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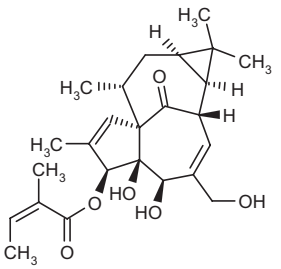
PEP-005

Treatment of Actinic Keratoses
Acute Myeloid Leukemia Therapy
Treatment of Basal Cell Carcinoma
Protein Kinase C Activator

3-Angeloylingenol
3-Ingenyl Angelate

2-Methyl-2(*Z*)-butenoic acid (1a*R*,2*S*,5*R*,5a*S*,6*S*,8a*S*,9*R*,10a*R*)-5,5a-dihydroxy-4-(hydroxymethyl)-1,1,7,9-tetramethyl-11-oxo-1a,2,5,5a,6,9,10,10a-octahydro-1*H*-2,8a-methanocyclopenta[*a*]cyclopropa[*e*]cyclodecen-6-yl ester

Ingenol 3-angelate



$C_{25}H_{34}O_6$
Mol wt: 430.5339

CAS: 075567-37-2

EN: 328987

Abstract

PEP-005 (ingenol 3-angelate) is a diterpene ester isolated from the plant *Euphorbia peplus*, the sap of which has been used to treat skin conditions, including warts, actinic keratoses and certain cancers, in traditional medicine. PEP-005 has topical antitumor activity against human cancer cell lines grown as subcutaneous tumors in mice and against actinic keratoses in humans. PEP-005 also appears to have antileukemic effects, inducing apoptosis in leukemia cell lines, as well as primary acute myeloid leukemia (AML) blasts. PEP-005 is a potent activator of protein kinase C (PKC), a serine threonine kinase consisting of 11 isozymes that differentially regulate a wide range of cellular functions, including differentiation and apoptosis. The antileukemic effects of PEP-005 were shown to be PKC-δ-dependent, with sensitivity of leukemia cell lines correlating with expression of PKC-δ. PKC-δ plays a key role in the regulation of apoptosis and is therefore a rational and novel potential target for anticancer therapies. Topical PEP-005 is in phase II development for the treatment of actinic keratoses *and basal cell carcinoma*, and a phase I trial is planned for an i.v. formulation for the treatment of AML in 2006.

Introduction

Protein kinase C (PKC) is a family of serine/threonine kinases consisting of 11 isozymes that have been grouped into 3 subfamilies depending on their structure and enzymatic properties. The classical PKC isozymes (α, βI, βII, γ) are all calcium-dependent and require the second messenger diacylglycerol (DAG) as a cofactor; the novel PKCs (δ, ε, η, θ) are calcium-independent but require DAG as a cofactor; and the atypical PKCs (ζ, λ/ι, μ) are also calcium-independent and appear to respond to alternative lipid activators such as phosphatidylinositol trisphosphate (1).

PKC enzymes play key regulatory roles in a number of important cellular processes, including proliferation, differentiation and apoptosis (2). In particular, a number of studies have suggested that PKC-δ plays a key role in the regulation of apoptosis and can have either a proapoptotic or an antiapoptotic function depending on the activating factor and the direction of its translocation within the cell (3). In particular, translocation of PKC-δ to the nucleus has been associated with apoptosis. Here it can cause apoptosis by inducing the disassembly of the nuclear lamina (4). PKC-δ has been shown to be activated in neutrophils undergoing spontaneous apoptosis and in various cell types in response to apoptotic stimuli such as H_2O_2, TNF-α, Fas ligation and treatment with chemotherapeutic agents (5). Furthermore, apoptosis in response to some of these stimuli can be inhibited using the PKC-δ-specific inhibitor rottlerin, or a dominant-negative mutant of PKC-δ. It has also been shown that PKC-δ is cleaved and activated by caspase-3 during apoptosis and that overexpression of the catalytic fragment of PKC-δ is sufficient to induce apoptosis in these cells (6). PKC-δ may therefore be involved in the early initiation phases of apoptosis and also in the effector phase.

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Modulators of PKC activity have already undergone clinical trials as potential anticancer agents, although to date these have been broad-spectrum activators of PKC isozymes, able to activate classical and/or novel PKC isozymes. Clinical trials using the PKC-activating phorbol ester phorbol myristate (PMA) indicated a therapeutic effect for this agent in patients with myelocytic leukemia (7). The PKC activator bryostatin 1 has also undergone clinical trials as an anticancer agent, but with mixed results (8). The predominant use of broad-spectrum PKC-modulating agents in clinical trials is partly due to a lack of agents able to selectively target the different PKC isozymes, although agents able to activate (bistratene A) or inhibit (rottlerin) PKC-δ have been isolated from natural sources.

Acute myeloid leukemia (AML) is a malignant disease of the bone marrow characterized by a block in the differentiation of hematopoietic stem cells to cells of the myeloid lineage, leading to an abnormal accumulation of immature precursors. Symptoms of AML include neutropenia, thrombocytopenia and anemia. Improvements in drug therapy and patient care have significantly improved prognosis in younger patients. The risk of AML, however, increases with age and remission rates in the elderly still remain very poor (9). The reason that success rates are lower for elderly patients includes the fact that AML occurring in elderly patients is less responsive to myelosuppressive chemotherapy, and the patients themselves are less tolerant to this form of therapy. In order to overcome these problems, novel adjunctive therapies need to be developed to improve tumor responses while not increasing the toxicity of established chemotherapies.

The plant *Euphorbia peplus*, commonly known as petty spurge, has a long history of use as a traditional remedy for a number of conditions, including warts, actinic keratoses and skin cancer. The active ingredient of the sap from this plant is the hydrophobic diterpene ester ingenol 3-angelate (PEP-005), which is being developed for clinical use by Peplin. A recent study has shown that topical application of PEP-005 cured a series of subcutaneous murine and human tumors established in C57BL/6 and *Foxn1*^{nu} mice (10), and in a phase I trial, PEP-005 was shown to have a clinically relevant impact on actinic keratosis (AK) lesions. AK is a skin lesion which can lead to skin cancer. PEP-005 has also been screened against the NCI panel of 60 different cancer cell lines for its cytostatic properties. This screen revealed potent antiproliferative effects against a number of leukemia cell lines. PEP-005 is currently in phase II clinical trials for the treatment of AK and basal cell carcinoma, and it has just completed preclinical testing for AML.

Pharmacological Actions

Single topical applications of PEP-005 (18 μg) on 3 consecutive days resulted in the elimination 1 month later of subcutaneous murine (B16 melanoma, LK2 squamous cell carcinoma) and human (DO4 melanoma) tumors established in C57BL/6 and *Foxn1*^{nu} mice. Treatment produced a mild but transient eythema and gave an excellent cosmetic outcome. *In vitro* studies with cell lines showed that the LD_{90} for PEP-005 was in the range 180-220 μM and that tumor killing both *in vitro* and *in vivo* involved primarily necrosis of tumor cells (10). There is also a biopsy-proven case of self-treatment of basal cell carcinoma with the sap of *E. peplus* (9).

In vitro studies to evaluate the antileukemic potential of PEP-005 assessed cytotoxicity against 4 leukemia cell lines (HL-60, U-937, NB4 and KG-1a) and blasts isolated from the bone marrow of patients with AML. A range of concentrations of PEP-005 were used (0.2, 2, 20 and 200 nM) and the effect on cell proliferation, differentiation and apoptosis was determined. After 4 days of treatment, PEP-005 caused significant apoptosis of up to 70% of cells in 3 of the 4 cell lines (HL-60, U-937 and NB4), with optimal effects at a concentration of 20 nM. PEP-005 had no effect on KG-1a cells even at concentrations as high as 20 μM. PEP-005 did not induce differentiation of the cell lines towards mature myeloid cells. PEP-005 was shown to activate PKC and analysis of PKC isozyme expression in the leukemia cell lines revealed that the unresponsive line KG-1a expressed very low levels of PKC-δ. Transfection of KG-1a with PKC-δ restored responsiveness to PEP-005, suggesting that expression of this PKC isozyme is required for PEP-005 action and that this may be a good marker for patient selection in clinical trials. Leukemic blasts from patients diagnosed with AML were cultured *in vitro* with a range of concentrations of PEP-005 (0.1, 1, 2, 10 and 20 nM). PEP-005 induced apoptosis (56-95%) in blasts isolated from 7 of 8 patients. The primary cells were more sensitive to PEP-005 than the cell lines and the optimum effective concentration was 2 nM. Importantly, after 48 h of treatment with PEP-005, normal myeloblasts isolated from cord blood and from adult peripheral blood following stem cell mobilization therapy were induced to differentiate, as assessed by loss of CD34, a marker of hematopoietic progenitor cells, but they did not undergo apoptosis and did not display the apoptotic morphology seen in primary AML blasts even at concentrations of PEP-005 as high as 200 nM. These data indicate a potentially broad therapeutic window for PEP-005 that spans at least 2 log concentrations (11).

Pharmacokinetics

Pharmacokinetic data obtained from rats administered PEP-005 daily at doses of 1.5, 7.5 and 15 μg/kg for 28 days showed that both C_{max} and AUC values increased with increasing dose. No apparent drug accumulation or gender differences were observed.

Toxicity

The toxicity of single and repeated i.v. doses of PEP-005 was used to determine the maximum tolerated dose (MTD) and to identify starting doses and doses safe for human exposure. Toxicity evaluations were carried out in

rats, rabbits and minipigs using PEP-005 at doses of 1-30 μg/kg. In rats, the minimum lethal dose for a single i.v. injection was found to be 20 μg/kg and it was in excess of this value for rabbits and pigs. The principal sign was tachypnea and most animals displayed miosis and lethargy. For repeated dosing, animals received i.v. injections for 28 consecutive days at doses of 1.5-15 μg/kg. The agent was well tolerated overall and the main effects, which were dose-dependent, were weight loss, respiratory disturbances and lethargy. The MTD was established in mice given bolus i.v. injections of PEP-005 for 7 consecutive days at 50 μg/kg.

Clinical Studies

In an open-label phase I clinical trial in 16 patients with discrete AK lesions, PEP-005 was shown to have a favorable safety profile. The most common local skin reaction was localized, mild erythema. The patients received a single topical application of 0.01% PEP-005 in gel form and there was a clinically relevant effect on the lesions within 21 days. PEP-005 has now progressed to phase II development for this indication in the U.S. This study will recruit between 13 and 34 patients and will have an open-label, dose-escalation format to determine the MTD for once-daily application or administration on 2 consecutive days with a 4-week follow-up. Secondary objectives of the trial are to evaluate the clinical efficacy of PEP-005 topical gel in clearing AK lesions and to assess any systemic absorption of PEP-005 at the MTD.

A phase II trial is also recruiting patients with basal cell carcinoma to determine the safety of two applications of PEP-005 0.0025%, 0.01% and 0.05% topical gel (12).

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Source

Peplin, Ltd. (AU).

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