Potential Role of PKC Inhibitors in the Treatment of Hematological Malignancies

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Abstract: The serine/threonine protein kinase C (PKC) family, the main target of tumor-promoting phorbol esters, is functionally associated to cell cycle regulation, cell survival, malignant transformation, and tumor angiogenesis. Although PKC isozymes represent an attractive target for novel anticancer therapies, our knowledge of PKC in tumorigenesis is still only partial and each PKC isoform may contribute to tumorigenesis in a distinct way. Specifically, PKC isoforms have wide and different roles, which vary depending on expression levels and tissue distribution, cell type, intracellular localization, protein-protein and lipid-protein interactions. Although PKC activation has been linked to tumor cell growth, motility, invasion and metastasis, other reports have shown that some PKC isoforms can also have opposite effects. Therefore, it will be necessary to analyze the relative contribution of each PKC isozymes in the development and progression of different tumors in order to identify therapeutic opportunities, using either PKC inhibitors or PKC activators as molecular tools of investigation. This minireview is focussed on the role of PKC signaling and on the perspective of PKC inhibition in hematological malignancies.

Key Words: PKC, hematological malignancies, apoptosis, tumorigenesis.

INTRODUCTION

Protein kinase C (PKC) isoenzymes are the major cellular target of phorbol esters (phorbol-12-myristate-13-acetate or 12-O-tetradecanoylphorbol 13-acetate), which are well-known powerful tumor promoters [1]. Therefore, it has been hypothesized approximately 30 years ago that PKC may play a pivotal role in key intracellular signaling pathways involved in tumor promotion [2, 3]. It was found soon after that the plasma cell membrane lipid diacylglycerol (DAG) and phorbol esters share the same binding site on PKC, which serve as hydrophobic anchors and stabilize the active PKC conformation [4]. These studies were the first to demonstrate that the bioactive lipid product DAG was able to regulate a kinase associated with tumorigenesis and that phorbol esters can substitute for DAG in activating PKC [5]. Therefore, PKC has immediately attracted considerable attention as potential target for anticancer therapy, and recent preclinical and clinical studies have confirmed the initial hypothesis, demonstrating promising activity of PKC inhibitors in a variety of tumors. Nevertheless, despite the intense research activity in the last three decades, our knowledge on the biology of the PKC family of protein kinases is still limited [6]. Indeed, the picture emerging is quite complex: many PKC isoforms mediate tumorigenic effects, while others can promote cell differentiation or even exert pro-apoptotic effects, depending on the expression levels in a given organ, the cell type or tissue distribution, the intracellular localization, and the interplay with other lipid-dependent intracellular pathways, such as in particular the PI 3 kinase/Akt [7-8]. A good example of the complex interplay between PKC isozymes and the regulation of key cellular events such as cell survival, proliferation and differentiation is provided by hematological malignancies [9-12].

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PKC Isoforms: Taxonomy and Mechanisms of Activation

The PKC family of serine/threonine kinases, comprising up to now 12 isoforms, is divided into 3 structurally and functionally distinct subgroups: conventional isoforms (cPKC; including PKC-α, PKC- βI, PKC-βII and PKC-γ); novel isoforms (nPKC; PKC-δ, PKC-ε, PKC-η and PKC-θ); and atypical isoforms (aPKC; including PKC-ζ and PKC-λ/ι). Moreover, PKC-μ and PKC-ν were recently added to the PKC super-family based on homology within the catalytic domain. PKC isoforms consist of an N-terminal regulatory domain and a C-terminal catalytic domain. The membrane targeting regulatory domains C1 and C2 of cPKCs confer binding to the lipid second messenger DAG, phorbol esters and phosphatidylserine (C1) as well as to Ca^{2+}. Similarly, nPKCs contain the C1 domain and a novel C2 domain; they are regulated by DAG but not by Ca^{2+}. In contrast, aPKCs are not regulated by either DAG or by Ca^{2+}.

The catalytic domain of all subgroups consists of the ATP binding (C3) and the kinase domains. Similar to cPKC and nPKC, PKC-μ is regulated by DAG and 12-*O*-tetradecanoylphorbol 13-acetate through a C1 domain. Additionally, it contains a transmembrane domain and a pleckstrin homology domain [13]. The production of the lipid second messenger DAG is predominantly initiated by stimulation of either tyrosine kinase receptors or G-protein-coupled receptors, followed by activation of phospholipase-C (PLC)-γ or PLC-β, respectively. In turn, PLC induces the generation of the second messengers inositol triphosphate and DAG.

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DAG then induces recruitment of cPKCs and nPKCs to the membrane *via* the C1 domains C1A and C1B, thereby inducing conformational changes that enable substrate binding and activation (see Fig. **1**). Furthermore, PKC stabilisation and activation are enhanced by transphosphorylation and autophosphorylation, allowing DAG-independent PKC activation [5]. PKC phosphorylation takes place in three well-conserved positions within cPKCs and nPKCs: the activation loop, as well as the carboxy-terminal turn and hydrophobic regions. In aPKCs, phosphorylation takes place only in one of the carboxy-terminal regions because to a lack of serine and threonine residues. Cell and stimuli-specific functions of PKC isoforms are mediated by their complex formation with a plethora of anchoring proteins and receptors, which explain their peculiar intracellular localization in several different cellular compartments.

Evidence for the Involvement of Different PKC Isoforms in Tumor Biology

The different PKC isoforms are expressed in a tissue- and cell type-specific manner. Specifically, PKC-α, PKC-δand PKC-ε are widely expressed, while the expression of all other isoforms is more restricted. Functionally, PKCs have been implicated in the regulation of cell survival, growth, differentiation, and migration both under physiologic conditions and during tumorigenesis [13-20]. Initially linked to phorbol ester-induced mitogenesis and tumor promotion, it has now become clear that members of the PKC family can act both as activators and inhibitors of tumorigenesis. Specifically, PKC-βand PKC-ε predominantly act as activators, while PKC-α, PKC-δ and PKC-η seems to act as inhibitors [13-15]. However, before ascribing a definite role to a specific isoform, it should be noticed that opposing effects have been reported for each isozyme depending on the expression levels and tissue distribution, cell type, stimuli, intracellular localization, protein-protein and lipid-protein interactions and the biologic environment. Therefore, it is critical to define the relative functional contribution of individual isozymes relative to these factors. Increased levels of PKC and/or increased activity have been described in various solid tumors [21-24]; and these increased levels of expression and/or activity are often linked to disease progression. Conversely, inhibition of PKC abrogates tumor growth in xenograft mouse models of solid tumors [25]. For the purpose of this review, however, it is noteworthy that several members of the PKC family have also been implicated in hematological malignancies. In particular, a significant over-expression of PKC-β has been reported in samples obtained from patients affected by progressive diffuse large B-cell lymphoma (DLBCL) and has been associated with poor prognosis [26, 27]. Elevated levels of PKC-β have also been associated to T-cell lymphomas and T-cell leukemias [28-30]. In the following sections, we will focus on the expression of several PKC isoforms in the context of hematological malignancies and we will consider the potential therapeutic application linked to inhibition of PKC signaling in a therapeutic perspective for the treatment of hematological malignancies.

PKC Isoforms in Multiple Myeloma (MM)

Several PKC isozymes have been found to be expressed by MM cell lines [31-34]. The significance of PKC-α, PKC-β and PKC-μ expression in MM cell lines has been linked to vascular endothelial growth factor (VEGF)- and Wnt-induced MM cell migration and metastatic potential [34, 35]. Conversely, over-expression of PKC-δ has been associated to the induction of MM cell apoptosis [36] and, together with PKC-η, to the control of interleukin (IL)-6 receptor-α shedding [32]. Of note, one of the strongest support to the hypothesis of using PKC inhibitors in the treatment of MM comes from studies demonstrating that a marked up-regulation of PKC-β represents a MM gene signature linked to the adverse prognostic t(4; 14)(p16; q32) translocation [37-38]. Dissecting more precisely the role of each isoform in MM, a pivotal role for PKC-α is described for integrin expression and growth factor-triggered signaling pathways in MM. In fact, VEGF-mediated MM cell migration on a fibronectin scaffold is associated with beta-1 integrin and phosphatidylinositol (PI) 3 kinase-dependent PKC-α activation [33, 39]. PKC-β is the major isoform expressed in normal and malignant B lymphocytes (see below); in MM micro-environment, an important role of PKC-β signaling is linked to VEGF-driven angiogenesis [40-44]. In this respect, it should be noticed that tumor angiogenesis plays a key role in promoting the progression of MM [35], and that anti-angiogenic effects of several PKC inhibitors have been characterized in both solid tumors as well as in MM [38, 45-47].

At variance to the previously mentioned PKC isoforms, PKC-δ is usually considered a pro-apoptotic isozyme, able to

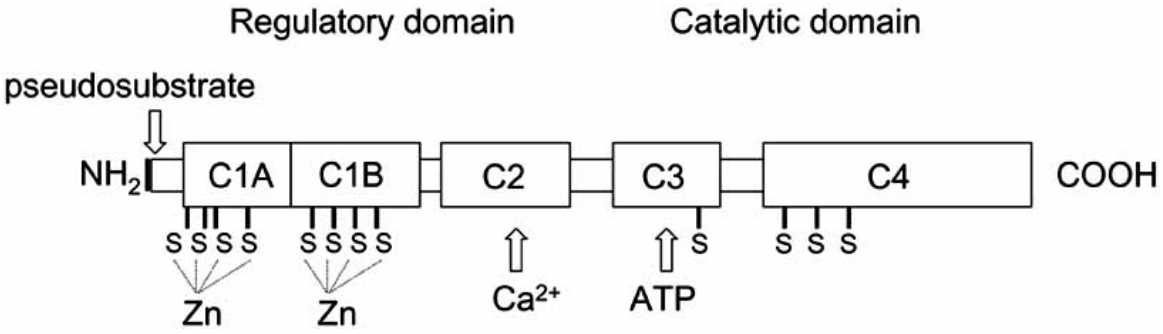

Fig. (1). Multidomain structure of PKC that enable it to serve as receptor for both tumor promoters and antitumor-promoting agents. C1: cysteine-rich constant region present in various isoenzymes of PKC. C1A: first zinc-fingers region in the C1 domain; C1B: second zinc-fingers region in the C1 domain; C2: Ca2+-binding domain; C3: ATP-binding region; C4: protein substrate-binding region; pseudosubstrate: autoinhibitory region of PKC that prevents the binding of protein substrate to the catalytic domain.

mediate tumor suppression [48]. The anti-cancer activity of PKC-δ seems to be mediated by a pro-apoptotic mechanism involving the caspase-3-dependent formation of a catalytic C-terminal fragment which phosphorylates nuclear PKC substrates, including DNA-dependent protein kinase, phospholipid scramblase-1 and lamin B, thereby stimulating apoptosis [49-54]. It has been demonstrated in MM cells that dexamethasone or anti-Fas exposure are able to trigger this cascade leading to overt apoptosis [36]. On the contrary, the entire PKC-δ isoform seems to exert a survival advantage in MM cells [55], clearly indicating that also the role of PKC-δ in controlling the survival/apoptosis of MM is much more complex than originally thought.

PKC Isoforms in B-Cell Lymphocytic Leukaemia (B-CLL)

Initial evidence has suggested that PKC-α confers resistance to the anti-leukemic therapy (by upregulating bcl-2 expression) [56, 57], and, similarly to what has been previously observed in MM, PKC activation has been associated to increased adhesiveness and to leukemic cell migration through an increased expression of integrins. Although these data suggest that PKC-α might be involved in leukemogenesis, a more recent and intriguing study has demonstrated that subversion of PKC-α signaling in normal murine hematopoietic progenitor cells results in a phenotype similar to human B-CLL [58], implying that a defective signaling through PKC-α might result in a defective differentiation of pre-B lymphocytes. Although the phenotypic similarities between human B-CLL and the B leukemia observed in this study [58] should be considered with caution, nevertheless this is the first report suggesting that a selective deficit of a given PKC isoform might be implicated in leukemogenesis. As previously demonstrated for MM, PKC-δ seems to play a dual role in regulating apoptosis in B-CLL cells. While the cleaved fragment of PKC-δ is able to promote apoptosis in a variety of different cell types [59], including leukemic cells, the intact isozyme seems to be a downstream target of the PI-3K/Akt pathway, able to mediate pro-survival signals and chemotherapeutic resistance in B-CLL [60].

New PKC Modulators Enrolled in Clinical Trials of Haematological Malignancies

Targeting PKC isozymes represents a novel target in tumor therapy, and recent preclinical and clinical studies have demonstrated promising activity of PKC inhibitors in a variety of tumors. In this paragraph, we will describe some of the more interesting compounds that have already shown a remarkable effect both *in vitro* and *in vivo* in preclinical and clinical studies: enzastaurin, midostaurin, bryostatin, curcumin and aplidin (Table **1**).

Enzastaurin

The macrocyclic bisindolylmaleimide enzastaurin (LY317615) is an orally available PKC inhibitor, which competes with ATP for the nucleotide triphosphate-binding site of PKC, thereby blocking its activation. The first and best characterized activity of enzastaurin is the inhibition of the PKC-β isoform. However, increasing experimental evidence clearly suggest that enzastaurin also potently inhibits other PKC isoforms, including PKC-α, PKC-δ, PKC-γ and PKC-ε, as well as the lipid dependent phosphatidylinositol 3-kinase/Akt signaling pathways [61]. Enzastaurin is primarily metabolized by CYP3A [62] and the generated metabolites are also PKC inhibitors with similar potency to native enzastaurin. Although initially developed for its potential antiangiogenic properties [63, 64], recent preclinical studies demonstrate significant anti-tumor activity against both solid tumors, as well as against different haematological malignancies, such as cutaneous T-cell lymphoma cells (HH, HuT-78) and leukemic cell lines (K562, MOLT-4 and HOP-92) cell lines [61, 65]. Preclinical studies in rats and dogs have demonstrated that enzastaurin was well tolerated [62]. Based on these promising preclinical data and the safety profile, a phase I dose-escalation and pharmacokinetic study of oral enzastaurin was initiated in patients with advanced cancers of the lung and the head and neck. This study showed minimal enzastaurin-related toxicity when used at a dose range of 20-700 mg/day and did not reach a maximal tolerated dose. Grade 1 chromaturia, fatigue and gastrointestinal toxicities (diarrhea, nausea and vomiting) were most com-

Table 1. Natural Products Modulators of PKC

Natural Product	Isolated from (Organism)	Active Costituent	Targeted PKC Isozyme	Ref.
metabolite	*Bugula neritina*	bryostatin	nPKCs	[75,76]
rhizome	*Curcuma spp.*	curcumin	broad	[94]
metabolite	*Aplidium albicans*	aplidine	δ	[106]
toad venom	*Secretia bufonis*	bufalin	β	[118]
root	*Angelica gigas*	decursin	α, β	[110,111]
root	*Panax ginseng*	ginsenoside-Rh2	β	[109]
fruit	*Gardenia jasminoides*	penta-acetyl geniposide	δ	[115]
sap	*Euphorbia peplus*	ingenol 3-angelate	δ	[107]
flower buds	*Daphne genkwa*	yuanhuacine	undefined	[119]
root tubers	*Trichosanthes kirilowii*	trichosanthin	δ (?)	[114]

mon; no clinically significant grade 3 or 4 toxicity occurred. Mean steady-state plasma levels of 2 μmol/L were achieved after oral administration of enzastaurin 525 mg/day. Clinical trials of enzastaurin, used alone or in combination with conventional chemotherapeutic drugs, are now ongoing in a variety of malignancies, including leukemias and lymphomas together with R-CHOP chemotherapy (rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone). Most prominently, enzastaurin is under evaluation for the treatment and prevention of relapse in DLBCL. Specifically, relapse of patients with high-risk non-Hodgkin's lymphoma/DLBCL usually occurs within 3 years and life expectancy is very short. Importantly, a multicenter phase II study has demonstrated that treatment with enzastaurin was well tolerated and associated with prolonged free from progression in a small subset of patients with relapsed or refractory DLBCL [66]. These promising preliminary data granted enzastaurin orphan-drug status by the European Medicines Agency for the treatment of patients with DLBCL. Enrolment into a phase III clinical trial is ongoing.

Some lines of evidence suggest that enzastaurin is able to inhibit PKC activation also in MM. In fact, the addition of enzastaurin *in vitro* results in the inhibition of cell survival, proliferation and migration of MM cell lines and primary tumor cells, obtained from patients affected by multidrug-resistant MM [37]. In a therapeutic perspective, it is particularly remarkable that enzastaurin overcomes also MM cell growth triggered by binding to bone marrow stromal cells. These *in vitro* findings were confirmed in an *in vivo* xenograft model of human MM, in which enzastaurin abrogated not only tumor cell growth and survival, but also tumor-associated angiogenesis. Since it is unlikely that enzastaurin will enter into the clinical practice as single pharmacological agent, it is particularly noteworthy that enzastaurin displayed a strong synergistic *in vitro* cytotoxicity when combined with bortezomib and a moderate synergistic or additive cytotoxicity when combined with melphalan or lenalidomide [37, 67]. Due to the extreme need of innovative therapeutic approaches in the treatment of MM, these preclinical results strongly support the clinical evaluation of enzastaurin, alone or in combination with other novel or conventional therapies for the treatment of MM. Besides being active in MM, some preliminary evidence indicate that enzastaurin also shows significant *in vitro* and *in vivo* antitumoral activity in Waldenstrom's macroglobulinemia, a low-grade lymphoplasmacytic lymphoma, in which PKC family members (e.g., PKC-β) are overexpressed. Moreover, enzastaurin enhances the *in vitro* antitumor activity of rituximab, bortezomib, fludarabine and dexamethasone, strongly supporting the potential therapeutic value of using enzastaurin in combination with these agents [47].

Midostaurin

The second pharmacological inhibitor of PKC, which had attracted interest in the scientific community is midostaurin (PKC-412, *N* -benzylstaurosporine), a derivative of staurosporine. Midostaurin or PKC412 is an oral multi-targeted kinase inhibitor, originally designed to inhibit both conventional Ca^{2+}-dependent (α, β1, β2, γ) as well as novel Ca^{2+}-independent (δ, ε, η), PKC isoforms. Interestingly, however, midostaurin/PKC412 also blocks the activation of the VEGF receptors Flt-1 and mutant Flt-3 (present in approximately one-third of acute myeloid leukemia patients), as well as of FGFR1, mutant FGFR3 and c-Kit [68]. The anti-tumoral activity of midostaurin/PKC412 has been reported in multiple preclinical models of hematologic malignancies (including B-CLL), acute myeloid leukemia, acute lymphoblastic leukemia, mast cell leukemia, pre-B-cell lymphoma, peripheral T-cell lymphoma [69, 70].

Ongoing clinical trials are evaluating the therapeutic potential of midostaurin/PKC-412 in patients with aggressive systemic mastocytosis, mast cell leukemia and newly diagnosed acute myeloid leukemia [71]. Significant activity of midostaurin/PKC412 was also observed in t(4;14) positive MM cells carrying either the single-activating kinase domain mutation K > 650 > E (OPM-1 cells) or the transmembrane mutation Y > 373 > C (KMS11 cells), as well as in MM cell lines RPMI8226, U266, MM.1S, MM.1R and NCI-929. The activity of midostaurin/PKC412 in MM is associated with JNK-dependent upregulation of c-Jun and downregulation of c-Fos. Conversely, JNK inhibition abrogates both c-Jun activation and apoptosis [70, 72, 73].

Bryostatin

Bryostatin-1 is a macrocyclic lactone derived from the marine invertebrate bryozoan *Bugula neritina* that potently binds the regulatory domain of PKC [74]. Similar to phorbol esters, bryostatin-1 is a modulator of PKC activity, but in contrast to phorbol esters, bryostatin-1 is not a tumor promoter [75, 76]. Bryostatin-1 may act either as an agonist or as an antagonist of PKC activity, depending on the cellular background and the exposure time. In this respect, it has been shown that short-term exposure to bryostatin-1 promotes PKC activation, whereas prolonged exposure significantly inhibits PKC. The unique activity of bryostatin-1 is due, at least in part, to its selectivity for the nPKC isozymes PKC-δ and PKC-ε, the slow kinetics of PKC translocation and the protection of PKC-δ from downregulation, as well as to its ability to induce leukemic differentiation *via* STAT1 activation [76-80].

Although several clinical phase I and II trials showed modest but promising activity of bryostatin-1, used as a single agent, in the treatment of refractory acute leukemias and indolent hematologic malignancies [81-83], its relative selectivity is interesting in light of its potential association with other drugs. In this respect, bryostatin-1 has been studied in association with TNF-related apoptosis inducing ligand (TRAIL) or with anti-TRAIL receptor agonistic antibodies [84]. In fact, it has been previously demonstrated that recombinant TRAIL displays both pro-maturative and pro-apoptotic effects in myeloid leukemic cells [85-87]. Since the intracellular levels of PKC-ε are critical determinants in regulating the survival/apoptotic response of hematopoietic cells [12], it will be of interest to evaluate whether combination of bryostatin-1 with recombinant TRAIL or anti-TRAIL receptor agonistic antibodies will allow overcoming the intrinsic resistance of most haematological malignancies to TRAIL [84].

The synergistic activity of bryostatin-1 with other drugs, such as the AS101 (ammonium trichloro-(dioxoethylene-0-0')-tellurate), has been valuated on human myeloid leukemia

cell differentiation *in vitro*, as well as in a mouse model. The combined use of AS101 with bryostatin-1 or with a low concentration of PMA resulted in the differentiation of HL-60 cell line towards the monocytic lineage, and a similar synergistic effect was found *in vivo*. In addition, the synergistic activity of these drugs *in vivo* resulted also in a reduced infiltration of leukemic cells into the spleen and the peritoneum of mice treated with both compounds, as well as in the sharply reduced number of the HL-60 colonies extracted from those organs. Remarkably, these anti-tumoral effects have been associated with significantly prolonged survival (100% for 125 days) of the treated mice. Regarding the mechanism of action, the antitumoral activity of bryostatin-1 plus AS101 involved an increased p21(waf1) expression levels independently of p53 activation. This upregulation of p21 (waf1) has been demonstrated to be necessary for HL-60 cell differentiation. Taken together, these data suggest that the combination of AS101 plus bryostatin-1 will probably enter future clinical trials for the treatment of myeloid leukemias [88].

The effect of bryostatin-1 has been tested also on the expression of CD20 in non-Hodgkin's lymphoma cells. Although the majority of B cell malignancies express the CD20 marker, only approximately 50% of patients respond to single-agent rituximab, a chimeric antibody directed against CD20 that induces apoptosis in targeted cells, and the available data suggest that a decreased CD20 expression could account for the lack of response observed in some patients treated with rituximab. Interestingly, Wojciechowski and coworkers using the DB and RAMOS B cell lines, as well as tumor cells derived from a chronic lymphocytic leukaemia (CLL) patient, demonstrated that bryostatin-1 enhanced the expression of both CD20 mRNA and protein. The effect of bryostatin-1 on CD20 expression in non-Hodgkin's lymphoma cells was mediated through the MAPK/ERK signal transduction pathway and involved PKC, but was independent of p38 MAPK, and was insensitive to dexamethasone. As expected, cells pretreated with bryostatin-1 were more susceptible to the proapoptotic effect of the anti-CD20 antibody rituximab. These data suggested that bryostatin-1 plus rituximab could be used in association in the treatment of B cell malignancies [89]. A phase II clinical trial is currently evaluating the efficacy of a combination including bryostatin 1 plus rituximab in the treatment of patients affected by B-cell non-Hodgkin's lymphoma or B-CLL, that have not responded to previous treatment with rituximab [71].

Preclinical studies suggest that bryostatin-1 might enhance the therapeutic effects of fludarabine in the treatment of hematological malignancies. A phase I study has been completed in patients with CLL or indolent lymphoma and demonstrated that bryostatin-1, when administered with full dose fludarabine, is moderately active in patients with persistent disease following prior treatment [90]. In view of the activity of monoclonal antibodies, such as rituximab in the treatment of CLL and indolent lymphomas, future clinical trials will take in consideration combinations including fludarabine plus bryostatin 1 plus rituximab [91]. In addition, a phase II study of bryostatin 1 and vincristine in patients with low or intermediate grade non-Hodgkin's lymphoma progressing or relapsing after a prior autologous bone marrow or stem cell transplant is ongoing [71].

Curcumin

Curcuma spp. contains turmeric, essential oils, and curcuminoids, including curcumin. Curcumin [1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] is regarded as the most biologically active constituent of the spice turmeric and it comprises 2-8% of most turmeric preparations. Various preclinical cell culture and animal studies suggest that curcumin has potential as an antiproliferative, anti-invasive, and antiangiogenic agent, as well as a chemopreventive agent. The pleiotropic activities of curcumin derive from its complex chemistry as well as its ability to influence multiple signaling pathways [92, 93]. Curcumin is a potent inhibitor of PKC, EGF-receptor tyrosine kinase, and IkappaB kinase. The tumor promoter PMA activates PKC by reacting with zinc thiolates present within the regulatory domain, whereas the oxidized form of cancer chemopreventive agent such as curcumin can inactivate PKC by oxidizing the vicinal thiols present within the catalytic domain. It has been demonstrated that curcumin-induced apoptosis is mediated through the impairment of the ubiquitin-proteasome pathway [94]. Curcumin is remarkably non-toxic and a pilot phase I clinical trials have shown curcumin to be safe even when consumed at a daily dose of 12 grams for 3 months [95]. Curcumin exhibits great promise as a therapeutic agent, and is currently investigated in human clinical trials in combination with bioperine, a pepper extract, in MM and myelodysplastic syndromes [96, 97, 71]. Other clinical trials suggest a potential therapeutic role for curcumin in a series of other inflammatory and chronic diseases [98-101].

Aplidin

Aplidin is a naturally occurring cyclic depsipeptide isolated from the Mediterranean tunicate Aplidium albicans that showed *in vitro* and *in vivo* antitumor activity [102-104]. Preclinical studies demonstrated that aplidin, at concentrations 10-100 nM, induced apoptosis in human leukemic cell lines and primary leukemic cell cultures from leukemic patients. Interestingly, primary cultures of normal human cells, including resting peripheral blood lymphocytes, were spared or weakly affected after aplidin treatment [105]. Aplidin effectively inhibits cell viability by triggering a canonical apoptotic program resulting in alterations in cell morphology, caspase activation, and chromatin fragmentation. Proapoptotic concentrations of aplidin induced early oxidative stress, which results in a rapid and persistent activation of both JNK and p38 MAPK and a biphasic activation of ERK. Inhibition of JNK and p38 MAPK blocks the apoptotic program induced by aplidin. JNK and p38 MAPK activation results in downstream cytochrome c release and activation of caspase-9 and -3 and PARP cleavage. Caspase-3 activated PKC-δ mediates the cytotoxic effect of aplidin. Remarkably, cells deficient in PKC-δ show enhanced survival upon drug treatment as compared to its wild type counterpart [106]. In phase I clinical trials that used the schedule of 24 hour intravenous continuous infusion (recommended dose of 3750 $\mu g/m^2$), a plasma concentration of 8 nM was achieved. Currently, a clinical phase II trial study of aplidin (plitidepsin) in subjects with relapsing or refractory multiple myeloma is now recruiting patients and will start soon (71). In light of the effects on PKC-δ, aplidin could have therapeutic potential in other hematological malignancies.

Other Natural Products that Affect PKC-Dependent Pathways and Show Potential Applications in Haematological Malignancies

As previously mentioned for curcumin and aplidin, naturally derived products, in particular herbal extracts, have been widely used in the past and are attracting considerable attention in modern medicine. Traditional Chinese medical herbs can be considered useful reservoirs of uncharacterized active principles, for centuries utilized to treat a variety of human diseases including cancer. Increasing literature reports the effects of natural products, or derivatives, as activator or inhibitor of PKC-dependent pathways. Based on preclinical data, we have summarized below the more interesting products with potential therapeutic interest in hematological malignancies (see Table **1** for PKC-isoform specificities).

Peplin

Peplin or ingenol 3-angelate (PEP005) is a selective small molecule activator of PKC-δ extracted from the plant *Euphorbia peplus*, whose sap has been used as a traditional medicine for the treatment of skin conditions including warts and cancer. Sap from the plant *Euphorbia peplus* has been used for many years in Australia as a folk remedy to treat a number of skin conditions. Peplin has potent antileukemic effects, inducing apoptosis in myeloid leukemia cell lines and primary acute myeloid leukemia (AML) blasts at nanomolar concentrations through a PKC-δ dependent mechanism. Of importance, this compound did not induce apoptosis in normal CD34+ cord blood hematopoietic progenitor cells at up to 2-log concentrations higher than those required to induce cell death in primary AML cells [107]. Up to now, despite to potential antileukemic effects, peplin has been tested in phase II clinical trials only on basal cell carcinomas and Bowen's disease.

Ginsenoside Rh2

Rh2 is a ginsenoside isolated from *Panax ginseng* that has drawn attention in some laboratories in Asian countries because of its potential tumor-inhibitory effect. Recently, it has been tested on many tumor cell lines for its effects on cell proliferation, induction of apoptosis, and potential interaction with conventional chemotherapy agents. The results showed that Rh2 inhibited cell growth by G1 arrest at low concentrations and induced apoptosis at high concentrations in a variety of tumoral cell lines, possibly through activation of caspases. The growth arrest and apoptosis may be mediated by two separate mechanisms. Apoptosis is not dependent on expression of the wild-type p53 nor the caspase 3, but is thought to be mediated by glucocorticoid receptors. Most interestingly, Rh2 can act either additively or synergistically with chemotherapy drugs on cancer cells. Since Rh2 is able to hypersensitize multidrug-resistant breast cancer cells to paclitaxel, it might be effective in the treatment of multidrug-resistant cancers, especially if used combination with conventional chemotherapy agents [108]. Interestingly, ginsenoside-Rh2 induced differentiation of HL-60 cells into morphologically and functionally granulocytes by PKC-dependent pathway. During differentiation, ginsenoside-Rh2 arrested the cell cycle at the G1/S phase and increased the activity of Ca^{2+}-dependent PKCs, in particular the PKC-η isoform [109].

Decursin

Decursin is a pyranocoumarin isolated from Angelica gigas that exhibits cytotoxic effects on various human cancer cell lines. Kim and co-workers demonstrated that decursin competed the binding of phorbol 12,13-dibutyrate to PKC-α and βII isozymes in human K562 leukemia cells and down-regulates PKC-α and βII isoforms activity in this cells [110-111]. The *in vivo* anti-tumor activity of decursin was investigated by administrating consecutively for 9 days 50-100 mg/kg i.p. in mice. Such treatment schedule showed a significant increase in life span and a significant decrease in the tumor weight and volume of mice inoculated with sarcoma-180 tumor cells [112]. Furthermore, decursin and chemically synthesized derivatives containing the coumarin structure have been tested on human K562 erythroleukemia and U937 myeloid leukemia cells and showed antiproliferative effects on both cell lines. Among these derivatives, Kim and co-workers identified compounds (1-3) and (4-6) that inhibited the proliferation of leukemic cells in a PKCβII-independent and dependent manner, respectively, indicating that the side chain of compounds determines its selectivity for the PKC-βII isoform [111]. Therefore, decursin should be considered a potential scaffold for future PKC-isozyme specific drugs.

Trichosanthin

Trichosanthin, an active component extracted from the root tubers of medical herb *Trichosanthes kirilowii*, that has long been traditionally used for mid-term abortion in ancient Chinese society for hundreds of years an now is gaining increasing interest as cancer therapeutic agents. Trichosanthin is a type I ribosome-inactivating protein that induces cell death in various cell types including several tumor cell lines. Recent preclinical results demonstrated that trichosanthin down-regulated p210(Bcr-Abl) in chronic leukemia cells at a time- and dose-dependent manners but also synergized with imatinib (STI571) to induce cell growth arrest, down-regulation of p210(Bcr-Abl) and its downstream signals [113]. Concerning its mechanisms of action, trichosanthin induces apoptosis in K562 cells *via* PKC inhibition. Interestingly, trichosanthin treatment induced a transient elevation in intracellular calcium concentration but calcium chelators had no effect on induced apoptosis, suggesting that calcium changes and Ca^{2+}-dependent PKC may not be involved in trichosanthin-mediated apoptosis in K562 cells. Furthermore, it was found that trichosanthin induced apoptosis in K562 cells by a caspase-3 mediated mechanism [114].

Penta-Acetyl Geniposide

A research study suggested that herbal-originated product penta-acetyl geniposide (PAG) could be developed as an antitumor drug, which induces apoptosis through activation of specific PKC isoforms. PAG treatment resulted in DNA fragmentation of C6 glioma cells and stimulated PKC-δ and PKC-ζ, which translocated to the cell membrane. PKC-δ inhibition blocked the PAG-induced apoptosis by decreasing the cell population of sub G1 peak. Since the mRNA levels of PKC-δ was not altered, these results suggested that PAG-induced apoptosis of tumor cells occurred through the activa-

tion of PKC-δ. In addition, it has been reported that PAG also induces the expression of p53 and apoptosis-related Bax protein [115].

Bufalin

Bufalin, a bufadienolide type steroid that is one of the major components of the toad venom-prepared traditional Chinese medicine called Ch'an Su or Senso, has potent differentiation activity in human leukemia ML1 cells. The cell growth was inhibited significantly at 10 nM bufalin and the cells arrested in the G2/M phase. In regards to its anticancer mechanism, it has been suggested that bufalin could be a topoisomerase inhibitors since it remarkably inhibited the activity of topoisomerase II in ML1 cells [116]. In addition, it was observed that bufalin also inhibited the proliferation of human leukemic HL-60 cells [117] and human monocytic leukemia THP-1 cells at nanomolar concentration [118]. The apoptotic effects of bufalin in human monocytic leukemia THP-1 cells involved PKC isozymes, as demonstrated by a PKC-specific but isozyme-nonselective inhibitor, Ro-31-8220. Furthermore, cPKC β- and nPKC δ-defective THP-1/TPA cells displayed strong resistance to the bufalin-induced DNA ladder formation and rottlerin, a nPKCδ-specific inhibitor, partially attenuated proapoptotic effects of bufalin through limited proteolysis of nPKCdelta and poly(ADP-ribose) polymerase, suggesting the involvement of these PKC isozymes in bufalin-induced apoptosis [118].

Yuanhuacine

Yuanhuacine is a diterpene ester isolated from the flower buds of *Daphne genkwa*. *In vitro* assay demonstrated that it is a selective antagonist of the phorbol ester receptor in PKC. This compound strongly inhibited the binding of 3H-phorbol-12, 13-dibutyrate (PdBu) to PKC with an IC_{50} value in the nanomolar range. Yuanhuacine inhibited the PdBu-stimulated PKC activity in the catalysis of the phosphorylation of Histone III-S with an IC50 of 2.82 nmol/L (PdBu = 10 micromol/L), while it had no effect on the basal and Ca^{2+}-stimulated PKC activity in the same assay system [119]. Yuanhuacine was found to induce apoptosis in human myelocytic HL-60 cells. This compound demonstrated to activate the apoptotic process, including DNA fragmentation, chromatin condensation, and sub-G1 hypodiploidy. The treatment resulted in the cleavage of procaspase-3 and PARP into active forms, suppressed expression of Bcl-2 and Bcl-XL and increased expression of the pro-apoptotic protein, Bax. In addition, yuanhuacine also suppressed tumor growth in Lewis lung carcinoma (LLC)-inoculated mouse model. The intraperitoneal administration of yuanhuacine in LLC-inoculated mice evidenced a significant inhibition of tumor size, with reductions of 24.2% and 45.8% at concentrations of 0.1 mg/kg and 0.5 mg/kg, as compared with the control mice. These results indicate that yuanhuacine is a potent antitumoral agent [120].

Innovative Approaches for Identifying Compounds with Binding Selectivity for Individual PKC Isozymes

Tumor promoters, such as phorbol esters, bind strongly to PKC isozymes to induce their activation. Since each PKC isozyme is involved in diverse biological events in addition to tumor promotion, the isozymes serve as promising therapeutic targets. Tumor promoters bind to the C1A and/or C1B domain of conventional and novel PKC isozymes. As these C1 domains play differential roles in PKC activation and their translocation in distinct cell compartments, the development of agents with binding selectivity for individual C1 domains represents a rationale approach for target-specific therapies. In this respect, a recent work proposed the use of a synthetic C1 peptide library of all PKC isozymes as a screening tool useful to identify promising lead compounds with binding selectivity for individual PKC [121]. The library has enabled to identify molecules that have been stated by the authors as promising lead compounds. By using this approach, indolactam and benzolactam, two compounds with some binding selectivity for the C1B domains of novel PKC isozymes, have been identified. Simpler in structure and higher in stability than other potent tumor promoters, a number of indolactam and benzolactam derivatives have been synthesized to develop new PKC isozyme modulators by several groups. Interestingly, this work established that the amide function of these compounds is involved in hydrogen bonding with the C1B domains of PKC-δ. Thus, trans-amide restricted analogues with a hydrophobic chain at an appropriate position are promising lead compounds with a high binding selectivity for novel PKC isozyme C1B domains [122].

CONCLUSIONS

Although intensive studies in the last 30 years, the relative contribution of individual PKC isozymes in development, growth, survival, progression and drug resistance of different cancers is still largely undefined. Hopefully, the use of more specific inhibitors of individual PKC isoforms (e.g., small interfering RNA technology) will elucidate PKC function in different tumors. Additional studies will find individual PKC isozyme functions relative to PKC-activating stimuli, subcellular localization and tumor microenvironment. Indeed, although PKCs are major targets for both phorbol esters as well as DAG, other molecules including protein kinase D, Ras guanyl nucleotide-releasing proteins, DAG kinases and Munc13 are also activated. Therefore, further studies are required to evaluate whether tumorigenic effects originally attributed to PKCs may instead be mediated *via* other molecules. In addition, potential cross-talks between PKCs and other intracellular signal transduction pathways remain to be better defined. Indeed, due to their multifaceted role within the tumor cell and its environment, PKCs represent attractive targets in cancer therapy and preclinical and clinical studies, using enzastaurin or midostaurin, demonstrate remarkable therapeutic potential of these PKC inhibitors, alone or in combination with other therapeutics, in a variety of hematologic malignancies. Further delineation of the relative contribution of PKC isozymes in the development and progression is required to identify therapeutic opportunities, using either more specific PKC inhibitors or combination regimens, to impair effectively tumor cell growth and survival. In future perspective, the use of a synthetic C1 peptide library of all PKC isozymes to screen natural extracts or its derivatives that activate apoptosis in hematological malignancies through PKC-dependent pathways will permit to identify more safe and active drugs that target specific PKC-isoforms.

REFERENCES

References 123-125 are related articles recently published.

[1] Berenblum I. The mechanism of carcinogenesis: a study of the significance of carcinogenic action and related phenomena. Cancer Res 1941; 1: 807-14.

[2] Castagna M, Takai Y, Kaibuchi K Sano K, Kikkawa U, Nishizuka Y. Direct activation of calcium-activated, phospholipid-dependent protein kinase by tumor-promoting phorbol esters. J Biol Chem 1982; 257: 7847-51.

[3] Nishizuka Y. The role of protein kinase C in cell surface signal transduction and tumour promotion. Nature 1984; 308: 693-8.

[4] Sharkey NA, Leach KL, Blimberg PM. Competitive inhibition by diacylglycerol of specific phorbol ester binding. Proc Natl Acad Sci USA 1984; 81: 607-10.

[5] Griner EM, Kazanietz MG. Protein kinase C and other diacylglycerol effectors in cancer. Nat Rev Cancer 2007; 7: 281-294.

[6] Mackay HJ, Twelves CJ. Targeting the protein kinase C family: are we there yet? Nat Rev Cancer 2007; 7: 554-62.

[7] Zauli G, Bassini A, Vitale M, Gibellini D, Celeghini C, Caramelli E, *et al.* Thrombopoietin enhances the alpha II beta 3-dependent adhesion of megakaryocytic cells to fibrinogen or fibronectin through PI 3 kinase. Blood 1997; 89: 883-95.

[8] Gibellini D, Bassini A, Pierpaoli S, Bertolaso L, Milani D, Capitani S, *et al.* Extracellular HIV-1 Tat protein induces the rapid Ser^{133} phosphorylation and activation of CREB transcription factor in both Jurkat lymphoblastoid T cells and primary peripheral blood mononuclear cells. J Immunol 1998; 160: 3891-8.

[9] Rossi F, McNagny KM, Smith G, Frampton J, Graf T. Lineage commitment of transformed haemopoietic progenitors is determined by the level of PKC activity. EMBO J 1996; 15: 1894-901.

[10] Zauli G, Visani G, Bassini A, Caramelli E, Ottaviani E, Bertolaso L, *et al.* Nuclear translocation of protein kinase C-alpha and -zeta isoforms in HL-60 cells induced to differentiate along the granulocytic lineage by all-trans retinoic acid. Br J Haematol 1996; 93: 542-50.

[11] Bassini A, Zauli G, Migliaccio G, Migliaccio AR, Pascuccio M, Pierpaoli S, *et al.* Lineage-restricted expression of protein kinase C isoforms in hematopoiesis. Blood 1999; 93: 1178-88.

[12] Mirandola P, Gobbi G, Ponti C, Sponzilli I, Cocco L, Vitale M. PKCepsilon controls protection against TRAIL in erythroid progenitors. Blood 2006; 107: 508-13.

[13] Nishizuka Y. Protein kinase C and lipid signaling for sustained cellular responses. FASEB J 1995; 9: 484-96.

[14] Nishizuka Y. Studies and perspectives of protein kinase C. Science 1986; 233: 305-12.

[15] Nishizuka Y. The Albert Lasker Medical Awards. The family of protein kinase C for signal transduction. JAMA 1989; 262: 1826-33.

[16] Asaoka Y, Nakamura S, Yoshida K, Nishizuka Y. Protein kinase C, calcium and phospholipid degradation. Trends Biochem Sci 1992; 17: 414-7.

[17] Dekker LV, Parker PJ. Protein kinase C - a question of specificity. Trends Biochem Sci 1994; 19: 73-7.

[18] Newton AC. Regulation of protein kinase C. Curr Opin Cell Biol 1997; 9: 161-7.

[19] Gamard CJ, Blobe GC, Hannun YA, Obeid LM. Specific role for protein kinase C-beta in cell differentiation. Cell Growth Differ 1994; 5: 405-9.

[20] Borgatti P, Mazzoni M, Carini C, Neri LM, Marchisio M, Bertolaso L, *et al.* Changes of nuclear protein kinase C activity and isotype composition in PC12 cell proliferation and differentiation. Exp Cell Res 1996; 224: 72-8.

[21] O'Brian C, Vogel VG, Singletary SE, Ward NE. Elevated protein kinase C expression in human breast tumor biopsies relative to normal breast tissue. Cancer Res 1989; 49: 3215-7.

[22] Schwarts GK, Jiang J, Kelsen D, Albino AP. Protein kinase C: a novel target for inhibiting gastric cancer cell invasion. J Natl Cancer Inst 1993; 85: 402-7.

[23] Gokmen-Polar Y, Murray NR, Velasco MA, Gatalica Z, Fields AP. Elevated protein kinase C-beta II is an early promotive event in colon carcinogenesis. Cancer Res 2001; 61: 1375-81.

[24] Da Rocha AB, Mans DR, Regner A, Schwartsmann G. Targeting protein kinase C: new therapeutic opportunities against high-grade malignant gliomas? Oncologist 2002; 7: 17-33.

[25] Dean N, McKay R, Miraglia L, Howard R, Cooper S, Giddings J, *et al.* Inhibition of growth of human tumor cell lines in nude mice by an antisense of oligonucleotide inhibitor of protein kinase C- alpha expression. Cancer Res 1996; 56: 3499-507.

[26] Shipp MA, Ross KN, Tamayo P, Weng AP, Kutok JL, Aguiar RC, *et al.* Diffuse large B-cell lymphoma outcome prediction by gene-expression profi ling and supervised machine learning. Nat Med 2002; 8: 68-74.

[27] Hans CP, Weisenburger DD, Greiner TC, Chan WC, Aoun P, Cochran GT, *et al.* Expression of PKC-beta or cyclin D2 predicts for inferior survival in diffuse large B-cell lymphoma. Mod Pathol 2005; 18: 1377-84.

[28] Felli MP, Vacca A, Calce A, Bellavia D, Campese AF, Grillo R, *et al.* PKC-theta mediates pre-TCR signalling and contributes to Notch3-induced T-cell leukemia. Oncogene 2005; 24: 992-1000.

[29] Gorelick G, Barreiro Arcos ML, Klecha AJ, Cremaschi GA. Differential expression of protein kinase C isoenzymes related to high nitric oxide synthase activity in a T lymphoma cell line. Biochim Biophys Acta 2002; 1588: 179-88.

[30] Villalba M, Kasibhatla S, Genestier L, Mahboubi A, Green DR, Altman A. Protein kinase C-theta cooperates with calcineurin to induce Fas ligand expression during activation-induced T cell death. J Immunol 1999; 163: 5813-9.

[31] Parant MR, Klein B, Vial H. Abnormal behavior of protein kinase C in the human myeloma cell line, RPMI 8226. FEBS Lett 1990; 269: 331-5.

[32] Thabard W, Collette M, Bataille R, Amiot M. Protein kinase C delta and eta isoenzymes control the shedding of the interleukin 6 receptor alpha in myeloma cells. Biochem J 2001; 358: 193-200.

[33] Podar K, Tai YT, Lin BK, Narsimhan RP, Sattler M, Kijima T, *et al.* Vascular endothelial growth factor-induced migration of multiple myeloma cells is associated with beta-1 integrin- and phosphatidylinositol 3-kinase-dependent PKC-alpha activation. J Biol Chem 2002; 277: 7875-81.

[34] Qiang YW, Walsh K, Yao L, Kedei N, Blumberg PM, Rubin JS, *et al.* Wnts induce migration and invasion of myeloma plasma cells. Blood 2005; 106: 1786-93.

[35] Podar K, Anderson KC. The pathophysiologic role of VEGF in hematologic malignancies: therapeutic implications. Blood 2005; 105: 1383-95.

[36] Chauhan D, Pandey P, Ogata A, Teoh G, Krett N, Halgren R, *et al.* Cytochrome C-dependent and -independent induction of apoptosis in multiple myeloma cells. J Biol Chem 1997; 272: 29995-7.

[37] Bergsagel PL, Kuehl WM, Zhan F, Sawyer J, Barlogie B, Shaughnessy J Jr. Cyclin D dysregulation: an early and unifying pathogenic event in multiple myeloma. Blood 2005; 106: 296-303.

[38] Podar K, Raab MS, Zhang J, McMillin D, Breitkreutz I, Tai YT, *et al.* Targeting PKC in multiple myeloma: *in vitro* and *in vivo* effects of the novel, orally available small-molecule inhibitor enzastaurin (LY317615.HCl). Blood 2007; 109: 1669-77.

[39] Podar K, Tai YT, Davies FE, Lentzsch S, Sattler M, Hideshima T, *et al.* Vascular endothelial growth factor triggers signaling cascades mediating multiple myeloma cell growth and migration. Blood 2001; 98: 428-435.

[40] Xia P, Aiello LP, Ishii H, Jiang ZY, Park DJ, Robinson GS, *et al.* Characterization of vascular endothelial growth factor's effect on the activation of protein kinase C, its isoforms, and endothelial cell growth. J Clin Invest 1996; 98: 2018-26.

[41] Takahashi T, Ueno H, Shibuya M. VEGF activates protein kinase C-dependent, but Ras-independent Raf-MEK-MAP kinase pathway for DNA synthesis in primary endothelial cells. Oncogene 1999; 18: 2221-30.

[42] Yoshiji H, Kuriyama S, Ways DK, Yoshii J, Miyamoto Y, Kawata M, *et al.* Protein kinase C lies on the signalling pathway for vascular endothelial growth factor-mediated tumor development and angiogenesis. Cancer Res 1999; 59: 4413-8.

[43] Suzuma K, Takahara N, Suzuma I, Isshiki K, Ueki K, Leitges M, *et al.* Characterization of protein kinase C beta isoform's action on retinoblastoma protein phosphorylation, vascular endothelial growth factor-induced endothelial cell proliferation, and retinal neovascularization. Proc Natl Acad Sci USA 2002; 99: 721-6.

[44] Wong C, Jin ZG. Protein kinase C-dependent protein kinase D activation modulates ERK signal pathway and endothelial cell proliferation by vascular endothelial growth factor. J Biol Chem 2005; 280: 33262-9.

[45] Sledge GW Jr, Gokmen-Polar Y: Protein kinase C beta as a therapeutic target in breast cancer. Semin Oncol 2006; 33: S15-8.

[46] Teicher BA, Menon K, Alvarez E, Liu P, Shih C, Faul MM. Antiangiogenic and antitumor effects of a protein kinase C-alpha inhibitor in human hepatocellular and gastric cancer xenografts. *In Vivo* 2001; 15: 185-93.

[47] Moreau AS, Jia X, Ngo HT, Leleu X, O'Sullivan G, Alsayed Y, *et al.* Protein kinase C inhibitor enzastaurin induces *in vitro* and *in vivo* antitumor activity in Waldenstrom's macroglobulinemia. Blood 2007; 109: 4964-72.

[48] Jackson DN, Foster DA. The enigmatic protein kinase C-delta: complex roles in cell proliferation and survival. FASEB J 2004; 18: 627-36.

[49] Emoto Y, Manome Y, Meinhardt G, Kisaki H, Kharbanda S, Robertson M, *et al.* Proteolytic activation of protein kinase C-delta by an ICE-like protease in apoptotic cells. EMBO J 1995; 14: 6148-56.

[50] Ghayur T, Hugunin M, Talanian RV, Ratnofsky S, Quinlan C, Emoto Y, *et al.* Proteolytic activation of protein kinase C delta by an ICE/CED 3-like protease induces characteristics of apoptosis. J Exp Med 1996; 184: 2399-404.

[51] Bharti A, Kraeft SK, Gounder M, Pandey P, Jin S, Yuan ZM, *et al.* Inactivation of DNA-dependent protein kinase by protein kinase Cdelta: implications for apoptosis. Mol Cell Biol 1998; 18: 6719-28.

[52] Yuan ZM, Utsugisawa T, Ishiko T, Nakada S, Huang Y, Kharbanda S, *et al.* Activation of protein kinase C delta by the c-Abl tyrosine kinase in response to ionizing radiation. Oncogene 1998; 16: 1643-8.

[53] Cross T, Griffiths G, Deacon E, Sallis R, Gough M, Watters D, *et al.* PKC-delta is an apoptotic lamin kinase. Oncogene 2000; 19: 2331-7.

[54] Frasch SC, Henson PM, Kailey JM, Richter DA, Janes MS, Fadok VA, *et al.* Regulation of phospholipid scramblase activity during apoptosis and cell activation by protein kinase Cdelta. J Biol Chem 2000; 275: 23065-73.

[55] Ni H, Ergin M, Tibudan SS, Denning MF, Izban KF, Alkan S. Protein kinase C-delta is commonly expressed in multiple myeloma cells and its downregulation by rottlerin causes apoptosis. Br J Haematol 2003; 121: 849-56.

[56] Kolch W, Heidecker G, Kochs G, Hummel R, Vahidi H, Mischak H, *et al.* Protein kinase C-alpha activates RAF-1 by direct phosphorylation. Nature 1993; 364: 249-52.

[57] Ruvolo PP, Deng X, Carr BK, May WS. A functional role for mitochondrial protein kinase C-alpha in Bcl2 phosphorylation and suppression of apoptosis. J Biol Chem 1998; 273: 25436-42.

[58] Nakagawa R, Soh JW, Michie AM. Subversion of protein kinase C-alpha signaling in hematopoietic progenitor cells results in the generation of a B-cell chronic lymphocytic leukemia-like population *in vivo*. Cancer Res 2006; 66: 527-34.

[59] Mischak H, Goodnight JA, Kolch W, Martiny-Baron G, Schaechtle C, Kazanietz MG, *et al.* Overexpression of protein kinase C-delta and -epsilon in NIH 3T3 cells induces opposite effects on growth, morphology, anchorage dependence, and tumorigenicity. J Biol Chem 1993; 268: 6090-6.

[60] Ringshausen I, Schneller F, Bogner C, Hipp S, Duyster J, Peschel C, *et al.* Constitutively activated phosphatidylinositol-3 kinase (PI-3K) is involved in the defect of apoptosis in B-CLL: association with protein kinase C-delta. Blood 2002; 100: 3741-8.

[61] Graff JR, McNulty AM, Hanna KR, Konicek BW, Lynch RL, Bailey SN, *et al.* The protein kinase C-beta-selective inhibitor, enzastaurin (LY317615.HCl), suppresses signalling through the AKT pathway, induces apoptosis, and suppresses growth of human colon cancer and glioblastoma xenografts. Cancer Res 2005; 65: 7462-9.

[62] Carducci MA, Musib L, Kies MS, Pili R, Truong M, Brahmer JR, *et al.* Phase I dose escalation and pharmacokinetic study of enzastaurin, an oral protein kinase C-beta inhibitor, in patients with advanced cancer. J Clin Oncol 2006; 24: 4092-9.

[63] Goekjian PG, Jirousek MR: Protein kinase C inhibitors as novel anticancer drugs. Expert Opin Investig Drugs 2001; 10: 2117-40.

[64] Keyes KA, Mann L, Sherman M, Galbreath E, Schirtzinger L, Ballard D, *et al.* LY317615 decreases plasma VEGF levels in human tumor xenograft-bearing mice. Cancer Chemother Pharmacol 2004; 53: 133-40.

[65] Green LJ, Marder P, Ray C, Cook CA, Jaken S, Musib LC, *et al.* Development and validation of a drug activity biomarker that shows target inhibition in cancer patients receiving enzastaurin, a novel protein kinase C-beta inhibitor. Clin Cancer Res 2006; 12: 3408-15.

[66] Robertson MJ, Kahl BS, Vose JM, de Vos S, Laughlin M, Flynn PJ, *et al.* Phase II study of enzastaurin, a protein kinase C-beta inhibitor, in patients with relapsed or refractory diffuse large B-cell lymphoma. J Clin Oncol 2007; 25: 1741-6.

[67] Rizvi MA, Ghias K, Davies KM, Ma C, Weinberg F, Munshi HG, *et al.* Enzastaurin (LY317615), a protein kinase C inhibitor, inhibits the AKT pathway and induces apoptosis in multiple myeloma cell lines. Mol Cancer Ther 2006; 5: 1783-9.

[68] Growney JD, Clark JJ, Adelsperger J, Stone R, Fabbro D, Griffin JD, *et al.* Activation mutations of human c-KIT resistant to imatinib mesylate are sensitive to the tyrosine kinase inhibitor PKC412. Blood 2005; 106: 721-4.

[69] Cools J, Stover EH, Boulton CL, Gotlib J, Legare RD, Amaral SM, *et al.* PKC412 overcomes resistance to imatinib in a murine model of FIP1L1-PDGFR-alpha-induced myeloproliferative disease. Cancer Cell 2003; 3: 459-69.

[70] Chen J, Lee BH, Williams IR, Kutok JL, Mitsiades CS, Duclos N, *et al.* FGFR3 as a therapeutic target of the small molecule inhibitor PKC412 in hematopoietic malignancies. Oncogene 2005; 24: 8259-67.

[71] www.clinicaltrials.gov, accessed March 18th 2008.

[72] Sharkey J, Khong T, Spencer A. PKC412 demonstrates JNK-dependent activity against human multiple myeloma cells. Blood 2007; 109: 1712-9.

[73] Bahlis NJ, Miao Y, Koc ON, Lee K, Boise LH, Gerson SL. N -Benzoylstaurosporine (PKC412) inhibits Akt kinase inducing apoptosis in multiple myeloma cells. Leuk Lymphoma 2005; 46: 899-908.

[74] Clamp A, Jayson GC. The clinical development of the bryostatins. Anticancer Drugs 2002; 13: 673-83.

[75] Smith JB, Smith L, Pettit GR. Bryostatins: potent, new mitogens that mimic phorbol ester tumor promoters. Biochem Biophys Res Commun 1985; 132: 939-45.

[76] Stone RM, Sariban E, Pettit GR, Kufe DW. Bryostatin 1 activates protein kinase C and induces monocytic differentiation of HL-60 cells. Blood 1988; 72: 208-13.

[77] Kraft AS, William F, Pettit GR, Lilly MB. Varied differentiation responses of human leukemias to bryostatin 1. Cancer Res 1989; 49: 1287-93.

[78] Drexler HG, Gignac SM, Jones RA, Scott CS, Pettit GR, Hoffbrand AV. Bryostatin 1 induces differentiation of B-chronic lymphocytic leukemia cells. Blood 1989; 74: 1747-57.

[79] Mohammad RM, Al-katib A, Pettit GR, Sensembrenner LL. Differential effects of bryostatin 1 on human non-Hodgkin B-lymphoma cell lines. Leuk Res 1993; 17: 1-8.

[80] Battle TE, Frank DA. STAT1 mediates differentiation of chronic lymphocytic leukemia cells in response to bryostatin 1. Blood 2003; 102: 3016-24.

[81] Cragg LH, Andreeff M, Feldman E, Roberts J, Murgo A, Winning M, *et al.* Phase I trial and correlative laboratory studies of bryostatin 1 (NSC 339555) and high-dose 1-B-D-arabinofuranosylcytosine in patients with refractory acute leukemia. Clin Cancer Res 2002; 8: 2123-33.

[82] Varterasian ML, Mohammad RM, Eilender DS, Hulburd K, Rodriguez DH, Pemberton PA, *et al.* Phase I study of bryostatin 1 in patients with relapsed non-Hodgkin's lymphoma and chronic lymphocytic leukemia. J Clin Oncol 1998; 16: 56-62.

[83] Varterasian ML, Mohammad RM, Shurafa MS, Hulburd K, Pemberton PA, Rodriguez DH, *et al.* Phase II trial of bryostatin 1 in patients with relapsed low-grade non-Hodgkin's lymphoma and chronic lymphocytic leukemia. Clin Cancer Res 2000; 6: 825-8.

[84] Zauli G, Secchiero P. The role of the TRAIL/TRAIL receptors system in hematopoiesis and endothelial cell biology. Cyt Growth Fact Rev 2006; 17: 245-57.

[85] Secchiero P, Gonelli A, Celeghini C, Mirandola P, Guidotti L, Visani G, *et al.* Activation of the nitric oxide synthase pathway represents a key component of tumor necrosis factor-related apoptosis-inducing ligand-mediated cytotoxicity on hematologic malignancies. Blood 2001; 98: 2220-8.

[86] Secchiero P, Gonelli A, Mirandola P, Melloni E, Zamai L, Celeghini C, *et al.* Tumor necrosis factor-related apoptosis-inducing ligand induces monocytic maturation of leukemic and normal

myeloid precursors through a caspase-dependent pathway. Blood 2002; 100: 2421-9.

[87] Secchiero P, Melloni E, Heikinheimo M, Mannisto S, Di Pietro R, Iacone A, *et al.* TRAIL regulates normal erythroid maturation through an ERK-dependent pathway. Blood 2004; 103: 517-22.

[88] Hayun M, Okun E, Hayun R, Gafter U, Albeck M, Longo DL, *et al.* Synergistic effect of AS101 and Bryostatin-1 on myeloid leukemia cell differentiation *in vitro* and in an animal model. Leukemia 2007; 21:1504-13.

[89] Wojciechowski W, Li H, Marshall S, Dell'Agnola C, Espinoza-Delgado I. Enhanced expression of CD20 in human tumor B cells is controlled through ERK-dependent mechanisms. J Immunol 2005; 174: 7859-68.

[90] Roberts JD, Smith MR, Feldman EJ, Cragg L, Millenson MM, Roboz GJ, *et al.* Phase I study of bryostatin 1 and fludarabine in patients with chronic lymphocytic leukemia and indolent (non-Hodgkin's) lymphoma. Clin Cancer Res 2006; 12:5809-16.

[91] Wendtner CM, Eichhorst BF, Hallek MJ. Advances in chemotherapy for chronic lymphocytic leukemia. Semin Hematol 2004; 41: 224-33.

[92] Liu JY, Lin SJ, Lin JK. Inhibitory effects of curcumin on protein kinase C activity induced by 12-O-tetradecanoyl-phorbol-13-acetate in NIH 3T3 cells. Carcinogenesis 1993; 145: 857-61.

[93] Mahmmoud YA. Modulation of protein kinase C by curcumin; inhibition and activation switched by calcium ions. Br J Pharmacol 2007; 150: 200-8.

[94] Lin JK. Molecular targets of curcumin. Adv Exp Med Biol 2007; 595: 227-43.

[95] Hatcher H, Planalp R, Cho J, Torti FM, Torti SV. Curcumin: from ancient medicine to current clinical trials. Cell Mol Life Sci 2008; 65(11): 1631-52.

[96] Bharti AC, Donato N, Aggarwal BB. Curcumin (diferuloylmethane) inhibits constitutive and IL-6-inducible STAT3 phosphorylation in human multiple myeloma cells. J Immunol 2003; 171: 3863-71.

[97] Bharti AC, Donato N, Singh S, Aggarwal BB. Curcumin (diferuloylmethane) down-regulates the constitutive activation of NF-kappaB and I-kappa B kinase in human multiple myeloma cells, leading to suppression of proliferation and induction of apoptosis. Blood 2003; 101: 1053-62.

[98] Surh YJ: Cancer chemoprevention with dietary phytochemicals. Nat Rev Cancer 2003; 3: 768-80.

[99] Singh S, Khar A. Biological effects of curcumin and its role in cancer chemoprevention and therapy. Anticancer Agents Med Chem 2006; 6: 259-70.

[100] Sharma RA, Steward WP, Gescher AJ. Pharmacokinetics and pharmacodynamics of curcumin. Adv Exp Med Biol 2007; 59: 453-70.

[101] Joe B, Vijaykumar M, Lokesh BR. Biological properties of curcumin-cellular and molecular mechanisms of action. Crit Rev Food Sci Nutr 2004; 44: 97-111.

[102] Depenbrock H, Peter R, Faircloth GT, Manzanares I, Jimeno J, Hanauske AR. *In vitro* activity of aplidine, a new marine-derived anti-cancer compound, on freshly explanted clonogenic human tumour cells and haematopoietic precursor cells. Br J Cancer 1998; 78: 739-744.

[103] Geldof AA, Mastbergen SC, Henrar RE, Faircloth GT. Cytotoxicity and neurocytotoxicity of new marine anticancer agents evaluated using *in vitro* assays. Cancer Chemother Pharmacol 1999; 44: 312-318.

[104] Erba E, Bassano L, Di Liberti G, Muradore I, Chiorino G, Ubezio P, *et al.* Cell cycle phase perturbations and apoptosis in tumour cells induced by aplidine. Br J Cancer 2002; 86: 1510-1517.

[105] Gajate C, An F, Mollinedo F. Rapid and selective apoptosis in human leukemic cells induced by aplidine through a Fas/CD95- and mitochondrial-mediated mechanism. Clin Cancer Res 2003; 9: 1535-45.

[106] García-Fernández LF, Losada A, Alcaide V, Alvarez AM, Cuadrado A, González L, *et al.* Aplidin induces the mitochondrial apoptotic pathway *via* oxidative stress-mediated JNK and p38 activation and protein kinase C delta. Oncogene 2002; 21: 7533-44.

[107] Hampson P, Chahal H, Khanim F, Hayden R, Mulder A, Assi LK, *et al.* PEP005, a selective small-molecule activator of protein kinase C, has potent antileukemic activity mediated *via* the delta isoform of PKC. Blood 2005; 106: 1362-8.

[108] Jia WW, Bu X, Philips D, Yan H, Liu G, Chen X, *et al.* Rh2, a compound extracted from ginseng, hypersensitizes multidrug-resistant tumor cells to chemotherapy. Can J Physiol Pharmacol 2004; 82: 431-7.

[109] Kim YS, Kim DS, Kim SI. Ginsenoside Rh2 and Rh3 induce differentiation of HL-60 cells into granulocytes: modulation of protein kinase C isoforms during differentiation by ginsenoside Rh2. Int J Biochem Cell Biol 1998; 30: 327-38.

[110] Kim HH, Ahn KS, Han H, Choung SY, Choi SY, Kim IH. Decursin and PDBu: two PKC activators distinctively acting in the megakaryocytic differentiation of K562 human erythroleukemia cells. Leuk Res 2005; 29: 1407-13.

[111] Kim HH, Sik Bang S, Seok Choi J, Han H, Kim IH. Involvement of PKC and ROS in the cytotoxic mechanism of anti-leukemic decursin and its derivatives and their structure-activity relationship in human K562 erythroleukemia and U937 myeloleukemia cells. Cancer Lett 2005; 223: 191-201.

[112] Lee S, Lee YS, Jung SH, Shin KH, Kim BK, Kang SS. Anti-tumor activities of decursinol angelate and decursin from Angelica gigas. Arch Pharm Res 2003; 26: 727-30.

[113] Zhang K, Xu J, Huang X, Wu L, Wen C, Hu Y, *et al.* Trichosanthin down-regulated p210Bcr-Abl and enhanced imatinib-induced growth arrest in chronic myelogenous leukemia cell line K562. Cancer Chemother Pharmacol 2007; 60: 581-7.

[114] Li J, Xia X, Nie H, Smith MA, Zhu X. PKC inhibition is involved in trichosanthin-induced apoptosis in human chronic myeloid leukemia cell line K562. Biochim Biophys Acta 2007; 1770: 63-70.

[115] Peng CH, Tseng TH, Liu JY, Hsieh YH, Huang CN, Hsu SP, *et al.* Penta-acetyl geniposide-induced C6 glioma cell apoptosis was associated with the activation of protein kinase C-delta. Chem Biol Interact 2004; 147: 287-96.

[116] Jing Y, Watabe M, Hashimoto S, Nakajo S, Nakaya K. Cell cycle arrest and protein kinase modulating effect of bufalin on human leukemia ML1 cells. Anticancer Res 1994; 14: 1193-8.

[117] Tian X, Wang PP, Liu YP, Hou KZ, Jin B, Luo Y, *et al.* Effect of bufalin-inducing apoptosis on Bcl-2 and PKC in HL-60 cells. Zhongguo Shi Yan Xue Ye Xue Za Zhi 2007; 15: 67-71.

[118] Kurosawa M, Tani Y, Nishimura S, Numazawa S, Yoshida T. Distinct PKC isozymes regulate bufalin-induced differentiation and apoptosis in human monocytic cells. Am J Physiol Cell Physiol 2001; 280: C459-64.

[119] Zhang DC, Cao CX, Zhang CJ, Zhou BN. Yuanhuacin A is a selective antagonist of phorbol ester receptor in protein kinase C. Sci China B 1993; 36: 803-8.

[120] Park BY, Min BS, Ahn KS, Kwon OK, Joung H, Bae KH, *et al.* Daphnane diterpene esters isolated from flower buds of Daphne genkwa induce apoptosis in human myelocytic HL-60 cells and suppress tumor growth in Lewis lung carcinoma (LLC)-inoculated mouse model. J Ethnopharmacol 2007; 111: 496-503.

[121] Irie K, Nakagawa Y, Ohigashi H. Toward the development of new medicinal leads with selectivity for protein kinase C isozymes. Chem Rec 2005; 5: 185-95.

[122] Irie K, Nakagawa Y, Ohigashi H. Indolactam and benzolactam compounds as new medicinal leads with binding selectivity for C1 domains of protein kinase C isozymes. Curr Pharm Des. 2004; 10: 1371-85.

[123] Borsello T, Forloni G. JNK signalling: a possible target to prevent neurodegeneration. Curr Pharm Des 2007; 13(18): 1875-86.

[124] Sathornsumetee S, Rich JN. Antiangiogenic therapy in malignant glioma: promise and challenge. Curr Pharm Des 2007; 13(35): 3545-58.

[125] Thaimattam R, Banerjee R, Miglani R, Iqbal J. Protein kinase inhibitors: structural insights into selectivity. Curr Pharm Des 2007; 13(27): 2751-65.