International Research Conference on Food, Nutrition, and Cancer

Cancer Inhibition by Inositol Hexaphosphate (IP_6) and Inositol: From Laboratory to Clinic[1,2]

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ABSTRACT Inositol hexaphosphate (IP_6) is a naturally occurring polyphosphorylated carbohydrate that is present in substantial amounts in almost all plant and mammalian cells. It was recently recognized to possess multiple biological functions. A striking anticancer effect of IP_6 was demonstrated in different experimental models. Inositol is also a natural constituent possessing moderate anticancer activity. The most consistent and best anticancer results were obtained from the combination of IP_6 plus inositol. In addition to reducing cell proliferation, IP_6 increases differentiation of malignant cells, often resulting in a reversion to normal phenotype. Exogenously administered IP_6 is rapidly taken into the cells and dephosphorylated to lower-phosphate inositol phosphates, which further interfere with signal transduction pathways and cell cycle arrest. Enhanced immunity and antioxidant properties can also contribute to tumor cell destruction. However, the molecular mechanisms underlying this anticancer action are not fully understood. Because it is abundantly present in regular diet, efficiently absorbed from the gastrointestinal tract, and safe, IP_6 holds great promise in our strategies for the prevention and treatment of cancer. IP_6 plus inositol enhances the anticancer effect of conventional chemotherapy, controls cancer metastases, and improves the quality of life, as shown in a pilot clinical trial. The data strongly argue for the use of IP_6 plus inositol in our strategies for cancer prevention and treatment. However, the effectiveness and safety of IP_6 plus inositol at therapeutic doses needs to be determined in phase I and phase II clinical trials in humans. J. Nutr. 133: 3778S–3784S, 2003.

KEY WORDS: • *prevention* • *treatment* • *differentiation* • *phytic acid*

Cancer remains a major health problem in the United States and in other developed countries (1). In our continuing effort to reduce the public health burden of cancer, there is a constant search for more effective cancer treatment, and increased interest in the concept of prevention, as a promising approach to the control of cancer (2).

A novel anticancer function of inositol hexaphosphate (IP_6;[4] also InsP_6 and phytic acid) has been shown both in vivo and in vitro (3–5). IP_6 is a polyphosphorylated carbohydrate, contained in high concentrations (0.4–6.4%) in cereals and legumes (6). *Myo*-inositol is a parent compound of IP_6. Only *myo*-inositol hexaphosphate has been found in plants; *neo*-, *chiro*-, and *scyllo*-inositol hexaphosphates have been isolated from soil (7). The phosphate grouping in positions 1, 2, and 3 (axial-equatorial-axial) is unique for IP_6, providing a specific interaction with iron to completely inhibit its ability to catalyze hydroxyl radical formation, making IP_6 a strong antioxidant, probably still the only role of IP_6 that is widely recognized and accepted.

Almost all mammalian cells contain IP_6 and much smaller amounts of its forms with fewer phosphate groups ($IP_{1\text{-}5}$), which are important for regulating vital cellular functions. Inositol occurs ubiquitously in cell membranes in conjugation with lipids, as phosphatidylinositol. Recently, inositol phospholipids in the plasma membrane have received much attention because of their biological significance for signal transduction systems. Phosphatidylinositol 4,5-bisphosphate (PIP_2), a phosphoinositide, is a precursor for several informational molecules in signal transduction—inositol 1,4,5-P_3 (IP_3), 1,2-diacylglycerol, and phosphatidylinositol 3,4,5-trisphosphate—linking receptor stimulation to Ca^{2+} mobilization (8). A second messenger role in intracellular Ca^{2+} homeostasis for IP_4 was also shown. It is now recognized that subsequent to PIP_2 hydrolysis a cascade of inositol phosphate metabolites are formed and that these multiple isomers show a complex pattern of interconversion (8–10). Inositol phosphates are versatile molecules with important roles in controlling diverse cellular

[1] Presented as part of a symposium, "International Research Conference on Food, Nutrition, and Cancer," given by the American Institute for Cancer Research and the World Cancer Research Fund International in Washington, D.C., July 17–18, 2003. This conference was supported by Balchem Corporation; BASF Aktiengesellschaft; California Dried Plum Board; The Campbell Soup Company; Danisco USA, Inc.; Hill's Pet Nutrition, Inc.; IP-6 International, Inc.; Mead Johnson Nutritionals; Roche Vitamins, Inc.; Ross Products Division; Abbot Laboratories; and The Solae Company. Guest editors for this symposium were Helen A. Norman and Ritva R. Butrum.

[2] Studies from the authors' laboratories were supported by the American Institute for Cancer Research, the Susan Komen Breast Cancer Foundation, the University of Maryland Designated Research Initiative Fund, and the University of Maryland Women Health Research Foundation.

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[4] Abbreviations used: Ins, inositol; IP_6, inositol hexaphosphate; IP_3, inositol 1,4,5-P_3; KS, Kaposi's sarcoma; PIP_2, phosphatidylinositol 4,5-bisphosphate.

activities (9,10). IP_6 may serve as a natural antioxidant (11) and possibly as a neurotransmitter (10). Different binding proteins for inositol polyphosphates have been isolated, indicating their importance for the cellular functions (12) such as effects on ion channels and protein trafficking (13,14), endocytosis (15), exocytosis (16), and efficient export of mRNA from the nucleus to the cell (17).

How can exogenously administered IP_6 affect tumor growth? Pioneering experiments showing this novel anticancer feature of IP_6 were performed by Shamsuddin et al. (18–20), who were intrigued by the epidemiologic data indicating that only diets containing a high IP_6 content (cereals and legumes) showed a negative correlation with colon cancer. Almost 15 y ago, Shamsuddin et al. hypothesized that IP_6 can be internalized by the cells and dephosphorylated to IP_{1-5} and then can enter into the intracellular inositol phosphate pool and inhibit tumor growth. It was also hypothesized that the addition of inositol, a precursor of inositol phosphates and also a natural carbohydrate, to IP_6 may enhance the anticancer function of IP_6 (18–20). Because inositol phosphates are common molecules involved in signal transduction in most mammalian cell systems, it was further hypothesized that the anticancer action of inositol phosphates would be observed in different cells and tissue systems (18–20). All these proposed hypotheses have been confirmed.

Contrary to the dogma and skepticism at that time, we showed that IP_6 is taken up by malignant cells (21) and that orally administered IP_6 can reach target tumor tissue distant from the gastrointestinal tract (22). Because of the highly charged nature of IP_6, it was a common misconception that it could not be transported into the cells. Analyzing absorption, intracellular distribution, and metabolism of IP_6 in HT-29 human colon carcinoma and cells of hematopoietic lineage (K-562, human erythroleukemia and YAC-1, mouse lymphoma cells), we found that IP_6 is rapidly taken up by mechanisms probably involving pinocytosis or receptor-mediated endocytosis, transported intracellularly, and dephosphorylated into inositol phosphates with fewer phosphate groups (21). Similar data were obtained when MCF-7 human breast cancer cells were incubated with [^{3}H]-IP_6 (SA 444 GBq/mmol, 370 Bq/10^6 cells): as early as 1 min after incubation, 3.1% of IP_6-associated radioactivity was taken up by MCF-7 cells, and 9.5% after 1 h. By differential centrifugation 86% radioactivity was recovered from the cell cytosol. Anion-exchange chromatography showed that 58% of the absorbed radioactivity was in IP_6 form. When [^{3}H]-IP_6 was administered intragastrically to rats, it was quickly absorbed from the stomach and upper intestine and distributed to various organs as early as 1 h after administration (22). Although the radioactivity isolated from gastric epithelium at this time was associated with inositol and IP_{1-6}, the radioactivity in the plasma and urine was associated with inositol and IP_1. These data indicate that the intact molecule was transported inside the gastric epithelial cells, wherein it was rapidly dephosphorylated, and that the metabolism of IP_6 was very rapid. In our preliminary studies, [^{3}H]-IP_6 was given via oral gavage to rats bearing 7,12-dimethylbenz[*a*]anthracene-induced mammary tumors. A substantial amount of radioactivity (19.7% of all radioactivity recovered in collected tissues) was found in tumor tissue as early as 1 h after administration, providing at least partial explanation for the antineoplastic activity of IP_6 at sites distant from the gastrointestinal tract. In this study only 50% of the radioactivity was excreted in urine within 72 h after administration; in addition feces accounted for another 10% of radioactivity, suggesting that at least 40% of the IP_6-associated radioactivity was distributed within the animal tissues. These data indicate that IP_6 can reach and concentrate at cellular targets. Chromatographic analysis of tumor tissue revealed the presence of inositol and IP_1, similar to plasma.

Using a novel and highly sensitive method combining gas chromatography–mass spectrometry analysis and HPLC, Grases et al. (23,24) were able to identify IP_6 in human urine and plasma and detect IP_6 and its less-phosphorylated forms (IP_{3-5}) in mammalian cells and in body fluids as they occur naturally. They also showed that the levels of IP_6 and its less phosphorylated forms fluctuate depending on the intake of IP_6.

That the extracellularly applied IP_6 enters the cell and that this intracellular delivery is followed by a dephosphorylation of IP_6 was recently confirmed by Ferry et al. (25).

Anticancer action of IP_6

As hypothesized, it was demonstrated that IP_6 is a broad-spectrum antineoplastic agent, affecting different cells and tissue systems. In vitro studies with IP_6 are summarized in **Table 1**.

IP_6 inhibited the growth of all tested cell lines in a dose- and time-dependent manner. The growth of cells of hematopoietic lineage was inhibited: human leukemic hematopoietic cell lines, such as K-562 (26,27) and human normal and leukemic hematopoietic cells (27). The antiproliferative activity of IP_6 was further reported in human colon cancer HT-29 cells (28), estrogen receptor–positive and estrogen receptor–negative human breast cancer cells (32), cervical cancer (25), prostate cancer (15,33,34), and HepG2 hepatoma cell lines (31). IP_6 also inhibited the growth of mesenchymal tumors, murine fibrosarcoma (39), and human rhabdomyosarcoma (38). However, cells from different origin have different sensitivity to IP_6 (the leukemic cell lines seem to be highly susceptible to IP_6), suggesting that IP_6 may affect different cell types through different mechanisms of action.

TABLE 1

Antitumor effect of inositol hexaphosphate (IP_6) in vitro

Organ or tissue	Species	Cell line	Investigator
Blood	Human	Erythroleukemia	Shamsuddin et al. (26)
		K562 cell line	
		K562 + human bone marrow	Deliliers et al. (27)
Colon	Human	Adenocarcinoma	Sakamoto et al. (28)
		HT-29 cell line	Yang & Shamsuddin (29)
Lung	Rat	Tracheal epithelium + B[a]P	Arnold et al. (30)
Liver	Human	HepG2 cells	Vucenik et al. (31)
Mammary	Human	Adenocarcinoma	Shamsuddin et al. (32)
		MCF-7, MDA-MB 231 cells	
Uterine cervix	Human	HeLa cells	Ferry et al. (25)
Prostate	Human	Adenocarcinoma	Shamsuddin & Yang (33)
		PC-3 cell line	
	Human	DU145 cells	Zi et al. (15) Singh et al. (34)
Skin	Mouse	JB6 cells	Huang et al. (35)
	Mouse	HEL-30 cells	Nickel & Belury (36)
Soft tissue	Mouse	3T3 fibroblast	Babich et al. (37)
	Human	Rhabdomyosarcoma, RD cells	Vucenik et al. (38)

The potential of IP_6 to induce differentiation and maturation of malignant cells, often resulting in reversion to the normal phenotype, was first demonstrated in K-562 hematopoietic cells (26). IP_6 was further shown to increase differentiation of human colon carcinoma HT-29 cells (28,29), prostate cancer cells (33), breast cancer cells (32), and rhabdomyosarcoma cells (38).

The cancer preventive activity of IP_6 in vitro was first tested in a benzo[*a*]pyrene-induced transformation in the rat tracheal cell culture transformation assay (30) and then was tested in a model using BALB/c mouse 3T3 fibroblasts (37) with modest efficacy. The observation that IP_6 impaired the transformation induced by epidermal growth factor or phorbol ester in JB6 (mouse epidermal) cells (35) strongly suggested the potential role of IP_6 as a cancer preventive agent, because this model has been a well-characterized cell system for studying the tumor promotion and molecular mechanisms of antitumor agents. Furthermore, IP_6 reduced 12-*O*-tetradecanoylphorbol-13-acetate–induced ornithine decarboxylase activity, an essential event in tumor promotion in HEL-30 cells, a murine keratinocyte cell line (36).

A summary of in vivo studies using IP_6 and inositol is shown in **Table 2**. Although experts in the field of nutrition and cancer have been performing in vivo experiments by adding IP_6 to the diet, in all our cancer prevention studies, IP_6 was given via drinking water in concentrations ranging from 0.4% to 2.0%. We were able to obtain comparable or even stronger tumor inhibition with much lower concentrations of IP_6 when it was given in drinking water. For example, much stronger tumor inhibition was achieved with 0.4% IP_6 in drinking water compared with the same amount given in a 20% high fiber diet (52).

The effectiveness of IP_6 as a cancer preventive agent was shown in colon cancer induced in different species (rats and mice) with different carcinogens (1,2-dimethylhydrazine and azoxymethane) (18–20,40–46). IP_6 was effective in a dose-dependent manner given either before or after carcinogen administration. The finding that IP_6 was able to reduce the development of large intestinal cancer 5 mo after carcinogen administration, when IP_6-treated animals demonstrated a significantly lower tumor number and size, has suggested its potential use as a therapeutic agent (20). IP_6 decreased the incidence of aberrant crypts when they were used as an intermediate biomarker for colon cancer (43,44). Studies using other experimental models showed that antineoplastic properties of IP_6 were not restricted to the colon. IP_6 significantly reduced experimental mammary carcinoma in Sprague-Dawley rats induced either by 7,12-dimethylbenz[*a*]anthracene (51–54) or *N*-methylnitrosourea (42). Using a two-stage mouse skin carcinogenesis model, Ishikawa et al. (55) investigated the effect of IP_6 on skin cancer and found a reduction in skin papillomas when IP_6 was given during the initiation stage but not when given during the promotion stage (55).

The therapeutic properties of IP_6 were demonstrated in the FSA-1 mouse model of transplantable and metastatic fibrosarcoma (39). After subcutaneous inoculation of mouse fibrosarcoma FSA-1 cells, mice were treated with intraperitoneal injections of IP_6 and a significant inhibition of tumor size and survival over untreated controls was observed. In this model experimental lung metastases are developed after intravenous injections of FSA-1 cells; intraperitoneal injections of IP_6 resulted in a significant reduction of metastatic lung colonies (39). A strong anticancer activity of IP_6 was also demonstrated against human rhabdomyosarcoma RD cells transplanted in nude mice (38), where the efficacy of IP_6 was tested on the tumor-forming capacity of RD cells. Peritumoral treatment with IP_6 (40 mg/kg) initiated 2 d after subcutaneous injection of rhabdomyosarcoma cells suppressed the tumor growth by 25–49-fold (38). IP_6 was also potent in inhibiting experimental hepatoma (31,48). We tested the effect of IP_6 on tumorigenicity and tumor regression in this model. A single treatment of HepG2 cells in vitro by IP_6 resulted in the complete loss of the ability of these cells to form tumors when inoculated subcutaneously in nude mice (48). Additionally, the preexisting

TABLE 2

Antitumor effect of IP_6 and inositol in vivo

Organ/Tissue	Species	Disease parameter	Mode	Investigator
Colon	Mouse	Carcinoma	in drink	Shamsuddin et al. (19)
	Rat	Carcinoma	in drink	Shamsuddin et al. (18,20)
	Rat	Carcinoma	in drink	Ullah & Shamsuddin (40)
	Rat	Carcinoma	in diet	Nelson et al. (41)
	Rat	Carcinoma	in diet	Shivapurkar et al. (42)
	Rat	Carcinoma	in diet	Pretlow et al. (43)
	Rat	Carcinoma	in diet	Challa et al. (44)
	Rat	Carcinoma	in diet	Jenab & Thompson (45)
	Mouse	Cell proliferation	in diet	Thompson & Zhang (46)
Liver	Rat	Hepatocellular Ca	in diet	Hirose et al. (47)
		HepG2 cell line	intratumoral	Vucenik et al. (48)
Lung	Mouse	Pulmonary adenoma	in diet	Estensen & Wattenberg (49) Wattenberg (50)
Mammary	Rat	Carcinoma	in drink	Vucenik et al. (51–53)
	Rat	Carcinoma	in diet	Shivapurkar et al. (42) Hirose et al. 1994 (54)
	Mouse	Cell proliferation	in diet	Thompson & Zhang (46)
Skin	Mouse	Papilloma two-step initiat→promotion	in drink in drink	Ishikawa et al. (55)
Soft Tissue	Rat	Fibrosarcoma Transplanted	in diet 12% Mg	Jariwalla et al. (56)
	Mouse	Fibrosarcoma Trans + Metast	i.p.	Vucenik et al. (39)
	Human	Rhabdomyosarcoma RD cell line	peritumoral	Vucenik et al. (38)

liver cancers regressed when they were treated directly with IP_6 (48).

Myo-inositol itself was also demonstrated to have anticancer function, albeit modest. It inhibited pulmonary adenoma formation in mice (49,50). We found that inositol alone or in combination with IP_6 can prevent the formation and incidence of several cancers in experimental animals: in soft tissue, colon, metastatic lung, and mammary cancers. Additionally, we showed that inositol potentiates both the antiproliferative and antineoplastic effects of IP_6 in vivo (3–5,19,39,51,52). Synergistic cancer inhibition by IP_6 when combined with inositol was observed in colon cancer (**Table 3**) (19) and mammary cancer studies (**Table 4**) (51,52). Similar results were seen in the metastatic lung cancer model (39). Thus, the combination of IP_6 and inositol was significantly better in different cancers than was either one alone.

Mechanisms of action of IP_6

The mechanisms involved in the anticancer activity of inositol compounds are not fully understood. It is known that virtually all animal cells contain inositol phosphates and that the inositol phosphates with fewer phosphate groups, especially IP_3 and IP_4, have an important role in cellular signal transduction, regulation of cell function, growth, and differentiation (8,9). We hypothesized that one of the several ways by which IP_6 plus inositol exerts its action is via lower-phosphate inositol phosphates. Measurement of intracellular inositol phosphates after IP_6 treatment showed an increased level of lower-phosphate inositol phosphates ($IP_{1\text{-}3}$) (21,24–26); their involvement in signal transduction pathways can affect cell cycle regulation, growth, and differentiation of malignant cells (3–5). Derivatives of phosphatidylinositol transmit cellular signals in response to extracellular stimuli, and enzymes responsible for the phosphorylation and hydrolysis of these signaling lipids play an important role in a broad range of biological effects. A central molecule is a phosphatidylinositol-3 kinase, which primarily phosphorylates the lipid phosphatidylinositol on the 3 position of the D-*myo*-inositol ring, yielding phosphatidylinositol-3-phosphate, but also can use phosphorylated forms of phosphatidylinositol as substrates. IP_6 inhibits phosphatidylinositol-3 kinase (35). This action is related to the IP_6 structure that is similar to D-3-deoxy-3-fluoro-PtdIns, an inhibitor of phosphatidylinositol-3 kinase (35). In addition to the blocking of phosphatidylinositol-3 kinase and activating protein-1 by IP_6 (35), protein kinase C (16,57) and mitogen-activated protein kinases (15,35) are involved in IP_6-mediated anticancer activity. The role of IP_6 among these multiple signaling pathways and their cross-talk in regulation of cell functions needs to be addressed in the future.

TABLE 3

Synergistic cancer inhibition by IP_6 when combined with inositol (Ins) 1,2-dimethylhydrazine (DMH)- induced colon carcinoma in mice

Experimental group	Tumor incidence (%)	Total number of tumors	No. of tumors/tumor bearing mice	Mitotic rate (%)
DMH	63[1]	22	12	1.92 ± 0.17
DMH + IP_6	47[2]	13	10	1.48 ± 0.15
DMH + Ins	30	9	6	1.01 ± 0.14
DMH + IP_6 + Ins	25	4	4	1.06 ± 0.13

[1] The difference in tumor incidence between DMH-only (carcinogen control group) and DMH + IP_6 + Ins is significant at $P < 0.001$.
[2] Between DMH + IP_6 and DMH + IP_6 + Ins at $p < 0.005$.
Adapted from Shamsuddin et al. (19).

TABLE 4

7,12-Dimethylbenz[a]nthracene (DMBA)-induced mammary carcinoma in rats

Experimental group	Tumor incidence (%)	Total number of tumors	No. of tumors/tumor-bearing rat	Rats with ≥5 tumors (%)
DMBA	92.5	113	3.1 ± 0.4[1]	17.5
DMBA + IP_6	71.5	69	2.5 ± 0.2[2]	5.3
DMBA + Ins	75.0	64	2.1 ± 0.2	2.5
DMBA + IP_6 + Ins	76.3	51	1.8 ± 0.1	0.0

[1] The difference in total number of tumors, tumor burden (No. of tumors/tumor-bearing rats) and tumor multiplicity (Rats with ≥5 tumors) between DMBA-only and DMBA + IP_6 is significant at $P < 0.05$.
[2] Between DMBA and DMBA + IP_6 + Ins for tumor burden and multiplicity at $P < 0.05$.
Adapted from Vucenik et al. (52).

IP_6 can also modulate cellular response at the level of receptor binding. IP_6, after sterically blocking the heparin-binding domain of basic fibroblast growth factor, disrupted further receptor interactions (58). This modulation in binding and the activity of basic fibroblast growth factor is thought to be due to the chair conformation of IP_6 mimicking that of the pyranose ring structure in heparin (58).

The observed anticancer effect of inositol compounds could be mediated through several other mechanisms. The antioxidant role of IP_6 is known and widely accepted; this function of IP_6 occurs by chelation of Fe^{3+} and suppression of ·OH formation (11). Therefore, IP_6 can reduce carcinogenesis mediated by active oxygen species and cell injury via its antioxidative function. This activity seems to be closely related to its unique structure. The phosphate grouping in positions 1,2,3 (axial-equatorial-axial) is unique to IP_6, specifically interacting with iron to completely inhibit its ability to catalyze hydroxyl radical formation, making IP_6 a strong antioxidant. This anticancer action of IP_6 may be further related to mineral binding ability; IP_6 by binding with Zn^{2+} can affect thymidine kinase activity, an enzyme essential for DNA synthesis, or remove iron, which may augment colorectal cancer (3–5,41,46).

Besides affecting tumor cells, IP_6 can act on a host by restoring its immune system. IP_6 augments natural killer cell activity in vitro and normalizes the carcinogen-induced depression of natural killer cell activity in vivo (59).

Value of IP_6 as a therapeutic and preventive agent for cancer

Safety. IP_6 is a natural compound and an important dietary component. Some concerns have been expressed regarding the mineral deficiency that results from an intake of foods high in IP_6 that might reduce the bioavailability of dietary minerals. However, recent studies demonstrate that this antinutrient effect of IP_6 can be manifested only when large quantities of IP_6 are consumed in combination with a diet poor in oligoelements (60–63). A long-term intake of IP_6 in food (60,61) or in a pure form (64) did not cause such a deficiency in humans. Studies in experimental animals showed no significant toxic effects on body weight, serum, or bone minerals (**Table 5**) or any

TABLE 5

Effect of inositol compounds on bone minerals

Treatment (*n*)	Ca^{2+} (*mg/g*)	Mg^{2+} (*mg/g*)	Zn^{2+} ($\mu g/g$)
Tap water (*n* = 6)	116.9 ± 13.9[1]	1.13 ± 0.14	109.2 ± 14.9
15 mM IP_6 (*n* = 3)	124.8 ± 11.3[2]	1.19 ± 0.14	127.4 ± 11.5
15 mM Ins (*n* = 4)	117.4 ± 14.2	1.10 ± 0.16	116.7 ± 14.5
15 mM IP_6 + 15 mM Ins (*n* = 5)	125.9 ± 9.0	1.14 ± 0.06	115.1 ± 9.9

[1] Values are mean ± SD.
[2] There was no statistical difference among groups in the levels of bone minerals.
Reprinted with permission from Vucenik et al. (52).

pathological changes in either male F344 or female Sprague-Dawley rats for 40 wk (40,51,52). Grases et al. (65) confirmed our findings and also reported that abnormal calcification was prevented in rats given IP_6.

IP_6 does not affect normal cells. The most important expectation of a good anticancer agent is for it to only affect malignant cells and not affect normal cells and tissues. That property was recently shown for IP_6. When the fresh $CD34^+$ cells from bone marrow was treated with different doses of IP_6, a toxic effect (inhibition of the clonogenic growth or as cytotoxicity on liquid cultures) was observed that was specific to leukemic progenitors from chronic myelogenous leukemia patients but no cytotoxic or cytostatic effect was observed on normal bone marrow progenitor cells under the same conditions (27). Recently, we (66) showed that IP_6 inhibited the colony formation of Kaposi's sarcoma (KS) cell lines, KS Y-1 (AIDS-related KS) and KS SLK (Iatrogenic KS), and CCRF-CEM (human adult T lymphoma) cells in a dose-dependent manner (66). However, in striking contrast to taxol, used as a control, IP_6 did not affect the ability of normal cells (peripheral blood mononuclear cells and T-cell colony-forming cells) to form colonies in a semisolid methylcellulose medium. Malignant and normal cells are known to have a different metabolism, growth rate, expression of receptors, etc., but the mechanism for this different selectivity of IP_6 for normal and malignant cells needs to be further investigated.

IP_6 acts synergistically with standard chemotherapeutics. Current cancer treatment recognizes the importance of using combination therapy to increase efficacy and decrease side effects of conventional chemotherapy. Another important aspect of cancer treatment is overcoming acquired drug resistance. Our recent data demonstrate that IP_6 acts synergistically with doxorubicin and tamoxifen, being particularly effective against estrogen receptor–negative and doxorubicin-resistant cell lines, both conditions that are challenging to treat (67). These data are particularly important because tamoxifen is usually given as a chemopreventive agent in the post-treatment period and doxorubicin has enormous cardiotoxicity and its use is associated with doxorubicin resistance.

IP_6 affects principal pathways of malignancy. Our goal is to identify agents that can target tumors at vulnerable sites and interrupt specific pathways of carcinogenesis. From the behavior and characteristics of malignant cells, several principal pathways of malignancy have been established, such as proliferation, cell cycle progression, metastases and invasion, angiogenesis, and apoptosis; interestingly, IP_6 targets and acts on all of them.

Uncontrolled proliferation is a hallmark of malignant cells, and IP_6 can reduce the cell proliferation rate of many different cell lines of different lineage and of both human and rodent origin (3–5,26,28,31–33,38). Although normal cells divide at a controlled and limited rate, malignant cells escape from the control mechanisms that regulate the frequency of cell multiplication and usually have lost the checkpoint controls that prevent replication of defective cells. IP_6 can regulate the cell cycle to block uncontrolled cell division and force malignant cells either to differentiate or go into apoptosis. IP_6 induces G_1 phase arrest and a significant decrease of the S phase of human breast (68,69), colon (69), and prostate (34) cancer cell lines. However, IP_6 causes the accumulation of human leukemia cells in the G_2M phase of the cell cycle; a cDNA microarray analysis showed a down-modulation of multiple genes involved in transcription and cell-cycle regulation by IP_6 (27).

One important characteristic of malignancy is the ability of tumor cells to metastasize and infiltrate normal tissue. A significant reduction in the number of lung metastatic colonies by IP_6 was observed in a mouse metastatic tumor model using FSA-1 cells (39). Using highly invasive MDA-MB 231 human breast cancer cells, we demonstrated that IP_6 inhibits metastasis in vitro through effects on cancer cell adhesion, migration, and invasion (70,71). Tumor cells emit substances known as matrix metalloproteinases that allow metastatic cells to pass into the blood vessels; IP_6 significantly inhibited secretion of MMP-9 from MDA-MB 231 cells (70).

Tumors depend on the formation of new blood vessels to support their growth and metastasis. Many tumors produce large amounts of vascular endothelial growth factor, a cytokine that signals normal blood vessels to grow. IP_6 inhibited the growth and differentiation of endothelial cells (66,72) and inhibited the secretion of vascular endothelial growth factor from malignant cells (27,66,72). IP_6 can also adversely affect angiogenesis as antagonist of fibroblast growth factor (58).

Apoptosis is a hallmark of action of many anticancer drugs. It has been reported that IP_6 induces apoptosis in vivo (45) and in vitro in prostate (34) and cervical cancer (25) cell lines, involving cleavage of caspase 3, caspase 9, and poly ADP-ribose polymerase, an apoptotic substrate, in a time- and dose-dependent manner.

Effectiveness of IP_6 as a cancer preventive agent. Possible mechanisms of the cancer preventive action of IP_6 include carcinogen blocking activities, antioxidant activities, and antiproliferation and antiprogression activities (73). Therefore, the strategy of chemoprevention is to use agents that will inhibit mutagenesis, induce apoptosis, induce maturation and differentiation, and inhibit proliferation (74). The antioxidant activity of IP_6 is widely accepted and indisputable (11), and IP_6 possesses antiproliferative and antiprogression activities. Its induction of terminal differentiation (26,28,29,32,33,38), restoration of immune response (59), modulation of growth factors (58), modulation of signal transduction pathways (15,16, 35,57), induction of apoptosis (25,34,45), and possibly inhibition of oncogene activity and restoration of tumor suppressor function are well documented. IP_6 not only inhibits the activities of some liver enzymes (75,76) but also significantly increases the hepatic levels of glutathione S-transferase (44,77), both of which indicate its possible role in carcinogen-blocking activities and cancer protection.

Although IP_6 may belong to almost all previously mentioned categories of cancer preventive drugs, affecting almost all phases of cancer prevention, it still appears that IP_6 is not a direct antagonist to the carcinogen because of its moderate efficacy in vitro when tested and compared with other chemopreventive agents (30) and a lack of dramatic decrease in cancer incidence when tested in vivo. However, because cancer prevention is a long process, a long administration of

cancer preventive agent is generally needed, requiring usually 10–40 y of continuous treatment (2,73), and, therefore, it is very important that cancer preventive agents have low or almost no toxicity. IP_6, a natural compound with virtually no toxicity, can satisfy this special and very important requirement for cancer prevention.

IP_6 plus inositol and patients

An enhanced antitumor activity without compromising the patient's quality of life was demonstrated in a pilot clinical trial involving six patients with advanced colorectal cancer (Dukes C and D) with multiple liver and lung metastasis (78). IP_6 plus inositol was given as an adjuvant to chemotherapy according to Mayo protocol. One patient with liver metastasis refused chemotherapy after the first treatment, and she was treated only with IP_6 plus inositol; her control ultrasound and abdominal computed tomography scan 14 mo after surgery showed a significantly reduced growth rate. A reduced tumor growth rate was noticed overall and in some cases a regression of lesions was noted. Additionally, when IP_6 plus inositol was given in combination with chemotherapy, side effects of chemotherapy (drop in leukocyte and platelet counts, nausea, vomiting, alopecia) were diminished and patients were able to perform their daily activities (78). Further controlled randomized clinical trials are necessary to confirm these observations.

Other biological effects of IP_6

In humans, IP_6 not only has almost no toxic effects, but it has many other beneficial health effects such as inhibition of kidney stone formation and reduction in risk of developing cardiovascular disease. IP_6 was administered orally either as the pure sodium salt or in a diet to reduce hypercalciuria and to prevent formation of kidney stones, and no evidence of toxicity was reported (64,65,79,80). A potential hypocholesterolemic effect of IP_6 may be very significant in the clinical management of hyperlipidemia and diabetes (75,76,81). IP_6 inhibits agonist-induced platelet aggregation (82) and efficiently protects myocardium from ischemic damage and reperfusion injury (83), both of which are important for the management of cardiovascular diseases.

Many potential beneficial actions of IP_6 have been described. The inclusion of IP_6 plus inositol in our strategies for prevention and treatment of cancer as well as other chronic diseases is warranted. However, the effectiveness and safety of IP_6 plus inositol need to be determined in Phase I and Phase II clinical trials in humans.

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