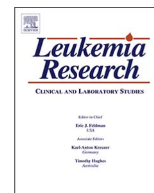
Leukemia Research 62 (2017) 64–69

Contents lists available at ScienceDirect

Leukemia Research

journal homepage: www.elsevier.com/locate/leukres



Research paper

The *in vivo* anti-leukemia activity of N-(1-Pyrenlyl) maleimide in a bioluminescent mouse model

Chi-Yuan Chen[a], Pei-Chi Chang[a], Tong-Hong Wang[a,b], Tzu-Chien V. Wang[c,*]

[a] *Graduate Institute of Health Industry Technology and Research Center for Industry of Human Ecology, College of Human Ecology, Chang Gung University of Science and Technology, Kwei-San, Tao-Yuan 333, Taiwan*

[b] *Tissue Bank, Chang Gung Memorial Hospital, Tao-Yuan, 333, Taiwan*

[c] *Department of Molecular and Cellular Biology, College of Medicine, Chang Gung University, Kwei-San, Tao-Yuan, 333, Taiwan*

ARTICLE INFO

Keywords:
N-(1-Pyrenyl) maleimide
Telomerase inhibitor
Anticancer drug
T-cell acute lymphoblastic leukemia
Bioluminescent mouse model

ABSTRACT

In a search for anticancer drugs by screening for inhibitors of telomerase, we have identified several small-molecule inhibitors that selectively inhibit telomerase in a cell-free system. Among these inhibitors, N-(1-pyrenyl) maleimide (NPM) induced apoptosis and displayed the greatest differential cytotoxicity against acute T cell leukemia-derived Jurkat cells cultured *in vitro*. In this work, the *in vivo* anti-leukemia activity of NPM was investigated using a bioluminescent mouse model. The luciferase–expressing Jurkat cells (Jurkat-Luc) were mixed with matrigel and injected subcutaneously into the nude mice. Drug treatment was commenced on day 7 after tumor implantation. The growth of xenografted tumors was significantly inhibited in the mice treated with NPM, which is comparable to the inhibitory effect of a classical anti-leukemia drug, cyclophosphamide. Combined treatment with NPM and cyclophosphamide further enhanced the growth inhibition of xenografted Jurkat-Luc cells. Immunohistochemistry staining with cleaved caspase 3 (cl-caspase 3) indicated a very heavy staining of cl-caspase 3 only in the tumor implants excised from the NPM-treated mice. We conclude that NPM induced apoptosis and inhibited the growth of xenografted Jurkat-Luc cells in nude mice, demonstrating that NPM displays anti-leukemia activity *in vivo*.

1. Introduction

Telomerase was recognized recently to be a central regulator of all of the hallmarks of cancer and thus becomes a strong strategic focus as a therapeutic target in human cancer [1]. Various telomerase-based therapeutic strategies have been explored in the past, including immunotherapy, hTERT-promoter–based gene therapy, and inhibition of telomerase activity [2,3]. Immunotherapy with hTERT-based vaccines has been extensively investigated and numerous clinical trials studied in the past decade have demonstrated only modest benefits [4]. In the gene therapy based on hTERT-promoter, both cytotoxic gene therapy and oncolytic virotherapy approaches have been employed to yield promising proof of principle data [5,6], yet no advanced clinical trials of this approach have been reported. In the inhibition of telomerase activity approach, although a number of small-molecule telomerase inhibitors have been discovered, none of these compounds has advanced to clinical trials [7]. As of today, the thio-phosphoramidate oligonucleotide inhibitor imetelstat (GRN163L), which targets the RNA template for hTERT, is the only anti-telomerase compound that has been extensively evaluated in clinical trials [8]. The advantages and limitations of these approaches have been reviewed [7,8].

In a search for anticancer drugs by screening for inhibitors of telomerase, we have identified several small-molecule inhibitors [9–11], including maleimide-derived compounds U-73122 and N-(1-Pyrenlyl) maleimide, that selectively inhibit telomerase *in vitro*. Among these inhibitors, NPM displayed the greatest differential cytotoxicity against acute T cell leukemia-derived Jurkat cells [9]. The primary cytotoxic effect of NPM against Jurkat cells manifests by induction of apoptosis, possibly through inhibition of telomerase and potentially modulation of uncharacterized targets. More recently, we have shown that the apoptosis induction by NPM is mediated by the induction of BAK oligomerization and mitochondrial dysfunction [12]. Therefore, despite this compound is a fluorescent reagent that is frequently used as a derivatization agent for the detection of thio-containing compounds [13,14], it is an attractive compound to be explored for its potential application in the treatment of leukemia *in vivo*. In this work, we employed a non-invasive bioluminescent mousemodel of human T-cell acute lymphoblastic leukemia (TALL) [15,16] to investigate the anti-leukemia activity of NPM against TALL *in vivo*.

* Corresponding author.
E-mail address: tcvwg@mail.cgu.edu.tw (T.-C.V. Wang).

http://dx.doi.org/10.1016/j.leukres.2017.09.004
Received 2 June 2017; Received in revised form 22 August 2017; Accepted 10 September 2017
Available online 12 September 2017
0145-2126/ © 2017 Elsevier Ltd. All rights reserved.

2. Materials and methods

2.1. Chemicals, antibodies, media and plasmids

RPMI-1640, fetal bovine serum (FBS), trypsin, penicillin, streptomycin, and amphotericin were from Gibco-BRL. N-(1-pyrenyl) maleimide and other chemicals were from Sigma Chemical Co. The antibiotic G418 was from InvivoGen. The plasmid.pGL4.51 containing the luciferase gene was purchased from Promega. Antibody against caspases 3 was purchased from Cell Signaling Technology.

2.2. Cell culture

Acute T cell leukemia-derived Jurkat cells were cultured in RPMI-1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, and antibiotics (100 U/ml penicillin, 100 U/ml streptomycin and 0.25 μg/ml amphotericin B). The peripheral blood lymphocytes (PBL) were isolated and cultured as previously described [17]. All cells were grown at 37 °C in a humidified incubator containing 5% CO2.

2.3. Construction of luciferase-expressing jurkat cells

The protocol for DNA transfection of unstimulated Jurkat cells followed that suggested by Amaxa Biosystems [18]. In brief, 1×10^6 Jurkat cells were suspended in 0.1 ml of human T cell nucleofector solution (Amaxa Biosystems, VPA-1002), mixed with 2 μg of pGL4.51 plasmid DNA, and transferred into an Amaxa certified cuvette. After placing the cuvette in the holder of Nucleofector™ (Amaxa, AAD-1001), the cells were electroporated using program V-24. The electroporated cells were quickly placed in supplemented RPMI 1640 medium containing 1 mg/ml G418 and cultured for two weeks to obtain stably transfected Jurkat cells (Jurkat-Luc) that express luciferase.

2.4. Assay for viability

Viability of cells was determined by staining the cells with trypan blue and/or MTS assay using a CellTiter 96^R AQ_{ueous} One Solution Cell Prliferation Assay kit (Promega), following the manufacturer's suggestions.

2.5. Assay for luciferase

The activity of luciferase was measured by the luciferase assay reagent (Bright-Glo™ Luciferase Assay System; Promega) following the manufacturer's instructions.

2.6. In vitro bioluminescence imaging

Serial dilutions of Jurkat-Luc cells ranging from 1×10^6 to 1×10^7 cells per well were plated in 100 μl in 96-well culture plates. Wells containing medium only were used to detect background fluorescence. Luciferase assay reagent (Bright-Glo™ Luciferase Assay System; Promega) 100 μl was added to medium immediately before bioluminescence imaging. Photon counts per second were recorded using an IVIS100 (Xenogen, Alameda, CA) imaging system.

2.7. A bioluminescent mouse model for human T-cell leukemia/lymphoma

The *in vivo* antitumor activity of NPM and cyclophosphoamide against human T-cell leukemia was studied using male nude mice, BALB/c nu/nu (supplied by the National Laboratory Animal Center, Taipei, Taiwan). The Jurkat-Luc cells (4×10^7 cells in 50 μl medium) were mixed with 50 μl matrigel and then injected subcutaneously into the nude mice. The luciferase-based bioluminescence imaging of xenografted tumors was found to be readily detected in all mice after 7 days of tumor implantation. Therefore, xenografted mice were randomly divided into groups of six (n = 6) on day 7 after tumor implantation and drug treatment was commenced. NPM treatment (4 mg per Kg of mice) was administered intraperitoneally three times per week whereas cyclophosphamide treatment (30 mg per Kg of mice) was administered intraperitoneally twice per week. The control group received injection of phosphate buffered saline (PBS) three times per week.

Luciferase-based bioluminescence imaging was performed with an IVIS100 imaging system equipped with a camera box and warming

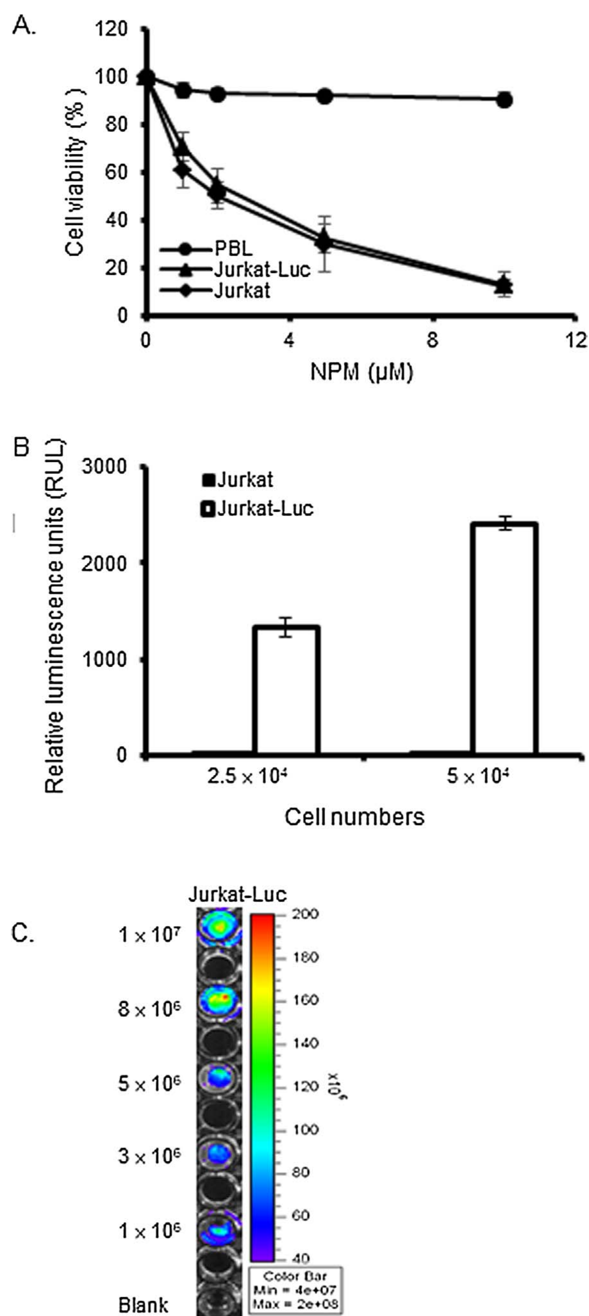


Fig. 1. Luciferase-expression and NPM-sensitivity of Jurkat-Luc cells.
(A) Sensitivity of Jurkat and Jurkat-Luc cells to NPM treatment. Cells were treated with different concentrations of NPM for 24 h. Viability of the treated cells were assayed by trypane blue method. The peripheral blood lymphocytes (PBL) served as the control for normal cells. The data shown are the means ± SD from three independent experiments. (B) The Jurkat and Jurkat-Luc cells were assayed for the expression of luciferase activity using Bright-Glo™ Luciferase Assay System of Promega. (C) The expression of luciferase activity was monitored by bioluminescence imaging of Jurkat-Luc cells using an IVIS100 imaging system.

Download English Version:

https://daneshyari.com/en/article/5527700

Download Persian Version:

https://daneshyari.com/article/5527700

Daneshyari.com