

Previous projects:

Studies on developing selenium incorporated chalcones as potential chemopreventive and chemotherapeutic agents of next generation

Because of the ability of natural occurring chalcones and various organoselenium compounds to prevent or reverse carcinogenesis or kill cancer cells with high selectivity without showing any genotoxicity and drug resistance, we initiated a pilot study that has been directed toward developing organoselenium compounds containing chalcone scaffold. We believe that such natural product-driven studies may provide important leads to develop an effective anti-cancer drug that has potential to supplement or replace current anti-cancer drugs which are known to produce adverse side effects, mutations leading to cancer and/or drug resistance.

Studies on polynuclear aromatic hydrocarbons, polynuclear sulfur heterocycles, and their metabolites

In our continuing effort to understand the mechanism by which environmental occurring polynuclear aromatic hydrocarbons and their heterocyclic analogs induce cancer, we are currently studying the metabolism of phenanthro[3,4-b]thiophene to its mutagenic/carcinogenic metabolites by liver and lung microsomes from various animal species as well as humans in order to have a better understanding of the carcinogenic potential of this and related carcinogens in various animals.

Identification of gene products modulated by benzopyrene (an environmentally present carcinogenic PAH) by cutting age microarray technique and in vitro analyses of the role of the particular gene in BP-induced signaling with a view to the development of biomarkers

We already have the microarray data of benzo[a]pyrene-induced gene expression in mouse epidermal JB6 cell line performed in Roswell Park Cancer Research institute. The data include expression level of 50,000 genes. We are now analyzing the data with the objective of identifying the biomarkers modulated in response to BP, an environmental carcinogen.

Long chain fatty acids as chemo-preventive agents against PAH-induced carcinogenesis

Studies undertaken include examination of the effect of long chain saturated fatty acids on PAH-induced tumorigenesis. In this context we will examine the regulation of fatty acid desaturase and AGPAT-9 which are involved in fatty acid metabolism.

Role of long chain saturated fatty acids in cellular protective response of apoptosis against PAH-induced carcinogenesis.

Efforts are in progress to decipher a new mechanistic insight with regard to the role of saturated fatty acids in PAH-induced apoptosis in p53-independent manner. In this context we will examine the effect of modulation of lipid metabolism on PAH-induced apoptosis response.

Identification of chemo-preventative targets in tumor promotion by tobacco smoke phenolic components.

Efforts are in progress to elucidate the signaling pathways involved in tumor promotion by tobacco smoke phenolic components. Role of protein kinase C (PKC) and p53 have been implicated in this regard. Ongoing studies include understanding of the role of PKC over-expression in chemo-prevention against tobacco smoke carcinogenesis using in vitro cell culture and in vivo animal models. (2012)

Mechanism of synergistic interactions of active TSC phenolic component(s) with polynuclear aromatic hydrocarbons (PAHs) (present in the environment) toward potentiation of carcinogenicity.

Efforts are in progress to identify the phenolic components in tobacco smoke phenolic fraction (TSCPhFr) having tumor promoting activity in PAH-initiated cells. Identification of the phenolic component will help development of chemo-preventive strategy through elimination of the respective phenolic component from tobacco leaf by genetic engineering. (2012)

Development of mechanism-based MMP inhibitors

This study was undertaken to develop a project in the area of chemoprevention. We are currently interested in developing strategy to prevent tumor metastasis which is the most common cause for cancer death. It is now growing evidence that the environmental pollutants including those found in Great Lakes are involved in this process of carcinogenesis. Our initial effort is to develop small organic molecules which are highly specific in inhibiting matrix metalloproteinase-9 (MMP-9) which appears to be specifically involved in the metastasis of prostate cancer. Our continuing effort is directed to develop synthesis of the potential inhibitors of MMP-9. (2010)

Mechanism-based CYP2A6 inhibitors as smoking cessation agents

Nicotine addiction is the primary cause for cigarette smoking which leads to high incidence of lung cancer and other diseases. CYP2A6 has been identified a principal cytochrome P-450 which is predominantly involved in the metabolism of nicotine to inactive products, thereby, removing active nicotine from body circulation. The smokers with high CYP2A6 in liver are prone to higher level of smoking to maintain the desired level of nicotine in the body. Thus CYP2A6 appears to be an excellent target for developing therapeutic agents for preventing cigarette smoking. Thus, our objective is to identify small organic molecules that can effectively inhibit CYP2A6 and, consequently, maintain clinical level of nicotine for a longer period in an effort to reduce frequency of cigarette smoking, especially, for chain smokers. (2010)

Effect of cigarette smoke components on nicotine addiction

This research is directed to identify chemical constituents in cigarette smoke that may be involved in promoting nicotine addiction by reversibly inhibiting the liver enzyme CYP2A6, a major enzyme involved in the metabolism of nicotine to inactive metabolites, during smoking. (2010)

Effect of heavy metals on PAH-induced genotoxicity

Studies directed to understand the mechanism(s) underlying the potentiating effect of cadmium, nickel and other heavy metals designated as environmental pollutants on the genotoxicity of PAHs, and thereby presents the carcinogenic risk to humans. We studied the effect of above metal pollutants on the protective signaling events (p53- dependent or independent cell cycle arrest and apoptosis) induced

in response to genotoxic stress by PAHs with a view to determine the biomarker(s) involved in metal toxicity. (2010)

Identification of phenolic component(s) present in the environment as well as in tobacco smoke condensate (TSC) as tumor promoter

We observed that the weakly acidic TSC phenolic fraction is a tumor promoter and increased the number of colonies of cells on soft agar (anchorage-independent cell growth). It possesses hundreds of phenolic components as determined by high pressure liquid chromatography (HPLC). The HPLC separated fractions as well as the crude TSC phenolic fraction are tested individually to examine their effect on anchorage-independent cell growth. We are in the process of determining the particular phenolic fraction(s) responsible for tumor promoting activity. (2010)

Mechanism of tumor promotion by TSC phenolic fraction

Attempts are pursued to understand the mechanism of synergistic interactions of active TSC phenolic component(s) with polynuclear aromatic hydrocarbons (PAHs) (present in the environment) toward potentiation of carcinogenicity. We determined the interference of TSC phenolic fraction with PAH-induced p53 response which is known to trigger the cellular protective machinery thereby justifying the possibility of p53's role in this regard. We are in a process to examine the role of p53 downstream signaling events e.g. NFkappaB and MAP kinases with a view to understand the underlying mechanism of tumor promotion by TSC phenolic fraction. (2010)

Gene expression in benzo[a]pyrene treated cells

We determined in vitro effect of the PAH benzo[a]pyrene on the cellular expression of several thousands of genes by cutting age Microarray technique. We observed up-regulation and down-regulation of many genes by benzo[a]pyrene. We are analyzing these thousands of gene expression data to sort out the role of particular gene product(s) in benzo[a]pyrene-induced cellular responses (both protective and tumorigenic) with a view to the development of biomarkers. (2010)

Tumor promoting effect of alcohol in polynuclear aromatic hydrocarbon (PAH)-induced carcinogenesis

Attempts are pursued to understand the mechanism of tumor promotion by ethanol in benzo[a]pyrene-induced tumorigenesis. Research is in progress to examine the effect of alcohol on cell cycle arrest and apoptosis induction in benzo[a]pyrene treated cell lines with a view to identify the signaling intermediates involved in alcohol-mediated tumor promotion in PAH-induced carcinogenesis. (2010)

Studies of the hydroxylated derivatives of polybrominated diphenyl ethers.

Recently we studied the metabolism and disposition of PBDEs in trouts under a grant funded by New York Sea Grant. PBDEs are emerging contaminants in Great Lakes Nation's other water ways. There is now increasing evidence that many of these chemicals cause endocrine disruption in aquatic and wildlife species. Our studies indicated that these compounds are bioaccumulated significantly in edible portion of trout muscle. Recently our laboratory is interested in studying the mechanism of by which two widely distributed PBDEs, namely, BDE-47 and BDE-99, induce their biological effects. Our effort was primarily focused on developing a proposal for identifying phenolic metabolites that are potentially involved in the endocrine disruption activities of the parent compounds using fish and mammalian models. A proposal related to these studies was submitted to National Institutes of Health. (2008)

Monitoring for toxins.

Trace analysis of organic compounds of environmental interest in various matrices. These studies include QA/QC for trace analysis of PAHs, PCBs, Polybrominated biphenyl ethers (PBBEs), chlorinated pesticides, and metals by HPLC-UV detector, HPLC-fluorescence detector, GC-FID, GC-ECD, and AA in water, sediments, and fish tissues.

Carcinogenesis.

Synergistic enhancement of carcinogenicity by tobacco smoke constituents

There is considerable evidence indicating that the weakly acidic phenolic fraction of tobacco smoke condensate (TSC), elicits strong tumor-promoting activity in polynuclear aromatic hydrocarbons (PAHs)-initiated animals. Pure phenols by themselves are weak tumor-promoters at very high dose and cannot account for the tumor-promoting activity of the whole phenolic fraction. The mechanism(s) underlying the tumor-promoting activity of the phenolic fraction is not known. We observed that TSC phenolic fraction at non-cytotoxic concentrations attenuates BPDE-induced (i) p53 accumulation and p21 expression in human lung small airway epithelial (SAE) cells and (ii) activation of ERKs and NF- κ B in mouse epidermal JB6 (P+) cells. Our ongoing effort in this respect is to understand whether TSC phenolic fraction inhibits p53 function by abrogating (i) DNA binding (in vitro and in vivo) and transcriptional activities of p53, (ii) p21 response, cell-cycle arrest, expression of G1 cyclins, activation of cdk, and phosphorylation of Rb protein, (iii) p53 stability, p53 phosphorylation/acetylation at serine and lysine residues respectively, p53-Mdm2 interaction, and PI3-K/Akt-mediated phosphorylation of Mdm2 which regulates p53-Mdm2 interaction (iv) the activation of DNA damage-induced kinases and their ability to phosphorylate p53 and (v) p53 transcription and NF- κ B activation. The data from these studies will help in assessing the health risk presented by tobacco smoke constituents.

Mechanism of carcinogenesis of polynuclear aromatic hydrocarbons and their aza- and thia-analogues

Polynuclear aromatic hydrocarbons and their heterocyclic analogs are ubiquitous environmental contaminants which are introduced in our environment through incomplete combustion of organic matters such as fossil fuels, tobacco, etc. It has been demonstrated that some of these compounds are potent carcinogens in laboratory animals, and may be responsible for causing majority of human cancers. There is now considerable evidence indicating that these environmental contaminants are metabolically activated to reactive metabolites which binds to DNA thereby inducing carcinogenesis. We are currently investigating various major biochemical pathways that may be involved in the bioactivation of these polynuclear aromatic compounds and their heterocyclic analogs. Our research is currently focused on:

- Synthesis of the potential metabolites of PAHs and their heterocyclic analogs.
- *vivo* and *in vitro* metabolism of PAHs and their heterocyclic analogs, and the characterization of the metabolites produced using modern instrumental techniques (HPLC, GC, GC-MS etc).
- In vitro and in vivo bioassay of carcinogens and their various metabolites. These studies involve (i) cell transformation activity of parent compounds and their metabolites using various bacterial strains, and animal or human cells, and (ii) tumorigenic activity in animal models (mouse skin etc).
- Characterization and evaluation of the potential role of DNA adducts produced by environmental carcinogens and their metabolites using various in vitro and in vivo models in order to understand the structural requirements and the function of these DNA adducts in transforming normal cell to cancerous cells.

- Characterization of specific enzymes, especially cytochrome P450s (in animals and humans) involved in the metabolic activation of chemical carcinogens. Cytochrome P450 is an important class of oxidative enzyme which play important role in the metabolic activation of environmental carcinogens. Cytochrome P450s exist in multiple forms and each form (isozyme) may differ significantly from others in its substrate specificity, regioselectivity, and stereoselectivity in the metabolism of carcinogens to their carcinogenic metabolites. Thus the characterization of specific P450s that are involved mainly in the metabolic activation of environmental carcinogens is of significant interest to cancer researchers.
- Potential role of non-carcinogenic metabolites on inhibitory and synergistic effect on the carcinogenicity of carcinogenic metabolites of carcinogens. This study is initiated to understand why certain environmental carcinogens (such as benzo[a]pyrene, dibenz[a,l]pyrene) exhibit high carcinogenic activity compared to their widely accepted ultimate carcinogens, bay-region diol epoxides.

The collective information produced from these and other mechanistic studies will help in assessing the health risk presented by various classes of environmental carcinogens, and in developing various strategies in the prevention of cancer and related diseases caused by these ubiquitous environmental carcinogens/toxicants.

Environmental metal pollutants, co-carcinogenesis, p53 signaling events and apoptosis

Cadmium is an environmental pollutant and is one of the major metal constituents of tobacco smoke. There is considerable evidence indicating that cadmium elicits synergistic enhancement of cell transformation when combined with benzo[a]pyrene (BP) or other PAHs. Smokers are particularly at a high risk of exposure to mixture of PAHs and cadmium. The mechanism of the synergistic interaction of heavy metals particularly cadmium with PAHs is not established. We observed that cadmium at non-cytotoxic concentrations attenuates PAH-induced (i) p53 accumulation, (ii) NF- κ B activation and (iii) DNA fragmentation (indicative of apoptosis) in different cell lines. We also observed that BPDE-induced NF- κ B activation and DNA fragmentation are inhibited by inhibitors of p53 and NF- κ B respectively. In order to examine whether the synergistic activity of cadmium is due to its interference with BP-induced signaling events which mediate p53 stability, p53 activation and p53 function toward apoptosis, we are investigating the effect of cadmium on (i) NF- κ B-mediated p53 transcription, (ii) activation of signaling pathways which regulate NF- κ B activation, (iii) the regulation of NF- κ B subunits which determines its apoptotic or anti-apoptotic function and (iv) the expression of anti- and pro-apoptotic proteins. The data obtained from these studies will help in assessing the associated health risk presented by tobacco smoke constituents and will be useful for therapeutic strategies in the prevention of cancer.

DNA damage by poly-nuclear aromatic hydrocarbons present in tobacco smoke and the mechanism of tumor promotion by phorbol ester

Polynuclear aromatic hydrocarbon (PAH) are ubiquitous environmental pollutants. There is considerable evidence showing that the prototype PAH benzo[a] pyrene (BP) induces p53 in human and mouse cells. DNA damage caused by benzo[a]pyrene (BP) or other PAHs induce p53 protein as a protective measure to eliminate the possibility of mutagenic fixation of the DNA damage. 12-O-tetradecanoylphorbol-13-acetate (TPA) inhibits p53 response induced by BP and other DNA-damaging agents and may cause tumor promotion. The molecular mechanism of attenuation of BP-induced p53 response by TPA is not known. We are investigating the interference of TPA with (i) the upstream regulation of p53 and (ii) the downstream signaling pathways which are involved in p53 function. In this regard the role of MAP kinase and NF- κ B signaling pathways in p53 stabilization and function has been focused.

Developing mechanism based chemopreventive agents

These studies include (i) developing mechanism-based suicide substrates or inhibitors of cytochrome P450s involved in the metabolic activation of P450s, (ii) developing various synthetic and naturally occurring compounds that potentially interfere with various signaling processes involved in cancer induction and its metastasis.

Emerging toxins.

Type-e Botulism In Lake Erie

Lake Erie has recently been the site of botulism type E outbreaks that have affected fish, waterfowl and mudpuppies. These epizootics were caused by a paralytic neurotoxin produced by the bacterium *Clostridium botulinum* type E. In our previous work, we found genetic evidence of *C. botulinum* type E in sediment as well as in dreissenid mussels, amphipods, oligochaetes and chironomids collected from Lake Erie. This evidence strongly suggests that aquatic invertebrates are links in the transmission of the toxin from the benthic food web compartment to higher trophic levels such as fish and birds. Continuing research is aimed at gaining a better understanding of the conditions that lead to growth of this bacterium and identification of pathways of bacterial toxin transmission.

Disposition and Metabolism of 2,4,2'4' tetrabromodiphenyl ether (BDE-47) in Rainbow Trout

Polybrominated diphenyl ethers, PBDEs, are an emerging environmental issue of concern. They are ubiquitous in the environment and levels in humans have increased by a factor of ~100 during the last 30 years. In New York, for example, PBDEs levels of at 135 parts per billion fat were found in breast milk. PBDEs can disrupt thyroid hormone balance, impair neural development, and impair immune response, especially during fetal and neonatal development in both humans, other mammals, and fish. In the Great Lakes, PBDEs have been detected in fish from all trophic levels, and their concentration in Great Lakes fish is doubling every three to four years, even though other toxics like PCBs and mercury are declining. In this experiment, trout were fed corn contaminated with BDE-47, and their tissues were analyzed for amounts of the original material and for any possible contaminants. These analyses showed that there was initial movement of BDE-47 from the stomach to the intestine and muscle tissue, with concentrations peaking at one week. Concentrations in adipose tissue started to increase in four weeks and was still accumulating after 10 weeks. No metabolites were detected in fish tissues during this study. However, consumption of contaminated tissue by higher vertebrates may form the basis for endocrine disruption due to subsequent metabolism by the consumer.

Estrogen mimic substances in Lake Erie

Endocrine-disrupting compounds, ranging from natural estrogens to industrial chemicals (PCBs and PBDEs) enter the Great Lakes and their tributaries through discharge of municipal wastewater treatment plants (WWTPs), industrial wastes, and agricultural drainage. Elevated levels of these compounds in aquatic systems affect fish and other organisms through their effect as endocrine regulators. The presence of estrogens in lakes has been linked to feminization of male fish, reproductive failure, and collapse of the fish population. This on-going project aims to determine the occurrence of estrogens of anthropogenic origin/activities in male fish of selected eastern Lake Erie species.

