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Effects of the kinase inhibitor CGP41251 (PKC 412) on lymphocyte activation and TNF-α production

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Abstract

CGP41251 is a serine/threonine and tyrosine kinase inhibitor that is a novel anticancer agent. Because the kinases that CGP41251 inhibits play important roles in T lymphocyte activation, we hypothesized that this compound may have useful immunomodulatory properties. Here we characterized the in vitro immunomodulatory effects of CGP41251. The effects of CGP41251 on lymphocyte proliferation, expression of T cell activation surface markers, and intracellular calcium response in peripheral blood mononuclear cells (PBMC's) were measured. Intracellular IL-2, TNF-α, IFN-γ expression in CGP41251-treated T cells stimulated by lectin was measured by flow cytometry. CGP41251 inhibited lectin-induced lymphocyte proliferation and upregulation of activation surface markers with a 50% inhibitory concentration (IC_{50}) of 0.1 μM. CGP41251, at micromolar concentrations, blunted the intracellular calcium response during PBMC activation. CGP41251 inhibited TNF-α production by T cells with an IC_{50} of 0.5 μM and did not significantly inhibit the production of IL-2 or IFN-γ. In conclusion, CGP41251 potently inhibits T lymphocyte activation and function and interferes with the proximal part of the T cell activation pathway. The ability of CGP41251 to selectively block T cell TNF-α production warrants the evaluation of this compound on other, e.g., monocyte, immune cells and in immunological conditions that are characterized by high TNF-α levels such as psoriasis and inflammatory bowel diseases.

Keywords: CGP41251; Kinase inhibitor; Lymphocyte activation; TNF-α

1. Introduction

The staurosporine analog CGP41251 was originally described as a small molecule inhibitor of the conventional protein kinase C isoforms α, β, γ and the calcium-independent protein kinase C isoforms δ, ε and η at nanomolar concentrations [1–3]. CGP41251 was shown to reversibly inhibit intracellular protein kinase C activity and downstream activation of the mitogen-activated protein kinase (MAPK) pathway [4–6]. In addition to its inhibitory effects on these protein kinase C isoforms, CGP41251 was also found to inhibit a

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variety of other kinases such as the vascular endothelial growth factor (VEGF) receptor kinase insert domain-containing receptor, c-*Src*, protein kinase A and c-*Syk* at submicromolar concentrations [1–3].

Because of its ability to inhibit proliferative signals mediated by multiple oncogenic kinases, tumor angiogenesis promoted by VEGF, and tumor multidrug resistance mediated by the protein P-glycoprotein, CGP41251 is being developed as an anticancer agent [1,7–9]. Indeed, there have been ample in vitro and preclinical in vivo demonstrations of the ability of CGP41251, alone or in combination with other antineoplastic agents, to inhibit tumor growth [10–13]. Furthermore, results of published Phase I and II studies with CGP41251 have shown that this orally available agent is safe with minimal toxicity and effectively inhibits tumor growth [8,10–14].

While the kinases that CGP41251 inhibits play key roles in carcinogenesis and cellular proliferation, these enzymes are also activated in other cell types. Indeed, kinases such as the conventional isoforms of protein kinase C, c-*Src*, protein kinase A and c-*Syk* are involved in the early events of T cell activation induced by engagement of the T cell receptor (TCR) [15–19]. Furthermore, previous studies have suggested that PKC may be involved in regulating survival of lymphocytes since staurosporine induced apoptosis in lymphocytes [20]. In light of these findings, we hypothesized that CGP41251 could potently suppress T cell activation and function, and thus might be useful in modulating alloimmune and autoimmune responses. This would further support our recent demonstration that specific kinase inhibition may affect T cell activation to the point of affording significant immunosupression in stringent preclinical conditions [21–25]. As a first step to explore this hypothesis, we performed in vitro investigations into the effects of CGP41251 on lymphocyte activation, proliferation and cytokine production.

2. Materials and methods

2.1. Materials, whole blood and peripheral blood mononuclear cells

Heparinized, whole blood and buffy coats from healthy donors obtained from the Stanford University Medical Center Blood Bank were centrifuged on Lymphoprep (Invitrogen, Carlsbad, CA) to obtain peripheral blood mononuclear cells (PBMC's). Experiments were performed on PBMC's isolated from at least 3 different donors. PBMC's were maintained in RPMI 1640 (Invitrogen) supplemented with 10% heat inactivated fetal bovine serum (FBS, Invitrogen), 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 1% nonessential amino acids, 1 mM sodium pyruvate and 100 μM β-mercaptoethanol at 37 °C and 5% CO_2 in humidified air. Media supplements were purchased from Sigma, St. Louis, MO unless indicated otherwise.

A stock solution of CGP41251 was made by diluting in DMSO (Sigma) and storing aliquots at −80 °C and thawing just prior to use. Aliquots of CGP41251 stock solutions were diluted in PBS or RPMI, and the final concentration of DMSO was no more than 0.01% (v/v) in the experiments. The calcium indicator dyes fluo-4/AM and Fura-red/AM (Molecular Probes, Eugene, OR) were diluted in DMSO. Concanavalin A, formalin and brefeldin were purchased from Sigma. Phytohemagglutinin (PHA) was purchased from Calbiochem-Novabiochem Corp. (San Diego, CA). All monoclonal antibodies (mAbs) were purchased from BD Biosciences Pharmigen (San Diego, CA). Components of the lysis and permeabilizing buffers were from Sigma.

2.2. PBMC proliferation assay

Using protocols previously reported in detail by our laboratory [26–28] human PBMC's were incubated with incremental concentrations of CGP41251 obtained by serial dilution of compound stock solution. Preincubation with drug was for 30 min in complete media. PBMC's were then stimulated with PHA (5 μg/ml) and subsequently dispensed into 96 well microtiter plates in quadruplicate fashion at a density of 10^5 cells/well. PBMC's were allowed to proliferate in growth conditions for 60 h, pulsed with 1 μCi/well [^{3}H] thymidine (Amersham Biosciences, Piscataway, NJ) and incubated for 12 more hours before harvesting cells on glass filtermats with a multichannel cell harvester (Wallac LKB Microbeta plus 1450, Turku, Finland). Filtermats were read on a scintillation counter and data recorded as counts per minute (cpm).