科技资料

Chemoimmuno Prevention of Cancer

1st International Conference, Vienna, Austria

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60 Illustrations, 77 Tables

1991
Georg Thieme Verlag
Stuttgart · New York

Thieme Medical Publishers, Inc.
New York

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Die Deutsche Bibliothek – CIP-Einheitsaufnahme

Chemoimmuno prevention of cancer : ... international conference ... – Stuttgart ; New York : Thieme ; New York : Thieme Med. Publ.

1. Vienna, Austria. – 1991

Printed in Germany by WB-Druck, D-8959 Rieden

ISBN 3-13-768901-5

Preface

In the last decade, chemoprevention research for cancer control and prevention has received tremendous impetus. The discovery of many biological response modifiers and new chemopreventive agents coupled with the understanding of complex regulatory processes at the molecular level, has given rise to the possibility of utilizing chemopreventive approaches as potential future treatment for high risk patients.

This book contains selected high quality manuscripts of oral presentations from the ChemoImmunoprevention meeting, CCPC-90, which was held in Vienna, Austria in August 1990.

The goal of this meeting was to bring together scientists, all of whom are working on different aspects of these multifaceted problems, to establish collaboration, share data and material, and discuss major issues of cancer prevention and control. This goal was amply met, demonstrated by the manuscripts derived from the selected talks as presented in this book.

We are indebted to Mucos Pharmaceutical Company and to the Vienna Academy of Medicine for sponsoring and organizing the CCPC-90 Conference.

Plans for the CCPC-92 Conference, which will be held in Berlin on August 1992, are under way.

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New Directions of Chemoprevention Research

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The opportunities developing for advances in cancer prevention in the 1990s are growing rapidly as a result of basic research discoveries. Molecular changes associated with cancer risk are increasingly accessible to study, and some of these findings will almost certainly lead to research on ways to reduce cancer incidence. New focuses of cancer prevention research are directed at identifying the cellular, molecular, and biochemical factors that can be used to predict future progression to neoplasia and at assessing the efficacy of intervention agents, including certain nutrients that may inhibit or prevent this progression. These biological or intermediate endpoint markers will expand the scope and add to the efficiency of future cancer prevention research.

The Cancer Prevention Research Program at the National Cancer Institute

A prevention goal at the National Cancer Institute (NCI) is to narrow the time between predicting cancer risk and implementing prevention strategies, in part by supporting research to understand the mechanisms related to cancer etiology and to transfer these findings into public health practices. The Cancer Prevention Research Program at NCI plans, promotes, and conducts extramural and intramural research directed toward the development and demonstration of existing and new approaches to cancer prevention. The extramural and intramural programs complement each other. The intramural program conducts research relating cancer to diet, nutrition, genetics, chemoprevention, and other cancer prevention strategies designed to lower human cancer incidence. A new intramural Laboratory of Nutritional and Molecular Regulation conducts research in basic science relevant to nutrition and cancer, emphasizing the basic mechanisms by which nutrients directly or indirectly alter the carcinogenic process. Complementary to this research area are two related extramural programs: Diet and Cancer and Chemoprevention. Studies aim at identifying persons at high risk of cancer and designing interventions to decrease that risk through dietary manipulation or administration of chemopreventive agents that may inhibit aspects of the carcinogenic process. These programs, discussed in the following section, bridge basic research to broad public applications.

Chemoprevention

In 1982, the Chemoprevention Program was established at the NCI to identify and evaluate the efficacy of specific micronutrients, non-nutrients, and drugs in reducing human cancer incidence. The program involves the integration of results from preclinical studies to identify candidate cancer-inhibiting agents

for testing in human chemoprevention trials. The NCI has developed a structured process for the testing of promising chemopreventive cancer-inhibiting agents, including research involving a broad range of modalities from tissue culture to clinical trials in humans [1]. An oversight committee selects agents that demonstrate chemopreventive potential. A series of stages designed to test systematically the efficacy and safety of the agent uses both *in vitro* and *in vivo* test systems. These systems reflect a range of carcinogenesis models chosen to provide the information that is most needed to consider human intervention.

At present, the preclinical chemoprevention program is studying 123 agents *in vitro*, 95 agents *in vivo*, and 5 agents undergoing animal toxicity testing. During the past year, a number of additional chemical and pharmaceutical agents have been shown to have potential to be further evaluated in clinical chemoprevention trials. As a group, the synthetic retinoids remain one of the most promising chemopreventives. Under investigation are the retinamide all-trans-N-(4-hydroxyphenyl) (4-HPR); a prostaglandin synthesis inhibitor, piroxican; a pharmaceutical, the ornithine decarboxylase inhibitor difluoromethylornithine (DFMO); and several other pharmaceuticals and naturally occurring constituents and trace minerals. Although a large number of agents may be evaluated initially, only a limited number meet the rigorous criteria required for clinical testing [2,3].

Diet and cancer

The Diet and Cancer Program at NCI parallels the Chemoprevention Program but focuses on dietary manipulation and modification as the intervention for inhibiting neoplastic progression. Epidemiologic and animal studies have demonstrated that diet may be one of the most important factors involved in the etiology of specific cancers and other chronic diseases. Among the dietary and nutritional risk factors that have been identified as disease-related are specific dietary components, various foods or food groups, and overall dietary patterns [4,5]. Dietary fat [6], implicated in cancer promotion, is a current research focus of this program.

There remains, however, a lack of consensus on the precise role of diet in the etiology and prevention of various cancers. Inconsistent findings have been difficult to interpret. Measurement errors of dietary intake in general and imprecision in methods for estimating food and nutrient intake may in part account for the inconsistencies.
Currently, for example, there is no biochemical measure that provides a good index of true total fat intake to validate self-reported dietary intake or to monitor adherence to diets differing in total fat content. The availability of biochemical/biological indicators of dietary exposure and compliance monitoring remains a challenge, and specific research recommendations are being made for the development of state-of-the-art methods for monitoring compliance and evaluating the efficacy of an intervention.

Two recent reports, the *Surgeon General's Report on Nutrition and Health* [7], and the National Research Council's report on *Diet and Health* [8], have identified the need for the development of markers of dietary intake and of exposure to dietary fats as specific research recommendations.

Research designs and strategies for studying the health consequences of dietary modification among women were explored at a July 1990 conference jointly sponsored by the NCI and the National Heart, Lung, and Blood Institute. Discussions focused on experimental and mechanistic research

and trial design strategies, issues of the feasibility of dietary modification and dietary compliance, and the methodologies available for assessing dietary change.

Discussants agreed that for studying the health consequences of dietary modification for cancer and cardiovascular disease prevention requires a broad program with a clinical trial as a focal point. A systematic effort was advocated that would also include observational studies within populations in the United States and other countries, intermediate marker studies, and laboratory studies that characterize the biochemical correlates of breast cancer incidence and heart disease. It was noted that the various dietary guidelines promulgated by the National Cancer Institute, the National Heart, Lung, and Blood Institute, the United States Department of Agriculture, and other groups are in general agreement; conflicting messages to the public of one heart disease diet and one cancer diet should be avoided.
Recommendations from the workgroup are to be used for the planning phases of this research.

Clinical metabolic studies

In an effort to lower human cancer risk, prevention research efforts have been initiated that build upon observational epidemiology and carcinogenesis research. Clinical metabolic studies are important components of cancer prevention research. They provide insights into cancer etiology, the kinetics and toxicity of specific preventive substances, and the monitoring of specific intermediate biological endpoints in cancer prevention research. The NCI sponsors Clinical Nutrition Research Units and has an interagency agreement with the United States Department of Agriculture for selected collaborative clinical metabolic studies. For example, clinical metabolical studies have been conducted to determine whether high dietary intakes of selenium may be associated with adverse health effects. Potential subclinical manifestations of even moderately high selenium intake should be examined carefully to ensure that the toxicity of this mineral, shown to be chemopreventive in animals, would not preclude its inclusion in a clinical protocol. In another example, lipid and lipoprotein responses were assessed in healthy volunteers. An experimental low-fat, low-saturated-fat, low-cholesterol, high-fiber diet significantly reduced plasma cholesterol, demonstrating that diet can be a significant modulator of plasma cholesterol levels.

Clinical Chemoprevention Trials

Human chemoprevention trials are a major area of emphasis in cancer prevention and control research. More than 20 clinical intervention trials supported by the NCI, mostly conducted in medical settings, are currently evaluating the efficacy of chemopreventive agents. Figure 1 shows the general strategy for flow of chemoprevention research, and Table 1 presents selected current chemoprevention intervention trials. Most of these trials are using biologic markers for measuring efficacy.

Phase I studies provide preliminary information on dose ranges, dose response, and efficacy. Examples of compounds undergoing phase I testing following confirmation of experimental chemopreventive efficacy are the ornithine decarboxylase inhibitor difluoromethylornithine (DFMO), a synthetic pharmaceutical that demonstrates chemopreventive activity in rat mammary and bladder target sites and mouse skin and mouse colon cancer sites, and the dithiolthione Oltipraz, an antioxidant in wide use as an antischistosomiasis drug. The drug enhances

Chemoprevention

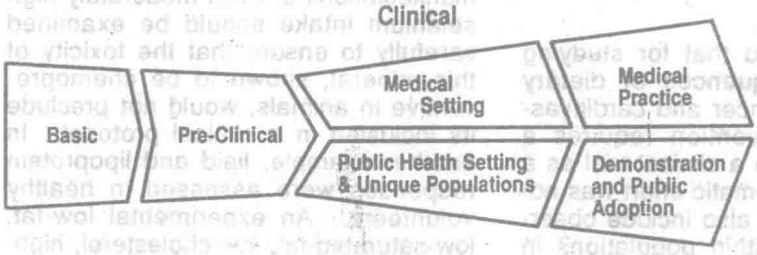

Fig. 1

Table 1. Selected Current Chemoprevention Intervention Trials*

Target Site	Target/Risk Group	Inhibitory Agents
Breast	Adenocarcinoma	4-HPR
Cervix	Mild/moderate cervical dysplasia	Trans-retinoic acid
Cervix	Cervical dysplasia	Folic acid
Colon	Previous colon adenoma	Wheat bran and calcium carbonate
Colon	Previous colon adenoma	Calcium
Colon	High-risk epithelial cell proliferation	Calcium
Colon	Previous adenomatous polyps	Beta-carotene
Colon	Previous adenomatous polyps	Piroxicam
Lung	Chronic smokers	13-cis-retinoic acid
Lung	Men, exposed to asbestos	Beta-carotene and retinol
Lung	Cigarette smokers	Beta-carotene and retinoids
Lung	Smoking males	Beta-carotene
Skin	Albinos in Tanzania	Beta-carotene
Skin	Previous basal cell carcinoma	Beta-carotene
Skin	Actinic keratoses patients	Retinol
Skin	Previous basal cell carcinoma	Retinol or 13-cis-retinoic acid
Oral cavity	Leukoplakia	13-cis-retinoic acid ± beta-carotene
Oral cavity	Leukoplakia	Beta-carotene
All sites	American physicians	Beta-carotene, aspirin

* National Cancer Institute, Division of Cancer Prevention and Control

electrophilic detoxification, increases pools of glutathione levels in tissues, and exhibits strong *in vivo* chemopreventive activity.

Phase II trial evidence for whether intermediate marker endpoints can be used to detect preneoplastic abnormalities is now being gathered for several tumor sites, notably lung and colon. One such trial is determining whether a marker of early lung carcinoma can be reliably identified and whether beta-carotene can modify the frequency of progression of this marker. Four hundred cigarette smokers will be studied to determine whether quantitative analysis of DNA in sputum epithelial cells can be used as a marker of premalignant abnormalities. The second endpoint in this trial is lung cancer incidence. A further intermediate marker endpoint trial is analyzing the incidence, prevalence, and modification of sputum and tissue atypia in high-risk asbestos workers. Enrollment is proceeding with subsequent randomization to chemoprevention groups receiving either beta-carotene, retinol, or placebo daily [3].

A randomized trial to evaluate the role of dietary wheat bran fiber and calcium supplements for modifying the risk of colon cancer through the prevention of recurring colon polyps is measuring several biochemical and biological markers as trial endpoints.

An important highlight of the past year has been the study published by Dr. Jerome Decosse and his group from the Cornell Medical Center [9]. This three-armed study of control, vitamins C and E alone, and vitamins C and E and a wheat fiber supplement showed a statistically significant reduction in colon polyps in the fiber/vitamin group. Using a polyp marker endpoint, not cancer incidence, this study is the first demonstration of an intervention trial that is randomized and blinded and that provides prospective evidence that adding a fiber supplement reduces a risk factor for colon cancer. Patients were sigmoidoscoped, and the number and diameter of colon polyps were measured as trial endpoints. When adjusted for patient compliance, the results were statistically significant for inhibition of benign large bowel neoplasia by the consumption of dietary fiber supplements [9]. In a more extensive study to be conducted in 1,000 subjects previously diagnosed with adenomatous polyps and 1,000 controls, NCI is initiating a trial to investigate reducing the recurrence of this precancerous lesion by dietary modification with a low-fat, high-fiber, and fruit- and vegetable-enriched diet.

Clinical trials, it should be noted, are also in progress in public health settings, and results will be available later in this decade. The study by Dr. Hennekens and his colleagues is notable, in which 22,000 healthy physicians are self-administering a beta-carotene supplement or placebo to determine the effect on overall cancer incidence [10]. The NCI and the National Public Health Institute of Finland are conducting a large-scale lung cancer prevention trial testing the oral administration of beta-carotene and alpha-tocopherol in a population of heavy smokers [11]. With a high lung cancer incidence coupled with marginal *per capita* intake of several micronutrients, Finland offers a unique environment for the study of lung cancer prevention. Four separate treatment groups are being evaluated in a population of 29,000 high-risk men, ages 50 to 69, using a 2 by 2 factorial design. The use of factorial designs, which evaluate two or more hypotheses in a single trial with a minimal increase in cost, is particularly suited to prevention trials. Clinical follow-up procedures for the Finland study will be used to diagnose cancers, and a reduction in cancer incidence, as demonstrated by

the trial, will be compared with national trends by monitoring Finland's unique government-operated health registers.

Many of the above trials shorten the intervention period by assessing alterations in precancerous lesions that may be associated with future malignancy, rather than measuring cancer incidence endpoints. In addition to lung and colon cancer, marker characterization is being applied to nutrition and chemopreventive interventions of the oral cavity, skin, esophagus, and cervix. There are also innumerable potentially useful serum markers for use alone or in combination with other data for assessing cancer risk potential or subject compliance with a study protocol.

The choice of a marker or precancerous lesion as an endpoint for a clinical intervention study is based on the strength of its association with a specific cancer, the prevalence of this lesion in a study population, its absence in a control group, and the ease with which the endpoint can be quantitatively evaluated [12]. In order to act as reliable indicators of future cancer incidence, these markers must be statistically validated in large clinical trials before researchers can be confident of their predictive value. Clinical intervention trials using intermediate endpoint markers have the advantage of requiring less time and resources than a standard prevention trial to achieve comparable statistical power.

The NCI continues to explore options for an explicit research agenda for reducing breast cancer incidence. One study under consideration for which there is growing interest, is using the antiestrogen Tamoxifen as a chemopreventive agent [13]. Based on preclinical work suggesting possible synergism, a factorial design testing both Tamoxifen and 4-hydroxyphenyl retinamide (4-HPR) might be considered for large-scale intervention. Experimental evidence in rats showed that following surgical resection of primary tumors, combined treatment with 4-HPR and Tamoxifen was consistently more effective than either single agent [14]. Although the antineoplastic efficacy of any single agent may be of clinical importance, the eventual combination of chemopreventive agents may show even greater clinical activity [15] and be particularly useful for selected high-risk groups.

Increased awareness of the future impact of biological markers in chemoprevention research and the hope that significant numbers of markers may be validated as reliable cancer predictors have resulted in the inclusion of these measures whenever possible as an additional facet of full-scale intervention trials. Positive results from these efforts may eventually expand medical practice to include the idea of early detection of susceptibility factors and precancer, with chemopreventive interventions of those so identified, as part of the regular practice of medicine. One potential goal that the medical practitioner and the public may look forward to is the possibility that a potentially high probability of risk may be titrated to lower individual cancer risk. Advances from chemoprevention and diet and cancer research may significantly contribute to these possibilities.

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Biological Significance of Autologous Tumor Killing in Human Cancer Patients and Its Modulation by Biological Therapy

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Cell-mediated cytotoxicity *in vitro* is considered to be one of the expressions of host defense against cancer. The outcome of the cytotoxicity assay depends on several factors: the immunological history of lymphocyte donors; the activation profile and composition of lymphocyte populations; the previous treatment of effector cells; the characteristics of target cells, which may represent a balance of the cytolytic potential of various effector cells, their subsets and antibodies with affinity of target recognition sites, and other factors contributing to innate target cell susceptibility [1]. Most studies on cell-mediated cytotoxicity against tumor have been performed by using of *in-vitro* cell lines as targets. It is, however, difficult to interpret the data on cytotoxicity against cultured tumor cells because tumor cells alter their susceptibility to cell-mediated lysis when cultured *in vitro* [2]. For a better understanding of the cytolytic function of lymphocytes against tumor in cancer patients, it is desirable to perform a cytotoxicity assay with autologous combination of fresh effector and target cells. Peripheral blood lymphocytes (PBL) from approximately 10% to 50% of human cancer patients, depending on histological types of tumors and metastatic status, expressed lysis of tumor cells freshly isolated from the same patients in a short-term assay [3-5]. The results we obtained from the studies on the population and at single-cell level indicate that $CD3^-CD16^+$ large granular lymphocytes (LGL) from the blood and tumor tissues of cancer patients lyse autologous, freshly isolated tumor cells [6,7] and release a novel cytotoxic factor with lytic activity against autologous and allogeneic fresh human tumor cells [8,9]. Autologous tumor cell killing (ATK) activity was also mediated by $CD3^+CD16^-$ T lymphocytes when patients had localized neoplasms, while it was observed primarily with LGL in patients with metastatic cancer [10,11]. In patients with metastatic cancer, LGL inhibited the proliferation of T lymphocytes and the development of their ability to kill autologous tumor cells in the autologous mixed lymphocyte-tumor culture (AMLTC) [12,13]. This is not the case with patients with localized tumors.

We have previously reported that systemic administration of OK432, a heat- and penicillin-treated pulverized preparation of the low virulent Su strain of streptococcus pyogenes of human origin, has been shown to prolong the survival rate in patients with advanced stages of cancer [14]. Our subsequent reports demonstrated that OK432 induces or augments ATK activity, NK activity and anomalous killer activity both *in vivo* and *in vitro* [5,15]. Here we describe the biological and clinical significance of blood ATK activity in human cancer patients and an *in-vivo* induction of ATK activity prior to surgery by