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In vitro evaluation of cannabidiol effects on cisplatin response in primary and metastatic human oral cancer cell models

Avaliação *in vitro* dos efeitos do canabidiol na resposta à cisplatina em modelos de células humanas de câncer oral primárias e metastáticas.

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Abstract
Introduction: *oral cancer ranks among the most aggressive and treatment-resistant neoplasms in humans. Although cisplatin-based chemotherapy remains one of the main therapeutic pillars, its efficacy is often compromised by the development of chemoresistance. Cannabidiol (CBD), a non-psychoactive phytocannabinoid derived from Cannabis sativa, has shown the ability to modulate several cellular pathways and enhance the activity of antineoplastic agents.* ***Objective:*** *to evaluate the effects of CBD on the response to cisplatin in cellular models of oral cancer.* ***Methodology:*** *this study utilised primary (HN4) and metastatic (HN12) human oral cancer cell lines. Cell viability, clonogenic growth, and migration were assessed following single or combined treatments.* ***Results:*** *CBD significantly enhanced cisplatin-induced cytotoxicity, leading to marked reductions in cell viability, colony-forming ability, and migration compared to cisplatin treatment alone.* ***Conclusion:*** *altogether, our results indicate that CBD may act as a promising adjuvant to sensitise resistant tumours and reduce aggressive features of oral cancer, supporting its potential use in combination with conventional therapies.*
Keywords: Cancer; CBD; cisplatin; chemoresistance; primary and metastatic cell models.

Resumo
Introdução*: O câncer oral está entre as neoplasias mais agressivas e de difícil tratamento em humanos. Embora a quimioterapia à base de cisplatina represente um dos principais pilares terapêuticos, sua eficácia é frequentemente comprometida pelo desenvolvimento de quimiorresistência. O canabidiol (CBD), um fitocanabinoide não psicoativo derivado da Cannabis sativa, tem demonstrado capacidade de modular diversas vias celulares e potencializar a ação de agentes antineoplásicos.* **Objetivo***: Avaliar os efeitos do CBD na resposta à cisplatina em modelos celulares de câncer oral.*
Metodologia*: Foram utilizadas linhagens celulares de câncer oral humano primário (HN4) e metastático (HN12). Foram avaliados a viabilidade celular, o crescimento clonogênico e a migração celular após tratamentos isolados ou combinados com CBD e cisplatina.*
Resultados*: Observou-se que o CBD intensificou significativamente a citotoxicidade induzida pela cisplatina, promovendo reduções expressivas na viabilidade celular, na capacidade de formação de colônias e na migração celular, quando comparado ao tratamento com cisplatina isoladamente.* **Conclusão***: Em conjunto, os achados indicam que o CBD pode atuar como um adjuvante promissor na sensibilização de tumores resistentes e na redução de características agressivas do câncer oral, reforçando o potencial de sua combinação com terapias convencionais.*
Palavras-chave: *Câncer; CBD; cisplatina; quimiorresistência; modelos celulares primário e metastático.*

INTRODUCTION

Oral cancer (OC), a subtype of head and neck squamous cell carcinoma (HNSCC), represents a major global health burden, characterised by distinctive anatomical and pathological features[1]. It primarily arises in the squamous epithelial cells lining the oral cavity and is closely associated with modifiable risk factors, including tobacco use, alcohol consumption, and human papillomavirus (HPV) infection[2]. The development of OC is often multifactorial, involving complex interactions between genetic predisposition, environmental exposures, and dysregulated immune responses[3] . Importantly, OC accounts for over 377,000 new cases and 177,000 deaths annually worldwide, ranking among the top three malignancies in South-Central Asia, where

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high-risk habits such as tobacco and betel quid chewing are common[4].

Clinically, OC often manifests as ulcerative or exophytic lesions, which can be easily overlooked or mistaken for benign conditions, highlighting the importance of careful clinical examination and early detection[1]. Despite significant advances in cancer therapies, early identification of precancerous lesions and accurate staging of oral malignancies remain critical for achieving optimal prognosis and initiating timely treatment, which may include surgery, radiation therapy, chemotherapy, or combinations thereof [2,5]. Nevertheless, the overall 5-year survival (OS) rate for OC remains around 50%, accompanied by high rates of recurrence and metastasis[6].

On the other hand, the OC treatment, which typically involves platinum-based regimens such as cisplatin, often faces resistance, leading to treatment failure, disease recurrence, and poorer prognosis. This resistance can arise through multiple mechanisms, including interactions with DNA repair enzymes, dysregulation of drug influx and efflux transporters, alterations in DNA repair pathways, changes in apoptotic signalling, activation of anti-apoptotic pathways, modulation of survival regulators, and variations in tumour heterogeneity[7-9]. Moreover, emerging evidence suggests that additional factors, such as cancer stem cells, tumour-associated stromal cells, and inflammatory mediators, may also contribute to cisplatin resistance and tumour recurrence, highlighting an area of ongoing investigation[10,11].

Furthermore, a comprehensive understanding of the molecular determinants of cisplatin resistance in OC is crucial for developing therapeutic strategies aimed at overcoming drug resistance, restoring chemosensitivity, and ultimately improving patient outcomes. Notably, alternative approaches such as combination therapies, targeted agents, immunotherapies, and nanoparticle-based drug delivery systems have emerged as promising strategies to counteract cisplatin resistance and enhance treatment efficacy[12,13] . Additionally, the implementation of predictive biomarkers, functional genomics, and systems biology approaches can help identify patient-specific determinants of cisplatin sensitivity, thereby advancing the field of precision oncology[14].

To address this knowledge gap, the therapeutic potential of cannabidiol (CBD), a non-psychoactive compound derived from Cannabis sativa, has been extensively explored. These approaches have attracted considerable interest in cancer research due to their ability to enhance chemosensitivity and improve treatment responses in patients[15]. Molecular studies investigating the chemo-potentiating effects of CBD have revealed multiple mechanisms, including the inhibition of drug efflux pumps, modulation of apoptotic signalling pathways, induction of oxidative stress, disruption of DNA repair processes, and alteration of tumour microenvironmental factors[16-19]. Importantly, CBD has also been reported to alleviate chemotherapy-induced adverse effects, such as nausea, vomiting, neuropathic pain, and loss of lean body mass, thereby potentially improving quality of life and treatment tolerability for patients undergoing cancer therapy [20,21].

Preclinical studies have documented a range of pharmacological effects of CBD, including anti-inflammatory activity, anti-proliferative and pro-apoptotic properties, and modulation of angiogenic factors[19,22-24] . Moreover, recent evidence suggests that CBD can enhance the efficacy of various chemotherapeutic agents, including cisplatin, doxorubicin, paclitaxel, and temozolomide, across a wide range of in vitro and in vivo models. Of note, these effects have been observed in multiple cancer types, including breast, lung, colon, prostate, and glioblastoma[16,25-27].

However, while CBD has demonstrated anticancer effects in various malignancies through mechanisms such as induction of apoptosis and inhibition of cell proliferation[28] , emerging evidence highlights its specific potential in OC. Recent in vitro studies show that CBD exerts potent dose – and time-dependent antitumor effects in OC cells, significantly reducing cell viability, inducing G0-G1 phase arrest, triggering apoptosis, and promoting DNA damage[29] . These effects are particularly noteworthy because CBD can modulate the endocannabinoid system, a pathway frequently dysregulated in oral carcinogenesis [30]. In addition, the compound's capacity to alleviate cancer-related pain and mitigate treatment-associated side effects further enhances its therapeutic promise, especially in OC, where such comorbidities substantially impact patient quality of life[31].

In this connection, the combined use of CBD with the conventional chemotherapeutic agent cisplatin aims to enhance treatment efficacy and potentially reduce the severity of side effects associated with therapy. Despite this promise, research in this area remains in its early stages, and additional clinical studies are required to establish the safety and effectiveness of such combinations, particularly in the context of OC.

In this study, for the first time, we pursued two main objectives: first, to investigate the cellular effects of CBD, and second, to assess its impact on chemoresistance through cell viability, clonogenic growth, and migration assays, especially when used in combination with cisplatin in human OC cells. To address these aims, experiments were conducted using two cell lines derived from primary (HN4) and metastatic (HN12) OC tumours, thereby encompassing different aspects of OC progression [32,33] .

METHODOLOGY

Cell lines and culture conditions

In this study, we used HN4 cells, originating from a primary OC of the tongue, HN12 cells, derived from a lymph node metastasis in the same patient [32,33], and the normal lung fibroblast cell line (MRC-5), from a human male, purchased from the American Type Culture Collection (ATCC), cat. Nº CCL-171. All cells were maintained in DMEM/F12 medium (Gibco™, Thermo Fisher®, Carlsbad, CA, USA),

supplemented with 10% Fetal Bovine Serum (FBS), 100U/ml penicillin, 100 µg/ml streptomycin, and kept in a humidified atmosphere containing 5% CO_2 at 37ºC.

Drugs and chemotherapeutic agents

The broad-spectrum CBD oil used in this study was generously provided by Thriftmaster Holding Group (TX, USA). The CBD formulation was identical to that described in our previous work[18,34] , with a purity exceeding 99%. For the experiments, CBD was directly added to the culture medium at the specified concentrations. On the other hand, cisplatin was purchased from Sigma-Aldrich [CAS No. 15663-27-1] and was dissolved in PBS (1 mg/mL stock) and further diluted in culture medium immediately before use, as recommended for stability and bioavailability. All experiments included matched vehicle controls (culture medium plus equivalent PBS volumes), confirming that there were no nonspecific effects on cell viability or morphology.

Cell viability assay

Viable cells (HN4, HN12, and MRC-5) were evaluated using AlamarBlue Cell Viability Assay Reagent (G-Biosciences, St. Louis, MO, USA). Briefly, the cells were seeded on 96-well plates (6 × 10^3 cells/well, CORNING) for 24 h at 37 °C and subsequently replenished with fresh medium with various concentrations of CBD (5µM, 10 µM, 15 µM, 20 µM) for 24-72h to MRC-5 cells and cisplatin (10µM, 20 µM, 30 µM, 40 µM, 50 µM, 60 µM and 70 µM) for 48h to HN4 and HN12 cells. Next, the results were obtained using a SpectraMax L Microplate Reader (fluorescence gain at 530 and 570 nm). The results represent the mean of three independent tests, each with three replicates for each cisplatin/CBD concentration. The half-maximal inhibitory concentration (IC_{50}) was determined as the concentration of cisplatin or CBD required to reduce cell growth by 50%. Dose-response curves were generated using GraphPad Prism 8 (GraphPad Software, La Jolla, CA, USA), applying a nonlinear regression model (log(inhibitor) vs. response – variable slope, four parameters). The model provided an excellent fit to the experimental data, with coefficient of determination (R^2) values consistently above 0.90 for all curves.

Colony formation assay

1 × 10^4 (HN4 and HN12) cells were plated into the 24-well (CORNING) culture plates. After 24h, the HN4 and HN12 cells were first treated with various concentrations of cisplatin alone (5µM, 10 µM, 15 µM, 20 µM, 25 µM, 30 µM, 35 µM, 40 µM, 45 µM and 50 µM) for 48h. Additionally, we treated HN4 and HN12 cells with the same concentrations of cisplatin and CBD at 2, 5, and 10 µM/ml for 48 hours. They remained in culture for ten days. Next, the colonies were fixed in a methanol-acetic acid (3:1) solution for 15 minutes, washed with PBS, and stained with a 0.1% crystal violet solution. The colonies were photographed. Quantification of cell colonies was done using ImageJ software. The colonies were calculated and normalised to the number of colonies in the untreated control group. The results were shown as the mean from each condition analysed in triplicate.

Cell migration by the wound healing assay

Cell migration was determined using a wound healing assay. Briefly, HN4 and HN12 cells were cultured under the same conditions previously described in 6-well plates (Corning) until they reached 90% confluence. Wounds were generated by scratching confluent cell monolayers with a sterile 200 µl pipette tip. Then, the shed cells were gently washed off with phosphate-buffered saline (PBS). Cells were then treated with CBD at concentrations of 2, 5, and 10 µM and allowed to migrate toward the denuded areas for 24-72 hours. Cell images at zero h, 24h, 48h, and 72h were taken under a light microscope (Olympus, Tokyo, Japan) at a magnification of ×100 in three random fields for each experimental setting. Migrating cells were photographed at the indicated times and compared to an untreated control group. The distances were measured in micrometres using ImageJ (NIH, Bethesda, MD, USA), and changes in cell migration were determined by calculating the percentage of wound area that remained open relative to the control. The assay was done in triplicate and repeated at least three times.

Statistical analysis

Data are presented as mean ± standard deviation. For comparisons between two or more groups, we used the student's t-test or Tukey post hoc test following one-way ANOVA for multiple group comparisons. A p-value <0.05 was considered statistically significant. All statistical analyses were performed using GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, USA).

RESULTS

CBD potentiates cisplatin response in primary and metastatic human OC Cells

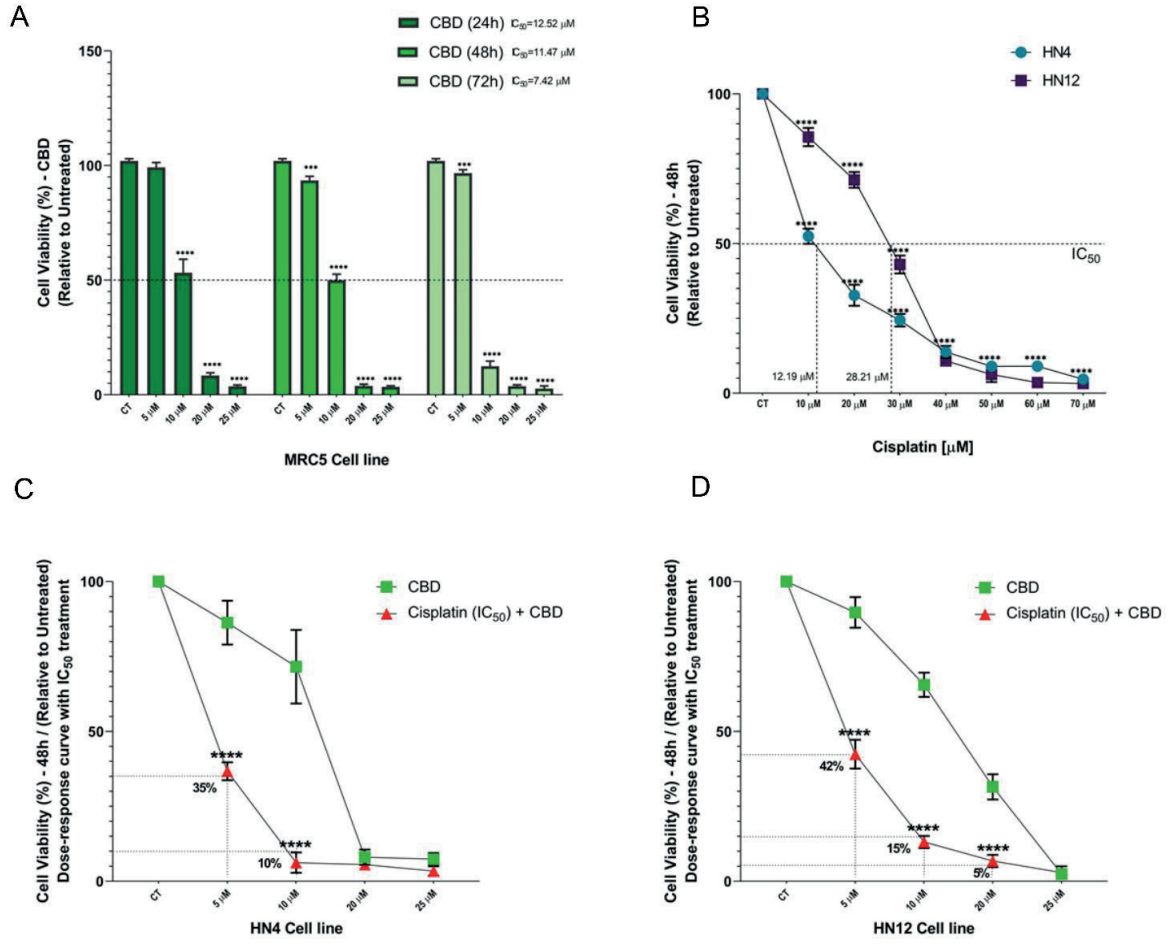
This study began by examining the impact of varying concentrations of CBD (5, 10, 20, and 25 µM) on MRC-5 normal lung fibroblast cells over different times (24, 48, and 72 hours). We found that CBD led to a significant reduction in cell viability, with IC50 values of 12.52 µM, 11.47 µM, and 7.42 µM at 24, 48, and 72 hours, respectively (Fig. 1A, $p < 0.05$). At this juncture, our objective was to assess the impact of the tested compound (CBD) on a normal cell line.

Subsequently, we also aimed to assess the potential of CBD to overcome cisplatin resistance in OC cells. To do this, HN4 and HN12 OC cell lines were treated with different concentrations of cisplatin (10, 20, 30, 40, 50, 60, and 70µM) for 48h. HN4 cells exhibited greater sensitivity

to cisplatin compared to HN12 cells, with IC_{50} values of 12.19 µM and 28.21 µM, respectively, over 48 hours (Fig. 1B, p<0,05). The subsequent phase involved assessing the effects of cisplatin treatment at doses of 12.19 µM for HN4 cells and 28.21 µM for HN12 cells, combined with CBD. In line with expectations, Fig. S01 illustrates that treatment with cisplatin at its corresponding IC_{50} values resulted in a reduction in cell viability, reaching approximately 50% (p < 0.001).

Notably, combining cisplatin at its respective IC_{50} concentrations with varying concentrations of CBD (5, 10, 20, and 25 µM) showed a significant reduction in cell viability in both HN4 and HN12 cells compared to cisplatin treatment alone. In HN4 cells, combining 12.19 µM cisplatin with five µM CBD resulted in a 65% inhibition of cell viability, while combining it with 10 µM CBD led to a 90% reduction (Fig. 1C, p<0,05). Similarly, in HN12 cells, combining five µM CBD with 28.21 µM cisplatin resulted in a 58% reduction, while combining it with 10 µM CBD led to an 85% reduction (Fig. 1D, p < 0.05). As a result, these findings suggest that CBD has the potential to mitigate resistance to cisplatin treatment in two tumour phenotypes of OC, including both primary and metastatic forms, as observed in these in vitro models.

Figure 1 – Cannabidiol (CBD) enhances cisplatin cytotoxicity in primary and metastatic oral cancer cells. *(A) Viability of MRC-5 normal lung fibroblasts treated with increasing concentrations of CBD (5–25 µM) for 24–72 h, showing time – and dose-dependent cytotoxicity. (B) Dose–response curves of cisplatin (10–70 µM) after 48 h treatment in HN4 (primary) and HN12 (metastatic) oral cancer cells, revealing differential sensitivity. (C–D) Combined treatment of CBD (5–25 µM) with cisplatin at respective IC_{50} concentrations (12.19 µM for HN4; 26.21 µM for HN12) resulted in a significant reduction in cell viability compared with either agent alone. Data represent mean ± SD of three independent experiments. *p<0.05. Three independent experiments were performed in triplicate. Data are expressed as means ± standard errors.*



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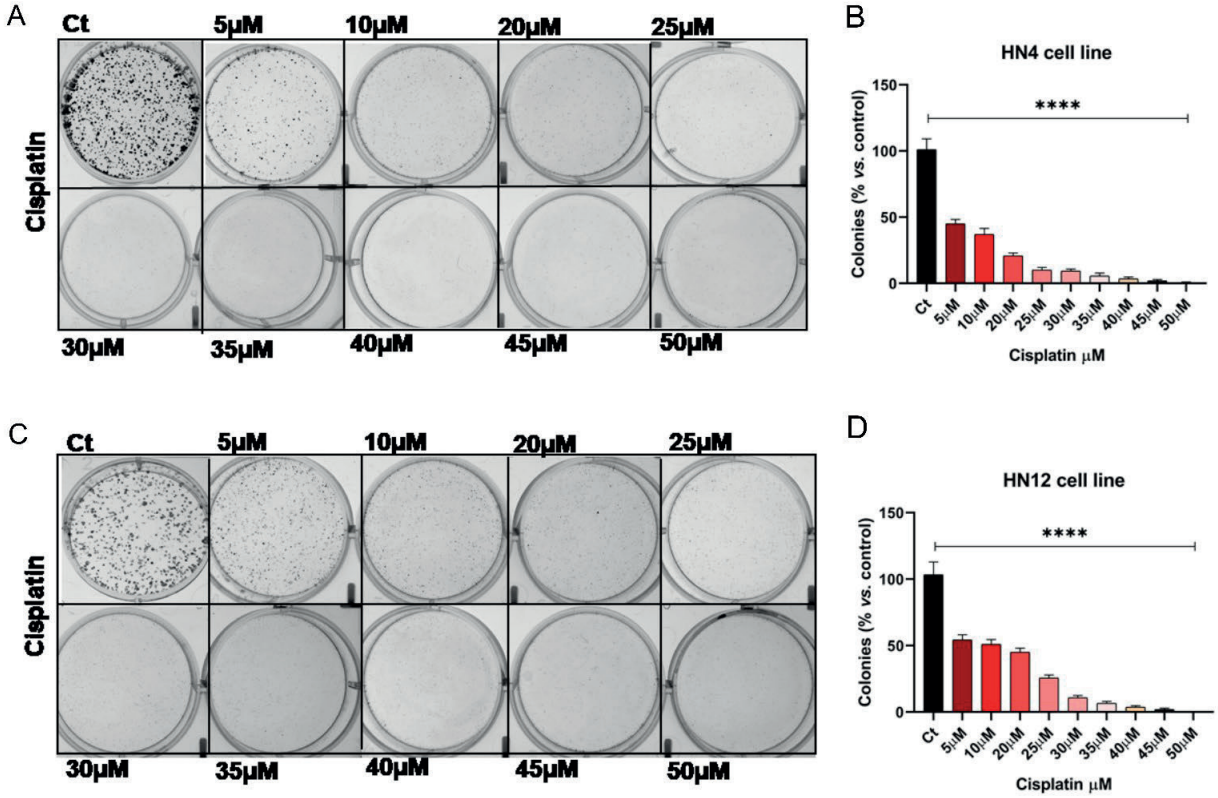
Combined cisplatin and cannabidiol treatment reduces colony formation in human oral cancer cells

Cisplatin resistance in oral cancer cells is a multifaceted phenomenon driven by numerous molecular mechanisms, which enable the evasion of cytotoxicity and ultimately bolster cancer cell survival[7]. To explore the potential impact of cisplatin and CBD combination therapy on cell survival, in vitro, we employed the clonogenic assay, regarded as the gold standard for measuring cell clonogenic survival[35] . Initially, we assessed the effects of varying concentrations of cisplatin alone.

Following treatment with cisplatin at concentrations ranging from 5 to 50 µM, reductions in both the number and size of colonies were observed in HN4 (Figs. 2A-B, p<0,001) and HN12 cells (Figs. 2C-D, p<0,001). There was a more pronounced effect in HN4 cells compared to HN12 cells following cisplatin treatment, indicating a greater reduction in the number of colonies at the same evaluated doses. However, colonies can still be observed up to a dose of 35 µM for both models studied. Next, we examined the combined treatment of cisplatin and CBD and its impact on oral cancer cell colony formation.

Figure 2 – Cisplatin reduces clonogenic survival in HN4 and HN12 oral cancer cells. *(A) Representative images of clonogenic assays following cisplatin treatment (5–50 µM) for 48 hours in HN4 oral cancer cells. (B) Quantification of colony formation (%) in HN4 cells after cisplatin exposure. (C) Representative images of clonogenic assays following cisplatin treatment (5–50 µM) for 48 hours in HN12 oral cancer cells. (D) Quantification of colony formation (%) in HN12 cells after cisplatin exposure. Data represent the mean ± standard error of three independent experiments performed in triplicate. Statistical analysis was conducted using Student's t-test; ****p < 0.0001 versus control (untreated). Three independent experiments were performed in triplicate. Data are expressed as means ± standard errors.*



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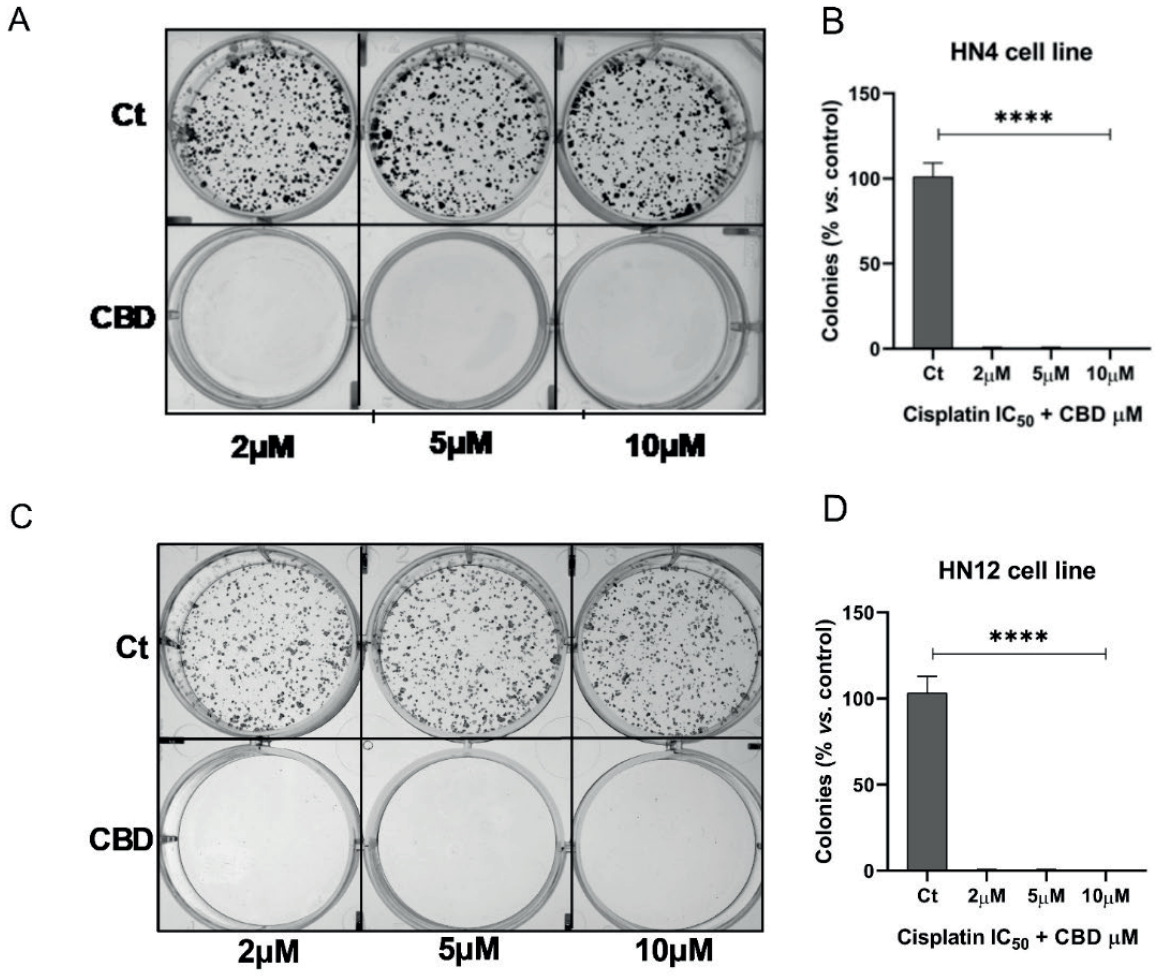
Then, the cisplatin was administered at doses corresponding to 12.19 µM and 28.21 µM (IC_{50} values) for HN4 and HN12 cancer cells, respectively. CBD treatment utilised doses of 2, 5, and 10 µM, selected because they were lower than the IC_{50} value determined for 48 hours in the MRC-5 lineage when evaluating CBD. As illustrated in Figs. 3A-B for HN4 cells and Figs. For HN12 cells, the combined treatment of cisplatin and CBD resulted in a substantial decrease in the clonogenic capacity of OC cancer cells, p < 0.0001. Remarkably, when 2µM CBD was combined with cisplatin doses of 12.19 µM for HN4 cells and 28.21 µM for HN12 cells for 48 hours, it efficiently reduced the clonogenic capacity of both in vitro models to zero. As a result, these findings suggest that this combined treatment exhibited a more significant inhibition of colony formation compared to cisplatin alone in the two OC cell lines.

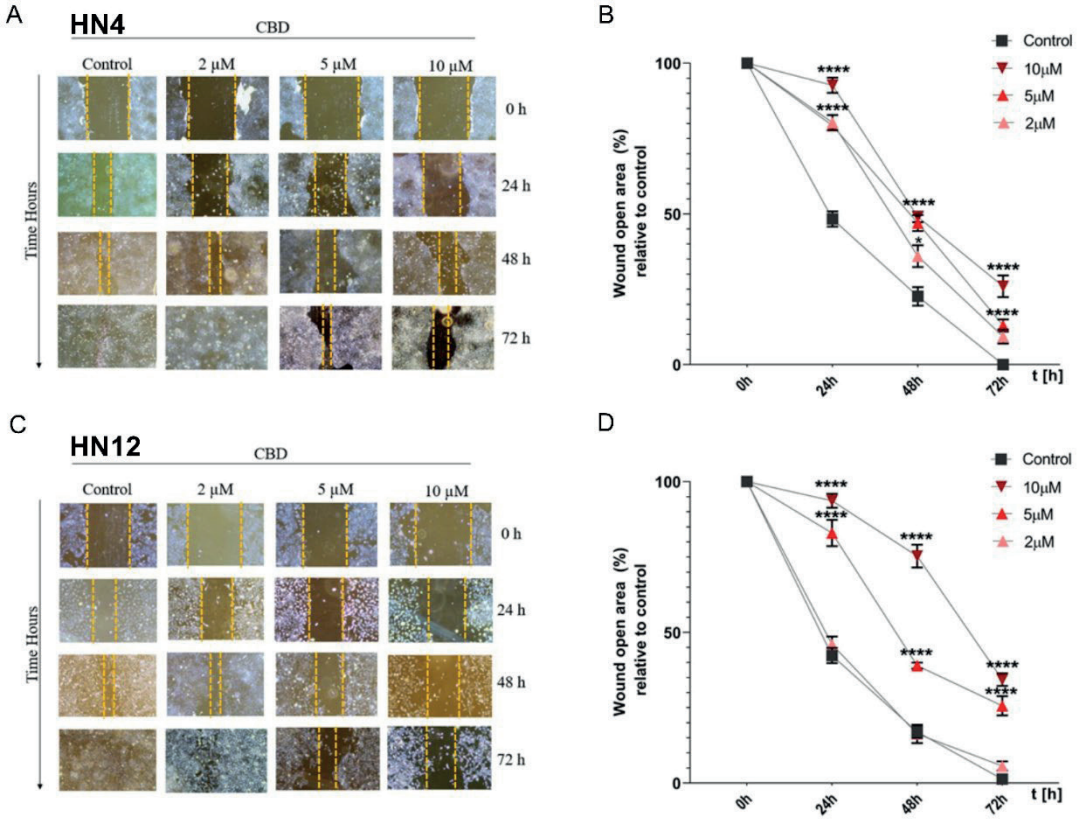
Figure 3 – The combination of CBD and cisplatin treatment exhibits enhanced reduction in colony formation compared to cisplatin treatment alone. *(A) Representative images of clonogenic assay following cisplatin treatment (12.19µM) for 48h plus CBD treatment (2, 5, and 10µM) in HN4 oral cancer cells. (B) Graphical representation of colony counts (%) in the HN4 cell line following this combined treatment. (C) Representative images of clonogenic assay following cisplatin treatment (26.21µM) for 48h plus CBD treatment (2, 5, and 10µM) in HN12 oral cancer cells. (D) Graphical representation of colony counts (%) in the HN12 cell line following this combined treatment. Data were analysed by Student's t-test. ****p <0.0001 versus the control group (untreated). Three independent experiments were performed in triplicate. Data are expressed as means ± standard errors.*



Cannabidiol inhibits migration of primary and metastatic oral cancer cells: evidence from scratch wound healing assays

To investigate whether CBD at 2, 5, or 10 µM without cisplatin had any influence on the migratory ability of the human oral cancer cell lines, scratch wound healing assays were performed. The wound healing assays demonstrated a significant inhibitory effect of CBD at concentrations of 2, 5, and 10 µM on the migration ability of HN4 cells (primary), as depicted in Figs. 4A-B, compared to the untreated control group (p< 0.001). As shown in Figs. 4C-D, CBD at concentrations of 5 and 10 µM exhibited inhibitory effects on the migration ability of HN12 cells (metastatic), compared to the untreated control group (p<0.001), highlighting its impact across distinct cancer phenotypes. These results provide supportive evidence of CBD's effectiveness in inhibiting the migration of oral cancer cells.

Figure 4 – Efficacy of CBD on cell migration in oral cancer cells. *Cell motility of HN4 and HN12 upon CBD treatment was determined by wound-healing assay at 2, 5, and 10μM for 24-72h. Representative photomicrographs (magnification, 100x of wound healing in (A-B) HN4 and (C-D) HN12 cells with their relatively quantified wound area (%). Three independent experiments were performed in triplicate. Tukey's post hoc test was performed following one-way ANOVA for multiple group comparisons. Differences were considered statistically significant at p < 0.05. **p < 0.0001 versus control (0 μM).*



DISCUSSION

Cisplatin, a widely used chemotherapeutic agent, is known for its DNA-damaging effects and is commonly employed in the treatment of OC[7] . However, despite its efficacy as a chemotherapy agent, many OC patients develop resistance to cisplatin over time, leading to treatment failure and disease progression. Consequently, cisplatin resistance poses a significant challenge in OC management[7,9] . Although the mechanisms underlying cisplatin resistance are complex and multifaceted, its resistance not only diminishes the effectiveness of cisplatin-based chemotherapy but also limits treatment options, necessitating the exploration of alternative therapeutic strategies[36] . Overcoming cisplatin resistance also requires a deeper understanding of the molecular mechanisms involved and the development of targeted therapies that can circumvent or reverse this resistance. Various approaches, including combination therapies, have been explored to enhance the effectiveness of cisplatin. Of note, these combined treatment approaches that include medicinal plants, natural products and nano-drug delivery systems, may help mitigate the impact of cisplatin resistance and improve outcomes in cancer management [37,38].

In this context, emerging evidence suggests that CBD, an active natural compound extracted from Cannabis sativa, has attracted attention for its potential therapeutic effects, including anti-inflammatory, analgesic, and anticancer properties, particularly in augmenting the activity of cisplatin in cancer treatment[17,39,40] . In addition, CBD's antiemetic and anxiolytic properties may also help alleviate the adverse effects associated with cisplatin treatment, improving patients' overall quality of life. However, when considering the combination of CBD and cisplatin, some research has indicated that CBD may have a synergistic effect with antineoplastic drugs, potentially enhancing this efficacy [41].

As an example, a study on bladder cancer cell lines suggested that CBD may have multiple effects when combined with cisplatin, inducing differential responses ranging from antagonistic to additive and synergistic effects[42] . Additionally, other studies have also indicated that CBD may enhance the effectiveness of primary agents utilised in the treatment of various cancers, notably prostate cancer. This is substantiated by recent evidence demonstrating CBD's capacity to inhibit the proliferation and invasiveness of prostate cancer cells,

thereby reinforcing its potential as a future chemotherapeutic agent[43]. Furthermore, CBD has been reported as a novel therapeutic agent targeting tumorigenesis in cisplatin-resistant non-small cell lung cancer due to its inhibitory effects, highlighting its efficacy in combating resistant cancer phenotypes[44] .

To investigate the interactions between cisplatin and CBD in OC treatment, Deng et al.[45] (2016) conducted a study that analysed the synergistic responses between cannabidiol (CBD) and DNA-damaging agents on the proliferation and viability of glioblastoma and neural progenitor cells in culture . Their research sheds light on the potential synergistic effects of CBD with DNA-damaging agents, providing insights into the interaction between CBD and cisplatin in the context of OC treatment.

The potential interaction between cisplatin and CBD for OC treatment is an area of growing interest and research. In the current study, we first investigated the dose response of CBD in a normal human cell line (fibroblast) to assess its impact in a combined treatment. Interestingly, we found that doses lower than 10 µM did not significantly impact more than 50% of the cell viability rate in this model. Consequently, in the subsequent phase of this study's design, we evaluated the impact of combining CBD with cisplatin on the cell viability of two in vitro tumour models of OC: one originating from primary tumours and the other from metastatic tumours. To our surprise, in this model, the combination of CBD with cisplatin significantly reduced the cell viability by more than 50% in both the primary and metastatic tumour models, surpassing the effects observed with cisplatin treatment alone. Continuing our investigation, when conducting clonogenic assays in this model, doses of up to 35 µM of cisplatin still resulted in the formation of a considerable number of cell colonies in vitro. However, upon combining cisplatin treatment with previously established IC_{50} doses of CBD at < 10 µM, a notable 100% reduction in colony numbers was observed, greatly surpassing the effects seen with cisplatin treatment alone. Last, we examined the impact of CBD alone on cell migration rates. Remarkably, CBD demonstrated an inhibitory effect, decreasing cell migration rates in these in vitro models of OC.

Beyond that, our study also highlights key differences in cisplatin sensitivity between primary and metastatic tumour models. While cisplatin remains a cornerstone of oral cancer treatment, resistance frequently emerges in metastatic lineages, a phenomenon well-documented in other cancers[46] . In our patient-derived models, metastatic cells exhibited reduced cisplatin sensitivity compared to their primary tumour counterparts, aligning with clinical observations that advanced OC tumours often develop chemoresistance to cisplatin through mechanisms such as enhanced DNA repair, drug efflux pumps, and apoptotic evasion[47,48] . Notably, the combination of CBD with cisplatin partially reversed this resistance in metastatic cells, suggesting that CBD may target pathways implicated in cisplatin tolerance, such as by modulating oxidative stress or inhibiting pro-survival signalling[17,39,49]. These findings underscore the translational potential of CBD to improve outcomes in treatment-refractory oral cancer, particularly for metastatic disease where therapeutic options are limited.

Molecular studies have shed light on the mechanisms underlying the synergistic effects of CBD and cisplatin in OC treatment. RNA-seq analysis has been instrumental in identifying changes in gene expression that are associated with DNA repair, cell division, and proliferation, which may contribute to the enhanced cytotoxicity of cisplatin when combined with CBD in OC treatment[39] . On the other hand, CBD has emerged as a potential therapeutic agent in OC treatment because CBD has been found to have oral absorption and bioavailability, which is relevant for its use in OC treatment[50] . Thus, in the context of OC treatment, the combination of CBD with cisplatin, a commonly used chemotherapeutic agent, has great promise.

Drug efflux pumps can contribute to cisplatin resistance in cancer treatment, and CBD has been found to inhibit their expression[51] . This provides a potential mechanism through which CBD may help overcome resistance mechanisms and enhance the efficacy of cisplatin in promoting cancer cell death. Additionally, CBD has been shown to reduce the expression of resistance-related proteins, as seen in the cases of doxorubicin and cisplatin in certain cancer cells[52]. These findings suggest that the ability of CBD to modulate drug efflux mechanisms could play a crucial role in sensitising cancer cells to chemotherapy agents like cisplatin.

While some studies have reported antagonistic interactions between CBD and cisplatin in certain cancer cell lines, other research has indicated that CBD may enhance the therapeutic effects of cisplatin in specific contexts[53]. This suggests that the interaction between CBD and cisplatin may vary depending on the specific cancer type and treatment regimen, and/or genetic background. However, when considering the interaction between CBD and cisplatin, it is essential to note that CBD may have a role in mitigating certain side effects associated with cisplatin treatment. For example, CBD has been found to attenuate vomiting and nausea-like symptoms induced by cisplatin through its interaction with serotonin receptors[54]. However, the effectiveness of CBD in reducing nausea may be limited in highly emetogenic therapies. Moreover, CBD has been investigated for its potential synergistic effects with DNA-damaging agents, such as cisplatin, in inhibiting the proliferation and viability of cancer cells[45]. Overall, these findings suggest that CBD may have a role in enhancing the therapeutic outcomes of conventional cancer treatments like cisplatin.

Our study makes a significant contribution by demonstrating that the combined treatment with cisplatin and CBD exhibits similar enhancing effects in both the primary and metastatic cell models studied. This finding is of great importance, particularly for patients with OC who present with metastases, as they typically have a poorer prognosis

with low rates of therapeutic success observed in clinical practice. Given this context, our results suggest that CBD combined with cisplatin could not only hold promise for patients classified as having a better prognosis but also offer potential alternative therapies to be explored in the future for patients who are in urgent need of new, safe options, especially those with metastatic disease. Our observations also suggest that combining CBD with cisplatin enables the use of lower doses of cisplatin to achieve a heightened cytotoxic effect on tumour cells. This translation from bench to bedside is yet to come, but it has the potential to improve the quality of life for patients undergoing and recovering from chemotherapy treatment protocols.

In our study, while we are demonstrating the therapeutic promise of CBD-cisplatin combination therapy in oral cancer models, there are several limitations that warrant discussion. First, by design, we prioritised functional assessments (viability, migration, and clonogenic capacity) to establish therapeutic potential, deliberately deferring mechanistic pathway analyses to subsequent in vivo studies employing transcriptomic approaches. Second, while our wound healing assays effectively modelled clinically relevant collective cell migration, they did not distinguish between migration and proliferation effects, an important nuance that needs to be addressed in future investigations. Finally, although our immortalised cell line models provided experimental consistency, they may not fully recapitulate the complexity of patient-derived tumour microenvironments. Importantly, these limitations are balanced by the study's key innovation: the first comparative evaluation of CBD-cisplatin effects in matched primary and metastatic tumour lineages, providing unique insights into overcoming metastatic resistance mechanisms. These foundational findings create a robust platform for future in vivo studies that will explore the molecular underpinnings of these observations.

While preclinical studies suggest promising synergistic effects between cisplatin and CBD in OC treatment, further research, including in vivo experiments and clinical trials, is crucial to validate these findings and establish optimal dosing regimens and treatment protocols. Additionally, careful consideration of drug interactions, potential side effects, and long-term impacts of combined therapy is essential. Individuals considering complementary therapies like CBD should discuss their options with their healthcare providers to evaluate potential interactions with conventional treatments and manage possible side effects. Furthermore, the legality and regulation of CBD products can vary by region, highlighting the importance of obtaining products from reputable sources that comply with local laws. Overall, CBD has potential as an adjunctive therapy for enhancing the chemotherapeutic response and improving clinical outcomes in cancer patients, underscoring the necessity for continued research in this rapidly evolving field.

However, our findings indicate that the CBD concentrations capable of reducing tumour cell viability are relatively close to those that also decrease viability in normal cells. This proximity suggests a potentially narrow therapeutic window, which warrants caution when considering the translational relevance of these results. Although consistent antitumor effects were observed, the impact of CBD on non-tumorigenic cells indicates that its selectivity may be limited, particularly at higher concentrations. These observations highlight the need for additional studies aimed at defining safe exposure thresholds and elucidating differential mechanisms of response between normal and malignant cells, including metabolic variability, basal oxidative stress levels, and signalling pathways associated with cellular resistance. Then, considering these factors is essential to better understand the therapeutic potential of CBD and to guide future approaches seeking to maximise antineoplastic efficacy while minimising undesirable cytotoxic effects.

CONCLUSION

In conclusion, while our study indicates that CBD, alone or in combination with cisplatin, may exert beneficial effects on oral cancer (OC) models by reducing cell viability, clonogenic capacity, and migration, these findings should be interpreted with caution. Importantly, we observed that the concentrations of CBD capable of exerting antitumor effects are close to those that also reduce the viability of non-tumorigenic cells, suggesting the possibility of adverse effects and a potentially narrow therapeutic window. This highlights the need for strategies aimed at minimising cytotoxicity in normal cells, as well as for a deeper investigation of mechanisms underlying differential sensitivity between healthy and malignant tissues. Therefore, although CBD shows promise as a complementary approach to enhance cisplatin-based chemotherapy, further studies, including dose-refinement, safety assessments, and well-designed clinical trials, are essential before any translational application can be considered. Such efforts will be crucial to determine the true therapeutic potential and safety of CBD in the management of OC.

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Supplementary

Figure 1 – Evaluation of the treatment with IC50 values of cisplatin in oral cancer cells. *Cell viability assay showing a reduction in HN4 and HN12 cell viability after cisplatin treatment at IC50 values of 12.19 µM and 26.21 µM for HN4 and HN12 cells, respectively. Three independent experiments were performed in triplicate. ***p<0.001.*