Protein kinase C: a target for anticancer drugs?

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Abstract

Protein kinase C (PKC) is a family of serine/threonine kinases that is involved in the transduction of signals for cell proliferation and differentiation. The important role of PKC in processes relevant to neoplastic transformation, carcinogenesis and tumour cell invasion renders it a potentially suitable target for anticancer therapy. Furthermore, there is accumulating evidence that selective targeting of PKC may improve the therapeutic efficacy of established neoplastic agents and sensitise cells to ionising radiation. This article reviews the rationale for targeting PKC, focuses on its role in breast cancer and reviews the preclinical and clinical data available for the efficacy of PKC inhibition.

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Introduction

The protein kinase C (PKC) family consists of at least 12 isozymes that have distinct and in some cases opposing roles in cell growth and differentiation (Blobe et al. 1994, Ron & Kazanietz 1999). Early observations that PKC isozymes are activated by tumour-promoting phorbol esters (Castagna et al. 1982) suggested a key role for PKC in tumour promotion and progression. This led to PKC being considered as a target for anticancer therapy. The presence of PKC isoforms with differential activation and tissue distribution (Way et al. 2000) raised the possibility of developing PKC isozyme-specific inhibitors with the potential for targeting specific intracellular pathways.

PKC was originally identified as a phospholipid- and calcium-dependent protein kinase (PK) (Takai et al. 1979). The subsequent classification of the isoforms is based on structural and activational characteristics (Nishizuka 1992, Newton 1995). The PKCs are divided into three subfamilies: conventional or classic PKCs (cPKC), non-classic or novel PKCs (nPKC) and atypical PKCs; their binding and activational characteristics are summarised in Fig. 1 (Schenk & Snaar-Jagalska 1999). Activation of PKC isoforms results in changes in their subcellular location. For example, PKCα and PKCζ translocate from the cytosol to the perinuclear membrane on activation (Distanik et al. 1994). Cell-specific isoform functions may be conferred by differences in subcellular localisation following translocation to specific anchoring proteins (Csuki & Mochley-Rosen 1999, Way et al. 2000).

The phospholipid diacylglycerol (DAG) plays a central role in the activation of PKC by causing an increase in the affinity of PKCs for cell membranes which is accompanied by PKC activation and pseudo-substrate release (Newton 1995). Activated PKC then phosphorylates and activates a range of kinases. Signals that stimulate G-protein-coupled receptors, receptor tyrosine kinases and non-receptor protein tyrosine kinases can all cause the production of DAG (Nishizuka 1992, Newton 1995). Several PKC isoforms are activated independently in a redundant manner through the phospholipase C (PLC) and phosphatidylinositol 3-kinase (PI3K) pathway (Newton et al. 1995). For example, platelet-derived growth factor activates PKCε through redundant and independent signalling pathways involving PLCγ and PI3K (Moriya et al. 1996). There is some evidence to suggest that there are functional differences between PKC activated through different pathways (Ohno 1997).

The downstream events following PKC activation are little known. The main pathway, which is activated by PKC, is the MEK-ERK pathway. PKCα phosphorylates and activates the serine/threonine kinase Raf1 both in vitro and in vivo (Kolch et al. 1993). It is, however, unclear how this phosphorylation contributes to activation of Raf1. In addition, It has been proposed that PKCα, δ and ε also activate the MEK-ERK pathway via Raf1 (Ueda et al. 1996, Cai et al. 1997). Activated Raf1 phosphorylates MEK1 and...
MEK2, which activate the mitogen-activated protein kinase cascade, ultimately resulting in transcription of genes involved in cell proliferation (Marshall 1996). PKCα, βI and γ specifically inactivate GSK-3β by phosphorylation, leading to derepression of the cJun transcription factor (Goode et al. 1992). PKC9 is reported to synergise with the Ca²⁺-dependent phosphatase calcineurin to stimulate JNK1 via Rac1 (Werlen et al. 1998).

Role of PKC in cancer

Early studies suggested a role for PKC isozymes in tumour promotion. PKC isozymes are selectively activated by tumour-promoting phorbol esters in a way similar to the endogenous activator DAG (Castagna et al. 1982). Subsequently a direct correlation between the ability of individual phorbol esters to promote tumours and to activate PKC has been demonstrated (Nishizuka 1984). Furthermore, other structurally unrelated tumour promoters (such as telocidin) also activate PKC at high concentrations (Nishizuka 1984).

Increased levels of PKC have been associated with malignant transformation in a number of cell lines including breast (O’Brien et al. 1989), lung (Takenaga & Takahashi 1996) and gastric carcinomas (Schwartz et al. 1993). In vivo, however, the relationship is less clear, with PKCβ expression being found to increase, remain the same or decrease in colon tumours when compared with normal epithelium. This may partially be explained by changes occurring in PKC expression during tumorigenesis. In colon cancer, PKCβII appears to be overexpressed early in the carcinogenic process with PKCα and βI expression decreasing later in tumour development (Gokmen-Polar et al. 2001). Studies with antisense PKCα oligonucleotides have demonstrated inhibition of tumour growth in nude mice bearing implanted human glioblastomas and human bladder, lung and colon carcinomas (Dean et al. 1996). In vitro experiments suggest that PKCα inhibition may result in apoptosis. PKCδ has also been implicated in the apoptotic response in human prostate cancer and myeloid leukaemia cell lines (Fujii et al. 2000, Majumder et al. 2000). Inhibition of PKC may have an impact on the invasive and metastatic potential of malignant cells. The PKC family is involved in cellular adhesion with PKC inducing the expression of adhesion proteins (Lane et al. 1989) and many cell adhesion receptors act as PKC sub-
strates (Herbert 1993). Atypical PKCs have been implicated in the evolutionarily conserved PAR protein complex and play a critical role in the development of junctional structures and apico-basal polarisation of mammalian epithelial cells (Suzuki et al. 2001). Furthermore, Wnt5a appears to be up-regulated in breast cancer by activated PKC and down-regulated by its inhibition (Jonsson et al. 1998). Thus PKC may act via the Wnt genes to affect the cytoskeleton and cellular adhesion.

Role of PKC in breast cancer

*In vitro* studies suggest a positive correlation between elevated PKC levels and both the invasive and chemotactic potential of human breast cancer cell lines (Blobe et al. 1994). One small study in nine patients examined PKC levels in surgical specimens of human breast tumours compared with normal breast tissue obtained from the same patient. PKC activity was significantly higher in the tumour tissue compared with the normal tissue ($P = 0.0003$) (O’Brian et al. 1989). PKC may also be associated with hormone dependence in breast cancer cells. Stable transfection of PKCα renders T47D human breast cancer cells hormone-independent *in vitro* and *in vivo* (Tonetti et al. 2000). Several oestrogen receptor (ER)-negative human breast cancer cell lines express significantly higher levels of PKC than ER-positive breast cancer cell lines (Fabbro et al. 1986, Platel et al. 1998). In two cell lines PKCδ appears to play a fundamental role in the regulation of growth in ER-positive breast cancer cells (Shanmugam et al. 2001). Furthermore, preliminary data suggest that PKCα overexpression may predict resistance to tamoxifen (Tonetti et al. 2002). These studies suggest that members of the PKC family have potential as targets in the treatment of breast cancer.

PKC inhibitors

The PKC family is undoubtedly an attractive target for therapeutic intervention given its role in tumorigenesis and the potential for enhancing cytotoxicity of existing drugs. The existence of different isozymes provides an opportunity to develop pharmacological agents that target specific PKC functions. Equally, however, there is no doubt that the complex nature of the many secondary messenger systems that involve PKC renders selective drug action difficult. Few of the currently available pharmacological agents exhibit a high degree of selectivity for a specific PKC isoform, and the majority also act on other PKs. The principal agents under investigation are listed in Table 1.

The microbial alkaloid staurosporine was identified 20 years ago as an antiproliferative agent and potent inhibitor of PKC. It probably acts by competing at PKC’s conserved ATP-binding sites. Although its specificity for PKC and its isoforms is poor, staurosporine has served as a lead compound from which many novel PKC inhibitors have been developed (Way et al. 2000). Drugs such as PKC412 (N-benzoyl-staurosporine) and UCN01 (7-hydroxystaurosporine) exhibit improved selectivity, with a potentially better therapeutic index *in vivo* and have been reported to enhance the effects of other cytotoxic agents.

UCN01

UCN01 was administered in phase I trials as either a 3 or 72 h i.v. infusion every 21–28 days (Fuse et al. 1998). The recommended phase II dose for UCN01 administered as a 72 h infusion is 42.5 mg/m$^2$ per day. UCN01 exhibits unusual pharmacokinetics in man compared with animal models with long elimination half-lives of between 253 and 1660 h. The unexpected results obtained with UCN01 in man can be explained by high binding to AAG resulting in $<0.02\%$ free UCN01, compared with 0.49–1.75% in animal studies (Fuse et al. 1998, 1999). Dose-limiting toxicities of pulmonary dysfunction, nausea and vomiting, and hyperglycaemia with metabolic acidosis were reported. Preliminary evidence suggests UCN01 modulation of both PKC substrate phosphorylation and the DNA damage-related G2 checkpoint (Sausville et al. 2001). Antitumour activity was observed against leiomyosarcoma, non-Hodgkin’s lymphoma (NHL), melanoma and lung cancer (Fuse et al. 1998).

PKC412

It seems likely that anticancer drugs acting as signal transduction inhibitors rather than as classic cytotoxics may require prolonged exposure to be effective. In this context PKC412 has the advantage of being administered orally. PKC412 was well tolerated in the phase I study, the main toxicities being nausea, vomiting, fatigue and diarrhoea. A formal maximum-tolerated dose (MTD) was not defined (Propper et al. 2001). Like UCN01, PKC412 has a longer half-life than would be predicted from preclinical studies due to altered gastrointestinal absorption and plasma protein binding (in particular binding to AAG) in patients with cancer (Propper et al. 2001). Given the lack of classic cytotoxic side-effects and unusual pharmacokinetics, assessing the inhibitory effects of PKC412 on PKC signalling pathways *in vivo* in parallel with standard pharmacokinetic studies was crucially important. The release of tumour necrosis factor (TNF) and interleukin (IL)-6 from phytohaemagglutinin-stimulated whole blood cells were both significantly inhibited in a time- and dose-dependent manner with the level of inhibition appearing to plateau at doses over 100 mg/day. In addition, down-regulation of extracellular signal-related kinase 2 (ERK2) was demonstrated across the 50–300 mg/day dose levels (Thavasu et al. 1999). The investigators concluded that, although a formal MTD had not been identified, the increased prevalence of symptomatic toxicities...
at 225 mg/day along with the apparent plateau in drug exposure and biological activity were such that 150 mg/day should be the recommended phase II dose (Propper et al. 2001). This dose was evaluated in a phase II trial of PKC412 in patients with accessible melanoma which incorporated tumour biopsies. This study failed to demonstrate consistent target inhibition or pharmacodynamic efficacy. Tumour cytosolic PKC inhibition varied from 7 to 91% in seven out of nine patients examined and tumour particulate PKC activity was reduced by 11–79% in four patients. In vitro testing demonstrated less than 10% inhibition of multi-drug resistance (MDR) (Propper et al. 2001). Although the phase I study results had been encouraging, these negative findings cast doubt on whether PKC412 achieves its intended effect in tumours. The development of PKC412 illustrates the difficulties inherent in selecting pharmacodynamic endpoints in clinical trials.

Bryostatin

The bryostatins are a family of at least 20 naturally occurring macrocyclic lactones derived from the marine bryozoan Bugula neritina. They have been shown to have promising antineoplastic and immunomodulatory activity in preclinical models. Bryostatin I is the prototype of this novel class of agents (Schaufelberger et al. 1991). They are potent activators of cPKC and nPKC subfamilies. However, in the presence of activating ligands, such as the tumour-promoting phorbol esters, bryostatins act as antagonists (Smith et al. 1985). The basis for the divergent activities of bryostatin I is
not known but may derive from differential isoform activation (Szallasi et al. 1994) or nuclear translocation (Hocevar & Fields 1991). Furthermore, bryostatin I potently downregulates cPKC/nPKC activity, probably via ubiquitin-directed proteasomal degradation of the enzyme (Lee et al. 1996). Bryostatin may also activate effector cells of the immune system and stimulate cytokine production (Mohr et al. 1987, Trenn et al. 1988).

Bryostatin I has been widely investigated in a series of phase I trials with infusion times varying from 1 to 24 h (Philip et al. 1993, Prendiville et al. 1993, Jayson et al. 1995). The dose-limiting toxicity in all studies has been myalgia with localised phlebitis a problem using the shorter infusion times. Antitumour activity has been demonstrated in patients with melanoma, epithelial ovarian carcinoma and NHL (Philip et al. 1993, Prendiville et al. 1993, Jayson et al. 1995).

Significant increases in plasma concentrations of TNF-α and IL-6 were observed when bryostatin, 50 µg/m², was administered as a 1 h infusion weekly for 3 weeks out of every 4 (Philip et al. 1993). A further study of a weekly 24 h infusion of bryostatin demonstrated significant changes in activated PKC in peripheral mononuclear cells during the infusion (Jayson et al. 1995). The previously reported elevation of IL-6 and TNF-α was not confirmed in this study. However, an increase in IL-2-induced proliferative response in peripheral blood lymphocytes and enhanced LAK activity was demonstrated.

Bryostatin showed some evidence of clinical activity in phase I trials but this was not confirmed in phase II trials of patients with pretreated metastatic melanoma (Propper et al. 1998) and metastatic colorectal cancer (Zonder et al. 2001). It had limited efficacy against relapsed low grade NHL (Varterasian et al. 1998).

**Tamoxifen**

The anti-oestrogen tamoxifen is a non-selective inhibitor of PKC. High, PKC inhibitory, doses of tamoxifen achieved disease stabilisation of childhood malignant gliomas in a phase I clinical trial (Couldwell et al. 1996, Pollack et al. 1997).

**ISIS3521**

Antisense oligonucleotides have been developed to achieve PKC isoform-specific inhibition by inhibiting expression of target mRNA sequences. ISIS3521, an antisense phosphorothioate oligonucleotide to PKCα, has been investigated in a phase I trial. Patients received 2 hourly i.v. infusions (0.15–6 mg/kg per day) three times per week. Drug-related toxicities included nausea, vomiting, fever, chills and fatigue. Haematological toxicity was limited to thrombocytopenia.

There was no relationship between dose, maximum concentration of drug, or area under the curve and coagulation times or complement levels. Dose escalation was discontinued because of the attainment of peak plasma concentrations which approached that associated with complement activation in primates. No dose-limiting toxicities were identified and two patients with NHL achieved complete responses (Neumannitis et al. 1999). A small phase II trial of ISIS3521 (2 mg/kg per day administered as a continuous infusion for 21 out of 28 days) in patients with metastatic breast cancer did not show any activity (Gradishar et al. 2001).

**The future for PKC inhibitors**

The PKC family undoubtedly represents an interesting and challenging target for the development of novel therapeutic agents. The current PKC inhibitors that have reached the clinic are relatively non-specific in their actions, do not fully exploit the potential for differential inhibition of PKC functions or specific isozymes and have encountered PK problems such as AAG binding. In the future it may be possible to develop agents that target a single isoform, different activating pathways or specific membrane interactions (specific RACKs or RICKs). Furthermore, as the downstream events resulting from PKC activation are better characterised it may be appropriate to target events further down the signalling pathways. Rational synthesis of molecules that target specific sites within the PKC isoforms is in progress. Peptide fragments that act as inhibitors or activators of translocation and antisense approaches are also being pursued (Way et al. 2000). Success with other signal transduction inhibitors such as Herceptin and STI571 have demonstrated that development of the right agent for the right target can be successful (Baselga 2001, Verweij et al. 2001). The role of PKC in tumorigenesis and apoptosis suggests that combining PKC inhibitors with conventional cytotoxics may be effective. Evidence from cell line data and the early phases of clinical trials suggests promising results for the combination of conventional cytotoxics with the existing PKC inhibitors (Alkan et al. 1993, Bunch & Eastman 1996, Wang et al. 1998, Ilson et al. 2000). In addition, PKC412 in vitro sensitises transformed murine fibroblasts to radiation-induced apoptosis (Zaug et al. 2001). It is not clear if this is due to PKC inhibition, but if it is the role of PKC isozyme inhibition in radiosensitisation would warrant further investigation.

Further translational research is needed to establish if inhibition of PKC isozymes will prove beneficial in altering the natural history of malignancy.

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