxic carcinogens, including polycyclic aromatic hydrocarbons (PAH) and aromatic amines, to genotoxic products, as measured by unscheduled DNA synthesis (Eldridge et al., 1992).

One route of aromatic amine bioactivation requires cytochrome P450-dependent oxidation of the amines to hydroxylamines; these intermediates are further activated by O-acetylation, catalyzed by the same enzyme which N-acetylates aromatic amines (Josephy et al., 1995). This pathway occurs in the liver, where both P450 and NAT activities are high. Involvement of this pathway in aromatic-amine-induced breast cancer, however, seems less likely, since the breast-carcinogenic effect of smoking is seen in slow acetylator women. Also, the levels of cytochrome P450 activity in (rat) mammary tissue are much lower than in the liver (Ritter and Malejka-Giganti, 1982; Davis et al., 1994). In cultured human mammary epithelial cells in vitro, aminohydroxylamines are cytotoxic and DNA-reactive, as assayed by 32P-postlabeling detection of DNA adducts (Fan et al., 1995). In contrast, these cells 'possessed no detectable capacity to metabolically activate [the parent AAs].'

**Peroxidases**

Peroxidases catalyze sequential one-electron oxidations of aromatic amines and diamines to generate reactive electrophiles which can bind to DNA (Josephy, 1985, 1989). Aromatic amines, the products of N-acetylation of aromatic amines, are poor peroxidase substrates (Josephy et al., 1983). Mammalian peroxidases have previously been implicated in toxicity and carcinogenesis: prostaglandin H synthase (cyclooxygenase-hydroperoxidase) in aromatic-amine-induced bladder carcinogenesis (Flammang et al., 1989); myeloperoxidase in the activation of aromatic amine drugs which cause idiosyncratic adverse reactions (Hofstra and Uetrecht, 1993).

Two peroxidases are found in milk (see below). Lactoperoxidase (LPO) is synthesized in the breast secretory epithelial cells. Milk also contains abundant neutrophils, which are rich in myeloperoxidase (MPO). Aromatic amine carcinogens (e.g. benzidine, 2-aminofluorene) can be activated by both LPO (Josephy et al., 1983; Malejka-Giganti et al., 1986) and MPO (Malejka-Giganti and Ritter, 1994), giving reactive metabolites which bind covalently to DNA in vitro.

**Mammary carcinogenesis**

What factors cause the mammary specificity of many aromatic amines? The high fat content of this organ leads to the accumulation of lipophilic compounds. However, many lipophilic carcinogens are not mammary-specific, so this factor alone cannot suffice. I believe that tissue-specific metabolic activation of carcinogens provides a plausible mechanistic basis for this organ specificity.

Milk, stored in the breast ducts (Mephem, 1987), is, potentially, an ideal bacterial growth medium. Therefore, elaboration of effective defenses against mastitis infections must have been an essential development in the evolution of mammals. Lactoperoxidase and neutrophils (MPO) are two of the key systems. I propose that LPO and MPO, secreted in milk, play critical roles in the bioactivation of aromatic amines in the breast. An attractive feature of this theory is that it traces the etiology of breast cancer to a biological and biochemical property peculiar to the mammary gland: the presence of antibacterial peroxidase systems. The ductal cells may be unique in their exposure to LPO, MPO, hydrogen peroxide generation, and aromatic amines. Most breast cancers are ductal cell carcinomas (Russo et al., 1990).

**LPO**

LPO is a soluble, glycosylated hemoprotein found in milk (Thomas et al., 1991; Chang et al., 1993; Ferrari et al., 1995).
Both the human and bovine LPO cDNAs have been cloned and sequenced (Dull et al., 1990). Bovine LPO and MPO share ~50% amino acid homology (Cals et al., 1991). Human LPO has been purified and characterized (Langbak and Flatmark, 1989). LPO is also found in other secretions, such as tears and saliva (Mansson-Rahmetulla et al., 1988).

The LPO system protects milk against the growth of bacteria (Reiter and Perraudin, 1991). The system consists of three components: LPO, thiocyanate anion (SCN⁻) and H₂O₂. SCN⁻ is derived from dietary sources (plant glucosinolates and glucosides) and from the rhodanese-catalyzed detoxification of cyanide. Hydrogen peroxide may be formed by the action of milk xanthine oxidase (Collins et al., 1988) or by the metabolic activity of bacteria, including Streptococcus and Lactobacillus (Thomas, 1985). These bacteria are, therefore, self-inhibitory in milk. LPO catalyzes the H₂O₂-dependent oxidation of SCN⁻, generating oxidants which inhibit the growth of microorganisms. Addition of hydrogen peroxide, to enhance the activity of the LPO system, is an alternative method for preserving milk, used in some developing countries where refrigerated transport is not available (Korhonen, 1980).

LPO-catalyzed oxidation of β-estradiol causes redox-cycling, oxidative stress and oxidation of reducing cofactors, such as glutathione and NADH (Sipe et al., 1994). This process could increase the availability of H₂O₂ for aromatic amine oxidation, and oxidative stress may also act as a tumor-promoting stimulus. Exposure to estrogens is regarded as an important risk factor for breast cancer (Marshall, 1993).

MPO

The respiratory burst oxidase in activated neutrophils converts O₂ to toxic species, such as superoxide and H₂O₂ (Korhonen and Reiter, 1983). Neutrophil MPO oxidizes Cl⁻ and Br⁻ to HOCl and HOBr, respectively. Neutrophils become activated by phagocytosis of opsonized microorganisms. These cells are also activated by phagocytosis of lipid vesicles in milk (Keeney et al., 1993). In summary, both the LPO and MPO systems are found in the breast and in milk, function in concert with H₂O₂-generating systems, and can activate aromatic amine carcinogens to reactive species which bind to DNA.

Increased neutrophil activity, resulting from inflammation or infection, should potentiate breast carcinogenesis. In fact, cigarette smoking is associated with mastitis as strongly as it is associated with breast cancer. Bundred et al. (1993) concluded that ‘smoking is directly implicated in the pathogenesis of periductal mastitis’; their case-control study showed a relative risk of 6.2 for smokers. They wrote that ‘Women with periductal mastitis should be warned that continued smoking may lead to recurrent mammary sepsis.’ Among four male patients with periductal mastitis, all smoked >10 cigarettes per day (Thomas et al., 1993). Mastitis and sepsis will lead to elevated levels of breast neutrophils, MPO and H₂O₂, thus enhancing the metabolic activation of cigarette-derived aromatic amines.

PAH and mammary cancer

Peroxidase-catalyzed activation may also explain breast carcinogenesis by PAH, which are also present in cigarette smoke (Phillips, 1983). Cavaliere and colleagues have established that PAH can be activated by peroxidase-catalyzed one-electron oxidation to reactive DNA-binding species (RamaKrishna et al., 1993). Some PAH (e.g., 7,12-dimethylbenz[a]anthracene) are potent mammary carcinogens in rats, while others (e.g., 5-methylchrysene) are mouse skin, but not rat mammary carcinogens (Cavaliere and Ragan, 1992). Cavaliere and Ragan (1985) studied the peroxidase-catalyzed oxidation of various PAHs and their carcinogenicities. They concluded that ‘only PAH with ionization potential low enough for activation by one-electron oxidation induce tumors in [rat mammary gland].’ (NAT-catalyzed acetylation is, of course, not a factor in PAH metabolism.)

Lactation and breast cancer

The negative association between breast cancer risk and lactation, observed in some epidemiological studies, may appear inconsistent with the imputed role for peroxidases in breast carcinogenesis. However, the risk reduction associated with lactation is small and, according to a recent study, limited to pre-menopausal women (Newcomb et al., 1994). Although the levels of LPO in non-lactating human breast are not known, the enzyme is present in non-lactating bovine udder (Brown and Mickelson, 1979). As Petrakis (1993) observed: ‘many of the chemical substances and disintegrating cellular products in the breast fluid are concentrated and can be retained within the ductal system for a long time. . . . In genetically predisposed women, the prolonged exposure to these high concentrations of steroid hormones, cholesterol epoxides, and probably other unidentified agents from the environment may initiate and promote benign and malignant breast disease.’ It is not clear to what extent (if at all) lactation would increase LPO-mediated activation of carcinogens, over a woman’s lifetime.

Indeed, lactation should decrease the amount of aromatic amine present in the fat reservoirs of the breast, albeit by passing these carcinogens onto the nursing child. During lactation, milk proteins, such as casein, are produced; these proteins bind aromatic amines (Yoshida and Ye, 1992). The potential for metabolic activation may well be greatest in non-lactating breast, because inactivating (binding) proteins are not present at high levels.

Further predictions arising from the theory

Several predictions of this theory of breast cancer causation can be tested. Human milk, LPO and MPO should activate aromatic amines in vitro. Aromatic amine levels should be elevated in the milk and breast tissue of women who smoke, and these compounds should include mammary carcinogens or their metabolites. Aromatic amine–DNA adduct levels should also be elevated in the breast tissue of smokers, especially in ductal cells, and the adducts should be consistent with peroxidative activation. Levels should be elevated further in slow-acetylator smokers. Peroxidase inhibitors (Divi and Doerge, 1994; Bandypadhyay et al., 1995) should inhibit mammary carcinogenesis by aromatic amines, whereas P₄₅₀ inhibitors should have little effect. [1-O-Hexyl-2,3,5-trimethylhydroquinone, a synthetic lipophilic hydroquinone antioxidant, which should be an excellent peroxidase substrate, inhibits PhIP-induced mammary carcinogenesis in rats (Hirose et al., 1995).] Finally, a clinical history of chronic or recurring mastitis should predispose women (especially smokers) to breast cancer.

Acknowledgements

I wish to thank Dr Christine Ambrosone for many helpful discussions and for bringing to my attention the work of Dr N.L. Petrakis. I also wish to thank Dr Ronald P. Mason for his encouragement and suggestions.
Aromatic amines and breast cancer


