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PaCaHa inhibits proliferation of human cancer cells in vitro

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Background/aim: The aim of this study was to investigate the antiproliferative and cytotoxic effects of a newly synthesized molecule named paracetamol acetohydroxamic acid (PaCaHa) on human neoplastic cell lines.

Materials and methods: A549, CRL 2923, HeLa, and ARPE were treated with various concentrations of PaCaHa and DMSO (vehicle control). The cytotoxic/cytostatic effects of PaCaHa were determined after a 24-h incubation period and compared to the DMSO control. The cytotoxic and antiproliferative effects were determined by the trypan blue dye exclusion and MTT methods.

Results: A higher susceptibility to PaCaHa was found in CRL 2923 and HeLa cells, while A549 and ARPE cells were less responsive to PaCaHa. The percent of cytotoxicity resulting from 400 µg/mL of PaCaHa were >90 for CRL-2923 and HeLa, 68 for A549, and 64 for ARPE cells. The cytotoxic difference between CRL-2923/HeLa and ARPE/A549 cells was significant ($P < 0.05$).

Conclusion: PaCaHa showed dose dependent cytotoxic and antiproliferative effects on three distinct human cancer cell lines. The differential effect of PaCaHa on different cancer cell lines suggests that PaCaHa could have a potential antitumor effect on specific cancer types. These results support further comprehensive studies on PaCaHa and its derivatives.

Key words: Paracetamol acetohydroxamic acid, cancer, MTT, CRL-2923, ARPE-19, A549, HeLa

1. Introduction

Uncontrolled proliferation of cells, resulting from genetic and epigenetic changes in DNA repair and apoptosis, leads to cancer (1,2). Cancer is currently the second leading cause of death after cardiovascular diseases in the world. According to the 2008 World Health Organization (WHO) cancer report, cancer is responsible for 7.6 million deaths each year. This report also estimated that worldwide deaths are likely to rise to over 11 million in 2030 (3). These statistics emphasize the need for new treatment strategies. Currently, the mortality and morbidity rates of cancer remain high despite advances in surgical, chemical, radiation and hormone therapies, with associated adverse reactions and resistance (4).

All these factors have led scientists to continue to develop alternative cancer treatment strategies (5). In this context, a number of synthetic compounds and natural products were evaluated for their efficacy. Paracetamol acetohydroxamic acid (PaCaHa) was selected for synthesis based on its similarity to compounds that inhibit cell growth (6,7). In the present study, we examined the in vitro cytotoxic effect of PaCaHa on human cancer cells (HeLa,

CRL-2923, and A549) and human diploid cells (ARPE-19) by using thiazolyl blue tetrazolium bromide (MTT) and trypan blue dye exclusion methods.

2. Materials and methods

2.1. Drug

PaCaHa was synthesized and kindly provided by Prof Dr Fatih Yılmaz (Recep Tayyip Erdoğan University, Rize, Turkey). The chemical structure of PaCaHa is shown in Figure 1.

2.2. Cell lines and cell culture

The adenocarcinomic human alveolar basal epithelial cell line (A549) and human cervical cancer epithelial cell line (HeLa) were kindly provided by Prof Dr Fikrettin Şahin (Yeditepe University, İstanbul, Turkey); the human endometrial adenocarcinoma cell line (CRL 2923) was a gift from Prof Dr Bedia Ağaçhan Çakmakoglu (İstanbul University, İstanbul, Turkey); and the diploid ARPE-19 retinal pigment epithelial cell line was kindly provided by Dr Muradiye Acar (Turgut Özal University, Ankara, Turkey). Dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-

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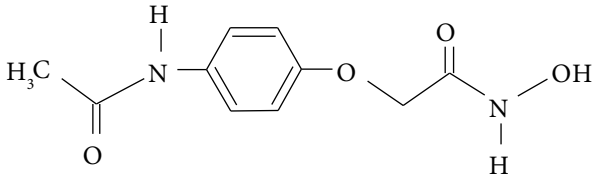


Figure 1. The chemical structure of PaCaHa.

2-yl)-2,5-diphenyltetrazolium bromide (MTT), taxol, D-PBS, and trypan blue (0.4%) were obtained from Sigma. Cell lines were maintained in RPMI-1640 (Hyclone) or DMEM (Gibco) with 10% fetal bovine serum (FBS) (Gibco) and antibiotics (100 µg/mL streptomycin + 100 U/mL penicillin) (Gibco) in T25 flasks at 37 °C in 5% CO₂. Confluent cells were detached using 0.25% trypsin-EDTA (Gibco) solution for serial passage. Cells were used in cytotoxicity assays as stated below.

2.3. Morphological studies and viability test

Stock solutions (50 mg/mL) of PaCaHa, made in DMSO (Sigma), were dissolved in a medium in order to generate working concentrations. A549 (1 × 10⁴ cells/well) and ARPE cells (5 × 10⁴ cells/well) were seeded into 24-well culture plates in 100 µL of growth medium in triplicate. After overnight incubation, various concentrations of PaCaHa (400, 200, 100, 50, and 25 µg/mL) or solely of the corresponding DMSO (max. 1.6%), were added to the wells. Taxol (5 nM) was used as a positive control. The cultures were maintained at 37 °C for 24 h. Then the cells were collected by trypsinization and the viable cells were counted by trypan blue exclusion with a hemocytometer. The percentage of viable cells was calculated using the following formula: [(Total cells counts – Dead cells counts)/Total cells counts] × 100. Cellular morphology was examined under an inverted light microscope with a 10× objective (Olympus).

2.4. Cytotoxic studies using the MTT assay

This assay was performed according to a slight modification of the procedure as reported by Mossmann (8). It was determined that MTT assay depended on the mitochondrial enzyme reduction of tetrazolium dye in determining cell viability. Briefly, 1 × 10⁴ cells/well were seeded into 96-well microtiter plates in 100 µL of growth medium in triplicate and allowed to adhere overnight. The next day, different concentrations of PaCaHa (400, 200, 100, 50, 25, and 12.5 µg/mL) or solely of the corresponding DMSO were added into the cells. After a 24-h incubation period, 10 µL of filter sterilized MTT (Sigma) solution (5 mg/mL in water) was added to each well and the cells were incubated for additional 4 h. After the medium was removed, formazan crystals formed in viable cells during the MTT treatment and these were dissolved by adding

100 µL of DMSO/well. The plates were then further incubated at 37 °C for another 20 min and the absorbance was measured at 570 nm using the ELISA microplate reader (Thermo, Multiskan GO) (8). All experiments were performed three times in triplicate.

2.5. Statistical analysis

Growth inhibition was calculated in terms of percentage by the formula: [(absorbance of control well – absorbance of sample well)/absorbance of control well] × 100. The results were analyzed using an independent sample t-test or unpaired t-test. The results were considered significant if the P value was lower than 0.05.

3. Results

3.1. Morphological effect

The cells used in this study were morphologically plastic adherent and exhibited a fibroblast appearance. To verify the effect of PaCaHa on cell morphology, A549, CRL-2923, HeLa, and ARPE-19 cells were exposed to different concentrations. The morphological features of A549, CRL-2923, HeLa, and ARPE-19 cells were examined under an inverted microscope after 24-h incubation with PaCaHa (400, 200, 100 µg/mL), taxol, or DMSO. We observed that morphological changes were directly proportional to PaCaHa concentration, as shown in Figure 2. No morphological changes were observed in cells treated with DMSO alone; however, those treated with taxol (5 nM) and PaCaHa (400 µg/mL) were rounded, detached from the surface, and in some cases showed small vacuoles in their cytoplasm (data not shown). In contrast, PaCaHa and taxol (used at the same concentration) had less of an effect on the morphology of the diploid ARPE cells (Figure 2). These data suggested that PaCaHa has potential anticancer activity on human cancer cells.

3.2. Viability results

To evaluate the viability of A549 and ARPE-19 more closely, we further tested them against five different concentrations of PaCaHa and DMSO. After 24-h incubation, the percentages of viable cells were determined by trypan blue dye exclusion (Table). The effect of PaCaHa on A549 and ARPE cells was dose-dependent. At 400 µg/mL, only 16% of A549 cells remained viable compared with 73.6% of ARPE-19 cells. Viability increased to 64% for A549 cells and 94.6% for ARPE-19 cells at 200 µg/mL (Table). We further observed that 100 µg/mL of PaCaHa resulted in 86% and 96% increase in viability of A549 and ARPE cells, respectively, indicating a gradual increase in cell viability. The presented data clearly indicate that PaCaHa has higher rates of growth inhibition effects on A549 cancer cells than on the control ARPE-19 cells.

3.3. Cytotoxic effect

In order to investigate the effects of PaCaHa on human cancer cell lines, PaCaHa, with five different concentrations

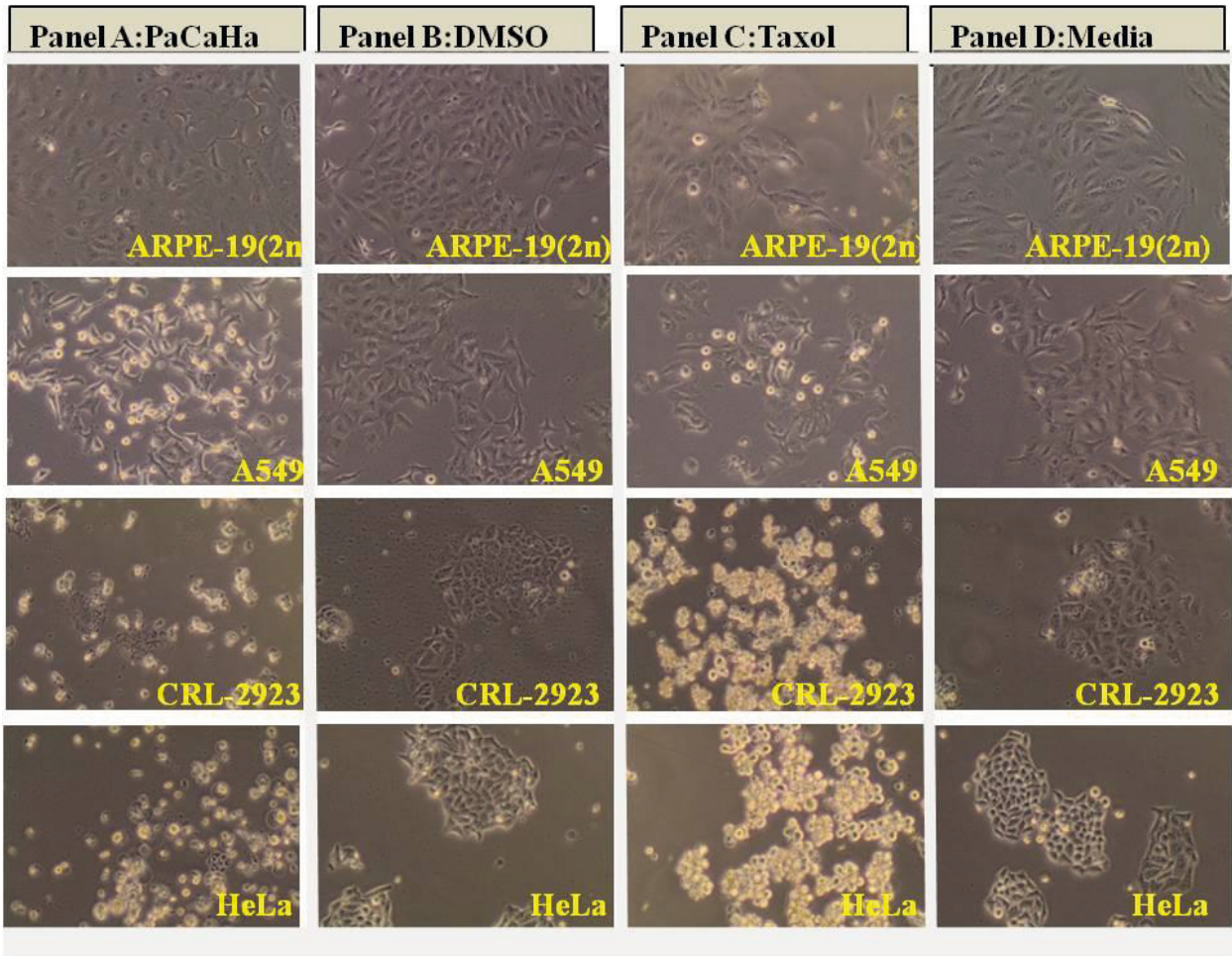


Figure 2. Cytotoxic effects of PaCaHa on human cancer and diploid cell lines. Cells were treated with PaCaHa, taxol, or DMSO for 24 h. Morphological changes were imaged by inverted microscopy. Panel A: PaCaHa (400 µg/mL), Panel B: DMSO (0.8%), Panel C: Taxol (5 nM), Panel D: Media alone.

Table. Antiproliferative effects of PaCaHa on A549 and ARPE-19 cells. Cells were treated with PaCaHa or DMSO for 24 h. Cell viability was calculated by trypan blue exclusion test.

Viability (%)				
	A549 cell lines		ARPE cell lines	
Concentrations	PaCaHa	DMSO	PaCaHa	DMSO
400 µg/mL	16	89.9	73.6	95.5
200 µg/mL	64	90	94.6	95.7
100 µg/mL	86	92	96	93.3
50 µg/mL	89.6	92.8	94.7	92.8
25 µg/mL	85	94	93.6	98

(between 12.5 and 400 µg/mL), and DMSO (control) alone were added to CRL-2923, HeLa, A549, and ARPE-19. Once added, cytotoxicity was performed using MTT assay.

As shown in Figure 3, we found that PaCaHa was cytotoxic in a dose-dependent manner and induced significant cell death in CRL-2923 and HeLa cell lines. It was determined

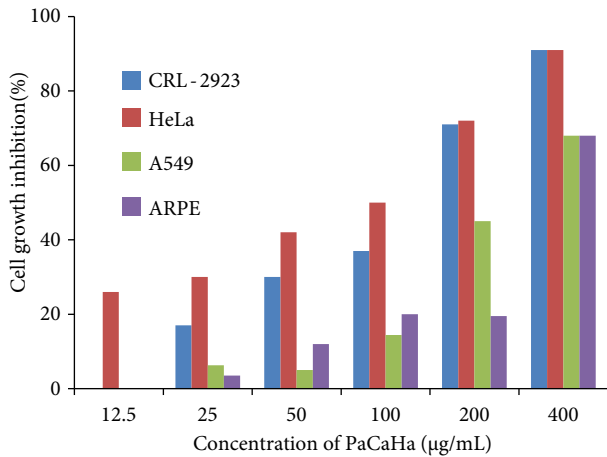


Figure 3. Cytotoxic effects of PaCaHa on human cancer and diploid cell lines. Cells were seeded on 96-well culture plates and treated with PaCaHa, taxol, or DMSO for 24 h. Cell growth inhibition was determined by MTT assay.

that 400 µg/mL PaCaHa was highly cytotoxic and led to >90% cell death in CRL-2923 and HeLa cells ($P < 0.05$). However, the same concentration of PaCaHa killed only 68% of A549 cancer cells. We also observed that the cytotoxic effects of PaCaHa at a concentration of 400 and 200 µg/mL on ARPE-19 cells were 64% and 19.5%, respectively. We further showed that there was a gradual decrease in cell cytotoxicity as the concentration of PaCaHa decreased (200, 100, 50, 25, and 12.5 µg/mL) with all three different cancer cell lines used in this study (Figure 3).

In terms of cytotoxicity, the ARPE-19 cells were significantly less sensitive than CRL-2923 and HeLa cells ($P = 0.025$ and $P = 0.05$, respectively) to PaCaHa, while the difference between ARPE-19 and A549 was not significant ($P = 0.517$). These results indicate that PaCaHa is more effective against cancer cells than normal cells and has a selective impact on different types of cancer cells (Figure 3).

4. Discussion

The 2008 World Health Organization (WHO) cancer report estimated that worldwide deaths are likely to rise to over 11 million in 2030; therefore, new, safe, and effective anticancer agents are needed (5). In recent years, the anticancer effects of synthetic chemicals and natural extracts of plants have gained prominence (9). In the past

few years, different varieties of compounds with cytotoxic and antiproliferative effects on different cancers have been identified (10–12). The most desired feature of an anticancer agent is for it to specifically target cancer cells with minimal or no toxicity in normal cells. There has been a continuous effort in the medical field to suppress the growth of cancer. The goal of the present study was to assess the cytotoxicity of a newly synthesized molecule PaCaHa in human neoplastic cell lines. The cytotoxic effects of PaCaHa were demonstrated by MTT and trypan blue exclusion assays. The trypan blue experiment showed that approximately 16% of A549 cells, with a concentration of 400 µg/mL of PaCaHa, survived after treatment, whereas 74% of the viable cells in the control group survived (ARPE) (Table). In the MTT assay, three cancer cell lines and one normal cell line were used to assess the cytotoxic potential of PaCaHa. The results showed that PaCaHa had significant cytotoxic effects on HeLa and CRL-2923 cancer cells compared with control ARPE cells ($P < 0.05$) (Figure 3). Interestingly, the observations also revealed the effects of PaCaHa to be more prominent in HeLa and CRL-2923 cells compared with A549 cancer cells (Figure 3). This selective effect on different cancer cells (HeLa and CRL-2923 vs. A549) suggests that this compound has potential antitumor effects. The specific mechanisms of the PaCaHa reaction responsible for its cytotoxic and antiproliferative activity are unknown, but there are similar compounds that exert similar effects by altering cell proliferation, cell differentiation, and gene expression (13). Additional PaCaHa derivatives will be tested to define the structural basis for its activity and selectivity against cancer cells, as well as its viability as an antitumor agent.

In conclusion, the overall findings of this study indicate that PaCaHa (12.5–400 µg/mL) increased cell cytotoxicity in a dose-dependent manner and caused selective cytotoxicity in specific cancer cells.

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