Background and Objectives. The staurosporine derivative PKC412 (CGP41251) is a more selective inhibitor of the conventional isoforms of protein kinase C (PKC) than is the parent compound. In addition to its growth inhibitory properties, PKC412 reverses the efflux function of the multidrug resistance (MDR)-1 gene product, P-glycoprotein (P-gp).

Design and Methods. The in vitro actions of PKC412 were investigated in peripheral blood lymphocytes (PBL) from 4 normal volunteers, B-cell isolates from 3 normal tonsils and 31 patients with B-cell chronic lymphocytic leukemia (B-CLL). Following incubation with PKC412 for 2 days, the viability of B-CLL cells was decreased relative to that of controls (63±23% at 1 µmole/L; 52±30% at 10 µmole/L; n=20). Normal PBL were significantly more resistant to the drug (91±5% viable cells at 1 µmole/L; 73±18% at 10 µmole/L; n=4). Thirteen of the B-CLL patients were treated with oral PKC412 in a phase II trial.

Results. PKC activity in malignant cells from these patients showed a reduction post-treatment of 25-96% of their respective pre-treatment levels. Morphologic analysis, as well as in situ assay for DNA strand breaks (TUNEL assay) showed that B-CLL cells were killed by an apoptotic mechanism. In B-CLL cells the mean IC50, for PKC412, as measured by the reduction of 3-(4,5-dimethylthiozol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), was 2.1 µmol/L in 16 samples in which the IC50 were below the maximum concentration of PKC412 used for the assay. In tonsillar B-cells, the mean IC50 was 11 µmol/L whereas PBL cells were resistant. Four of eight and 1/3 B-CLL samples that were resistant to chlorambucil and fludarabine, respectively, were sensitive to PKC412. In 15/31 B-CLL samples a dose-dependent reversal of P-gp-mediated drug efflux by PKC412 was observed. A statistically significant correlation (p<0.001) was observed between P-gp protein expression as measured by FACScan analysis and the reversal of efflux activity by either PKC412 or verapamil. PKC412 increased the sensitivity of B-CLL cells to 2’-chlorodeoxyadenosine and chlorambucil.

Interpretation and Conclusions. This study establishes the in vitro cytotoxic and multidrug resistance (MDR) modulatory properties of PKC412 towards malignant cells from B-CLL patients. The direct anti-tumor activity combined with the potential for P-gp modulation make PKC412 an attractive drug for the treatment of malignancies expressing the MDR phenotype, or in combination with conventional drugs.

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Key words: B-chronic lymphocytic leukemia, protein kinase C, PKC412, multidrug resistance, apoptosis.
PKC functions to oppose apoptosis, particularly in hematopoietic cells and various PKC inhibitors are potent inducers of apoptosis in neoplastic cells. The role of PKC in the regulation of apoptosis may involve induction of bcl-2 mRNA and protein, phosphorylation of bcl-2, or modulation of intracellular pH. Upregulation of unidentified NFκB inducible genes may also involve PKC. Moreover, there is substantial evidence that inhibition of PKC may increase the susceptibility of neoplastic cells to cytotoxic drug-induced apoptosis. The important roles played by PKC in the regulation of cell proliferation and apoptosis and thereby malignancy suggest that it is a potential target for the development of anti-cancer agents.

Considerable indirect evidence suggests that P-glycoprotein (P-gp), the product of the multidrug resistance (MDR) 1 gene, is phosphorylated by PKC and that phosphorylation increases its ability to extrude cytotoxic drugs from malignant cells. The MDR phenotype of most cancer cells correlates with the level and activity of PKC. PKC activity is elevated in a number of selected MDR cell lines. Treatment of cells expressing P-gp with PKC activators has been shown to increase drug resistance. By contrast, PKC inhibitors decrease drug resistance. PKC is a positive regulator of P-gp ATPase activity and this may account for the increased drug resistance observed in P-gp overexpressing cells following activation of PKC. Co-immunoprecipitation studies have shown that P-gp interacts selectively with the α, β, γ, ε and θ PKC isoforms.

The naturally occurring compound staurosporine is a potent inhibitor of PKC and displays high antiproliferative and apoptosis-inducing activity. However, it does not selectively inhibit specific PKC isoforms. A derivative of staurosporine, PKC412 (N-benzoyl staurosporine) is a less potent PKC inhibitor but shows a higher degree of selectivity towards conventional PKC isoforms α, β, and γ. The proliferation of mitogenically stimulated normal human peripheral blood T-lymphocytes is inhibited by staurosporine at an IC50 of <0.01 μmole/L and by PKC412 at an IC50 of 0.092 μmole/L.

PKC412 has high affinity for P-gp and sensitizes P-gp-expressing cells to cytotoxic agents. It was more efficient than staurosporine in the reversal of anthracycline efflux in the anthracycline-resistant subline of ovarian carcinoma. In the same study with multidrug resistant human tumor cell lines, the PKC412 inhibited the cytotoxic action of adriamycin. The synergistic action was less pronounced or not observed when the P-gp-negative parental cell line was studied.

Preliminary studies show that PKC412 was well absorbed after oral administration and well tolerated by healthy adult male volunteers (unpublished observations) and by patients with advanced cancer. Therefore, PKC412 may have therapeutic value in combination with conventional drugs in the treatment of tumors expressing an MDR phenotype.

Previous studies on the actions of PKC412 have been on either normal or malignant human cell lines. Studies have shown that P-gp can be detected in chronic lymphocytic leukemia (CLL) cells and that its expression increases with advancing stage post-therapy and in patients treated with P-gp transportable drugs. We have previously investigated apoptosis in cells from B-CLL and MDR in relation to clinical outcome in blasts from acute leukemia patients. The effect of PKC412 as a cytotoxic agent and as a modulator of MDR was therefore, investigated in B-CLL cells in vitro.

**Design and Methods**

**Patient material**

Peripheral blood was collected in preservative-free heparin from 31 patients with B-CLL. Their age ranged from 46 - 87 (mean 66) years, 17 were male and 14 were female. Their Binet stages were A=13, B=10 and C=8. Thirteen had received no previous therapy (Binet stage A=8, B=5) while 18 (Binet stage A=5, B=5, C=8) had received previous therapy. The white cell count ranged from 18.7 to 182.5 × 10⁹/L. No patient had received chemotherapy or radiotherapy in the three months prior to this study.

Mononuclear cells were separated by density gradient centrifugation on Lymphoprep (Nygaard, Norway). They were depleted of monocytes by adherence to plastic and T-cells were removed by rosetting with sheep erythrocytes. The final B-CLL cell purity was assessed by FACScan analysis (Becton Dickinson, UK) using CD5 and CD2 and found to be greater than 90%.

Peripheral blood lymphocytes (PBL) were also obtained from five normal individuals and three tonsils removed at surgery. The tonsillar cells were teased and T-cells depleted to give a final B-cell population of >90% purity.

**Assessment of cell viability and apoptosis**

PBL and B-CLL cells were incubated at 2 × 10⁶ cells/mL in RPMI 1640 medium supplemented with antibiotics and 15% fetal calf serum (Life Technologies, UK). Following 48h incubation, cell via-
In vitro cytotoxicity

In vitro cytotoxicity was studied using the MTT assay at six concentrations of PKC412 or of cytotoxic drugs. Three cytotoxic drugs conventionally used in the treatment of CLL were studied, i.e., 2'-chlorodeoxyadenosine, chlorambucil and fludarabine (all from Aldrich-Sigma, UK). The cytotoxic drugs were studied either alone or in combination with 100 nM of PKC412. Results are expressed as the concentration of drug that decreased cell viability by 50% in the MTT assay compared with controls incubated in the absence of drug (IC50, calculated by IC50 software). PKC412 was dissolved in DMSO and diluted stepwise in RPMI medium. Further serial dilutions were performed in RPMI and the highest concentration achieved in culture without reprecipitation was 10 mmole/L with 0.5% DMSO. This concentration of DMSO did not affect cell viability in the MTT assay when added alone.

MDR profile

P-gp, multidrug resistance associated protein (MRP) and lung resistance protein (LRP) expression was studied on a FACScan (Becton Dickinson) using monoclonal antibodies MRK16 (P-gp, TCS Biologicals, UK), MRm6 (MRP, Monosan, UK) and LRP56 (LRP, Monosan, UK). The results are expressed as a ratio of mean cell fluorescence (MCF) of the specific antibody relative to the MCF of isotype-matched control serum.

The modulation of drug efflux by 10 μmole/L verapamil (VPM, Aldrich-Sigma, UK) or PKC412 at 10, 50, and 100 nmole/L was studied on a FACScan using the fluorescent dye DiOC, an established substrate for P-gp. A limited number of samples were also studied with 1 μmole/L PKC412. The results are expressed as a ratio of mean cell fluorescence (MCF) values obtained in the presence and absence of the modulator.

PKC activity

PKC activity in malignant cells was assayed as described earlier. Essentially, activity was measured in cell extracts with protamine sulphate as activator and substrate in the presence of 32P-γ-ATP. Activity was expressed as cpm/μg of protein from cell extracts before and after treatment with PKC412.

Statistical methods

Data were analyzed by Student’s t test for paired samples.

Results

Malignant cells from B-CLL patients are more sensitive to apoptosis induction by PKC412 than are normal peripheral blood lymphocytes

The actions of PKC412 on normal PBL from five donors are shown in Figure 1. A significant decrease in viability following 2 days of incubation was observed only at a drug concentration of 10 μmole/L (Figure 1A). Although PKC412 induced an increase in the percentage of apoptotic cells, this was not statistically significant at any of the concentrations tested (Figure 1B). The TUNEL assay also failed to reveal a significant induction of apoptosis by 10 μmole/L PKC412 (data not shown).

Malignant cells isolated from different patients showed highly variable sensitivity to the cytotoxic actions of PKC412. When mean values were compared, a significant decrease in viability was observed even at 0.1 μmole/L PKC412 (Figure 1A). However, some isolates were almost entirely resistant to killing by even 10 μmole/L drug while others showed >90% of dead cells at this concentration (Figure 1A). The induction of apoptosis, as detected by morphologic criteria (Figure 1B) or by the TUNEL assay (Figure 1C), was also highly variable. When mean values were compared, significant apoptosis induction was detected at 1 and 10 μmole/L PKC412. However, some isolates were almost entirely resistant to apoptosis induction even at 10 μmole/L drug, whereas others showed a >15% increase in apoptotic cells at 1 μmole/L PKC412 (Figures 1A and 1B). An inverse correlation was observed between percent surviving and percent apoptotic cells (data not shown). No significant correlation was observed between sensitivity to PKC412 in vitro and Binet stage, white cell count or previous treatment history. Incubation of CLL cells in vitro with human plasma in place of FCS resulted in a substantial decrease in spontaneous and PKC412 induced apoptosis at concentrations up to 10 μmole/L, the maximum concentration that could be achieved in vitro (data not shown).
PKC412 is cytotoxic to tonsillar B-cells and B-CLL cells but not to normal PBL

The cytotoxic effect of PKC412 was additionally studied in PBL, tonsillar B-cells and B-CLL cells using the MTT assay (Figure 2). Tonsillar B-cells were sensitive to PKC412 with a mean IC\textsubscript{50} value of 2.1 \(\mu\text{mole/L}\) (range 0.9–8.1 \(\mu\text{mole/L}\)). The remaining samples had IC\textsubscript{50} values >10 \(\mu\text{mole/L}\), the maximum concentration achieved in vitro (Figure 2C). PKC412 at a concentration of 100 nmole/L had no cytotoxic effect on B-CLL cells. However, no correlation was observed between drug sensitivity and...
stage of disease, age or previous treatment. No significant difference was observed in IC50 values when the samples were divided into P-gp-positive (MFI >1.2) and P-gp-negative (MFI<1.2) subgroups, or into subgroups showing PKC412 dose-dependent reversal of efflux (Δ) or concentration dependent (Ο) or concentration independent. Means and SEM bars are indicated for each series of data except for P-gp in (A).

PKC412 inhibits PKC activity in B-CLL cells

In 8/13 patients treated with oral PKC412 for 14 days, the PKC activity in the malignant B-cells post-treatment was reduced by 25-96% compared to their respective pre-treatment values. The pre-treatment PKC activity ranged from 496-5684 cpm/µg protein and the post-treatment activity ranged from 331-600 cpm/µg protein.

PKC412 reverses P-gp-mediated efflux of DiOC in B-CLL cells

The role of PKC412 as a modulator of P-gp was investigated in PBL, tonsillar B-cells and B-CLL cells by FACScan analysis of the efflux of DiOC. There was no significant modulation of efflux by either PKC412 or VPM, the latter a known modulator of
P-gp in either PBL or tonsillar B-cells. In B-CLL cells the mean MCF ratio for reversal of efflux by VPM was 1.92. With PKC412 a dose-dependent reversal of efflux was observed with mean MCF ratios of 1.01, 1.18 and 1.34 at 10, 50 and 100 nmole/L respectively. There was a significant correlation between reversal of efflux by PKC412 and that by VPM (p < 0.001, r = 0.72). A significant correlation was also observed between P-gp expression and reversal of efflux by VPM (p < 0.001, r = 0.85) and by 100 nmole/L PKC412 (p < 0.001, r = 0.60). When the samples were subdivided on the basis of either P-gp expression (Figure 3A) or dose-dependent reversal of efflux (Figure 3B), the correlation was greatly enhanced.

There was no correlation between efflux modulation by PKC412 at any concentration and expression of either MRP or LRP. Furthermore there was no correlation between P-gp, MRP and LRP expression (data not shown).

PKC412 sensitizes B-CLL cells to some conventional cytotoxic drugs

The mean IC₅₀ values for 2'-chlorodeoxyadenosine and chlorambucil showed a small decrease in the presence of 100 nmole/L PKC412 in vitro (Figures 4A and 4B). This decrease was not statistically significant (2'-chlorodeoxyadenosine alone vs with PKC412 p = 0.052). This effect was not observed for fludarabine (Figure 4C). The mean IC₅₀ value for daunorubicin, a substrate for P-gp, also showed a decrease in the presence of PKC412 (Figure 4D) but the decrease was not statistically significant. Although the mean decrease in IC₅₀ values with PKC412 was small, subsets of patients were identified (9/18 for 2'-chlorodeoxyadenosine, 5/16 for chlorambucil, 5/19 for fludarabine, 4/11 for daunorubicin) in whom the decrease in IC₅₀ values ranged from 32-87%. Additionally, 3/8 and 1/3 samples that were resistant to chlorambucil and fludarabine, respectively, were sensitive to 1.0-1.6 µmole/L PKC412 alone. On the other hand, two samples resistant to chlorambucil and fludarabine were also resistant to PKC412 and one of these samples was also resistant to 2'-chlorodeoxyadenosine and daunorubicin.

Discussion

Previous studies on the actions of PKC412 have been carried out mainly in human and animal tumor cell lines. Studies of its in vitro actions on a pure malignant cell population from patients have not been described. This study reports the cytotoxic action and MDR reversal properties in vitro on a purified leukemia cell population from patients with B-CLL.

In agreement with previous studies in cell lines, PKC412 had cytotoxic activity in B-CLL cells in vitro at concentrations of 1 µmole/L and above. Although the mean IC₅₀ value for PKC412 in B-CLL cells was 19.1 µmole/L, nearly half the samples had IC₅₀ values of less than 2 µmole/L, including some with high lymphocyte counts. In a wide range of cell lines the IC₅₀ varied from 15 nmole/L to > 1 µmole/L (and unpublished data). In a phase I dose escalation study of 12.5-300 mg/d PKC412 in patients with advanced cancer, the maximal observed plasma concentration of PKC412 was in the range of 0.3-7.0 µmole/L. In addition to the unchanged drug, a metabolite of the drug showing biological activity in vitro was also found in patients' plasma at concentrations greater than 10 µmole/L. The phase I study also confirms that although the drug binds to plasma proteins it still had biological activity on cells and tissues in vivo. Although PKC activity was not evaluated in the in vitro study samples, some of these patients went on to be treated with oral PKC412. The malignant cells from these patients treated with 25-225 mg/d of PKC412 over 2-8 weeks showed a significant reduction in PKC activity (Virchis et al., submitted) confirming that PKC412 can inhibit PKC activity in B-CLL cells. B-CLL cells are non-proliferative and are blocked in the G₀/G₁ phase of the cell cycle. The effect of PKC412 on these cells is, therefore, not attributable to actions on the cell cycle but are consistent with a direct action on the apoptotic machinery.

The decrease in spontaneous and PKC412-induced apoptosis in vitro in the presence of human plasma may be attributed to at least two factors. First, plasma has been shown to contain unidentified factors that block apoptosis by signaling via the phosphatidylinositol 3'-kinase/AKT pathway. Second, human plasma α1 acid glycoprotein (AAG) binds PKC412 avidly thus blunting its cytotoxic actions. While these in vitro observations might suggest that PKC412 may not be effective against B-CLL cells in vivo, it is has been shown that sustained exposure resulting from sequential doses in patients results in cytotoxicity of B-CLL cells (Virchis et al., submitted), malignant tissues and circulating white cells.

PKC412 at 100 nmole/L displayed a negligible cytotoxic effect on B-CLL cells. Consequently, this concentration was used for studies on in vitro reversal of efflux of DiOC in these cells. At this con-
centration PKC412 showed significant reversal of P-gp function in B-CLL cells. Even lower concentrations showed reversal of efflux and a clear dose-dependent reversal was observed in 50% of the samples. The dose-related reversal of efflux also suggests that PKC412 may affect P-gp directly, resulting in retention of DiOC. When samples were grouped on the basis of PKC412 dose-dependent efflux, there was significant correlation with both reversal of efflux induced by VPM and with elevated expression of P-gp. In two samples, a higher concentration of PKC412 (1 µmole/L) was also used in the reversal of efflux studies. The MCF ratio at this higher concentration was comparable to that obtained with 10 µmole/L VPM (data not shown). However, 1 µmole/L PKC412 is not toxic and is well tolerated (unpublished data) compared to VPM at 10 µmole/L, a level at which severe cardiac toxicity has been observed in clinical trials.

Incubation of P-gp overexpressing KB 8511 epidermoid carcinoma cells with non-cytotoxic concentrations of PKC412 sensitizes the cells to vinblastine with complete reversal of the MDR phenotype, resulting in a dose-dependent accumulation of the fluorescent dye rhodamine-123. Our preliminary studies with the CEM cell line and its resistant variant VLB using 100nmole/L PKC412 showed similar results, although a complete reversal was not achieved probably due to higher P-gp expression (data not shown). A similar concentration of PKC412 has been used by others for MDR reversal studies in cell lines. Non-toxic concentrations of PKC412 significantly enhanced the cytotoxic properties of doxorubicin, actinomycin D, vinblastine and vincristine but not 5-flourouracil. However, the enhanced intracellular concentrations of doxorubicin did not change P-gp protein expression in human breast carcinoma (MCF-7), murine colon adenocarcinoma (CT-26) or their MDR variant cell lines. PKC412 efficiently reversed anthracycline efflux in an anthracycline-resistant subline (A2780/ADR) of ovarian carcinoma. Furthermore, the antitumor activity of doxorubicin against drug resistant murine carcinoma cells was enhanced by oral administration of PKC412.

The non-significant sensitization of B-CLL cells in vitro with a combination of PKC412 and conventional drugs was not surprising. None of these drugs (2′-chlorodeoxyadenosine, chlorambucil and fludarabine) is a substrate for P-gp. However, in a number of samples there was considerable decrease in the IC50 value for all the drugs tested. It is, therefore, possible that PKC412 can sensitize B-CLL cells from a subgroup of patients to conventional drugs by mechanisms unrelated to P-gp modulation. It is also possible that consistent sensitization results may be achieved by longer exposure of these cells to PKC412 (see last paragraph). However, a decrease in the IC50 value for daunorubicin, a known substrate for P-gp with PKC412, was also not significant. This was surprising considering that a significant correlation was observed between increased P-gp expression and reversal of DiOC efflux in the cohort of samples investigated. This difference may be due to the greater sensitivity of the DiOC efflux assay compared to the MTT assay. However, there was no correlation between PKC412 IC50 values and P-gp expression in B-CLL cells. This may be attributed to the narrow concentration window within which PKC412 acts as a modulator of P-gp without causing direct cytotoxic effects.

Although chlorambucil and fludarabine are not substrates for P-gp, treatment with either of these agents in combination with PKC412 can nevertheless be beneficial since PKC may regulate the expression of anti-apoptotic proteins. These proteins may oppose the induction of apoptosis by chlorambucil and fludarabine. Signal transduction inhibitors which decrease expression of these proteins via blockade of PKC activity may, therefore, augment the cytotoxic activity of conventional drugs in an additive or synergistic manner. Recent reports also suggest that P-gp in addition to acting as an efflux pump, may also have effects on apoptosis by mechanisms which are yet unclear. In these studies, NIH 3T3 cells transfected with the mdr-1 gene conferred resistance to UV radiation-induced cell death by apoptosis which was reversed by P-gp modulators, VPM or a monoclonal antibody to P-gp.

Since B-CLL cells are non-proliferative, it is not clear whether the cytotoxic effect of PKC412 is via inhibition of PKC. Inhibition of PKC may, however, play a role in the regulation of apoptosis observed in these B-CLL cells. Alternatively, a recent study shows that PKC412 can block in vivo signaling pathways in cancer patients by suppressing cytokine release. How this contributes towards apoptosis in B-CLL cells needs investigation. The mechanism of the modulatory effect of PKC412 on MDR is also not clear; it may act directly as a competitive or non-competitive inhibitor of P-gp or may modulate MDR via inhi-
bition of PKC. In cells from AML patients, a highly significant positive correlation was found between MDRI and PKCβ possibly via modulation of the phosphorylation of P-gp.34,38

At present, other PKC inhibitors are being studied in vitro and in vivo in B-CLL and other malignancies. Bryostatin 1 has completed phase I studies in non-Hodgkin’s lymphoma (NHL) and B-CLL; in this context 11/29 patients achieved stable disease for 2-19 months.39 UCN-01 (7-hydroxy staurosporine) is being tested in phase I clinical trials in patients with refractory neoplasms43 and has been suggested as a combination agent with fludarabine to improve overall survival in B-CLL.41 UCN-01 enhances cellular sensitivity to 5-fluorouracil by suppressing thymidylate synthase via downregulation of E2F-1.42 In vitro studies have shown that Safingol enhances the cytotoxic effect of mitomycin C in gastric cancer cells by promoting drug-induced apoptosis.43 Out of 17 patients with various tumors, minor responses were observed in 3/6 patients with pancreatic cancer and 1/3 with sarcoma in a pilot clinical trial with Safingol (L-threo-dihydrospingosine).44 Potentiation of 1-β-D-arabinofuranosylcytosine (ara-C) by Safingol or bryostatin 1 by interference with the mitogen-activated protein kinase (MAPK) cascade has also been demonstrated.45 However, none of these PKC inhibitors has an effect on the MDR phenotype.

PKC412, an agent with antitumour activity in vitro and in vivo, offers an attractive combination agent in the treatment of malignancies expressing the MDR phenotype. In addition, its ability to sensitize tumor cells may allow the use of lower concentrations of conventional drugs thus reducing their toxic side effects. In a recent study PKC412 has been shown to sensitize murine cells to ionizing radiation.46 Sensitization is further confirmed in our studies in cells from B-CLL patients treated with oral PKC412 over a period of 2-8 weeks, such that a significant lowering of the in vitro IC50 was observed for chlorambucil and fludarabine compared to pre-treatment samples.47 In a review on PKC targeting, Jarvis and Grant confirmed the accumulating evidence that selective targeting of PKC improves the efficacy of conventional cytotoxic agents.48 The statistical discordance between in vitro and in vivo studies may be attributed to the continuous exposure of B-CLL cells to PKC412 in vivo where a significant reduction in circulating malignant cells was observed (Virchis et al., A novel treatment approach for low grade lymphoprolif-erative disorders using PKC412, a protein kinase C inhibitor, submitted).

Contributions and Acknowledgments

KG and RGW: responsibility for the integrity of work from inception to laboratory analysis, data analysis and data interpretation to submission of manuscript. DTJ, MG, SMH: contributed to a significant proportion of the laboratory work. AEV, HGP, AVH: contributed towards collection and analysis of the clinical data. AM, KC, KCz: contributed towards providing PKC412 data from source and analyzing current data. ABM: contributed towards initial planning of the study and collection of clinical data.

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Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

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Peer Review Outcomes

What is already known on this topic
Inhibitors of protein kinase enzymes involved in cell sig-
naling represent an innovative therapeutic strategy in
leukemia. Synthetic PKC inhibitors have been developed
for clinical use and shown to be active.

What this study adds
Selective inhibition of B-CLL acts through an apoptotic
mechanism which apparently spares normal PB lym-
phocytes.

Manuscript processing
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tion was taken jointly by Professor Lo Coco and the Edi-
tors. Manuscript received August 14, 2001; accepted

Potential implications for clinical practice
Ongoing phase-II clinical trials should better define the
advantages of this naturally occurring agent, particular-
ly in B-CLL cases resistant to chlorambucil and fludara-
bine. Its role as a chemosensitizer is also clinical inter-
est.