The exogenous administration of CB2 specific agonist, GW405833, inhibits inflammation by reducing cytokine production and oxidative stress

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Abstract. The present study aimed to investigate the role of cannabinoid 2 (CB2) receptors in a rat model of acute inflammation. Therefore, the potential of anti-inflammatory effects of CB2 receptor agonist (GW405833), CB2 receptor antagonist (AM630), and diclofenac, were investigated in carrageenan induced paw oedema in rats: as were assessed by measuring paw oedema; myeloperoxidase (MPO) activity in paw tissue; malondialdehyde (MDA) concentration; glutathione (GSH) level in paw tissue for oxidant/antioxidant balance; cytokine (interleukin-1β, IL-1β; tumour necrosis factor-α, TNF-α) levels in serum; histopathology of paw tissue for inflammatory cell accumulations. The results showed that GW405833 or diclofenac significantly reduced carrageenan-induced paw oedema. GW405833 also inhibited the increase of MPO activity, the recruitment of total leukocytes and neutrophils, and MDA concentration during carrageenan-induced acute inflammation, along with reversed nearly to the normal levels the increased of TNF-α, and IL-1β in serum. AM630 did not affect inflammation alone however clearly reversed the effects of agonist when co-administered. The mechanism of GW405833's suppression of inflammation is supported by these results, which are achieved by the inhibition of neutrophil migration, which regulates the reduction of oxidative stress, TNF-α and IL-1β levels. Finally, the activation of CB2 receptor, by selective agonist, has a major role in peripheral inflammation, and in the near future, targeting the peripheral cannabinoid system as a promising alternative to treat inflammation diseases may be considered a novel pharmacologic approach.

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Introduction

There is an increasing interest in the activation mechanisms of cannabinoid (CB) receptors as experimental or clinical therapeutic strategies with recent reviews (1). The main receptors of the endocannabinoid system are the CB1 and CB2 cannabinoid receptors: Although CB1 receptors are highly expressed in the brain (2), and also present nerve terminal of peripheral tissues (3), the CB2 receptors are expressed in inflammatory and immune cells, and in various peripheral tissues under normal physiological or pathophysiological conditions (4). 2-arachidonoylglycerol (2-AG) is the endogen ligand of CB2, arachidonic acid-derived endogenous bioactive lipid, its production from cell membrane lipid precursors is activity-dependent and its action is terminated by specific lipase, monoacylglycerol lipase (MAGL) (3). The high levels of CB2 express within the immune system including B cells, natural killer cells, monocytes, neutrophils, CD8 lymphocytes, CD4 lymphocytes (5,6).

Inflammation is a pathophysiological response to disturbed homeostasis caused by infection, injury and trauma. Cell migration into the site of injury, erythema, oedema, pain, and fever are clinical signs of inflammation and symptoms. The release of pro-inflammatory cytokines, such as tumour necrosis factor-α (TNF-α), activated by macrophages, leads to vascular endothelial cell activation and causes leukocytes to migrate to the inflamed region (7).

It is well known that carrageenan-induced paw oedema model result in oxidative stress with the increase lipid peroxidation or the depletion of antioxidant sulfhydryl pool such as glutathione, during the acute phase of inflammation in cells (8-10).

A lot of studies suggest an involvement of CB2 receptors in inflammatory process (11,12). A recent study demonstrates that the selective MAGL inhibitor inhibits carrageenan inflammation with attenuated the development of paw oedema in mice; the activation of CB2 receptors mediated its

anti-oedematous actions (13). The selective MAGL inhibitor, URB602, is to prevent an acute inflammatory disease without producing adverse psychoactive effects, so the data contributed to clarify the physiological role of 2-AG in respect to inflammatory reactions, suggesting its protective role in the body (14). Studies have identified an anti-inflammatory role for the endocannabinoid system by its inhibition of TNF-α release. The other researches indicate the controversial results. Lunn *et al* (15), interestingly suggest that CB2 antagonism by means of inverse agonist of CB2 receptor is inhibit leukocyte trafficking *in vivo* rodent model induced by chemokines or by antigen challenge, so it may provide immunotherapeutic treatment of inflammatory diseases.

We hypothesized that the administered CB2 specific receptor agonist could induce an anti-inflammatory effect in a carrageenan-induced inflammatory model by stimulating CB2 receptor activity on peripheral cells, decreasing the release of cytokines, and regulating the oxidant/anti-oxidant balance. This model of inflammatory was chosen because it is widely used to study acute inflammation in rodents. Therefore, the aim of this study was to investigate: i) presence of CB2 receptor in paw tissue; ii) a pharmacologic role of CB2 receptor; iii) effects of CB2 receptor agonist and antagonist exogenous administration on inflammation, and iv) a distinctive mechanism for inflammatory processes via CB2 receptors of cannabinoid system in carrageenan induced inflammatory paw oedema in rats.

Materials and methods

Chemicals. Chemicals including Carrageenan, GW405833, AM630, and other reagents including dimethyl sulfoxide (DMSO), trichloroacetic acid (TCA), thiobarbituric acid (TBA), dithiobisnitrobenzoate (DTNB), hexadecyltrimethylammonium bromide (HETAB), and o-dianisidine were obtained from Sigma-Aldrich; Merck KGaA (Darmstadt, Germany). All other chemicals were obtained from analytical grade and standard commercial suppliers. Diclofenac was obtained from Abdi Ibrahim Ilaç San Tic AŞ (Turkey). GW405833, and AM 630 were dissolved in 2% DMSO. TNF-α and IL-1β Elisa (Novex) kits useful for rat were purchased from Thermo Fisher Scientific, Inc., Waltham, MA, USA.

Animals. Wistar-albino rats weighing 200-250 g were randomly housed in appropriate cages at 22±2˚C under a 12/12-h dark/light cycle with free access to tap water and commercial rat chow. Ethics committee approval was obtained from Mustafa Kemal University with protocol no. 2013/7-17. Procedures were in accordance with the Guide for Care and Use of Laboratory Animals.

Experimental Protocol and Carrageenan induced paw oedema . Both CB2 agonist (GW 405833) and CB2 antagonist (AM630) were given in doses of 3 and 1 mg/kg, respectively, by an intravenous route from rat tail after dissolving in 2% DMSO. The doses of CB2 agonist and antagonist compounds were chosen according to literature (16,17). After non-selective cyclooxygenase (COX) inhibitor drug, diclofenac, and carrageenan were dissolved in 1% sterile saline, diclofenac was administered at a dose of 10 mg/kg. The rats were randomly assigned 7 groups, with 6 animals in each group (n=6): 1: Saline control, 2: CAR (carrageenan), 3: CAR+DIC (carrageenan plus diclofenac), 4: CAR+AGO (carrageenan plus CB2 receptor agonist), 5: CAR+ANTA (carrageenan plus CB2 receptor antagonist), 6: CAR+AGO+ANTA (carrageenan plus CB2 receptor agonist plus CB2 receptor antagonist), 7: CAR+Vehicle (carrageenan plus DMSO). Antagonist was administered at 5 min. prior to agonist. Diclofenac was applied intraperitoneally, carrageenan was applied as intraplantar injection.

Ten minutes after the last drug administration, into the plantar surface of the right hind paw of all rats, except saline control group, were given a 0.1 ml volume of the previously prepared 1% carrageenan solution, resulting in the include of the paw oedema. All local injections were made at same volume. The oedema formation was assessed as an increase in paw thickness at the dorsal-planter axis at the metatarsal level using a calliper. Basal thickness was determined before animals were given the drug. The changes in the paw thickness were measured at 0, 1, 2, 3, and 4 h. Relation between paw thickness and basal values was found and noted. To determine the anti-inflammatory potency, the relation between all drug-given groups and 1% carrageenan group was expressed as %.

Animals were anesthetized with ketamine (50 mg/kg, IP) and xylazine (5 mg/kg, IP). They were monitored for loss of the tail reflex, which was defined as loss of the twitching or movement of the tail pinched using the fingers.

Blood was collected by cardiac puncture and centrifuged at 3000 rpm for 10 min to obtain serum. Serum samples were stored at -80˚C up to analysis. Animals were killed by neck dislocation and soon paw tissue and skin samples were taken for histopathological evaluation and biochemical analyses.

Cytokine measurements in serum. IL-1β and TNF-α levels were determined using an ELISA kits for rats and this process was performed 2 times, according to the procedures supplied by the manufacturer (Thermo Fisher Scientefic, Inc.) and the results expressed as pg/ml of serum.

Malondialdehyde (MDA) measurements in paw tissue. The lipid peroxide levels, as MDA concentrations, in the paw tissues were according to the method previously described (18). Briefly the tissue samples were homogenized in an ice bath, ice-cold TCA by adding 10 ml of 10% TCA per g of tissue, with an ultrasonic tissue homogenizer. After two consecutive centrifugations at 3,000 g for 15 min, 500 μl supernatant was mixed with equal volume of 0.67% TBA and heated to 100˚C for 15 min. The absorbances of the samples were then measured spectrophotometrically at 535 nm. Each assay was performed in duplicate.

Glutathione (GSH) levels in paw tissue. GSH contents of the paw tissue samples were measured by a modified Elman method (19). To the 0.5 ml of supernatant obtained by using the same homogenization procedure as described above, 2 ml 0.3 M Na2HPO4 solution was added. A 0.2 ml solution of DTNB was added into the mixture, and the absorbance at 412 nm was measured immediately after vortexing. Each assay was performed in duplicate.

Myeloperoxidase (MPO) activity. To determine MPO activity, an index of polymorphonuclear cell accumulation, exactly 100 mg of tissue was weighed and homogenized in potassium buffer containing 0.5% hexadecyltrimethylammonium bromide (HETAB). To obtain tissue pellet, homogenates were centrifuged at 4,500 g for 15 min at 4˚C and then they were resuspended, and MPO activity was assayed by measuring the change in absorbance at 460 nm using o-dianisidinedihydrochloride and hydrogen peroxide (20). Each assay was performed in duplicate. One unit of MPO activity was defined as that degrading 1 μmol of peroxide per min at 25˚C. The activity was then normalized as unit per mg of tissue (U/mg).

Histopathological analyses. The paw tissue and skin samples were fixed in 10% formaldehyde solution for 10 days. The samples were dehydrated, cleared and embedded in increasing alcohol series (EMD Millipore, Billerica, MA, USA), in xylene series (EMD Millipore) and in the paraffin blocks, respectively. To evaluate histological analyses, before these blocks were stained with hematoxylin and eosin (H&E) (EMD Millipore) the blocks were cut into 10 μm thick sections and mounted on glass slides. After than this sections were evaluated with a camera attachment microscope (Carl Zeiss Axiocam ERc5s; Carl Zeiss AG, Oberkochen, Germany). All histological analyses were performed by an observer blinded to the treatment protocol. To calculate both the results and the mean of the numerical intensity of inflammation, in each H&E section approximately 700 inflammatory cells were evaluated. The degree of inflammation was evaluated with a score from 0 to 5, defined as follows: 0=no inflammation, 1=weak inflammation, 2=mild inflammation, 3=moderate inflammation, 4=moderate/severe inflammation and 5=severe inflammation.

Statistical analysis. For 6 animals per group, the data are expressed as mean value SEM. For the present study, one-way analysis of variance (ANOVA) was used and Bonferroni post hoc test for multiple comparisons was followed. The pharmacological roles of CB2 receptor agonist and/or antagonist on carrageenan-induced inflammatory processes were compared with that in the diclofenac and carrageenan groups. P<0.05 was considered to indicate a statistically significant difference.

Results

Macroscopic results of anti-oedematous activity. The anti-inflammatory activities of CB2 receptor agonist, CB2 receptor antagonist, and diclofenac on carrageenan-induced acute paw oedema were shown in Fig. 1. Intraplantar injection of carrageenan into the rat paws caused the clear thickening of the paw at 4 h after the administration. Carrageenan-induced paw oedema was been reduced in the diclofenac and CB2 agonist given group. The anti-oedematous activity of CB2 receptor agonist was comparable to that of diclofenac. When the antagonist was given alone, it had no any anti-inflammatory activity. Moreover, when administered in combination with the agonist, antagonist reversed anti-inflammatory effect of the agonist.

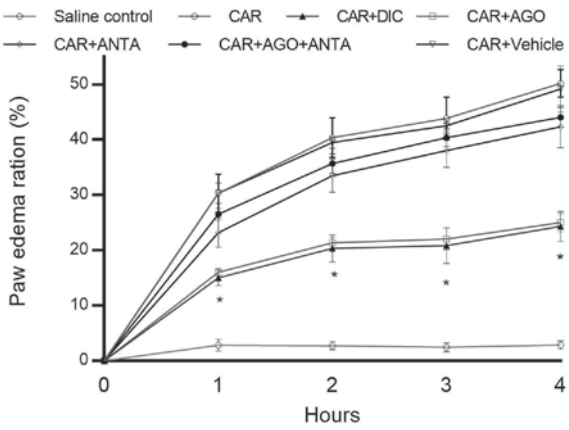

Figure 1. Effects of GW405833 (CAR+AGO), AM630 (CAR+ANTA), their combination (CAR+AGO+ANTA) and diclofenac (CAR+DIC) on the carrageenan-induced paw edema formation. Rats were evaluated for paw edema at 0, 1, 2, 3 and 4 h post-carrageenan injection. Results were expressed as percentage increase in paw thickness. Each point represents the mean ± SEM of six rats. *Statistically significant compared with carrageenan group at P<0.05. AGO, agonist; ANTA, antagonist; DIC, diclofenac; CAR, carrageenan.

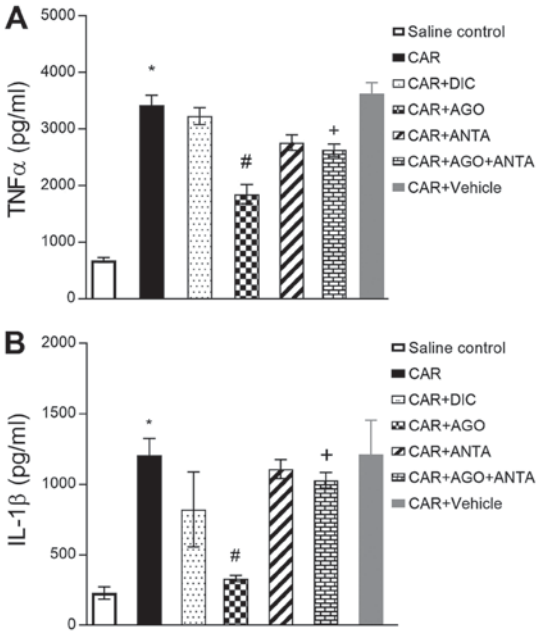

Figure 2. Effects of GW405833 (CAR+AGO), AM630 (CAR+ANTA), their combination (CAR+AGO+ANTA) and diclofenac (CAR+DIC) on serum (A) TNF-α and (B) IL-1β activities in carrageenan-induced paw inflammation of rat (4th hour). Each point represents the mean ± SEM of rats (n=6). *Statistically significant compared with saline control group, #statistically significant compared with carrageenan group, and +statistically significant compared with agonist-treated group at P<0.05. TNF-α, tumor necrosis factor-alpha; IL-1β, interleukin 1β. AGO, agonist; ANTA, antagonist; DIC, diclofenac; CAR, carrageenan.

Cytokine, IL-1β, and TNF-α, serum measurements. Serum cytokine levels in the group treated with carrageenan were seen to significantly increase (Fig. 2). These levels were observed to significantly decrease in the CB2 agonist or diclofenac given groups. However, these decreases in cytokine levels were not seen in the antagonist group. As reversed anti-inflammatory effect, the effects of agonist were reversed by CB2 antagonist on serum cytokine levels.

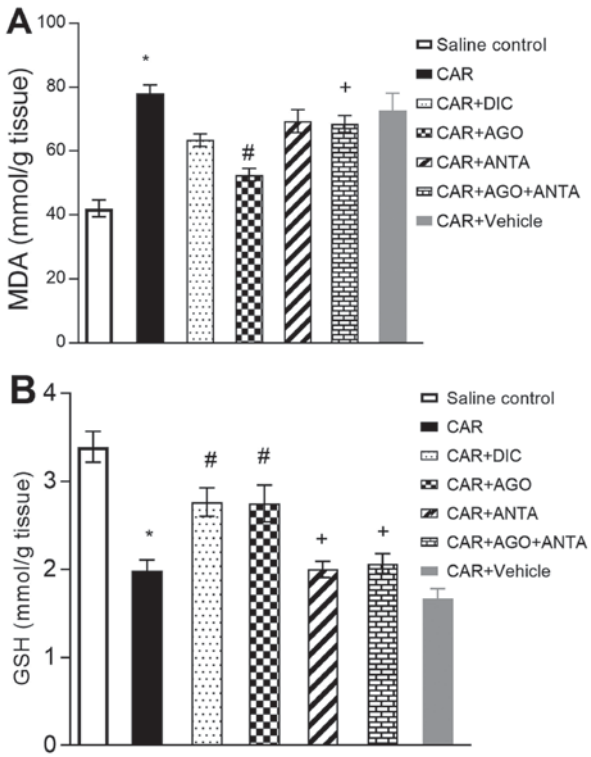

Figure 3. Effects of GW405833 (CAR+AGO), AM630 (CAR+ANTA), their combination (CAR+AGO+ANTA) and diclofenac (CAR+DIC) on (A) MDA and (B) GSH levels in carrageenan-injected paw tissues (4th hour). Each point represents the mean ± SEM of rats (n=6). *Statistically significant compared with saline control group, #statistically significant compared with carrageenan group, and +statistically significant compared with agonist-treated group at P<0.05. GSH, glutathione; MDA, malondialdehyde. AGO, agonist; ANTA, antagonist; DIC, diclofenac; CAR, carrageenan.

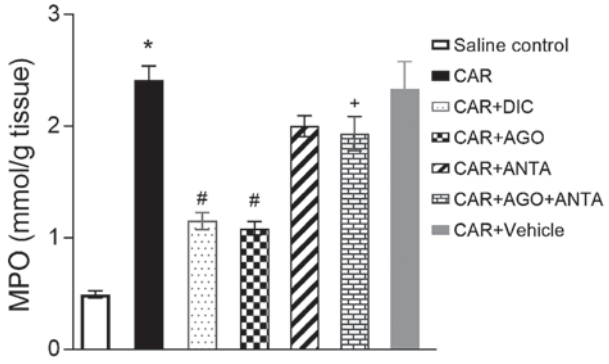

Figure 4. Effects of GW405833 (CAR+AGO), AM630 (CAR+ANTA), their combination (CAR+AGO+ANTA) and diclofenac (CAR+DIC) on MPO activity in carrageenan-injected paw tissues (4th h). Each point represents the mean ± SEM of rats (n=6). *Statistically significant compared with saline control group, #statistically significant compared with carrageenan group, and +statistically significant compared with agonist-treated group at P<0.05. MPO, myeloperoxidase. AGO, agonist; ANTA, antagonist; DIC, diclofenac; CAR, carrageenan.

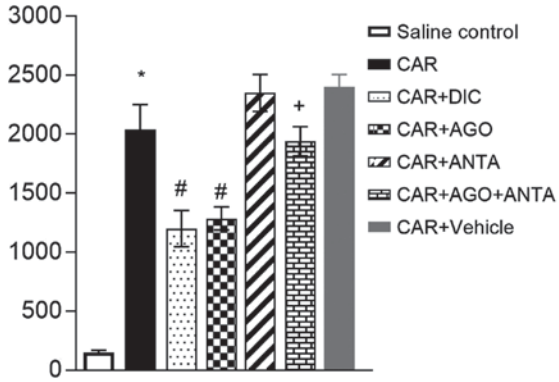

Figure 5. Mean numerical density of inflammatory cells in rat paw tissues (cell/mm^2). Each point represents the mean ± SEM of rats (n=6). *Statistically significant compared with saline control group, #statistically significant compared with carrageenan group, and +statistically significant compared with agonist-treated group at P<0.05.

Oxidant and antioxidant activity in paw tissue. Carrageenan application was found to increase the MDA concentration in rat paw tissues. The MDA concentration was significantly lower in the diclofenac and agonist group than in the carrageenan group. An increase in the inducer effect of carrageenan in the antagonist group alone was no found to alteration, while the beneficial effect of agonist was found to be caused by the combination of antagonist and agonist. It was decreased GSH level in inflammatory tissue, while level of GSH was increased in both diclofenac and agonist-given group (Fig. 3).

MPO activity in paw tissue. The development of histological damage was associated with increased infiltration of neutrophils as shown by an increase in MPO activity, a peroxidase enzyme released by neutrophils and considered a marker of neutrophilic infiltration. The administration of either cannabinoid 2 agonist (GW405833, 3 mg/kg) or diclofenac (10 mg/kg) each significantly reduced MPO activity (Fig. 4), although cannabinoid 2 antagonist, AM630, was not significantly effect.

Histopathological results. Histological changes in oedema paws 4 h after injection of carrageenan. Light micrograph of rat paw in all studied groups. Paws were harvested 4 h after injection of carrageenan and subjected to histochemical staining of paw tissues. Saline control group was showing no histopathological changes. The inflammation score for the experimental groups (carrageenan, antagonist, and vehicle) was more than that of the saline control group and treatment groups (agonist or diclofenac). Carrageenan-treated and Vehicle group showing marked (+++++) inflammatory reaction associated with intermuscular infiltration with massive number of neutrophils. CAR+DIC group and CAR+AGO group are showing weak (+) inflammatory reactions. CAR+ANTA group is showing (++++) inflammatory reaction. CAR+AGO+ANTA group is showing mild (++) inflammatory reaction (Fig. 5).

Histopathological pictures of all groups are summarized in Fig. 6. In the paw samples of the CAR, CAR+ANTA, CAR+AGO+ANTA, and CAR+Vehicle groups, the inflammatory infiltrates were abundant. In the all these groups, collagen and elastic bundles, more fibroblast profiles, capillaries, dense lymphocyte, white blood cells (especially neutrophil), macrophage-like cells were found, as compared with saline control or groups treated with agonist or diclofenac.

Saline control group results: Histologically, the structures of the epidermis, stratified squamous epithelial cells and dermis were normal. The fibers and connective tissue cells also were normal.

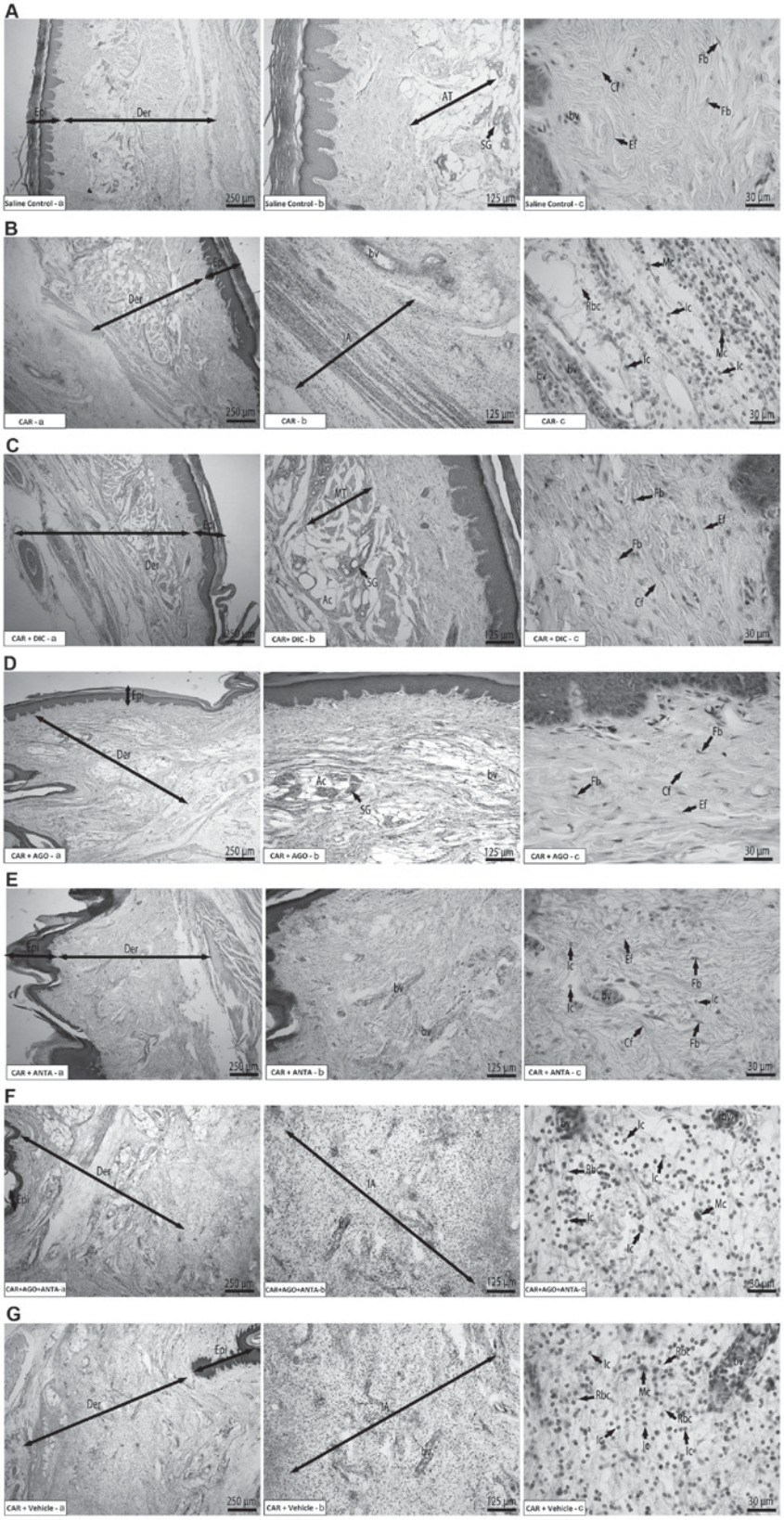

Figure 6. Histological changes in oedema paws 4 h after injection of carrageenan. Light micrograph of rat paw in all studied groups. Paws were harvested 4 h after injection of carrageenan and subjected to histochemical staining of paw tissues. Histopathological pictures of all groups are summarized in Fig. 6. Saline control group was showing no histopathological changes. The amount of inflammatory cells in the carrageenan group was higher than that of the saline control group and agonist or diclofenac groups. There is a slight difference between saline control and treatments group in term of tissue content. The amount of inflammatory cells in the antagonist and agonist+antagonist groups were higher than that of the saline control group and agonist or diclofenac groups. The amount of inflammatory cells in the vehicle group was higher than that of the saline control group and agonist or diclofenac groups. H&E staining, the magnifications of images a, b, and c are at x4, x10 and x40, respectively. Epi, epidermis; Der, dermis; AT, adipose tissue; SG, Sweet gland; Fb, fibroblast; bv, blood vessel; Ef, elastic fiber; Cf, collagen fiber. IA, inflammation area; Rbc, red blood cell; Ic, inflammatory cell; MC, macrophage like cell. Ac, adipose cell; MT, muscle tissue. (A) Saline control group; (B) carrageenan model group; (C) diclofenac group; GW405833, (D) CB2 agonist group; (E) AM630, CB2 antagonist group; (F) CB2 agonist+antagonist group; and (G) Vehicle group. AGO: Agonist, ANTA, antagonist; DIC, diclofenac; CAR, Carrageenan, Vehicle, dimethyl sulfoxide.

CAR group results: Observation of inflammatory cells in the dermis and hypodermis was evident. The inflammation score for this group was more than saline control, CAR+AGO, and CAR+DIC groups. In addition, macrophage like cells was observed in the hypodermis, around the vessels.

CAR+DIC group results: The appearance of the dermis, hypodermis, epidermis and other tissue components, including fibers and connective tissue cells were similar to those in the saline control group.

CAR+AGO group results: The tissues in this group were similar to those in the healthy group. In term of epidermis, dermis, hypodermis and other tissue components, including fibers and connective tissue cells, no significant difference was found between the tissues in this group and either the saline control or diclofenac group.

CAR+ANTA group results: Compared with the carrageenan group, dermis and hypodermis were found to have fewer inflammatory cells, but the inflammation cell density in this group was higher than the saline control and treatment group.

CAR+AGO+ANTA group results: The appearance of the epidermis, dermis, hypodermis and other tissue components, including fibers and connective tissue cells were similar to the carrageenan group. There was a significant difference compared to the saline control and diclofenac group in term of inflammatory cells density. In addition, but in the epidermis around the vessels, macrophage-like cells were observed.

CAR+Vehicle group results: This group is associated with a large number of inflammatory cells in dermis and hypodermis when compared with the saline control and treatment groups. Moreover, in the hypodermis around the vessels, macrophage-like cells were observed.

Discussion

Although the role of the CB2 receptor in the regulation of inflammation has been recently researched, its mechanism has not been fully understood. The results of this study confirm the effects of exogenous CB2 agonist, and a pharmacologic role for CB2 receptor on carrageenan-induced paw oedema in rats. We demonstrate that pre-treatment of rat with CB2 selective receptor agonist GW405833 decreases paw oedema, tissue inflammatory cell infiltration and increased MPO level, tissue lipid peroxidation, and serum TNF-α level. CB2 receptor activation also increases the tissue GSH level.

The process of inflammation is actually a response to various stimuli against those who have given organism. Some of these stimuli are trauma, infection, ischemia/reperfusion and immunological reactions (21,22). Carrageenan-induced paw oedema is a well-established model for evaluating acute inflammation and anti-inflammatory effect of candidate drugs (9). That oedema involves both neurogenic and non-neurogenic mechanisms which have been associated with arachidonic acid cascade which leads to the formation of eicosanoids, prostaglandin, thromboxane, leukotriene, reactive oxygen species, cytokines and other inflammatory mediators, which were mediated by activity of 5-lipoxygenase and COX enzymes (8). To date, several previous studies showed endocannabinoid system have been major roles in nociception or inflammatory reactions by receptor activation (23,24) or exogenous agonist administrations (25-30) or inhibition of metabolized enzyme of 2-AG ligand (13,31,32). In this study we showed that both CB2 receptor selective agonist GW405833 and non-specific COX inhibitor drug diclofenac, clearly inhibited the development of carrageenan-induced oedema. Elmes *et al* speculate that CB2 agonist JWH-133 on paw volume been comparable to the effect of systemic post-administration of morphine and rofecoxib (25). Ghosh *et al* declared that JZL184, selective MAGL inhibitor, reduces carrageenan-induced paw oedema were mediated through CB2 receptors (13). Findings of a study can be indirectly support our clear observations (33). Because of that results, related to CB1 receptor way, said do not support a role for anandamide (AEA), CB1 receptor ligand, in preventing carrageenan-induced oedema, while palmitoylethanolamide (PEA), an endogenous fatty acid, administration and the catabolic enzyme fatty acid amide hydrolase (FAAH) blockade elicited anti-oedema effects of an equivalent magnitude as produced by, dexamethasone, and diclofenac. Due to confirm the occurrence of CB2 receptor, we administered CB2 receptor antagonist AM630 plus to CB2 receptor agonist in carrageenan-induced inflammatory animals. CB2 receptor antagonist clearly reversed the anti-oedematous effect of agonist in paw tissue. However CB2 receptor antagonist has no effect on inflammation response.

At the end of the histopathological examinations; it was found that there was a significant decrease in the number of inflammatory cells in the agonist and diclofenac treated groups, although there were a large number of inflammatory cells in the tissue samples in the antagonist and carrageenan groups. As seen in macroscopic analysis, the effect of the COX inhibitor diclofenac was similar to anti-inflammatory effect of CB2 agonist. Haruna *et al* (34), have showed that the S-777469, (CB2) agonist, reduced the epidermal thickness and the number of mast cells infiltrating skin lesions of DNFB-induced ear swelling in mice. MAGL inhibition in diseased mice increased 2-arachidonoylglycerol levels, leading to a reduction of macroscopic and histological colon alterations in the trinitrobenzene sulfonic acid (TNBS)-induced colitis (31). Administration of antagonist alone did not contribute to anti-inflammatory effect. This data suggests that the CB2 receptor antagonist reversed the effect of the CB2 receptor agonist.

In pathological conditions and triggers inflammatory signals, the ability of diclofenac to control carrageenan-induced paw oedema is through the inhibition of COX, which is overexpressed in inflammation. Our data show that CB2 receptor agonist is as effective as the COX inhibitor to control the development of carrageenan induced paw oedema. CB2 receptor agonist may be indirectly and/or directly decreases prostaglandin and other pro-inflammatory mediator production through enhanced COX-2 expression. This data is supported by recent studies. 2-AG limits COX-2 elevation in neuronal inflammation (35). MAGL inhibition by KML29 induced a decrease in prostaglandin levels in most peripheral tissues (32). On the other hand, COX activity is induced by the endocannabinoids, specifically by the ligand AEA for CB1 and by inverse agonism of CB2 (36). Another data is that cannabinoid receptor 2 suppresses leukocyte inflammatory migration by modulating the lipoxygenase pathway (37).

Cell migration is an important step of the inflammatory process of the injured tissue. So, the anti-inflammatory effects of CB2 agonists to be understood that involve the inhibition of cell migration especially neutrophil migration, we measured the MPO activity in the tissue for this purpose. The prominent enzyme of neutrophils is MPO, which are released in the extracellular space or with their activation. The more neutrophil chemotaxis and migration into the inflamed tissue, the more MPO activity increases (38,39). As it can be seen from the data we have obtained; the CB2 agonist inhibits neutrophil infiltration, which was evident from the reduced MPO activity. This demonstrates that neutrophil migration to the inflamed site is inhibited by the CB2 agonist. JWH015, selective CB2 agonist, is decrease leukocytes infiltration in both submucosa and mucosa, as well as the myeloperoxydase activity, in LPS treated mice (28).

During acute pancreatitis, an upregulation especially of CB2 on apoptotic cells is occur (40). Cannabinoids may limit hepatic injury by modulating the expression of adhesion molecules and the infiltration and activation of inflammatory cells by CB2-dependent mechanisms (4). Carrageenan-induced paw inflammation is dependent on PMN infiltration. TNF-α acts as a continuous stimulator for neutrophil infiltration in the inflammatory tissues and it also up-regulates the production of cell-type specific leukocyte chemo attractants, known as chemokines, which have also been shown to cause up-regulation of cell adhesion molecules and neutrophil activation (41). We focused on TNF-α since it is proposed to play an early and crucial role in the cascade of pro-inflammatory cytokines and the subsequent inflammatory processes (39). We have proved inflammation induction with carrageenan led to an increase in serum levels of TNF-α and IL-1β. It supported our findings in previous studies (28,31,42,43).

Pro-inflammatory cytokines such as TNF-α and IL-1β are important mediators associated with certain inflammatory diseases such as rheumatoid arthritis etc (44). Thus, an effective way to treat various inflammatory diseases can be a form of treatment for the inhibition of these mediators. In our study, pre-treatment with CB2 agonist significantly reversed this significant increase, suggesting that CB2 agonist exerted an anti-inflammatory action and inhibited polymorph nuclear cell migration, probably by decreasing TNF-α and IL-1β levels.

One of the major damages caused by inflammation is free oxygen radicals. Reactive oxygen species are molecules that are highly unstable and highly reactive (45). Acute inflammatory processes can cause leukocyte migration occurs involve inflammation mediators including neutrophil-derived active oxygen species and free radicals (45). To understand the working mechanism of agonist and antagonist, the lipid peroxidation product, MDA, and antioxidant, GSH, parameter in paw tissue were studied. The end-product of lipid peroxidation is MDA. It refers to the imbalance between oxidant and antioxidant system. It is well known that the tissue lipid peroxidation levels can affected and the antioxidant levels in paw tissues can reduced in carrageenan inflammation studies. Previous studies have showed that in carrageenan-induced paw oedema, the elimination of toxic oxygen metabolites depends on the efficacy of the antioxidants (8-10). GSH is actually the main component included in the endogenous antioxidant system. GSH reduces the cytotoxic effects of these toxic oxygen metabolites (45). In the present study, the GSH level was significantly higher in the agonist group than in carrageenan group. When agonist was given with carrageenan, it caused high MDA level to drop significantly, and so we speculated that GW405833 decreases oxidative stress caused by carrageenan inflammation. We think that the CB2 receptor activation in peripheral tissues may be decrease the carrageenan paw inflammation by reducing oxidative stress. It is mean that CB2 receptor agonists may be used as anti-inflammatory agents to prevent inflammation. This study has shown that GW405833 may contribute to the reduction of oxidative stress in a variety of inflammatory diseases due to potential therapeutic effects. The inflammation of paw tissue by reducing oxidative stress is inhibited by CB2 agonist when administered to exogenous. So, it can benefit from the activation of the CB2 receptor in order to prevent inflammation. It has been suggested in our study that CB2-specific agonists can be used to inhibit the production of both oxidative stress and pro-inflammatory cytokines in inhibit of various inflammatory diseases. JWH133, CB2 agonist, has the potential to treat bacterial translocation and various relevant abnormalities through inhibition of systemic/intestinal oxidative stress, inflammatory cytokines and TNF-α release in cirrhosis (29).

We worked GW405833 as CB2 selective agonist to control carrageenan-induced paw inflammation in rats. Because it has highly selective on CB2 receptors, a lot of researchers had used in the designed inflammatory models as sepsis (46), osteoarthritis (47), and inflammatory pain (48). The presence of CB2 receptors is continuing to discuss while information about whether they are present in peripheral tissues is limited. The protective effects of the CB2 receptor stimulation caused us to hypothesize that the presence of these receptors in the paw tissue and that the expression of these receptors increases with inflammatory stimuli.

In summary, our results demonstrate that CB2 agonist has not only anti-inflammatory activity in the models of acute inflammation but also the inhibition of neutrophil migration. This effect, at least in part, is due to reduce IL-1β and TNF-α levels and oxidative stress.

This results support the conclusion that CB2 receptor activation in the pathogenesis of acute inflammatory in response to carrageenan. This study demonstrated that CB2 receptors are present in the paw and that expression of CB2 receptors by inflammatory stimulation is increased. The CB2 receptor agonist, GW405833, exerted significant anti-inflammatory effects such as diclofenac in anti-inflammatory effect. Co-administration of the CB2 receptor antagonist AM630 with the CB2 agonist reversed this effect pharmacologically. The anti-inflammatory effect of the CB2 receptor agonist may be due to decrease both in serum cytokine levels and/or in oxidative stress and increase in unbalanced antioxidant system. These findings forcefully suggest that targeting CB2 cannabinoid receptors may represent a novel strategy in protecting against inflammatory of peripheral cells. In conclusion, In the prevention of inflammatory diseases, activate of CB2 receptors may be a promising therapy.

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Availability of data and materials

The datasets used and/or analysed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

AP, SAC, HK and MFD conceived and designed the experiments, and provided the relevant materials and analytical tools. AP and MFD performed the experiments. AY and EE performed histological analysis. FU, MKO and SOA analyzed the data. SOA wrote the manuscript.

Ethics approval and consent to participate

Ethics committee approval was obtained from Mustafa Kemal University with protocol number 2013/7-17. Procedures were in accordance with the Guide for Care and Use of Laboratory Animals.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Rom S and Persidsky Y: Cannabinoid receptor 2: Potential role in immunomodulation and neuroinflammation. J Neuroimmune Pharmacol 8: 608-620, 2013.
2. Zogopoulos P, Vasileiou I, Patsouris E and Theocharis SE: The role of endocannabinoids in pain modulation. Fundam Clin Pharmacol 27: 64-80, 2013.
3. Witkamp R and Meijerink J: The endocannabinoid system: An emerging key player in inflammation. Curr Opin Clin Nutr Metab Care 17: 130-138, 2014.
4. Bátkai S, Osei-Hyiaman D, Pan H, El-Assal O, Rajesh M, Mukhopadhyay P, Hong F, Harvey-White J, Jafri A, Haskó G, *et al*: Cannabinoid-2 receptor mediates protection against hepatic ischemia/reperfusion injury. FASEB J 21: 1788-1800, 2007.
5. Núñez E, Benito C, Pazos MR, Barbachano A, Fajardo O, González S, Tolón RM and Romero J: Cannabinoid CB2 receptors are expressed by perivascular microglial cells in the human brain: an immunohistochemical study. Synapse 53: 208-213, 2004.
6. Yao BB, Hsieh G, Daza AV, Fan Y, Grayson GK, Garrison TR, El Kouhen O, Hooker BA, Pai M, Wensink EJ, *et al*: Characterization of a cannabinoid CB2 receptor-selective agonist, A-836339 [2,2,3,3-tetramethyl-cyclopropanecarboxylic acid [3-(2-methoxy-ethyl)-4,5-dimethyl-3H-thiazol-(2Z)-ylide ne]-amide], using in vitro pharmacological assays, in vivo pain models, and pharmacological magnetic resonance imaging. J Pharmacol Exp Ther 328: 141-151, 2009.
7. Marques TH, Marques ML, Medeiros JV, Silva RO, dos Reis Barbosa AL, Lima TC, de Sousa DP and de Freitas RM: Cyane-carvone, a synthetic derivative of carvone, inhibits inflammatory response by reducing cytokine production and oxidative stress and shows antinociceptive effect in mice. Inflammation 37: 966-977, 2014.
8. Albayrak A, Halici Z, Cadirci E, Polat B, Karakus E, Bayir Y, Unal D, Atasoy M and Dogrul A: Inflammation and peripheral 5-HT7 receptors: The role of 5-HT7 receptors in carrageenan induced inflammation in rats. Eur J Pharmacol 715: 270-279, 2013.
9. Büyükokuroğlu ME: Anti-inflammatory and antinociceptive properties of dantrolene sodium in rats and mice. Pharmacol Res 45: 455-460, 2002.
10. Uzkeser H, Cadirci E, Halici Z, Odabasoglu F, Polat B, Yuksel TN, Ozaltin S and Atalay F: Anti-inflammatory and antinociceptive effects of salbutamol on acute and chronic models of inflammation in rats: Involvement of an antioxidant mechanism. Mediators Inflamm 2012: 438912, 2012.
11. Conti S, Costa B, Colleoni M, Parolaro D and Giagnoni G: Antiinflammatory action of endocannabinoid palmitoylethanolamide and the synthetic cannabinoid nabilone in a model of acute inflammation in the rat. Br J Pharmacol 135: 181-187, 2002.
12. Iwamura H, Suzuki H, Ueda Y, Kaya T and Inaba T: In Vitro and in vivo pharmacological characterization of JTE- 907, a novel selective ligand for cannabinoid CB 2 receptor. J Pharmacol Exp Ther 296: 420-425, 2001.
13. Ghosh S, Wise LE, Chen Y, Gujjar R, Mahadevan A, Cravatt BF and Lichtman AH: The monoacylglycerol lipase inhibitor JZL184 suppresses inflammatory pain in the mouse carrageenan model. Life Sci 92: 498-505, 2013.
14. Comelli F, Giagnoni G, Bettoni I, Colleoni M and Costa B: The inhibition of monoacylglycerol lipase by URB602 showed an anti-inflammatory and anti-nociceptive effect in a murine model of acute inflammation. Br J Pharmacol 152: 787-794, 2007.
15. Lunn CA, Fine JS, Rojas-Triana A, Jackson JV, Fan X, Kung TT, Gonsiorek W, Schwarz MA, Lavey B, Kozlowski JA, *et al*: A novel cannabinoid peripheral cannabinoid receptor-selective inverse agonist blocks leukocyte recruitment in vivo. J Pharmacol Exp Ther 316: 780-788, 2006.
16. Rivers-Auty JR, Smith PF and Ashton JC: The cannabinoid CB2receptor agonist GW405833 does not ameliorate brain damage induced by hypoxia-ischemia in rats. Neurosci Lett 569: 104-109, 2014.
17. Hayakawa K, Mishima K, Nozako M, Hazekawa M, Irie K, Fujioka M, Orito K, Abe K, Hasebe N, Egashira N, *et al*: Delayed treatment with cannabidiol has a cerebroprotective action via a cannabinoid receptor-independent myeloperoxidase-inhibiting mechanism. J Neurochem 102: 1488-1496, 2007.
18. Casini AF, Ferrali M, Pompella A, Maellaro E and Comporti M: Lipid peroxidation and cellular damage in extrahepatic tissues of bromobenzene-intoxicated mice. Am J Pathol 123: 520-531, 1986.
19. Sedlak J and Lindsay RH: Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem 25: 192-205, 1968.
20. Bradley PP, Priebat DA, Christensen RD and Rothstein G: Measurement of cutaneous inflammation: Estimation of neutrophil content with an enzyme marker. J Invest Dermatol 78: 206-209, 1982.
21. Arslan SO, Gelir E, Sayan H and Ozacmak VH: L-Arginine and melatonin interaction in rat intestinal ischemia-reperfusion. Fundam Clin Pharmacol 19: 533-535, 2005.
22. Arslan SO: Morphine modulates microvascular leakage dose-dependently in the airway of ovalbumin-sensitized rats. Turk J Med Sci 40: 279-286, 2010.
23. Ashton JC and Glass M: The cannabinoid CB2 receptor as a target for inflammation-dependent neurodegeneration. Curr Neuropharmacol 5: 73-80, 2007.
24. Berger ND, Gadotti VM, Petrov RR, Chapman K, Diaz P and Zamponi GW: NMP-7 inhibits chronic inflammatory and neuropathic pain via block of Cav3.2 T-type calcium channels and activation of CB2 receptors. Mol Pain 10: 77, 2014.
25. Elmes SJ, Winyard LA, Medhurst SJ, Clayton NM, Wilson AW, Kendall DA and Chapman V: Activation of CB1 and CB2 receptors attenuates the induction and maintenance of inflammatory pain in the rat. Pain 118: 327-335, 2005.
26. Valenzano KJ, Tafesse L, Lee G, Harrison JE, Boulet JM, Gottshall SL, Mark L, Pearson MS, Miller W, Shan S, *et al*: Pharmacological and pharmacokinetic characterization of the cannabinoid receptor 2 agonist, GW405833, utilizing rodent models of acute and chronic pain, anxiety, ataxia and catalepsy. Neuropharmacology 48: 658-672, 2005.

27. Harvey BS, Nicotra LL, Vu M and Smid SD: Cannabinoid CB2 receptor activation attenuates cytokine-evoked mucosal damage in a human colonic explant model without changing epithelial permeability. Cytokine 63: 209-217, 2013.
28. Tambaro S, Antonietta M, Mastinu A and Lazzari P: Evaluation of selective cannabinoid CB(1) and CB(2) receptor agonists in a mouse model of lipopolysaccharide-induced interstitial cystitis. Eur J Pharmacol. Elsevier 729: 67-74, 2014.
29. Yang YY, Hsieh SL, Lee PC, Yeh YC, Lee KC, Hsieh YC, Wang YW, Lee TY, Huang YH, Chan CC and Lin HC: Long-term cannabinoid type 2 receptor agonist therapy decreases bacterial translocation in rats with cirrhosis and ascites. J Hepatol 61: 1004-1013, 2014.
30. Smoum R, Baraghithy S, Chourasia M, Breuer A, Mussai N, Attar-Namdar M, Kogan NM, Raphael B, Bolognini D, Cascio MG, *et al*: CB2 cannabinoid receptor agonist enantiomers HU-433 and HU-308: An inverse relationship between binding affinity and biological potency. Proc Natl Acad Sci USA 112: 8774-8779, 2015.
31. Alhouayek M, Lambert DM, Delzenne NM, Cani PD and Muccioli GG: Increasing endogenous 2-arachidonoylglycerol levels counteracts colitis and related systemic inflammation. FASEB J 25: 2711-2721, 2011.
32. Pasquarelli N, Porazik C, Hanselmann J, Weydt P, Ferger B and Witting A: Comparative biochemical characterization of the monoacylglycerol lipase inhibitor KML29 in brain, spinal cord, liver, spleen, fat and muscle tissue. Neuropharmacology 91: 148-156, 2015.
33. Wise LE, Cannavacciulo R, Cravatt BF, Martin BF and Lichtman AH: Evaluation of fatty acid amides in the carrageenan-induced paw edema model. Neuropharmacology 54: 181-188, 2008.
34. Haruna T, Soga M, Morioka Y, Imura K, Furue Y, Yamamoto M, Hayakawa J, Deguchi M, Arimura A and Yasui K: The inhibitory effect of S-777469, a cannabinoid type 2 receptor agonist, on skin inflammation in mice. Pharmacology 99: 259-267, 2017.
35. Zhang J and Chen C: Endocannabinoid 2-arachidonoylglycerol protects neurons by limiting COX-2 elevation. J Biol Chem 283: 22601-22611, 2008.
36. Kim J and Watkins BA: Cannabinoid receptor antagonists and fatty acids alter endocannabinoid system gene expression and COX activity. J Nutr Biochem 25: 815-823, 2014.
37. Liu YJ, Fan HB, Jin Y, Ren CG, Jia XE, Wang L, Chen Y, Dong M, Zhu KY, Dong ZW, *et al*: Cannabinoid receptor 2 suppresses leukocyte inflammatory migration by modulating the JNK/c-Jun/Alox5 pathway. J Biol Chem 288: 13551-13562, 2013.
38. Nussbaum C, Klinke A, Adam M, Baldus S and Sperandio M: Myeloperoxidase: A leukocyte-derived protagonist of inflammation and cardiovascular disease. Antioxid Redox Signal 18: 692-713, 2013.
39. Winterbourn CC, Kettle AJ and Hampton MB: Reactive oxygen species and neutrophil function. Annu Rev Biochem 85: 765-1092, 2016.
40. Michler T, Storr M, Kramer J, Ochs S, Malo A, Reu S, Göke B and Schäfer C: Activation of cannabinoid receptor 2 reduces inflammation in acute experimental pancreatitis via intra-acinar activation of p38 and MK2-dependent mechanisms. Am J Physiol Gastrointest Liver Physiol 304: G181-G192, 2013.
41. Neri M, Fineschi V, Di Paolo M, Pomara C, Riezzo I, Turillazzi E and Cerretani D: Cardiac oxidative stress and inflammatory cytokines response after myocardial infarction. Curr Vasc Pharmacol 13: 26-36, 2015.
42. Li YY, Li YN, Ni JB, Chen CJ, Lv S, Chai SY, Wu RH, Yüce B and Storr M: Involvement of cannabinoid-1 and cannabinoid-2 receptors in septic ileus. Neurogastroenterol Motil 22: e350-e388, 2010.
43. Silva RO, Sousa FB, Damasceno SR, Carvalho NS, Silva VG, Oliveira FR, Sousa DP, Aragão KS, Barbosa AL, Freitas RM and Medeiros JV: Phytol, a diterpene alcohol, inhibits the inflammatory response by reducing cytokine production and oxidative stress. Fundam Clin Pharmacol 28: 455-464, 2014.
44. Gui H, Tong Q, Qu W, Mao CM and Dai SM: The endocannabinoid system and its therapeutic implications in rheumatoid arthritis. Int Immunopharmacol 26: 86-91, 2015.
45. Arslan SO, Zerin M, Vural H and Coskun A: The effect of melatonin on bleomycin-induced pulmonary fibrosis in rats. J Pineal Res 32: 21-25, 2002.
46. Gui H, Sun Y, Luo ZM, Su DF, Dai SM and Liu X: Cannabinoid receptor 2 protects against acute experimental sepsis in mice. Mediators Inflamm 2013: 741303, 2013.
47. Schuelert N, Zhang C, Mogg AJ, Broad LM, Hepburn DL, Nisenbaum ES, Johnson MP and McDougall JJ: Paradoxical effects of the cannabinoid CB2 receptor agonist GW405833 on rat osteoarthritic knee joint pain. Osteoarthr Cartil 18: 1536-1543, 2010.
48. Whiteside GT, Gottshall SL, Boulet JM, Chaffer SM, Harrison JE, Pearson MS, Turchin PI, Mark L, Garrison AE and Valenzano KJ: A role for cannabinoid receptors, but not endogenous opioids, in the antinociceptive activity of the CB2-selective agonist, GW405833. Eur J Pharmacol 528: 65-72, 2005.