

Potential cytotoxic effect of cannabidiol against human glioblastoma cells *in vitro*

Potencjalny efekt cytotoksyczny kannabidiolu względem ludzkich komórek glejaka wielopostaciowego

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Słowa kluczowe: kannabidiol, glejak wielopostaciowy, hodowle komórkowe.

Abstract

Introduction: In addition to its antiepileptic and anti-inflammatory properties, cannabidiol (CBD), a non-psychoactive component of cannabis, also has potential anticancer properties.

Aim of the research: Evaluation the effect of CBD on the cell viability of 2 human glioblastoma lines.

Material and methods: LN18 and LN229 cell lines relative to the physiological primary oligodendrocyte line MO3.13 were included in the study. The cells were exposed to CBD at increasing doses of 5, 10, 15, 20, 25, and 30 μM for 24 and 48 h. Cell viability after the planned incubation period was assessed using the commercial MTT test. Statistical calculations were evaluated with usage of Statistics Kingdom web application, and the IC_{50} value was assessed with usage of Quest Graph™ IC_{50} Calculator AAT Bioquest, Inc.

Results: A dose-dependent cytotoxic activity of CBD was found in reducing the viability of each of the tested cell types. The cytotoxic effect was comparable after 24 and 48 h of incubation. The IC_{50} value was the lowest for LN229 line cells and amounted to 8.9 μM and 8.8 μM after 24 and 48 h of incubation, respectively, and the highest for the MO3.13 line and amounted to 12.4 μM and 13.5 μM after 24 and 48 h of incubation, respectively.

Conclusions: CBD exerts a cytotoxic effect against glioblastoma cells. However, the reduction of viability also of physiological cell line suggests that the observed effect may not be selective for glioblastoma.

Streszczenie

Wprowadzenie: Oprócz właściwości przeciwpadaczkowych i przeciwzapalnych, kannabidiol (CBD), niepsychoaktywny składnik konopi ma również potencjalne właściwości przeciwnowotworowe.

Cel pracy: Ocena wpływu CBD na żywotność komórek dwóch linii komórkowych ludzkiego glejaka.

Materiał i metody: Do badania włączono linie komórkowe LN18 i LN229, porównując je z fizjologiczną linią pierwotnych oligodendrocytów MO3.13. Komórki poddano działaniu CBD w rosnących dawkach 5, 10, 15, 20, 25 i 30 μM przez 24 i 48 godzin. Żywotność komórek po okresie inkubacji oceniono przy użyciu komercyjnego testu MTT. Obliczenia statystyczne przeprowadzono przy użyciu aplikacji internetowej Statistics Kingdom, a wartość IC_{50} oceniono przy użyciu kalkulatora Quest Graph™ IC_{50} AAT Bioquest, Inc.

Wyniki: Stwierdzono, że zależna od dawki aktywność cytotoksyczna CBD zmniejsza żywotność każdego z badanych typów komórek. Efekt cytotoksyczny był porównywalny po 24 i 48 godzinach inkubacji. Wartość IC_{50} była najniższa dla komórek linii LN229 i wynosiła odpowiednio 8,9 μM i 8,8 μM po 24 oraz 48 godzinach inkubacji, a najwyższa dla linii MO3.13 i wynosiła odpowiednio 12,4 μM i 13,5 μM po 24 oraz 48 godzinach inkubacji.

Wnioski: Wyniki wskazują, że CBD wywiera efekt cytotoksyczny na komórki glejaka. Jednak zauważony wpływ na zmniejszenie żywotności fizjologicznej linii komórkowej sugeruje, że obserwowane oddziaływanie może nie być wybiórcze względem komórek glejaka.

Introduction

Cannabidiol (CBD) is a non-psychoactive component of cannabis [1]. Unlike tetrahydrocannabinol (THC), the use of CBD does not raise any major medical, ethical, or legal controversies worldwide

[2]. Preparations containing CBD, most often available in the form of oil or dried substances, are used as sedatives, relieving pain or muscle tension, although the indications for the use of CBD seem to be much broader [3]. CBD is also used in veterinary medicine as an analgesic and anti-anxiety agent [4]. Approved

for use by the US Food and Drug Administration and the European Medicines Agency, a drug containing CBD (Epidyolex, 100 mg/ml) is used in the treatment of rare, life-threatening, and chronically debilitating types of drug-resistant epilepsy [5]. However, single reports also indicate that CBD may have anti-cancer effects (for review see [6]). Glioblastoma (GBM) is one of the most lethal central nervous system (CNS) tumours in adults. Standard therapy for GBM (maximum safe surgical resection followed by adjuvant radiotherapy and chemotherapy) is still unsatisfactory, but it has remained unchanged for almost 20 years [7]. Therefore, the search for new methods of treatment and new substances that can be used in GBM therapy is highly desirable. Natural substances of animal or plant origin have great potential to become anticancer drugs [8]. A previous report revealed that CBD administered by inhalation seems able not only to limit GBM growth but also to alter the dynamics of the tumour micro-environment [9]. A retrospective study of a cohort of 15 consecutive, unselected patients with GBM suggested that 400–600 mg CBD orally in addition to standard therapy extends the average survival time by approximately 3 months [10]. It is not clear whether CBD has a cytotoxic effect directly on cancer cells or whether its effect is related to other mechanisms indirectly leading to the inhibition of tumour progression.

Aim of the research

The aim of our work was to investigate the potential cytotoxic effect of CBD on selected GBM cell lines under *in vitro* conditions.

Material and methods

Cell culture

The human glioblastoma cell lines were obtained from ATCC (LN229 – ATCC CRL-2611; LN18 – ATCC CRL-2610). As control cells, MO3.13 (TebuBio), an immortal human-human hybrid cell line that express phenotypic characteristics of primary oligodendrocytes, was used. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% (v/v) foetal bovine serum, penicillin (10,000 U/ml), and streptomycin (10 mg/ml). The cells were incubated at 37°C, 5% CO₂ atmosphere. The cells were maintained in the logarithmic growth phase by regular passage at 80% confluence.

Cell viability assay

The cytotoxicity of the CBD was studied against human glioblastoma cell lines (LN229 and LN18) vs. primary oligodendrocytes cell line (MO3.13) used as a control line. After 24-h incubation in growth medium with the addition of 10% foetal bovine serum (FBS) on 96-well plates, the cells were treated with the fol-

lowing concentrations of CBD: 0 (vehicle), 5, 10, 15, 20, 25, and 30 µM in medium without FBS. The cells were cultured at 37°C in the presence of 5% CO₂-air for the next 24 and 48 h. CBD concentrations were selected on the basis of other studies available in the literature [11], which was verified during experiment by incubation with CBD concentrations > 30 µM resulting in rapid death of the vast majority of cells (data not shown). The CBD cytotoxicity was evaluated using the MTT colorimetric method based on the ability of viable cells to the transformation of yellow, soluble tetrazolium salts [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, MTT] to purple insoluble formazan, by cellular dehydrogenases. After incubation with different CBD concentrations, cell cultures were supplemented with 10 µl per well of 5 mg/ml MTT (Sigma-Aldrich, Saint Louis, MO, USA) stock in PBS, and the incubation was continued for 4 h at 37°C. Next, the medium with MTT was removed, and the formed crystals were dissolved in 100 µl of DMSO. The solution absorbance was measured at 570 nm, using a spectrophotometric plate reader Epoch, BioTek Instruments (Vermont, United States). The relative cytotoxic activity was determined as the amount of CBD capable of reducing 50% of cell viability (IC₅₀ value). The experiment was performed 4 times with 6 replicates for each concentration.

Statistical analysis

The results of cells viability were expressed as median values with first and third quartiles. Statistical analysis was performed using available on-line free software. The statistical significance of the differences between the control vehicle vs. groups incubated with differ CBD concentrations through 24 or 48 h was evaluated with usage of the Kruskal-Wallis Test calculator (Statistics Kingdom, 2017. web application, <https://www.statskingdom.com/kruskal-wallis-calculator.html>). Non-linear regression (curve fit) was used to establish IC₅₀ values of CBD after 24 and 48 h of incubation for all 3 cell lines, assessed with use of "Quest Graph™ IC50 Calculator" (AAT Bioquest, Inc., 15 Jan 2024, <https://www.aatbio.com/tools/ic50-calculator>). Values were considered significant with $p < 0.05$.

Results

A dose-dependent cytotoxic activity of CBD was found in reducing the viability of each of the tested cell types. This effect was observed even for the lowest tested CBD concentration (5 µM), but the result was not statistically significant for either of the incubation periods. A CBD concentration of 10 µM resulted in a statistically significant reduction in the viability of LN229 cells by 43% ($p < 0.001$) and MO3.13 cells by 23% ($p < 0.05$). Higher CBD concentrations (15–30 µM) significantly reduced the viability of all analysed

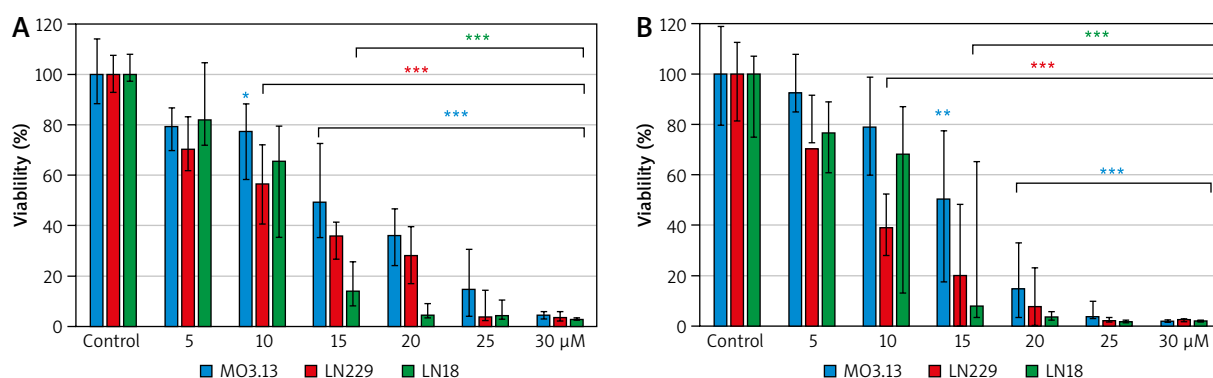


Figure 1. Viability of analysed cell lines (%) for 24 h (A) and 48 h (B) of incubation periods. Values were expressed as median and first and third quartiles. All CBD concentrations were compared to the control. Kruskal-Wallis test with Dunn's post-hoc test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

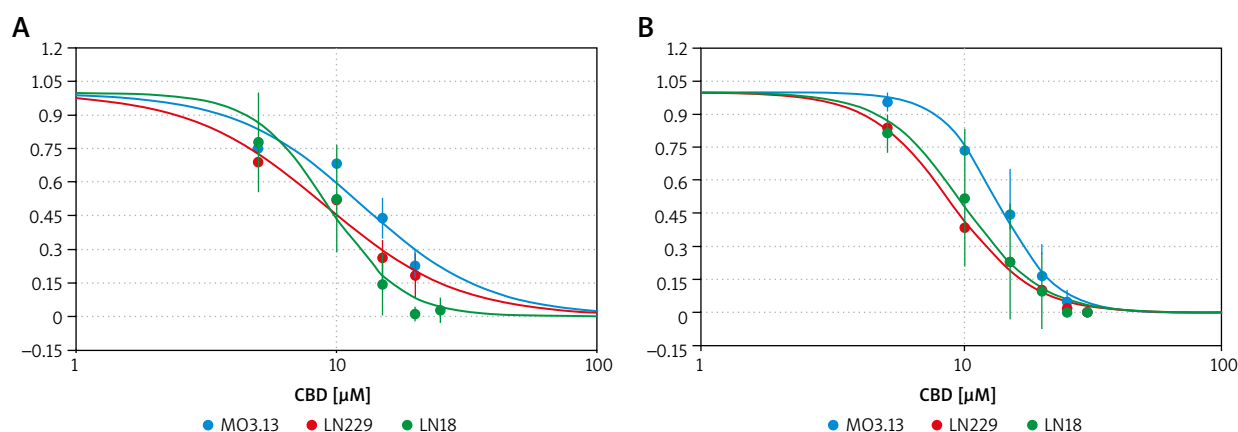


Figure 2. The IC₅₀ values (µM) regarding MO3.13, LN229, and LN18 cells after 24 (A) and 48 h (B) of incubation with CBD. Values were expressed as mean and standard error bars

cell lines in a dose-dependent manner ($p < 0.001$). In the case of 48 h of incubation, the decrease in viability obtained for LN229 cells was statistically significant at a CBD concentration of 10 µM (reduction by 61%, $p < 0.001$) and above, while for MO3.13 and LN18 cells at a concentration of 15 µM and above (reduction by 49% $p < 0.01$ and 92% $p < 0.001$ for MO3.13 and LN18, respectively, Figures 1 A, B).

The viability assessment was used by calculating the IC₅₀ value for each incubation period. The IC₅₀ value for CBD within of 24 h of incubation was 12.418 µM, 8.900 µM, and 9.185 µM for cell lines MO3.13, LN229, and LN18, respectively. In the case of 48 h of incubation, the calculated IC₅₀ was 13.512 µM, 8.810 µM, and 9.685 µM for MO3.13, LN229, and LN18 cells, respectively. The results are shown in Figures 2 A, B.

Discussion

Anticancer compounds exert various mechanisms of action, directly damaging tumour cells or indirectly by, for example, facilitating the penetration of chemotherapeutics into the tumour [12]. Our goal was to

check the effect of CBD on 2 human glioblastoma cell lines under *in vitro* conditions. The study showed that CBD has a dose-dependent cytotoxic effect on both evaluated lines: LN229 and LN18. Unfortunately, CBD showed comparable effects towards the MO3.13 line used in the experiment as a physiological control line. The results indicate that CBD may not selectively affect glioblastoma cells. Demonstrating the differences between the effects of CBD on physiological and pathological lines at this stage would indicate interesting properties of this compound that are potentially applicable in therapy. However, previous studies report that the IC₅₀ value was not always lower for cancer cells compared to healthy cells. In a study by Shokrzadeh Madieh *et al.* the IC₅₀ value of temozolomide was 29.19 µg/ml for the U-87 MG line (GBM line) compared to the IC₅₀ value against non-cancer WI-38 cells (human fibroblasts) of 4.94 µg/ml [13]. Another study assessing the effect of CBD on cancer lines (ovarian cancer A2780 and A2780/CP70) showed that IC₅₀ values for CBD were 2–3 times lower compared to non-cancerous lines (normal prostate epithelial PNT2 and

human retinal pigment ARPE19) indicating partially selective cytotoxicity of CBD against cancer cells [11]. In our study the IC_{50} of CBD against GBM lines were 10–15 times higher than the IC_{50} values obtained by Carmichael *et al.* for cisplatin against small cell lung cancer line [14]. An interesting observation was that the IC_{50} values for CBD were 4–8 times lower than another natural compound, curcumin, against GBM cell lines, suggesting higher cytotoxic potential of CBD vs. curcumin [15]. From the above results, it can be concluded that CBD may not be remarkably cytotoxic to all glioblastoma cell lines. However, observation does not rule out this substance as a potential candidate for further research because the cytotoxicity towards the physiological cell line was not greater than that of both cancer lines, but further studies should evaluate the effect of CBD on glioblastoma cells *in vivo*.

Another question is whether the CBD concentrations used in our experiment are achievable *in vivo*. Previous study showed, that in post-menopausal women taking CBD 100 mg or 300 mg daily, serum CBD concentrations ranged from 10 to 50 ng/ml, respectively [16]. Wang *et al.* revealed the maximum CBD concentration in cats' serum was about 300 ng/ml at a dose of 1.37 mg/kg body weight [17]. Other study showed that the plasma CBD concentration in dogs reaches up to 1000 ng/ml at a dose of 2.5 mg/kg body weight (when converted to molar concentration it gives up to 3 μ M) [18]. The concentration of CBD in rats' plasma reaches above 1000 ng/ml and in the brain; it reached over 2000 ng/g of tissue [19]. These results show that CBD is present in the blood and within CNS during therapy in relatively high doses, but lower than those used in our experiment. However, the 10-fold higher concentration (30 μ M compared to Ref [18]) used in our study reduced the viability of all analysed cell lines to several per cent, which suggests that very high doses of CBD may be toxic to cells *in vivo*. An important aspect of many studies testing substances of plant or animal origin is the limitation of using components in pure form, with a minimal addition (depending on the purity of the purchased reagent) of other substances naturally occurring in this case, in cannabis. We do not mean the so-called entourage effect, i.e. the common beneficial effect of all cannabis components when used together, because the presence of this effect is uncertain. However, the mutual relations of various substances, e.g. CBD reducing the psychoactive effect of THC, are known in pharmacology [20, 21]. Therefore, theoretically, a single substance may not have the same effect as, for example, full-spectrum CBD oil containing other non-psychoactive cannabinoids and terpenes. This issue certainly requires further research especially in terms of anti-cancer properties.

CBD is a non-psychoactive component of cannabis. Due to the controversy caused by the possibility of widespread use of hemp containing, among others, psychoactive THC, substances such as CBD seem attrac-

tive for research. Previous clinical observations as well as preclinical studies indicate that further research on the anti-cancer properties of CBD should be continued.

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Ethical approval

Not applicable.

Conflict of interest

The authors declare no conflict of interest.

References

1. Radwan MM, Chandra S, Gul S, ElSohly MA. Cannabinoids, phenolics, terpenes and alkaloids of cannabis. *Molecules*. 2021; 26(9): 2774.
2. Wheeldon J, Heidt J. Cannabis, research ethics, and a duty of care. *Res Ethics*. 2023; 19(3): 250-287.
3. O'Sullivan SE, Jensen SS, Nikolajsen GN, Bruun HZ, Bhuller R, Hoeng J. The therapeutic potential of purified cannabidiol. *J Cannabis Res*. 2023; 5: 21.
4. Corsato Alvarenga I, Wilson KM, McGrath S. Tolerability of long-term cannabidiol supplementation to healthy adult dogs. *J Vet Intern Med*. 2024; 38(1): 326-335.
5. Calapai F, Esposito E, Ammendolia I, Mannucci C, Calapai G, Currò M, Cardia L, Chinou I. Pharmacovigilance of unlicensed cannabidiol in European countries. *Phytother Res*. 2023; 38(1): 74-81.
6. Seltzer ES, Watters AK, MacKenzie Jr D, Granat LM, Zhang D. Cannabidiol (CBD) as a promising anti-cancer drug. *Cancers (Basel)*. 2020; 12(1): 3203.
7. Xiong Z, Raphael I, Olin M, Okada H, Li X, Kohanbash G. Glioblastoma vaccines: past, present, and opportunities. *EBioMedicine*. 2024; 100: 104963.
8. Małek A, Kocot J, Mitrowska K, Posyniak A, Kurzepa J. Bee venom effect on glioblastoma cells viability and gelatinase secretion. *Front Neurosci*. 2022; 16: 792970.
9. Khodadadi H, Salles ÉL, Alptekin A, Mehrabian D, Rutkowski M, Arbab AS, Yeudall WA, Yu JC, Morgan JC, Hess DC, Vaibhav K, Dhandapani KM, Baban B. Inhalant cannabidiol inhibits glioblastoma progression through regulation of tumor microenvironment. *Cannabis Cannabinoid Res*. 2021; 8(5): 824-834.
10. Likar R, Koestenberger M, Stutschnig M, Nahler G. Cannabidiol may prolong survival in patients with glioblastoma multiforme. *Cancer Diagn Progn*. 2021; 1(2): 77-82.
11. Sooda K, Allison SJ, Javid FA. Investigation of the cytotoxicity induced by cannabinoids on human ovarian carcinoma cells. *Pharmacol Res Perspect*. 2023; 11(6): e01152.
12. Tilsed CM, Fisher SA, Nowak AK, Lake RA, Lesterhuis WJ. Cancer chemotherapy: insights into cellular and tumor

- microenvironmental mechanisms of action. *Front Oncol.* 2022; 12: 960317.
13. Shokrzadeh Madieh N, Tanna S, Alqurayn NA, Vaideanu A, Schatzlein A, Brucoli F. Aminobenzofuran-containing analogues of proximicins exhibit higher antiproliferative activity against human UG-87 glioblastoma cells compared to temozolomide. *RSC Adv.* 2023; 13(12): 8420-8426.
 14. Carmichael J, Mitchell JB, DeGraff WG, Gamson J, Gazdar AF, Johnson BE, Glatstein E, Minna JD. Chemosen-sitivity testing of human lung cancer cell lines using the MTT assay. *Br J Cancer.* 1988; 57(6): 540-547.
 15. Alexandru O, Georgescu AM, Ene L, Purcaru SO, Serban F, Popescu A, Brindusa C, Tataranu LG, Ciubotaru V, Dri-cu A. The effect of curcumin on low-passage glioblastoma cells in vitro. *J Cancer Res Ther.* 2016; 12(2): 1025-1032.
 16. Kulpa J, Harrison A, Rudolph L, Eglit GML, Turcotte C, Bonn-Miller MO, Peters EN. Oral cannabidiol treatment in two postmenopausal women with osteopenia: a case series. *Cannabis Cannabinoid Res.* 2023; 8(S1): S83-S89.
 17. Wang T, Zakharov A, Gomez B, Lyubimov A, Trotter NL, Schwark WS, Wakshlag JJ. Serum cannabinoid 24 h and 1 week steady state pharmacokinetic assessment in cats using a CBD/CBDA rich hemp paste. *Front Vet Sci.* 2022; 9: 895368.
 18. McGrath S, Bartner LR, Rao S, Packer RA, Gustafson DL. Randomized blinded controlled clinical trial to assess the effect of oral cannabidiol administration in addition to conventional antiepileptic treatment on seizure frequency in dogs with intractable idiopathic epilepsy. *J Am Vet Med Assoc.* 2019; 254(11): 1301-1308.
 19. Greco R, Francavilla M, Demartini C, Zanaboni AM, Sodergren MH, Facchetti S, Pacchetti B, Palmisani M, Franco V, Tassorelli C. Characterization of the biochemical and behavioral effects of cannabidiol: implications for migraine. *J Headache Pain.* 2023; 24: 48.
 20. Williamson EM, Evans FJ. Cannabinoids in clinical practice. *Drugs.* 2000; 60(6): 1303-1314.
 21. Al-Khazaleh AK, Zhou X, Bhuyan DJ, Münch GW, Al-Dalabeeh EA, Jaye K, Chang D. The neurotherapeutic arsenal in cannabis sativa: insights into anti-neuroinflammatory and neuroprotective activity and potential entourage effects. *Molecules.* 2024; 29(2): 410.

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