IP_6 & Inositol in Cancer Prevention and Therapy

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Abstract: Inositol and its phosphorylated form – inositol hexaphosphate (IP_6) are naturally occurring carbohydrates, abundantly found in certain high-fiber diets, such as cereals and legumes. They, as well as other inositol phosphates with fewer phosphate groups (IP_{1-5}) are contained in almost all mammalian cells, although in much smaller amounts, where they are important in regulating vital cellular functions. A striking anticancer action of IP_6 was demonstrated in different experimental models. Although inositol possesses a modest anticancer activity, the most consistent and best anticancer results were obtained from the combination of Inositol + IP_6. In addition to reducing cell proliferation, IP_6 increases differentiation of malignant cells, often resulting in reversion to normal phenotype with decreasing production of tumor markers such as CEA, PSAP, and AFP. Exogenously administered IP_6 is rapidly taken into the cells and dephosphorylated to lower inositol phosphates, which further affect signal transduction pathways resulting in cell cycle arrest. Enhanced immunity and antioxidant properties also contribute to tumor cell destruction. Because it is abundantly present in regular diet, efficiently absorbed from the gastrointestinal tract, and safe, Inositol + IP_6 holds great promise in our strategies for both prevention and therapy of cancer. Inositol + IP_6 enhances the anticancer effect of conventional chemotherapy, controls cancer metastases, and improves the quality of life, as shown in pilot clinical studies. Emerging clinical and rather vast amount of laboratory data accumulated so far strongly suggest its role either as an adjuvant or as an "alternative" to current chemotherapy for cancer. In addition to cancer, Inositol + IP_6 has great potential in prevention of kidney stones, diabetic complications and atherosclerotic cardiovascular diseases, ailments that afflict people throughout the world.

Keywords: Cancer prevention, cancer treatment, differentiation, phytic acid, InoCell.

INTRODUCTION

It is widely accepted that diet changes are a powerful means to prevent and modulate cancer. Dietary supplements are becoming increasingly popular in Western society. The efficiency of dietetic supplements in cancer prevention and treatment is a popular, but still controversial subject of research.

Inositol and inositol hexaphosphate (IP_6), abundant in cereals and legumes have demonstrated a novel anticancer action. Available as dietary supplement, Inositol + IP_6 (InoCell™) is consumed by many cancer patients for its several health benefits. In this review, we summarize the evidence showing that inositol and IP_6 are the active components of certain types of high fiber diet playing a unique role in cancer prevention and treatment as well as in reducing the risk of chronic diseases of modern society.

OCCURRENCE, DISTRIBUTION, AND METABOLISM OF INOSITOL AND IP_6

Inositol, a simple 6-carbon carbohydrate, is considered to be an essential nutrient and a member of the B vitamins. When all of its 6 carbons are attached to phosphate groups, it is known as inositol hexaphosphate (InsP_6 or IP_6). Contained in high concentrations (0.4–6.4%) in cereals and legumes, IP_6 has shown a novel anticancer function [1-5]. *Myo*-inositol is also shown to have similar, albeit a weaker anticancer action is considered to be the parent compound of IP_6; the latter can be converted to inositol by removing all of the 6 phosphates, as is done commercially. Thus the relationship between the two are like the "chicken and the egg." Only *myo*-inositol hexaphosphate has been found in plants; *neo*-, *chiro*-, and *scyllo*-inositol hexaphosphates have been isolated from soil [6]. 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.

Almost all mammalian cells contain IP_6 and much smaller amounts of its forms with fewer phosphate groups (IP_{1-5}), which are important for regulating vital cellular functions such as cell division, cellular differentiation, exocytosis, endocytosis, etc. Thus, this ubiquitous molecule appears to come from the soil to our cells *via* the cereal grains, staple for much of the world population.

Inositol is present in cell membranes in conjugation with lipids, as phosphatidylinositol. Due to their important role in cellular signal transduction systems, inositol phospholipids in the plasma membrane have received much attention in recent days. 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 [7]. 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

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are formed and that these multiple isomers show a complex pattern of inter-conversion [7–9]. The sequential dephosphorylation of IP_3 and IP_4 produces multiple inositol phosphate isomers in a cyclic metabolic pathway that results in inositol returning to the phosphoinositide pool. Furthermore, in a continual cycle of phosphorylation and dephosphorylation, IP_6 pool is rapidly turning over. Inositol phosphates are versatile molecules with important roles in controlling diverse cellular activities [8,9]. IP_6 may serve as a natural antioxidant [10] and possibly as a neurotransmitter [9]. Different binding proteins for inositol polyphosphates have been isolated, indicating their importance for the cellular functions [11] such as effects on ion channels and protein trafficking [12,13], endocytosis [14], exocytosis [15], and efficient export of mRNA from the nucleus to the cell [16].

Based on epidemiological data indicating that only diets containing a high IP_6 content (cereals and legumes) showed a negative correlation with colon cancer, Shamsuddin and his co-workers [17–19], performed pioneering experiments showing novel anticancer feature of IP_6. Nearly 20 years ago, against the prevalent dogma that IP_6 cannot be absorbed through the gastrointestinal system, Shamsuddin hypothesized that IP_6 given orally would be absorbed into our body, internalized by the cells and dephosphorylated to IP_{1-5} and enter into the intracellular inositol phosphate pool to inhibit tumor formation. It was also hypothesized that the addition of inositol to IP_6 would enhance the anticancer function of IP_6 [17–19]. And since inositol phosphates are common molecules involved in signal transduction in almost all mammalian cell systems, it was further hypothesized that the anticancer action of inositol phosphates would be observed in different cells and tissue systems [17–19]. All of these proposed hypotheses have been tested and confirmed in subsequent experiments. In this review, we present the state of the art knowledge of the anticancer and other health benefits of inositol and IP_6.

In testing the hypothesis that the anticancer action of IP_6 may be mediated *via* lower phosphorylated forms of inositol, our experiments showed that IP_6 is not only absorbed from the gastrointestinal tract, but also taken up by malignant cells [20]. Indeed orally administered IP_6 can reach target tumor tissue distant from the gastrointestinal tract [21]. Exogenous IP_6 is rapidly taken up by mechanisms involving pinocytosis or receptor-mediated endocytosis, transported intracellularly, and dephosphorylated into inositol phosphates with fewer phosphate groups [20]. 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 following administration [21]. While 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. When [^{3}H]-IP_6 was given *via* oral gavage to rats bearing 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 anti-neoplastic activity of IP_6 at sites distant from the gastrointestinal tract. Thus, IP_6 can reach and concentrate in cellular targets. Chromatographic analysis of tumor tissue revealed the presence of inositol and IP_1, similar to that in the plasma. One of the vexing problems in this nascent field of IP_6 research has been the lack of robust technology to determine non-radiolabeled IP_6 in biological tissues and fluids. Using newer technologies of Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES), Professor Grases and his coworkers in Spain [22,23] 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. Ferry *et al.* [24] recently added further confirmation to these data and our original hypothesis as well as our pioneering observation that the externally applied IP_6 enters the cell followed by dephosphorylation. Thus not only one of the key misconceptions about this molecule is nullified, but we have demonstrated that IP_6 is an essential nutrient whose level in plasma and urine fluctuates following deficiency or replenishment [22,23]. In essence, it has all the characteristics of a vitamin.

A BROAD-SPECTRUM ANTICANCER ACTIVITY OF IP_6

The vast amount of laboratory data accumulated so far confirms another one of our original hypotheses - that IP_6 is indeed a broad-spectrum antineoplastic agent, effective against cancers of different cells and tissue systems. The mechanisms underlying the anticancer activity of IP_6 and inositol may involve changes in pathways leading to cell growth and cell death. These include hormone and growth factor signaling, regulatory mechanisms of cell cycle progression, cell differentiation, and apoptosis. Examples of effect of IP_6 on some of these pathways will be described.

In vitro studies with IP_6 are summarized in Table **1**, while those of *in vivo* studies are in Table **2**.

The two basic cellular mechanisms of this anticancer action are normalization of the elevated rate of cell proliferation and increased differentiation of malignant cells. IP_6 inhibited the growth of all tested cell lines in a dose- and time-dependent manner irrespective of whether they were epithelial or mesenchymal. Reduction of the elevated rate of cell proliferation to normal level has been observed both *in vivo* in intact animals as well as *in vitro* in culture of malignant cell [mostly human]. *In vitro* IP_6 inhibited the growth of human leukemia cells [25,26], human colon cancer cells [27], both estrogen receptor-positive and estrogen receptor-negative human breast cancer cells [28], cervical cancer [24], prostate cancer [14,29,30], and hepatoma cell lines [31]. Examples of IP_6 inhibition of the growth of mesenchymal tumors include murine fibrosarcoma [32], and human rhabdomyosarcoma [33]. However, cells from different origin have different sensitivity to IP_6 (hepatoma and leukemic cell lines seem to be highly susceptible to IP_6), suggesting that IP_6 affects different cell types through different mechanisms of action.

Along with this reduction in cell proliferation IP_6 induces differentiation and maturation of malignant cells, often resulting in reversion to the normal phenotype, as demonstrated in K-562 hematopoietic cells [25], human colon carcinoma HT-29 cells [27,34], prostate cancer cells

Table 1. Antineoplastic Activity of IP_6 in Various Cancer Models *In Vitro*

Organ or tissue	Species	Cell line	Investigator
Blood	Human	Erythroleukemia	Shamsuddin *et al.* 1992 [25]
		K562 cell line	
		K562 + human bone marrow	Deliliers *et al.* 2002 [26]
Colon	Human	Adenocarcinoma	Sakamoto *et al.* 1993 [27]
		HT-29 cell line	Yang & Shamsuddin 1995 [34]
Lung	Rat	Tracheal epithelium	Arnold *et al.* 1995 [35]
		+ B[a]P	
Liver	Human	HepG2 cells	Vucenik *et al.* 1998 [31]
Mammary	Human	Adenocarcinoma	Shamsuddin *et al.* 1996 [28]
		MCF-7, MDA-MB 231 cells	Tantivejkul *et al.* 2003 [63,64,69]
Uterine cervix	Human	HeLa cells	Ferry *et al.* 2002 [24]
Prostate	Human	Adenocarcinoma	Shamsuddin & Yang 1995 [29]
		PC-3 cell line	
	Human	DU145 cells	Zi *et al.* 2000 [14]
			Singh *et al.* 2003 [30]
Skin	Mouse	JB6 cells	Huang *et al.* 1997 [37]
	Mouse	HEL-30 cells	Nickel & Belury 1999 [38]
Soft tissue	Mouse	3T3 fibroblast	Babich *et al.* 1993 [36]
	Human	Rhabdomyosarcoma, RD cells	Vucenik *et al.* 1998 [33]

[29], breast cancer cells [28], and rhabdomyosarcoma cells [33]. We have observed that IP_6 treatment of human hepatoma cell line causes a dramatic reduction in secretion of the tumor marker α-fetoprotein (AFP) (Table **3**) [31]. A practical application of this effect in the clinical setting will be to monitor patients with cancer for reduction of tumor markers by laboratory tests; and there are emerging evidence of that happening clinically [please see "Inositol + IP_6 and Patients" below].

Aside from the chemoprevention studies done in *in vivo* colon and mammary cancer models (see below), cancer preventive activity of IP_6 was also tested *in vitro,* in a benzo[*a*]pyrene-induced transformation of rat tracheal epithelial cells [35], and in BALB/c mouse 3T3 fibroblasts [36]. In an *in vitro* model of skin carcinogenesis, IP_6 impaired the transformation induced by epidermal growth factor or phorbol ester in JB6 (mouse epidermal) cells [37], thus strongly suggesting its role as a preventive agent for skin cancer. Furthermore, IP_6 reduced 12-*O*-tetradecanoylphorbol-13-acetate–induced ornithine decarboxylase activity, an essential event in tumor promotion in HEL-30 cells, and a murine keratinocyte cell line [38]. Additionally, Gupta *et al.* showed in a mouse carcinogenesis model that IP_6 causes a reduction in the number of skin tumor formation [39].

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) [17–19,40-46]. 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 months after carcinogen administration, when IP_6-treated animals demonstrated a significantly lower tumor number and size, had suggested its potential use as a therapeutic agent [19]. IP_6 decreased the incidence of aberrant crypts, often used as an intermediate biomarker for colon cancer [43,44]. Studies using other experimental models showed that the antineoplastic properties of IP_6 were not restricted to colon. IP_6 significantly reduced experimental mammary carcinoma in Sprague-Dawley rats induced either by 7,12-dimethylbenz[*a*]anthracene [46-50] or *N*-methylnitrosourea [42]. Aside from the study of Gupta *et al.* [39], showing prevention of skin carcinogenesis mentioned above, using a 2-stage mouse skin carcinogenesis model, Ishikawa *et al.* [51] investigated the effect of IP_6 on skin cancer. They also found a reduction in skin papillomas when IP_6 was given during the initiation stage but not when given during the promotion stage [51]. In a very recent report Lee *et al.* [52] have shown that dietary administration of IP_6 and inositol prevents chemically induced rat hepatocarcinogenesis.

The therapeutic properties of IP_6 were demonstrated in the FSA-1 mouse model of transplantable and metastatic fibrosarcoma [32]. 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

Table 2. Antineoplastic Activity of IP_6 and Inositol in Various Cancer Models *In Vivo*

Organ or Tissue	Species	Disease parameter	Mode	Investigator
Colon	Mouse	Carcinoma	in drink	Shamsuddin *et al.*
	Rat	Carcinoma	in drink	1988-1989 [17–19]
	Rat	Carcinoma	in drink	Ullah & Shamsuddin 1990 [40]
	Rat	Carcinoma	in diet	Nelson *et al.* 1989 [41]
	Rat	Carcinoma	in diet	Shivapurkar *et al.* 1996 [42]
	Rat	Carcinoma	in diet	Pretlow *et al.* 1992 [43]
	Rat	Carcinoma	in diet	Challa *et al.* 1997 [44]
	Rat	Carcinoma	in diet	Jenab & Thompson 2000 [45]
	Mouse	Cell proliferation	in diet	Thompson & Zhang 1991 [46]
Liver	Rat	Hepatocellular Ca	in diet	Hirose *et al.* 1991 [54]
	Mouse	HepG2 cell line	intratumoral	Vucenik *et al.* 1998 [55]
	Rat	Carcinoma	in drink	Lee *et al.* 2005 [52]
Lung	Mouse	Pulmonary adenoma	in diet	Estensen & Wattenberg 1993 [57]
				Wattenberg 1995 [58]
Mammary	Rat	Carcinoma	in drink	Vucenik *et al.* 1993,1995,1997 [47-49]
	Rat	Carcinoma	in diet	Shivapurkar *et al.* 1996 [42]
				Hirose *et al.* 1994 [50]
	Mouse	Cell proliferation	in diet	Thompson & Zhang 1991 [46]
Prostate	Mouse	DU145 cells	in drink	Singh *et al.* 2004 [56]
Skin	Mouse	Papilloma 2-step	in drink	Ishikawa *et al.* 1999 [51]
	initiat→promotion	in drink		
	Mouse	Carcinoma	topical	Gupta *et al.* 2003 [39]
Soft Tissue	Rat	Fibrosarcoma	in diet	Jariwalla *et al.* 1988 [53]
		Transplanted	12% Mg	
	Mouse	Fibrosarcoma	i.p.	Vucenik *et al.* 1992 [32]
		Trans + Metast		
	Human	Rhabdomyosarcoma	peritumoral	Vucenik *et al.* 1998 [33]
		RD cell line		

developed after intravenous injections of FSA-1 cells; intraperitoneal injections of IP_6 resulted in a significant reduction of metastatic lung colonies [32]. Adding much higher amounts to the diet, Jariwalla *et al.* [53] in a rat fibrosarcoma tumor model have also reported similar results. A strong anticancer activity of IP_6 was also demonstrated against human rhabdomyosarcoma RD cells transplanted in nude mice [33], 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 days after subcutaneous injection of rhabdomyosarcoma cells suppressed the tumor growth by 25 – 49-fold [33]. IP_6 was also potent in inhibiting experimental hepatoma [31,54,55]. 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 [55]. Additionally, the pre-existing liver cancers regressed when they were treated directly with IP_6 [55]. Studies from Professor Agarwal's laboratory also demonstrated the efficacy of orally administered IP_6 in drinking water against *in vivo* growth of human prostate cancer xenografts in nude mice [56].

Table 3. α-fetoprotein (AFP) Secretion by HepG2 Cells After IP_6 Treatment

Experimental group	AFP secretion in culture media (ng/mL)	AFP secretion (pg/cell)
Culture media	1379.0 ± 65.7*	27.6 ± 1.3
250 μM IP_6	275.5 ± 44.6	10.2 ± 1.7
500 μM IP_6	63.0 ± 24.0	5.3 ± 2.0
1000 μM IP_6	1.9 ± 0.2	0.3 ± 0.0

* The difference in AFP secretion between culture media and 1000 μM IP_6 is significant at $p < 0.0001$ (*t*-test).
Adapted from Vucenik *et al.* 1989 [31].

Table 4. Synergistic Cancer Inhibition by IP_6 When Combined with Inositol (Ins) in 1,2-Dimethylhydrazine (DMH) - Induced Colon Carcinoma in Mice

Experimental group	Tumor incidence (%)	Total number of tumors	No. of tumors/ tomor bearing mice	Mitotic rate (%)
DMH	63*	22	12	1.92 ± 0.17
DMH + IP_6	47**	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

*The difference in tumor incidence between DMH-only (carcinogen control group) and DMH + IP_6 + Ins is significant at $p < 0.001$.
**Between DMH + IP_6 and DMH + IP_6 + Ins at $p < 0.005$.
Adapted from Shamsuddin *et al.* 1989 [18].

Myo-inositol itself was also demonstrated to have anticancer function, albeit modest. It inhibited soft tissue, colon, mammary, and lung tumor formation [18,32,47,48,57,58]. Additionally, as hypothesized, we showed that inositol potentiates both the antiproliferative and antineoplastic effects of IP_6 *in vivo* [1-4,18,32,47,48]. Synergistic cancer inhibition by IP_6 when combined with inositol was observed in colon cancer (Table 4) [18] and mammary cancer studies [47,48]. Similar results were seen in the metastatic lung cancer model [32]. Of note is that in certain models some tumor parameters may either remain unchanged or, worse exacerbated by inositol or IP_6 alone. But not only the combination of IP_6 and inositol was significantly better in different cancers than was either one alone, but it also consistently reduced *all* the tumor parameters. Thus, in clinical settings, one must not use IP_6 or inositol alone since individually they are neither optimally efficient, nor sufficient.

MECHANISMS OF ANTICANCER ACTIVITY OF INOSITOL AND IP_6

Though we know that the cellular mechanisms involved in the anticancer activity of inositol and IP_6 involve cell proliferation and differentiation, we are now only beginning to understand the biochemical pathways. 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 [7,8]. We hypothesized that one of the several ways by which inositol + IP_6 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_{1-3}) [20,23–25]; their involvement in signal transduction pathways can affect cell cycle regulation, growth, and differentiation of malignant cells [1–4].

Reversible phosphorylation of specific intracellular proteins is believed to be an important and versatile mechanism for regulating their biological activity, which, in turn, controls a variety of cellular functions. Despite the fact that IP_6 is the most abundant inositol metabolite in cells, its cellular function is still an enigma. In addition to the blocking of phosphatidylinositol-3 kinase (PI-3K) and activating protein-1 (AP-1) by IP_6 [37], protein kinase C (PKC) [15,59] and mitogen-activated protein kinases (MAPK) [14,37] are involved in IP_6-mediated anticancer activity. Moreover, very recently it was shown that IP_6 could operate *via* a direct control of protein phosphorylation [60]. Interestingly, although inositol phosphate-regulated phosphorylation was shown for IP_6, no activated phosphorylation was observed using the lower inositol phosphate in particular IP_3 or IP_4 [60]. Thus, the role of IP_6 among these multiple signaling pathways and their cross talk in regulation of cell function, needs to be addressed in the future. 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 [61].

The observed anticancer effect of inositol compounds could be mediated through several other mechanisms. The antioxidant role of IP_6 is widely recognized; this function of IP_6 occurs by chelation of Fe^{3+} and suppression of •OH formation [10]. Therefore, IP_6 can reduce carcinogenesis mediated by active oxygen species and cell injury *via* its antioxidative function. 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. Similarly, excessiron, which may augment colorectal cancer formation, can be removed by IP_6 [1-4,41,46].

Besides affecting tumor cells, IP_6 can act on host by restoring its immune system. IP_6 augments natural killer cell activity *in vitro* and normalizes the carcinogen-induced depression of natural killer (NK) cell activity *in vivo* [62]. Figure 1 shows that there is an inverse relationship between NK activity and tumor incidence, an increased incidence is correlated with a decreased NK cell activity. The animals on IP_6 and inositol enjoyed a lower incidence of cancer and had a concomitantly enhanced NK cell activity. But those animals that received the combination of Inositol + IP_6 had the highest NK activity and lowest tumor incidence [62].

IP_6 INHIBITS PROLIFERATION, CELL CYCLE PROGRESSION, METASTASIS AND INVASION, ANGIOGENESIS, AND INDUCES APOPTOSIS

It has been shown that IP_6 affects some principal pathways of malignancy.

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 them all.

Uncontrolled proliferation is a hallmark of malignant cells, and as discussed before, IP_6 can reduce the cell

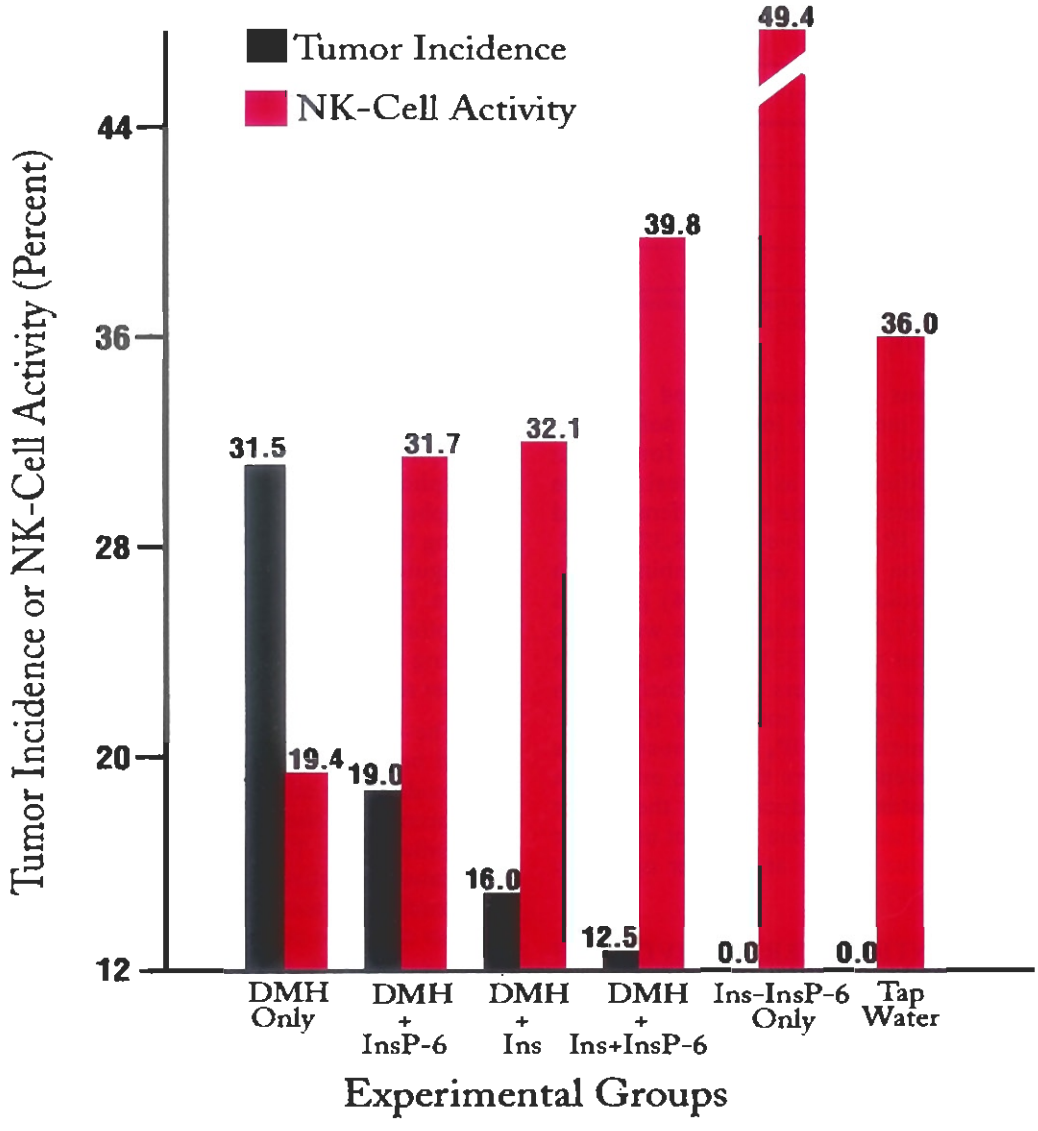

Fig. (1). Modulation of NK-cell activity by inositol and IP_6.

Note that the carcinogen DMH results in tumor formation with reduced NK activity. Mice treated with inositol or IP_6 show decreased incidence of tumor correlated with enhanced NK activity; but the best results are seen with combination of Inositol + IP_6

proliferation rate of many different cells of different lineage and of both human and rodent origin [1–4,25,27-29,31,33]. 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 to go into apoptosis. IP_6 induces G_1 phase arrest and a significant decrease of the S phase of human cancer cell lines [30,63,64]. However, in leukemia cells, IP_6 causes the accumulation of cells in the G_2M phase of the cell cycle; cDNA microarray analysis showed a down-modulation of multiple genes involved in transcription and cell cycle regulation by IP_6 [26].

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 [32]. 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 [65,66]. 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 [65].

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

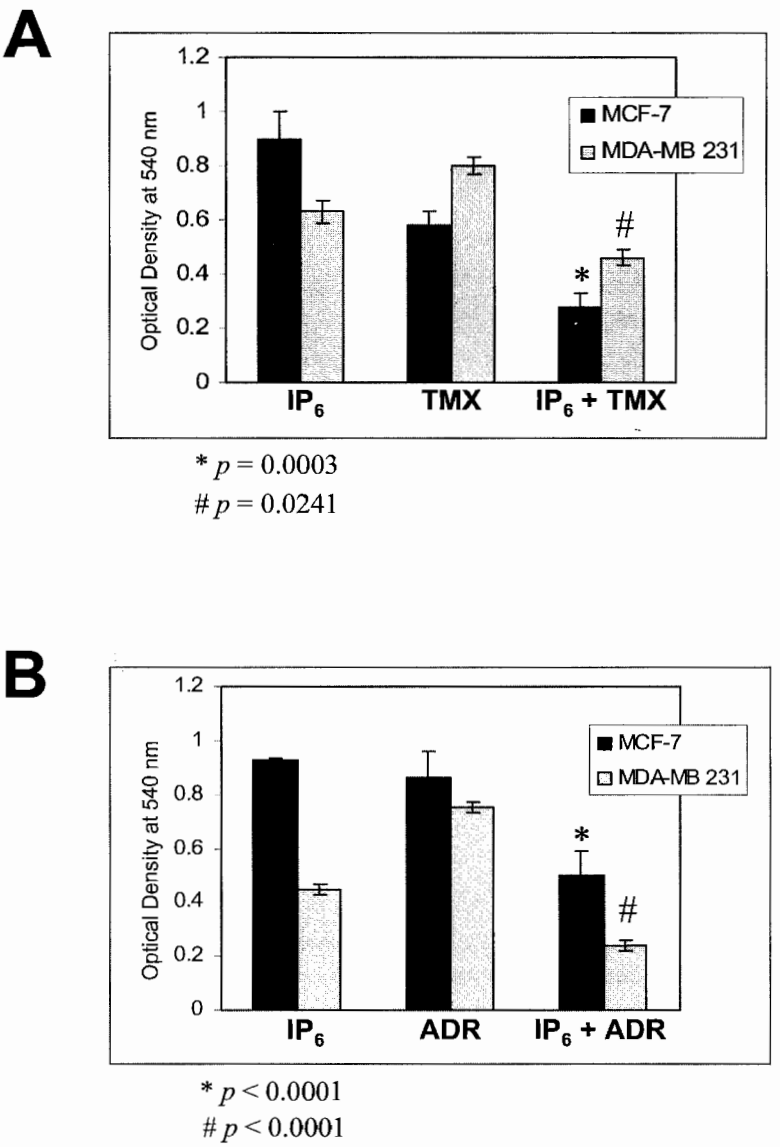

Fig. (2). IP_6 enhances the anti-proliferative effects of tamoxifen and adriamycin.

Simultaneous or sequential treatment of MCF-7 (estrogen receptor-positive) and MDA-MB 231 (estrogen receptor-negative) human breast cancer cell lines with IP_6 + tamoxifen (TMX) (**A**) or IP_6 + adriamycin (ADR) (**B**) yielded synergism. Adapted from Tantivejkul *et al.* 2003 [70].

inhibited the growth and differentiation of endothelial cells [67,68] and inhibited the secretion of vascular endothelial growth factor from malignant cells [26,56,67,68]. IP_6 can also adversely affect angiogenesis as antagonist of fibroblast growth factor [61].

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 [30], breast [63], cervical cancer [24], and Kaposi's sarcoma (KS) cell lines [67]), involving cleavage of caspase 3, caspase 9, and poly ADP-ribose polymerase, an apoptotic substrate, in a time- and dose-dependent manner. Moreover, the role of inositol pyrophosphates (IP_7 and IP_8) in cell death has been suggested. Inositol pyrophosphates occur physiologically, and are formed from IP_6 by a family of three inositol hexaphosphate kinases. Recent study by Nagata *et al.* [69] provided compelling evidence that one of these kinases, inositol hexakiphosphate kinase-2, by generating IP_7, provides physiologic regulation of the apoptotic process

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

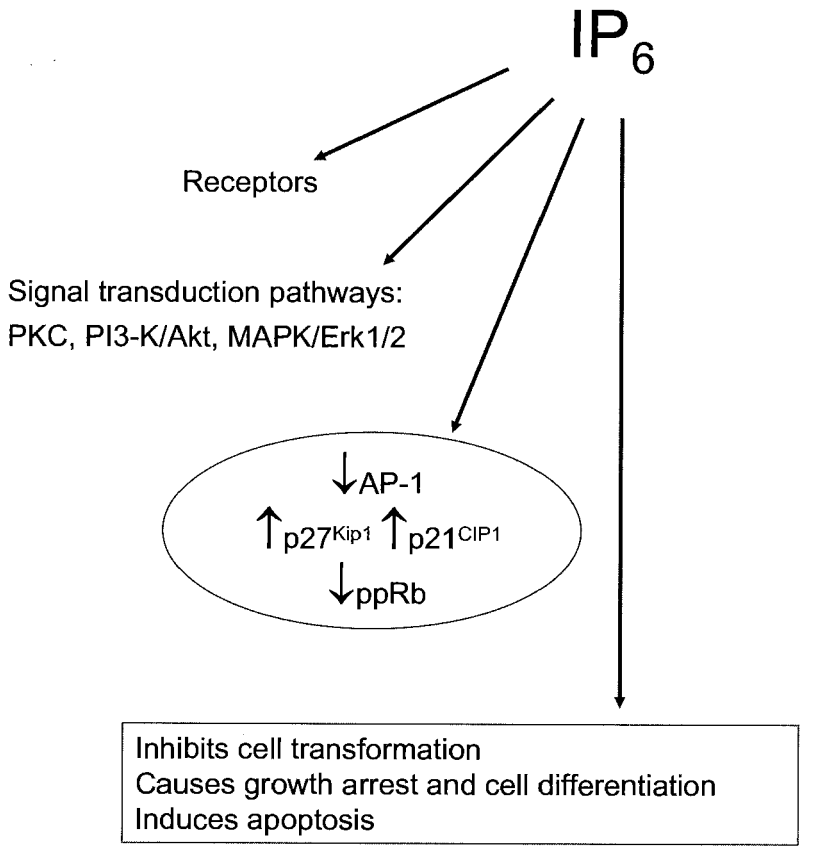

Fig. (3). Proposed mechanism of anticancer action of IP_6.

Above-summarized studies clearly suggest that acting at receptor levels, affecting signal transduction pathways and cell cycle regulators, IP_6 inhibits transformation, growth, survival, and induces differentiation program and apoptosis.

particularly effective against estrogen receptor–negative and doxorubicin-resistant cell lines, both conditions that are challenging to treat (Fig. **2**) [70]. 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.

The proposed mechanism of anticancer action of IP_6 is summarized in Fig. **3**.

IP_6 IS SAFE AND SELECTIVE

IP_6 is a natural compound and an important dietary component. In the past, some concerns have been expressed regarding 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 [71-74]. Long-term intake of IP_6 in food [71,72] or in a pure form [75] did not cause such a deficiency in humans. Studies in experimental animals showed no significant toxic effects on body weight, serum, or bone minerals or any pathological changes in either male F344 or female Sprague-Dawley rats for their lifetime [40,48,49]. Grases *et al.* [76] not only confirmed our findings but also reported that abnormal calcification was prevented in rats given IP_6, clearly an added health benefit. Moreover, in a very recent study Grases *et al.* [77] monitored the effect of IP_6 on mineral status during long period up to a second generation of rats, evaluating possible effects related to the pregnancy and lactations periods. No decrease in mineral bioavailability was observed, except for an indication of lower zinc concentrations in bone; however, animals fed with the equilibrated purified diet ± IP_6 had around 10-fold higher zinc levels in bone compared to the common, non-purified rat chow [77].

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 - to be selective and not to affect normal cells. 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 [26]. Recently, we showed that IP_6 inhibited the colony formation of Kaposi Sarcoma cell lines, KS Y-1 (AIDS-related KS cell line) and KS SLK (Iatrogenic KS), and CCRF-CEM (human adult T lymphoma) cells in a dose-dependent manner [67]. 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 [67]. 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.

INOSITOL + IP_6 AND PATIENTS

An enhanced antitumor activity without compromising the patient's quality of life was demonstrated in a pilot clinical trial involving 6 patients with advanced colorectal cancer (Dukes C and D) with multiple liver and lung metastasis [78]. Inositol + IP_6 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 Inositol + IP_6; her control ultrasound and abdominal computed tomography scan after 14 months after surgery showed a significantly reduced growth rate. Overall a reduced tumor growth rate was noticed and in some cases even tumor regression was noted. Additionally, when Inositol + IP_6 was given in combination with chemotherapy, side-effects of chemotherapy (drop in leukocyte and platelet counts, nausea, vomiting, and alopecia) were diminished and patients were able to perform their daily activities [78]. The same group of clinicians have been following up 22 patients with colorectal carcinoma (Dukes B2, C and D) surgically operated and submitted to adjuvant polychemotherapy, Mayo protocol and radiotherapy, in addition to Inositol + IP_6, and found that these patients manifested diminished side-effects of chemotherapy with improved quality of life [79]. Beneficial effect of Inositol + IP_6 was further observed in the treatment of ductal invasive breast carcinoma [80,81]. Not only that chemotherapy was never interrupted, but combination of Inositol + IP_6 contributed to diminished chemotherapy-related side-effect, improved quality of live, and prolonged survival of patients with metastatic recurrence of breast cancer [80,81]. A long-term survival of a patient with advanced non-small cell lung cancer treated with Inositol + IP_6 combined with chemo-radiotherapy was also recently reported [82]. However, further prospective and controlled randomized clinical trials are necessary to confirm these observations.

CONCLUSIONS

The preceding review shows that inositol and IP_6 are ubiquitous in nature, they are essential for vital cellular function and their deficiency results in diseases. Replacement by healthy diet or by supplementation may reverse some, if not all of the deficiency diseases. Not only is Inositol + IP_6 is safe but it also is effective against a variety of diseases. At the time of submission of this paper we are not aware of any negative data, and all reports published so far have indicated that these compounds have effects not only on cancer prevention and treatment, but also can improve many different diseases and conditions, such as reducing the risk of diabetes [13,83,84], hyperlipidemia [85-88], cardiovascular disease [89-92], kidney stone formation [74,75,93-95], and ulcer [96]. Quite interestingly and conveniently, prophylactic dosage of Inositol + IP_6 is low (1.5 – 2.0 g/day) and the cancer therapeutic dosage may be as high as 8-12 g/day. In the absence of a dose-determination study in humans, these values were extrapolated from animal data. And orally, it has been found to be safe at dosage several fold higher than the maximum therapeutic dose. Thus, inclusion of this safe and effective combination of Inositol + IP_6 in our strategies for prevention and therapy of cancer as well as other chronic diseases is warranted.

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ABBREVIATIONS

AP-1	=	Activating protein-1
Ins	=	Inositol
IP_6	=	Inositol hexaphosphate
IP_3	=	Inositol 1,4,5-P_3
PIP_2,	=	Phosphatidylinositol 4,5-bisphosphate
MAPK	=	Mitogen-activated protein kinases
NK	=	Natural killer
PI-3K	=	Phosphatidylinositol-3 kinase
PKC	=	Protein kinase C

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