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Therapeutic potential of protein kinase C inhibitors

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Abstract

The serine/threonine protein kinase, protein kinase C (PKC) is a family of closely related isoforms which are physiologically activated by diacylglycerol generated by the binding of a variety of agonists to their cellular receptors. Free fatty acids may also play a role in activating PKC. The enzyme apparently mediates a wide range of signal transduction processes in cells and, therefore, inhibitors directed selectively against PKC may have wide-ranging therapeutic potential. This review highlights the evidence that inappropriate activation of PKC occurs in a number of disease states. Such evidence, however, is often seriously flawed because it relies on the use of phorbol esters, which are potent and direct PKC activators but may not mimic the physiological triggering of the enzyme in cells, or on the use of non-selective protein kinase inhibitors such as H7 and staurosporine. A new generation of bis-indolylmaleimides, derived from the lead provided by staurosporine, shows a high degree of selectivity for PKC over closely related protein kinases and such agents may provide more appropriate tools to investigate the role of PKC in cellular processes.

Introduction

Protein kinase C (PKC) is a family of serine/threonine specific protein kinases, consisting of at least eight isoforms, which plays a key role in a range of signal transduction processes (Fig. 1) [1]. Activation of PKC induces phosphorylation of many proteins in cells causing alterations to a number of biological systems thought to underlie several pathological states. The prospects for pharmacological manipulation, directed selectively against PKC, appear quite good. This review highlights the evidence that inappropriate activation of PKC occurs in a number of disease states. However, much of this research should be interpreted with caution, in particular that which relies on the use of non-selective protein kinase inhibitors such as H7 and staurosporine. Definitive evidence will require the use of potent and selective inhibitors of PKC.

PKC inhibitors and immunopathology

Immune reactions have a critical part to play in the defence of the host against infectious agents. There are, however, many cases in which the immune reactions of the host produce tissue damage or disease. A loss of tolerance to self-antigens may result in autoimmune disease. Exposure to external non-infectious allergens can result in anaphylactic reactions leading to conditions such as asthma, whilst artificial stresses placed on the system cause massive hypersensitivity reactions, for example, transplant rejection. Since PKC activity is pivotal to signal transduction pathways in many inflammatory cell types, a role can be envisaged for the involvement of the enzyme in many of these disease conditions, identifying them as potential candidates for treatment with inhibitors of PKC.

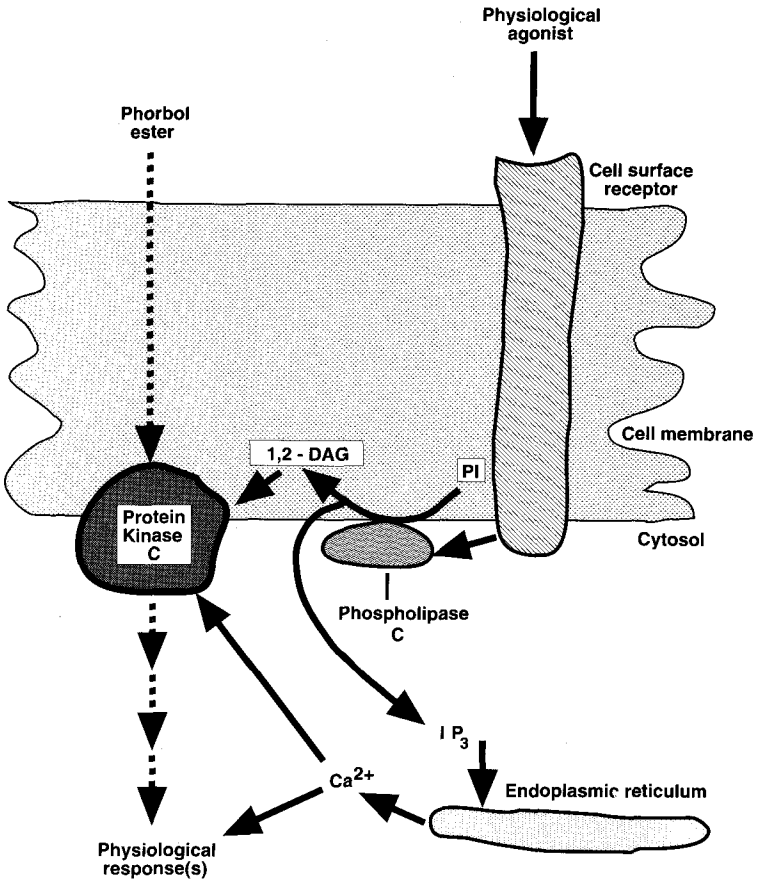


Figure 1
Protein kinase C-mediated signal transduction. Extracellular agonists bind specifically to a cell surface receptor. These then activate the enzyme phospholipase C, either by direct phosphorylation if the receptor is a tyrosine-specific protein kinase or via a G-protein. Phospholipase C acts on phosphatidylinositol (PI) to release inositol triphosphate (IP_3) and diacylglycerol (DAG). IP_3 mobilizes Ca^{2+} stored in the endoplasmic reticulum which, together with DAG, activates PKC. PKC catalyses the ATP-dependent phosphorylation of substrate proteins, ultimately resulting in various cellular responses.

Rheumatoid arthritis

Rheumatoid arthritis is a systemic syndrome in which inflammation of the joints associated with immune complex deposition is a major feature. Early synovitis often progresses to a hyperplastic stage with a chronic inflammatory cell infiltrate and resulting damage to articular tissue. Cartilage damage is caused by both the actions of metalloproteases released by a variety of cells found in inflamed joints and by free radicals formed as a result of a respiratory burst in phagocytic cells.
The role of PKC in rheumatoid arthritis has been previously described [2]. Inappropriate proliferation of T cells in response to antigen is an early and important event which may well underlie this auto-immune disease. Antigen-driven T cell proliferation may be regarded as a two-stage process. In the first stage, the antigenic signal delivered via the T cell receptor–CD3 complex results in increased surface expression of the IL-2 receptor and in production of IL-2. In the second stage, binding of IL-2 to its receptor triggers cell division. The involvement of PKC in antigen-driven T cell activation resulting in enhanced expression of the high-affinity receptor for IL-2 has been demon-

strated [3]. The case for PKC involvement in IL-2-induced T cell proliferation rests on the IL-2-induced recruitment of PKC to the T cell membrane [4] and on the similarity of phosphorylation patterns induced by IL-2 and by a combination of phorbol esters, specific activators of PKC and Ca^{2+} ionophore. However, T cells in which PKC has been down-regulated by prolonged phorbol ester treatment [5] and a T cell line which apparently does not express PKC both still respond to IL-2 [6]. Despite this lack of clarity, the balance of evidence suggests that although PKC is required for antigen-driven T cell proliferation, this enzyme is not involved in IL-2-induced proliferation. PKC-mediated down-regulation of surface receptor molecules involved in T cell activation (CD3 and CD4) suggests that activation of PKC may also limit this response and, thus, the enzyme may play a dual response in T cell activation. There is increasing evidence that the neutrophil oxidative burst is mediated through PKC-dependent pathways [7, 8] and the enzyme has also been implicated in the release of histamine from basophils and mast cells [9, 10].

Systemic lupus erythematosus (SLE)

SLE is caused by auto-antibodies produced to a wide variety of host cellular antigens, particularly nuclear antigens. The fatal form is a systemic disease featuring high fever, skin rash, nephritis, polyarthritis and CNS symptoms. The role of PKC in this condition is unclear. T lymphocytes isolated from SLE patients showed a decrease in proliferation in response to phorbol ester but not to phytohemagglutinin (PHA), suggesting a defect in the PKC-mediated signal transduction pathway [11]. The total PKC activity in these cells is significantly lower than in the T cells from normal individuals. IL-2 production by PHA-stimulated cells from SLE patients was significantly depressed compared to control values, with a correlation between degree of depression and disease activity. The depressed IL-2 activity can be completely reversed by the addition of phorbol ester or partially by calcium ionophore [12]. The cells also had significantly a lower peak increase in intracellular calcium after stimulation by a monoclonal antibody against the CD3 antigen. This suggests that an alteration in the signal transduction mechanism after activation of the antigen receptor complex occurs in patients with SLE and that inhibition of PKC may further exacerbate the disease condition. The B cell hyperfunction causes an increased secretion of autoantibodies and this is also a hallmark of SLE. Normal human peripheral blood mononuclear cells can be stimulated to produce large quantities of IgG by treatment with the PKC activator, 1-oleoyl-2-acetylglycerol (OAG) [13]. The non-selective protein kinase inhibitor 1-[5-isoquinolinesulphonyl]-2-methylpiperazine (H7) blocked spontaneous IgG production in cells from active SLE patients, suggesting that, in these cells, PKC may have a part to play in mediating the progress of the disease.

Diabetes mellitus

Diabetes mellitus is a general term for a group of diseases which have as a common denominator abnormalities in carbohydrate metabolism, usually due to a deficiency in the production or utilization of insulin. Immunogenic factors associated with the onset of the conditions include the production of auto-antibodies to either insulin, insulin receptors, islet cell cytoplasm or to islet cell surface antigen. There is some weak evidence that PKC is down-regulated in diabetic neuropathy and vascular damage [14, 15]. However, enhanced PKC activation may play an important role in the signal transduction processes leading to the auto-immune responses which probably underlie this disease and, therefore, PKC inhibitors would be expected to have a beneficial effect on the condition.

Asthma

Asthma presents clinically as reversible acute respiratory distress, caused mainly by constriction of the smooth muscles of the small bronchi. Allergic or extrinsic asthma is caused by the activation of IgE-sensitized mast cells and the subsequent release of inflammatory mediators into the bloodstream. Non-allergic or intrinsic asthma is probably due to an imbalance of the physiological control of smooth muscle tone. Antigen challenge may activate PKC by cross-linking membrane-bound immunoglobulin molecules in the leucocytes of sufferers from extrinsic asthma; it has been speculated that a biochemical lesion may be responsible for increased PKC activity in non-allergic asthmatics [16]. Experiments using membrane preparations from lymphocytes of allergic patients have shown that β-adrenergic-receptor-stimulated adenylate

cyclase activity is reduced after donors are exposed to allergen [16, 17]. A similar non-specific desensitization of adenylate-cyclase-linked receptors occurs in human lymphocytes incubated *in vitro* with phorbol ester [17]. PKC activation stimulates cyclooxygenase activity and augments Ca^{2+}-dependent lipoxygenase products [18]. Ca^{2+}-stimulated synthesis of 5-lipoxygenase products has been shown to be increased in cells taken from both extrinsic and intrinsic asthmatics [19]. PKC-dependent superoxide production by neutrophils is also stimulated in cells from intrinsic asthmatics. Thus, a number of lines of evidence suggests that PKC activation may be involved in the pathology of both intrinsic and extrinsic asthma.

Transplantation

Transplant rejection is caused by an immune-mediated tissue inflammatory reaction provoked by specifically sensitized T cells. The reaction is initiated by a perivascular accumulation of lymphocytes around the site of the antigen. Sensitized cells cause a massive infiltration of non-sensitized cells into the area which are then responsible for subsequent tissue destruction. The success of immunosuppressive agents, such as cyclosporin A, in preventing transplant rejection [20] indicates that other compounds which inhibit T cell proliferation may have a role to play in this area. Evidence that inhibitors of PKC can block T cell proliferation has been described above. A role for the enzyme in transplant rejection has also been indicated by work investigating myocyte damage following cardiac transplant. Lymphocyte-mediated myocyte injury was prevented if the lymphocytes were pre-treated with phorbol ester to deplete PKC before being introduced into an *in vitro* model of cellular damage [21].

Multiple sclerosis

Multiple sclerosis (MS) is an inflammatory disease of the CNS characterized by focal T cell and macrophage infiltration into white matter. The inflammatory lesions are distributed around small ventricles in the white matter, preferentially in the spinal cord and cerebellum. This results in neurological dysfunction, paralysis, sensory deficits and visual problems. There is now strong evidence that the disease is autoimmune in nature [22, 23]. A counterpart animal disease, experimental allergic encephalomyelitis, can be induced in the rat by transferring lymphocytes from actively sensitized animals to naive recipients confirming this to be an immune cell-mediated condition. Immunosuppressive agents, such as cyclosporin A and FK-506, suppress both the active [24] and adoptively induced disease [25], possibly by down-regulating IL-2 gene transcription and preventing T cell proliferation. Whilst cyclosporin A and FK-506 are believed to act by blocking the translocation of the cytosolic component of nuclear factor of activated T cells (NF-AT) to the nucleus, the other component of NF-AT required for DNA binding activity and IL-2 transcription is synthesized in the nucleus via a PKC-mediated pathway [26]. This suggests that compounds, such as PKC inhibitors, which block T cell proliferation may be suitable therapeutic agents for the treatment of MS and related conditions.

PKC inhibitors and CNS disease

Diseases of the central nervous system provide another potential target for therapy with PKC inhibitors.

Alzheimer's disease

PKC may be involved in the pathogenesis of the major lesion of Alzheimer's disease (AD), within the CNS. Thus, high PKC immunoreactivity has been demonstrated in neuritic (senile) plaques including the diffuse plaques which are considered to be an early marker of AD pathology [27, 28]. Also, the neurofibrillary tangle, a pathological cytoskeletal structure found in diseased neurons of AD patients, contains proteins which are excessively phosphorylated. It is possible, though not yet demonstrated, that PKC may be responsible for the phosphorylation of one or more of these proteins [29]. Phosphorylation of amyloid precursor peptide by PKC has been demonstrated *in vitro* [30] and it has been speculated that pharmacologic manipulation of the state of phosphorylation of this peptide may modify the cerebral amyloidosis associated with AD. There is also a link between PKC and amyloid beta-protein, the major protein of plaque amyloid which is considered to be a primary factor in the pathology of AD, in that the protein can, at different concentrations, either stimulate or inhibit the enzyme [31]. Clearly, much work remains to be done in order to elucidate the role of PKC in AD

and this will be aided by the use of selective PKC inhibitors, which may ultimately also find therapeutic utility in this debilitating disease.

Malignant glioma

In brain tumours such as malignant glioma there is evidence that PKC may be involved in the proliferation of the glial cells, although the precise role for the enzyme is still somewhat confused. Thus, there are conflicting reports as to the effect of phorbol esters on the growth of glial cells in culture, with some workers suggesting stimulation [32, 33], but others showing a reduction of thymidine incorporation following stimulation with 12-*O*-tetradecanoyl-13-phorbol acetate (TPA) [34, 35]. Similarly confusing are the reports of an increased PKC enzyme activity in malignant glioma cell lines [34], but low phorbol ester binding capacity in brain glial tumours in comparison to tissues not undergoing malignant transformation [36]. One area in which there does seem to be agreement, however, is that structurally unrelated compounds which inhibit the activity of isolated PKC, all inhibit thymidine incorporation and proliferation of glial cells *in vitro* [32, 33, 37, 38], although this does not preclude another common mechanism of action. Recent results demonstrating improved survival in rats with gliomas, following treatment with another non-selective protein kinase inhibitor, staurosporine, encourage the belief that PKC inhibitors will be therapeutically useful in this condition and have been used as the rationale for testing tamoxifen in human patients [39].

PKC inhibitors and oncology

PKC is important in cellular differentiation and growth control and alterations in the activity of the enzyme are implicated in malignant transformation processes [40–43]. It is not surprising, therefore, that both inhibitors (see below) and activators (e.g. bryostatin) of PKC have been explored as potential therapeutic agents for the treatment of cancer.

Fibroblasts transfected with the PKC-β gene [44] or PKC-γ gene [45] overproduce PKC and express enhanced tumorigenicity. In addition, a mutated PKC gene cloned from murine UV-induced fibrosarcoma shows oncogenic potential both *in vitro* and *in vivo* [46]. Experimental and clinical studies suggest that alterations of PKC activity are involved in colorectal carcinogenesis [41]. Chemically induced experimental tumours (e.g. 1,2-dimethylhydrazine in colonic cancer) show an activation of DAG and an initial increase in membrane bound PKC in the preneoplastic colon followed by a reduction [47].

How PKC modulates cell growth and differentiation is not clear, but the enzyme is known to phosphorylate DNA methyltransferase (an enzyme which alters gene expression by changing DNA methylation patterns) [48] and transcription factors (e.g. AP-1) [49]. The results of other studies suggest that PKC plays an important role in the transformation processes downstream to *ras* oncogenes [50] and is involved in controlling the c-erbB-2 protein that leads to Jun/Fos-mediated transcription in nuclei [51].

The metastatic ability of B16 melanoma sublines correlates well with the basal level of membrane bound PKC indicating that PKC is also involved in the dissemination of tumour cells [52]. Moreover, TPA increases formation of metastases by SP1 mouse mammary carcinoma cells and, subsequently, inhibits formation of metastases when PKC is down-regulated [53].

To date some of the most powerful evidence for the potential utility of PKC inhibitors as anticancer agents has come from the PKC selective staurosporine analogues, CGP41251 and UCN-01. Both compounds are antiproliferative against a variety of transformed cell lines and exhibit antitumour activity *in vivo* [54, 55]. Other inhibitors based upon phospholipid analogues (e.g. BM41440) and sphingosine analogues (e.g. kynacyte) have also been claimed to increase the efficacy of cancer chemotherapies, such as adriamycin or cisplatin, with no apparent increase in toxicity [56]. These data suggest that potent and highly selective PKC inhibitors could provide effective cancer chemotherapy.

PKC inhibitors and virology

Viral entry into target cells is a key event for infection and replication and there have been numerous reports on the requirement of PKC for this process. Most of these data, however, have been generated using the non-selective protein kinase inhibitors H7 and staurosporine [57, 58] and should be viewed with caution. Human immunodeficiency virus (HIV) has also been claimed to require PKC activation for successful viral entry by

inducing phosphorylation of CD4 on target cells [59]; however, there has also been a report demonstrating that the cytoplasmic domain of CD4 is not required for HIV infection [60].

TPA has been shown to enhance HIV-1 replication in chronically infected MOLT-4 HIV cell lines [61] and there is evidence that this works by induction of a cellular transcription factor NF-κB which binds to the enhancer region of HIV LTR [61]. However, DNA topoisomerase II phosphorylation state and activity also correlates well with HIV production. Inhibition of the phosphorylation with PKC inhibitors (*O*-alkylglycerophospholipid analogues) results in reduction of HIV production [62]. Furthermore, other PKC activators, OAG and bryostatin-1, induce HIV expression in chronically infected U1 cells [63].

HIV spends a large part of the viral life cycle as a latent provirus integrated into the host genome. Since the HIV-1 tat protein increases gene expression during productive infection by up to 100-fold, it is likely that the regulation of tat activity is critical to the establishment and maintenance of latency. There is evidence that PKC depleted cells exhibit a marked reduction in HIV-1 transactivation without any significant effect on the synthesis of tat protein. Transactivation in these PKC deficient cells can be restored by transfection with a wild type PKC expression vector [64]. Clearly, PKC inhibitors could be of value for the treatment of HIV-1 disease both by suppressing the activation of latent HIV-1 and ongoing viral replication.

Activation of PKC may also be required for the expression of Epstein-Barr virus genome in latently infected cells, since treatment of infected cells with TPA or OAG leads to marked expression of early antigens [65].

Recently, evidence has come to light that PKC mediates transactivation of the hepatitis B virus [66, 67]. The recent development of more selective PKC inhibitors will permit the evaluation of such compounds as antiviral agents.

PKC inhibitors and cardiovascular disease

Several cardiovascular diseases may be dependent on alterations in cellular PKC activity. Many of these related diseases may stem from the changes in a few common molecular mechanisms involving PKC (e.g. altered Na^+/H^+ exchange in hypertension and in cardiac hypertrophy; inappropriate gene expression leading to mitosis in cardiac hypertrophy and atherosclerosis).

Hypertension

Hypertension is an important predisposing factor in the pathogenesis of ischaemic heart disease and myocardial infarction and is the most common risk factor associated with stroke. The increased contractile responsiveness of arteries from hypertensive rats to phorbol esters [68–72] has been used to suggest that signal transduction pathways are sensitized in hypertensive vasculature [73]. This contributes to the heightened vascular reactivity and, hence, to the elevated resistance seen in hypertension. These vasoconstrictive responses and mediator release associated with them are blocked by the protein kinase inhibitor H7 [74]. Additionally, PKC activity in tissue extracts from aortas of SH rats [75] and in platelets [76] and erythrocytes [77] from patients with essential hypertension is significantly higher than that of normotensive controls.

Cardiac Hypertrophy

Left ventricular hypertrophy usually develops in hypertension and refers to the compensating increase in cardiac mass which occurs to deal with increased cardiac load. Several lines of evidence suggest that PKC is involved in the myocyte proliferation which occurs in response to mechanical loading and which underlies cardiac hypertrophy. In some instances, tissue-associated PKC is increased in this condition and, in myocytes subjected to "stretching", expression of specific genes associated with proliferation (e.g. c-fos) is enhanced and is blocked by H7 [78]. Furthermore, PKC may also be involved in the enhanced phosphorylation of cardiac myosin light chain which accompanies cardiac hypertrophy [79].

Ischaemia

A decrease in total PKC activity (membrane-bound and cytosolic) has been detected in neuronal tissue subjected to ischaemia [80–83]. This may, however, represent a deregulation of PKC activity resulting from its conversion from a cofactor-dependent (PKC) to a cofactor-independent form (PKM) [84]. Subcellular redistribution of PKC also occurs in some tissues [84]; however, the

picture is complicated since enzyme translocation appears to occur in a cell-specific [85] and isotype-specific [86, 87] manner which does not clearly relate to the damage inflicted by the ischaemic insult and subsequent reperfusion. Conflicting data exist on the effects of inhibitors on post-ischaemic neuronal damage; some studies claim that protein kinase inhibitors aggravate neuronal damage [88], others that staurosporine prevents neuronal cell death [89] and inhibits the post-ischaemic impairment of working memory in rats exposed to cerebral ischaemia [90].

Free-radical-induced tissue damage plays an important role in reperfusion-induced damage following cardiac ischaemia. Studies with inhibitors have implicated PKC activation in the signal transduction pathways leading to free-radical production from neutrophils [7, 8] and macrophages. Additionally, H7 improves cardiac function (left ventricular relaxation) following ischaemia [91].

Atherosclerosis

Hyperplasia of arterial smooth muscle cells probably plays a critical role in the development of the atherosclerotic plaques which cause vascular damage in this disease. Direct stimulation of PKC by phorbol esters will cause proliferation of vascular smooth muscle cells and the proliferative response to physiological agonists such as PDGF is partially inhibited by prolonged treatment with phorbol esters which cause down-regulation of PKC [92]. Additionally, the non-selective protein kinase inhibitors H7 and staurosporine antagonise the proliferative response to some agonists (prolactin [93], serum [94]).

PKC inhibitors and other indications

Septic shock

Septic shock due to gram-negative bacteraemia or endotoxaemia is a major problem in surgical patients. Tumour necrosis factor (TNF) is believed to be a primary inflammatory mediator of septic shock. Experiments carried out with TPA suggest that activation of PKC is required in the signal transduction pathway of lipopolysaccharide-stimulated TNF release in Kupffer cells [95]. Inhibitors of PKC could, therefore, prove to be of therapeutic value in treatment of septic shock.

Dermatology

Psoriasis is a chronic skin disease which is characterized by epidermal hyperproliferation and inflammation. There are a number of lines of evidence which link PKC activity to the development of the psoriatic lesion. For example, it is well known that application of a phorbol ester such as TPA to animal skin induces a cutaneous inflammation with several similarities to the psoriatic lesion. Also, membrane-associated PKC is significantly increased in fibroblasts from both involved and non-involved psoriatic skin, compared to normal controls [96, 97]. The use of compounds such as tiflucarbine, W7 and staurosporine suggests that the proliferation of cultured human keratinocytes is driven by PKC-dependent pathways, since their inhibition of this proliferation parallels their potencies as inhibitors of PKC rather than their activities against other enzymes which they also inhibit [98]. Taken in conjunction with the anti-inflammatory effects, which would be expected of PKC inhibitors, these findings bode well for the utility of PKC inhibitors as a treatment for psoriasis.

Cystic fibrosis

There is some limited evidence, obtained from studies using skin fibroblasts [99], that there is abnormal PKC regulation of macromolecule secretion in cystic fibrosis patients. Whether or not this defect is susceptible to correction by inhibitors of PKC awaits further study.

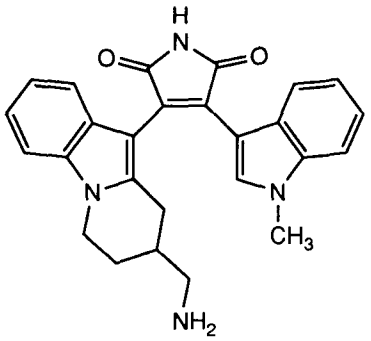
Concluding remarks

From the foregoing discussion it would seem likely that a very wide range of potential therapeutic applications exists for PKC inhibitors; however, much of the evidence which has been used to implicate PKC contains serious flaws. The ability to stimulate a physiological process with direct activators of PKC such as phorbol esters does not preclude alternative pathways which might be used by physiological agonists. The increased concentrations of PKC, the translocation of PKC and/or altered phosphorylation patterns observed in cells from diseased tissues may actually represent a compensating mechanism attempting to redress the imbalance caused by the initial lesion. Studies in which PKC is down-regulated by prolonged treatment with phorbol esters offer circumstantial evidence if the cellular response disappears in response

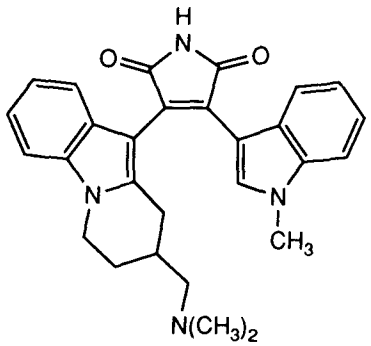
to this treatment. However, if the response is maintained this is inconclusive, since phorbol esters differentially down-regulate certain PKC isotypes [100]. Cell lines in which PKC expression is selectively deleted have not been widely used in these studies to support other lines of evidence but would seem to offer an alternative avenue of investigation.

In the light of these observations, the therapeutic potential for PKC inhibitors can best be explored by using highly selective compounds. Studies with H7, K252a and staurosporine are inconclusive since these agents lack the required selectivity for PKC over closely related protein kinases (Table 1) [101, 102]. Furthermore, H7 is ineffective as a PKC inhibitor in healthy cellular systems except at concentrations exceeding 100 μM [102]. A new generation of bis-indolylmaleimide inhibitors, derived from the lead provided by staurosporine show a high degree of selectivity for PKC over a range of closely related protein kinases (Table 2) [103, 8]. These agents have been extensively investigated *in vitro* and *in vivo* as immunomodulators. Results obtained using the most potent of this series, Ro 31-8425 (Fig. 2), suggest that, although PKC activation is an essential step in the induction of IL-2, the enzyme is not required for the subsequent IL-2 receptor stimulation and T cell proliferation (A. Lamont, personal communication). Ro 31-8830, an orally active compound, inhibits a phorbol ester-driven paw inflammation in rodents and selectively blocks the secondary inflammation which develops during adjuvant-induced arthritis in rats (Fig. 3) [104]. Ro 31-8830 is inactive in a whole range of models of acute inflammation suggesting a selective action which could not be readily predicted from *in vitro* and *in vivo* studies with K252a [105] and staurosporine.

The availability of these potent and highly selective inhibitors of PKC represents a turning point in the attempt to elucidate the role of this enzyme in fundamental biological processes and will enable a re-evaluation of the evidence for a role for PKC in the conditions described above. Nevertheless, the prospect that these inhibitors will be of therapeutic



Ro 31-8425



Ro 31-8830

Figure 2
Structures of Ro 31-8425 and Ro 31-8830.

Table 1
Inhibitory profile of staurosporine, K252a and H7.

	IC_{50} (nM)		
Enzyme	Staurosporine	K252a	H7
Serine/threonine kinases			
Rat brain protein kinase C	9	470	18 000
Bovine brain protein kinase A	120	200	16 000
Rabbit muscle phosphorylase kinase	0.5	1.7	65 000
Turkey gizzard myosin light chain kinase	55	3500	n.d.

Table 2
Inhibitory profile of Ro 31-8425, Ro 31-8830 and staurosporine.

Enzyme	IC_{50} (n*M*)		
	31-8425	31-8830	staurosporine
Serine/threonine kinases			
Rat brain protein kinase C	8	42	9
Bovine brain protein kinase A	2860	8600	120
Rabbit muscle phosphorylase kinase	1270	1135	0.5
Turkey gizzard myosin light chain kinase	3700	12 750	55
Rat liver casein kinase II	> 100 000	> 30 000	7000
Tyrosine specific protein kinases			
Human $p56^{lck}$	> 100 000	> 100 000	3000
Human $p60^{src}$	> 100 000	> 100 000	2000

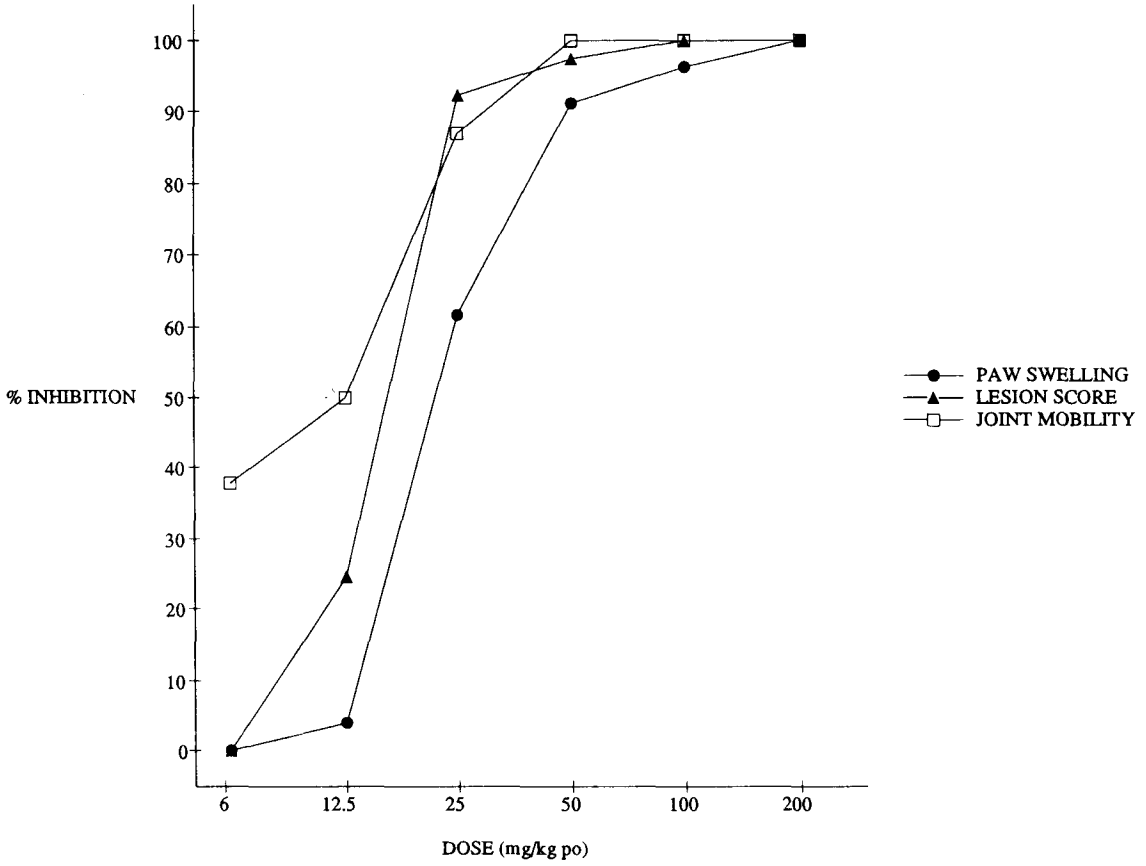


Figure 3
The effects of Ro 31-8830 (200 mg/kg p.o.) on a model of developing adjuvant arthritis in the rat. Adjuvant arthritis was induced in groups of rats by injection of 0.1 ml of *Mycobacterium tuberculosis* in liquid paraffin (5 mg/ml) into the right hand paw. Groups of 5 rats were dosed daily from day 0 with Ro 31-8830 or vehicle for 14 days. Results were determined as the percentage inhibition of the change from control to normal levels. Paw volume was measured as the change in volume of the left (non-injected paw) from days 7–14, lesion score was determined visually on day 14 and joint mobility was measured as the difference between the degree of flexion and extension.

benefit across a wide range of diseases remains exciting. Such enormous potential provides the driving force behind the vast efforts which are currently underway to put these compounds into the clinical arena as soon as possible.

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