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Cannabinoid 2 receptor activation reduces leukocyte adhesion and improves capillary perfusion in the iridial microvasculature during systemic inflammation

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Abstract.
BACKGROUND: Leukocyte adhesion to the endothelium and decreased microvascular blood flow causing microcirculatory dysfunction are hallmarks of systemic inflammation. We studied the impact of cannabinoid receptor activation on the iridial microcirculation, which is accessible non-invasively *in vivo*, in systemic inflammation induced by endotoxin challenge.
METHODS: 40 Lewis rats were used in the experiments. Endotoxemia was induced by 2 mg/kg i.v. lipopolysaccharide (LPS). Cannabinoid receptors (CBRs) were stimulated by i.v. administration of WIN 55212-2 (WIN; 1 mg/kg). CB_1R antagonist (AM281; 2.5 mg/kg i.v.) or CB_2R antagonist (AM630; 2.5 mg/kg i.v.) treatment prior to WIN was applied to identify the anti-inflammatory effects underlying each CBR subtype. Leukocyte-endothelial interactions were examined in rat iridial microvas culature by intravital microscopy at baseline and 1 and 2 h post-LPS. Additionally, systemic (mean arterial pressure, heart rate) and local (laser Doppler flow) hemodynamic variables were measured prior to and during cannabinoid treatments.
RESULTS: Endotoxemia resulted in severe inflammation as shown by significantly increased numbers of adherent leukocytes at 1 and 2 h observation time post-LPS challenge and decreased microcirculatory blood flow at 2 h within the iridial microcirculation. WIN treatment significantly reduced leukocyte adhesion in iridial microvessels with a diameter greater and less than 25 μm during endotoxemia ($p < 0.05$). Pre-treatment of animals by CB_1R antagonist, AM281, did not affect WIN effects on LPS-induced leukocyte adhesion. When pre-treated with the CB_2R antagonist, AM630, a reversal of the WIN-induced reduction in leukocyte adhesion was noticed in vessels with a diameter of less than 25 μm ($p < 0.05$). Cannabinoid treatment significantly increased the local iridial microcirculatory blood flow 2 hours after systemic LPS administration ($p < 0.05$).
CONCLUSIONS: Systemic administration of the CBR agonist, WIN, decreased leukocyte-adhesion and improved iridial microvascular blood flow. This effect is most likely mediated by CB_2R activation. Our findings indicate that the iris microvasculature can serve as a model to study the microcirculation during systemic inflammation and help to identify potential therapies to treat microcirculatory dysfunction in diseases such as sepsis.

Keywords: Endotoxemia, microcirculation, cannabinoid receptor, WIN 55212-2

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Abbreviations

CBR	cannabinoid receptor
CB_1R	cannabinoid 1 receptor
CB_2R	cannabinoid 2 receptor
CNS	central nervous system
DMSO	Dimethyl Sulfoxide
IMBF	iridial microvascular blood flow
IVM	intravital microscopy
LDF	laser Doppler flowmetry
LPS	lipopolysaccharide
MAP	mean arterial pressure
WIN	WIN 55, 212-2

1. Introduction

Systemic inflammation occurs in several acute and chronic medical conditions. Typically, immune cells are activated globally causing a cytokine storm and release of other inflammatory mediators such as nitric oxide and prostaglandins. Finally, alterations of the microcirculatory blood flow and therefore, the oxygen and nutrient supply to the tissues can be observed [4, 21, 29, 36, 42, 51].

Studies investigating microcirculatory dysfunction in systemic inflammation have experimental constraints due to limited access to microcirculatory beds. Often, access to these areas involves surgical procedures that can induce additional local inflammation or diminish the integrity of the microvasculature. The eye, due to its anatomical location and the translucent cornea provides a unique opportunity for direct, non-invasive observation of the microcirculation *in vivo* [51]. Methods such as intravital microscopy (IVM) and laser Doppler flow metry (LDF) can be employed to study the microvasculature of the iris during systemic inflammation [7, 51]. The iridial microcirculation can be used to model other microvascular beds experiencing dysfunction in systemic inflammatory conditions such as sepsis. Furthermore, these techniques provide the ability to evaluate the efficacy of potential therapeutic candidates *in vivo* including cannabinoid ligands.

The endocannabinoid system (ECS) is a bioactive lipid signaling system that is comprised of two G-protein coupled receptors, the cannabinoid 1 receptor (CB_1R) and the cannabinoid 2 receptor (CB_2R), together with endogenous ligands (endocannabinoids) and enzymes responsible for their synthesis and degradation [4]. CB_1R is expressed throughout the central nervous system (CNS)and activation of presynaptic neuronal CB_1R inhibits neurotransmitters release [12, 27]. Additionally, modulation of non-neuronal CB_1R is associated with an anti-inflammatory response in the CNS [46]. The anti-inflammatory effects of CB_1R have been linked to a decrease in inflammatory mediators and reduced leukocyte migration [44]. It has also been reported that CB_1R influences hemodynamics, which may also affect the immune response [9, 25, 53]. CB_2R is primarily expressed on immune cells and activation of this receptor has been documented to be immunosuppressive [4, 37]. CB_2R activation decreases levels of pro-inflammatory mediators including cytokines (TNF-α, IL-1β, IL-6), chemokines (CCL2, CCL5, CXCL2) and adhesion molecules (ICAM-1, VCAM-1 and integrin β1) [43, 45, 51, 56]. CB_2R-mediated reductions in pro-inflammatory mediators have been attributed to decreased levels of mRNA for transcription factors

NF-κB and AP-1 [43, 51]. In support of an immunosuppressive role for CB_2R, selective CB_2R agonists decrease migration of specific leukocyte populations both *in vitro* and *in vivo* during an inflammatory response [38, 45]. Additionally, it has been found that CB_2R agonists have vasodilatory effects resulting in local increase in the blood flow [33]. Taken together, this evidence suggests that activation of the endocannabinoid system may decrease inflammation and microcirculatory dysfunction. WIN 55,212-2 (WIN) is an aminoalkylindole synthetic cannabinoid that activates both CB_1R and CB_2R [34]. Selective inhibition of its receptor targets allows independent investigation of therapeutic benefits of both CB_1R and CB_2R activation.

The purpose of the present study was 1) to determine if the iridial microcirculation shows the typical signs of immune cell activation in systemic inflammation and therefore, could be used as an accessible model for dysfunctional microvascular beds in diseases such as sepsis, and 2) to examine the impact of both CB_1R and CB_2R activation by WIN on microcirculatory dysfunction due to endotoxin challenge. Specifically, we investigated leukocyte-endothelial interactions in vessels of varying diameters within the iridial microvasculature as well as macro- and microhemodynamic parameters including mean arterial pressure, heart rate, and iridial microcirculatory blood flow.

2. Methods

2.1. Animals

All animal procedures were approved by the Dalhousie University Committee on Laboratory Animals and complied with the Canadian Council for Animal Care guidelines (http://www.ccac.ca/). All studies involving animals are in accordance with the ARRIVE (Animal Research: Reporting *In Vivo* Experiments) guidelines (http://www.nc3rs.org.uk/).

Male Lewis rats (250–400 g, $n = 8$/group; Charles River Laboratories International Inc., Wilmington, MA, USA) were maintained on a light/dark cycle (07:00–19:00), and fed ad libitum. Animals were anesthetised by intraperitoneal (i.p.) injection of sodium pentobarbital (65 mg/kg; CevaSanteAnimale, Montreal, QC, Canada). Depth of anesthesia was assessed by toe-pinch every 15 min. The femoral vein and artery were catheterized using non-radiopaque polyethylene tubing (PE 50; Clay Adams, Sparks, MD, USA). Both catheters were attached to infusion pumps (Lifecare 5000 Plum Infusion Pump; Abbott, IL, USA). The venous catheter was used for the administration of drugs, anaesthetic agents and fluorochromes. The arterial catheter was used to monitor the vital signs with the Marquette Eagle 4000 Patient Monitor (General Electric, North Bergen, NJ, USA). Animals were sacrificed at the end of the experiment, under anaesthesia, by intravenous (i.v.) injection of KCl (0.142 mL/kg).

2.2. Endotoxemia

Rats were administered 2 mg/kg lipopolysacharide i.v. (LPS, Escherichia coli, 026:B6, Sigma-Aldrich, Oakville, ON, Canada) dissolved in normal saline (0.9% sodium chloride, Hospira, Montreal, QC, Canada) to generate endotoxemia. Control animals received an i.v. bolus of normal saline at an equal volume. Animals were studied for 2 h following LPS challenge as described previously [6, 48].

2.3. Drugs

The non-selective cannabinoid agonist, WIN (WIN 55,212-2, (R)-(+)-[2,3-Dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone mesylate), was

administered i.v. at 1 mg/kg. The CB_1R antagonist, AM281 (1-(2,4-Dichlorophenyl)-5-(4-iodophenyl)-4-methyl-N-4-morpholinyl-1H-pyrazole-3-carboxamide) was administered i.v. at 2.5 mg/kg, and the CB_2R antagonist, AM630 (6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1H-indol-3-yl](4-methoxyphenyl)methanone) was administered i.v. at 2.5 mg/kg. Drug treatments were given 15 min after LPS. When antagonists were administered, they were given prior to WIN. All cannabinoids were obtained from Tocris Bioscience (Ellisville, MO, USA). All drugs were prepared in Dimethyl Sulfoxide (DMSO; Sigma-Aldrich Canada Ltd., Oakville, ON, Canada) and diluted 1:1 with saline, and given i.v over a 15 min period. The dose of WIN was determined by previous studies described in the literature which demonstrated anti-inflammatory effects [15, 49]. WIN has a K_i value of 5.05 nM for the CB_1R and 3.13 for the CB_2R [22]. The doses of the CB_1R antagonist, AM281 (2.5 mg/kg), and the CB_2R antagonist, AM630 (2.5 mg/kg), were based on previous published studies [28, 36, 50].

2.4. Intravital microscopy

The iridial microcirculation was observed with the Olympus OV100 Small Animal Imaging System (ON, Canada). The OV100 contained a MT-20 light source and a DP70 CCD camera. Fluorescence excitation was generated by xenon lamp (150 W), and 8 position excitation filters to block for rhodamine-6G (excitation 515–560 nm, emission 590 nm) and FITC (450–490 nm, emission 520 nm). Images were captured in real-time and recorded in a digital format by the software, Wassabi (Hamatsu, Herrsching, Germany). Each rat was placed in a stereotactic frame (Kopf, CA, USA), and Tear-Gel (Novartis Pharmaceuticals Canada Inc., Dorval, QC, Canada) was applied on the cornea to prevent dehydration of the tissue throughout the experiment. Rhodamine-6G (1.5 ml/kg; Sigma-Aldrich, ON, Canada) and FITC-albumin (1 ml/kg; Sigma-Aldrich, ON, Canada) were injected i.v. 15 min before initiating IVM. The fluorescent dye Rhodamine-6G tags leukocytes within the vessels, and FITC-albumin allowed for the visualization of blood flow through the vasculature.

Animals were placed on a heating pad to maintain body temperature at 37°C and IVM was carried out in four regions of interest, each in a different quarter of the iridial microcirculation. Each region of interest was recorded for 30 s and four randomly selected vessels (diameter <100 μm) were observed hourly. For IVM studies, data were analysed off-line in a blinded manner using the imaging software ImageJ (National Institute of Health, USA). The length measured between points of branching and diameter of the vessel was used to calculate the endothelial area. Adhering leukocytes were defined as white blood cells that attached to the vessel wall and were immobile for a 30 s observation period; adhering cells were reported as the number of cells per mm^2 of endothelial surface. Leukocytes which interacted with the endothelium at a slower velocity than midstream blood cell velocity past an arbitrary line perpendicular to the endothelial wall during the 30 s were quantified as rolling leukocytes. Examination of vessels was divided into those that were greater or less than 25 μm in diameter; vessels of greater than 25 μm are confluent vessels in the rat microvasculature. No measurements were conducted in vessels larger than 100 μm.

2.5. Hemodynamics

Haemodynamic variables, including mean arterial pressure (MAP) and heart rate (HR) were measured from the femoral artery catheter every 15 min. Animals with a MAP below 70 mm Hg were excluded from the study [11].

2.6. Laser doppler flowmetry

Laser Doppler flowmetry (LDF) was used to measure the iridial microvascular blood flow (IMBF). This method quantifies IMBF by the detection of erythrocyte movement by the Doppler effect. A glass fibre laser Doppler probe (diameter 120 μm, wave length 810 nm, resulting penetration depth about 1–2 mm) is used to monitor infrared light from a fibre optic cable to measure the light scatter by stationary tissue and moving blood cells within a region of interest approximately 1 mm^3 in area. Scattered light from moving cells results in a Doppler frequency shift of the infrared light while stationary tissue does not [24]. This Doppler frequency shift is then quantified by analysis of the backscatter of light proportional to the flow of blood cells within the region of interest.

LDF readings were taken from rat eyes following placement of a glass fiber laser Doppler probe 1-2 mm away from the cornea at a 90° angle so as to not come in contact with any tissue. The laser Doppler probe was focused on the iridial vasculature and IMBF measurements were taken for 180 sec at 5 min before systemic injection of either saline or LPS, and at 60 and 120 min after injection. The LDF signal was recorded using a PeriFlux System 5000 (Perimed Inc., Ardmore, PA, USA) DIAdem 8.0.1 software (National Instruments, Vaudreuil-Dorion, Quebec, Canada). All LDF values were calculated after normalization to baseline blood flow readings as 100% for each animal.

2.7. Statistics

Individual animals in each of the treatment groups ($n = 8$/group) were coded and data was derived in a non-biased fashion.The D'Agostino and Pearson omnibus normality test was used to confirm normality of the data. All data are presented as means ± SEM and were analysed with the statistical software Prism v.6 (GraphPad Software Inc., San Diego, CA, USA). IVM data was analysed by two-way ANOVA with Dunnett's *post hoc* test, comparing all experimental groups to the LPS treated group in vessels of all sizes. Additional analysis by a one-way ANOVA with Dunnett's *post hoc* test was conducted to decipher if cannabinoid treatments affected leukocyte adhesion in different regions of the microvasculature. As with IVM, hemodynamic variables were analysed at –15 min, 1 h and 2 h by two-way ANOVA with Dunnett's *post hoc* test with respect to LPS administration. LDF data was analyzed after baseline blood flow of each animal was set to 100% to eliminate variation in iridial blood flow in the microcirculation due to systemic hemodynamic variables. Changes of blood flow during the experiments are shown in percentage of baseline and observations from each treatment group were averaged per time point. Prior to LDF measurements being normalised to 100% at baseline, a one-way ANOVA was conducted to ensure that there were no significant differences in ocular blood flow prior to injection of saline controls or LPS. Differences were considered significant at $p < 0.05$.

3. Results

3.1. Leukocyte adherence

Systemic administration of LPS significantly increased the number of leukocytes adhering to iridial microvascular endothelium at 1 and 2 h after induction compared to saline treated control animals ($p < 0.05$, Fig. 1 A&B). Administration of the vehicle, DMSO + saline, did not significantly alter the number of adherent leukocytes throughout the experiment compared to LPS (data not shown). WIN

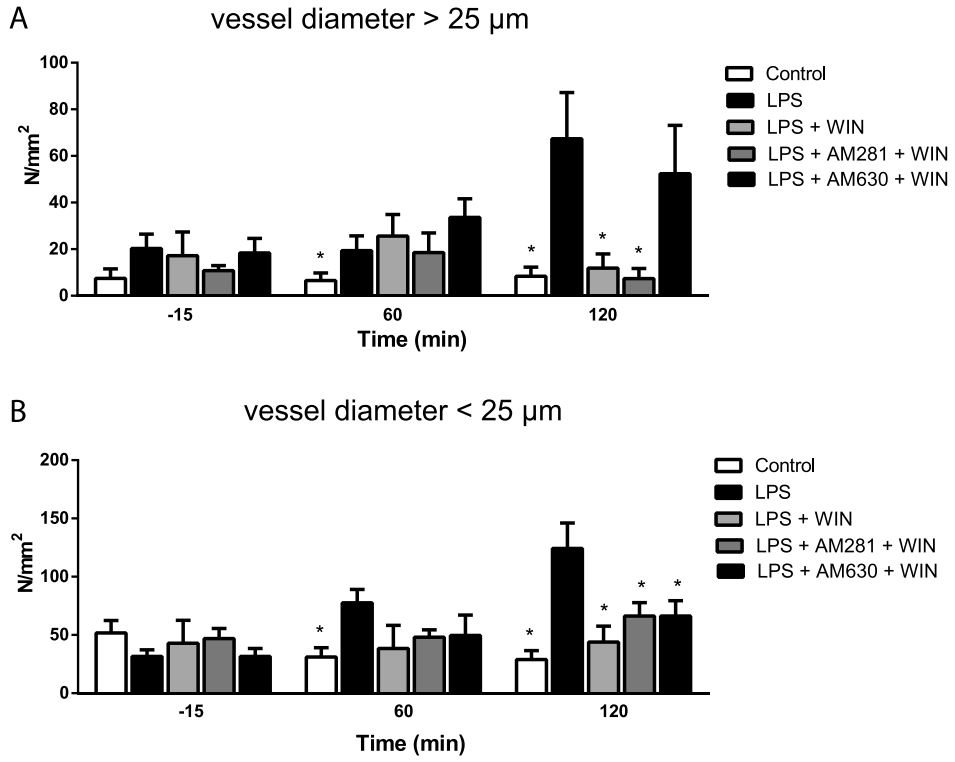

Fig. 1. Bar graph representing adherent leukocytes in vessels of a diameter greater (A) or less (B) than 25 μm at –15, 60 and 120 min. Treatment with LPS + WIN and LPS + AM281 + WIN significantly decreased the number of leukocytes adhering to the endothelium in all the vessels (A, B) compared to untreated LPS animals while as treatment with LPS + AM630 + WIN also significantly decreased the number of leukocytes adhering to the endothelium compared untreated LPS animals in the vessels of a diameter <25 μm. Values represent mean ± SEM. $^{*}P < 0.05$ compared to the LPS group.

treatment - in absence or presence of the CBR antagonists, respectively - did not significantly attenuate leukocyte adhesion to the iridial microvasculatureat 1 h after LPS challenge. However, at 2 h after LPS administration, WIN significantly reduced the number of leukocytes adhering to vessels of all diameter in the iridial microvasculature compared to untreated LPS animals. Pre-administration of the CB_1R or CB_2R antagonists (AM281 and AM630, respectively) did not prevent the WIN-induced reduction in leukocytes adhering to the endothelium in vessels with a diameter <25 μm, although block of either CBR did reduce WIN's effectiveness. However, treatment with the selective CB_2R antagonist, AM630, but not the CB_1R antagonist AM281 prior to WIN, abolished the decrease in leukocytes adherence in the vessels larger than 25 μm produced by WIN.

3.2. Leukocyte rolling

There were no significant changes in leukocyte rolling 1 or 2 h after LPS administration, as compared to saline control. Treatment of animals with cannabinoid ligands, WIN, AM281 + WIN and AM630 + WIN, did not result in any significant changes in leukocytes rolling, as compared to LPS treated group, in all categories of vessels analyzed (Table 1).

Table 1
Number of rolling leukocytes per min after different treatment regimens at 120 min. All values are expressed as mean ± SEM

	Control	LPS	LPS + WIN	LPS + AM281 + WIN	LPS + AM630 + WIN
All vessels	0.723 ± 0.483	0.772 ± 0.219	1.913 ± 1.218	0.437 ± 0.101	0.574 ± 0.114
>25 μm	0.268 ± 0.011	0.634 ± 0.177	0.233 ± 0.145	0.089 ± 0.089	0.309 ± 0.172
<25 μm	0.617 ± 0.021	0.889 ± 0.072	0.726 ± 0.087	0.659 ± 0.120	0.524 ± 0.075

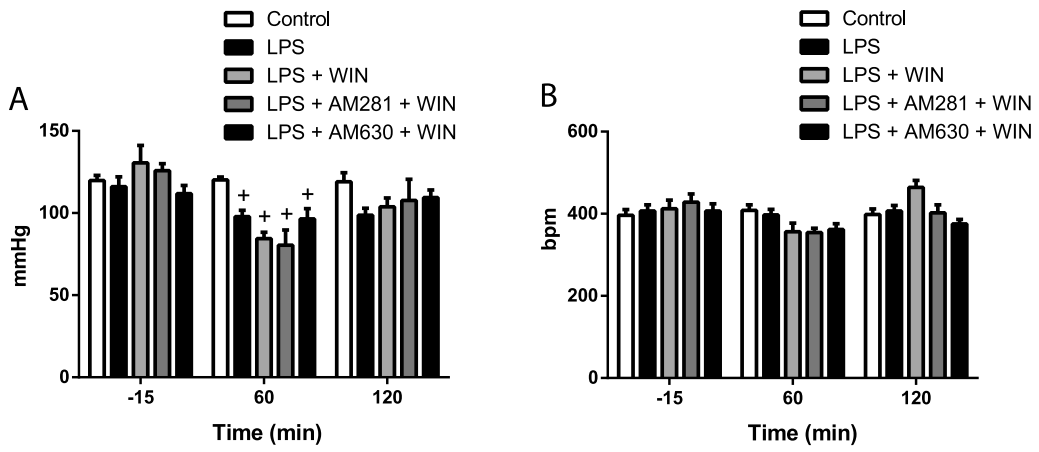

Fig. 2. Hemodynamics. (A) Mean arterial pressure (mmHg). (B) Heart rate (beats per minute). Groups: control, LPS, LPS + WIN, LPS + AM281 + WIN, LPS + AM630 + WIN. All groups treated with LPS had a significant but transient decrease in blood pressure at 1 h. At 2 h no significant changes were observed. No significant changes in heart rate were observed. Values represent mean ± SEM. $^{+}P < 0.05$ compared to control group.

3.3. Hemodynamics

No significant differences in MAP were observed between any group at 15 min before saline or LPS injections (Fig. 2A). At 1 h post LPSall animal with endotoxemiahad a significantly decreased MAP in comparison to the control group ($p < 0.05$), while at 2 h this difference was abolished. There was no significant difference in MAP between animals which received LPS and those that were treated with cannabinoids (WIN, WIN+AM281, WIN+AM630) at 1 or 2 h.

No significant difference in heart rate (HR) was observed between any groups at –15 min before induction of endotoxemia or 1 and 2 h post-LPS challenge.

3.4. IMBF

Local blood flow in the iris was not significantly altered at 1 h following LPS compared to saline treated animals ($p < 0.05$). Treatment with WIN, AM281 + WIN or AM630 + WIN did not significantly alter the blood flow in comparison to untreated LPS animals. By 2 h IMBF significantly decreased by 46% in LPS treated animals compared to control animals ($p < 0.05$). All cannabinoid treatments: WIN (145%, $p > 0.05$), and WIN in combination with AM281 (137%, $p > 0.05$), or AM630 (116%, $p > 0.05$), significantly increased IMBF flow at 2 h post-LPS injection in comparison to untreated LPS animals (Fig. 3).

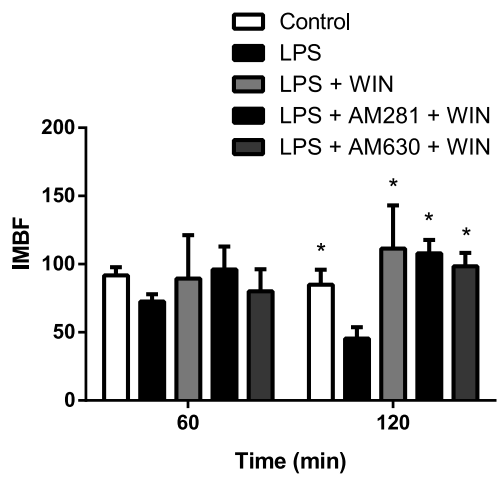

Fig. 3. Iridial microvascular blood flow (% of baseline) in the following treatment groups: control, LPS, LPS + WIN, LPS + AM281 + WIN, LPS + AM630 + WIN. A significant decrease in IMBF was observed in animals with endotoxemia by 2 h. Treatment with CBR agonists during LPS challenge reversed decreased IMBF. Values represent mean ± SEM. $^{*}P < 0.05$ compared to the LPS group.

4. Discussion

4.1. Iridial microcirculation as a model for the microcirculatory dysfunction during systemic inflammation

This study demonstrates the possibility of using the eye to non-invasively study microcirculatory dysfunction in systemic inflammation. Here in, we have provided evidence that both leukocyte activation and microcirculatory blood flow changes can be observed in the iris 2 h after systemic LPS challenge. Our results of increases in leukocyte-endothelial interactions and alterations in iridial microvascular blood flow are consistent with other investigations of systemic inflammation [10, 13, 19, 20, 28, 54].

We report that reduction in IMBF by 46%occurs within 2 h after systemic LPS administration. Previous reports have speculated upon the heterogeneity of the microcirculatory blood flow during critical illness [10, 19, 42]. Hiltebrand et al. [19] showed that jejunal mucosal blood flow was not significantly decreased during peritonitis and septic shock, however there was approximately a 50% reduction in regional blood flow in the stomach mucosa, colon, liver, pancreas and kidney [19]. These results indicate that the iridial microcirculation more dominantly represents the microcirculation of organs undergoing shunting of blood and potential failure during sepsis and septic shock. Once we established that the iridial microcirculation responded similarly to other regions of microvasculature in systemic inflammation we investigated the ability for cannabinoid ligands to decrease inflammation and microcirculatory blood flow.

4.2. Cannabinoid effects on leukocyte-endothelial interactions

The present study describes that, in particular, activation of CB_2R decreases leukocyte-endothelial interactions within the iridial microcirculation after systemic LPS administration. It has been documented that both CB_1R and CB_2R agonists play a role in the immune response and regulation of the immune system.

Activation of both cannabinoid receptors by the non-selective cannabinoid WIN has been demonstrated to attenuate leukocyte-endothelial interactions in a model of autoimmune encephalomyelitis [39]. Another study with WIN reported a dose-dependent decreased release of TNF-α after LPS stimulation in murine immune cells [14]. The effects of WIN could be compound specific, however these responses may be elicited by selective activation of the CB_1R and CB_2R.

Here we report that selective activation of the CB_2R by administration of WIN and CB_1R inhibition by AM281 decreased leukocyte adhesion in all vessels of the iridial microcirculation during endotoxemia. This observation corroborates earlier reports of the anti-inflammatory effects of CB_2R activation in systemic inflammation [5, 16, 28, 52]. Similarities between the anti-inflammatory effects of CB_2R agonists in the eye and other microcirculatory beds provides further reason to accept the use of the iris as a model to study microcirculatory inflammation during sepsis.

We have documented that selective CB_1R activation, by administration of WIN and CB_2R inhibition by AM630 decreases leukocyte adhesion to the iridial microvasculature with a diameter less than 25 μm. Several reports attribute CB_1R anti-inflammatory effects to central as well as peripheral CB_1R modulation of cytokine-related pathways. WIN delayed the release of chemoattractants (KC and MIP-2) in mice with peritonitis, consequently inhibiting neutrophil migration. When these animals were pretreated with selective CB_1R antagonist, SR141716A, neutrophil migration returned to normal [49]. Similar results were found by Sacerdote et al. [47] in which the non-selective cannabinoid receptor agonist, CP55, 940 and a CB_2R antagonist reduced macrophage recruitment [47]. Krustev et al. [26] found that inhibiting endocannabinoid degradation reduced leukocyte-endothelial interactions in experimental joint inflammation, and this effect was mediated by both CB_1R and CB_2R activation [26].

In systemic inflammation, actions of the CB_1R have been focused on CB_1R inhibition to improve GI motility [31] and implications of CB_1R mediated hypotension [53]. In our investigation in endotoxemia, activation of the CB_1R attenuated leukocyte-endothelial adhesion within the sub-population of the microvasculature which had a diameter of less than 25 μm. These results may be explained by CB_1R vasodilatory effects. Wagner et al. [55] demonstrated that several selective CB_1R agonists differently altered cardiac output and total peripheral resistance [55]. Many of the measurable outcomes due to vasodilation are focused on macro-hemodynamics. One focus includes decreases in systemic blood pressure [55]. This alteration in systemic blood pressure does not necessarily equate to changes in localised blood flow [35]. Vasodilation within the microvasculature contributes to increasing local blood flow; reviewed by Bagher and Segal [3]. Increased blood flow strengthens shear forces, inhibiting adhesion of leukocytes to the endothelium [8]. These effects of shear forces and reduced leukocyte adhesion may deteriorate as microvasculature becomes larger (>25 μm).

4.3. Iridial microvascular blood flow

In the present study administration of LPS resulted in a significant decrease in the blood flow within the iridial microcirculation. This observation is consistent with reports of decreased blood flow during sepsis or septic shock in several microcirculatory beds such as the stomach mucosa, pancreas, liver, colon, and kidney [2, 19]. Cannabinoid treatment was able to significantly increase the IMBF. Since this increase was not reversible by CB_1R or CB_2R antagonists, respectively, CBR independent effects may also be involved. These actions could have occurred by non-CB_1R/CB_2R activation at GPR18, GPR55 or TRPV1 receptors which can be activated by cannabinoid ligands with the potential to have vasomodulatory actions [1, 23, 32, 40].

As discussed previously, activation of the CB_2R is documented to be immunomodulatory, having effects on cytokine, chemokine and adhesion molecule regulation decreasing the number of leukocytes recruited to the area of inflammation [17]. Relationships between leukocytes and platelets play key roles in blood coagulation [41]. If leukocytes, platelets and coagulation factors are activated within the microvasculature they decrease and consequently inhibit microcirculatory blood flow [30]. A decrease in leukocytes recruitment by CB_2R activation attenuating the release of inflammatory mediators would inhibit platelet-leukocyte interactions and resultant coagulation factors that would intrinsically decrease vascular blood flow [30, 36, 38, 43, 45, 56]. Therefore, the increases that are seen in microcirculatory blood flow could be consequences of CB_2R activation.

It was previously eluded to that CB_1R activation can increase the blood flow of the iridial microcirculation by its vasoactive effects. WIN, amongst other CB_1R agonists, has been shown to be a vasodilator [55]. Within the ocular microcirculation vasodilators have been observed to increase the microcirculatory flow by LDF. Hétu et al. showed in rats administered with potent vasodilator, CO_2, a transient increase in blood perfusion in the ocular microcirculation. The opposite was also true, when animals were exposed to O_2, vasoconstriction occurred and blood flow in the microcirculation of the choroid and retina transiently decreased [18]. These observations in conjunction with decreasing leukocyte adhesion could be working together as a positive feedback loop reinforcing the beneficial effects of each other.

5. Conclusions

In conclusion, the present study has demonstrated microcirculatory dysfunction in the iris during systemic inflammation. Leukocyte activation was attenuated and IMBF was improved by the non-selective CBR agonist WIN. This effect is most likely mediated by CB_2R activation. The iris microcirculation could be used as a model for changes in those microvascular beds that cannot be investigated by non-invasive techniques in systemic inflammation.

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Authors' contributions

JTT, CL, and MEM conceived the study design. JTT and RM conducted IVM and systemic hemodynamic experiments. JZ established the animal model of iris IVM and edited the manuscript. JTT conducted LDF measurements, data analysis and preparation of the manuscript. CL, MEM and AMS edited the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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