Meeting Report

Third International Conference on Mechanisms of Antimutagenesis and Anticarcinogenesis

Silvio De Flora, Giorgio Bronzetti, and John H. Weisburger


Abstract

This conference, attended by scientists from 27 countries, focused on the most recent advances in the field of antimutagenesis and anticarcinogenesis. Particular emphasis was given to the mechanistic approach, which is believed to be an essential prerequisite for a safer and more effective implementation of chemoprevention of cancer and of mutation-related diseases. The arrangement of the six regular sessions basically followed and updated the detailed classification of mechanisms of inhibitors of mutagenesis and carcinogenesis proposed by S. De Flora and C. Ramel (Mutat. Res., 202: 285-306, 1988), covering both extracellular and cellular mechanisms involved in the prevention of mutations and cancer initiation, as well as in the modulation of later stages of the carcinogenesis process. In addition, a workshop was devoted to methodological aspects concerning the modulation of the genotoxic and carcinogenic response.

The present report covers the main themes of overview lectures or research communications presented by more than 60 speakers. Most presentations were multi-authored, as the result of collaborative studies, in several cases at the international level, but only the names of speakers will be given.

Received 7/3/91.

Mechanistic Approaches to Antimutagenesis and Anticarcinogenesis

Session 1 included three introductory lectures on general mechanisms. Silvio De Flora (Institute of Hygiene and Preventive Medicine, University of Genoa, Italy) highlighted the role of antimutagenesis and anticarcinogenesis in the primary prevention of mutations and cancer, as related to other intervention strategies. He discussed the problems involved in chemoprevention and stressed the importance of a mechanistic approach. Based on an updated classification, he provided an analysis of mechanisms of inhibitors exploitable for preventive purposes. R. C. von Borstel (Department of Genetics, University of Alberta, Edmonton, Alberta, Canada) defined the major mechanisms involved in the origin of spontaneous mutations, i.e., the three R's (recombination, replication, and repair) in DNA metabolism. Further protection against spontaneous mutations may be afforded by inhibiting intracellular free radicals and formation of alkylating agents, as well as DNA depurinations and imbalances of nucleotide pools. Several inhibitors are known or, more often, suspected to work through multiple mechanisms, and Luigi M. De Luca (Laboratory of Cellular Carcinogenesis and Tumor Promotion, National Cancer Institute, Bethesda, MD) emphasized the ability of retinoids to profoundly affect tumorigenesis and discussed the underlying biological mechanisms, with special reference to their role in the maintenance of normal cell differentiation. This property involves the nuclear retinoid acid receptors, which belong to the superfamily of steroid/thyroid receptors. The three introductory lectures stressed the possible occurrence of adverse effects of inhibitors, under certain conditions, and the double-edged nature of several mechanisms.

Inhibition of Mutagenesis and Carcinogenesis by Extracellular Mechanisms

An interesting approach to cancer chemoprevention is to intercept harmful agents when they are still in extracellular spaces. A promising method, reported by Helmut Bartsch (International Agency for Research on Cancer, Lyon, France), is to prevent the endogenous formation of N-nitroso compounds from nitrite and amino precursors in the acidic gastric environment but also in other sites at neutral pH, due to catalysis by bacterial enzymes. Inhibitors, such as vitamins C and E, plant phenols, and complex mixtures in the diet, were reviewed along with an evaluation of their mechanisms and efficacy, also supported by epidemiological evidence and by studies in humans. The protective mechanism of dietary fiber which has been associated with a lower risk of colon cancer and breast cancer in animal models and humans, was reviewed by John H. Weisburger (American Health
Modulation of Metabolism and Blocking of Reactive Species

A variety of mechanisms of inhibition can be involved in the intracellular environment. Session 3 dealt with cytoplasmic processes affecting the metabolic activation or detoxification of mutagens/carcinogens, or blocking reactive species, thereby preventing their interaction with critical cell macromolecules. Thomas Kensler (Johns Hopkins University) focused on inducers of electrophile detoxication enzymes, such as the glutathione S-transferases, UDP-glucuronol transferases, and NAD(P)H:quinone one reductase. Chemoprevention can be achieved in many target tissues by administering monofunctional inducers, such as dithiolthiones, which enhance Phase II enzymes without stimulating cytochrome P-450 activities. Peter Moldéus (Department of Toxicology, Karolinska Institute, Stockholm, Sweden) pointed out the protective properties of GSH and of the enzymes involved. A number of GSH analogues and precursors have been studied. The most important is N-acetylcysteine, which effectively evokes glutathione biosynthesis, protects against drug-induced toxicity, and displays a broad array of antimitageneic and anticarcinogenic properties. Michael G. Simic (National Institute of Standards and Technology, Gaithersburg, MD) stressed the importance of reduction of oxidative damage in the overall process of cancer prevention and delineated the mechanism of action of antioxidants, also depending on their redox potential and cell localization. Electron transfer and H-atom transfer from phenolic antioxidants to oxidizing intermediates were evaluated in experimental systems.

These introductory lectures were followed by presentations of original research data. Four of them dealt with the protective effects and mechanisms of sulfur compounds, especially thiols. Giovanni Brambilla (Institute of Pharmacology, University of Genoa, Genoa, Italy) investigated the ability of thiols to reduce DNA fragmentation, as assessed by viscosimetry, induced in rat liver by low doses of N-nitrosodimethylamine. Disulfiram was the most effective of 10 thiols. Roberto Barale (Department of Evolutionary Biology, University of Ferrara, Ferrara, Italy) reported the ability of N-acetylcysteine to inhibit bacterial mutagenicity as well as the enhancement of sister chromatid exchanges in cultured human lymphocytes induced by diesel exhausts. Yasushi Yamazoe (Department of Pharmacology, Keio University, Tokyo, Japan) investigated the ability of thiols to modulate the activity of acetyltransferase and sulfotransferase involved in rat liver metabolism of the heterocyclic amine Glu-P-1. David J. Meyer (CRC Molecular Toxicology Research Group, University College and Middlesex School of Medicine, London, England) demonstrated the induction of GSH S-transferase subunits in the liver cytosol of rats fed with the anticarcinogenic drug oltipraz. Subunits 1b and 3 seemed to detoxify aflatoxin oxide, and subunits 7-7 detoxified benzo(a)pyrene-7,8-diol-9,10-oxide by GSH conjugation. However, the analogy with tumor-promoting agents triggering similar mechanisms was also pointed out. Three presentations reported on the protective effects of antioxidants in different experimental systems. Masao Hirose (First Department of Pathology, Nagoya City University, Nagoya, Japan) described extensive carcinogenicity studies in rats with BHA and several naturally occurring antioxidants, displaying organ-specific activity, especially via stimulation of cell proliferation. Using the oxygen radical generating system of xanthine/xanthine oxidase, Diana Anderson (BIBRA Toxicology International, Carshalton, England) observed that vitamin C was more efficient than vitamin E in inhibiting malformations in whole rat embryo cultures and detachment of germ cells cocultivated with Sertoli cells. Michael J. Plew (Institute for Environmental Studies, University of Illinois at Urbana-Champaign, IL) explored the mechanisms responsible for blocking the activation of aromatics amines by peroxidase inhibitors, such as diethylthiobismarbamate. Cultured tobacco cell suspensions were used as the activating system and bacteria as the genetic indicator organism.

Modulation of DNA Repair and Control of Gene Expression

Session 4 was devoted to intranuclear mechanisms involved in DNA replication and repair in the control of gene expression in mammalian cells, prokaryotes, and oncogenic viruses, David A. Boothman (Department of Radiation Oncology, University of Michigan, Ann Arbor, MI) introduced the complex subject of modulation of DNA repair. Inhibition of DNA repair, which preferentially occurs in areas of the genome undergoing high levels of transcription, leads to a variety of consequences for damaged mammalian cells. A possible use of DNA repair inhibitors is to prevent carcinogenesis by enhancing lethality in damaged cells, as investigated with β-lapachone, a DNA repair inhibitor and topoisomerase I modulator. Curtis C. Harris (Laboratory of Human Carcinogenesis, National Cancer Institute) dealt with modulating effects on oncogenes, tumor suppressor genes, and antimetastasis genes, with emphasis on suppressor genes, their mechanisms, and their involvement in human carcinogenesis. The possibility of modulating gene expression with the goal of cancer prevention is supported by evidence that somatic cell hybrids between normal human bronchial epithelial cells and lung carcinoma lines have a finite life span. The control of the
expression of human papillomavirus in neoplasia was addressed by Joseph A. DiPaolo (Laboratory of Biology, National Cancer Institute), who generated immortalized cell lines derived from human epithelial cervical cells containing integrated, rearranged, and transcriptionally active human papillomavirus genomes. Assays with transforming growth factor β1 and β2, α- and γ-interferon, and leukoregulin established that hepatitis B virus gene expression is differentially regulated by specific cytokines produced by cervical epithelia or by leukocytes infiltrating cervical dysplasia and carcinoma.

The modulation of DNA repair in bacteria was presented by Takehiko Nohmi (National Institutes of Hygienic Sciences, Tokyo, Japan), who suggested that the samAB and the umuDC51 operons play an important role in UV and chemical mutagenesis in Salmonella typhimurium, and by Dietrich Schulte-Frohlinde (Max-Planck Institut für Strahlenkemie, Müllheim a.d. Ruhr, Germany), who showed that misrepair leading to deletion mutation in Escherichia coli-transforming plasmids is strongly influenced by base sequences vicinal to the restriction site.

George Kopsidas (Department of Microbiology, La Trobe University, Melbourne, Australia) found that the frameshift mutagenesis of 9-aminoacridine in S. typhimurium, involving intercalation of its planar molecule into DNA, is modulated by the type of carbon source in the medium, with a significant depression by glucose. Francesco Feo (Institute of General Pathology, University of Sassari, Sassari, Italy) reported on the chemopreventive effects of 5-adenosyl-l-methionine in rat hepatocarcinogenesis models and on the mechanisms involved. S-Adenosyl-l-methionine can counteract hypomethylation and overexpression of c-Ha-ras, c-Ki-ras, and c-myc appearing early during tumor promotion and persisting in neoplastic nodules. Hiroshi Kasai (National Cancer Center Research Institute, Tokyo, Japan) spoke on an international collaborative study (involving Japan, Korea, France, and the United States) on one of the major products of DNA base damage, 8-hydroxyguanine, induced by oxygen radicals, in turn stemming from the action of diverse carcinogens or radiation. Chlorogenic acid extracted from plants inhibited its in vitro formation due to lipid peroxide. Formation of 8-hydroxyguanine in the liver of γ-irradiated mice decreased with time, due to repair by a specific endonuclease, that has been purified. Sahdu Devaki Nandan (Department of Physiology and Pharmacology, Texas A & M University) observed that retinoidic acid increased c-Ha-ras mRNA levels in a rat hepatoma cell line, as well as in rat aortic smooth muscle cells treated in vitro with benzo(a)pyrene.

Mechanisms of Inhibition of Tumor Promotion, Progression, Invasion, and Metastases

Peter Cerutti (Department of Carcinogenesis, Swiss Institute for Experimental Cancer Research, Epalinges, Switzerland) described the fine balance of the multiple components of the antioxidant defense, as a response to growth promotion by oxidants. The oxidative stress triggers (patho)physiological reactions playing a role in inflammation, fibrosis, and tumorigenesis. The importance of cellular antioxidant defenses was demonstrated by the decrease in inducibility of the protooncogene c-fos in transfectants of mouse epidermal cells overproducing superoxide dismutase or catalase. An overview of inhibitors of tumor promotion and progression in the multi-stage mouse skin model was presented by Barbour S. Warren (University of Texas System Cancer Center, Smithville, TX). A variety of mechanisms may be associated with the inhibition of tumor promotion by different agents, e.g., scavenging of free radicals, antiproliferative or antinflammatory effects, alterations of cell differentiation, protein processing or calcium regulation, blocking of prostaglandin synthesis or ornithine decarboxylase induction, cytotoxic effects, etc. Free radical scavenging may be involved in prevention of tumor progression, as shown by the protective effect from the topical application of GSH. Charles W. Boone (Chemoprevention Branch, National Cancer Institute) described the intraepithelial neoplasia (or dysplasia) which precedes invasive epithelial cancer. Cancer chemopreventive agents can inhibit the progression of the intraepithelial neoplasia by two basic mechanisms, i.e., mutagen blocking and proliferation suppression. Whatever the mechanism, inhibitors are expected to prolong the latency period before the appearance of cancer.

These introductory lectures were followed by research reports. Ann R. Kennedy (Department of Radiation Oncology, University of Pennsylvania, Philadelphia, PA) dealt with the anticarcinogenic effects of protease inhibitors, especially the soybean-derived Bowman-Birk inhibitors. Multiple mechanisms are involved, including the normalization of some carcinogen-induced effects, such as increased levels of proteolytic activities, gene amplification, or c-myc expression. Minako Nagao (National Cancer Center Research Institute, Tokyo, Japan) highlighted the role of PP involved in the signal transduction of malignant transformation, as shown, e.g., by an enhanced mRNA expression of PP-2Aα in hepatocellular carcinomas induced by carcinogens produced during cooking. Okadaic acid, a specific inhibitor of PP-1 and PP-2, yielded flattened forms of activated c-ras and retTPC

Transformants of NIH 3T3 cells. Masami Suganuma (National Cancer Center Research Institute, Tokyo, Japan) discussed the antipromoting properties of natural compounds, i.e., sarcophytol A, epigallocatechin gallate, and cryptoporic acids D and E, isolated from a soft coral, green tea, and a fungus, respectively, which partly act by suppressing oxyradicals. She proposed a combination of various antitumor promoters for cancer chemoprevention. Patrizia Hrelia (Department of Pharmacology, University of Bologna, Bologna, Italy) reported on the effects of α- and β-interferons on benzo(a)pyrene metabolism and clastogenicity. Treatment of mice with interferons results in a temporary depression of P-450 isozymes and of hepatic oxidative drug metabolism, as well as of benzo(a)pyrene-induced clastogenicity in bone marrow cells. Vladimir Krutovskikh (International Agency for Research on Cancer, Lyon, France) observed that functional gap junctional intercellular communications and cell adhesion molecules are necessary for efficient tumor suppression and anticarcinogenesis. Adriana Albini (National Institute for Cancer Research, Genoa, Italy) used an in vitro reconstituted basement membrane as a model to study tumor cell invasion and metastases. Invasion of basement membranes can be inhibited by several compounds such as TIMP-2, a member of the tissue inhibitor of metalloproteinase (TIMP) family, inhibitors of collagenase IV, and a cysteine-containing synthetic peptide. Moreover, invasiveness of oncogene-transformed cells was inhibited by interferons.
Prospects for the Chemoprevention of Mutations and Cancer

Session 6 was devoted to applied aspects of antimutagenesis and anticarcinogenesis, but with emphasis on the mechanistic approach. Charles W. Boone described the advances in chemopreventive drug development at the Chemoprevention Branch of the National Cancer Institute. So far, 11 high-priority agents have been identified, i.e., the retinoids isoretinoin and fenretinide, piroxicam, ibuprofen, calcium, difluromethylornithine, glucaric acid, olibraz, glycyrrhetinic acid, N-acetylcysteine, and a dehydroepiandrosterone analogue. Hikoya Hayatsu (Faculty of Pharmaceutical Sciences, Okayama University, Okayama, Japan) reviewed the mechanisms of inhibitors in the diet. He emphasized the role of the diet as a source of antimutagens and anticarcinogens, illustrated by food-borne inhibitors, and of the possible mechanisms involved. On the general subject of prevention of mutation and cancer, Mark Shigenaga (Division of Biochemistry and Molecular Biology, University of California, Berkeley, CA) dealt with the practical importance of an adequate intake of antioxidants in the prevention of the degenerative diseases associated with aging, such as cancer, heart disease, and cataracts. He also stressed the role of mitogenesis in carcinogenesis and consequently the possibility of preventing cancer by means of anti-proliferative agents.

Cesare Maltoni (Istituto Oncologico Addari, Bologna, Italy) performed large-scale experiments in rodents to study the inhibition of both spontaneous and induced cancer. He discussed extrapolation to humans and stressed the usefulness of these approaches to cancer chemopreventive strategies. Piero Dolara (Department of Pharmacology, University of Florence, Florence, Italy) demonstrated a protective role of starch against 1,2-dimethylhydrazine-induced colon carcinogenesis. Compared with an isocaloric sucrose diet, starch decreased the proliferation and the number of dysplastic crypts in the colon, accompanied by a higher concentration of short-chain fatty acids. Using the rainbow trout, George Bailey (Department of Food Science and Technology, Oregon State University, Corvallis, OR) proposed that the anticarcinogenicity of indole-3-carbinol toward aflatoxin B1, N-nitrosodiethyamine, and 7,12-dimethyl-benzanthracene may be a function of its in vivo conversion, at low stomach pH, into potent inhibitors of P-450-1A1 catalytic activity. Anticarcinogenicity of chlorophyllin in the same model, toward aflatoxin B1, IQ, and Trp-P-2, may involve several mechanisms, including formation of chlorophyllin-carcinogen complexes. Siegfried Knasmüller (Institute of Tumor Biology and Cancer Research, University of Vienna, Vienna, Austria) investigated the antigenotoxic effects of dietary constituents toward N-nitroso compounds, using a host-mediated assay in several organs of mice carrying repair-proficient or -deficient E. coli strains. R. J. Sram (Psychiatric Centre, Prague, Czechoslovakia) reported that dietary α-tocopherol and ascorbic acid supplementation in two communities of elderly people had beneficial effects, including a lowered death rate and protection against age-dependent increase in unscheduled DNA synthesis and lipid peroxidation.

A special session dealt with the general subject of chemoprevention. Monroe E. Wall (Research Triangle Institute, Research Triangle Park, NC) isolated antimutagenic agents with novel structures, by the large-scale screening of natural products of terrestrial or marine origin. The antimutagenic effects of dietary leafy vegetables were ascribed by Ebata Junko (Faculty of Science of Living, Osaka City University, Osaka, Japan) to ·OH radical scavenging and catalase activity. C. Ioannides (Division of Toxicology, University of Surrey, Guildford, England) described the conflicting results obtained with anthraflavic acid, a plant-derived compound, which in vitro was antimutagenic toward the food carcinogen IQ, but in rats induced P450 activity in liver that gave higher mutagenic activity with IQ. Norbert Frank (German Cancer Research Center, Heidelberg, Germany) showed that prolinedithiocarbamate is a less toxic and more effective inhibitor of N-nitrosodiethyamine carcinogenesis in rats than disulfiram, decreasing the metabolic activation of the carcinogen and modulating the cellular immune system. Hideki Mori (Department of Pathology, Gifu University, Gifu, Japan) demonstrated that dietary magnesium hydroxide influences the cell cycle in the large bowel and protects against colon carcinogenesis induced by methylazoxymethanol and 1,2-dimethylhydrazine.

Poster Presentations

Approximately 60 posters, dealing with a variety of research data related to antimutagenesis and anticarcinogenesis, were displayed at ICMAA-III. A plenary poster discussion was chaired by John H. Weisburger (American Health Foundation, Valhalla, NY) and Giorgio Cantelli Forti (Department of Pharmacology, University of Bologna, Bologna, Italy). The opportunity was given not only to experienced scientists but also to younger investigators, speaking for the first time to an international audience, to discuss their findings and conclusions in a lively and friendly atmosphere.

Assessment of Antimutagenicity and Anti-carcinogenicity: End Points and Systems

A special workshop was aimed at discussing methodological aspects, which not only are relevant for the assessment of protective effects in mutagenesis and carcinogenesis but also provide suitable tools for exploring the mechanisms involved. Frits H. Sobels (Department of Radiation Genetics and Chemical Mutagenesis, State University of Leiden, Leiden, The Netherlands) introduced this workshop, to be published in Mutation Research.

Michael D. Waters (U.S. Environmental Protection Agency, Research Triangle Park, NC) illustrated the concept of activity profiles. After successful use in a display of mutagenicity data, activity profiles have been adapted to show antimutagenicity data from short-term tests. Italo Barrai (Department of Evolutionary Biology, University of Ferrara, Ferrara, Italy) described statistical methods, based on multiple regression and tables of proportions, to evaluate the combined activity of different agents in reversion systems. Silvio De Flora (Institute of Hygiene and Preventive Medicine, University of Genoa, Genoa, Italy), dealing with the modulation of the mutagenic response in prokaryotes, described methodological variations of mutagenicity and DNA repair tests in bacteria for assessing antigenotoxic effects. He stressed the role of these techniques in understanding the mechanisms of inhibitors but cautioned against oversimplification and
technical inadequacies. Giorgio Bronzetti (CNR Institute of Mutagenesis and Differentiation, Pisa, Italy) discussed short-term test systems in yeasts for assessing the antimutagenicity of compounds reacting with mutagens or interfering with cellular functions, and gave examples of mechanisms of inhibitors of physical or chemical mutagens in strain D7 of *Saccharomyces cerevisiae*. Yukiaki Kuroda (Azabu University, Sagamihara, Japan) discussed the methodologies used for assessing antimutagenic effects, with special reference to the evaluation of 6-thioguanine-resistant mutations in Chinese hamster V79 cells, and using pre- or posttreatment techniques. Erich Gebhart (Institute of Human Genetics, University Erlangen-Nürnberg, Nürnberg, Germany) reviewed in detail test systems evaluating anticlastogenicity in cultured mammalian cells. The results are considerably influenced by the experimental conditions as well as by the end point investigated, e.g., chromosome aberrations or sister chromatid exchanges.

The second part of the workshop was devoted to in vivo test systems. Claes Ramel (Department of Genetic and Cellular Toxicology, University of Stockholm, Stockholm, Sweden) discussed the modulation of genotoxicity in *Drosophila* test systems, in particular the somatic mutation and recombination tests (SMART), a useful model for elucidating the mechanisms of inhibition of recombinogenic and mutagenic effects. Lester Mitscher (Department of Medicinal Chemistry, Kansas University, Lawrence, KS) commented on methodologies for isolating and screening antimutagens in pure form from higher plants. An interesting system, according to George S. Bailey (Department of Food Science and Technology, Oregon State University, Corvallis, OR), is fish, such as rainbow trout, since as many as 10,000 animals can be used, thereby providing high statistical power as to protective or adverse effects toward various end points (biochemical indices, carcinogen-DNA adducts, neoplastic lesions). Charles W. Boone (Chemoprevention Branch, National Cancer Institute) described chemically induced autochthonous tumor systems to assess the efficacy of candidate chemopreventive agents, namely chemically induced mouse skin papillomas, rat mammary adenocarcinoma, hamster tracheal squamous cell carcinoma and lung adenocarcinoma, rat or mouse colon adenocarcinoma, and mouse bladder carcinoma.

Four reports dealt with the monitoring of mutation or cancer protective effects in humans. Mortimer L. Mendelsohn (Lawrence Livermore National Laboratory, Livermore, CA) discussed the estimation of somatic mutational effects, feasible in humans for five assay-gene combinations. He commented on the hypoxanthine phosphoribosyltransferase and glycophorin A assays for evaluating antimutagenic effects in humans, which were used to find either a reduction of background levels or a modulation of response to known mutagens. Michael G. Simic (National Institute of Standards and Technology, Gaithersburg, MD) proposed the application of urinary biomarkers for evaluating the protective effects of antioxidants in the diet or as chemopreventive additives. These markers involve detection in urines of thymidine glycol and 8-hydroxydeoxyguanosine, two products of ·OH radical reactions with DNA bases. Miriam P. Rosin (Cell Biology Unit, Simon Fraser University, Burnaby, British Columbia, Canada) discussed the evaluation of anticlastogenicity in humans, especially the micronucleus test on exfoliated cells in individuals in populations at elevated risk for cancer. This technique was particularly useful as an intermediate end point during treatment with chemopreventive agents. Charles W. Boone reviewed chemoprevention trials by the National Cancer Institute Chemoprevention Branch. Third-generation trials, now in progress, evaluate the chemoprevention of cancer at various sites (skin, breast, lung, colon, and cervix) in high-risk individuals.
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