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Anti-cancer effect of clofazimine as a single agent and in combination with cisplatin on U266 multiple myeloma cell line

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ABSTRACT

Multiple Myeloma (MM) is a malignant neoplasm of bone marrow plasma B cells with high morbidity. Clofazimine (CLF) is an FDA-approved leprostatic, anti-tuberculosis, and anti-inflammatory drug that was previously shown to have growth suppression effect on various cancer types such as hepatocellular, lung, cervix, esophageal, colon, and breast cancer as well as melanoma, neuroblastoma, and leukemia. The objective of this study was to evaluate the anticancer effect and mechanism of CLF on U266 MM cell line. CLF (10 μM, 24 h) treatment resulted up to 72% growth suppression on a panel of hematological cell lines. Dose-response study conducted on U266 MM cell line revealed an IC_{50} value of 9.8 ± 0.7 μM. CLF also showed a synergistic inhibition effect in combination with cisplatin. In mechanistic assays, CLF treatment caused mitochondrial membrane depolarization, change in cell membrane asymmetry and increase in caspase-3 activity; indicating to an intrinsic apoptosis mechanism. This study provides new evidence for the anticancer effect of CLF on U266 cell line. Further *in vivo* and clinical studies are warranted to evaluate its therapeutic potential for MM treatment.

1. Introduction

Multiple Myeloma (MM) is a malignant neoplasm of bone marrow plasma B cells. It accounts for 1% of all types of cancers and 13% of all hematologic malignancies [1]. Approximately 86,000 new worldwide cases of MM occur annually. In the past decade, one of the major advances in the management of MM has been the introduction of novel agents, bortezomib, thalidomide, and lenalidomide, as a part of the frontline treatment. These drugs have markedly improved the rate of complete response without increasing toxicity substantially [1–5]. The survival rate in MM, which previously ranged from 3 to 5 years, now exceeds 10 years as a result of the advent of high-dose therapy with novel chemotherapeutic agents in combination with autologous stem cell transplantation [6]. However, the prognosis is variable and MM is still a disease with high morbidity and mortality [7].

Clofazimine (CLF) is a riminophenazine agent and an FDA-approved leprostatic, anti-tuberculosis, and anti-inflammatory drug listed in the World Health Organization Model List of Essential Medicines. Since the 1960s, broad-range anti-bacterial and strong immunosuppressive effects of CLF have been shown in various cases. Activation of T lymphocytes plays a major role in the immunomodulatory action of CLF [8] and it is used as a general anti-inflammatory agent, especially for infectious skin diseases [9].

Since 1998, *in vitro* and *in vivo* anticancer effects of CLF have also been studied on a large variety of cancer types such as hepatocellular, lung, cervix, esophageal, colon, and breast cancers as well as melanoma, leukemia, and neuroblastoma. CLF has been shown to reverse multidrug-resistance (MDR) in various cell lines [10–15], have tumoricidal properties by causing cell membrane lysophospholipid content increase [15], inhibit respiratory function and energy metabolism in yeast and transformed fibroblasts, inhibit tumor energy metabolism of a chemoresistant human non-small-cell bronchial carcinoma cell line [16], inhibit both respiratory function and tumor energy metabolism, activate the intrinsic apoptotic pathway in a Kv1.3-dependent and Bax/Bak-independent way

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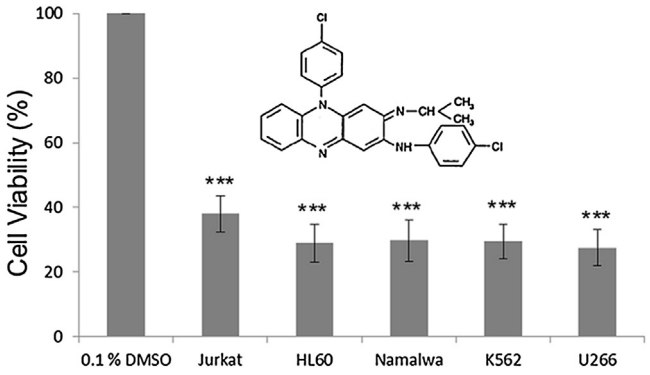

Fig. 1. Effect of 10 μM CLF (structure shown) on the viability of five hematological cell lines (Jurkat, U266, Namalwa, K562, and HL60) after 24 h. Asterisks denote statistical significance at $p < 0.001$ ($n = 4$).

[17,18], cause poly (ADPribose) polymerase (PARP) cleavage [17], trigger phospholipase A2-mediated oxidative and nonoxidative mechanisms [15], and finally inhibit the Wnt signaling pathway [19]. It was also used in two different phase II clinical studies and in both cases, it was well tolerated and had minimal toxicity, leading to the conclusion that it has therapeutic potential for the treatment of hepatocellular carcinoma [11,12].

Based on its previously proven promising anticancer potential, we aimed to evaluate the anticancer potency and mechanism of CLF on U266 MM cell line.

2. Materials and methods

2.1. Chemicals

Clofazimine (C8895) and Cisplatin (C2210000) were purchased from Sigma-Aldrich (St. Louis, MO, USA). CLF (10 mM stock solution) was prepared in 0.2% dimethyl sulphoxide (DMSO) or 100% EtOH (in combination studies) and stored at −20 °C. Cisplatin (1 mM stock solution) was prepared in 0.9% NaCl and stored at room temperature.

2.2. Cell culture

HL60 (acute promyelocytic leukemia), Namalwa (Burkitt lymphoma), K562 (chronic myelogenous leukemia), Jurkat (T-cell leukemia), and U266 (multiple myeloma) cells were kindly provided by Prof. Yusuf Baran (Department of Molecular Biology and Genetics, İzmir Institute of Technology, İzmir, Turkey). All cells were cultured in RPMI-1640 growth medium containing 10% fetal bovine serum and 1% penicillin–streptomycin at 37 °C in a 5% CO_2 incubator.

2.3. Cell viability assays

Toxicity, potency (IC_{50}) determination, time-response and combination assays were done using the CellTiter Blue Cell Viability Assay (Promega, Madison, WI, USA). For each sample, five technical replicates were used. Cells were treated at a density of 1,000,000

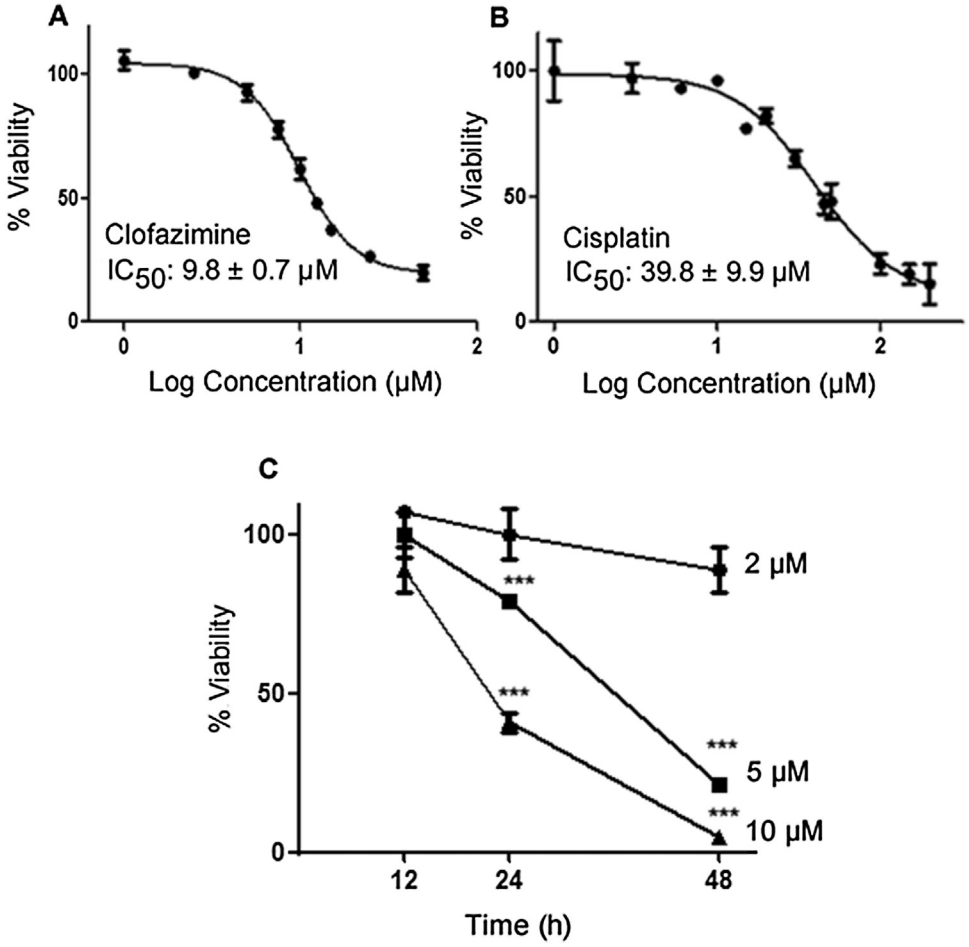

Fig. 2. Dose and time response of CLF treatment on U266 cells. IC_{50} determination of (A) CLF and (B) cis. (C) Time-response of CLF treatment (2, 5, and 10 μM) at 12, 24, and 48 h, respectively. Asterisks denote statistical significance at $p < 0.001$ ($n = 3$).