



# **SEEKING FOR STRATEGIES TO SUPPORT THE BLOOD-BRAIN BARRIER IN THE PROTECTION AGAINST INVADING CELLS**

Summary of the Ph.D. Thesis

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## INTRODUCTION

During inflammatory brain diseases, elevated number of immune cells infiltrate the central nervous system (CNS) across the brain vasculature which is a serious danger to the functioning brain. Besides leukocytes implicated in neuroinflammation, cancer cells can also migrate across the cerebral vessels to form brain metastases. The most frequent origins of brain metastases are primary tumors of lung, breast and skin (melanoma). Of all primary tumors, melanoma shows the highest predilection to spread to the brain. Brain metastases of melanoma carry a poor prognosis with a median survival of approximately four months.

The brain microvasculature forming the blood-brain barrier (BBB) allows the passage of nutrients and metabolites required by the CNS with passive diffusion or active transport and also prevents the entry of cells, potentially harmful molecules or ions which interfere with neuronal communications. Therefore, by controlling the movement of solutes and cells between the blood stream and the brain parenchyma, the BBB has a crucial role in maintaining the homeostasis of the CNS.

Endothelial cells, lining the cerebral microvasculature, form the anatomic basis of the BBB. Special characteristics of these cells establish the barrier function of the cerebral vasculature. Tight junctions (TJs) between endothelial cells form a physical barrier restricting the paracellular way of transport and transmigration. Beyond endothelial cells, the BBB consists of astrocyte end-feet, pericytes and the basal lamina and has an intimate relationship with neurons and microglia. These perivascular cells and structures contribute to the maintenance and modulation of the barrier characteristics of endothelial cells.

Since the CNS lacks a classical lymphatic system, leukocytes and metastatic tumor cells have to pass the blood vessels to reach the brain parenchyma. Cancer cell migration across the brain endothelium is a less studied process than leukocyte diapedesis. Presumably, the diapedesis of leukocytes and extravasation of metastatic tumor cells share similar mechanisms, but there are significant differences as well.

The pathology of neuroinflammatory diseases and brain metastases involves several cellular and molecular mechanisms. For effective therapies, several steps of these multifactorial diseases should

be targeted, including the diapedesis of leukocytes or cancer cells. New molecular biological findings and chemical compounds discovered during the last few decades might provide new perspectives for interfering with these steps. Among these, cannabinoid receptor 2 (CB2) agonists and lignans might be promising compounds to target immune and metastatic cell migration into the brain.

The cannabinoid system is known mainly for its psychoactive effects; however, it has been previously shown that its activation may induce anti-inflammatory and neuroprotective processes as well. Cannabinoids exert their effects mainly through two receptor types: cannabinoid receptor 1 (CB1) and CB2 which mediate distinct effects. The psychoactive effect is triggered by CB1, whereas the anti-inflammatory effects are mainly induced by the activation of CB2. Selective CB2 agonists, such as JWH133 and O1966, provide new perspectives to study the role of specific activation of CB2 in several physiological and pathological processes, without activating the CB1 that may induce psychoactive side-effects. These selective agonists are also clinical candidates for the treatment of several diseases associated with inflammation.

Lignans are polyphenolic substances constituting one of the major classes of phytoestrogens. These chemical compounds are widely distributed throughout the plant kingdom and have structural similarity to the endogenous hormone 17 $\beta$ -estradiol. There is an evergrowing interest in neuroinflammatory disease or cancer treatment with natural compounds, such as phytoestrogens including lignans. Several lignans have been shown to possess anti-inflammatory effects in different pathological conditions, including neuroinflammatory diseases. Experimental evidence has demonstrated the inhibitory effect of some lignans on cancer growth and metastasis formation using animal models.

## **AIMS**

Entry of leukocytes or cancer cells across the brain endothelium into the CNS is a key event during neuroinflammation or formation of brain metastases. In our studies we addressed the effect of CB2 agonists and lignans from *Heliopsis helianthoides* var. *scabra* on BBB function

and cell migration across the brain endothelium. Our investigations had the following specific aims:

1. To investigate CB and CB-like receptor expression in the brain endothelium and melanoma cells.
2. To examine the effect of CB2 agonists on leukocyte-endothelial cell adhesion using in vivo and in vitro models.
3. To observe the effect of CB2 agonists on the barrier properties of the brain endothelium in vivo and in vitro.
4. To determine the role of CB2 activation in the adhesion of melanoma cells to the cerebral endothelium and transmigration of these cells through the BBB.
5. To evaluate the effect of lignans from *Heliopsis helianthoides* var. *scabra* on melanoma brain metastasis formation and on the barrier function of brain endothelial cells in vitro.

## **MATERIALS AND METHODS**

### ***Cell culture and treatments***

The following cell cultures were used for our in vitro experiments: human microvascular cerebral endothelial cell line (hCMEC/D3; abbreviated as D3), A2058 human melanoma cell line, human primary brain microvascular endothelial cells (BMVECs), primary human monocytes, primary rat brain endothelial cells (RBECs).

JWH133 from Tocris and O1966 acquired from Organic were used as CB2 agonists. Lignans from *H. helianthoides* var. *scabra* was acquired from our collaborators, Zsanett Hajdú et al. (Department of Pharmacogeny, University of Szeged, Szeged, Hungary).

### ***RT-PCR***

Total RNA was isolated using TRIzol reagent (Life Technologies). RNA was transcribed into cDNA using the SuperScript III reverse transcription kit (Life Technologies). The amplification was performed on a BioRad iQ5 instrument using Maxima SYBR Green Mix (Fermentas). PCR products were electrophoresed on 1.5% agarose gels stained with ethidium bromide.

### ***Immunofluorescence studies and phalloidin staining***

CB2 and ZO-1 protein expression was evaluated by immunofluorescence on monolayers of BMVECs. Primary antibodies and dilutions used included polyclonal antibodies to ZO-1 (1:25; Invitrogen) and CB2 (1:100; Thermo Scientific) applied on the samples for 24 h. Alexa-488 conjugated goat anti-rabbit and Alexa-594 conjugated goat anti-mouse (Invitrogen) secondary antibodies were used at 1:400 for 1 h.

Monolayers of D3 cells and RBECs were treated with different lignans and studied with immunofluorescent staining. Fixed cells were incubated with primary rabbit antibody against ZO-1 (Invitrogen) overnight. The stainings were visualized with Cy3-conjugated goat anti-rabbit antibody (Jackson ImmunoResearch Laboratories).

Following treatment for 2 h with Hel-6 or Hel-11 (5  $\mu$ M), A2058 melanoma cells were stained with Alexa488-phalloidin.

### ***Adhesion of monocytes to BMVECs***

Confluent monolayers of BMVECs were exposed to the indicated experimental treatments. All treatments were removed from endothelial cells before adding monocytes (labelled with 5  $\mu$ M of the fluorescent tracer calcein AM (Invitrogen)) at  $2.5 \times 10^5$  cells per well in a 24-well plate. Monocytes and endothelial cells were incubated together for 15 min and then rinsed three times with PBS to eliminate nonadherent monocytes. Finally, fluorescence of adherent monocytes was measured.

### ***Adhesion of melanoma cells to D3 cells***

Confluent monolayers of D3 endothelial cells and melanoma cells were pretreated with the mentioned drugs for 4 h.  $10^5$  melanoma cells/well (in a 24-well plate) were loaded onto the endothelial monolayer in serum-free medium and left for 90 min. After washing, cells were fixed. Melanoma cells attached to endothelial cells were counted.

### ***Transmigration assay***

Confluent layers of RBECs grown on 8  $\mu$ m pore size filter inserts were pretreated with the CB2 agonist, then melanoma cells receiving the same pretreatments were plated onto the endothelial monolayer. 5 h later

cells were fixed and cells from the upper compartment were wiped off with a cotton swab. Transmigrated melanoma cells were counted.

### ***Wound healing assay***

Monolayers of D3 endothelial cells or A2058 melanoma cells were wounded by scratching with a pipette tip. Cells were monitored over 24 h and phase contrast images were taken. The wound healing effect was quantified by averaging the number of migrating cells counted in 5 wounded areas.

### ***Intravital microscopy***

Intravital microscopy for in vivo leukocyte adhesion and vascular permeability was performed on adult male mice that underwent craniotomy and cranial window implantation. At the start of the procedures, the animals were placed under anesthesia [mixture of ketamine (100 mg/ml, i.p.) and xylazine (20 mg/ml, i.p.) at a dose of 1 ml/kg]. Observation of surface cerebral vessels through the cranial window was performed with a Stereo Discovery V20 epifluorescence microscope (Carl Zeiss Microimaging). For deep-tissue imaging, we used a Leica TCS SP5 II MP multiphoton microscope. For evaluation of adhesion, leukocytes were stained in vivo with an intravenous bolus injection of 50  $\mu$ l of a 0.01% solution of rhodamine 6G (Sigma-Aldrich). Identification of the brain vasculature and evaluation of vessel permeability was performed using intravenously injected 40 kDa FITC-conjugated dextran. Adherent leukocytes were scored as the number of cells per square millimeter of the vascular surface area, calculated from the diameter and standardized length (100  $\mu$ m) of the vessel segment under observation. For multiphoton imaging, randomly selected cortical postcapillary venules (20–30  $\mu$ m in diameter and down to 600  $\mu$ m below the cortical surface) were selected for imaging.

### ***Western blot experiments***

Low-passage BMVECs were used for the Western blots. BMVEC cellular fractions were collected using the ProteoExtract kit (EMD Chemicals) Protein extracts were resolved by SDS-PAGE on 4–20% precast gradient gels (Thermo Scientific). Proteins were transferred to nitrocellulose membranes and incubated overnight with polyclonal



antibodies against CB2 (1:1000; Cayman Chemicals), occludin (1:500; Invitrogen), claudin-5 (1:300; Invitrogen), and sodium potassium ATPase (1:1000; Abcam). Secondary antibodies conjugated to HRP (Thermo Scientific) were then added for 1 h and detected using Supersignal West Pico chemiluminescence substrate (Thermo Scientific).

### ***Measurement of transendothelial electrical resistance***

To determine the integrity of BMVEC monolayers, transendothelial electrical resistance (TEER) measurements were performed using the 1600R electric cell-substrate impedance sensing (ECIS) system (Applied Biophysics). The readings were acquired continuously at 30 min intervals for 24 or 36 h.

To measure the TEER of RBEC monolayers, the CellZscope instrument (nanoAnalytics) was used. After TEER had reached plateau, endothelial cells were treated with Hel-6 (5  $\mu$ M) or Hel-11 (5  $\mu$ M) and TEER was followed for 2 h.

### ***Measurement of BBB permeability ex vivo***

Changes in BBB permeability were assessed using the fluorescent tracer sodium fluorescein (SF). Briefly, animals were injected intravenously with 50  $\mu$ l of 2% SF in PBS. The tracer was allowed to circulate for 30 min. The mice were transcardially perfused with PBS and the brains quickly isolated. Brain tissue was weighed and homogenized in a 10 x volume of 50% trichloroacetic acid. The homogenate was centrifuged and SF fluorescence was measured.

## **RESULTS**

### **Expression of cannabinoid receptors and cannabinoid-like receptors in brain endothelial cells and melanoma cells**

As the first step, expression of CB2 of brain endothelial cell was evaluated by immunofluorescence using confluent cultures of BMVECs. We performed double immunostaining for CB2 and ZO-1, a highly expressed TJ protein in barrier-forming BMVECs. Moderate

immunostaining for the CB2 was observed with strong intercellular expression of ZO-1.

Given the important role the BBB plays during inflammation, we sought to characterize whether the expression level of CB2 changes in the brain endothelium under inflammatory insult. CB2 protein in membrane fractions was evaluated by Western blot from BMVECs exposed to various pro-inflammatory stimuli (IL1 $\beta$ , TNF $\alpha$  or LPS).

As a next step, we determined the expression pattern of cannabinoid and cannabinoid-like receptors in CECs and melanoma cell lines, applied for our following experiments, by using RT-PCR. Our results demonstrate that D3 human brain endothelial cells and A2058 human melanoma cells express the CB2A transcriptional variant of the CB2, but not the CB2B. RBECs were found to express variants 1 and 2 of CB2. In addition, D3 cells express CB1, GPR18 and GPR55, whereas the presence of GPR119 was not detectable in this cell line. Furthermore, we detected the presence of CB1, GPR18, GPR55 and GPR119 in A2058 melanoma cells.

## **Effect of CB2 activation on leukocyte-brain endothelial cell interactions and BBB dysfunction under inflammatory conditions**

### ***CB2 activation decreases leukocyte adhesion in pial vessels***

We used intravital microscopy via cranial window to allow the direct observation of cerebral vascular changes and leukocyte–endothelial cell interactions in real time. LPS was injected to induce systemic inflammation and cytokine production. In a subset of animals, selective CB2 agonist (JWH133) was injected simultaneously with LPS. At 4 h and 24 h after LPS injection, leukocyte adhesion was markedly increased. Administration of JWH133 in combination with LPS attenuated leukocyte adhesion by 56% 4 h after and by 65% 24 h after injection. We also sought to test the same in vivo parameters with a novel resorcinol derivative CB2 agonist (O1966) in a similar fashion. O1966 also markedly reduced adhesion of leukocytes to cerebral vessels at 4 and 24 h (by 50 and 60%, respectively).

### ***CB2 activation decreases leukocyte adhesion on deep cortical ascending postcapillary venules***

Because conventional wide-field fluorescence microscopy is limited with respect to the depth to which an object can be imaged, analysis of leukocyte adhesion in brain parenchymal vessels was visualized with multiphoton microscopy. Leukocytes and the vasculature were concomitantly imaged with rhodamine 6G and high-molecular-weight FITC-dextran, respectively. As expected, the LPS insult induced significant immune–endothelial interaction. Introduction of JWH133 or O1966 with LPS reduced the number of adherent cells, thus confirming the previous results obtained in surface vessels.

### ***Endothelial CB2 activation decreases the number of monocytes adhering to the brain endothelium***

We performed experiments in primary human BMVECs to determine whether the observations *in vivo* could be confirmed in human brain endothelial cells and also to distinguish the effects of the CB2 agonist on endothelial cells from that on leukocytes. BMVEC monolayers were stimulated with TNF $\alpha$  (20 ng/ml) for 4 h with increasing concentrations of JWH133 or O1966. Primary human monocytes were placed on the BMVECs to initiate adhesion only after all treatments were removed and the medium was changed. Unlike in the *in vivo* experiments, the change in medium before the monocyte introduction allows for the evaluation of the effects of the CB2 agonist on BMVECs only. The results from the adhesion assays indicate that human brain endothelial cells respond to CB2 agonists in preventing immune cell adhesion. In addition, the effects with either CB2 agonist are dose dependent for the reduction in adhesion.

### ***CB2 agonists prevent BBB dysfunction *in vivo* and *in vitro****

LPS exposure induces an acute opening of the BBB, which is potentiated by the presence of inflammatory mediators and activated immune cells. In order to test the effect of CB2 activation on inflammation-induced BBB opening, animals were treated with LPS and some of them were co-treated with O1966. The leakage of FITC-labelled dextran was visualized by intravital microscopy. There was no leakage of FITC-dextran from the cerebral vessels at baseline. In contrast, 4 h

after LPS injection, a more fuzzy vessel staining was observed due to the considerable vascular permeability. However, in the presence of O1966 the vessel staining was more clear and SF content of brains was significantly lower than of those derived from animals treated with LPS only, indicating that CB2 activation attenuated the LPS-enhanced permeability of the brain vessels.

As a more precise quantitative comparison of permeability, animals were injected with the small molecular tracer SF (376 Da), and the brain tissue content of SF was measured. Tracer content in the whole brain was elevated by threefold in the LPS condition compared to naive animals. Animals administered with either O1966 or JWH133 showed reduced LPS enhanced permeability by 50% when compared to the LPS animals. Taken together, the results indicate that CB2 agonists provide a barrier protective effect.

In vitro, BBB leakiness and integrity was assessed via measurement of TEER. Treatment of BMVEC monolayers with LPS induced a rapid drop in resistance (80% of control) over time, which was significantly attenuated by O1966. These data indicate that CB2 stimulation can provide BBB protection under inflammatory conditions, supporting our in vivo observations.

Our next set of experiments was designed to understand whether CB2 activation could increase barrier structural integrity under physiological conditions. Application of the highly selective CB2 agonists (O1966 or JWH133) resulted in dose- and time-dependent augmentation of tightness of BMVEC monolayers (increase in TEER with 11–15%).

Although various signaling mechanisms can influence BBB tightness, the culminating effect is on TJ proteins located between brain endothelial cells assuring tightness of the BBB. Therefore, TJ protein amount in BMVEC membrane fractions was evaluated after treatment with CB2 agonists. Four h stimulation of CB2 with O1966 or JWH133 caused a 2.2- to 2.7-fold increase in the amount of occludin and claudin-5 present in the membranous fraction of BMVEC lysates.

## **Effect of CB2 activation on melanoma-brain endothelial cell interactions**

Since activation of CB2 inhibited adhesion of leukocytes to the brain endothelium, we aimed at testing whether CB2 activation can also inhibit tumor-endothelial interactions.

### ***CB2 activation decreases the number of melanoma cells adhering to the brain endothelium***

In order to understand the role of CB2 in tumor metastasis formation, we tested whether activation of CB2 with its agonist JWH133 can affect the attachment of melanoma cells to brain endothelial cells. Treatment of human brain endothelial cells (D3) or human melanoma cells (A2058) with JWH133 during the adhesion assay did not alter adhesion of melanoma cells to the endothelium. However, a 4 h long pretreatment of both endothelial cells and melanoma cells with JWH133 and treatment with the same compound during the adhesion assay significantly reduced the number of adhered melanoma cells compared to the untreated control. These results suggest that activation of CB2 on both endothelial cells and melanoma cells contribute to the adhesion reducing effect of JWH133.

### ***CB2 agonist attenuates transmigration of melanoma cells through brain endothelial cell layers***

Next experiments were conducted to understand whether CB2 activation can interfere with the transendothelial migration of melanoma cells as well. Transendothelial migration of A2058 cells was tested on primary RBECs. Pretreatment of brain endothelial cells with JWH133 reduced the migration rate of melanoma cells. A more potent reduction in the number of transmigrated melanoma cells was observed when both cell types were pretreated with the CB2 agonist, which was also applied during the transmigration assay.

## **Potential antimetastatic effect of lignans from *Heliopsis helianthoides* var. *scabra* in the brain**

Besides CB2 agonists, lignans might also elicit anti-metastatic effects. Therefore, we tested the effects of lignans isolated from

*Heliopsis helianthoides* var. *scabra* in our in vitro brain metastasis model.

### ***Compound structure elucidation of lignans from Heliopsis helianthoides var. scabra***

All of the lignans were identified from the chloroform-soluble fraction of the methanol extract of the fresh root of *Heliopsis helianthoides* var. *scabra*. Their identification and structural investigation were performed by Zsanett Hajdú et al. (Department of Pharmacognosy, University of Szeged, Szeged, Hungary). Six lignans from the fresh roots of *H. helianthoides* var. *scabra* were identified: two new arylbenzofuran-type neolignans, 1''-dehydroegonol 3''-methyl ether (Hel-2) and egonol 3''-methyl ether (Hel-3), and four known lignan derivatives, namely helioxanthin (Hel-6), (7E)-7,8-dehydroheliobupthalmin (Hel-11), heliobupthalmin (Hel-12) and 7-acetoxyhinokinin (Hel-19).

### ***Hel-6 and Hel-11 induce morphological changes in melanoma cells and inhibit their migration***

Phase contrast microscopy images revealed that two of the six lignans, Hel-6 and Hel-11 changed the morphology of the melanoma cells. To visualize the morphological and cytoskeletal changes, cells treated with Hel-6 or Hel-11 (each 5  $\mu$ M) were stained with Alexa-488-conjugated phalloidin. Exposure to Hel-6 resulted in many cells with elongated morphology with actin-rich protrusions, filopodia and actin stress fibers. In contrast, the presence of Hel-11 changed the morphology of melanoma cells leading to a more expanded shape without large filopodia.

A wound healing assay was used to investigate the effects of Hel-6 and Hel-11 on the directional migration of melanoma cells. Both lignans reduced the rate of migration of A2058 cells. Migration is a key property in the process of cancer cell metastasis, so identifying new inhibitory compounds might be useful to find anti-metastatic agents.

### ***Hel-6 reduces the adhesion of melanoma cells to brain endothelial cells***

As the next step, the ability of the isolated compounds to inhibit the attachment of melanoma cells to brain endothelial cells was investigated. For this, D3 human brain endothelial cells and A2058 human melanoma cells were pretreated with the different lignans for 3 h and then treated with the same lignan during the adhesion assay. Hel-6 (2  $\mu\text{M}$ ) decreased the number of melanoma cells adhering to the endothelial layer, while the other compounds had no effect.

In order to determine the cell-type-specific impact of Hel-6, either melanoma or endothelial cells were pretreated with the compound and adhesion experiments were performed in the absence of the lignan. Preincubation of the melanoma cells with 5  $\mu\text{M}$  Hel-6 did not have any effect on the adhesion. However, when the brain endothelial cells were pretreated with 2  $\mu\text{M}$  Hel-6, the extent of adhesion of the melanoma cells to the brain endothelium decreased significantly but to a lesser extent than that observed when Hel-6 was present during the adhesion experiment. In the latter case, pretreatment of A2058 was not able to reduce the adhesion further. These findings indicate a specific role of the endothelial changes induced by Hel-6 in reducing the adhesion, and that these changes are reversible.

### ***Hel-6 and Hel-11 improve the barrier function of CECs***

The BBB integrity was evaluated via measurement of the TEER, which is a widely used marker of the tightness of the intercellular junctions. 2 h after the treatment of a monolayer of primary RBECs with 5  $\mu\text{M}$  Hel-6, the TEER was significantly elevated by about 15-20% relative to the control. A minor elevation of the TEER was likewise observed in the presence of Hel-11 (5  $\mu\text{M}$ ) as well.

Continuous lines of TJs between brain endothelial cells are essential for the integrity of the BBB. Therefore, expression and localization of ZO-1 was investigated, which is an important protein component of TJs. Immunofluorescence studies revealed a stronger ZO-1 staining at the cell-cell contacts between adjacent endothelial cells (D3 and RBEC cultures) in the presence of Hel-6, and a similar effect was seen when the cells were treated with Hel-11.

### ***Hel-6 and Hel-11 mitigate migration of endothelial cells***

An important step in the growth of metastases is the vascularization of the tumor. In this angiogenic process, migration of endothelial cells is a key element. As the next step, therefore, effects of Hel-6 and Hel-11 on the migration of brain endothelial cells were assessed by using the wound healing assay. Both Hel-6 and Hel-11 led to a significant reduction in endothelial cell migration.

### **SUMMARY**

Trafficking of damage-causing immune cells or metastasis forming cancer cells across the BBB are pivotal steps in the formation of severe and often incurable neuroinflammatory disorders or brain metastases, respectively. Therefore, new treatment strategies inhibiting and preventing the entry of these cells into the brain are needed to improve the clinical outcomes, such as cure rate and also the quality of life of the patients suffering from these diseases.

In our studies we addressed the effects of CB2 agonists and lignans from *Heliopsis helianthoides* var. *scabra* on BBB integrity and cell movements across the brain endothelium. First, the expression of CB2 was detected in brain endothelial cells and in melanoma cells. To note, CB2 expression had been shown on several types of leukocytes before. We found enhanced CB2 expression in human brain endothelial cells exposed to various pro-inflammatory stimuli. We also identified the presence of other cannabinoid and cannabinoid-like receptors, such as CB1, GPR18 and GPR55 receptors, but not GPR119 in the D3 human brain endothelial cell line, while A2058 melanoma cells expressed CB1, GPR18, GPR55 and GPR119.

To evaluate the effects of CB2 activation on the elevated leukocyte–endothelial cell adhesion, we tested CB2 agonists, in an LPS-induced encephalitis model in both surface and ascending cortical vessels. Administration of CB2 agonists could significantly reduce the leukocyte adhesion in our model. Inhibitory effect of CB2 activation on leukocyte adhesion to the endothelial layer was also observed in vitro.

Similar to leukocytes, activation of CB2 reduced the adhesion of melanoma cells to the layer of brain endothelial cells. CB2 agonism



decreased the transendothelial migration rate of melanoma cells as well. Our results showed a BBB protective effect of CB2 activation which was due to the upregulated expression of tight junction proteins.

Similar to CB2 activation, two compounds from the six tested lignans, namely helioxanthin (Hel-6) and dibenzylbutane dehydroheliobuphthalmin (Hel-11) enhanced the barrier function and increased the expression of the TJ protein ZO-1 at the junctions of the brain endothelial cells. These two lignans inhibited the migration of both melanoma and brain endothelial cells, and helioxanthin also reduced the adhesion of melanoma cells to the brain endothelium.

Our findings suggest that CB2 agonists and the lignans Hel-6 and Hel-11 are potential therapeutic candidates for preventing and curing diseases associated with immune cell or cancer cell trafficking across the BBB.

## LIST OF PUBLICATIONS

*Full papers directly related to the subject of the thesis*

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