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Review

Methyl jasmonate: A plant stress hormone as an anti-cancer drug

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ABSTRACT

Jasmonates act as signal transduction intermediates when plants are subjected to environmental stresses such as UV radiation, osmotic shock and heat. In the past few years several groups have reported that jasmonates exhibit anti-cancer activity *in vitro* and *in vivo* and induce growth inhibition in cancer cells, while leaving the non-transformed cells intact. Recently, jasmonates were also discovered to have cytotoxic effects towards metastatic melanoma both *in vitro* and *in vivo*.

Three mechanisms of action have been proposed to explain this anti-cancer activity. The bio-energetic mechanism – jasmonates induce severe ATP depletion in cancer cells via mitochondrial perturbation. Furthermore, methyl jasmonate (MJ) has the ability to detach hexokinase from the mitochondria. Second, jasmonates induce re-differentiation in human myeloid leukemia cells via mitogen-activated protein kinase (MAPK) activity and were found to act similar to the cytokinin isopentenyladenine (IPA). Third, jasmonates induce apoptosis in lung carcinoma cells via the generation of hydrogen peroxide, and pro-apoptotic proteins of the Bcl-2 family.

Combination of MJ with the glycolysis inhibitor 2-deoxy-D-glucose (2DG) and with four conventional chemotherapeutic drugs resulted in super-additive cytotoxic effects on several types of cancer cells. Finally, jasmonates have the ability to induce death in spite of drug-resistance conferred by either p53 mutation or P-glycoprotein (P-gp) over-expression.

In summary, the jasmonates are anti-cancer agents that exhibit selective cytotoxicity towards cancer cells, and thus present hope for the development of cancer therapeutics.

Contents

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1. Introduction

The jasmonate family which consist of *cis*-jasmone (CJ), jasmonic acid (JA), and methyl jasmonate (MJ) (Sembdner, 1993), are fatty acid-derived cyclo pentanones that occur ubiquitously in the plant kingdom (Fig. 1).

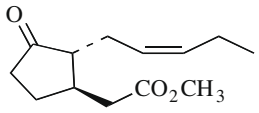
They were first isolated from the jasmine plant, and are a class of plant stress hormones similar to the salicylates.

The biosynthetic pathway of jasmonates was elucidated in the 1980's and those experiments showed that exogenous jasmonates exert effects on a wide spectrum of physiological processes. Today, jasmonates are recognized to be among the most potent and important signals for the regulation of defense-related genes in different species of the plant kingdom. It was discovered that constitutive activation of jasmonate signaling results in enhanced resistance to herbivores. In addition to anti-herbivore activity, genetic studies in *Arabidopsis* have shown that jasmonate signaling promotes direct defense responses to fungal pathogens (Davis, 2004; Samaila et al., 2004). Additionally, the jasmonate family acts as signal transduction intermediate when plants are subjected to environmental stresses such as UV radiation, osmotic shock, cytotoxic drugs and heat (Wang et al., 2007). The role of JA is the intracellular signaling response to injury, while MJ causes induction of proteinase inhibitor, which accumulates in response to wounding or to pathogenic attacks (Farmer and Ryan, 1990). The jasmonates have been reported to be involved in plant programmed cell death in a mechanism which resembles mammalian apoptosis (Wang et al., 2007). In addition to their role in plants, jasmonates were also found to have effects on cultured animal cells. These effects are anti-cancer activities which were exhibited both *in vitro* and *in vivo* (Flescher, 2007). Jasmonates and some of their synthetic derivatives, were shown to inhibit the proliferation and to induce cell death in various human and murine cancer cell lines, including breast, prostate, melanoma, lymphoblastic leukemia and lymphoma cells (Fingrut and Flescher, 2002), and exhibited selective cytotoxicity towards cancer cells even when they were a part of a mixed population of leukemic and normal cells drawn from the blood of chronic lymphocytic leukemia (CLL) patients (Fingrut and Flescher, 2002; Flescher, 2005). Furthermore, survival studies showed that jasmonates increased the life span of T-cell lymphoma-bearing mice (Fingrut and Flescher, 2002).

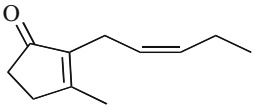
There are about one thousand species of plants that possess significant anti-cancer action. This action could be preventive and/or therapeutic. The first and most famous plant stress hormone that has been studied for many years is salicylate. Salicylic acid and its synthetic derivative – acetyl salicylic acid, i.e., aspirin, exhibit anti-cancer activity. Salicylate suppressed the proliferation of various types of cancer cells, including lymphoblastic leukemia, prostate, breast and melanoma human cancer cells (Fingrut and Flescher, 2002; Sotiriou et al., 1999), and also induced apoptosis in human myeloid leukemia cell lines (Klampfer et al., 1999), colorectal cancer cells (Elder et al., 1996; Lee et al., 2003), gastric cancer cells (Chung et al., 2003) and human glioblastoma cells (Amin et al., 2003). Aspirin, the synthetic salicylate, suppressed the proliferation of metastatic murine and human melanoma cells, human prostate cancer cell lines, and colon cancer cells (Fingrut and Flescher, 2002; Sotiriou et al., 1999).

Salicylates and jasmonates are both well known important plant signals, which share function but not structure (Fingrut and Flescher, 2002; Ryals et al., 1996). They both can cause systemic acquired resistance (SAR) to pathogens and injury in plants (Ryals et al., 1996), and share similarities in their anti-cancer activity towards mammalian cancer cells.

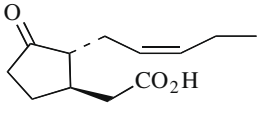
In the last 5 years we have published three reviews concerning the anti-cancer effects of MJ and its suggested mechanisms of action (Flescher, 2005, 2007; Goldin et al., 2007). This review will focus particularly on the cytotoxic effects of MJ as discovered recently, including their effect on metastatic melanoma and on the mitochondria deficient parasite – *Trichomonas vaginalis.*



Methyl jasmonate



***Cis* - jasmone**



Jasmonic acid

Fig. 1. Structures of naturally-occurring jasmonates.

2. The anti-cancer effects of MJ

We have previously reported that jasmonates can suppress the proliferation of various cancer cells and induce their death. Jasmonates were discovered for having the two desirable characteristics of anti-cancer drugs, which is to be highly selective towards cancer cells and ineffective towards normal cells, and to have the ability to act against drug resistant cells. In the case of the leukemic cell line (MOLT-4), MJ was proven to be significantly more cytotoxic towards this malignant cell line than towards normal lymphocytes (Fingrut and Flescher, 2002). The other characteristic was demonstrated using a pair of B-lymphoma clones of the same line differing in their p53 expression; wild type versus mutant p53. These clones differ drastically in their response to the cytotoxic drug Bleomycin and the radiomimetic neocarzinostatin (NCS), i.e., the mutant p53-expressing clone is by far less susceptible to these agents (Fingrut and Flescher, 2002; Flescher, 2007). In contrast, jasmonates were equally active against either clones. In the wild type cells, MJ induced mostly apoptotic death whereas in the mutant cells it induced necrotic death (Fingrut and Flescher, 2002; Flescher, 2007). This finding indicates that jasmonates can circumvent the resistance of mutant p53-expressing cells towards chemotherapy by inducing a non-apoptotic mode of cell death (Fingrut et al., 2005).

2.1. The anti-cancer effects of MJ on prostate and breast cancer cells

Prostate and breast cancers are the most common malignancies among males and females, respectively (Lopez-Otin and Diamandis, 1998). The accepted hypothesis is that breast and prostate cancer may represent, in some aspects, homologous cancers in females and males, respectively. These two cancers are considered to be the most common with a roughly equal lifetime risk. Both of these tumors are influenced strongly by steroid hormones – estrogen in the case of breast cancer and androgen in the case of prostate cancer. Evidence from 1954 showed that there is a significantly higher frequency of prostate cancer among relatives of breast cancer patients and proposed for the first time that prostate cancer may be the male equivalent of some female breast cancers (Macklin, 1954). Recently, Yeruva et al. (2008) have demonstrated similar anti-cancer effects of jasmonates on both prostate and breast cancers (Yeruva et al., 2008a,b).

In the case of prostate cancer the authors used two prostate cancer cell lines (PC-3 and DU-145) that do not express p53, but express high levels of the anti-apoptotic protein Bcl_2 (Copeland et al., 2007; Mori et al., 2006; Mujoo et al., 2005). However, when the mitochondria of these cells are damaged it activates the intrinsic apoptotic pathway and overcomes the effects of the anti-apoptotic protein Bcl_2 (Nachshon-Kedmi et al., 2004; Nutt et al., 2002; Oh et al., 2006; von Haefen et al., 2002). Their results have shown that MJ inhibited the proliferation of both prostate cell lines, induced S-phase arrest in PC-3 cells and G_0/G_1 block in DU-145 cells (Yeruva et al., 2008b). Furthermore, treatment with MJ resulted in activation of apoptosis hallmarks such as DNA fragmentation and caspase 3 and increased the death receptor protein tumor necrosis factor receptor 1 (TNFR1), which indicates an extrinsic apoptotic signaling in cancer cells (Yeruva et al., 2008b). Their study on breast cancer cells has shown similar effects of MJ on the two human breast cancer cell lines, MDA-MB-435 and MCF-7. Here also MJ inhibited the growth of these cells, caused cell-cycle arrest and apoptosis. MJ increased the expression of TNFR1, caused G_0/G_1 phase arrest, activated caspase-3 in MDA-MB-435 cells, and decreased mitochondrial membrane potential (Yeruva et al., 2008a). In another study on the human prostate cell line PC-3 conducted by Ezekwudo et al., they have studied the combined effect of MJ and γ-radiation (Ezekwudo et al., 2008). This combination of MJ and γ-radiation yielded a more than additive effect, compared to single treatment. The cytotoxic effect of radiation alone on these cancer cells was minimal given the dose and exposure time, resulting in little or no caspase-3 activity. However, adding MJ following irradiation resulted in a fivefold increase in caspase-3 activity compared to the radiation treated group, and 1.5-fold increase compared to the MJ treated group (Ezekwudo et al., 2008).

2.2. The cytotoxic effect of MJ on various human cervical carcinoma cell lines

Development of cervical cancer is strongly associated with infection by certain types of human papillomavirus (HPV), and more than 90% of the cervical cancers contain HPV DNA. The major viral oncogenes HPV E6 and E7 are always present and continuously expressed in HPV positive cancers (DiMaio and Liao, 2006; Zur Hausen, 2002).

The ability of the oncogenic proteins E6 and E7 to interact with and facilitate the degradation of cellular proteins that regulate the cell cycle and apoptosis such as p53 and pRb, respectively, is a potential mechanism by which these viral proteins induce tumors (DiMaio and Liao, 2006; Zur Hausen, 2002). The major problem with the current treatment for cervical cancer stems from the fact that many anti-cancer agents act through the induction of apoptosis (Bohm and Schild, 2003; Strasser et al., 2000). However, due to overexpression of the viral proteins in these cells, they express inactivated p53 and pRb. Therefore, the fact that the cytotoxic effects of jasmonates were shown to be independent of transcription, translation and p53 expression (Fingrut et al., 2005) can render them a potential treatment for this type of cancer. Indeed, we have recently shown the cytotoxic effect of MJ towards a range of cervical carcinoma cell lines (Kniazhanski et al., 2008). We found that MJ induced cell death that displayed mixed features of apoptosis and necrosis. Apoptotic indicators were: condensed chromatin and low content of DNA (sub-G_1), exposure of phosphatidylserine on the outer leaflet of the cell membrane, decline in procaspase-9 and 3 levels, and activation of poly (ADP-ribose) polymerase (PARP) cleavage by caspase 3. Furthermore, MJ induced upregulation of Bax and the survival protein Bcl-2 in HeLa cells and decreased p21 levels in HeLa and SiHa cells (Kniazhanski et al., 2008).

In summary, the effect of MJ on these four cervical cancer cell lines was mostly the induction of cell death. The cell death was independent of p53 activity.

2.3. The cytotoxic effect of MJ on human neuroblastoma cell lines

The anti-cancer effects of MJ were also studied, for the first time, on human neuroblastoma cells (Tong et al., 2008a,b). MJ was shown to suppress the growth of cultured neuroblastoma cells in association with downregulation of proliferating cell nuclear antigen (PCNA), and to induce apoptosis via modulation of expression of two anti-apoptotic proteins, XIAP (X-linked inhibitor of apoptosis protein) and survivin (Tong et al., 2008a,b). MJ induced G_0/G_1 arrest in SK-N-SH and BE(2)-C cells (Tong et al., 2008a), and G_2/M phase arrest in SH-SY5Y cells (Tong et al., 2008b). According to previous studies (Prosperi, 1997) PCNA modulates cell cycle via combination with many kinds of cyclin-dependent kinase/cyclin complexes, and downregulation of PCNA via antisense oligonucleotides facilitates the cell-cycle arrest at G_0/G_1 phase. Here the authors suggest that in the neuroblastoma cell line MJ downregulates PCNA and thus induces cell-cycle arrest.

Furthermore, MJ was found to suppress N-myc expression in SH-SY5Y neuroblastoma cells. N-myc is a proto-oncogene, overexpressed in approximately 25–30% of primary untreated neuroblastomas and associated with advanced stage disease, rapid progression, and poor prognosis (Brodeur, 2003). Its suppression by MJ might be correlated with the jasmonate-mediated anti-cancer activity on these cells.

2.4. MJ and metastasis

After witnessing the anti-cancerous effect of jasmonates on various murine and human tumors, including breast and prostate carcinoma, melanoma, lymphoblastic leukemia, etc. it was interesting to further investigate whether jasmonates have the ability to inhibit the metastatic process (Reischer et al., 2007). Invasion and metastases are the two major problems of conventional anti-cancer treatment. In the phase of metastatic disease cancer cells move within tissues by their own motility and can invade and localize distant organs. Once there is a metastatic disease, local therapy alone can no longer cure the patients and there is a need for an alternative treatment in order to control the migration of cancer cells.

Therefore we have decided to evaluate the potential anti-metastatic activities of jasmonates in the model of metastatic melanoma.

Melanoma accounts for approximately 4% of all cancers diagnosed in the USA. Depth of invasion of the primary lesion and the initial treatment are the two major factors that affect the prognosis of melanoma patients. In the case of metastatic melanoma there is no treatment that reliably affects the course of disease

(Morton et al., 2003). This is due to the resistance of melanoma cells towards various chemotherapeutic drugs (Rockmann and Schadendorf, 2003). Recently we have investigated the anti-metastatic effect of MJ on the murine B16 melanoma cells.

Our *in vitro* results reveal that MJ is capable of interfering with cell motility at concentrations that neither reduce cell number nor affect the cell ATP stores. *In vivo* studies have shown that MJ significantly suppressed the development of melanoma growth in murine lungs, at a dose of 75 mg/kg which is the highest non-toxic concentration. Moreover, when testing the effects of MJ on variant B16 cells which are highly metastatic drug-resistant melanoma cells, we found that those cells respond to MJ in a very similar manner to the parental cells, thus suggesting that MJ affects B16 melanoma cells independently of their drug-resistance status.

These findings show that besides inducing suppression of proliferation and death in cancer cells (Reischer et al., 2007), MJ has the ability to suppress various cellular functions, such as motility, which are essential to the metastatic process.

2.5. Combinations of MJ with other anti-cancer drugs exhibit synergistic or additive effects

Almost all curative chemotherapy regimens for cancer that are accepted today employ multi-agent drug combinations (Frei et al., 2003) this is basically due to the advantages multi-agent treatments have in comparison to single-agent treatments. Combining different anti-cancer drugs can maximize tumor cell toxicity while minimizing host toxicities. Additionally, combined therapy may overcome tumor cells which are resistant to a specific treatment, and can also prevent or slow the development of newly resistant tumor cells (Takimoto et al., 2005).

Consequently, we have decided to evaluate the combined effects of MJ and various other anti-cancer agents, as well as 2-deoxy-D-glucose (2DG), searching for super-additive interactions. 2DG is a glycolysis inhibitor that when combined with MJ was shown to have an additive effect on ATP depletion in B-lymphoma cells expressing either wild type (wt) or mutant p53 (Fingrut et al., 2005). The basis for this additive effect is probably due to the inhibitory effects jasmonates and 2DG have on two cellular pathways that generate ATP, oxidative phosphorylation and glycolysis, respectively.

Four different chemotherapeutic drugs in routine clinical usage were chosen based on their mode of action, which differs from that of MJ. The drugs BCNU (Carmustine) and Cisplatin belong to the family of alkylating agents, which impair cell functions by forming covalent bonds with the amino, carboxyl, sulfhydryl and phosphate groups in biologically important molecules. Taxol belongs to the family of Taxanes, which are semisynthetic derivatives of extracted precursors from the needles of yew plants. The taxenes promote microtubular assembly and stability, therefore blocking the cell cycle in mitosis (Takimoto et al., 2005). Adriamycin is an anti-tumor antibiotic that intercalates DNA at guanine-cytosine and guanine-thymine sequences, resulting in spontaneous oxidation and formation of free oxygen radicals that cause strand breakage (Takimoto et al., 2005).

Even though the mechanism of these drugs is different from that of MJ, their cytotoxic effect is mediated, though indirectly, via mitochondrial perturbation. We have shown previously that the mitochondria are the direct target organelle of MJ (Rotem et al., 2005). BCNU was shown to induce mitochondrial DNA damage (Cai et al., 2005). Taxol induces mitochondrial membrane depolarization resulting in translocation of apoptosis-inducing factor (AIF) from the mitochondria to the cytosol (Ahn et al., 2004). Cisplatin induces mitochondrial membrane depolarization and cytochrome *c* release (Wang et al., 2006). Adriamycin increases intra-cellular levels of reactive oxygen intermediates, followed by mitochondrial membrane depolarization, cytochrome *c* release and caspase 3 activation (Tsang et al., 2003).

Our results revealed cooperative effects of this combined treatment in six malignant cell lines *in vitro*, i.e., breast, lung, prostate and pancreas carcinomas as well as leukemia. Furthermore, an *in vivo* study revealed that combination of MJ and Adriamycin is synergistic against BCL1 leukemia in mice (Heyfets and Flescher, 2007).

MJ was shown to drastically lower the IC50 values of these chemotherapeutic drugs, pointing towards the potential of reducing unwanted side effects. Combination of MJ and 2DG has had a super-additive cytotoxic effect on carcinoma cells, probably due to the cooperation between inhibition of both ATP biosynthesis pathways.

In the case of the drug BCNU, when applied as a single agent on pancreas carcinoma cell line, it had no cytotoxic effect. However, combination with MJ yielded super-additive effect, obviously implying that the drug had an effect, even if not cytotoxic. We therefore hypothesized that the effects of BCNU on mitochondria (Cai et al., 2005; Wang et al., 2006) render these organelles hyper-sensitive to perturbation by MJ, resulting in super-additive effects.

3. Jasmonates: the mechanism of action

The mechanism of action of jasmonates is still not fully defined. Up till now, three mechanisms have been proposed to explain their anti-cancer activities, including induction of severe ATP depletion in cancer cells via mitochondrial perturbation, induction of re-differentiation in human myeloid leukemia cells via mitogen-activated protein kinase activity, and induction of reactive oxygen species-mediated apoptosis in lung carcinoma cells via generation of hydrogen peroxide and pro-apoptotic proteins of the Bcl-2 family.

3.1. The bio-energetic mechanism

Mitochondria are widely recognized as pivotal to life and death decisions in cells. Indeed, both apoptotic and necrotic death may result from mitochondrial perturbation.

During the last few years we have investigated the cytotoxic effect of MJ on mitochondria isolated from cancer cells, and the anti-mitochondrial effects of MJ on intact normal and cancer cells. The anti-mitochondrial effects of MJ include mitochondrial membrane depolarization, swelling and cytochrome *c* release. We have demonstrated that jasmonates induced mitochondrial perturbation in intact CLL leukemic cells and in mitochondria isolated from these cells. However, MJ acted selectively on the cancer cell mitochondria and it did not affect mitogenically stimulated normal human lymphocytes (Rotem et al., 2005). Cancer mitochondria differ from normal mitochondria in several aspects. This includes higher mitochondrial membrane potential in cancer cells, possible modulation of the expression of permeability transition pore complex (PTPC) components which include the adenine nucleotide translocator, cyclophilin D and the voltage-dependent anion channel (VDAC), and enhanced rates of ATP generation through glycolysis rather than oxidative phosphorylation (Warburg effect) in cancer cells (Debatin et al., 2002; Newmeyer and Ferguson-Miller, 2003; Chen, 1988; Dang and Semenza, 1999; Warburg and Dickens, 1930). In light of the above, we hypothesize that the impaired ability of cancer cell mitochondria to generate ATP would render them more sensitive to the rapid ATP depletion induced by MJ. Indeed, we had shown that MJ reduced the ATP cellular levels in various cancer cells long before any sign of cytotoxic effect was observed (Goldin et al., 2007). In accordance with mitochondria being the target organelles of MJ, such decreases in ATP levels were found to be

independent of pyruvate and oligomycin, a substrate and an inhibitor of oxidative phosphorylation, respectively (Fingrut et al., 2005). On the other hand, glucose protected the cells against MJ-induced ATP decreased levels and combination of the glycolysis inhibitor 2DG and MJ showed synergistic effects (Fingrut et al., 2005; Heyfets and Flescher, 2007). Once we had discovered the anti-cancer potential of MJ our goal was to locate the target molecule of MJ in cancer cell mitochondria. This was later discovered to be the enzyme hexokinase.

Hexokinase, the target molecule of MJ, is the initial enzyme in the glycolytic pathway. Its isozymes, hexokinase type I and hexokinase type II can bind to the mitochondrial VDAC through a hydrophobic interaction (Nakashima et al., 1986). In cancer cells, mitochondria-bound hexokinase and VDAC are overexpressed (Pedersen, 2002), this overexpression along with its glucose phosphorylation activity, are suggested to play a pivotal role in cancer cell growth rate and survival (Bustamante and Pedersen, 1977; Hammerman et al., 2004).

We have shown (Goldin et al., 2008) that MJ binds specifically to mammalian hexokinase and disrupts its interaction with VDAC, leading to detachment of hexokinase from the mitochondria followed by cytochrome *c* release. MJ-induced detachment of mitochondria-bound hexokinase perturbs mitochondrial permeability and overall cellular bio-energetics.

3.1.1. The involvement of the PI3K/Akt pathway in the cytotoxic effect of MJ

The phosphatidylinositol 3-kinase (PI3K)/Akt pathway regulates fundamental cellular functions and is often overactivated in a wide range of tumor types (Osaki et al., 2004; Sroka et al., 2007; Vivanco and Sawyers, 2002). This pathway plays a major part in the resistance of tumor cells to conventional anti-cancer therapies (West et al., 2002). According to several studies elevated Akt activity attenuates the sensitivity of cancer cell lines toward different chemotherapeutic agents such as Vincristine, Staurosporine, and TRAIL (Mookherjee et al., 2007; Nesterov et al., 2001; VanderWeele et al., 2004), and phospo-Akt (pAkt) expression level has been found to be a significant prognosticator in patients with different cancer types such as breast carcinoma (Perez-Tenorio and Stal, 2002), gastric carcinoma (Murakami et al., 2007), and soft-tissue sarcomas (STSs) (Tomita et al., 2006). Furthermore, according to a study on rat fibroblasts there is a connection between Akt signaling and mitochondrial hexokinase in the regulation of cell death (Gottlob et al., 2001; Majewski et al., 2004a,b).

Given all of the above, blocking the PI3K/Akt pathway can serve as a good target in anti-cancer therapy as it results in growth inhibition of tumor cells and sensitizes them toward different cytotoxic agents.

We have recently identified a strong correlation between the susceptibility of cells to MJ and the basal pAkt levels in sarcoma cell lines (Elia and Flescher, 2008). We found that treatment with MJ resulted in an increase in pAkt levels in two sarcoma cell lines (murine and human) and we showed that combination of PI3K/Akt pathway inhibitors with MJ, blocks MJ-induced activation of Akt. This blockage causes sensitization of the cell toward the cytotoxic effect of MJ leading to a synergistic cytotoxic effect (Elia and Flescher, 2008).

Akts' ability to promote survival and inhibit cell death depends on glucose availability (Rathmell et al., 2003). Given the role of Akt in MJ-induced cytotoxicity we examined the involvement of glucose metabolism in this process. Our results showed that modifying the glycolytic pathway and modifying the Akt pathway resulted in similar effects.

The combination of MJ with 2DG yielded a synergistic cytotoxic effect; cells grown in glucose-free media were more susceptible to the cytotoxic effect of MJ. Finally, 2-DG abrogated the MJ-induced elevation in pAkt levels and was sufficient to inhibit the anti-apoptotic effects of Akt thus rendering the cells more sensitive to the cytotoxic effects of MJ (Elia and Flescher, 2008). These results point out the advantages in using combinations of MJ and Akt inhibitor/2-DG as a novel multicomponent anti-cancer therapeutic modality for sarcomas.

3.1.2. The effect of MJ on the amitochondriate parasite T. vaginalis

T. vaginalis is a human urogenital tract parasite which causes a sexually transmitted disease called trichomoniasis. *T. vaginalis* is characterized for having no mitochondria and therefore can provide an answer as to whether mitochondria are essential for rendering the cells susceptible to the cytotoxic effect of MJ.

According to our findings on a variety of mammalian cancer cell types, there is a positive correlation between the susceptibility of a given cell type to the cytotoxic effect of MJ and the degree of ATP depletion induced in that cell (Goldin et al., 2007). However, in contrast to the effect of MJ on cancer cells (Fingrut et al., 2005; Goldin et al., 2007), its cytotoxic effect on *T. vaginalis* parasitic cells was not preceded by a drop in cellular levels of ATP, suggesting a different cytotoxic mechanism from the one through which it affects cancer cells (Ofer et al., 2008). Moreover, MJ was shown to induce a G_2/M phase cell-cycle arrest and non-apoptotic cell death, as indicated by the lack of apoptosis hallmarks; i.e., lack of caspase 3 activity, DNA laddering, and sub-G_1 peak (Ofer et al., 2008).

Although it was proven that the mitochondria are target organelles of MJ, here (Ofer et al., 2008) we have shown that MJ is also capable of damaging cells lacking mitochondria, i.e., mitochondria-deficient *T. vaginalis* parasites.

3.2. The re-differentiation mechanism

Re-differentiation occurs when cancer cells are being genetically and phenotypically modified according to a genetic program. This modified state proliferates at a slower rate and loses its earlier neoplastic attributes. Retinoids are a family of natural and synthetic nuclear receptor ligands (Brtko and Thalhamer, 2003), which are considered to act via this mechanism. All-*trans* retinoic acid (ATRA) induces re-differentiation of acute promyelocytic leukemia cells (APL) (Zhang et al., 2000), but has limited success as a single agent in the treatment of other hematopoietic malignancies (Ohno et al., 1993). The Cytokinin family of plant hormones was also found to induce re-differentiation in leukemic cells and in the novel study of Tsumura et al. (2008) they have shown that the cytokinin Isopentenyladenine (IPA) and MJ induced several differentiation markers in the human myeloid leukemia cells, HL-60. MJ and IPA both induced nitro blue tetrazolium (NBT) reduction (Flescher, 2007; Tsumura et al., 2008), and they both caused upregulation of the *S100P* gene, which encodes for calcium binding protein (Tsumura et al., 2008). In previous studies, MJ was shown to upregulate the *S100P* gene expression in other myeloid leukemia cells, such as NB4 and U937 (Flescher, 2007). On the other hand, CD14 (monocyte-specific surface antigen) expression and α-naphthyl acetate esterase activity were induced by MJ (Flescher, 2007; Ishii et al., 2004) but hardly by IPA (Ishii et al., 2002, 2005). According to Tsumura et al. (2008) recent results, based on a cDNA microarray analysis of the HL-60 leukemic cells, MJ and IPA-induced differentiation share a similar mode of action (Tsumura et al., 2008).

3.3. The reactive oxygen species (ROS) – mediated apoptosis

The third proposed anti-cancer mechanism of MJ is the involvement of ROS in MJ-induced apoptosis. In the work of Oh et al. (2005) MJ was shown to induce heat shock protein 72 (HSP72) in C6 glioma cells via heat shock factor I (Oh et al., 2005). This MJ-in-

duced expression was prevented by specific inhibition of hydrogen peroxide and hydroxyl radicals (Oh et al., 2005). Moreover, in the human non-small cell lung cancer (NSCLC) cells (A549), MJ was shown to induce apoptosis via a cascade involving hydrogen peroxide generation and an increase in the pro-apoptotic proteins Bax and Bcl-X_S (Kim et al., 2004). Further investigating the effect of MJ on A549 cells revealed the involvement of more pro-apoptotic proteins.

In the study of Yeruva et al. (2006) on the two NSCLC cells, A549 and H520, MJ inhibited the long-term proliferation as was demonstrated in survival assays, and induced G_2/M block in both cell lines (Yeruva et al., 2006). Treatment with MJ increased the overall amount of apoptotic cells and increased both the pro-apoptotic protein Bax and the anti-apoptotic protein Bcl_2 in H520 cells (Yeruva et al., 2006). Earlier studies reported that MJ caused phosphorylation of mitogen-activated protein kinases (MAPK) in A549 and leukemia cells (Kim et al., 2004; Rotem et al., 2003), in agreement with the results of Yeruva et al. (2006). The two NSCLC cell lines, A549 and H520 differ in their p53 expression (functional versus non-functional, respectively). p53 is a tumor suppressor gene and is a direct transcriptional activator for p21 and the pro-apoptotic Bax. Therefore, cells lacking the wt-p53 gene are considered to be more resistant to apoptotic inducers. However, this study revealed the opposite when the mutant-p53 expressing cells, H520 were found to be more susceptible to MJ than the wt-p53 expressing cells, A549 (Yeruva et al., 2006). Their results also indicated that the level of Bcl_2 in H520 cells was higher than in A549 cells, thus suggesting that the greater sensitivity of the former cell to jasmonates does not depend on the Bcl_2 level. Furthermore, in H520 cells phosphorylation of p38, a MAPK that has the ability to phosphorylate p53 and increase its activity, increased 2 h after exposure to MJ whereas in A549 cells the phosphorylation decreased at least 8 h after exposure.

In conclusion, the cytotoxic effects of MJ towards NSCLC cells are independent of p53 expression and of p38 phosphorylation, but do depend on the presence of Bcl_2 proteins, regardless of their level.

4. MJ suppresses proliferation in *Arabidopsis*

Apart from their role in inducing apoptosis and cell-cycle arrest in mammalian cancer cells, jasmonates were also found to be responsible for inhibiting mitosis and growth in plants.

A recent study of Zhang and Turner (2008) has demonstrated that repeated wounding activates jasmonate synthesis, a growth inhibitor which eventually causes a reduction in organ size. Bonsai plants are an extreme example for such leaf size reduction. They have shown that repeated wounding of leaves of the *Arabidopsis* plant resulted in growth reduction that was mediated through endogenous production of jasmonates. This conclusion was arrived at by using *Arabidopsis* mutants, which were unable to synthesize jasmonates or were unable to respond to jasmonates, and exhibited significantly less wound-induced growth inhibition than their wild type parents. They have shown that this stunted growth did not result from reduced cell size, but from reduced cell number which was associated with a reduction in mitotic index, as revealed by the reduced expression of *cycB1;2*.

5. Methyl jasmonate-rich plants as a source of anti-cancer preparations

Approximately 40% of Americans use alternative remedies, including herbal medicine, for disease prevention and therapy. The presence of certain phytochemical constituents supports the pharmacological and physiological efficacy of some ethnomedical treatment regimens. Of particular interest, considerable epidemiological and experimental evidence has been accumulated indicating risk reduction for numerous cancers (Park and Pezzuto, 2002). While studies focus on either prevention or therapy, this distinction is not always meaningful. The current understanding of carcinogenesis suggests that cancer cells are the final product of a series of genetic changes. Thus, chemoprevention may actually be carried out by killing of pre-neoplastic cells through mechanisms very similar, or even identical, to those by which phytochemicals kill mature cancer cells.

A great deal of evidence has suggested that a diet rich in fruits and vegetables protects against various neoplastic diseases. Overall, diets high in vegetables and fruits (more than 400 g/day) may prevent at least 20% of all cancers (Park and Pezzuto, 2002). Consequently, while this section will attempt to rationally associate methyl jasmonate content with the anti-cancer activities of certain plants, one should bear in mind that consumption of plants results in the administration of a huge variety of chemicals. Thus, a given phytochemical may only exert its positive effects in concert with other constituents of the plant of source.

We searched available databases and publications, and hereby provide the following list of plants containing relatively high levels of methyl jasmonate: olive, *Jasminum*, *Cymbidium goeringii*, *Polianthes tuberose*, *Chloranthus spicatus*, Ginger, *Boronia megastigma*, *Lonicera japonica* (Honeysuckle), *Artemisia tridentate*, and *Rosmarinus officinalis* L. (ESO 00 Database of essential Oils, 1999; Ikeda et al., 1994; Meshack Afitlhile et al., 2005; Ruiz Del Castillo, 2007). We found evidence in the literature for anti-cancer activities ascribed to five of these plants. These will be discussed below.

The anti-cancer effects of olive have been described extensively (Menendez and Lupu, 2006; Hashim et al., 2005; Stoneham et al., 2000; Newmark, 1997; Escrich et al., 2007). Olive oil contains a vast range of substances such as monounsaturated free fatty acids (e.g., oleic acid), hydrocarbon squalene, tocopherols, aroma components, and phenolic compounds (Hashim et al., 2005). An ecological study comprising 28 countries from four continents reported that 76% of the inter-country variation in colorectal cancer incidence rates was explained by three significant dietary factors – meat, fish, and olive oil – in combination. Meat and fish were positively associated, and olive oil was negatively associated (Stoneham et al., 2000). Epidemiological studies of breast and pancreatic cancer in several Mediterranean populations have demonstrated that increased dietary intake of olive oil is associated with a small decreased risk or no increased risk of cancer, despite a higher proportion of overall lipid intake. Experimental animal model studies of high dietary fat and cancer also indicate that olive oil has either no effect or a protective effect on the prevention of a variety of chemically induced tumors (Newmark, 1997). Different mechanisms for the modulatory actions of olive oil and other dietary lipids on cancer have been proposed. Among them, there is experimental evidence for influence on the hormonal status, cell membranes structure and function, signal transduction pathways, gene expression and the immune system (Escrich et al., 2007).

Many components of olive oil such as oleic acid have been suggested to be responsible for its anti-oncogenic effect. Newmark (1997) suggested that the high squalene content of olive oil, as compared to other human foods, is a major factor in the cancer risk-reducing effect of olive oil. We propose that methyl jasmonate contributes to the anti-cancer effect of olive oil.

Obviously, jasminum plants are a rich source of methyl jasmonate. Oral administration of ethanolic extract of *Jasminum grandiflorum* flowers to 7,12-dimethylbenz[α]anthracene (DMBA)-injected animals, completely prevented the formation of mammary tumors in the pre-initiation period (Kolanjiappan and Manoharan, 2005). Another approach taken was to study the effects of jasmine tea on the induction of tumors in rats. Esophageal tumors were induced by *N*-nitrosomethylbenzylamine and the rats were given

jasmine tea. Jasmine-treated rats exhibited a tumor incidence of 44% versus 90% in the untreated group (Chen, 1992).

Ginger is also a plant to which many reports attribute anti-cancer activities (Manju and Nalini, 2005; Park and Pezzuto, 2002; Rhode et al., 2007; Shukla and Singh, 2007). Ginger rhizome (*Zingiber officinale*), known commonly as ginger, is consumed worldwide in cookeries as a spice and a flavoring agent. An ethanol extract of ginger mediated anti-tumor promoting effects in a mouse skin tumorigenesis model. Pre-application of ginger extract on the skin of SENCAR mice resulted in significant inhibition of tetradecanoyl phorbol acetate (TPA)-induced epidermal ornithine decarboxylase, cyclooxygenase and lipoxygenase activity. In a long-term study, ginger extract also significantly protected against skin tumor incidence (Park and Pezzuto, 2002). In an additional study, rats were given 1,2-dimethylhydrazine (DMH) to induce colon carcinogenesis. When ginger was given to the rats at the initiation and post-initiation stages of carcinogenesis, the number of tumors as well as the incidence of cancer was significantly decreased. In addition, ginger supplementation significantly reduced circulating lipid peroxidation and significantly enhanced the enzymic and non-enzymic anti-oxidants as compared to non-supplemented DMH-treated rats (Manju and Nalini, 2005). *In vitro*, ginger inhibited growth and modulated secretion of angiogenic factors in ovarian cancer cells. Indeed, ginger treatment resulted in inhibition of NF-κB activation as well as diminished secretion of VEGF and IL-8 (Rhode et al., 2007).

Evidence for anti-cancer effects of *L. japonica* is scarce. The volatile constituents of the flowers of *L. japonica* Thunb, (Japanese honeysuckle), were isolated and analyzed. Sixty compounds were identified. Linalool was the major constituent. Nevertheless, 4-terpineol, nerolidol, *cis*-jasmone, *cis*-3-hexenyl tiglate, methyl palmitate and *trans*-linalool oxide, were detected in lesser but appreciable quantities. The volatiles showed a notable cytotoxic activity on the brain cancer cell line U251 and were found to be more potent than cisplatin. In addition, the volatiles showed a considerable cytotoxic activity on the liver cancer cell line Hep-G2 (El-Kashoury et al., 2007). In addition, a patent claims that a herbal preparation containing several plants including *L. japonica* provides anti-cancer effects, especially for breast cancer (Patent, U.S., 20070082072).

Finally, from among the methyl jasmonate-rich plants, *R. officinalis* L. is also widely considered to be endowed with anti-cancer effects, e.g. Cheung and Tai (2007), Huang et al. (1994), Sancheti and Goyal (2006), Sharabani et al. (2006), Singletary and Nelshoppen (1991), and Slamenova et al. (2002). Application of a methanol extract of the leaves of the *R. officinalis* L. (rosemary) to mouse skin inhibited the covalent binding of benzo(a)pyrene [B(a)P] to epidermal DNA and inhibited tumor initiation by B(a)P and DMBA. Application of rosemary to mouse skin also inhibited TPA-induced ornithine decarboxylase activity, TPA-induced inflammation, arachidonic acid-induced inflammation, TPA-induced hyperplasia, and TPA-induced tumor promotion (Huang et al., 1994). In a different approach, supplementation of a semi-purified diet containing 1% of rosemary extract decreased significantly DMBA-induced mammary tumor incidence (Singletary and Nelshoppen, 1991). The effects of rosemary extract were also studied at the *in vitro* level to further elucidate its mechanism of action. An ethanol extract from rosemary reduced the genotoxic activity of H_2O_2 and of visible light-excited methylene blue, in colon cancer CaCo-2 cells, and in hamster lung V79 cells. The authors suggest that the protective effect against oxidative damage to DNA is a consequence of scavenging of both OH radicals and singlet oxygen (Slamenova et al., 2002). In a recent study, crude ethanolic rosemary extract exhibited anti-proliferative effects on human leukemia and breast carcinoma cells. Interestingly, the extract also had anti-oxidant activity (Cheung and Tai, 2007). Finally, rosemary extract proved efficient in reducing side effects of vitamin D analogs. Combined treatment with 1% dry rosemary extract (mixed with food) and 1,25-dihydroxy-16-ene-5,6-*trans*-cholecalciferol resulted in a strong cooperative delay in tumor appearance and reduction in tumor size, in a myeloid leukemia model, without inducing hypercalcemia (Sharabani et al., 2006).

In conclusion, the different plants discussed above contain significant levels of methyl jasmonate. Here we put forward for the first time the hypothesis that methyl jasmonate is contributing to the reported anti-cancer effects of these plants. Consequently, it is warranted to evaluate epidemiologically and experimentally whether other methyl jasmonate-rich plants are also capable of suppressing cancer growth. The concrete question whether MJ contributes to the anti-cancer effects of the above mentioned plants can be addressed experimentally. One approach might be to develop genetically modified plants that will contain extremely high levels of MJ and compare them to their wild type counterparts in terms of cancer prevention. If indeed MJ plays a role in cancer prevention, one would expect the genetically modified plants to confer increased resistance towards cancer development. A different approach would be to measure the actual quantities of MJ consumed when the MJ-rich plants are eaten, administer pure MJ at those levels, and study its anti-cancer effects. However, as stated earlier, MJ may only exert its therapeutic effect in combination with other plant constituents.

6. Conclusions

MJ and salicylic acid are both stress hormones exhibiting anti-tumor activities (Fingrut and Flescher, 2002), as well as being established inducers of systemic acquired resistance (SAR). Over the years MJ was proven to have cytotoxic effect against various tumors both *in vitro* and *in vivo*. Its cytotoxicity against transformed cells is highly selective, thus suggesting low levels of side-effects usually encountered with existing cytotoxic drugs. This manuscript summarizes the recently discovered data considering the cytotoxic effects of MJ. This includes inhibition of proliferation and induction of cell-cycle arrest on various breast and prostate carcinoma cell lines, as well as neuroblastoma cell lines. Additionally, MJ was proven to have anti-metastatic effects on murine metastatic melanoma cells, both *in vitro* and *in vivo*. Apart from MJ anti-cancerous effect on mammalian cells, MJ was found to be cytotoxic towards the amitochondriate parasite *T. vaginalis* and to inhibit growth in the *Arabidopsis* plant.

A recent study showed that the derivatives of benzothiadiazole-7-carboxylates, a family of plant SAR activators, inhibit the growth of leukemia and lung cancer cells (Zhu et al., 2008). This finding supports further our initial hypothesis that plant stress hormones are endowed with anti-cancer activities, and present new hope for the development of cancer therapeutics.

6.1. Future directions

Two major directions for future research present themselves. First, attempts should be made to develop MJ as a clinical tool for the therapy of human cancer. This would of course require complete pre-clinical development (pharmacology, toxicology, etc.), as well as actual clinical trials. Preliminary first-in-man experiments suggest that MJ is bio-available, but thorough pharmacokinetic studies are still to be performed (Flescher, personal communication). Second, *in vivo* trials aimed at using MJ as a preventive agent are warranted. In such experiments, animals will be fed with pure MJ, tumors will be induced by carcinogens (chemical and/or physical) and the effect of MJ on oncogenesis will be monitored. Such experiments are admittedly long and costly, but are essential in order to evaluate the full anti-cancer potential of MJ.

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