Jasmonates–a new family of anti-cancer agents

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Since salicylate, a plant stress hormone, suppresses the growth of various types of cancer cells, it was deemed of interest to investigate whether the jasmonate family of plant stress hormones is endowed with anti-cancer activities. Cell lines representing a wide spectrum of malignancies, including prostate, breast and lung, exhibit sensitivity to the cytotoxic effects of methyl jasmonate (MJ). Jasmonates induced death in leukemic cells isolated from the blood of chronic lymphocytic leukemia (CLL) patients and increased significantly the survival of lymphoma-bearing mice. Among the naturally occurring jasmonates, MJ is the most active, while the synthetic methyl-4,5-didehydrojasmonate, was approximately 29-fold more active than MJ. The cytotoxic activity of MJ is independent of transcription and translation. Studies have suggested several mechanisms of action. It appears that while prolonged exposures to relatively low concentrations of jasmonates induce growth arrest and re-differentiation in myeloid leukemia cells, higher concentrations of MJ induce direct perturbation of cancer cell mitochondria, leading to the release of cytochrome *c* and eventual cell death. A most important characteristic of jasmonates is their ability to selectively kill cancer cells while sparing normal cells. Even within a mixed population of normal and leukemic cells derived from the blood of CLL patients, MJ killed preferentially the leukemic cells. In conclusion, jasmonates present a unique class of anti-cancer compounds which deserves continued research at the basic and pharmaceutical levels in order to yield novel chemotherapeutic agents against a range of neoplastic diseases. *Anti-Cancer Drugs* 16:911–916 © 2005 Lippincott Williams & Wilkins.

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Introduction

Plants face a myriad of challenges that perturb their homeostatic existence. Such stressful conditions include drought, radiation, bird and insect bites, as well as various types of infections. In response, biomolecules collectively designated as plant stress hormones are synthesized. These hormones induce signaling cascades and gene expression in plant cells, the final outcome of which differs. Just like the stress response in animals, cells may overcome stress-induced damage and regain homeostasis. Alternatively, cells may in effect commit suicide through apoptotic or necrotic cell death pathways.

The plant stress hormone most widely studied in mammalian systems is undoubtedly salicylic acid and its synthetic derivative acetyl salicylic acid, i.e. aspirin. The anti-inflammatory effects of salicylates have been studied and reviewed [1,2]. Since the anti-cancer effects of the plant stress hormones belonging to the jasmonate family are the focus of this review, I will only briefly refer to the anti-cancer effects of salicylates.

Salicylate suppressed the proliferation of lymphoblastic leukemia, prostate, breast and melanoma human cancer cells [3,4]. Furthermore, salicylate induced apoptosis in human myeloid leukemia cell lines [5], colorectal cancer cells [6,7], gastric cancer cells [8] and human glioblastoma cells [9].

Aspirin at a plasma-attainable and non-toxic level suppressed the proliferation of metastatic murine melanoma cells and human melanoma cells [10]. Additionally, incubation with plasma-attainable concentrations of aspirin for 3 days reduced cellular proliferation by up to 35–55% in human prostate cancer cell lines [11]. Aspirin was found to exert similar cytostatic effects on colon cancer cells [12–15]. Finally, both aspirin and salicylate induced apoptosis in B cell chronic lymphocytic leukemia (CLL) cells [16].

Another plant stress hormone, abscisic acid, has also been reported to induce death in mouse leukemia cells [17].

Based on the known anti-cancer effects of salicylates and abscisic acid, we hypothesized that this capacity might be shared by other plant stress hormones as well. Consequently, an investigation of the anti-cancer potential of the major plant stress hormone family, i.e. jasmonates, was initiated.

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Jasmonates and their role in stress responses of plants

Jasmonates, originally found as major constituents in the etheric oil of jasmine, are potent lipid regulators that mediate responses to mechanical and infectious stresses throughout the plant kingdom. These compounds are derived principally from linolenic acid and are structurally similar to certain prostaglandins. Stresses, such as wounding, can cause a rise in jasmonate biosynthesis which, in turn, results in specific programs of gene expression. Among these genes are those encoding stress-protective and pathogenesis-related proteins [18,19]. Programmed cell death often accompanies the anti-microbial response of plants, resulting in the formation of a zone of dead cells around the infection site. This zone is thought to function as a barrier that inhibits proliferation and spread of the pathogen [20–22]. Thus, cellular suicide is a characteristic of the stress response in both plants as well as animals.

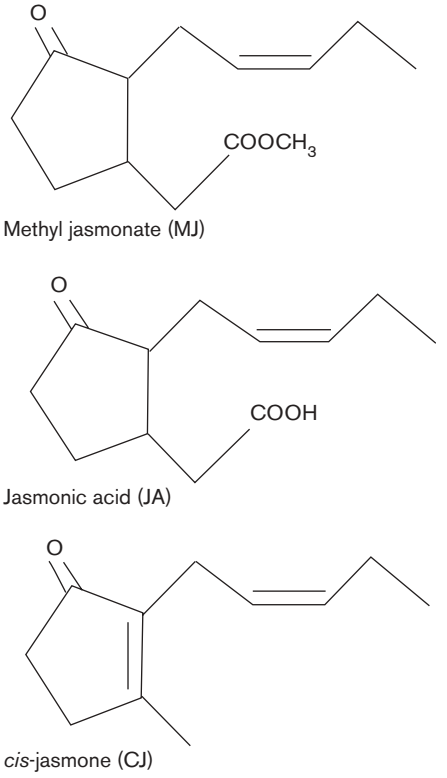
Anti-cancer effects of methyl jasmonate (MJ)

Naturally occurring jasmonates include several molecular species (Fig. 1 depicts three that have been studied as anti-cancer agents). MJ is the most active among them and has thus been studied in numerous cellular systems. Therefore, this section will focus on MJ; the other derivatives will be discussed in another section.

Neoplastic cells representing major solid tumors and hematogenic cancers have been studied as targets for MJ action. Table 1 presents the findings to date, most of which have been acquired using human cells (except for EL-4 lymphoma cells which are of murine origin). As can be seen, a wide spectrum of malignancies, including three of the most important human cancers, i.e. prostate, breast and lung, exhibit sensitivity to the cytotoxic effects of MJ. When cell death is indicated as the mode of toxicity, it is well established by various assays. However, suppression of proliferation is defined by a decrease in cell numbers (in comparison to the appropriate control) which may in fact represent suppression of proliferation and/or cell death. The exceptions are the results with HL-60 cells [25] where the actual lack of cell death has been established. Two of the cell lines were studied by two groups each (MCF7 breast carcinoma and A549 lung carcinoma). In each case, only one of the groups established in a straightforward manner that MJ induced cell death. Nevertheless, as mentioned above, the other group did not actually determine whether cell death occurred and there is therefore no contradiction between the studies. The mechanism of action will be discussed below.

MJ exhibited a cytotoxic effect on cancer cells freshly isolated from *ex vivo* blood samples drawn from CLL patients [23]. These patients suffered from B lymphocyte CLL and exhibited diverse responses. No correlation was found between the extent of the response with a given blood sample and their age, sex, stage of disease or medication. However, the percentage of leukemic cells within a given sample was in a direct and strong correlation with the degree of cytotoxicity exerted by MJ. This finding was further analyzed and will be discussed in a section on the selectivity of jasmonate anti-cancer activity (below).

Fig. 1



Structures of naturally occurring jasmonates.

In addition to *in vitro* studies, MJ was tested in a syngeneic mouse model of T lymphoma (EL-4). Administration of MJ p.o. daily increased significantly the survival of the lymphoma-bearing C57Bl mice [3]. Thus, MJ was shown to suppress the growth of cancer cells *in vivo* as well as *in vitro*.

Comparative effects of jasmonate derivatives

Naturally occurring, as well as synthetic, jasmonate derivatives have been compared as to their anti-cancer effects. The naturally occurring MJ and jasmonic acid (JA, see Fig. 1) have been compared *in vitro*, using leukemia, lymphoma, breast, prostate and melanoma cancer cells as targets [3]. In general, MJ has been found to be superior

Table 1 Cancer cell lines affected by MJ

Cell type	Suppression of proliferation	Cell death	Mechanism of action	Reference
Acute lymphoblastic leukemia Molt-4		+	direct effect on mitochondria via PTPC opening	[3,23]
Melanoma SK-28		+	ND	[3]
Prostate adenocarcinoma LNCaP		+	ND	[3]
Prostate adenocarcinoma PC-3	+		ND	[24]
Prostate adenocarcinoma HTB-81	+		ND	[24]
Breast carcinoma MCF7		+	ND	[3]
	+		ND	[25]
T lymphoma EL-4		+	ND	[3]
Liver carcinoma Hep 3B		+	direct effect on mitochondria via PTPC opening	[23]
Human myeloid leukemia HL-60	+		induction of differentiation	[25]
Monocytoid leukemia U937	+		induction of differentiation	[25]
Monocytoid leukemia THP-1	+		induction of differentiation	[25]
Promyelocytic leukemia NB4	+		induction of differentiation	[25]
Lung adenocarcinoma PC9	+		ND	[25]
Lung adenocarcinoma PC14	+		ND	[25]
Lung adenocarcinoma A549	+		ND	[25]
		+	induction of H_2O_2 production	[26]

ND=not done; PTPC=permeability transition pore complex.

to JA in terms of cytotoxicity and caspase-3 activation (a marker of apoptosis). In accordance, the sensitivity of two prostate cancer cell lines was found to be in the following order: MJ > *cis*-jasmone (CJ) ≥ JA [24]. For the structure of CJ, see Fig. 1. Interestingly, although samples from different CLL patients exhibit a spectrum of intensities in their response to jasmonates, their relative sensitivities can also be generally summed up as: MJ > CJ ≥ JA [23]. Thus, the methyl group appears to contribute considerably to the cytotoxic effect of MJ. The basis for this finding, e.g. improved permeation of cells, higher affinity to a putative target molecule, etc., remains to be established.

Ishii *et al.* [25] have tested a series of synthetic jasmonate derivatives and compared them with MJ. Only one derivative, dihydrojasmone, was significantly less cytotoxic than MJ, while all the other seven derivatives exhibited smaller IC_{50}s in comparison to MJ. The IC_{50} of the most active derivative, methyl-4,5-didehydrojasmonate, was approximately 29-fold smaller that that of MJ, as determined by suppression of the growth of HL-60 cells. It therefore appears that introduction of a double bond between positions 4 and 5 of the cyclopentanone ring has a significant effect on the anti-cancer activity of MJ, and may provide a hint as to additional modifications that could yield a more efficacious jasmonate.

Proposed mechanisms of action of jasmonate

Prior to discussing specific mechanisms of action, we will refer to the issue of apoptotic versus necrotic cell death. As can be seen in Table 1, several groups have determined MJ to induce cell death. The apoptotic nature of cell death was established by a series of assays, including fluorescence microscopy, caspase-3 activity and flow cytometry [3,26]. In accordance, MJ was found to induce DNA fragmentation characteristic of apoptotic cells in callus plant cells [27]. Nevertheless, careful analysis of the flow cytometry data [3] suggests that MJ treatment of Molt-4 leukemic cells induces the appearance of necrotic cells either before or concomitantly with apoptotic cells. Thus, MJ may induce necrosis, as well as apoptosis, in leukemic cells.

Given the role of MJ in plant stress responses, it was only natural to test the hypothesis that MJ induces stress signaling leading to cell death in cancer cells. Indeed, MJ was found to induce the activation of two stress-regulated mitogen-activated protein kinases, c-Jun N-terminal kinase (JNK) and p38, in leukemic [28] and lung carcinoma [26] cells. Furthermore, it resulted in downstream nuclear signaling measured as the activation of the AP-1 transcription factor [28]. However, using inhibitors of JNK and p38, it was found that these enzymes do not mediate the cytotoxic effect of MJ [28]. Since MJ induced the transcription factor AP-1, the role of transcription and translation in MJ cytotoxicity was studied. Employing cycloheximide (a protein synthesis inhibitor) and actinomycin D (an mRNA synthesis inhibitor), it was established that MJ induces cell death in a manner independent of *de novo* gene expression [28].

MJ and some of its derivatives induce differentiation in myeloid leukemia cells [25], as judged by nitroblue tetrazolium-reducing activity, morphology, α-naphthyl acetate esterase activity and surface antigens. The differentiation resulted in the appearance of cells exhibiting monocytic and granulocytic characteristics. Induction of differentiation is bound to involve a drastic modification of the gene expression program, and is thus dependent on alterations of transcription and translation. Thus, while the cytotoxic effects of MJ are independent of mRNA and protein synthesis, its differentiation-inducing capacity is dependent on macromolecular biosynthesis. One way of explaining this difference is that differentiation was induced in myelogenous leukemia

cells [25], while the cytotoxicity was demonstrated in acute lymphoblastic leukemia cells [3]. However, a more illuminating explanation may be the fact that in the differentiation experiments, lower concentrations of jasmonates, and much longer periods of exposure, were employed, in comparison to the cytotoxic assay. This analysis raises the possibility, which deserves further investigation, that jasmonates may have more than one mechanism of anti-cancer action, depending on the dosage and schedule of administration.

Recent studies have analyzed the mechanism through which jasmonates induce cell death. Mitochondria were found to play a pivotal role in the mechanism of action of jasmonate. Jasmonates induced mitochondrial membrane depolarization and cytochrome *c* release in intact cancer cells [23]. More importantly, MJ induced swelling and cytochrome *c* release in mitochondria isolated from human leukemia and hepatoma cell lines, as well as leukemic cells from CLL patients [23]. Thus, MJ has direct mitochondriotoxic effects, strongly suggesting that mitochondria are target organelles of jasmonates. In support of this contention, inhibitors of the opening of the mitochondrial permeability transition pore complex (PTPC, a pore-mediating mitochondrial perturbation resulting in cell death) reduced significantly the toxic effects of MJ on cancer cells and on mitochondria isolated from these cells. These studies [23] show that jasmonates kill cancer cells in a PTPC-dependent manner and act directly on the mitochondria of these cells. This attribute of jasmonates should endow them with the ability to bypass pre-mitochondrial anti-apoptotic mutations, thereby making this class of anti-cancer agents potentially active against a variety of drug-resistant tumors. Studies of lung carcinoma cells [26] also suggest the involvement of mitochondria in MJ-induced apoptosis, possibly via hydrogen peroxide generation and the subsequent induction of the Bcl-2 family pro-apoptotic proteins Bax/Bcl-X_S in the process.

Preferential effects of jasmonates on transformed cells

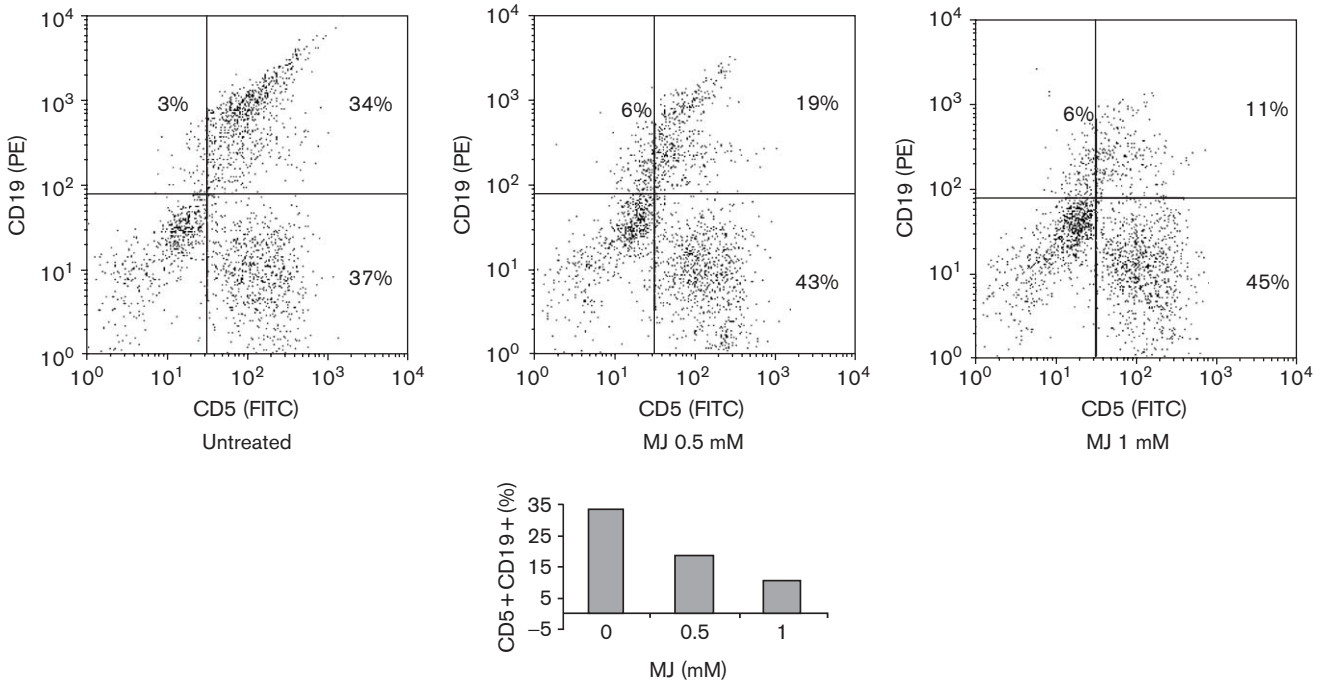
A major characteristic expected of a potential anti-cancer agent is its ability to selectively affect cancer cells while sparing normal ones. Since normal blood cells are easily obtainable, a comparison was performed between the effect of jasmonates on leukemic cells and on their normal counterparts. Both JA and MJ were preferentially cytotoxic towards the leukemic cells [3]. Accordingly, jasmonates did not induce cytochrome *c* release or swelling in mitochondria isolated from normal lymphocytes, whereas these events were recorded in mitochondria isolated from leukemic cells [23]. It thus appears that the difference between the normal and cancer cells exists at the mitochondrial level. Interestingly, jasmonates did not induce swelling in mitochondria isolated from immortal, but non-transformed, 3T3 human fibroblasts [23], suggesting that neoplastic transformation renders the mitochondria susceptible to jasmonates. However, caution should be exercised in interpreting differences between normal and cancer cells as a possible basis for the observed selective cytotoxicity. A case in point is the difference in the extent and kinetics of activation by MJ, of the stress-regulated kinases JNK and p38, in normal lymphocytes versus leukemic cells [28]. Using inhibitors of these kinase cascades we excluded a role for JNK and p38 in the cytotoxic mechanism of MJ. Thus, while normal lymphocytes exhibited a different protein kinase response to MJ, in comparison with leukemic cells, this did not constitute the basis for the differential susceptibility of these cells to the cytotoxic activity of MJ.

In addition to normal lymphocytes, peripheral blood erythrocytes [29] and human sperm cells (E. Flescher, personal communication) were also found to be resistant to jasmonate cytotoxicity. Again, these results strongly support the conclusion that jasmonates target specifically transformed cells. A recent experiment highlights this point vividly. Blood leukocytes were isolated from CLL patients and exposed to MJ overnight. Since the CLL leukemic cells aberrantly express both a B cell (CD19) and a T cell (CD5) marker, they can be easily distinguished from normal cells in the same blood sample. FACS analysis revealed that MJ induced a drop in the percentage of leukemic cells in a dose-dependent manner (Fig. 2). The most straightforward interpretation of these results is that MJ kills preferentially the leukemic cells in the sample derived from the CLL patient.

Conclusions and future directions

The structures of jasmonates are different from any group of currently available anti-cancer agents. Their activity and selectivity, suggesting low levels of side-effects usually encountered with existing cytotoxic drugs, pos ition jasmonates as the very first of a new class of drug of major interest for oncologists and for their patients, whose chemotherapeutic promise should be realized through preclinical and clinical development. While many anti-cancer agents are of plant origin, the actual function of these compounds in the plant is often unknown. The fact that jasmonates regulate stress responses in plants as well as in mammalian cancer cells suggests that identification of plant-derived molecules with known roles in plant cell death may provide novel candidates for use in clinical oncology. Potential future research directions include structure–function analysis in order to identify the actual jasmonate target molecule(s) and discovery of new derivatives with superior therapeutic index.

Fig. 2



Preferential killing of leukemic cells from the peripheral blood of a CLL patient. Peripheral blood lymphocytes drawn from a CLL patient were incubated overnight with different concentrations of MJ. Three-color FACS analysis was performed, in which dead cells (propidium iodide-positive) were excluded, and the living cells were analyzed as to their expression of a T cell (CD5) and/or B cell (CD19) markers. The upper right-hand corners of each of the upper schemes contain the $CD19^+CD5^+$ leukemic cells. While the untreated samples contain 34% leukemic cells, this value drops to 19 and 11% upon treatment with MJ overnight (as depicted graphically in the bottom figure).

In addition to anti-cancer activity, jasmonates exhibit anti-parasitic activities, inducing the death of *Plasmodium falciparum* and *Schistosoma mansoni* [29], and anti-fungal activity against *Aspergillus flavus*, inducing inhibition of growth and of aflatoxin production [30]. These activities present additional potential applications of jasmonates.

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