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The effects of cannabinoids on glioblastoma growth: a systematic review with meta-analysis of animal model studies

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Abstract

Glioblastoma multiforme (GBM) is the most frequent and aggressive malignant brain tumour, with a poor prognosis despite available surgical and radio-chemotherapy, rising the necessity for searching alternative therapies. Several preclinical studies evaluating the efficacy of cannabinoids in animal models of GBM have been described, but the diversity of experimental conditions and of outcomes hindered definitive conclusions about cannabinoids efficacy.

A search in different databases (Pubmed, Web of Science, Scopus and SciELO) was conducted during June 2019 to systematically identify publications evaluating the effects of cannabinoids in murine xenografts models of GBM. The tumour volume and number of animals were extracted, being a random effects meta-analysis of these results performed to estimate the efficacy of cannabinoids. The impact of different experimental factors and publication bias on the efficacy of cannabinoids was also assessed.

Nine publications, which satisfied the inclusion criteria, were identified and subdivided in 22 studies involving 301 animals. Overall, cannabinoid therapy reduced the fold of increase in tumour volume in animal models of GBM, when compared with untreated controls. The overall weighted standardized difference in means (WSDM) for the effect of cannabinoids was -1.399 (95% CI: -1.900 to -0.898; P-value<0.0001). Furthermore, treatment efficacy was observed for different types of cannabinoids, alone or in combination, and for different treatment durations. Cannabinoid therapy was still effective after correcting for publication bias.

The results indicate that cannabinoids reduce the tumour growth in animal models of GBM, even after accounting for publication bias.

Keywords: cannabinoids, glioblastoma multiforme, animal model studies, systematic review, meta-analysis

1. Introduction

The incidence in adults of newly diagnosed glioblastomas is 0.59-3.69 cases per 100,000- person life-years (Dumitru et al., 2018). Glioblastoma multiforme (GBM), also known as grade IV astrocytoma, is simultaneously the most common class of malignant brain tumours and one of the most aggressive types of cancer. Therefore, after the diagnostic, patients usually live just more 6-12 months, which is mostly related with the high invasiveness and proliferation rate of GBM (Velasco et al., 2007). The existing guidelines for therapeutic approaches to treat GBM (surgical resection and focal radiotherapy) are simply palliative (Guzmán et al., 2006). Several chemotherapeutic compounds, such as alkylating agents (e.g. temozolomide - TMZ) and nitrosoureas (e.g. carmustine) have also been assessed, but increase in survival of patients was only moderate (Guzmán et al., 2006). Only TMZ showed some clinical efficacy in a phase III clinical trial (Stupp et al., 2005). Furthermore, GBM presents a high-level of resistance to the standard chemotherapy and radiotherapy (Torres et al., 2011). For that reason, the search for new promising compounds to treat GBM is essential.

Cannabinoids, the bioactive compounds of *Cannabis sativa* L., exert their effects mostly by activating certain types of G-protein coupled receptors (GPCRs), which are usually triggered by a group of endogenous ligands, the endocannabinoids (Blázquez et al., 2008). The endocannabinoid system was found when studying the main bioactive compound of *C. sativa*, Δ^9-tetrahydrocannabinol (THC) (Allister et al., 2005). Two cannabinoid receptors are identified, CB1 and CB2 which are activated by most cannabinoids including THC. These receptors are coupled to $G_{i/o}$, leading to inhibition of adenylyl cyclase. Other targets, as the transient receptor potential vanilloid (TRPV) channels, peroxisome proliferator activated receptors (PPARs) and a number of GPCRs like GPR12, GPR6, GPR3 and GPR55 are also activated by some cannabinoids such as cannabidiol (CBD) which is has low affinity for cannabinoid receptors (Dumitru et al., 2018; O'Sullivan, 2016). The endocannabinoid system also plays an important role in several diseases.

Several preclinical experiments indicate that drugs mimicking the endocannabinoid system may be applied to prevent the growth of cancer (Rocha et al., 2014). In fact, it was demonstrated that cannabinoids can regulate both the cell growth and death in various types of cancer (Allister et al., 2005). The first studies demonstrating the anti-tumour effects of several cannabinoids in animal models of glioma were published in the early 2000s (Massi et al., 2004; Recht et al., 2001; Sánchez et al., 2001a). These studies encouraged the first pilot phase I clinical trial including a reduced number of patients (Guzmán et al., 2006), which showed safety of THC administration and indicated its anti-proliferative activity. Since then, several preclinical studies using animal models were published, most of them reporting the capacity of cannabinoids in reducing the progression of GBM (Dumitru et al., 2018; Erices et al., 2018; McAllister et al., 2015; Rocha et al., 2014).

The use of animal models is of major importance in research aiming the improvement of human health care (Hooijmans et al., 2014). Although some recent reviews had been published reporting animal studies of anti-tumour effect of cannabinoids on GBM (Dumitru et al., 2018; Erices et al., 2018), a meta-analysis of these studies was not performed yet. There are several benefits in conducting meta-analyses on data from animal studies; they can be used to inform clinical trial design, or to explain discrepancies between preclinical and clinical trial results (Vesterinen et al., 2014).

The objective of this work was to perform a systematic review, complying with the PRISMA (Preferred Reported Items for Systematic Reviews and Meta-Analysis) statement, followed by meta-analysis of results obtained using animal models on the effects of cannabinoids in GBM growth, to clarify the therapeutic potential of those compounds.

2. Materials and methods

2.1. Search strategy, inclusion criteria and study selection

The electronic search for this systematic review was undertaken on various databases (Pubmed, Web of Science, Scopus and SciELO) during June 2019. The databases were queried using the Boolean operator tools, with the following strategy: (cannabinoid* OR cannabi*) AND (glioblastoma OR astrocytoma OR glioma OR oligodendroglioma OR GBM OR glioblastoma multiforme). The references of the articles considered relevant were also verified to find additional works. Following the PRISMA statement (Moher et al., 2015, 2009), titles and abstracts of the selected articles were firstly screened and the full texts of those considered important were then analysed in detail. The literature selection procedure was performed independently by two authors, being a third consulted in case of disagreements. To be included in this systematic review, studies must accomplish the following criteria: to use human-derived cells in animal models (xenografts), to present a control group (vehicle), to show the result of the outcome (tumour volume) at the beginning and at the end of the treatment with cannabinoids, and to indicate the standard deviation (S.D.) of the measurements and the animal number per group.

2.2. Risk of bias assessment

The methodological quality of the included studies was evaluated by a 9-item quality checklist adapted from the CAMARADES (Collaborative Approach to Meta-Analysis and Review of Animal Data in Experimental Studies) published criteria, which comprise: 1) publication in a peer-reviewed journal; 2) reporting the number of tumour cells implanted; 3) reporting the randomized allocation of tumour-bearing animals to treatment and control groups; 4) blinded assessment of outcome; 5) sample size calculation; 6) compliance with animal welfare regulations; 7) potential conflicts of interest; 8) number of animals originally inoculated with tumour cells; and 9) explanation of any treated animals excluded from analysis (J. A. Hirst et al., 2014; T. C. Hirst et al., 2014).

2.3. Data extraction and synthesis

After the selection of the studies, the included ones were carefully analysed and the following data were extracted and summarized: first author's last name, year of publication, type of GBM cells and intervention, tumour implantation site, outcome analysed, model used, dose of cannabinoid(s) and duration of the treatment. The revision and extraction of the data were independently performed by two authors applying a prespecified protocol, being a third reviewer consulted to analyse discrepancies in data extraction. The results extracted were both initial and post-intervention mean values of tumour volume with the corresponding S.D. and were then converted in terms of fold of increase. The results of tumour volume were generally reported in figures in the original studies, and for that reason the Inkscape program (Version 0.92.4) was used to obtain the numerical values to perform the statistical analysis.

2.4. Statistical analyses

The present meta-analysis was performed to clarify the effects of cannabinoids on GBM growth by summarizing the results of studies in which the cannabinoids were administered in animals inoculated with human-derived GBM cells. For the outcome of interest, an assessment was performed on the pooled effect of the treatment with cannabinoids in terms of weighted standardized difference in means (WSDM) between the change from pre- and post-treatment mean values of the intervention and control groups. Data statistical analysis was undertaken using the Comprehensive Meta-Analysis software (Version 2.0) by introducing the number of animals, the fold of increase and respective S.D. values of the outcome for intervention and control groups, being the random effects model employed (Borenstein et al., 2009). Forest plots were generated to illustrate the study-specific effect sizes along with a 95% confidence interval (CI). The statistic I^2 of Higgins was used as a measure of inconsistency across the findings of the included studies. The scale of I^2 has a range of 0 to 100% and values on the order of 25%, 50% and 75% are considered low, moderate and high heterogeneity, respectively (Higgins et al., 2003). Subgroup analysis was performed on the outcome under study, per the model used, type of cannabinoids and duration of the treatment, in order to evaluate the impact of these experimental factors on the cannabinoid effect size and to explore potential sources of heterogeneity. The Chi-square test was employed to assess whether there is homogeneity between the different subgroups with respect to the effect under study.

Three different analyses were used to assess the potential impact of publication bias on the present meta-analysis: 1) Funnel plot (Light et al., 1994; Light and Pillemer, 1984); 2) Egger's regression test (Borenstein et al., 2009; Egger et al., 1997); 3) Duval and Tweedie's Trim and Fill approach (Duval and Tweedie, 2000a, 2000b), which allows the best estimate of the unbiased pooled effect size to be obtained and creates a funnel plot that includes both the observed studies (shown as blue circles) and the necessary imputed studies (shown as red circles) to obtain the absence of bias.

The sensitivity analysis was also achieved by eliminating each study one at a time to evaluate the stability of the results.

3. Results

3.1. Search and selection of studies

Among the 40 articles initially identified, 9 met all the inclusion criteria for this systematic review. Fig. 1 shows the detailed steps of the article selection process. From the 16 full-text articles assessed for eligibility, 7 were excluded. The reasons for exclusion were mostly the inconsistency in presenting the results (tumour perimeter, weight, diameter) (Duntsch et al., 2006; Recht et al., 2001; Silva et al., 2019), different study designs (Aguado et al., 2007; Singer et al., 2015; Soroceanu et al., 2013) and different summary statistics (median) (Fisher et al., 2016). Six of the 9 included studies were divided into different experiments. Finally, 22 studies, totalizing 301 animals, were included in this systematic review and meta-analysis.

3.2. Included studies and characteristics

The principal characteristics of the included studies are outlined in Table 1. The studies cover a broad spectrum of cannabinoids both natural and synthetic, together with several types of human-derived GBM cells, which were applied in different types of animal models (xenografts). Furthermore, the cannabinoids were administered to the animals alone or in combination with each other at different doses. Such variables were included in this meta-analysis to explore potential sources of heterogeneity.

3.3. Risk of bias

The Supplementary Table 1 shows the study quality scores assessed using the CAMARADES checklist. All the included studies are peer-reviewed publications, reported the number of tumour cells implanted and referred the randomization of the animals for both treatment and control groups. However, none of the studies reported the blind of outcome assessment and have calculated the sample size. Overall, the global quality of the included studies is good (quality scores superior to 4 in a total of 9).

3.4. Effects of cannabinoids on GBM growth

The meta-analysis results of the effects of cannabinoids on GBM growth are graphically reported on Fig. 2, being the overall results presented in Table 2. It is possible to verify that cannabinoids were able to significantly reduce (P-value<0.0001) the mean fold of increase of tumour volume (WSDM: -1.399; 95% CI: -1.900 to -0.898), indicating that, in fact, these compounds acted against GBM. It should be noted that, nevertheless, moderate heterogeneity was observed (I^2=72%).

3.5. Subgroup and sensitivity analyses

A subgroup analysis was also performed (Table 3) to evaluate the influence of the model used, type of cannabinoids and treatment duration. Regarding the model used, only for subcutaneous xenografts was obtained a significant reduction (P-value<0.0001) of the mean fold of increase of tumour volume (WSDM: -1.512; 95% CI: -2.060 to -0.965). However, for intracranial xenografts only 2 studies were considered, which may explain the absence of statistical significance in this subgroup. Nevertheless, the model used did not account for a significant proportion of the observed heterogeneity

(Chi^2=1.082; P-value=0.298). Concerning the type of cannabinoids, all of them were able to significantly reduce the fold of increase of tumour volume, except the cannabinoid KM-233, but in this case the number of studies, two, is too low to draw definitive conclusions. Regarding the heterogeneity between the types of cannabinoid, it was low for cannabidiol (CBD) but high for THC studies. In fact, the type of cannabinoid explained a significant proportion of the observed heterogeneity, according to the Chi-square test (Chi^2=14.219; P-value=0.007). Concerning the treatment duration, it did not account for a significant heterogeneity (Chi^2=1.535; P-value=0.675), but it is difficult to establish a definitive conclusion because only one study was considered for both treatments with 8 and 35 weeks. For treatments with 12-15 weeks and 22-27 weeks, significant reduction of the fold of increase of the tumour volume was observed. The sensitivity analysis was also performed by excluding one or more studies from the analysis to see how this affected the results. The results showed that the pooled effects of cannabinoids on GBM growth did not change substantially if a single or a few studies were omitted (Fig. 3). Overall, the sensitivity analysis demonstrated that the findings of this meta-analysis are robust.

3.6. Publication bias

To analyse the publication bias, a funnel plot was generated for the outcome considering the Trim and Fill adjustment (Fig. 4). It was observed that there are more studies on the right than on the left, and for that reason 2 studies were inputted on the left to adjust the funnel plot to the absence of publication bias. The WSDM both observed and adjusted were reported on Tables 2 and 3.

The presence of publication bias was further explored using Egger's regression test. This test indicates evidence of publication bias for the impact of cannabinoids administration on GBM growth. (Table 4).

4. Discussion

In this systematic review with meta-analysis of 9 publications, subdivided in 22 studies and involving 301 animals, it was found that the overall cannabinoid therapy reduced tumour volume in murine xenografts models of GBM. Furthermore, treatment efficacy was observed for different types of cannabinoids, alone or in combination, and different treatment durations.

Several previous *in vitro* and *in vivo* pre-clinical studies in animal models and pilot studies in human patients (Allister et al., 2005; Guzmán et al., 2006; Ladin et al., 2016) had reported the therapeutic potential of cannabinoids on GBM, based on reduction of tumour growth. However, to the best of our knowledge, the present work is the first systematic review with meta-analysis performed regarding the effects of cannabinoids on GBM.

In the present meta-analysis, the outcome analysed was the fold of increase from initial tumour volume before treatment, rather than median survival time, since most of the studies reported the initial and final volume, or the fold of increase in tumour volume, together with the respective S.D. or standard error of mean (S.E.M.).

Regarding the site of tumour inoculation, most of the studies included in the present meta-analysis used heterotopic subcutaneous xenografts, with only 2 studies using orthotopic intracranial xenografts. Only for the subcutaneous xenograft model, a significant reduction of tumour volume by cannabinoids was found. Nevertheless, there was no significant variation in cannabinoids effect between tumour models.

The subgroup analysis for different cannabinoids, revealed that most cannabinoids, either natural or synthetic and either alone or in combination, were able to reduce tumour volume of murine GBM models, except for the synthetic cannabinoid KM-233. However, the effect of the different cannabinoids varied, and the type of cannabinoid showed to be a significant source of heterogeneity. Concerning the duration of treatment with cannabinoids, a significant decrease of tumour volume was obtained for the 12-15 weeks and for the 22-27 weeks treatment periods. There was no significant variation between different treatment duration.

In the present analysis, only the studies reporting animals inoculated with tumour cells of human origin were considered. This choice aimed to reduce the heterogeneity among the studies. On the other hand, using cells of human origin constitute a more reliable model/construct of GBM and previous studies suggest that human-derived tumours are more sensitive to chemotherapy than those originated in rodents (Amarasingh et al., 2009).

The overall quality of the studies included in the present meta-analysis was good. The publication bias of the present meta-analysis was also assessed, and the results indicate its presence, which is usually due to the fact of neutral studies often remain unpublished or take longer to get published than those reporting statistically significant results, as previously mentioned (Sena et al., 2014). However, probably this was not the case for the studies considered in the present meta-analysis, since, after correcting for publication bias, the adjusted WSDM was more negative, suggesting a stronger reduction on tumour volume induced by cannabinoids, than the non-adjusted value. However, we cannot exclude that other confounding effects of certain aspects of studies design (including randomization, allocation concealment and blinded outcome assessment) might also constitute source of bias, as commonly happens with animal studies (Amarasingh et al., 2009).

In the present meta-analysis, the results in general presented moderate or high heterogeneity, even after subgrouping for site of cell tumour inoculation, type of cannabinoid or treatment duration. This is common in meta-analysis dealing with data obtained from animal models (Hooijmans et al., 2014), where the cause of heterogeneity is difficult to identify due to experimental differences between studies. Nevertheless, animal studies are crucial to the understanding of disease mechanisms and for testing interventions for safety and efficacy.

The promising results obtained in animal models of GBM, led to 3 pilot clinical trials to assess the efficacy of cannabinoids in GBM patients (Dall'Stella et al., 2019; Guzmán et al., 2006; Kenyon et al., 2018). The first study, performed in 2006 and including 9 patients, showed safety of THC; however, no clear activity of THC on tumour progression was reported (Guzmán et al., 2006). The study of Kenyon, et al 2018 (Kenyon et al., 2018), enrolled 7 patients treated with CBD and reported extended survival in 4 and slowed disease progression in 3 of the patients. The study of Dall'Stella, et al 2019 (Dall'Stella et al., 2019) enrolled only 2 patients submitted to chemoradiation followed by a multiple drug regimen (procarbazine, lomustine, and vincristine) plus CBD, both patients showed no signs of disease progression for at least 2 years.

The chemotherapeutic options to treat GBM are, in fact, limited. Only TMZ showed clinical efficacy, although modest, in a phase III clinical trial (Stupp et al., 2005), the median survival increasing from 12.1 months with radiotherapy alone to 14.6 months with radiotherapy plus TMZ. Therefore, the potential use of cannabinoids, alone or in combination with other drugs or radiotherapy, to treat GBM deserves further investigation.

Preclinical studies using animal models of GBM, showed that cannabinoids in combination with TMZ produced a stronger anti-tumoural effect than the effect of each drug alone (Blázquez et al., 2008; López-Valero et al., 2018a, 2018b). In fact, a phase II clinical trial of 21 patients had been recently conducted. This trial showed that patients treated with a combination of THC and CBD in addition to TMZ had a median survival of >662 days compared with 369 days in the group treated with TMZ alone (Schultz and Beyer, 2017).

In vitro studies showed that cannabinoids may reduce tumour growth by: 1) inducing apoptosis and cytotoxic autophagy); 2) inhibiting cell proliferation, and 3) inhibiting-angiogenesis (Dumitru et al., 2018). Cannabinoid-induced activation of the intrinsic apoptotic pathway and of autophagy in GBM cells, seems to be mediated by increased ceramide production (Dumitru et al., 2018). Another mechanism by which cannabinoids induce GBM cell apoptosis involves increased reactive oxygen species production and oxidative stress (Massi et al., 2010). Increased reactive oxygen species-production also showed to mediate cannabinoids-induced inhibition of glioma stem cells self-renewal (Singer et al., 2015). On the other hand, THC inhibits the cell cycle progression in GBM by decreasing the levels of E2F1 and Cyclin A while increasing the level of the cell cycle inhibitor p16 (Galanti et al., 2008). Furthermore, cannabinoids also showed to inhibit angiogenesis by decreasing VEGF levels (Blázquez et al., 2008). Additionally, cannabinoids have a role in the treatment of cancer as palliative interventions against nausea, vomiting, pain, anxiety, and sleep disturbances; and today's scientific results suggest that cannabinoids could play an important role in palliative care of brain tumor patients (Likar and Nahler, 2017).

Concerning the type of receptor and mechanism involved in the anti-tumour actions of cannabinoids, it depends on the type of cannabinoid. For THC, a partial agonist for CB1 and CB2 receptors, both cannabinoid receptors shown to mediate the cytotoxic effect of THC on GBM cell lines (Torres et al., 2011; Lorente et al., 2011; Carracedo et al., 2006). Selective agonists of CB1 receptors such as KM-233 (Gurley et al., 2012) and of CB2 receptors such as JWH-133 (Sánchez et al., 2001) produced cytotoxicity on GBM cells and reduced tumour growth in rat GBM xenografts, respectively. The anti-tumour effects on GBM produced by both CB1 and CB2 receptors activation seems to be mediated by ceramide production, leading to autophagy and apoptosis (Dumitru et al. 2018). On the other hand, CBD anti-tumour effect on GBM is only partially mediated by CB2 receptor activation (Massi et al., 2004) and does not involve ceramide production or TRPV activation, but rather involves reactive oxygen species formation and consequent apoptosis (Torres et al., 2011; Massi et al., 2004).

5. Conclusions

Cannabinoids are effective in reducing tumour growth in animal models of GBM, particularly in subcutaneous xenograft models. Besides, treatment efficacy was observed for different types of cannabinoids, alone or in combination, and different treatment durations. The results also showed the presence of publication bias, which, however, do not invalidate the efficacy of cannabinoids. These results in experimental GBM models are promising and highlights the importance of cannabinoid translational research which may lead to clinically relevant studies.

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Table 1: Characteristics of the 22 included studies in this systematic review with meta-analysis.

Study[a]	Year	Cells	Intervention	Outcome analysed	Model used	Dose (per day)	Duration of the treatment
López-Valero, et al **A) 1)**	2018	Human GBM line (U87MG)	Evaluation of the effect of cannabidiol (CBD) and tetrahydrocannabinol (THC) alone or in combination (CBD+THC), and in combination with temozolomide (TMZ) in apoptosis, migration, animal survival and tumour volume in tumour xenografts (mice inoculated with U87MG cells)	Tumour volume	Subcutaneous xenografts	CBD (15 mg/kg)	15 days
López-Valero, et al **A) 2)**	2018				Subcutaneous xenografts	THC:CBD (1:4) (THC 6.5 mg/kg + CBD 24.5 mg/kg)	14 days
López-Valero, et al **A) 3)**	2018				Intracranial xenografts	THC:CBD (1:4) (THC 6.5 mg/kg + CBD 24.5 mg/kg)	14 days
López-Valero, et al **B) 1)**	2018	Human GBM line (U87MG)	Evaluation of the effect of CBD+THC (1:1) in combination of TMZ on tumour volume and animal survival in tumour xenogrfts (mice inoculated with U87MG cells)	Tumour volume	Subcutaneous xenografts	THC (15 mg/kg)	15 days
López-Valero, et al **B) 2)**	2018				Subcutaneous xenografts	THC:CBD (1:1) (THC 15 mg/kg + CBD 15 mg/kg), peritumoural administration	12 days
López-Valero, et al **B) 3)**	2018				Subcutaneous xenografts	THC:CBD (1:1) (THC 15 mg/kg + CBD 15 mg/kg),	12 days

						oral administration	
López-Valero, et al **B) 4)**	2018				Subcutaneous xenografts	THC:CBD (1:1) (THC 45 mg/kg + CBD 45 mg/kg), oral administration	12 days
López-Valero, et al **B) 5)**	2018				Intracranial xenografts	THC:CBD (1:1) (THC 7.5 mg/kg + CBD 7.5 mg/kg)	7 days
Ossa, et al **1)**	2013	Human GBM line (U87MG)	Evaluation of the effect of CBD, THC or CBD+THC (1:1), in solution or microparticles on apoptosis, migration, angiogenesis and on tumour volume of tumour xenografts (mice inoculated with U87MG cells)	Tumour volume	Subcutaneous xenografts	THC (15 mg/kg)	22 days
Ossa, et al **2)**	2013				Subcutaneous xenografts	CBD (15 mg/kg)	22 days
Ossa, et al **3)**	2013				Subcutaneous xenografts	THC:CBD (1:1) (THC 7.5 mg/kg + CBD 7.5 mg/kg)	22 days
Gurley, et al **1)**	2012	Human GBM line (U87MG)	Evaluation of the effect of the cannabinoid KM-233 on tumour volume of tumour xenografts (mice inoculated with U87MG cells)	Tumour volume (model D-08-0673 MG)	Subcutaneous xenografts	KM-233 (24 mg/kg)	35 days
Gurley, et al **2)**	2012			Tumour volume (model D-09-0363 MG)	Subcutaneous xenografts	KM-233 (24 mg/kg)	15 days
Torres, et al **1)**	2011	Human GBM lines (U87MG and T98G)	Evaluation of the effect of CBD, THC, alone or in combination with TMZ on	Tumour volume	Subcutaneous xenografts	THC (15 mg/kg)	15 days

Torres, et al **2)**	2011		viability/proliferation, apoptosis and tumour volume of tumour xenografts (mice inoculated with U87MG cells)		Subcutaneous xenografts	CBD (7.5 mg/kg)	15 days
Torres, et al **3)**	2011				Subcutaneous xenografts	THC (7.5 mg/kg)	15 days
Torres, et al **4)**	2011				Subcutaneous xenografts	THC:CBD (1:1) (THC 7.5 mg/kg + CBD 7.5 mg/kg)	15 days
Lorente, et al **1)**	2011	Human GBM lines (GOS3, U87MG, A172, SW1783, U118MG, U373MG, T98G and SW1088)	Evaluation of the effect of THC on viability, apoptosis and tumour volume on tumour xenografts. Influence of expression levels of midkine/ALK on THC efficacy	Tumour volume	Subcutaneous xenografts (derived from T98 cells)	THC (15 mg/kg)	15 days
Lorente, et al **2)**	2011				Subcutaneous xenografts (derived from T98 cells)	THC (15 mg/kg)	15 days
Massi, et al	2004	Human GBM lines (U86MG and U373)	Evaluation of the effect of CBD on proliferation, apoptosis and tumour volume on tumour xenografts (mice inoculated with U87MG cells)	Tumour volume	Subcutaneous xenografts	CBD (0.5 mg/mouse)	23 days
Sánchez, et al	2001	Human tumour cells prepared from a grade IV astrocytoma	Evaluation of the effect of JWH-133 on tumour size of tumour xenografts	Tumour size	Subcutaneous xenografts	JWH-133 (50 µg injected	25 days

			(mice immunotolerant - Rag-$2^{-/-}$)			intratumourally/day)	
Carracedo, et al	2006	Human GBM line (U87MG) and mice embrionary fibroblasts (MEF)	Evaluation of the effect of THC on viability, apoptosis and tumour volume on tumour xenografts (mice inoculated with U87MG cells and MEF)	Tumour volume	Subcutaneous xenografts (derived from U87MG cells)	THC (15 mg/kg)	14 days

[a]The numbers in unpaired parenthesis indicate the division of each work in several studies.

Table 2: Effects of cannabinoids on GBM growth.

Outcome analysed	**Number of studies**	**Number of animals**	**WSDM observed (95% CI)**	**P-value**	**I^2 (%)**	**Model used**	**WSDM adjusted for absence of bias (95% CI)**
Tumour volume (fold of increase)	22	301	-1.399 (-1.900 to -0.898)	<0.0001[a]	72	Random	-1.606 (-2.135 to -1.077)

WSDM – weighted standardized difference in means; CI – confidence interval; [a]Indicates a significant result.

Table 3: Subgroup analysis of the effects of cannabinoids on GBM growth.

Variable	GBM growth			
	Number of studies	**95% CI**	**P-value**	**I^2 (%)**
Total	22	-	-	-
WSDM observed	-	-1.399 (-1.900 to -0.898)	<0.0001[a]	72
WSDM adjusted for absence of bias	-	-1.606 (-2.135 to -1.077)	-	-
Model used				
subcutaneous xenografts	20	-1.512 (-2.060 to -0.965)	<0.0001[a]	74
intracranial xenografts	2	-0.738 (-2.091 to 0.616)	0.286	55
Cannabinoids				
CBD	4	-1.075 (-2.082 to -0.069)	0.036[a]	15
JWH-133	1	-6.641 (-9.972 to -3.310)	<0.0001[a]	0
KM-233	2	-0.103 (-1.456 to 1.251)	0.882	0
THC	7	-1.757 (-2.571 to -0.944)	<0.0001[a]	77
THC+CBD	8	-1.301 (-2.039 to -0.564)	0.001[a]	62
Duration of the treatment (days)				
8	1	-1.489 (-3.995 to 1.017)	0.244	0

12-15	15	-1.495 (-2.128 to -0.862)	<0.0001[a]	73
22-27	5	-1.480 (-2.598 to -0.362)	0.009[a]	76
35	1	-0.008 (-2.294 to 2.277)	0.994	0

WSDM – weighted standardized difference in means; CI – confidence interval; [a]Indicates a significant result.

Table 4: Assessment of publication bias for the impact of cannabinoids administration on GBM growth.

Outcome analysed	**Egger's regression test**			
	95% CI	***t***	**df**	**P-value**
Tumour volume (fold of increase)	-9.783 to -5.451	7.337	20	<0.00001[a]

CI – confidence interval; df – degrees of freedom; [a]Indicates a significant result.

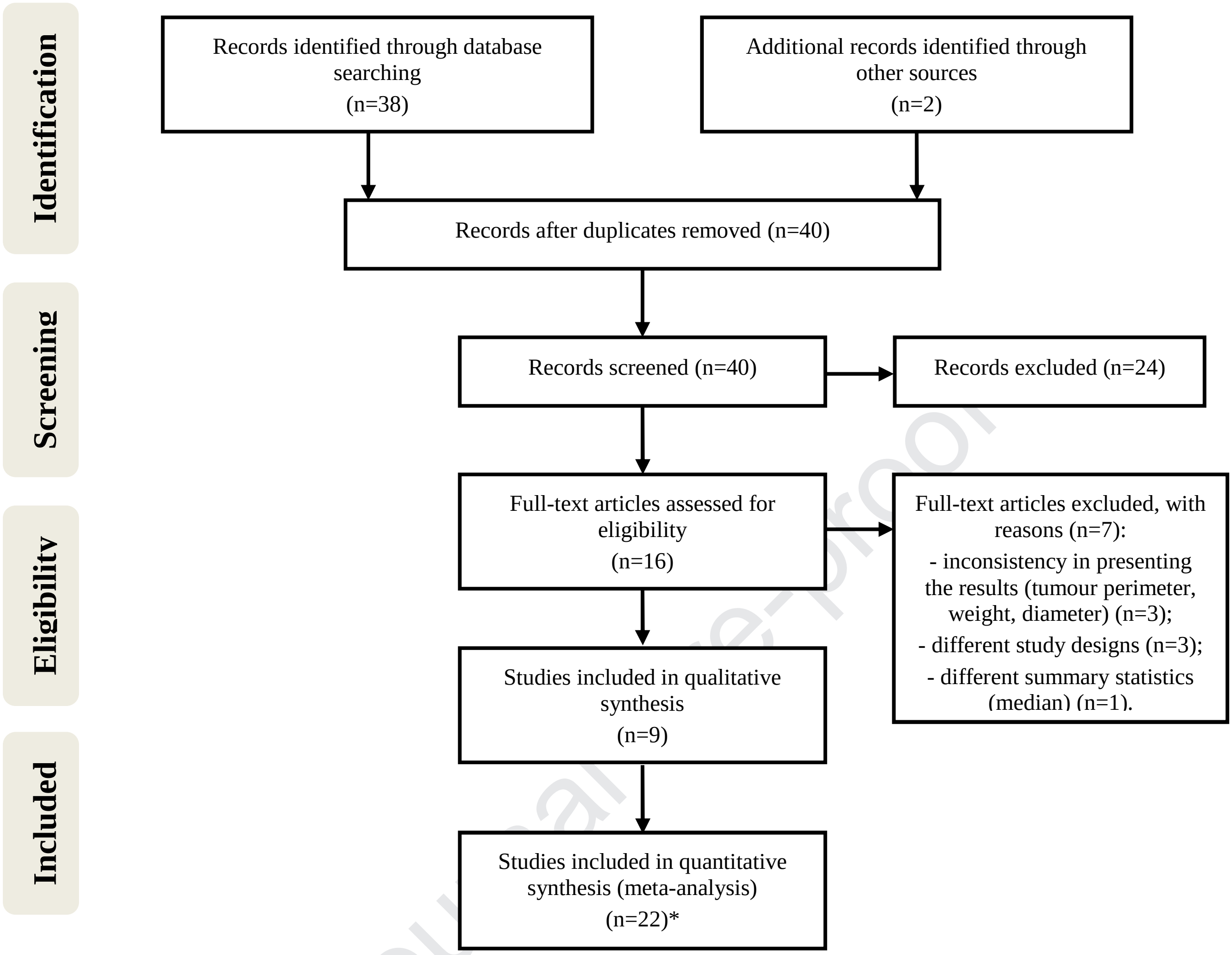
Fig. 1: Flow-diagram of database search, study selection and articles included in this systematic review with meta-analysis.

Fig. 2: Forest plot of comparisons of the effects of cannabinoids on GBM growth.

Fig. 3: Results of sensitivity analysis.

Fig. 4: Funnel plot of standard error by difference in means (publication bias tests) of the effects of cannabinoids on GBM growth.

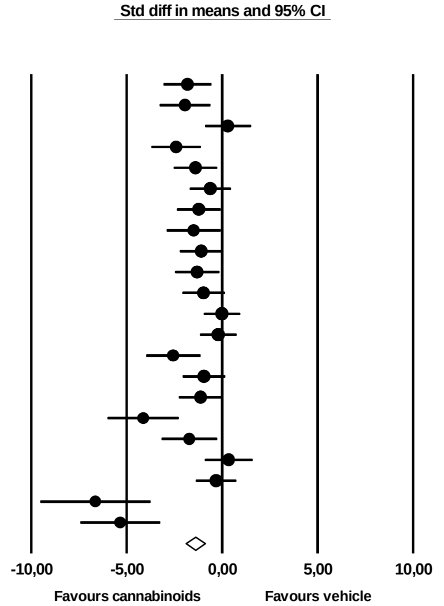
Fig. 1



*The work of López-Valero, et al 2018 B) was divided into 5 different studies. The work of Torres, et al 2011 was divided into 4 different studies. The works of López-Valero, et al 2018 A) and Ossa, et al 2013 were divided into 3 different studies. The works of Gurley, et al 2012 and Lorente, et al 2011 were divided into 2 different studies. (The division of each work in several studies is indicated by the numbers in unpaired parenthesis in Table 1)

Fig. 2

Study name	Standard error	Std diff in means	Lower limit	Upper limit	Z-Value	P-Value	Relative weight
López-Valero, et al A) 1)	0,635	-1,815	-3,060	-0,570	-2,858	0,004	4,68
López-Valero, et al A) 2)	0,675	-1,945	-3,267	-0,623	-2,883	0,004	4,51
López-Valero, et al A) 3)	0,609	0,306	-0,887	1,500	0,503	0,615	4,79
López-Valero, et al B) 1)	0,657	-2,410	-3,698	-1,123	-3,669	0,000	4,58
López-Valero, et al B) 2)	0,577	-1,398	-2,529	-0,267	-2,423	0,015	4,92
López-Valero, et al B) 3)	0,547	-0,618	-1,691	0,454	-1,130	0,258	5,05
López-Valero, et al B) 4)	0,582	-1,218	-2,359	-0,077	-2,093	0,036	4,90
López-Valero, et al B) 5)	0,715	-1,489	-2,890	-0,088	-2,083	0,037	4,34
Ossa, et al 1)	0,573	-1,087	-2,210	0,035	-1,899	0,058	4,94
Ossa, et al 2)	0,589	-1,305	-2,459	-0,151	-2,217	0,027	4,87
Ossa, et al 3)	0,565	-0,969	-2,076	0,139	-1,715	0,086	4,98
Gurley, et al 1)	0,486	-0,008	-0,961	0,944	-0,017	0,986	5,31
Gurley, et al 2)	0,487	-0,197	-1,152	0,758	-0,404	0,686	5,31
Torres, et al 1)	0,721	-2,561	-3,974	-1,147	-3,551	0,000	4,32
Torres, et al 2)	0,564	-0,949	-2,054	0,156	-1,683	0,092	4,98
Torres, et al 3)	0,575	-1,127	-2,254	0,001	-1,958	0,050	4,93
Torres, et al 4)	0,947	-4,135	-5,991	-2,279	-4,367	0,000	3,46
Lorente, et al 1)	0,740	-1,720	-3,170	-0,269	-2,323	0,020	4,24
Lorente, et al 2)	0,637	0,345	-0,903	1,594	0,542	0,588	4,67
Massi, et al	0,538	-0,317	-1,371	0,737	-0,589	0,556	5,09
Sánchez, et al	1,473	-6,641	-9,528	-3,753	-4,507	0,000	2,06
Carracedo, et al	1,068	-5,336	-7,429	-3,244	-4,998	0,000	3,06
	0,255	-1,399	-1,900	-0,898	-5,475	0,000	



Heterogeneity: Tau^2=0.993; Chi^2=74.427; df=21; P-value<0.0001; I^2=72%

Test for overall effect: Z=-5.975; P-value<0.0001

Fig. 3

Study name	Statistics with study removed					
	Point	Standard error	Lower limit	Upper limit	Z-Value	P-Value
López-Valero, et al A) 1)	-1,384	0,266	-1,905	-0,863	-5,206	0,000
López-Valero, et al A) 2)	-1,377	0,265	-1,896	-0,859	-5,206	0,000
López-Valero, et al A) 3)	-1,479	0,258	-1,984	-0,974	-5,737	0,000
López-Valero, et al B) 1)	-1,349	0,260	-1,859	-0,838	-5,179	0,000
López-Valero, et al B) 2)	-1,408	0,269	-1,935	-0,881	-5,237	0,000
López-Valero, et al B) 3)	-1,448	0,268	-1,974	-0,922	-5,398	0,000
López-Valero, et al B) 4)	-1,417	0,269	-1,945	-0,890	-5,268	0,000
López-Valero, et al B) 5)	-1,401	0,266	-1,923	-0,880	-5,265	0,000
Ossa, et al 1)	-1,425	0,269	-1,952	-0,897	-5,289	0,000
Ossa, et al 2)	-1,413	0,269	-1,939	-0,886	-5,255	0,000
Ossa, et al 3)	-1,431	0,269	-1,959	-0,903	-5,312	0,000
Gurley, et al 1)	-1,475	0,261	-1,987	-0,963	-5,647	0,000
Gurley, et al 2)	-1,469	0,265	-1,987	-0,950	-5,551	0,000
Torres, et al 1)	-1,344	0,259	-1,852	-0,836	-5,185	0,000
Torres, et al 2)	-1,432	0,269	-1,960	-0,904	-5,316	0,000
Torres, et al 3)	-1,422	0,269	-1,950	-0,895	-5,283	0,000
Torres, et al 4)	-1,283	0,245	-1,764	-0,803	-5,236	0,000
Lorente, et al 1)	-1,390	0,265	-1,909	-0,870	-5,244	0,000
Lorente, et al 2)	-1,479	0,258	-1,984	-0,974	-5,739	0,000
Massi, et al	-1,461	0,266	-1,982	-0,940	-5,498	0,000
Sánchez, et al	-1,267	0,235	-1,728	-0,807	-5,392	0,000
Carracedo, et al	-1,247	0,234	-1,705	-0,789	-5,338	0,000
	-1,399	0,255	-1,900	-0,898	-5,475	0,000

Std diff in means (95% CI) with study removed

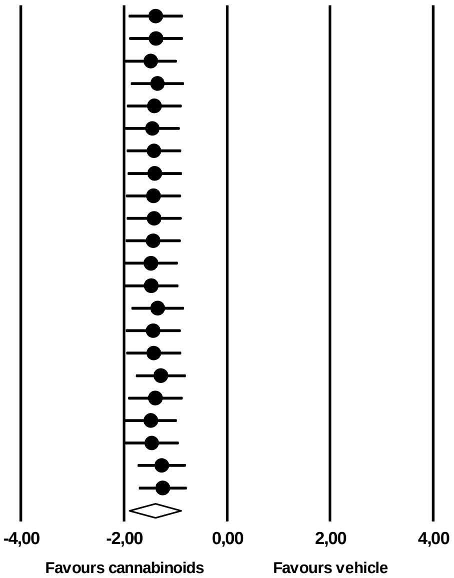
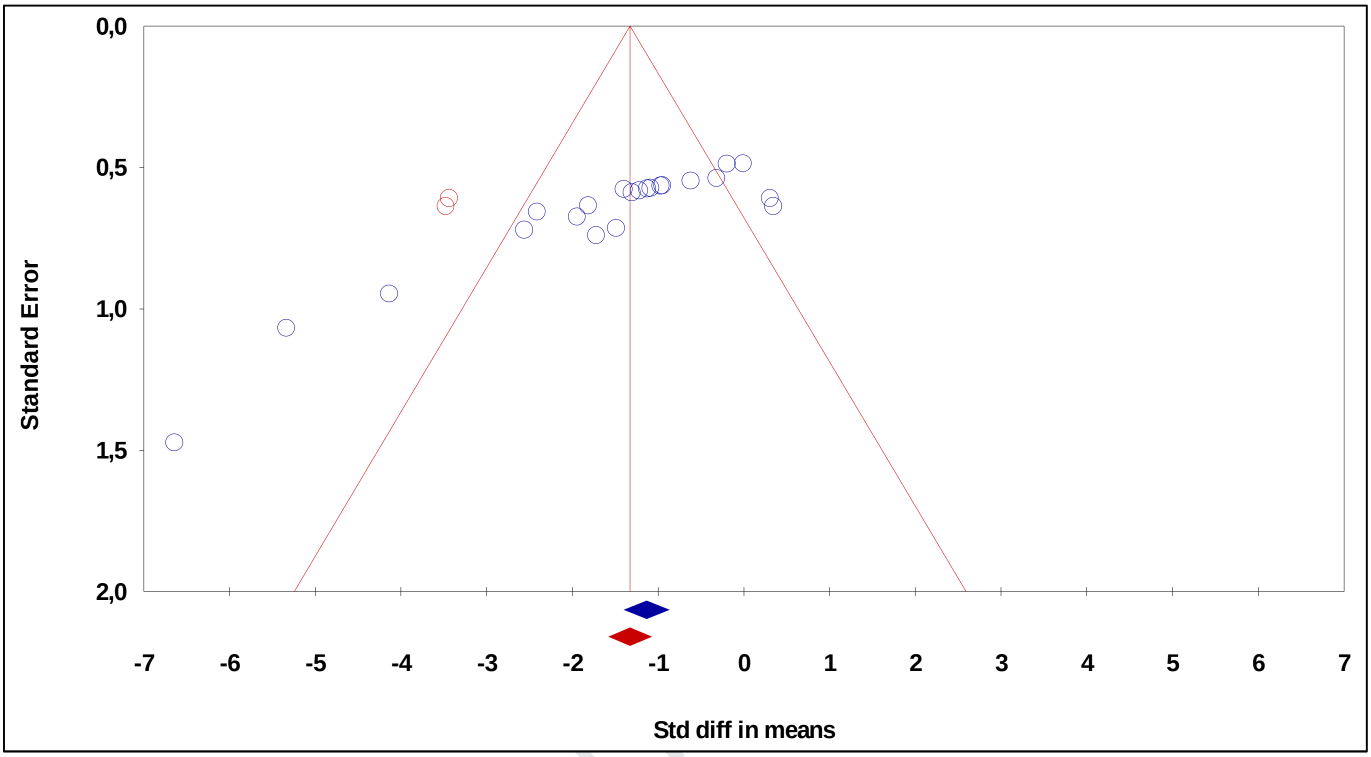


Fig. 4



The blue circles indicate the observed studies and the red circles indicate the necessary imputed studies to obtain absence of bias.