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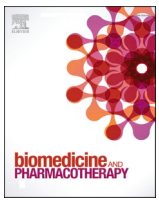

Cannabis-derived products antagonize platinum drugs by altered cellular transport

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ABSTRACT

Cannabinoids, a class of compounds derived from *Cannabis sativa* L., have recently become more widely accessible for public consumption in the form of diverse cannabis products, in parallel with weakening the measures that so far restricted their availability. The US Food and Drug Administration has approved several cannabis-derived drugs for management of various diseases as well as chemotherapy-induced nausea and vomiting. Besides the attenuation of adverse effects of chemotherapy, numerous reports about cannabinoid-mediated anticancer effects further motivate cancer patients to support their therapy with such products. Here we present a set of preclinical data with human cell culture models, suggesting that cannabidiol and cannabis extracts may effectively counteract the anticancer effects of the clinically widely used standard-of-care platinum-based drugs. We show that even low concentrations of cannabinoids reduced the toxicity of cisplatin, oxaliplatin, and carboplatin, an effect which was accompanied by decreased platinum adduct formation and a set of commonly used molecular markers. Mechanistically, our results excluded the possibility that the observed enhanced survival of cancer cells was mediated transcriptionally. Instead, trace metal analyses strongly indicate an inhibitory impact of cannabinoids on intracellular platinum accumulation, thereby implicating changes in cellular transport and/or retention of these drugs as the likely cause of the observed biological effects. Our study raises the possibility that the desirable effect of counteracting adverse effects of chemotherapy might, at least for some cannabinoids, reflect impaired cellular availability, and consequently attenuation of the anticancer effects of platinum drugs.

Data availability: All data supporting the conclusions are available in the article and supplementary files. Raw data are available upon request from the corresponding author.

1. Introduction

Current standard-of-care cancer chemotherapy protocols commonly include cisplatin or other platinum-derived drugs as their key components [1]. Cisplatin was the first such compound, approved by the FDA in 1978 for the treatment of ovarian and testicular cancers [1], with the application spectrum later extended to cover also head and neck, esophageal, gastric, colon, bladder, and cervical cancers as the first-line therapy, as well as many other types of malignancies, including lung cancer, as the second-line therapy, often combined with other chemotherapeutics and/or other treatment modalities [2,3]. Mechanistically, cisplatin becomes hydrolyzed in cells to become a potent electrophile that reacts with cysteine-rich proteins and purine bases at the N7 position. Ensuing DNA damage is the most widely accepted mechanism to which the antineoplastic properties of cisplatin have been attributed. Indeed, cisplatin forms DNA adducts and crosslinks, which can hinder DNA replication and hence cell division, inducing cell death [3].

However, cisplatin has multiple shortcomings, including dose-

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limiting side effects and the development of resistance. Neuro-, nephron-, oto-, and gastrointestinal toxicity with myelosuppression are some of the most common negative side effects impacting patients, with nephrotoxicity as the major dose-limiting factor [2,4]. Although carboplatin, the second generation of platinum-based drugs, solved some of the challenging issues of cisplatin in oncology, carboplatin also has serious side effects, including dose-limiting myelosuppression [2,3]. Moreover, despite some improved attributes, carboplatin is unsuitable for certain cancers that can be treated by cisplatin but is approved for the treatment of ovarian and testicular cancer, as well as numerous other tumor types in the context of combined therapies [2,2,5]. Compared to cisplatin, the carboplatin molecule contains a bidentate dicarboxylate ligand instead of two chlorine atoms. The ligand provides carboplatin with new properties, including slower hydrolysis, lower reactivity, and longer retention. Although the reaction products of carboplatin are analogous to those of cisplatin, substantially higher concentrations of carboplatin are needed to achieve the same toxicity [3]. Furthermore, cross-resistance is inevitable due to the close similarity of these two substances [2,6].

Oxaliplatin, the third-generation platinum-based drug, was developed to respond to acquired resistance induced by cisplatin and carboplatin. Its ability to overcome resistance is attributable to the different constitution of the molecule, which contains a 1,2-diaminocyclohexane ligand instead of amine groups, and oxalate as a leaving group [5,6]. This composition makes oxaliplatin lipophilic, which provides additional routes for cellular transport and alters its reactivity and mechanism of action. Oxaliplatin has lower reactivity with DNA, but its toxicity and anticancer properties are strongly exacerbated by its ability to interfere with ribosome biogenesis and protein synthesis [2,5,6]. Oxaliplatin is commonly used to treat colorectal cancers [2,6]. However, similar to previous platinum drug generations, oxaliplatin causes severe adverse effects with dose-limiting neurotoxicity [2,4].

While many patients initially benefit from platinum-derived chemotherapy, a large proportion of tumors relapse, and the acquired resistance to further chemotherapy often results in treatment failure. Though far from being fully understood, such drug resistance mechanisms include modulation of drug transport, glutathione and metallothionein pathways, enhanced DNA-damage repair, inhibition of cell death, and altered cancer cell metabolism [6].

In this study, we identify cannabidiol and cannabis plant extracts as substances interfering with the toxicity of all three of the above-mentioned platinum-based drugs. Cannabinoids are a family of substances with a broad pharmacological profile, potentially applicable to numerous health conditions, from neurological to inflammatory and autoimmune diseases [7–9]. Importantly, cannabis products are clinically highly relevant due to their extensive use among cancer patients [10–12]. Cannabis products are generally accepted to attenuate chemotherapy-induced unwanted side effects. Several cannabinoid-based drugs, including Dronabinol and Nabilone, are indeed being prescribed for chemotherapy-induced nausea and vomiting [13,14]. The motivation of oncological patients to use cannabis-based products is further boosted by numerous studies that suggest their anticancer properties via various mechanisms of action. It has also been speculated that simultaneous consumption of cannabinoids may support ongoing anticancer therapy through some direct anticancer effects [7,9,15]. However, antagonistic effects were also suggested [16,17]. Indeed, our present results call for caution with regard to such combinatorial use, as the positive effects of cannabinoids on cancer chemotherapy may not be universally applicable. Indeed, when combined with some chemotherapeutics, such as platinum-derived drugs, cannabis may undermine their anticancer effects and thereby attenuate the treatment efficacy.

2. Materials and methods

2.1. Cell lines

Human osteosarcoma U-2-OS (ATCC), human lung carcinoma NCI-H1299 (ATCC), and human lung fibroblast MRC-5 (ATCC) cells were maintained in DMEM (Lonza) supplemented with 10% fetal bovine serum (Thermo Fisher Scientific) and 1% penicillin/streptomycin (Sigma-Aldrich). MRC-5 cells were further supplemented with 1% non-essential amino acids (MEM NEAA, Gibco).

2.2. Crystal violet assay

Cells were seeded on a 96-well plate with a seeding density of 5000 cells per well. On the same day, the cells were pretreated with 2.5 μM cannabinoid. After 17 h, the cells were treated with increasing concentrations of the platinum-based drug. Cisplatin (CisPt) and oxaliplatin (OxPt) were used in concentrations 5, 10, 20, and 40 μM. Carboplatin (CarboPt) was used in concentrations 50, 100, 200, and 400 μM. In the wash-out experiment, the CBD (cannabidiol) pretreatment was removed by washing with new DMEM media before treatment with platinum-based drugs. After 72 h of treatment, the cells were fixed with 70% cold ethanol for 15 min and labeled with crystal violet solution (5 g Crystal Violet, Sigma Aldrich, C6158; 200 mL 96% ethanol, 800 mL H_2O) for 20 min. Next, the 96-well plates were thoroughly washed under running water and left to dry. The cell-incorporated crystal violet dye was solubilized by phosphate buffer saline containing 0.2% Triton X-100 and measured at 590 nm using a spectrometer (TECAN, Infinite M200PRO). The results are shown as the mean value and standard deviation from three independent experiments. Five technical repeats were performed per experiment.

2.3. Colony formation assay

Cells were seeded on a 12-well plate with a seeding density of 100 cells per well. On the day of seeding, the cells were pretreated with 2.5 μM CBD, and after 17 h, the cells were treated with 2.5 μM CisPt, 5 μM CarboPt, or 5 μM OxPt. After 12 days, the colonies were fixed with 70% cold ethanol and labeled with crystal violet solution. Cells were washed under running water and air dried. For better contrast, the wells were filled with powdered white edible sugar and scanned using a tabletop scanner (Epson Perfection V750 PRO). The mean area and colony count were evaluated using ImageJ (Wayne Rasband) software. The results are shown as the mean value and standard deviation from three independent experiments. Three technical repeats were performed per experiment.

2.4. Immunofluorescence staining and quantitative microscopy

The cells were seeded on glass coverslips or a 24-well glass bottom plate (Cellvis, P24-1.5H-N). Cells were pretreated with 10 μM CBD on the day of seeding, before treating with 10 μM platinum-based drug for 24 h. In the case of treatment with cycloheximide (Cycloheximide solution, Sigma Aldrich, C4859), the treatment was shortened to 17 h and chemicals (CBD, cycloheximide, platinum-based drugs) were administrated concomitantly. The cells were fixed with 4% cold formaldehyde for 15 min, followed by permeabilization with phosphate buffer saline containing 0.5% Triton X-100 for 5 min. Subsequently, the cells were blocked with DMEM media containing 10% fetal bovine serum (FBS, Gibco) for 1 h, before staining with primary antibody dissolved in blocking media overnight at 4 °C. Secondary antibodies were added for 1 h at room temperature. Nuclei were stained with DAPI (Sigma) (1 $\mu g \cdot mL^{-1}$) for 5 min. Samples were visualized and acquired using fluorescence microscopes (Olympus IX81 ScanR and/or Zeiss LSM 980). Quantitative analysis was performed in ScanR Analysis software (Olympus).

For cisplatin-DNA adduct staining, the cells were blocked in 5% bovine serum albumin and incubated in 2 M HCl for 10 min at 37 °C consecutively. The remainder of the protocol was performed as described above.

The results are shown as the mean value and standard deviation from three independent experiments.

2.5. Immunoblotting

The cells were seeded on a 6-cm Petri dish, pretreated with 10 μM CBD on the day of seeding, and then treated with 10 μM platinum-based drug for 24 h. The cells were lysed in Laemmli sample buffer. The protein concentration was measured by Bradford assay (Pierce Detergent Compatible Bradford Assay Kit, Thermo Scientific, 1863028), and approximately 10 μg of protein was loaded into a precast gel (Mini-PROTEAN TGX Stain-Free Gels, Bio-Rad, 4568093) and run under the conditions with constant 20 mA per gel. The separated proteins were transferred to nitrocellulose membrane, which was blocked in 5% milk (edible dried low-fat milk) dissolved in Tris-buffer saline containing 0.1 Tween 20 for 1 h. Next, the membrane was incubated with primary antibodies overnight at 4 °C, followed by secondary antibodies for 1 h at room temperature. Proteins were visualized by HRP substrate (Immobilon Forte Western HSR Substrate, Merck Millipore, WBLUF0500), and images were acquired using the ChemiDoc imaging system (Bio-Rad). The figure used in the manuscript represents one of three independent experiments.

2.6. Determination of total platinum and zinc content

The cells were seeded in a 15-cm Petri dish and were pretreated with 10 μM CBD on the day of seeding. Subsequently, the cells were treated with 20 μM CisPt for indicated time-points or 20 μM OxPt and 200 μM CarboPt for 24 h. The cells were then quickly washed in phosphate buffer saline and removed to 2-mL Eppendorf tubes. The samples were normalized according to the protein concentration. To determine the protein concentration, the samples were frozen at −80 °C to rupture the cells and then centrifuged at 20,000 g for 10 min at 4 °C. The protein concentration was measured from the supernatant using the BCA assay (Pierce BCA Protein Assay Kit, Thermo Scientifics, 23225).

The total Pt and Zn contents were determined by solution ICP-MS using an external calibration in the range of 5–100 $\mu g \cdot L^{-1}$ for Zn and 0.1–100 $\mu g \cdot L^{-1}$ for Pt. The aliquots of cell cultures were digested with 4 mL of concentrated nitric and hydrochloric acids (1:1, v/v) in the UltraWAVE digestion unit (Milestone, Italy) using a general temperature-control digestion method for biological samples. Following mineralization, the digests were quantitatively transferred to a 10-mL volumetric flask and filled with ultrapure water. The samples were subjected to ICP-MS analysis using the ORS-ICP-MS 7700x instrument (Agilent Technologies, Japan) in helium mode to overcome spectral interferences. The following isotopes were selected for quantitative ICP-MS analysis: ^{66}Zn, ^{194}Pt, and ^{195}Pt; and ^{89}Y and ^{209}Bi as internal standards. The regular measurement of independently prepared quality control samples at the concentration level of 50 $\mu g \cdot L^{-1}$ for Zn and 5 $\mu g \cdot L^{-1}$ for Pt was adopted to ensure the reliability of the ICP-MS results. The results are shown as the mean value and standard deviation from three independent experiments.

2.7. 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide

For the XTT assay, 5000 cells per well were seeded on a 96-well plate. The cells were pretreated with 10 μM CBD and, after 17 h, treated with increasing concentrations of platinum-based drugs. After 72 h, the XTT assay was performed according to the manufacturer's instructions (AppliChem) and measured using a spectrometer (TECAN, Infinite M200PRO). The results are shown as the mean value and standard deviation from three independent experiments. Five technical repeats were conducted per experiment.

2.8. RNA interference

siRNA against MTF1 (OriGene, SR302991) was used for RNA interference. The cells were transfected with siRNA using Lipofectamine RNAiMAX (Invitrogen, 13778) according to the manufacturer's instructions. The cells were reseeded to the required plate or dish 24 h after transfection. Treatments were started 72 h after transfection.

2.9. Cultivation of cannabis plants

Two genotypes of cannabis (*Cannabis sativa* L.) were used in this study. The genotype "Kosher Haze" (KH; Dutch Passion®, the Netherlands) was classified as chemotype I, which was THC dominant and grown from regular seeds. The seeds were cleaned with 0.03% v/v hydrogen peroxide and germinated on wet paper, in the dark, at laboratory temperature. During the vegetative phase of 8 weeks, the photoperiod was maintained at 18 h light/6 h dark, with a temperature of 24/18 °C, and relative humidity of 50%. During this phase, the plants underwent transplanting from germination soil (Florcom®, Czechia) to coconut coir (BioBizz, Spain) and topping. The genotype "Fantasy Bud" was classified as chemotype III, which was CBD dominant and grown from cuttings purchased from Konopex company (Ostrava, Czechia). Rooted clones were introduced directly into the flowering phase with 12 h of light. Both genotypes were cultivated in coconut coir (BioBizz, Spain). Plagron Cocos A + B, Power Roots, and Pure Zym (Plagron, the Netherlands) fertilizers were used for plant nutrition during the vegetative phase according to the manufacturer's instructions. Green sensation (Plagron, the Netherlands) was added to the nutrition scheme during the flowering stage. Full-spectrum 300 W LED Attis lights (Lumatek, UK) were used for illumination. Upon maturation (approx. 9 weeks), inflorescences were harvested and dried at 25 °C in the dark for 7 days, before curing and storing in glass jars in the dark at laboratory temperature.

2.10. Cannabis extraction

The dried inflorescences were sieved through a 1-mm mesh and decarboxylated for 30 min at 121 °C. Ethanolic extracts were prepared by adding 60 mL of 96% EtOH v/v to 6 g of decarboxylated material and sonicating for 30 min at 40 kHz. Upon centrifugation, the extraction solvent (1 mL supernatant aliquots) was evaporated on a centrifugal evaporator (Labconco, USA) at 40 °C. Phytocannabinoid profiling was accomplished according to methods recommended by the USP Cannabis expert panel [18] and according to the Dutch OMC (Office on Medicinal Cannabis, Analytical monograph Cannabis Flos (flowers/granulated); Version 7.1; Office of Medicinal Cannabis, Ministry of Health Welfare and Sport: Netherlands, 2014). The phytocannabinoid profiles of the extracts are shown in detail in ST1.

2.11. Statistical analysis

XY graphs comprising error bars are plotted as mean values and standard deviation from three independent experiments, each presented by five technical replicates. Interleaved and stacked bar graphs are plotted as mean values and standard deviation from three independent experiments. The scatter plot presents the mean values and standard deviation from three independent experiments, each presented by quantitative data obtained from ScanR Analysis software. Ordinary one-way ANOVA was used to assess statistical significance, and the resulting P-value is shown in the graphs. All graphical and analytic processing, including the calculation of the IC50 and IC75, were performed in GraphPad Prism 9.4.1.

2.12. Chemicals and antibodies

The following antibodies were used for immunofluorescence labeling for microscopy: anti-cisplatin modified DNA (1:1000, Abcam, ab103261), γH2AX (1:500, Millipore, 05-636), anti-nucleolin (1:1000, Abcam, ab70493), p53 (1:500, Santa Cruz, sc-6243), Alexa Fluor 488 goat anti-mouse IgG (1:500, Invitrogen, A11001), Alexa Fluor 568 goat anti-rabbit IgG (1:500, Invitrogen, A11036), and Alexa Fluor 568 goat anti-rat IgG (1:500, Abcam, ab175476).

The following antibodies were used for immunoblotting: anti-b-actin (1:1000; Santa Cruz Biotechnology, sc-47778), anti-MTF1 (1:1000; NOVUS Biologicals, NBP1-86380), anti-p53 (1:500, Santa Cruz, sc-126), anti-SMC1 (1:1000, Abcam, ab9263), goat-anti mouse IgG-HRP (1:1000; GE Healthcare, NA931), and goat-anti-rabbit (1:1000; GE Healthcare, NA934).

The following cannabinoids were used for the abovementioned assays: CBD (-(-)cannabidiol, 10 mM stock solution in DMSO; Abcam, ab120448), Δ^9-THC (Δ^9-Tetrahydrocannabinol solution 1 mg·mL^{-1} in methanol; Sigma Aldrich, T4764), and CBG (Cannabigerol solution 1 mg·mL^{-1} in methanol; Sigma Aldrich, C-141). Extract 1 (CBD enriched) and extract 2 (Δ^9-THC enriched) were prepared and characterized at the Crop Research Institute (Olomouc, Czech republic) as stated in the Material and Methods. Information on the composition of the extracts is described in ST1. The extract was dissolved in DMSO to achieve a 10 mM solution of the dominant cannabinoid.

The following platinum-based drugs were used for the abovementioned assays: Cisplatin (Selleckchem, S1166), carboplatin (Selleckchem, S1215), and oxaliplatin (Selleckchem, S1224). Cisplatin and carboplatin were dissolved in water, with stock solutions of 1 mg·mL^{-1} and 10 mg·mL^{-1}, respectively. Oxaliplatin was dissolved in N, N-dimethylformamide (Sigma Aldrich, 227056) at a 5 mg·mL^{-1} stock concentration.

The following chemicals were used for ICP-MS: Certified reference material of aqueous calibration solution, ASTASOL® Pt and Zn (1000.0 ± 2.0 mg·L^{-1}), nitric acid (69%, ANALPURE® grade), internal standards mix, INT-MIX 1 Sc, Y, In, Tb, and Bi (10.0 ± 0.1 mg·L^{-1}) were purchased from Analytika, Ltd., Czech Republic. Ultrapure water with a resistivity of 18.2 MΩ cm was produced by a Milli-Q Reference purification system (Millipore Corporation, Molsheim, France).

3. Results

3.1. CBD protects cells from the toxicity of three clinically used platinum-based drugs

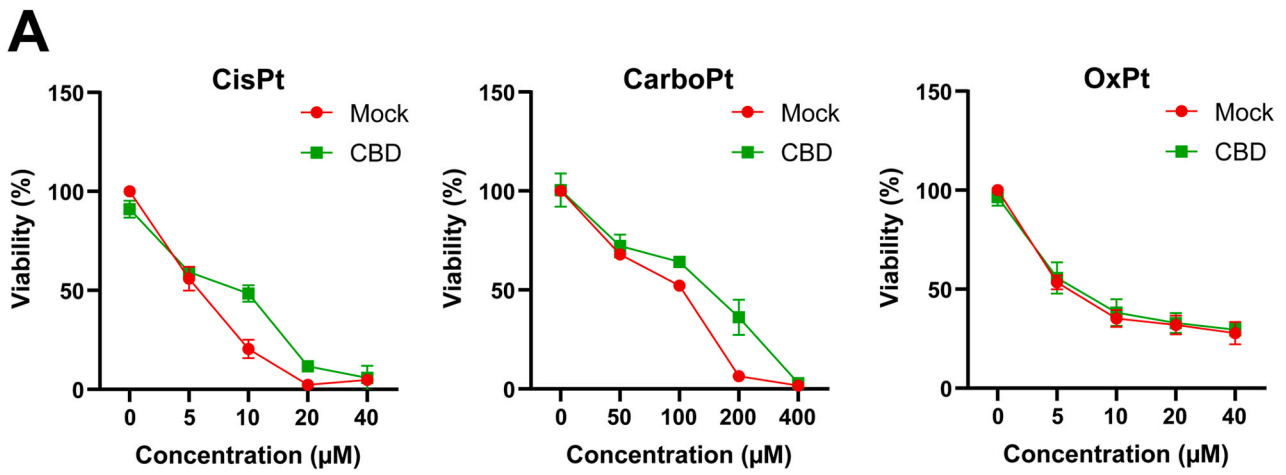
We have recently reported that cannabidiol (CBD)-triggered expression of metallothioneins can protect cancer cells against copper-containing substances such as CuET (an anticancer metabolite of disulfiram) [17]. Metallothioneins, in general, are cysteine-rich proteins that can protect cells against the toxic effects of various metals and metal-containing drugs. Here, we wished to determine whether a similar metallothionein-mediated resistance mechanism could be relevant for platinum-based drugs. First, we performed cytotoxicity tests with CBD combined with each of the three clinically used platinum cytostatics: cisplatin (CisPt), carboplatin (CarboPt), and oxaliplatin (OxPt), respectively. As the assay readout, we used the crystal violet staining analysis in human U-2-OS osteosarcoma cells pretreated with CBD and then treated with increasing concentrations of the 3 platinum drugs. As shown in Fig. 1A, even relatively low concentrations of CBD effectively interfered with the toxic effects of CisPt and CarboPt, while the toxicity profile of OxPt remained unchanged. The experiment was also performed with a human lung carcinoma HCI-H1299 cell line and with primary MRC-5 lung fibroblasts, respectively. Overall, the results obtained with all three cell models showed a similar trend (Figs. 1A and S1). Next, we employed a colony formation assay using the U-2-OS cells, to corroborate the CBD-protective effects in an approach that involves long-term exposure to the drugs. In this case, the CBD-mediated protective effect was even more pronounced and also confirmed in the OxPt-treated cells (Fig. 1B). Next, we analyzed the response using commonly used cellular stress markers, which partly differ across the platinum drugs [19–21]. In the case of CisPt, we analyzed the DNA damage using the γH2AX marker [19], the level of which was significantly reduced in the CBD-pretreated cells (Figs. 2A and S2) in a concentration-dependent manner (S3). Moreover, using quantitative fluorescence microscopy, we confirmed the CBD-mediated attenuation of p53 levels after CarboPt treatment (Figs. 2A and S2). In cells treated with OxPt, CBD pretreatment caused lower levels of nucleolar stress, as reflected by NCL-protein localization (Figs. 2A and S2). Interestingly, for the diploid MRC-5 cells, the extent of nucleolar stress remained unchanged upon pretreatment with CBD, suggesting that the protective effect of CBD is even greater in cancer cells compared to normal cells. We also performed a western blot analysis of the p53 protein as the shared stress response marker induced by all platinum-based drugs [22]. Consistently with the other markers, in all models, the CBD cotreatment resulted in an attenuation of the platinum drug-induced p53 accumulation (Fig. 2B, S4). For CisPt, we also quantified the signal from CisPt-modified DNA using specific antibodies for these structures, thus assessing directly the extent of the DNA adducts caused by the drug. This immunofluorescence method enables direct visualization of cis-platinated DNA, a readout that was found to be significantly decreased after CBD pretreatment (Fig. 2C), suggesting that the protective effect of CBD against CisPt-mediated toxicity is directly linked to the ability of the drug to reach its molecular target.

3.2. CBD affects the cellular transport of platinum drugs

Metallothioneins induced by CBD serve as intracellular heavy metal chelators and detoxificators, and therefore they might offer a potential explanation for the protective effects described above. To investigate this possibility, we employed an RNAi-mediated knockdown of MTF1, a transcription factor required for metallothionein expression, whose depletion abolished the CBD-triggered resistance of human cancer cells towards the copper-containing anticancer metabolite of disulfiram in our previous work [17]. In MTF1 deficient cells, the CBD-mediated protective effect remained unchanged for CisPt and CarboPt (S5Fig. 5A, B), thereby disproving the hypothesis that metallothionein could be responsible for the observed CBD-induced resistance to platinum drugs. Next, we included a washing step in the CBD pretreatment toxicity experiment to determine whether the protective effect employed other transcriptional responses; the idea being that any such transcriptional response should be relatively stable, and therefore an acute removal of CBD from the culture medium immediately after pretreatment should minimally impact the protective effect of CBD. However, upon CBD wash-out, U-2-OS cells lost the resistance against platinum-based drugs (S6). We further excluded the transcription-promoted rescue effect of CBD by cotreatment with cycloheximide (CHX), an inhibitor of protein synthesis. Indeed, cells cotreated with CHX retained their resistance against CisPt in the presence of CBD, as shown by lower induction of γH2AX and a reduced formation of CisPt-DNA adducts in the nuclei (Fig. 3). These data indicate that CBD may rather affect the cellular influx and/or efflux of platinum drugs. To address this possibility, we used the ICP-MS method to measure the intracellular levels of platinum after CBD pretreatment. Our results demonstrated significantly lower levels of intracellular platinum in CBD-cotreated cells, supporting the hypothesis that altered cellular transport may underlie the CBD-induced resistance to platinum drugs (Fig. 4).

3.3. Observed protective effect of CBD against platinum-based drugs is shared by cannabis plant extracts

Instead of pure CBD, many patients use more or less defined cannabis



	Mock		CBD			Mock		CBD			Mock		CBD	
CisPt	IC50	IC75	IC50	IC75	CarboPt	IC50	IC75	IC50	IC75	OxPt	IC50	IC75	IC50	IC75
	5.54	8.82	8.78	16.83		88.25	148.70	129.50	249.00		4.79	39.88	6.56	50.03

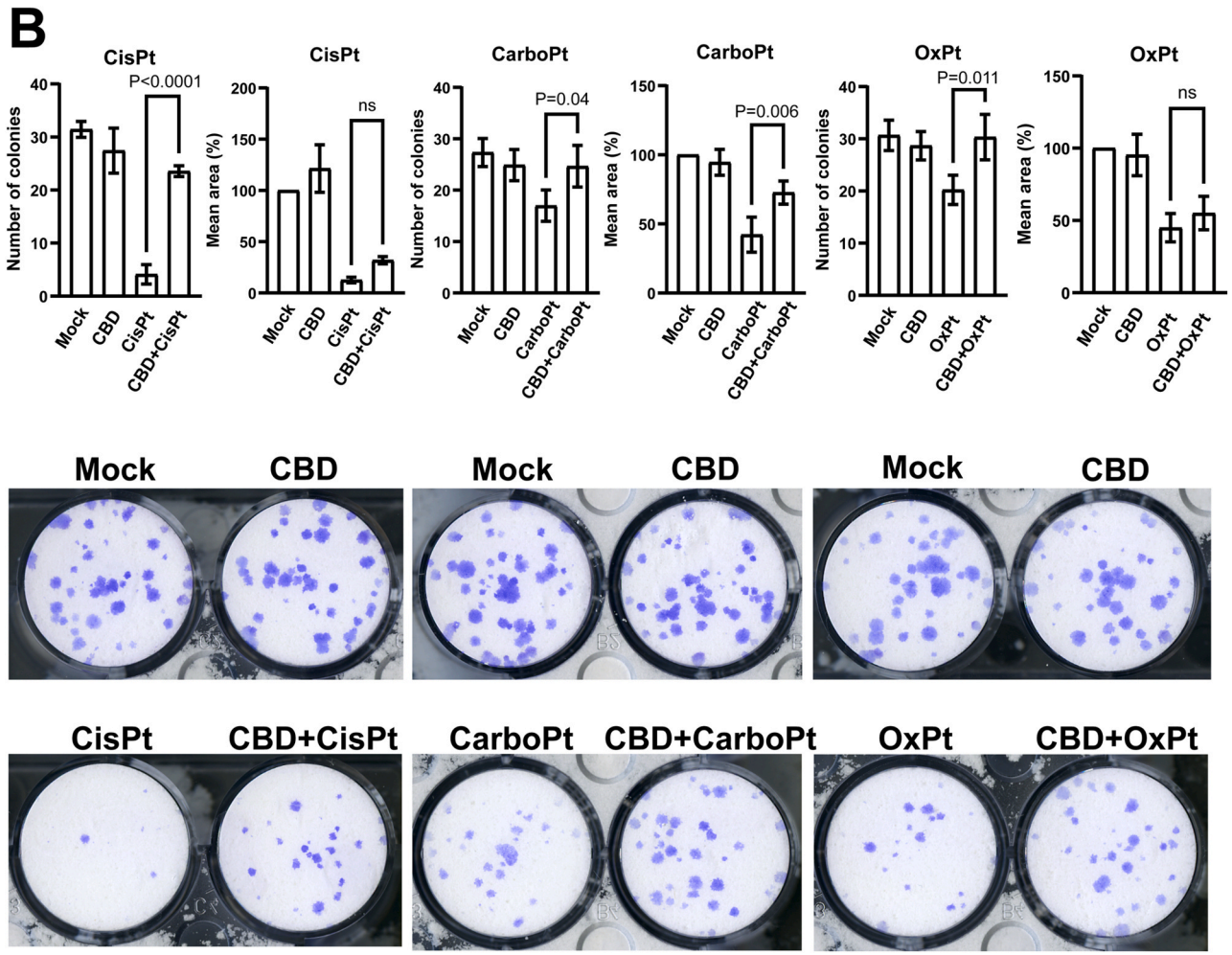


Fig. 1. Cannabidiol (CBD) protects U-2-OS cells against platinum-based drugs. A) Cells pretreated with 2.5 μM CBD and treated with increasing concentrations of cisplatin (CisPt) and carboplatin (CarboPt) were more resistant to platinum-based drugs compared to mock-pretreated cells. Cells were pretreated for 17 h and treated for 72 h. The result was assessed by crystal violet assay. The IC50 and IC75 values (μM), shown in the table below the graphs were calculated using logarithmic transformation and nonlinear regression. The final graphs present the mean and standard deviation of three independent experiments (n = 3). B) The results of the 12-day colony formation assay reveal substantial interference of CBD with all three tested platinum-based drugs (including oxaliplatin [OxPt]). Cells were pretreated with 2.5 μM CBD for 17 h, before treating with CisPt (2.5 μM), CarboPt (5 μM), or OxPt (5 μM) and grown for 12 days. The number of colonies and mean colony area were plotted on the graphs. For bar graphs, one-way ANOVA was used to calculate the P-value. The result represents the mean and standard deviation of three independent experiments (n = 3).

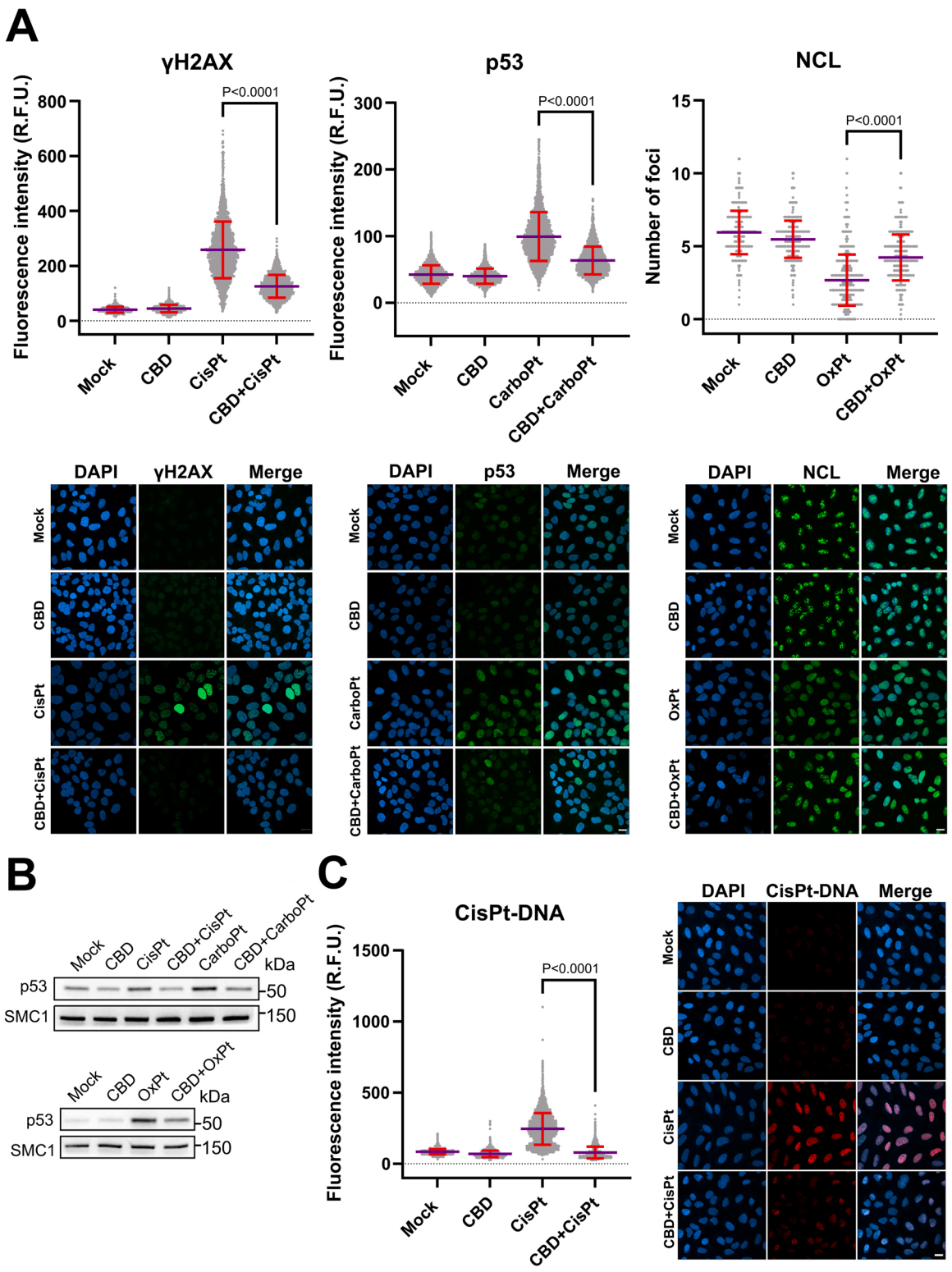


Fig. 2. Cannabidiol (CBD) protects U-2-OS cells from platinum-based drug-induced stress responses. A) CBD decreased DNA damage (measured as γH2AX) after cisplatin (CisPt) treatment, attenuated induction of p53 after carboplatin (CarboPt) treatment, and increased the number of compact nucleoli after oxaliplatin (OxPt) treatment (as evaluated by nucleolin localization). The cells were pretreated with 10 μM CBD for 17 h, before treating with CisPt (10 μM), CarboPt (100 μM), or OxPt (10 μM) for 24 h. The cells were analyzed using fluorescence microscopy (scale bar: 20 μm). For scatter plots, one-way ANOVA was used to calculate the P-value. The result represents the mean and standard deviation of three independent experiments (n = 3). B) Western blot analysis shows that CBD-pretreated cells accumulated less p53 after platinum-based drug treatment. Cells were pretreated with 10 μM CBD for 17 h, before treating with CisPt (10 μM), CarboPt (100 μM), and OxPt (10 μM) for 24 h. The result represents one of three independent experiments. C) CBD decreased CisPt-DNA adducts in CisPt-treated cells compared to mock-pretreated and CisPt-treated. Cells were pretreated with 10 μM CBD for 17 h, before treating with CisPt (20 μM) for 24 h, and analyzed by quantitative immunofluorescence microscopy (scale bar: 20 μm). For scatter plots, one-way ANOVA was used to calculate the P-value. The result represents the mean and standard deviation of three independent experiments (n = 3).

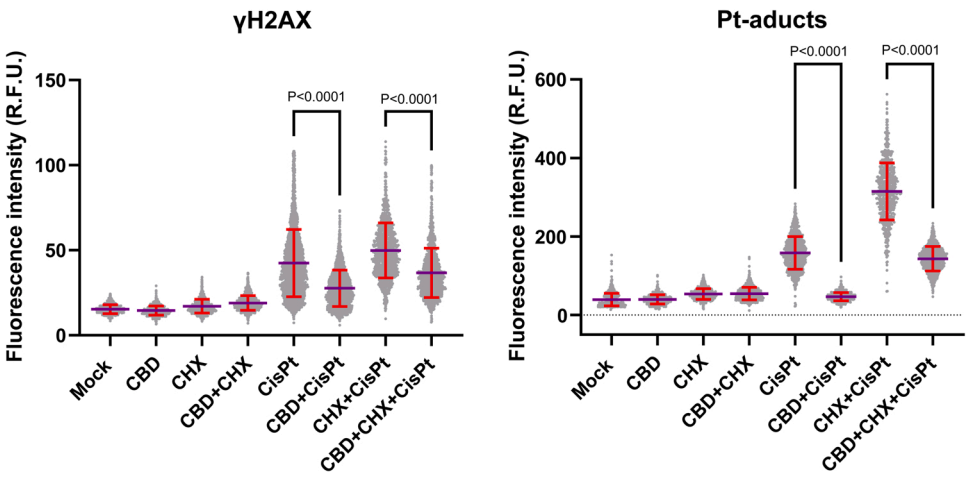


Fig. 3. Inhibited translation does not limit the protective effect of cannabidiol (CBD) against cisplatin (CisPt) in U-2-OS cells. Coadministration of cycloheximide (CHX) does not affect the CBD-promoted rescue effects of cis-platinum measured either as DNA damage (via γH2AX signal) or as the presence of cis-platinated DNA. Cells were treated with 50 μg/mL of CHX, 10 μM CBD, and 20 μM CisPt (10 μM CisPt for γH2AX detection) concomitantly for 17 h. Cells were fixed, stained, and analyzed using quantitative immunofluorescence microscopy. For scatter plots, one-way ANOVA was used to calculate the P-value. The result represents the mean and standard deviation of three independent experiments (n = 3).

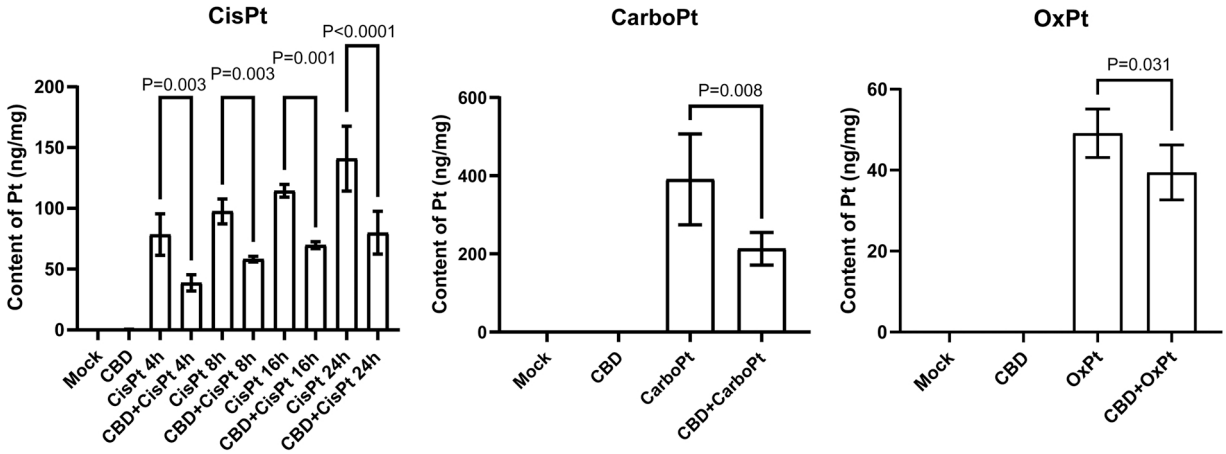


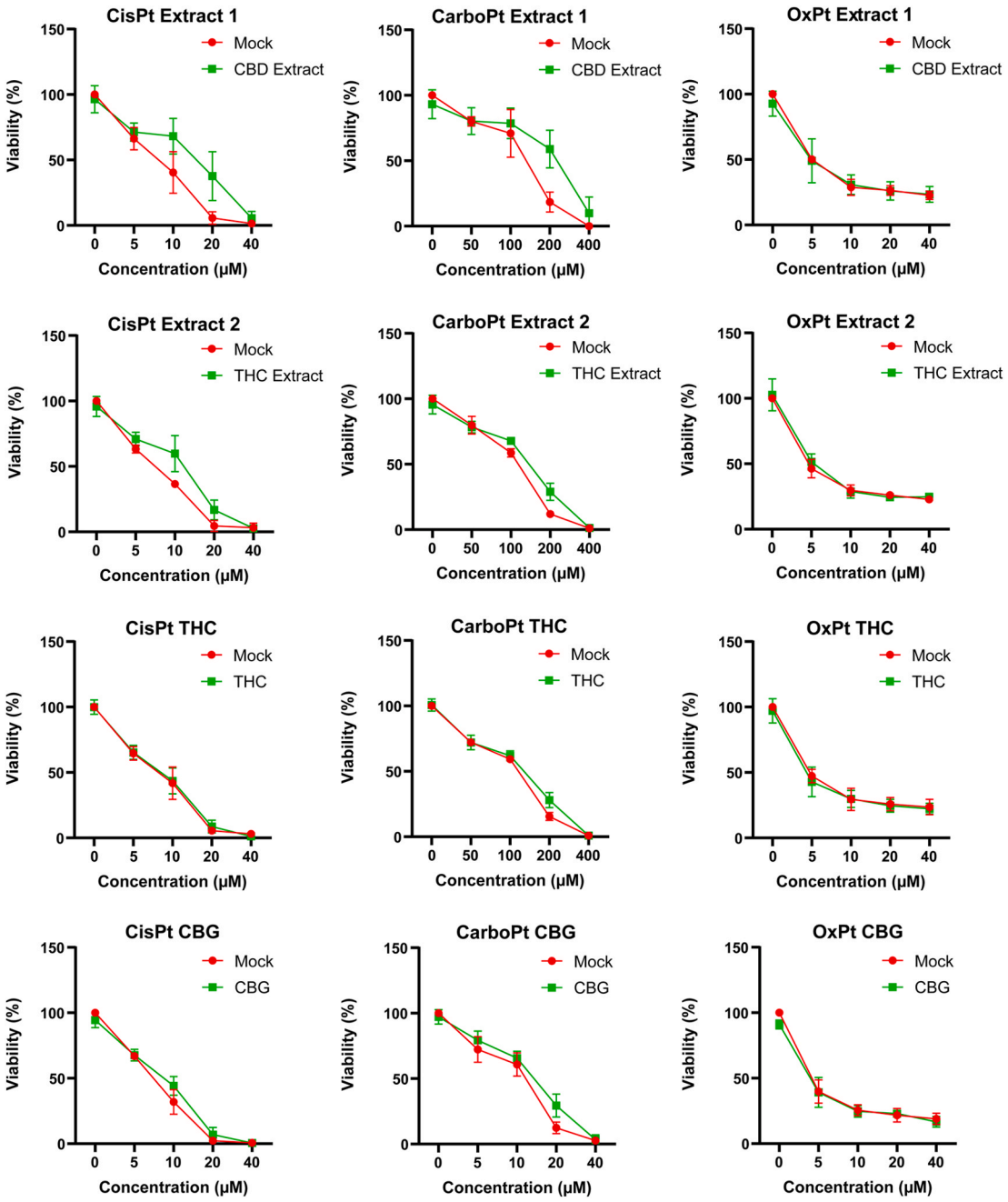
Fig. 4. U-2-OS cells pretreated by cannabidiol (CBD) internalize less platinum. Trace elemental analysis showed a lower platinum content in cells subjected to combined treatment compared to those subjected to platinum-based drug only. Cells were pretreated with 10 μM CBD for 17 h, before treating with CisPt (20 μM) for 4, 8, 16, and 24 h, or CarboPt (200 μM) and OxPt (20 μM) for 24 h. The content of platinum was re-counted at 1 mg of protein. For bar graphs, one-way ANOVA was used to calculate the P-value. The result represents the mean and standard deviation of three independent experiments (n = 3).

plant extracts containing additional biologically active cannabinoids and other compounds. Therefore, we took advantage of the availability of cannabis extracts produced from two contrasting chemotypes (CBD dominant versus THC dominant), which were grown under defined conditions (see material and methods for more details and ST1). The protective effect against platinum drug toxicity that we observed for the CBD dominant variant was equally or even more potent than cell cotreatment by CBD-alone (Fig. 5). However, the extract from the Δ^9-THC dominant variant was considerably less protective, suggesting that Δ^9-THC itself may not be involved in the CBD-induced drug resistance. Consistently, further experiments with Δ^9-THC pretreatment alone revealed no impact on the cellular sensitivity against platinum drugs (Fig. 5). Finally, we also tested the effect of cannabigerol (CBG), another cannabinoid typically present in cannabis plant extracts. CBG pretreatment displayed a significant, albeit less pronounced induction of resistance against CisPt and CarboPt (Fig. 5), indicating that protection against platinum-based drugs may be shared among diverse cannabinoids.

4. Discussion

Our present study sheds more light on the causes of acquired chemoresistance, one of contemporary oncology's most essential and challenging topics. Specifically, we have identified a so-far unrecognized interference of CBD and cannabis extracts with the anticancer effects of three clinically commonly used platinum-based chemotherapeutics, resulting in acutely induced resistance of several human cancer cell models to these anticancer drugs. Furthermore, we also provide mechanistic insights into the observed cannabis product-induced chemoresistance. First, our functional assays aiming at better understanding this cannabinoid-induced chemoresistance phenomenon first excluded metallothioneins, a class of proteins that cause resistance to some other anticancer compounds [17] and which are transcriptionally induced by cannabinoids through the activity of the MTF1 transcription factor, as a potential mediator of resistance to platinum-derived drugs. Second, we further excluded that the acutely induced resistance to platinum drugs heavily relies on either ongoing transcription or protein translation in the target cells. Overall, our results narrowed the resistance mechanism using ICP-MS to cellular drug transport and/or retention in a translational-independent manner. We propose that cannabinoids limit the cellular uptake or accelerate the elimination of platinum-derived drugs from human cells and that this mechanism provides a major contribution to, though not necessarily the entire explanation for, the observed robust resistance to cytotoxic effects of clinically used platinum-derived chemotherapy in preclinical model experiments.

Previous studies of platinum-based medications examined a range of potentially involved membrane transporters, with OCT1–3, MATE1/2,



	Mock		Ex 1	
CisPt	IC50	IC75	IC50	IC75
	7.49	12.67	15.41	27.47
	Mock		Ex 2	
	IC50	IC75	IC50	IC75
	6.91	11.85	11.37	18.76
	Mock		THC	
	IC50	IC75	IC50	IC75
	7.43	12.95	7.72	13.61
	Mock		CBG	
	IC50	IC75	IC50	IC75
	6.92	10.87	8.59	14.00

	Mock		Ex 1	
CarboPt	IC50	IC75	IC50	IC75
	135.40	187.70	242.60	325.20
	Mock		Ex 2	
	IC50	IC75	IC50	IC75
	109.30	163.70	144.90	222.70
	Mock		THC	
	IC50	IC75	IC50	IC75
	105.80	176.80	116.50	213.70
	Mock		CBG	
	IC50	IC75	IC50	IC75
	107.40	174.00	137.50	225.10

	Mock		Ex 1	
OxPt	IC50	IC75	IC50	IC75
	3.78	22.76	4.67	28.93
	Mock		Ex 2	
	IC50	IC75	IC50	IC75
	2.93	23.31	3.60	21.80
	Mock		THC	
	IC50	IC75	IC50	IC75
	3.09	23.69	2.50	23.34
	Mock		CBG	
	IC50	IC75	IC50	IC75
	1.88	14.83	2.58	17.58

Fig. 5. Cannabis extracts and cannabigerol (CBG) provide similar rescue effects to cannabidiol (CBD) against cisplatin (CisPt) and carboplatin (CarboPt) in U-2-OS cells. CBD-enriched extract (Extract 1) induced a strong rescue effect against CisPt and CarboPt. Δ^9-THC enriched extract (Extract 2) had a moderate protective effect against CisPt and CarboPt. Δ^9-tetrahydrocannabinol (Δ^9-THC) alone did not modulate cell sensitivity against platinum-based drugs. CBG had a protective effect on cells treated with CisPt and CarboPt, which was comparable to that observed with Extract 2. Cells were pretreated with extracts to reach a 2.5 μM concentration of the primary cannabinoid for 17 h, followed by platinum-based drugs for 72 h. The result was assessed by crystal violet assay. The IC50 and IC75 values were calculated using logarithmic transformation and nonlinear regression. The final graphs present the mean and standard deviation of three independent experiments (n = 3).

CTR1, ATP7A/7B, and MRP2/4 among the most studied [6,23,24], and even more have been considered recently [25,26]. Whether or to what extent any of these transporters are direct CBD targets remains to be elucidated. Potential mechanisms by which CBD may influence the transport of platinum-based drugs in a translation-independent manner include direct interaction with transporters, modulation of transporter phosphorylation, and altered transporter localization. Direct interaction with transporters could occur through competition with substrates, as has been shown for CBD and prazosin in the case of the BCRP transporter [27]. Posttranslational modification of transporters is another proposed mechanism by which CBD could interfere with transport. For example, Δ9-THC has been shown to indirectly modulate the phosphorylation of the divalent metal transporter-1[28]. Lastly, altered transporter localization is another possibility. It has been shown that CBD can affect membrane composition [29]. Interestingly, our ICP-MS data revealed that CBD significantly increases the intracellular amount of zinc (S7). However, whether and how this phenomenon is related to platinum drug uptake requires further research. Nevertheless, the resulting increase in intracellular zinc levels provides a mechanistic explanation for the CBD-mediated expression of metallothioneins reported previously [30], linking this intriguing effect of cannabinoids with the known fact that increased intracellular zinc levels trigger MTF1-mediated expression of metallothioneins [31–33].

Importantly and highly relevant for oncology, our data suggest that simultaneous use of cannabis products, mainly those containing high amounts of CBD, has the potential to negatively affect the treatment of cancer patients with the widely used standard-of-care platinum-based drugs. This result is crucial because many cancer patients search for ways to support their treatment and/or relieve the adverse effects of standard chemotherapy, and cannabis products are often considered in such a context as an increasingly available option to provide both properties. Indeed, cannabis has been demonstrated to lessen several adverse conditions associated with cancer treatment, such as pain, nausea, vomiting, depression, and weight loss [16,34,35]. Moreover, direct anticancer effects of cannabis, through different proposed mechanisms, have also been suggested [7,9,15,16]. Two products, Nabilone (a synthetic cannabinoid) and Dronabinol (synthetic Δ^9-THC), have been approved by the FDA for attenuation of chemotherapy-induced nausea and vomiting [14]. Additionally, Sativex (Δ^9-THC:CBD) is a medicine under evaluation for relieving cancer-related pain [36–40]. In light of our data, cotreatment with these products under ongoing chemotherapy with platinum drugs should better be avoided, or at least Dronabinol should be the preferred choice in such clinical settings.

The potential interplay of platinum-based drugs with cannabis products has been studied to some extent at the preclinical level, including efficacy modulation. However, the results so far have been limited and inconsistent. For example, in line with our observation, CBD had been reported to attenuate CarboPt- and CisPt-mediated cytotoxic effects in canine urothelial carcinoma cells [41] and malignant melanoma cell lines [42], respectively. In contrast, some studies suggested that combined treatment might be more effective in regimens containing CisPt [43], while oxaliplatin combination with CBD was proposed to overcome OxPt resistance in colorectal cancer cell lines [44]. Furthermore, some relevant clinical studies that are presently at the early planning stage (NCT04585841 [45], NCT03607643 [46], NCT04582591 [47], NCT04398446 [48]) aim to evaluate CBD-mediated attenuation of OxPt-induced side effects. There is also a retrospective analysis suggesting the beneficial effects of cannabis on neuropathic pain in OxPt-treated patients [49]. However, our present results strongly suggest that this area of cancer research should first be very carefully evaluated before any widespread clinical applications should be recommended. Indeed, in the light of our present dataset, it is plausible that the attenuation of platinum-based drug-induced adverse effects in patients might reflect (at least partially) the overall decreased intracellular availability of the drugs, resulting in reduced treatment efficacy and, as a consequence, also reduced adverse effects.

Overall, we hope that our findings will motivate further research to identify the cellular transporter(s) affected by cannabinoids that are involved in platinum-based drug transport. Simultaneously, our results should serve as a warning for patients and physicians to carefully reconsider and better avoid combining platinum-based therapy and cannabis products containing high levels of CBD, at least until this drug interference phenomenon becomes better assessed in both preclinical and especially clinical settings.

5. Conclusion

In this study, we identified clinically relevant negative interactions between cannabis and the three most commonly used platinum-based anticancer chemotherapeutics. Additionally, we found that such cannabinoid-induced chemoresistance is translationally independent and reflects altered cellular drug transport. Our data should serve as a warning relevant for concurrent usage of some cannabis products by patients undergoing platinum-based treatment, to avoid (until more information on this issue becomes available) a potential negative impact of such combination on therapy outcome.

CRediT authorship contribution statement

Tereza Buchtova, Martin Mistrik, and Jiri Bartek designed the experiments, interpreted the data, and write the manuscript; Tereza Buchtova performed most of the cellular experiments; Lucie Beresova contributed to the cytotoxicity tests, microscopic experiments, and colony formation assays; Katarina Chroma, Martin Mistrik contributed to the microscopic experiments and cytotoxicity tests, Tomas Pluhacek performed measurements of platinum content, Tibor Beres, Dominika Kaczorova, and Petr Tarkowski prepared and analyzed cannabis extracts.

Conflicts of interest statement

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

Acknowledgement

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Ethics approval and consent to participate

Not applicable.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2023.114801.

References

[1] E. Wiltshaw, Cisplatin in the treatment of cancer, Platin. Met. Rev. 23 (1979) 90–98.

[2] D. Tsvetkova, S. Ivanova, Application of approved cisplatin derivatives in combination therapy against different cancer diseases, Molecules 27 (2022) 2466, https://doi.org/10.3390/molecules27082466.

[3] S. Dasari, P.B. Tchounwou, Cisplatin in cancer therapy: molecular mechanisms of action, Eur. J. Pharm. 740 (2014) 364–378, https://doi.org/10.1016/j.ejphar.2014.07.025.

[4] R. Oun, Y.E. Moussa, N.J. Wheate, The side effects of platinum-based chemotherapy drugs: a review for chemists, Dalton Trans. 47 (2018) 6645–6653, https://doi.org/10.1039/c8dt00838h.

[5] S. Dilruba, G.V. Kalayda, Platinum-based drugs: past, present and future, Cancer Chemother. Pharm. 77 (2016) 1103–1124, https://doi.org/10.1007/s00280-016-2976-z.

[6] J. Zhou, Y. Kang, L. Chen, H. Wang, J. Liu, S. Zeng, L. Yu, The drug-resistance mechanisms of five platinum-based antitumor agents, Front Pharm. 11 (2020) 343, https://doi.org/10.3389/fphar.2020.00343.

[7] F. Pellati, V. Borgonetti, V. Brighenti, M. Biagi, S. Benvenuti, L. Corsi, Cannabis sativa L. and nonpsychoactive cannabinoids: their chemistry and role against oxidative stress, inflammation, and cancer, Biomed. Res Int 2018 (2018) 1691428, https://doi.org/10.1155/2018/1691428.

[8] F.F. Peres, A.C. Lima, J.E.C. Hallak, J.A. Crippa, R.H. Silva, V.C. Abílio, Cannabidiol as a promising strategy to treat and prevent movement disorders? Front Pharm. 9 (2018) 482, https://doi.org/10.3389/fphar.2018.00482.

[9] S. Pisanti, A.M. Malfitano, E. Ciaglia, A. Lamberti, R. Ranieri, G. Cuomo, M. Abate, G. Faggiana, M.C. Proto, D. Fiore, C. Laezza, M. Bifulco, Cannabidiol: State of the art and new challenges for therapeutic applications, Pharm. Ther. 175 (2017) 133–150, https://doi.org/10.1016/j.pharmthera.2017.02.041.

[10] M.C. Weiss, J.E. Hibbs, M.E. Buckley, S.R. Danese, A. Leitenberger, M. Bollmann-Jenkins, S.W. Meske, K.E. Aliano-Ruiz, T.W. McHugh, S.L. Larson, E.H. Le, N. L. Green, P.B. Gilman, V.G. Kaklamani, R.T. Chlebowski, D.M. Martinez, A. Coala-T-Cannabis, Survey Study of breast, Cancer patients' Use Cannabis , , Treat., Cancer 128 (2022) 160–168, https://doi.org/10.1002/cncr.33906.

[11] S.A. Pergam, M.C. Woodfield, C.M. Lee, G.-S. Cheng, K.K. Baker, S.R. Marquis, J. R. Fann, Cannabis use among patients at a comprehensive, Cancer Cent. a State Leg. Med. Recreat. Use, Cancer 123 (2017) 4488–4497, https://doi.org/10.1002/cncr.30879.

[12] S. Pisanti, M. Bifulco, Medical Cannabis: A plurimillennial history of an evergreen, J. Cell Physiol. 234 (2019) 8342–8351, https://doi.org/10.1002/jcp.27725.

[13] M.E. Badowski, P.K. Yanful, Dronabinol oral solution in the management of anorexia and weight loss in AIDS and cancer, Ther. Clin. Risk Manag 14 (2018) 643–651, https://doi.org/10.2147/TCRM.S126849.

[14] B. Todaro, Cannabinoids in the treatment of chemotherapy-induced nausea and vomiting, J. Natl. Compr. Canc Netw. 10 (2012) 487–492, https://doi.org/10.6004/jnccn.2012.0048.

[15] P. Massi, M. Solinas, V. Cinquina, D. Parolaro, Cannabidiol as potential anticancer drug, Br. J. Clin. Pharm. 75 (2013) 303–312, https://doi.org/10.1111/j.1365-2125.2012.04298.x.

[16] T. Buchtova, D. Lukac, Z. Skrott, K. Chroma, J. Bartek, M. Mistrik, Drug–Drug Interactions of Cannabidiol with Standard-of-Care Chemotherapeutics, Int. J. Mol. Sci. 24 (2023) 2885, https://doi.org/10.3390/ijms24032885.

[17] T. Buchtova, Z. Skrott, K. Chroma, J. Rehulka, P. Dzubak, M. Hajduch, D. Lukac, S. Arampatzis, J. Bartek, M. Mistrik, Cannabidiol-induced activation of the metallothionein pathway impedes anticancer effects of disulfiram and its metabolite CuET, Mol. Oncol. (2021), https://doi.org/10.1002/1878-0261.13114.

[18] N.D. Sarma, A. Waye, M.A. ElSohly, P.N. Brown, S. Elzinga, H.E. Johnson, R. J. Marles, J.E. Melanson, E. Russo, L. Deyton, C. Hudalla, G.A. Vrdoljak, J. H. Wurzer, I.A. Khan, N.-C. Kim, G.I. Giancaspro, Cannabis Inflorescence for Medical Purposes: USP Considerations for Quality Attributes, J. Nat. Prod. 83 (2020) 1334–1351, https://doi.org/10.1021/acs.jnatprod.9b01200.

[19] P.L. Olive, J.P. Banáth, Kinetics of H2AX phosphorylation after exposure to cisplatin, Cytom. B Clin. Cytom. 76 (2009) 79–90, https://doi.org/10.1002/cyto.b.20450.

[20] S. Siemer, D. Ornskov, B. Guerra, B. Boldyreff, O.G. Issinger, Determination of mRNA, and protein levels of p53, MDM2 and protein kinase CK2 subunits in F9 cells after treatment with the apoptosis-inducing drugs cisplatin and carboplatin, Int J. Biochem Cell Biol. 31 (1999) 661–670, https://doi.org/10.1016/s1357-2725(99)00020-5.

[21] T. Ozdian, D. Holub, Z. Maceckova, L. Varanasi, G. Rylova, J. Rehulka, J. Vaclavkova, H. Slavik, P. Moudry, P. Znojek, J. Stankova, J.B. de Sanctis, M. Hajduch, P. Dzubak, Proteomic profiling reveals DNA damage, nucleolar and ribosomal stress are the main responses to oxaliplatin treatment in cancer cells, J. Proteom. 162 (2017) 73–85, https://doi.org/10.1016/j.jprot.2017.05.005.

[22] S. Schoch, S. Gajewski, J. Rothfuß, A. Hartwig, B. Köberle, Comparative study of the mode of action of clinically approved platinum-based chemotherapeutics, Int J. Mol. Sci. 21 (2020) 6928, https://doi.org/10.3390/ijms21186928.

[23] S. Harrach, G. Ciarimboli, Role of transporters in the distribution of platinum-based drugs, Front Pharm. 6 (2015) 85, https://doi.org/10.3389/fphar.2015.00085.

[24] H. Burger, W.J. Loos, K. Eechoute, J. Verweij, R.H.J. Mathijssen, E.A.C. Wiemer, Drug transporters of platinum-based anticancer agents and their clinical significance, Drug Resist Updat 14 (2011) 22–34, https://doi.org/10.1016/j.drup.2010.12.002.

[25] D. Wu, T. Liu, S. Deng, R. Han, Y. Xu, SLC39A4 expression is associated with enhanced cell migration, cisplatin resistance, and poor survival in non-small cell lung cancer, Sci. Rep. 7 (2017), https://doi.org/10.1038/s41598-017-07830-4.

[26] M. Sobczak, M. Strachowska, K. Gronkowska, A. Robaszkiewicz, Activation of ABCC Genes by Cisplatin Depends on the CoREST Occurrence at Their Promoters in A549 and MDA-MB-231 Cell Lines, Cancers (Basel) 14 (2022) 894, https://doi.org/10.3390/cancers14040894.

[27] L.L. Anderson, M.G. Etchart, D. Bahceci, T.A. Golembiewski, J.C. Arnold, Cannabis constituents interact at the drug efflux pump BCRP to markedly increase plasma cannabidiolic acid concentrations, Sci. Rep. 11 (2021) 14948, https://doi.org/10.1038/s41598-021-94212-6.

[28] Y.A. Seo, R. Kumara, H. Wetli, M. Wessling-Resnick, Regulation of divalent metal transporter-1 by serine phosphorylation, Biochem J. 473 (2016) 4243–4254, https://doi.org/10.1042/BCJ20160674.

[29] P. Bielawiec, S. Dziemitko, K. Konstantynowicz-Nowicka, A. Chabowski, J. Dzięcioł, E. Harasim-Symbor, Cannabidiol improves muscular lipid profile by affecting the expression of fatty acid transporters and inhibiting de novo lipogenesis, Sci. Rep. 13 (2023) 3694, https://doi.org/10.1038/s41598-023-30872-w.

[30] A. Juknat, N. Rimmerman, R. Levy, Z. Vogel, E. Kozela, Cannabidiol affects the expression of genes involved in zinc homeostasis in BV-2 microglial cells, Neurochem Int 61 (2012) 923–930, https://doi.org/10.1016/j.neuint.2011.12.002.

[31] B. Zhang, O. Georgiev, M. Hagmann, Ç. Günes, M. Cramer, P. Faller, M. Vasák, W. Schaffner, Activity of Metal-Responsive Transcription Factor 1 by Toxic Heavy Metals and H2O2 In Vitro Is Modulated by Metallothionein, Mol. Cell Biol. 23 (2003) 8471–8485, https://doi.org/10.1128/MCB.23.23.8471-8485.2003.

[32] F. Otsuka, Transcription Factor MTF-1 Involved in the Cellular Response to Zinc, Yakugaku Zasshi 141 (2021) 857–867, https://doi.org/10.1248/yakushi.20-00247.

[33] V. Günther, U. Lindert, W. Schaffner, The taste of heavy metals: Gene regulation by MTF-1, Biochim. Et. Biophys. Acta (BBA) - Mol. Cell Res. 1823 (2012) 1416–1425, https://doi.org/10.1016/j.bbamcr.2012.01.005.

[34] T.L. Mortimer, T. Mabin, A.-M. Engelbrecht, Cannabinoids: the lows and the highs of chemotherapy-induced nausea and vomiting, Future Oncol. 15 (2019) 1035–1049, https://doi.org/10.2217/fon-2018-0530.

[35] N. Montero-Oleas, I. Arevalo-Rodriguez, S. Nuñez-González, A. Viteri-García, D. Simancas-Racines, Therapeutic use of cannabis and cannabinoids: an evidence mapping and appraisal of systematic reviews, BMC Complement Med Ther. 20 (2020) 12, https://doi.org/10.1186/s12906-019-2803-2.

[36] J.R. Johnson, M. Burnell-Nugent, D. Lossignol, E.D. Ganae-Motan, R. Potts, M. T. Fallon, Multicenter, double-blind, randomized, placebo-controlled, parallel-group study of the efficacy, safety, and tolerability of THC:CBD extract and THC extract in patients with intractable cancer-related pain, J. Pain. Symptom Manag. 39 (2010) 167–179, https://doi.org/10.1016/j.jpainsymman.2009.06.008.

[37] R.K. Portenoy, E.D. Ganae-Motan, S. Allende, R. Yanagihara, L. Shaiova, S. Weinstein, R. McQuade, S. Wright, M.T. Fallon, Nabiximols for opioid-treated cancer patients with poorly-controlled chronic pain: a randomized, placebo-controlled, graded-dose trial, J. Pain. 13 (2012) 438–449, https://doi.org/10.1016/j.jpain.2012.01.003.

[38] J.R. Johnson, D. Lossignol, M. Burnell-Nugent, M.T. Fallon, An open-label extension study to investigate the long-term safety and tolerability of THC/CBD oromucosal spray and oromucosal THC spray in patients with terminal cancer-related pain refractory to strong opioid analgesics, J. Pain. Symptom Manag. 46 (2013) 207–218, https://doi.org/10.1016/j.jpainsymman.2012.07.014.

[39] M.T. Fallon, E. Albert Lux, R. McQuade, S. Rossetti, R. Sanchez, W. Sun, S. Wright, A.H. Lichtman, E. Kornyeyeva, Sativex oromucosal spray as adjunctive therapy in advanced cancer patients with chronic pain unalleviated by optimized opioid therapy: two double-blind, randomized, placebo-controlled phase 3 studies, Br. J. Pain. 11 (2017) 119–133, https://doi.org/10.1177/2049463717710042.

[40] A.H. Lichtman, E.A. Lux, R. McQuade, S. Rossetti, R. Sanchez, W. Sun, S. Wright, E. Kornyeyeva, M.T. Fallon, Results of a Double-Blind, Randomized, Placebo-Controlled Study of Nabiximols Oromucosal Spray as an Adjunctive Therapy in Advanced Cancer Patients with Chronic Uncontrolled Pain, J. Pain. Symptom Manag. 55 (2018) 179–188.e1, https://doi.org/10.1016/j.jpainsymman.2017.09.001.

[41] J.M. Inkol, S.E. Hocker, A.J. Mutsaers, Combination therapy with cannabidiol and chemotherapeutics in canine urothelial carcinoma cells, PLoS One 16 (2021), e0255591, https://doi.org/10.1371/journal.pone.0255591.

[42] P. Marzęda, P. Wróblewska-Łuczka, M. Drozd, M. Florek-Łuszczki, K. Załuska-Ogryzek, J.J. Łuszczki, Cannabidiol Interacts Antagonistically with Cisplatin and Additively with Mitoxantrone in Various Melanoma Cell Lines-An Isobolographic Analysis, Int J. Mol. Sci. 23 (2022) 6752, https://doi.org/10.3390/ijms23126752.

[43] O. Marinelli, M.B. Morelli, D. Annibali, C. Aguzzi, L. Zeppa, S. Tuyaerts, C. Amantini, F. Amant, B. Ferretti, F. Maggi, G. Santoni, M. Nabissi, The Effects of Cannabidiol and Prognostic Role of TRPV2 in Human Endometrial Cancer, Int J. Mol. Sci. 21 (2020) 5409, https://doi.org/10.3390/ijms21155409.

[44] S. Jeong, B.G. Kim, D.Y. Kim, B.R. Kim, J.L. Kim, S.H. Park, Y.J. Na, M.J. Jo, H. K. Yun, Y.A. Jeong, H.J. Kim, S.I. Lee, H.D. Kim, D.H. Kim, S.C. Oh, D.-H. Lee, Cannabidiol Overcomes Oxaliplatin Resistance by Enhancing NOS3- and SOD2-Induced Autophagy in Human Colorectal Cancer Cells, Cancers (Basel) 11 (2019), E781, https://doi.org/10.3390/cancers11060781.

[45] J.R. Andersen, The Effect of Medical Cannabidiol on Lean Body Mass in Patients Receiving Oxaliplatin or Paclitaxel Based Chemotherapy, clinicaltrials.gov, 2022. https://clinicaltrials.gov/ct2/show/NCT04585841 (accessed January 16, 2023).

[46] Leaf Vertical Inc, Randomized Double-Blind, Placebo-Controlled Parallel Multi-Center Study to Assess the Efficacy of Cannabidiol (BRCX014) Combined With Standard-Of-Care Treatment in Subjects With Multiple Myeloma, Glioblastoma Multiforme, and GI Malignancies, clinicaltrials.gov, 2018. https://clinicaltrials.gov/ct2/show/NCT03607643 (accessed April 18, 2022).
[47] Zealand University Hospital, A Phase II Trial of Cannabidiol for Prevention of Chemotherapy-induced Peripheral Neuropathy in Patients Receiving Oxaliplatin or Paclitaxel Based Chemotherapy, clinicaltrials.gov, 2022. https://clinicaltrials.gov/ct2/show/study/NCT04582591 (accessed January 16, 2023).
[48] Effect of Hemp-CBD on Patients With CIPN - Tabular View - ClinicalTrials.gov, n.d. https://clinicaltrials.gov/ct2/show/record/NCT04398446 (accessed January 18, 2023).
[49] B. Waissengrin, D. Mirelman, S. Pelles, F. Bukstein, D.T. Blumenthal, I. Wolf, R. Geva, Effect of cannabis on oxaliplatin-induced peripheral neuropathy among oncology patients: a retrospective analysis, Ther. Adv. Med Oncol. 13 (2021), https://doi.org/10.1177/1758835921990203.