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Evaluation of cannabidiol nanoparticles and nanoemulsion biodistribution in the central nervous system after intrathecal administration for the treatment of pain

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Abstract

We investigated how the biodistribution of cannabidiol (CBD) within the central nervous system (CNS) is influenced by two different formulations, an oil-in-water (O/W) nanoemulsion and polymer-coated nanoparticles (PCNPs). We observed that both CBD formulations administered were preferentially retained in the spinal cord, with high concentrations reaching the brain within 10 min of administration. The CBD nanoemulsion reached C_{max} in the brain at 210 ng/g within 120 min (T_{max}), whereas the CBD PCNPs had a C_{max} of 94 ng/g at 30 min (T_{max}), indicating that rapid brain delivery can be achieved through the use of PCNPs. Moreover, the $AUC_{0-4\ h}$ of CBD in the brain was increased 3.7-fold through the delivery of the nanoemulsion as opposed to the PCNPs, indicating higher retention of CBD at this site. Both formulations exhibited immediate anti-nociceptive effects in comparison to the respective blank formulations.

Keywords: Intrathecal; Cannabidiol; Nanoemulsion; Polymer-coated nanoparticles; Drug-delivery
Data availability: Raw data created during this research are openly available from the corresponding author (maria.marlow@nottingham.ac.uk) and at the University of Nottingham Research Data Management Repository (https://rdmc.nottingham.ac.uk/) http://doi.org/10.17639/nott.7283.

Introduction

Drug delivery is a major challenge in the effective treatment of central nervous system (CNS) disorders, such as neuropathic pain. Systemic drug administration via parenteral routes often leads to very low concentrations of active therapeutics agents within the CNS, mostly attributable to the blood-brain barrier (BBB).[1,2] This is therefore a limiting step in the development of novel treatments for neuropathic pain, and one of the reasons why advancements have not been rapid in this therapeutic area.

Intrathecal (IT) delivery is an approach which bypasses CNS barriers and successfully attains high concentrations of therapeutics in the cerebrospinal fluid (CSF) whilst minimising off-target effects.[3] Nonetheless, IT drug administration still poses difficulties due to limited drug solubility, inadequate drug distribution in the CNS and poor pharmacokinetic profiles.[4] This is especially true for lipophilic drugs, such as cannabidiol (CBD), which have been shown to possess analgesic properties in the treatment of neuropathic pain.[5,6]

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CBD has been previously reported to induce analgesia as a result of its activity on transient receptor potential cation channel subfamily V member 1 ($TRPV_1$,), μ and δ opioid receptors in the CNS.[7–9] However, its efficacy is limited due to its extensive first-pass metabolism and poor penetration into the CNS when administered orally, intravenously or through inhalation.[10] Nonetheless, although IT delivery circumvents first pass metabolism, the high lipophilicity of CBD would still lead to poor solubility, binding to matrix proteins and partitioning into fatty cell membranes of the subarachnoid space (SAS) when delivered directly to the CNS. Therefore, these factors would significantly limit the distribution of CBD away from the site of injection in the lumbar segment of the spinal cord and into the brain.[11,12] Henceforth, nanocarrier formulation strategies may be used in conjunction with the IT administration technique to aid drug solubilisation and enhance biodistribution in the CNS.

Colloidal formulations have received much attention in the last few decades as a result of their potential to enhance and improve drug delivery. Nanocarriers allow for therapeutic agents to be effectively encapsulated and released over time, alongside surface and size optimisation which may lead to differential distribution and transport across biological barriers.[13] It was demonstrated that drugs formulated within nanocarriers possess extended half-lives when administered IT as opposed to the free form, therefore leading to differentiated pharmacokinetic profiles based on formulation strategies.[2]

In this work, the delivery of CBD directly to its site of action in the CNS via a lumbar IT injection was proposed. Subsequently, it was aimed to investigate how two distinct nanocarrier formulations influence its anti-nociceptive effects and biodistribution within the CNS, as this has not been previously described in the literature. It was hypothesised that a CBD nanoemulsion formulation would be preferentially retained at the site of injection in the spinal cord as a result of its lipophilicity and size,[14,15] whereas this retention would be overcome through the formulation of CBD polymer coated nanoparticles (PCNPs). Moreover, it was hypothesised that the PEGylated hydrophilic polymer coating on the CBD PCNPs would minimise interactions with the CNS matrix, thus facilitating their movement away from the injection site.[16] Furthermore, it was also hypothesised that both formulations would elicit anti-nociceptive effects after IT delivery.

It is thought that one of the most critical features influencing the behaviour of nanocarriers in vivo is their size, as this directly influences the interaction of the nanoparticle material with biological structures.[17] Nanocarrier size is particularly important for IT delivery, because the SAS consists of a mesh of trabeculae made of fibroblasts, collagen fibrils and various other extracellular matrix components which can affect their distribution.[18]

Two different CBD nanocarriers were formulated, an oil-in-water (O/W) nanoemulsion and PCNPs which were administered to healthy rats via a direct lumbar IT injection. These formulations were chosen due to their different physicochemical properties (either lipid based or polymer-coated nanocrystal), size (286.8 nm vs 121.8 nm), stability and monodispersity. The anti-nociceptive effects of the CBD formulations were evaluated through electrophysiological measurements of the limb-withdrawal to noxious mechanical stimuli, and tissue biodistribution was quantified by high performance liquid chromatography (HPLC). Therefore, this study explores the prospect of optimising nanocarrier technology in order to influence the biodistribution of drugs within the CNS after lumbar IT administration.

Methods

Preparation of CBD Nanoemulsion

Stock solutions of CBD (100 mg/mL) were prepared in propylene glycol – ethanol (9:1, v/v), and further diluted in Intralipid® nanoemulsion to 2 mg/mL final CBD concentration. Control samples were prepared with nanoemulsion spiked with blank propylene glycol – ethanol (9:1, v/v). Formulations were incubated at 37 °C for 1 h (stirring at 170 rpm). Thereafter, the density of the nanoemulsion was modified to 1.1 g/mL with KBr. Saline solution (0.9 % NaCl) served as a standard solution with a density of 1.0046 g/mL. Gradient solutions with densities of 1.019 and 1.063 g/mL were prepared from the 0.9 % NaCl solution by the addition of KBr. A density gradient was then built and the CBD-containing nanoemulsion was separated from free drug by ultracentrifugation (Sorvall® TH-641 Rotor, Thermo Fisher Scientific, 20,000 rpm, at 15 °C for 30 mins). The top 1 mL layer was collected and vortexed immediately. Samples were stored at 4 °C until further analysis.

Preparation of PCNPs

CBD PCNPs were formulated by a double nanoprecipitation technique[44,45] based on a previously developed procedure by Styliari et al., 2020[31] with some additional modifications. A triblock star co-polymer 3-arm PEG_{1014}-$(LA)_{100}$ (tPEG) previously developed[32] was synthesised and characterised by NMR and GPC (refer to S1 for detailed methods of polymer synthesis and characterisation) and used to coat the surface of CBD nanocrystals. Briefly, CBD was dissolved in 1 mL acetone (5 mg/mL stock concentration) and then added to 5 mL deionised water in a dropwise manner over 90 s. The solution was left stirring for 4 h at 550 rpm to allow for acetone evaporation. Subsequently, tPEG dissolved in 1 mL acetone (5 mg/mL stock concentration) was added to water containing CBD in a similar dropwise manner over 90 s. The final formulation containing a CBD to tPEG ratio of 1:1 was allowed a minimum of 4 h stirring at 550 rpm for acetone to evaporate and CBD PCNPs to form. Excess CBD was removed by centrifugation at 4000 *g* for 15 min (Eppendorf 5430, Barkhausenweg, Germany). Samples were evaporated over N_2 air to a desired concentration and were subsequently stored at 4 °C until further analysis.

Physical Characterisation of CBD Nanoemulsion and PCNPs

The particle size (d. nm) and polydispersity index (PDI) were determined by dynamic light scattering (DLS) on Malvern Zetasizer Nano ZS series (Malvern Instruments, UK) using back-scatter detection at 173° scattering angle (He—Ne laser wavelength of 633 nm). Samples were diluted by a factor of 1/1000 with deionised water and measured at 25 °C (refractive index set to 1.47 and 1.46 for soybean oil and tPEG respectively and 1.330 for the water dispersant). The Zeta Potential (ZP) was also

measured on Malvern Zetasizer Nano ZS at 25 °C through determination of electrophoretic mobility (dilution factor 1/1000). ZP measurements involved 3 scans consisting of 12 runs each. Data was processed using Malvern DTS software to calculate the mean particle size value and PDI.

Quantification of CBD in Nanoemulsion and PCNPs

The concentration of CBD in formulations was determined by reverse-phase HPLC (Waters Alliance 2695 separations module) coupled to a photodiode array ultraviolet (UV) detector (Waters 996). Analytical conditions used were previously published[24] and applied with slight modifications. Briefly, the mobile phase consisted of acetonitrile (ACN): water (62:38, v/v), flow rate of 1 mL/min and oven temperature of 55 °C. The stationary phase was comprised of ACE C18-PFP column (150 × 4.6 mm, 3 μm) coupled with an ACE C18-PFP 3 μm guard cartridge (Hichrom Ltd., Reading, UK). Dichlorodiphenyltrichloroethane (DDT) was used as an internal standard.

Quantification of CBD in Nanoemulsion

CBD was extracted through ethanol dilutions and subsequently diluted in mobile phase spiked with internal standard DDT (5 μg/mL final concentration), and 40 μL injected into the HPLC system for analysis. Calibration curves were linear in the range of 0.5 μg/mL to 10 μg/mL ($r^2 > 0.99$) and the limit of quantification for these analytical conditions was reported to be 10 ng/mL.[24]

Quantification of CBD in PCNPs

Samples were flash frozen in liquid nitrogen and freeze-dried for 12 h. CBD was quantified by reverse-phase HPLC to calculate encapsulation efficiency (EE %) and drug loading (DL %). Methanol was used to reconstitute 1 mg of lyophilised material. Samples were vortexed and centrifuged (Eppendorf 5430, Barkhausenweg, Germany) at 4000 g for 5 min through filtered Eppendorf tubes to separate precipitated polymer. Filtrate was collected and diluted further in methanol followed by mobile phase. After filtration through 0.22 μm syringe filters, 40 μl of sample was injected into HPLC for quantification. Calibration curves were linear ($r^2 > 0.99$) in the range of 0.5 μg/mL to 50 μg/mL.

The following equations were used to calculate EE % and DL % in PCNPs.

$$\frac{Drug\ present\ in\ NPs\ (mg)}{Initial\ drug\ added\ (mg)} \times 100 = EE\%$$

$$\frac{Drug\ present\ in\ NPs\ (mg)}{Polymer\ and\ drug\ after\ freeze\ drying\ (mg)} \times 100 = DL\%$$

Stability of Nanoemulsion and PCNPs

Nanoemulsion and PCNPs stability was analysed by DLS periodically, following storage at 4 °C.

Experimental Animals

Sprague-Dawley male adult rats (200–225 g) were obtained from Charles River (Kent, UK). They were maintained on 12-h light / day cycle at ambient temperature with free access to food and water. All animal experiments were carried out in line with the Home Office Animals (Scientific Procedures) Act 1986 adopting the principles of the 3Rs (replacement, reduction and refinement) and under approved Home Office project and personal licences.

Anaesthesia

Rats were anaesthetised with 3 % isoflurane (Baxter, UK) delivered in 100 % oxygen at 1 L/min rate in a plexiglass induction chamber. Anaesthesia was maintained by mechanical ventilation (model number 50–9703, Harvard Apparatus, Holliston, USA), set to 80 breaths / min and lung volume of 1 mL / 100 g. After the loss of reflexes, the rat was removed from the induction chamber and placed in a supine position, at which point anaesthesia was maintained at 3 % isoflurane via a glass nose-cone.

Tracheostomy Surgery

Cannulation of the trachea was performed via a tracheostomy surgery, to allow for very precise mechanical ventilation, which is essential to ensure complete control over anaesthetic delivery for electrophysiology recordings.[43] The fur over the throat of the rat was removed using scissors. The skin and the muscle layers on top of the trachea were then bluntly dissected using rat-tooth forceps to expose the trachea underneath. The trachea was elevated by placing a pair of sharp forceps beneath it to separate it from other surrounding layers of tissue. Two loose ligatures (2.0 suture silk) were placed underneath the trachea and tied 1 cm apart. The tracheostomy was carried out in the middle of the ligatures with a no. 11 blade and scalpel. Once a cut was made, a cannula was inserted with the bevel facing upwards, at a 3 mm depth into the tracheal opening. The cannula was made of 5–6 cm of curved tubing (1.57 mm interior and 2.08 mm exterior; Portex). Once the cannula was inserted, the ligatures tightened at the bottom and top of the trachea. After the cannula was secured on the trachea, it was connected to anaesthetic tubing.

Stereotaxic Placement

All rats were moved onto the stereotaxic frame in a prone position, fitted in conventional ear bars and bite bars after the trachea was cannulated. A rectal temperature probe connected to a thermostatically controlled heat blanket was inserted to maintain a core body temperature of 37 ± 1 °C.

Laminectomy Surgery

Once the rat was placed onto the frame, isoflurane was reduced to 2% for the remainder of the surgery. An incision through the fur and skin alongside the spinal cord from the middle of the shoulder-blades all the way to the hip was made (no. 11 blade and scalpel). After the musculature beneath the skin

was exposed and connective tissue was removed, incisions on either side of the spinal column starting from the shoulder blades were made (approx. 1 mm from the spinal column and 2–3 cm in length). Once the incisions (approx. 5 mm in depth) were performed, the column was elevated using rat-tooth forceps and clamped into position for the laminectomy. A scalpel was used to make an incision in the muscle layer overlaying the spinal cord (T13 and L-1 vertebrae). This layer of tissue was removed by rongeurs, exposing two spinal processing underneath. Rongeurs were used to insert into the space between L1 – L2 vertebrae which were then removed exposing the L4 – L5 region of the spinal cord for intrathecal injection. Anaesthetic level was reduced to 1.1 or 1 % isoflurane over the course of 45 min to allow for anaesthetic stabilisation.

Electromyography (EMG) Recordings

Electrophysiological techniques were previously used in the literature to assess the effects of intrathecal $GABA_A$ receptor antagonists on mechanical pain thresholds.[20] In this study, EMG recordings were carried out as described previously.[20,21] A bipolar EMG electrode (modified 27-gauge needle with two wires running through; Ainsworks, UK) was inserted into the right biceps femoris muscle. The electrode was connected to a NeuroLog head-stage (module NL100AK; Digitimer, Welwyn Garden City, UK) and the raw signals were amplified x 2000 (module NL104A) before being filtered through a band-pass at 10 – –1000 Hz (module NL125). Data was analysed by LabChart software through a PowerLab acquisition unit (AD Instruments, Sydney, Australia).

Mechanical Stimulation

Once anaesthetic level was stabilised at 1.1 or 1%, von Frey (VF) filaments were used to establish baseline responses. This was achieved by applying VF filaments to the plantar surface of the hind whilst recording the EMG response to the mechanical stimulation. The EMG responses were displayed as the raw signal and the integral of the root mean square (RMS) of the raw EMG response (an example EMG trace shown in Supplementary materials). The area under the curve (AUC) of the RMS was calculated and plotted against the VF filament used to establish the withdrawal response of both the baseline (before injection) and after injection of each formulation. The AUC of the EMG response (represented as a change relative to baseline) elicited at each time point tested across all VF filament weighs was plotted and the responses elicited after the injection of a blank and CBD formulations were compared. Following the IT injection, EMG recordings were carried out every 10 min for 30 mins, by stimulating the plantar surface of the hind paw with 100, 180 and 300 g VF filaments.

Intrathecal Injection

After baseline EMG responses were established, a Hamilton syringe coupled with 30 gauge-needle was used to inject 15 μL of CBD nanoemulsion and PCNPs in the IT space on the contralateral side of the spinal cord, beneath the L4 – L5 segments. To minimise injection variability, a micromanipulator was used and the formulation was injected over the course of 1–2 min to reduce back-flow. Following the injection, the needle was left at the injection site for up to 5 min to minimise loss of formulation.

Tissue Biodistribution Analysis

Rats were sacrificed by pentobarbital overdose (intraperitoneal injection, 200 mg/kg). Death was confirmed by exsanguination and tissues (brain, spinal cord, liver, spleen, deep cervical lymph nodes) and serum were collected for biodistribution analysis. Tissues were placed on ice straight away and stored at −80 °C until analysis by HPLC using a previously published method.[23–25] Blood was collected during exsanguination in an Eppendorf tube (1 mL) and was immediately centrifuged at 4000 *g* for 5 min to separate out the serum. The upper serum layer was then collected and stored at −80 °C until analysis.

Statistical Analysis

All data were expressed as mean ± SD or SEM. All statistical analyses were performed by GraphPad Prism 7 (GraphPad Software, UK). Data were tested for normal distribution. If three or more groups were analysed a two-way ANOVA was carried out with a post-hoc Tukey's test or Sidak's multiple comparisons test. If two groups were analysed a two-tailed t-test was performed. Differences considered statistically significant at $*p < 0.05$.

Results

CBD Intralipid® Nanoemulsion

The addition of CBD to Intralipid® nanoemulsion did not cause a significant change in particle size nor PDI as analysed by DLS (Fig. 1A). The size of CBD nanoemulsion was 286.8 ± 13.46 nm with a PDI of 0.135, indicative of a homogenous formulation. The nanoemulsion was stable for 70 days at 4 °C storage, with no significant physical changes (286.8 ± 13.46 nm on the day of formulation and 294.1 ± 1.38 nm 70 days later) (Fig. 1B). The ZP of the CBD nanoemulsion (ranging from −54.1 to −53.2 mV throughout 70 days) was not significantly different from the blank nanoemulsion (−59.5 to −54.0 mV) highlighting the stability of the formulation (Fig. 1C).

CBD PCNPs

CBD PCNPs formed by the double precipitation method were physically characterised by DLS (Fig. 2). It was clear the uncoated CBD formed aggregates with very large PDI and particle size (2222 ± 537.5 nm, 0.412 PDI), whereas when tPEG was added to the formulation the PCNPs formed were significantly smaller in size and had significantly less aggregation (121.8 ± 1.1 nm, PDI 0.079; Fig. 2A). tPEG alone self-assembled into polymeric micelles with a particle size diameter of 92.28 ± 1.16 nm and PDI of 0.156. In the presence of CBD nanocrystals, tPEG coats the drug leading to a significant increase in particle size (121.8 ± 1.1 nm) ($p < 0.001$), but not PDI (0.079 ± 0.014) (Fig. 2A). PCNPs were analysed periodically by DLS after storage at 4 °C and were shown to be stable for 31 days (Fig. 2B).

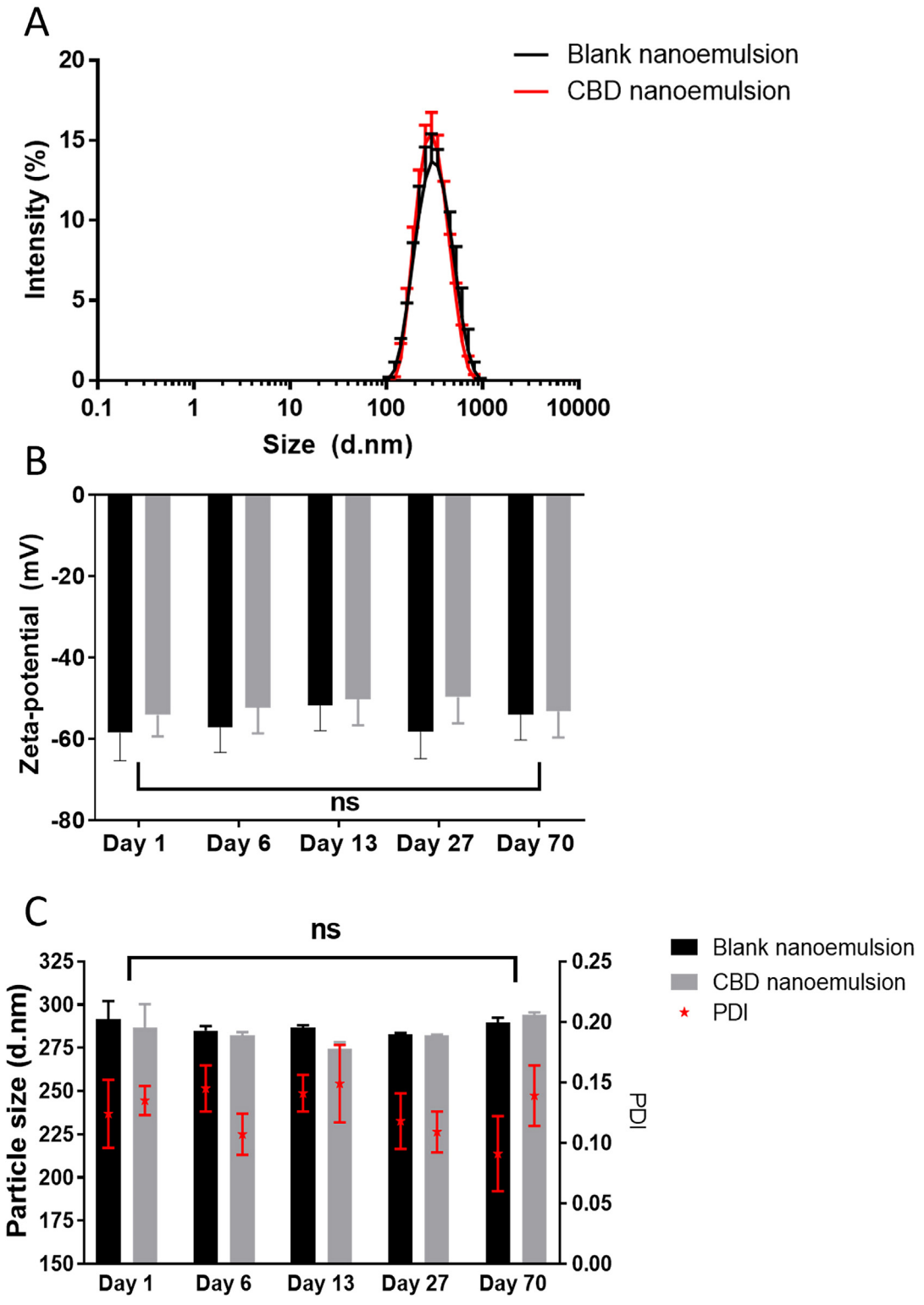

Fig. 1. Physical characterisation of CBD and blank nanoemulsions. **A.** Particle size (d.nm) and PDI of commercial Intralipid® nanoemulsion before and after CBD association determined by DLS (n = 3, means ± SD). No significant difference in size between blank nanoemulsion and after CBD association with nanoemulsion (291.5 nm ± 10.5 nm to 286.8 m, ± 13.46). PDI exhibited no significant changes from 0.124 ± 0.028 (blank nanoemulsion) to 0.135 ± 0.013 (CBD nanoemulsion). **B.** CBD nanoemulsion stability (n = 3, mean ± SD). Formulation remained stable at 4 °C over the course of 70 days, with no significant differences in size on the day of formulation (286.8 nm ± 13.46 nm) to 70 days later (294.1 ± 1.38). There were no significant differences in the PDI of the CBD nanoemulsion on day of formulation (0.135 ± 0.012) to 70 days later (0.139 ± 0.025). **C.** ZP of the blank and CBD nanoemulsion analysed by electrophoretic light scattering over the course of 70 days following storage at 4 °C (n = 3, mean ± SD). No significant difference in ZP measurements between CBD nanoemulsion (−54.1 mV to −53.2 mV) and blank nanoemulsion (−58.5 mV to −54.0 mV) over the time course tested.

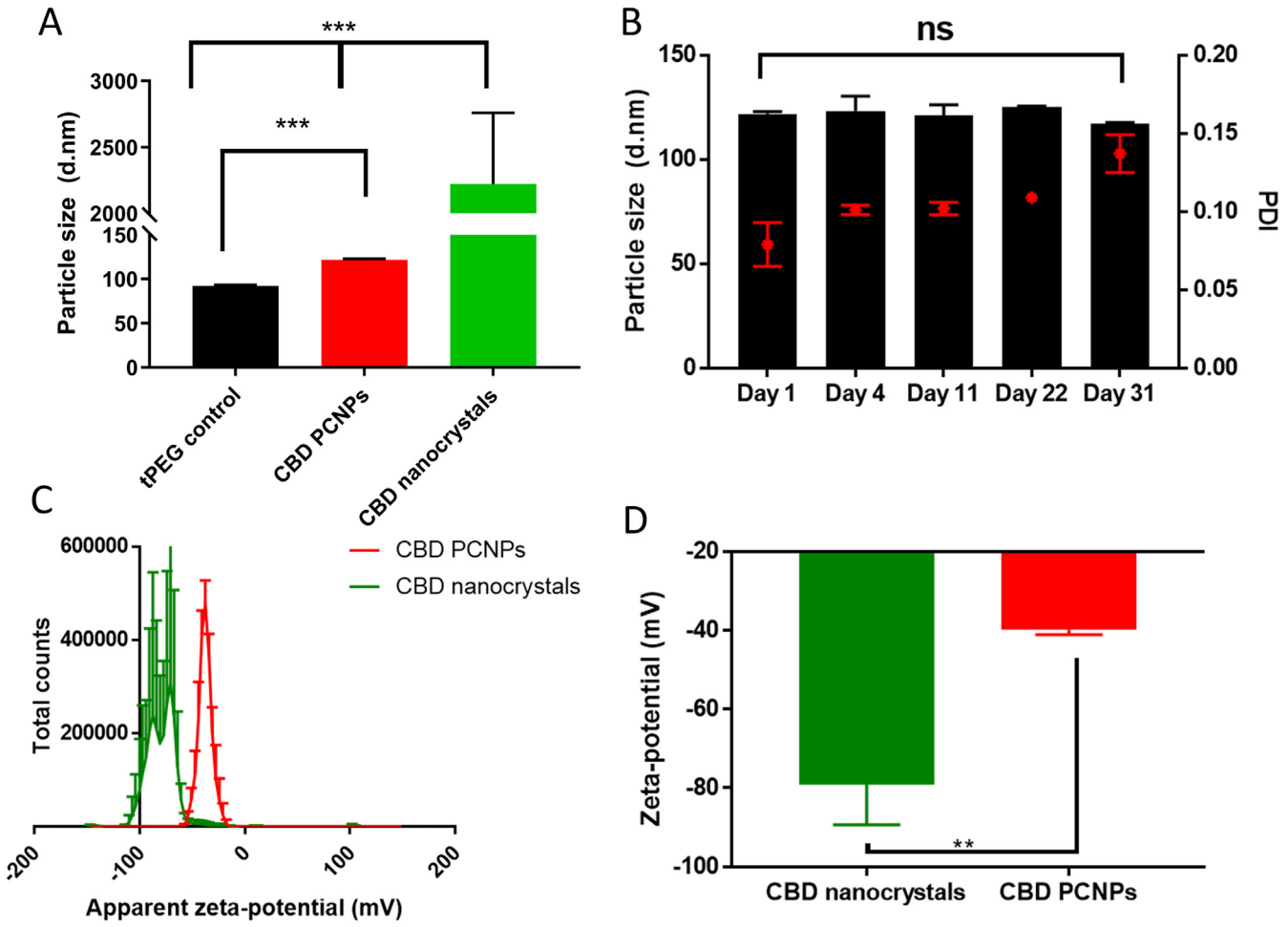

Fig. 2. Physical characterisation of CBD PCNPs. **A.** The particle size (d.nm) of CBD PCNPs formulated by a double nanoprecipitation method and analysed by DLS (n = 3, displayed as mean ± SD). tPEG alone self-assembles into micelles (92.28 nm ± 1.16 nm, PDI of 0.156) and coats CBD leading to a significant increase in particle size (121.8 nm ± 1.1) but not PDI (0.079 ± 0.014). CBD nanocrystals without coating are unstable and aggregate, leading to a significantly larger particle size 2222 nm ± 537.5 nm and 0.412 PDI. **B.** The stability of PCNPs stored at 4 °C was analysed periodically by DLS (n = 3, mean ± SD). 1 mg/mL CBD: 1 mg/mL tPEG PCNPs were stable at 4 °C for at least 31 days, with no significant change in particle size (121.8 ± 1.08 nm on the day of formulation to 116.9 ± 0.24 nm 31 days later) nor PDI (0.079 ± 0.014 to 0.137 ± 0.036). **C.** ZP of uncoated CBD nanocrystals was variable and stabilised showing only one peak when CBD PCNPs were formulated. **D.** ZP of CBD PCNPs (−39.9 mV) was significantly higher than CBD nanocrystals (−79.9 mV).

There were no significant changes in the particle sizes nor PDI of the CBD PCNP, which remained below 0.2 throughout, appropriate for parenteral delivery.[19] The ZP of CBD PCNPs was significantly higher at −39.9 mV ($p < 0.01$) than the ZP of uncoated CBD nanocrystals, which were highly unstable (−79.9 mV) (Fig. 2C and D).

Drug Loading and Association of CBD Formulations

CBD association with the nanoemulsion was calculated as the amount of CBD loaded per unit volume of formulation quantified by HPLC. The initial concentration of CBD added to nanoemulsion was 2 mg/mL and following separation of free drug by ultracentrifugation, the amount of CBD quantified was 1.3 mg/mL (65.1 % association). CBD encapsulation efficiency in PCNPs was calculated as the amount of CBD quantified in 1 mg of lyophilised material divided by the theoretical amount of CBD in the formulation. Drug loading efficiency was calculated in a similar manner, by dividing the amount of CBD quantified in 1 mg by the theoretical quantity of both CBD and polymer added to the formulation. This method achieved high CBD encapsulation and loading efficiencies of 46.04 % and 28.52 % respectively (Table 1). The formulation was evaporated under N_2 air to a pre-determined volume to concentrate CBD to 1 mg/mL concentration.

CBD Nanoemulsion and PCNPs Inhibit EMG Activity to Light Noxious Stimuli within 10 Mins of IT Injection in Healthy Rats

To test the efficacy of our formulations in vivo, we examined the effect of the CBD formulation on withdrawal responses after mechanical stimuli using EMG. The withdrawal reflex is a sensory behaviour which protects animals from surrounding danger.

The area under the curve (AUC) for the integral of the EMG signal was taken as a response to paw stimulation with VF

Table 1
Encapsulation efficiency (%), drug loading (%) and amount of drug loaded in CBD PCNPs and CBD nanoemulsion.

Formulation	Initial CBD concentration (mg/mL)	tPEG concentration (mg/mL)	Association/Encapsulation efficiency (%)	Drug loading (%)	Final CBD concentration (mg/mL)
PCNPs	1	1	46.04	28.52	0.46
Nanoemulsion	2	–	65.1	–	1.3

filaments of various strengths (100, 180 and 300 g) after IT injection and compared it to the AUC generated prior to injection. To control for the different sensitivities to isoflurane in rats and variations in the anaesthetic cycle over time, we established the EMG baseline prior to injection and set this as 100 %. All subsequent measurements after injections were expressed as a percentage change from each individual baseline, as previously described.[20–22] The AUC for all VF filaments was calculated and plotted against the time after injection of each formulation. The AUC of the EMG response (represented as change relative to baseline) elicited after the injection of CBD formulations showed a significant inhibition within 10 min of administration as compared to the respective blank controls (Fig. 3). There was a clear anti-nociceptive effect of both CBD formulations as measured by the decrease in EMG AUC after mechanical stimulation with all VF filaments 10 min after injection. These results confirm the successful IT injection in our in vivo model, and also indicate that CBD has an immediate therapeutic effect within the CNS after IT administration into the lumbar segment of healthy rats. No significant effects were observed at later time points.

Biodistribution of CBD in the CNS after IT Injection

The biodistribution of the CBD formulations in the CNS and in the spleen, liver, deep cervical lymph nodes and serum was quantified by HPLC through a previously established method.[23–25] Tissues were sampled over the course of 4 h, as previous CBD pharmacokinetic data suggests that T_{max} (time of maximum concentration) is achieved between 1 and 4 h.[10] Moreover, this was also coupled with non-recovery experimental time constraints. No CBD was detected in the liver, spleen or deep cervical lymph nodes, or in serum at any time-point tested after the administration of the nanoemulsion and PCNPs. Considering the differences in tissue weight between the brain and spinal cord, it was decided to evaluate the mass of CBD in the CNS as opposed to its concentration, thus taking in consideration the dilution factor in the brain (~ 4-fold difference between brain and spinal cord weight).

The highest mass of CBD found in the spinal cord after the administration of the nanoemulsion was at the 2 h time-point (3651 ng; Fig. 4A). A significantly lower proportion of CBD was found 10 min after injection of the nanoemulsion (404 ng) in contrast to PCNPs (7761 ng). The highest mass of CBD after the administration of PCNPs was within 10 min of delivery, after which it significantly decreases at 30 mins, with the lowest concentration at 4 h (Fig. 4A).

CBD was detected in the brain immediately after the injection of both formulations, ranging in concentrations from 8 to 210 ng/mL, and was detected up to 4 h after administration (Supplementary materials). The fact that no CBD was detected outside of the CNS indicates that the IT administration technique achieves localised drug delivery with the highest concentrations of drug in the spinal cord and a fraction of the drug reaching the brain within 10 min of injection of both formulations (Fig. 4B). No detectable CBD concentrations were found clearing from the CNS into the spleen and liver at any time-points tested. The dose of CBD administered in both formulations in this study was

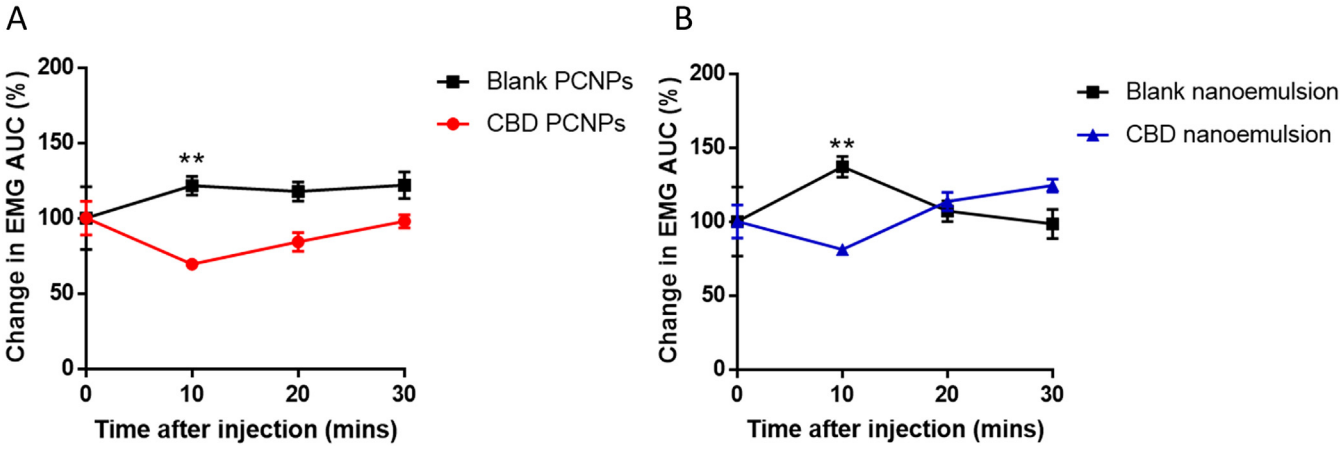

Fig. 3. EMG recordings calculated as the area under the curve (AUC) of the 100, 180 and 300 g VF filaments at 10, 20 and 30 min after injection of formulations. **A.** AUC of EMG response after the injection of CBD nanoemulsion compared to blank nanoemulsion (mean ± SEM, n = 5 for CBD nanoemulsion and n = 4 for blank). Injection of CBD nanoemulsion significantly decreased AUC at 10 min (**$p < 0.01$) in comparison to the EMG AUC after the injection of blank nanoemulsion **B.** AUC of EMG response after the injection of CBD PCNPs compared to blank PCNPs (mean ± SEM, n = 5 for CBD PCNPs and n = 4 for blank PCNPs). Injection of CBD PCNPs significantly decreased EMG AUC at 10 (**p < 0.01) in comparison to the EMG AUC after the injection of blank PCNPs. Statistical analysis by 2-way ANOVA with Sidak's multiple comparisons test.

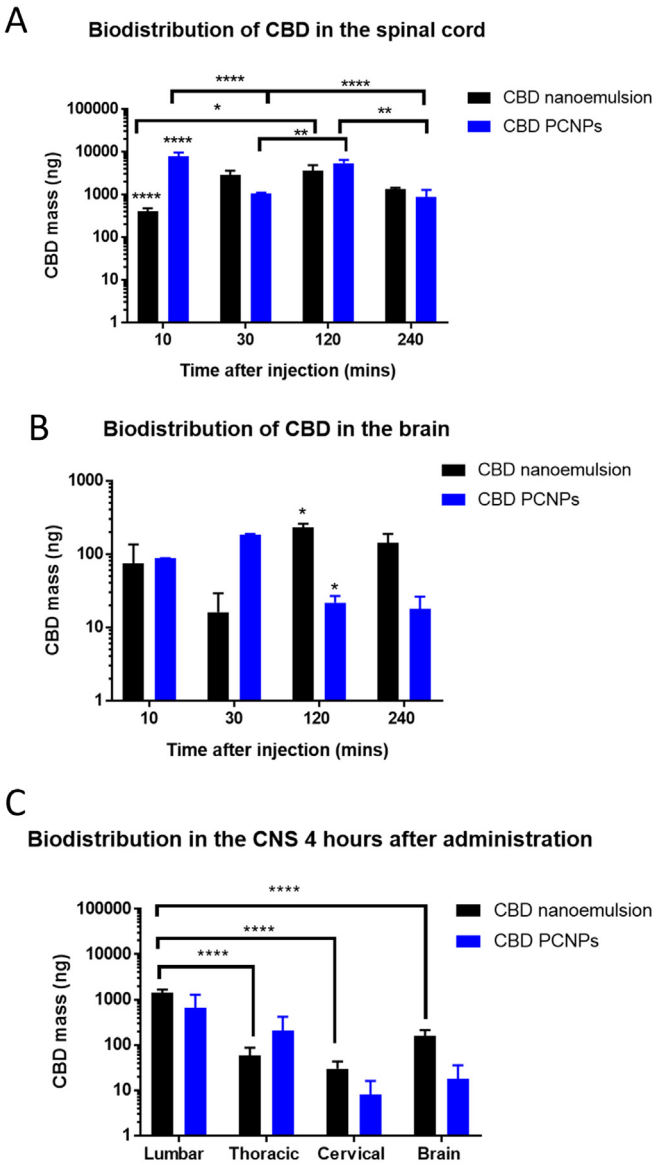

Fig. 4. Biodistribution of CBD formulations in the CNS after IT administration of 0.05 mg/kg dose. **A.** Biodistribution of CBD mass in the spinal cord (n = 3). CBD mass was significantly higher in the spinal cord after administration of CBD PCNPs within 10 min (****$p < 0.001$). CBD mass after injection of PCNPs was highest at the 10 min time-point (7761 ng), significantly higher than at 30 min and 4 h (****$p < 0.001$) and lowest at 4 h after injection (867 ng). The mass of CBD after administration of the CBD nanoemulsion was the lowest 10 min after administration (404 ng), peaking with a significantly higher mass at 2 h (3651 ng) (*$p < 0.05$). **B.** Biodistribution of CBD mass in the brain after the administration of CBD nanoemulsion and PCNPs (n = 3). There was a significantly higher mass of CBD detected in the brain 2 h after injection of CBD nanoemulsion (228 ng) as compared to CBD PCNPs (21 ng) (*$p < 0.05$). No other significant differences were observed, with total the CBD mass found in the brain ranging from 16 ng to 228 ng over the course of 4 h. **C.** Biodistribution of CBD formulations in the CNS, 4 h after IT injection (n = 6 for nanoemulsion and n = 3 for PCNPs). Mass of CBD in brain, cervical segment and thoracic segment were significantly lower (****$p < 0.001$) than the mass of CBD in the lumbar segment (site of injection) 4 h after the administration of CBD nanoemulsion (n = 6). No significant differences in the mass of CBD in any of the other CNS tissues were observed after the administration of CBD PCNPs (values expressed as mean ± SEM).

0.05 mg/kg. The highest C_{max} in the brain was achieved upon administration of the CBD nanoemulsion at T_{max} 120 min (Table 2).

Discussion

The lipid nanoemulsion Intralipid® licenced as a parenteral nutrition method was chosen as a formulation for CBD due to previous reports of suitability and low toxicity in clinical settings.[26] Nanoemulsions have been previously used to aid the delivery of insoluble lipophilic drugs or therapeutics that are prone to chemical instability, such as enzymatic degradation or hydrolysis.[27] Furthermore, the dispersion of therapeutics in lipids is also a known method of enhancing their delivery by increasing their subsequent residence at the site of injection.

Table 2
Pharmacokinetic parameters of CBD formulations in the brain.

PK parameter	CBD nanoemulsion	CBD PCNPs
C_{max}* (ng/g)	210	94
T_{max}† (min)	120	30
AUC‡ $_{0–4\ h}$ (ng/g)	27,337	7341

* Peak plasma concentration.
† Time to reach C_{max}.
‡ Area under the curve.

A variety of drug-containing nanoemulsions have been brought to the market, with numerous others under evaluation preclinically.[28] Intralipid® has been used in the past as a nanocarrier for the delivery of paclitaxel, a poorly water soluble chemotherapeutic.[29] In this case, an increase in the droplet size of Intralipid® was noted upon the loading of the therapeutic (294.6 nm with 6 mg/mL paclitaxel as opposed to 283.3 nm for the blank nanoemulsion), but the PDI remained below 0.2. These results also highlight the inherent stability of the nanoemulsion, evidencing its suitability for use as a nanocarrier for lipophilic drugs. CBD has been previously formulated in lipid-based systems preclinically, mostly for oral administration.[23,25,30] Nonetheless, to our knowledge, its biodistribution within the CNS after direct lumbar administration formulated as a CBD nanoemulsion has not been previously studied.

For our alternative formulation, a simple and reproducible method for the coating of CBD nanocrystals with an amphiphilic PEG_{1014}-$(LA)_{100}$ polymer was employed.[31] The polymer of choice was previously developed by another research group[32] and was synthesised and characterised herein by the same procedures. tPEG was selected due to its biodegradable and biocompatible properties ideal for IT delivery. This was based on studies on Genexol-PM, a polymeric NP formulation of paclitaxel and monomethoxy poly (ethylene glycol)-block-poly (D,L-lactide (mPEG-PDLLA)) approved for the treatment of breast cancer in South Korea.[33] In the literature, CBD has been previously formulated in PLC microspheres through an emulsion-solvent evaporation technique by Ossa et al., 2012[34]; however, the size ranged between 20 and 50 μm, which is not appropriate for parenteral administration, as the size should ideally be below 100 nm to manoeuvre through the SAS.[16] To our knowledge this is the first CBD PCNP system developed, as previous CBD formulations have mainly focused on either lipid-based systems (lipid nanocapsules) or polymeric microparticles.[30,34–37]

We employed EMG recordings to assess the excitability of the dorsal horn to noxious mechanical stimuli after the IT administration of the CBD formulations. VF filaments used in this study ranged from 100 to 300 g bending force in order to test the anti-nociceptive potential of the formulations at various degrees of noxious mechanical stimulation. The increase in EMG signal after injection of blank nanoemulsion could be indicative of sensitisation as a result of penetrance of the SAS, which might also lead to a shallower level of anaesthesia. Either way, it provides evidence that, in both formulations, CBD has an immediate anti-nociceptive effect in the dorsal horn of healthy rats, indicating its partitioning from the vehicle and its subsequent activity on nociceptive signalling in the spinal cord.[9] In previous studies, Genaro et al., 2017[6] injected CBD (10 to 50 nmol in 0.25 μL total volume; dissolved in 100 % grape seed oil) directly to the rostral anterior cingulate cortex in rats. They showed a dose-dependent decrease in mechanical allodynia which lasted 120 mins.[6] Interestingly, this decrease in mechanical allodynia started 20 min after injection, which possibly indicated a slower therapeutic onset when injected directly to the brain. However, this could also be due to the fact that CBD in that study was dissolved in pure oil as opposed to a nanoemulsion. Taken together, previous literature indicates that CBD is effective in reducing pain,[38–40] and our study also demonstrates its inhibition of EMG activity to noxious mechanical stimuli within 10 min of IT delivery.

Bujedo et al., 2017[41] have found that after IT administration, hydrophilic drugs such as morphine reached the CSF at higher concentrations in comparison to lipophilic opioids, which were sequestered in the epidural fat.[41] Moreover, fentanyl and alfentanil accumulated in epidural fat at 32- and 20-fold higher concentrations than morphine, respectively, leading to decreased quantities reaching the receptors expressed on the spinal cord.[41] This effect might also be observable with the CBD nanoemulsions, given the fact that the lipid droplets may display an affinity towards the fatty tissues of the CNS. Ummenhofer et al., 2000[11] have also demonstrated that the tissue distribution of opioids after administration into the SAS of pigs is largely governed by their lipid solubility, with sufentanil rapidly partitioning out of the CSF into more hydrophobic environments, such as the lipids which make up the non-aqueous portion of the white matter.[11] Therefore, this phenomenon decreases the amount of drug reaching the spinal cord opioid receptors.

The dose of CBD administered in both formulations in this study was 0.05 mg/kg. The highest C_{max} in the brain was achieved upon administration of the CBD nanoemulsion at T_{max} 120 min (Table 2). Deiana et al., 2012[42] have administered CBD in a cremophor and solutol formulation at a 120 mg/kg dose, through oral gavage and IP administration. The highest C_{max} achieved in the brain of rats was 12.6 μg/g through oral delivery.[42] Equivalence of the dose we administered intrathecally to the one in this study results in a 2400-fold difference, therefore, assuming linear pharmacokinetics, the C_{max} achieved through oral administration would be 5.25 ng/g at the same dose as in the current work. This study therefore demonstrates that significantly higher brain concentrations can be achieved through IT delivery, and the C_{max} can be enhanced through the use of a nanoemulsion. On the other hand, T_{max} was reached earlier when PCNPs were administered (30 min as opposed to 120 mins), indicating that the use of PCNPs leads to the faster distribution of CBD from the lumbar segment of the spinal cord to the brain. Whereas, the interactions of the nanoemulsion lipid droplets with the SAS components (and epidural fat) might hinder the movement through the IT space. This phenomenon was also evidenced by the $AUC_{0–4h}$ of the nanoemulsion in the brain, which was 3.7-fold higher than that of the PCNPs. These data suggest that the residence time of the CBD in the brain is increased through the delivery of a nanoemulsion as opposed to PCNPs (i.e., clearance is slower). Householder et al., 2019[14] have shown that 100 nm fluorescent PEGylated NPs administered to the cisterna magna of healthy mice rapidly distributed alongside the entire neuraxis,

whilst being retained in the leptomeninges for over 3 weeks. They also investigated larger 10 μm microparticles which did not exhibit as widespread distribution as the NP counterparts.[14] Nonetheless, in the brain, our formulations display different T_{max} values. The CBD nanoemulsion leads to a higher C_{max} of 210 ng/g at T_{max} of 120 mins, whereas the PCNPs exhibited a C_{max} of 94 ng/g at T_{max} of 30 mins. This might be attributable to the clearance of the smaller NPs via perivascular spaces in the CSF or through the cribriform plate into the nasal mucosa.[2,14]

Therefore, in this study it was shown that CBD reaches the brain following the administration of both formulations, demonstrating that IT delivery is a potential delivery technique for efficacious CNS targeting. Moreover, it was also demonstrated that widespread CBD distribution in the CNS after administration of both formulations at all time-points was attained (Supplementary materials). Nonetheless, there was significant retention of the CBD nanoemulsion at the lumbar segment of the spinal cord (site of injection) in comparison to the cervical and thoracic segments ($p < 0.001$), 4 h after administration (Fig. 4C). No significant differences were observed in the distribution of the CBD PNCPs across the spinal cord segment at this time-point. These results might be indicative of CBD retention at the site of injection over-time, with decreasing concentrations of therapeutics reaching the more distant spinal segments. This suggests that retention of CBD at the site of injection in the nanoemulsion after 4 h might be due to partitioning into fatty tissues due to its lipophilicity, which was overcome through formulation of PCNPs.

A potential limitation associated with this analytical methodology is that due to whole tissue homogenisation it was not possible to detect whether the drug penetrated the brain parenchyma or was simply bound to the surface of the brain. Further studies investigating the penetration of drugs into the brain parenchyma would be tremendously beneficial to the field, most notably in the treatment of brain tumours deeply embedded into the brain. As the literature suggests that entry of a drug into the brain parenchyma is minimal, mediated by diffusion and no deeper than 1–2 mm from the surface of the brain.[4] It would be interesting to investigate if the CBD formulations were able to penetrate deep into the brain parenchyma or if there would be altered biodistribution in different structures of the brain. This could be achieved via co-registration of mass spectrometry imaging (i.e., using orbiSIMS or ToF-SIMS) with histological staining on post-sacrificial tissue, to provide high resolution in situ data of drug penetration within brain parenchyma.

The results in our study suggest that the CBD nanoemulsion was initially retained within the CNS fatty tissues whilst acting as a depot, releasing CBD over time, with its mass in the spinal cord peaking 2 h after injection. Nonetheless, CBD attained brain delivery within 10 min of administration, reaching a C_{max} of 210 ng/g and a T_{max} of 120 min when delivered as a nanoemulsion. In contrast, upon delivery of CBD PCNPs, the mass of CBD was the highest in the spinal cord 10 min after injection, decreasing at the 30 min time-point, followed by a subsequent return in concentration after 120 mins. CBD PCNPs also attained brain delivery within 10 min with a C_{max} of 94 ng/g reaching T_{max} at 30 mins. In light of these data, the initial hypothesis that the CBD nanoemulsion would be preferentially retained at the site of injection as a result of its size and lipophilicity will be rejected. This is because we have observed a 3.7-fold higher $AUC_{0–4\ h}$ and higher C_{max} in the brain upon the delivery of the nanoemulsion as opposed to PCNPs. This suggests that more CBD was delivered to the sites furthest away from the lumbar segment (i.e., the brain) when a lipid-based nanoemulsion was used. This occurred even though a significant amount of CBD was retained at the site of injection, especially at the 4 h time-point (Fig. 4C). Interestingly, we observed that T_{max} in the brain was reached earlier with the delivery of PCNPs, probably resulting from the smaller, PEGylated nature of this formulation. Nonetheless, the PCNPs were quickly cleared away from this site, therefore the accumulation of CBD in the brain when administered as PCNPs was reduced in comparison to the administration of the nanoemulsion. In contrast, we can accept our hypothesis that CBD PCNPs led to more spread from the site of injection, as we have observed that a higher mass proportion of CBD reached the adjacent thoracic segment in comparison to the CBD nanoemulsion. Nonetheless, this spread was only applicable to the spinal cord, as when PCNPs reached the most distal site, the brain, their accumulation was hindered, possibly due to their rapid clearance of the PCNPs. Moreover, we witnessed an almost immediate anti-nociceptive effect of both CBD formulations 10 min after administration, thus accepting our initial hypothesis that both formulations would exhibit anti-nociceptive effects in vivo. These results provide essential information for the development of CBD therapies for neuropathic pain and provide rationale for nanocarrier design to aid their delivery to the CNS by IT administration.

These results suggest that the CBD delivered in a nanoemulsion formulation is preferentially retained at the site of injection, however it still attains brain delivery within 10 min of injection. When PCNPs were administered, CBD delivery to the brain was more rapid, possibly due to the PEGylated nature of the formulation. Nonetheless, we observed that accumulation of CBD in the brain when PCNPs were injected was lower in comparison to the nanoemulsion, possibly due their rapid clearance. Moreover, we witnessed an almost immediate anti-nociceptive effect of both CBD formulations 10 min after administration, thus accepting our initial hypothesis that both formulations would exhibit anti-nociceptive effects in vivo. These results provide essential information for the development of CBD therapies for neuropathic pain and provide rationale for nanocarrier design to aid their delivery to the CNS by IT administration.

Disclosure Statement

All authors state that they have no conflicts of interest to disclose.

CRediT Authorship Contribution Statement

Paula Muresan: The conception and design of the study, planning, experimental investigation, data curation and visualisation, data analysis and writing of the original draft.

Stephen Woodham: Animal experimentation (including laminectomy surgery, anaesthesia and electromyography (EMG) recordings), data analysis, manuscript drafting and reviewing.

Fiona Smith: Experimental investigation of the nanoparticles, data analysis, manuscript drafting and reviewing.

Vincenzo Taresco: Polymer synthesis and characterisation, data analysis, manuscript drafting and reviewing.

Jaymin Shah: Conception and design of the study (emulsions/nanoparticles), manuscript review.

Mei Wong: Conception and design of the study (emulsions/nanoparticles), manuscript review.

Victoria Chapman: Design of the animal studies and animal license holder, manuscript review.

Stuart Smith: Conception and design of the study, manuscript review.

Gareth Hathway: Conception and design of the animal studies, data analysis/review, manuscript review.

Ruman Rahman: Conception and design of the study, supervision, manuscript reviewing.

Pavel Gershkovich: Conception and design of the study, supervision, manuscript reviewing.

Maria Marlow: Conception and design of the study, supervision and project administration, manuscript reviewing and editing.

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Appendix A. Supplementary data

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References

1. Cook RL, Householder KT, Chung EP, Prakapenka AV, DiPerna DM, Sirianni RW. A critical evaluation of drug delivery from ligand modified nanoparticles: confounding small molecule distribution and efficacy in the central nervous system. *J Control Release* 2015;**220**(Pt A):89-97.
2. Fowler MJ, Cotter JD, Knight BE, Sevick-Muraca EM, Sandberg DI, Sirianni RW. Intrathecal drug delivery in the era of nanomedicine. *Adv Drug Deliv Rev* 2020;**165–166**:77-95.
3. Pflepsen KR, Peterson CD, Kitto KF, Vulchanova L, Wilcox GL, Fairbanks CA. Detailed method for intrathecal delivery of gene therapeutics by direct lumbar puncture in mice. *Methods Mol Biol* 2019;**1937**:305-12.
4. Pardridge W. Drug transport in the brain via the cerebrospinal fluid. *Fluids Barriers CNS* 2011;**8**:7.
5. Russo EB. Cannabinoids in the management of difficult to treat pain. *Ther Clin Risk Manag* 2008;**4**(1):245-59.
6. Genaro K, Fabris D, Arantes ALF, Zuardi AW, Crippa JAS, Prado WA. Cannabidiol is a potential therapeutic for the affective-motivational dimension of incision pain in rats. *Front Pharmacol* 2017;**21**(8):391.
7. Kathmann M, Flau K, Redmer A, Tränkle C, Schlicker E. Cannabidiol is an allosteric modulator at mu- and delta-opioid receptors. *Naunyn-Schmiedeberg'sArchives of Pharmacology* 2006;**372**(5):354-61 372.
8. Laun AS, Shrader SH, Brown KJ, Song Z-H. GPR3, GPR6, and GPR12 as novel molecular targets: their biological functions and interaction with cannabidiol. *Acta Pharmacologica Sinica* 2018;**3**(40):300-8.
9. Urits I, Gress K, Charipova K, Habib K, Lee D, Lee C, et al. Use of cannabidiol (CBD) for the treatment of chronic pain. *Best Pract Res Clin Anaesthesiol* 2020;**34**(3):463-77.
10. Millar SA, Stone NL, Yates AS, O'Sullivan SE. A systematic review on the pharmacokinetics of cannabidiol in humans. *Front Pharmacol* 2018;**26**(9):1365.
11. Ummenhofer WC, Arends RH, Shen DD, Bernards CM. Comparative spinal distribution and clearance kinetics of intrathecally administered morphine, fentanyl, alfentanil, and sufentanil. *Anesthesiology* 2000;**92**(3):739-53.
12. Flack SH, Bernards CM. Cerebrospinal fluid and spinal cord distribution of hyperbaric bupivacaine and baclofen during slow intrathecal infusion in pigs. *Anesthesiology* 2010;**112**(1):165-73.
13. Barua S, Mitragotri S. Challenges associated with penetration of nanoparticles across cell and tissue barriers: a review of current status and future prospects. *Nano Today* 2014;**9**(2):223-43.
14. Householder KT, Dharmaraj S, Sandberg DI, Wechsler-Reya RJ, Sirianni RW. Fate of nanoparticles in the central nervous system after intrathecal injection in healthy mice. *Sci Rep* 2019;**9**(1):12587.
15. Wolf DA, Hesterman JY, Sullivan JM, Orcutt KD, Silva MD, Lobo M, et al. Dynamic dual-isotope molecular imaging elucidates principles for optimizing intrathecal drug delivery. *JCI Insight* 2016;**1**(2)e85311.
16. Nance EA, Woodworth GF, Sailor KA, Shih TY, Xu Q, Swaminathan G, et al. A dense poly(ethylene glycol) coating improves penetration of large polymeric nanoparticles within brain tissue. *Sci Transl Med* 2012;**4**(149)149ra119.
17. Blanco JA, Romero IA, Benoit JP, Torres Suarez AI. Nannocannabinoids for brain tumor drug delivery. *An Real Acad Farm* 2019;**85**(2):198-216.
18. Saboori P, Sadegh A. Histology and morphology of the brain subarachnoid trabeculae. *Anat Res Int* 2015;**2015**279814.
19. Danaei M, Dehghankhold M, Ataei S, Hasanzadeh Davarani F, Javanmard R, Dokhani A, et al. Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. *Pharmaceutics* 2018;**10**(2):57.
20. Hathway G, Harrop E, Baccei M, Walker S, Moss A, Fitzgerald M. A postnatal switch in GABAergic control of spinal cutaneous reflexes. *Eur J Neurosci* 2006;**23**:112-8.
21. Hathway GJ, Fitzgerald M. Time course and dose-dependence of nerve growth factor-induced secondary hyperalgesia in the mouse. *J Pain* 2006 Jan;**7**(1):57-61.
22. Koch SC, Fitzgerald M, Hathway GJ. Midazolam potentiates nociceptive behavior, sensitizes cutaneous reflexes, and is devoid of sedative action in neonatal rats. *Anesthesiology* 2008 Jan;**108**(1):122-9.
23. Feng W, Qin C, Chu Y, Berton M, Lee JB, Zgair A, et al. Natural sesame oil is superior to pre-digested lipid formulations and purified triglycerides in promoting the intestinal lymphatic transport and systemic bioavailability of cannabidiol. *Eur J Pharm Biopharm* 2021 May;**162**:43-9.
24. Zgair A, Wong JC, Sabri A, Fischer PM, Barrett DA, Constantinescu CS, et al. Development of a simple and sensitive HPLC-UV method for the simultaneous determination of cannabidiol and Δ(9)-tetrahydrocannabinol in rat plasma. *J Pharm Biomed Anal* 2015 Oct;**10**(114):145-51.
25. Zgair A, Lee JB, Wong JCM, Taha DA, Aram J, Virgilio DD, et al. Oral administration of cannabis with lipids leads to high levels of cannabinoids in the intestinal lymphatic system and prominent immunomodulation. *Sci Rep* 2017;**7**:14542.
26. Fortner CL, Grove WR, Bowie D, Walker MD. Fat emulsion vehicle for intravenous administration of an aqueous insoluble drug. *Am J Hosp Pharm* 1975;**32**(6):582-4 PMID: 1155469.
27. Bonferoni MC, Rossi S, Sandri G, Ferrari F, Gavini E, Rassu G, et al. Nanoemulsions for "nose-to-brain" drug delivery. *Pharmaceutics* 2019;**11**(2):84.
28. Hippalgaonkar K, Majumdar S, Kansara V. Injectable lipid emulsions-advancements, opportunities and challenges. *AAPS PharmSciTech* 2010 Dec;**11**(4):1526-40.

29. Najlah M, Kadam A, Wan KW, Ahmed W, Taylor KM, Elhissi AM. Novel paclitaxel formulations solubilized by parenteral nutrition nanoemulsions for application against glioma cell lines. *Int J Pharm* 2016;**506** (1–2):102-9.
30. Cherniakov I, Izgelov D, Domb AJ, Hoffman A. The effect of pro Nano-Lipospheres (PNL) formulation containing natural absorption enhancers on the oral bioavailability of delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) in a rat model. *Eur J Pharm Sci* 2017;**15**(109):21-30.
31. Styliari I, Taresco V, Theophilus A, Cameron A, Garnett M, Laughton.. Nanoformulation-by-design: an experimental and molecular dynamics study for polymer coated drug nanoparticles. *RSCAdvances* 2020;**10**:19521-33.
32. Vasey C, Pearce A, Sodano F, Cavanagh R, Abelha T, Cuzzucoli Crucitti V, et al. Amphiphilic tri- and tetra-block co-polymers combining versatile functionality with facile assembly into cytocompatible nanoparticles. Biomaterials. *Science* 2019;**7**.
33. Werner ME, Cummings ND, Sethi M, Wang EC, Sukumar R, Moore DT, et al. Preclinical evaluation of genexol-PM, a nanoparticle formulation of paclitaxel, as a novel radiosensitizer for the treatment of non-small cell lung cancer. *Int J Radiat Oncol Biol Phys* 2013 Jul 1;**86**(3):463-8.
34. Pérez Hernán, de la Ossa D, Ligresti A, Gil-Alegre ME, Aberturas MR, Molpeceres J, et al. Poly-ε-caprolactone microspheres as a drug delivery system for cannabinoid administration: development, characterization and in vitro evaluation of their antitumoral efficacy. *J Control Release* 2012;**161**(3):927-32.
35. Aparicio-Blanco J, Romero IA, Male DK, Slowing K, García-García L, Torres-Suárez AI. Cannabidiol enhances the passage of lipid nanocapsules across the blood-brain barrier both in vitro and in vivo. *Mol Pharm* 2019 May 6;**16**(5):1999-2010.
36. Aparicio-Blanco J, Sebastián V, Benoit JP, Torres-Suárez AI. Lipid nanocapsules decorated and loaded with cannabidiol as targeted prolonged release carriers for glioma therapy: in vitro screening of critical parameters. *Eur J Pharm Biopharm* 2019 Jan;**134**:126-37.
37. Lorente M, Gil-Alegre ME, Torres S, García-Taboada E, Aberturas Mdel R, Molpeceres J, et al. Local delivery of cannabinoid-loaded microparticles inhibits tumor growth in a murine xenograft model of glioblastoma multiforme. *PLoS One* 2013;**8**(1)e54795.
38. Xiong W, Cui T, Cheng K, Yang F, Chen SR, Willenbring D, et al. Cannabinoids suppress inflammatory and neuropathic pain by targeting α3 glycine receptors. *J Exp Med* 2012 Jun 4;**209**(6):1121-34.
39. Costa B, Trovato AE, Comelli F, Giagnoni G, Colleoni M. The non-psychoactive cannabis constituent cannabidiol is an orally effective therapeutic agent in rat chronic inflammatory and neuropathic pain. *Eur J Pharmacol* 2007 Feb 5;**556**(1–3):75-83.
40. Izzo AA, Borrelli F, Capasso R, Di Marzo V, Mechoulam R. Non-psychotropic plant cannabinoids: new therapeutic opportunities from an ancient herb. *Trends Pharmacol Sci* 2009 Oct;**30**(10):515-27.
41. Bujedo B. Physiology of spinal opioids and its relevance for pain management selection. *Open J Pain Med* 2017;**1**(1):021-6.
42. Deiana S, Watanabe A, Yamasaki Y, Amada N, Arthur M, Fleming S, et al. Plasma and brain pharmacokinetic profile of cannabidiol (CBD), cannabidivarine (CBDV), Δ9-tetrahydrocannabivarin (THCV) and cannabigerol (CBG) in rats and mice following oral and intraperitoneal administration and CBD action on obsessive-compulsive behaviour. *Psychopharmacology (Berl)* 2012 Feb;**219**(3):859-73.
43. Rampil IJ, King BS. Volatile anesthetics depress spinal motor neurons. *Anesthesiology* 1996 Jul;**85**(1):129-34.
44. Fessi H, Puisieux F, Devissaguet JP, Ammoury N, Benita S. Nanocapsule formation by interfacial polymer deposition following solvent displacement. *Int J Pharm* 1989;**55**:R1-4.
45. McCrorie P, Mistry J, Taresco V, et al. Etoposide and olaparib polymer-coated nanoparticles within a bioadhesive sprayable hydrogel for post-surgical localised delivery to brain tumours. *Eur J Pharm Biopharm* 2020 Dec;**157**:108-20.