Naunyn-Schmiedeberg's Arch Pharmacol (2017) 390:1201–1209
DOI 10.1007/s00210-017-1418-8

ORIGINAL ARTICLE

A synthetic cannabinoid JWH-210 reduces lymphoid organ weights and T-cell activator levels in mice via CB_2 receptors

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Received: 18 May 2017 / Accepted: 10 August 2017 / Published online: 22 August 2017

Abstract The problem of new psychoactive substances (NPS) is emerging globally. However, the immunotoxicity of synthetic cannabinoids is not evaluated extensively yet. The purpose of the present study was to investigate whether synthetic cannabinoids (JWH-210 and JWH-030) induce adverse effects on lymphoid organs, viability of splenocytes and thymocytes, and immune cell activator and cytokines in mice. JWH-210 (10 mg/kg, 3 days, i.p.) is more likely to have cytotoxicity and reduce lymphoid organ weight than JWH-030 of ICR mice in vivo. We also demonstrated that JWH-210 administration resulted in the decrease of expression levels of T-cell activator including Cd3e, Cd3g, Cd74p31, and Cd74p41, while JWH-030 increased Cd3g levels. In addition, JWH-210 reduced expression levels of cytokines, such as interleukin-3, interleukin-5, and interleukin-6. Furthermore, we demonstrated that a CB_2 receptor antagonist, AM630 inhibited JWH-210-induced cytotoxicity, whereas a CB_1 receptor antagonist, rimonabant did not in primary cultured splenocytes. These results suggest that JWH-210 has a cytotoxicity via CB_2 receptor action and results in decrement of lymphoid organ weights, T-cell activator, and cytokine mRNA expression levels.

Keywords New psychoactive substances · Immune system · Cytokine · T-cell activator · Lymphoid organ

Introduction

New psychoactive substances (NPS) have diverse adverse effects including cardiovascular, neurological, gastrointestinal, and pulmonary. Currently, there is a dearth of knowledge on many types of NPS. Most of the available data on NPS-induced toxicity are derived from retro- or prospectively analyzed cases of intoxication as well as interviews with drug users and, therefore, are of limited scientific value (Hohmann et al. 2014). Therefore, to evaluate the adverse effect of NPS, preclinical studies are required to study their toxicity. However, most preclinical research studies have investigated the dependence potential and neuropsychiatric effects of NPS. Synthetic cannabinoids are one of most abused NPS and have dependence liability potential that is similar to that of natural and botanical components. Research on the cannabinoids has revealed several hundred agonists that might be abused, with varying affinity for the CB_1 and CB_2 receptors (Fattore and Fratta 2011). The endocannabinoid system participated in the regulation of physiological process such as caloric balance and the control of arterial smooth muscle tone (Hohmann et al. 2014). CB_1 receptors are found mainly in the nervous system, as well as on particular types of neurons (Seely et al. 2011). Synthetic cannabinoids are potent CB_1 agonist and exert a delta-9-tetrahydrocannabinol (THC)-like effect, with alteration of mood, perception, sleep and wakefulness, body temperature, and cardiovascular function (Hermanns-Clausen et al. 2013). Their side effects are more

Electronic supplementary material The online version of this article (doi:10.1007/s00210-017-1418-8) contains supplementary material, which is available to authorized users.

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varied and more severe than those of THC, with the more common ones being tachycardia, arterial hypertension, hyperglycemia, hypokalemia, hallucinations, and agitation (Hohmann et al. 2014). Because of expression patterns of CB receptors in the immune system, it can be assumed that cannabinoids have significant effects on immune response. It has been reported that cannabinoids significantly hampered the resistance to infection in human as well as several animal species (Friedman et al. 2003). However, the exact harmful effects of synthetic cannabinoids on the immune system are not fully understood yet. In the present study, we investigated the effects of the synthetic cannabinoids, which have differences in CB receptor binding affinity on the immune system in vivo and ex vivo. JWH-030 is regarded as agonists at both the CB_1 and CB_2 with Ki values of 87 and 320 nM, respectively. JWH-210 has a higher binding affinity with Ki values of 0.46 nM at CB_1 and 0.69 nM at CB_2 (Showalter et al. 1996; Griffin et al. 1998; Huffman et al. 2005; Rieder et al. 2010). The cytotoxicity of the synthetic cannabinoids was evaluated in thymocytes and splenocytes. We also measured lymphoid organ weight and T-cell activator and cytokine levels of lymphoid organs in mice after administration of JWH-210 and JWH-030. Furthermore, to clarify a mechanism underlying synthetic cannabinoid-induced toxicity, a pharmacological rescue study of the cytotoxicity of the agents was performed using CB_1 and CB_2 receptor antagonists.

Materials and methods

Animals

All experimental procedures were approved by the Animal Ethics Committee, National Institute of Food and Drug Safety Evaluation and complied with the Guide for the Care and Use of Laboratory Animals (Institue of Laboratory Animal Resources (U.S.) 1996).

All efforts were made to minimize animal distress and prevent suffering. Male and female ICR mice (4 weeks old) were obtained from the Ministry of Food and Drug Safety (Association for Assessment and Accreditation of Laboratory Animal Care, AAALAC member, Osong, Republic of Korea). The mice were maintained in an animal facility where they were transferred to cages on arrival and were housed in adequate group sizes. They were allowed to acclimatize for 1 week before being used in the experiments. The animal holding rooms were maintained at a temperature of 21–24 °C and 40–60% relative humidity with a 12-h light/dark cycle (lights on at 08:00 to 20:00). The animals received a solid diet, tap water ad libitum.

Materials

All the synthetic cannabinoids tested (JWH-210 and JWH-030 illustrated in Fig. 1) were purchased from Cayman Chemical (Ann Arbor, MI, USA). Rimonabant hydrochloride and AM630 were purchased from Sigma-Aldrich (St. Louis, MO, USA). The other routine chemicals were purchased from Sigma-Aldrich unless otherwise specified.

Lymphoid organ weight

ICR mice were weighted every day during injection period and just before being euthanized. JWH-210 or JWH-030 was administered (10 mg/kg, i.p.) for 3 days. Vehicle is 0.5% DMSO and 0.5% Tween80 in saline. Mice were sacrificed by cervical dislocation, and spleen and thymus were removed 24 h after last administration. Body weight and selected absolute organ weights were scaled, and relative organ to body weight ratios were calculated for spleen and thymus.

Splenocyte and thymocyte culture

Spleen and thymus were isolated from mice. A 100-μm-pore cell strainer (Falcon, USA) was used for dividing single cells. Single cell suspensions of spleen and thymus were prepared by gently pressing the tissues with sterilized slide glass. Splenocytes and thymocytes were pooled from isolated organs and seeded in plates with the RPMI (Gibco, Waltham, MA, USA) supplemented with β-mercaptoethanol (50 μM), HEPES (10 mM), FBS (5%), L-glutamine (1 mM), and antibiotics/antimycotics (Invitrogen, Waltham, MA, USA).

Cell viability (in vivo administration)

Splenocytes were cultured from JWH-210- (10 mg/kg, 3 days, i.p.) or JWH-030- (10 mg/kg, 3 days, i.p.) treated mice as mentioned previously. The cells were seeded in 96-well plates (5×10^5 cells/well) with 100 μL media. After seeding single cell suspensions, the plates were incubated for 6 h in 95% air:5% CO_2 using standard culture methods. Cell viability was measured using LIVE/DEAD viability/cytotoxicity kit (Thermo Fisher, Waltham, MA, USA) according to the manufacturer's manual. In brief, prior to the assay, the cells were washed twice with phosphate-buffered saline (PBS) and added 100 μL PBS to each well with supplied EthD-1 and calcein AM. The cells were incubated for 30 min at room temperature in the dark, and cell viability was measured by microplate-based cell cytometer (Celigo, Lawrence, MA, USA). The data of Live (% corrected) was used to analyze cell viability.

Cell viability (ex vivo treatment)

Splenocytes were cultured from naïve mice as mentioned previously. The cells were seeded in 96-well plates (5×10^5 cells/well) with 100 μL media. After seeding single cell suspensions, the plates were incubated for 6 h in 95% air:5% CO_2 using standard culture methods. To confirm cell viability by

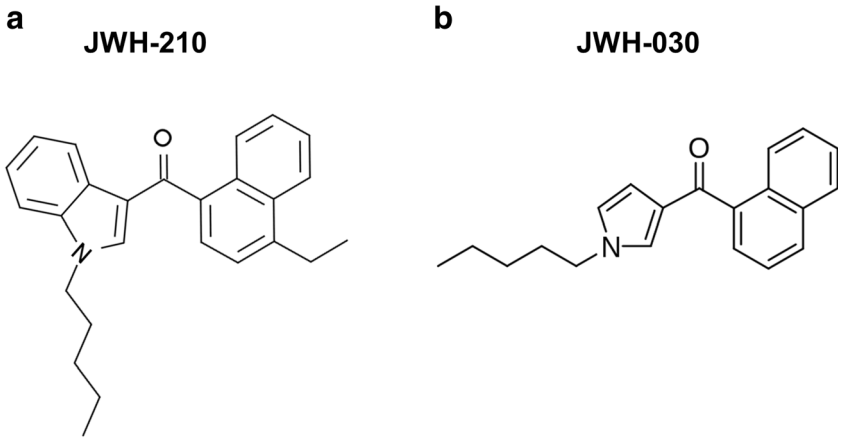

Fig. 1 Chemical structures of synthetic cannabinoids. (**a**) Structure of JWH-210. (**b**) Structure of JWH-030

synthetic cannabinoids, the splenocytes were treated with synthetic cannabinoids (0, 1, 10, and 100 μM) for 16 h. To observe relationship between JWH-210 and CB receptors, the splenocytes were treated with media or JWH-210 or JWH-210/CB receptor antagonist (rimonabant or AM630) for 4 h. Cell viability was measured as mentioned previously.

T-cell and B-cell activation-related gene profiling and quantitative PCR

Spleen were isolated from mice that were injected with JWH-210 (10 mg/kg, i.p., 3 days). Total RNA was extracted by easy-spin DNA free total RNA extraction kit (Intron, Seoul, Republic of Korea). Complementary DNA (cDNA) was synthesized from total isolated RNA using a SuperScript III first-strand synthesis system (Invitrogen) for quantitative PCR (qPCR). RT^2 Profiler™ PCR Array Mouse T-Cell & B-Cell Activation (PAMM-053Z, QIAGEN, Valencia, CA, USA) was used for pathway-focused T-cell and B-cell activation-related gene expression analysis. Subsequent qPCR was performed with an iCycler iQ5 real-time detection system (Bio-Rad, Hercules, CA, USA) using the SYBR Green PCR master mix (Thermo Fisher Scientific, Waltham, MA, USA). For the polymerase activation, the initial incubation was conducted at 50 °C for 2 min followed by 95 °C for 10 min. After that, 40 cycles were performed at 95 °C for 15 s and then at 60 °C for 1 min. cDNA was included in a 25-μL-volume PCR reaction with the following components: 0.125 μL each of forward and reverse primer, 12.5 μL SYBR green, and 0.5 μg of cDNA with sterilized water. The primers used were as follows: mouse Cd3ε, forward 5′-CGTCCGCCATCTTGGTAGAG-3′, reverse 5′-ATTC AATGTTCTCGGCATCGT-3′; mouse Cd3γ, forward 5′-TGGAGAAGCAAAGAGACTGACA-3′, reverse 5′-GCCA TCCACTTGTACCAAATTC-3′; mouse Cd74p41, forward 5′-TTCCTCACACCAAGAGCCG-3′, reverse 5′-TGTC CAGTGGCTCACTGCAG-3′; mouse Cd74p31, forward 5′-ACCGAGGCTCCACCTAAAGAG-3′, reverse 5′-TTGA CCCAGTTCCTGCCTG-3′; mouse IL-3 (N-4011, Bioneer, Daejeon, Republic of Korea); mouse IL-5 (N-4012, Bioneer, Daejeon, Republic of Korea); mouse IL-6 (N-4013, Bioneer, Daejeon, Republic of Korea); mouse TNF-α (N-4015, Bioneer, Daejeon, Republic of Korea); mouse TNF-β (N-4018, Bioneer, Daejeon, Republic of Korea); mouse IL-1β (N-4009, Bioneer, Daejeon, Republic of Korea); mouse IL-10 (N-4014, Bioneer, Daejeon, Republic of Korea); mouse GAPDH (used as an internal control), forward 5′-TGTC

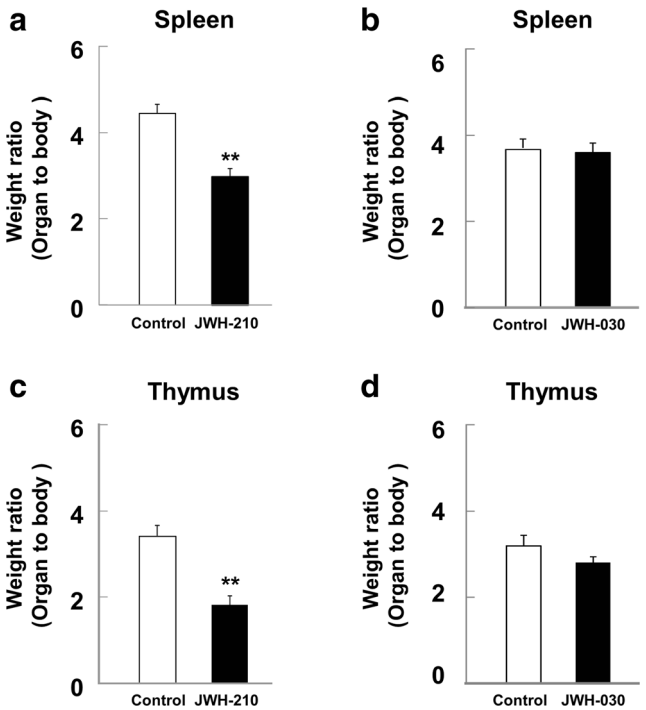

Fig. 2 Effects of synthetic cannabinoids on spleen and thymus. The organ weight was scaled and presented relatively to body weight. (**a**) JWH-210 administration reduced the organ weight of spleen, while (**b**) JWH-030 had no effects. (**c**) JWH-210 administration reduced the organ weight of thymus, while (**d**) JWH-030 had no effects. Data are shown as the means ± S.E. ($n = 8$). $^{**}p < 0.01$ vs each vehicle-treated control group (Student's t test)

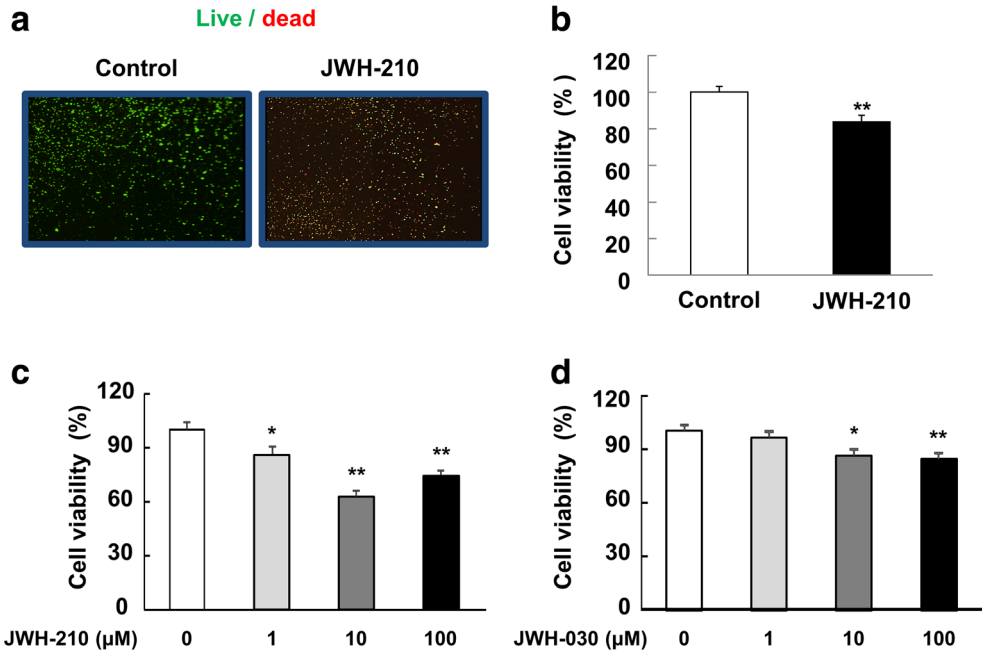

Fig. 3 Effects of JWH-210 on cell viability of splenocytes and thymocytes. (**a**) Representative photographs show live (green) and dead (cell) cell images of splenocytes isolated from JWH-210 treated mice (10 mg/kg, 3 days, i.p.) and control mice. (**b**) JWH-210 administration reduced the cell viability of splenocytes. Data are shown as the means ± S.E. ($n = 4$). $^{**}p < 0.01$ vs each vehicle-treated control group (Student's t test). Cell viability was measured after treatment of JWH-210 (**c**) in splenocytes and JWH-030 (**d**) in thymocytes isolated from naïve mice at indicated concentrations. JWH-210 and JWH-030 reduced cell viability of splenocytes and thymocytes. Data are shown as the means ± S.E. ($n = 8$). $^{*}p < 0.05$, $^{**}p < 0.01$ vs each vehicle-treated control group (one-way ANOVA, Bonferroni's)

AAGCTCATTTCCTGGT-3′, reverse 5′-CTTA CTCCTTGGAGGCCATG-3′.

Immunofluorescence

The ICR mice were treated with JWH-210 (10 mg/kg, 3 days, i.p.), and then, the mice were perfused with phosphate-buffered saline (PBS, pH 7.4) with heparin under inhaled CO_2 anesthetization. The mice spleen were immediately fixed with 4% paraformaldehyde for 3 days at 4 °C and transferred to 30% sucrose solutions at 4 °C. After being transferred to 30% sucrose solutions, the mice spleen were cut into 16 μm sections by using a cryostat microtome (Leica CM 1850; Leica Microsystems, Seoul, Republic of Korea) and mounted on MAS-GP type A-coated slides. After two 10-min washes in PBS (pH 7.4), endogenous peroxidase activity was quenched by incubating prepared spinal cord sections in 3% hydrogen peroxide in PBS for 20 min, followed by an additional two 10-min washes in PBS. Sections were blocked for 1 h in 5% bovine serum albumin (BSA) and incubated overnight at 4 °C with a mouse polyclonal antibody against CD3 (1:200; Santa Cruz Biotechnology, CA, USA) and a rat polyclonal antibody against CD8 (1:200; eBioscience, USA). Sections were then washed three times (10 min each) in PBS and incubated for 2 h at room temperature with a secondary antibody conjugated to Alexa Fluor 488 and 594 (Invitrogen Molecular Probes, Carlsbad, CA, USA). Sections were then washed three times (10 min each) in PBS, incubated for 30 s at room temperature in the dark for DAPI staining, and mounted with VectaMount™ AQ (Vecta Laboratories, Burlingame, CA, USA). IF images were acquired using an inverted Zeiss Axiovert 200 M fluorescent microscope (Microscope Axio Imager.A2, Carl Zeiss, Oberkochen, Germany) (×200).

Measurement of CD3/CD4- and CD3/CD8-positive cell

Splenocytes and thymocytes were cultured from mice that were injected with JWH-210 (10 mg/kg, i.p., 3 days) as mentioned previously. After seeding single cell suspensions, the plates were incubated for 6 h in 95% air:5% CO_2 using standard culture methods. The cultured cells were fixed with 4% formaldehyde for 15 min. After two washes in PBS, endogenous peroxidase activity was quenched by incubating prepared cells in 0.3% hydrogen peroxide and 0.2% Triton X-100 in PBS for 15 min, followed by an additional two washes in PBS. The cells were blocked for 1 h in 5% bovine serum albumin (BSA) and incubated overnight at 4 °C with the following primary antibodies: CD3 (1:100, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), CD4 (1:100, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), and CD8 (1:100, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). The cells were washed three times (10 min each) in PBS and incubated for 2 h at room temperature in the dark with the following secondary antibodies conjugated to Alexa Fluor 488 and 594 (1:500, Invitrogen Molecular Probes, Carlsbad, CA, USA). The cells were washed three times (10 min each) in PBS and incubated for 5 min at room temperature in the dark with DAPI (2 mg/mL stock, 1:1000, Sigma-Aldrich, St. Louis, MO). Fluorescent intensity was measured by microplate-based cell cytometer (Celigo, Lawrence, MA, USA), and cell populations were identified using color-coding overlay methods according to the manufacturer's instructions.

Table 1 Gene profiling results of T-cell and B-cell activators

No	Gene symbol	Fold regulation	Sub-classification	Functional grouping
1	Thy1	− 14.42	Regulator of T-cell activation	T-cell activation
2	Icam1	− 8.1681		
3	Cd3g	− 6.2767		
4	Lag3	− 5.8563		
5	Cd1d1	− 5.278		
6	Cd8b1	− 4.2575		
7	Cd47	− 3.9724		
8	Cblb	− 3.4343		
9	Cd8a	− 3.1602		
10	Dpp4	− 3.1167		
11	Spp1	− 4.6913	T-cell proliferation	
12	Cd3e	− 4.6589		
13	Casp3	− 3.7321		
14	Ripk2	− 3.605		
15	Il18	− 3.2043		
16	Cxcr4	− 3.1167		
17	Socs1	− 9.9866	T-cell differentiation	
18	Apc	− 7.5162		
19	Cd4	− 5.9381		
20	Cd74	− 5.6178		
21	Flt3	− 4.5631		
22	Cdkn1a	− 3.8371	B-cell proliferation	B-cell activation
23	Rag1	− 8.6939	B-cell differentiation	
24	H60a	− 6.9163	Macrophage activation	Other immune cell activation
25	Cd48	− 3.8906	Mast cell activation	

Twenty-five genes were downregulated in splenocytes by JWH-210 administration (10 mg/kg, 3 days, i.p.). One to 21 genes are T-cell activation-related genes. There were no upregulated genes

Data analysis

The data represent the mean ± S.E. Statistically significant differences from the values in vehicle-treated group were analyzed by Student's *t* test, one-way or two-way ANOVA followed by Bonferroni's test for equal variance data, and followed by Dunnett's rank test for non-equal variance data using SigmaPlot 13 software (SigmaPlot, Chicago, IL, USA). Probabilities less than 5% ($p < 0.05$) were considered to be statistically significant.

Results

JWH-210 reduced the weight of spleen and thymus

To investigate the effect of synthetic cannabinoids on lymphoid organ, ICR mice were injected with JWH-210 or JWH-030 (10 mg/kg, i.p. 3 days). We observed that JWH-210 decreased the weight of spleen and thymus, whereas JWH-030 had no effects in organ weight (Fig. 2).

JWH-210 reduced cell viability of splenocytes and thymocytes

We aimed to determine whether JWH-210, which has reduced organ weight, induced cytotoxicity. JWH-210 (10 mg/kg, i.p. 3 days) reduced cell viability in splenocytes (Fig. 3a, b), while JWH-030 (10 mg/kg, i.p. 3 days) had no effects on cell viability (Supplementary Fig. 1). In addition, JWH-210 (1, 10, and 100 μM) or JWH-030 (10 and 100 μM) treatments induced cytotoxicity in splenocytes and thymocytes (Fig. 3c, d). These results suggest that JWH-210 induced cytotoxicity of lymphoid organ.

JWH-210 reduced the expression of T-cell activators in mice spleen

To investigate the effects of JWH-210 on immune system activator levels, mouse T-cell and B-cell activation PCR array was performed. The gene profiling results showed that 25 genes out of 85 genes were downregulated, and no gene was upregulated in the splenocytes of JWH-210-treated mice

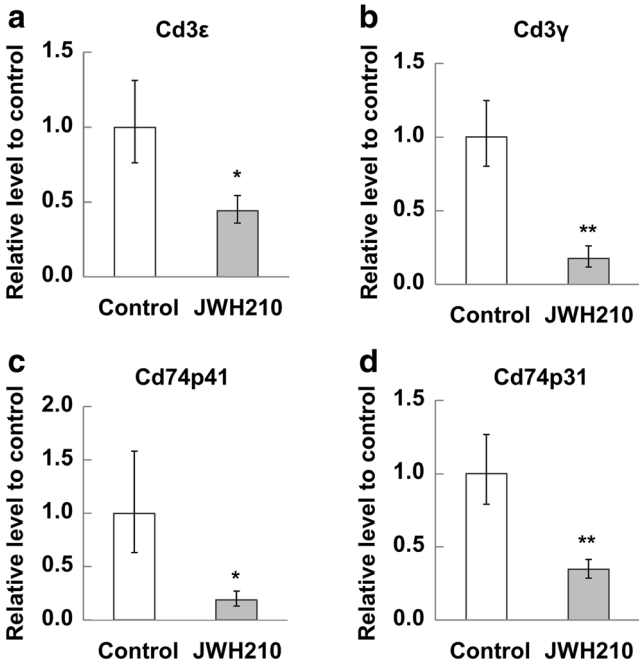

Fig. 4 Effects of JWH-210 on mRNA levels of T-cell activators. JWH-210 reduced mRNA levels of (**a**) Cd3ε, (**b**) Cd3γ, (**c**) Cd74 p41, and (**d**) Cd74 p31 in splenocytes. Data are shown as the means ± S.E. ($n = 6$). *$p < 0.05$, **$p < 0.01$ vs each vehicle-treated control group (Student's t test)

(Table 1 and Supplementary Fig. 2). Most of the down-regulated genes were related with T-cell activation. Among those genes, qPCR assay demonstrated that CD3 antigen epsilon polypeptide (Cd3ε), CD3 antigen gamma polypeptide (Cd3γ), Cd74 antigen (invariant polypeptide of major histocompatibility complex, class II antigen-associated) p31, and Cd74 p41 genes were downregulated significantly in the splenocyte of JWH-210-treated mice (Fig. 4). These results suggest that JWH-210 may affect the immune system via the regulation of T-cell activator levels.

JWH-210 reduced the expression of T-cell marker in spleen

To confirm the expression of T-cell marker (CD8 and CD3), we used immunohistochemistry. The results showed that JWH-210-treated mice decreased the expression of these markers in comparison with the control (Fig. 5a). We also measured CD3/CD4- and CD3/CD8-positive cells in mice splenocytes. Both cells were decreased by treatment of JWH-210 in mice splenocytes compared with the control (Fig. 5b–e). These results

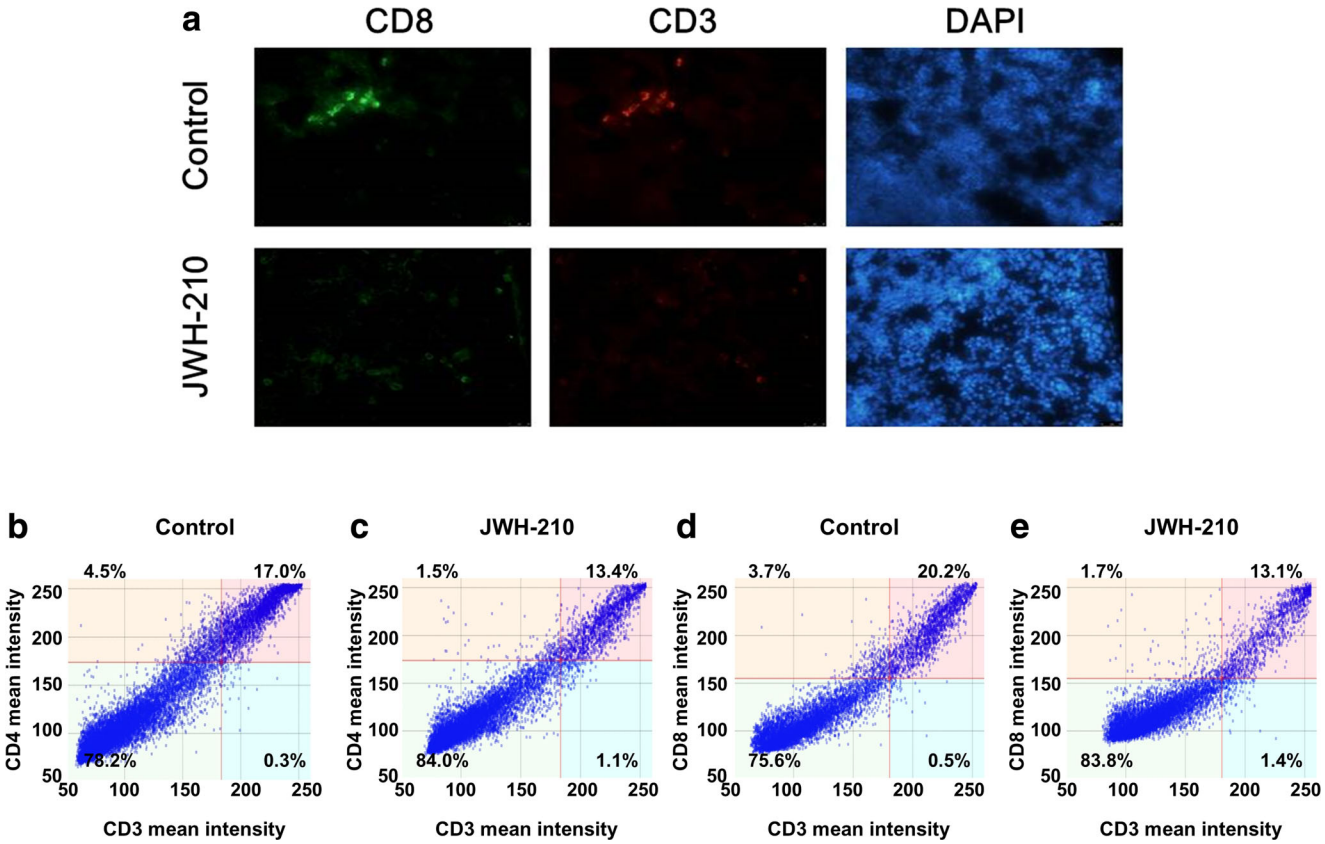

Fig. 5 Effects of JWH-210 on T-cell marker. (**a**) Expression of CD8- and CD3-positive cells in mice spleen. (**b**)–(**c**) Expression of CD3/CD4-positive cells in mice splenocytes. (**d**)–(**e**) Expression of CD3/CD8-positive cells in mice splenocytes

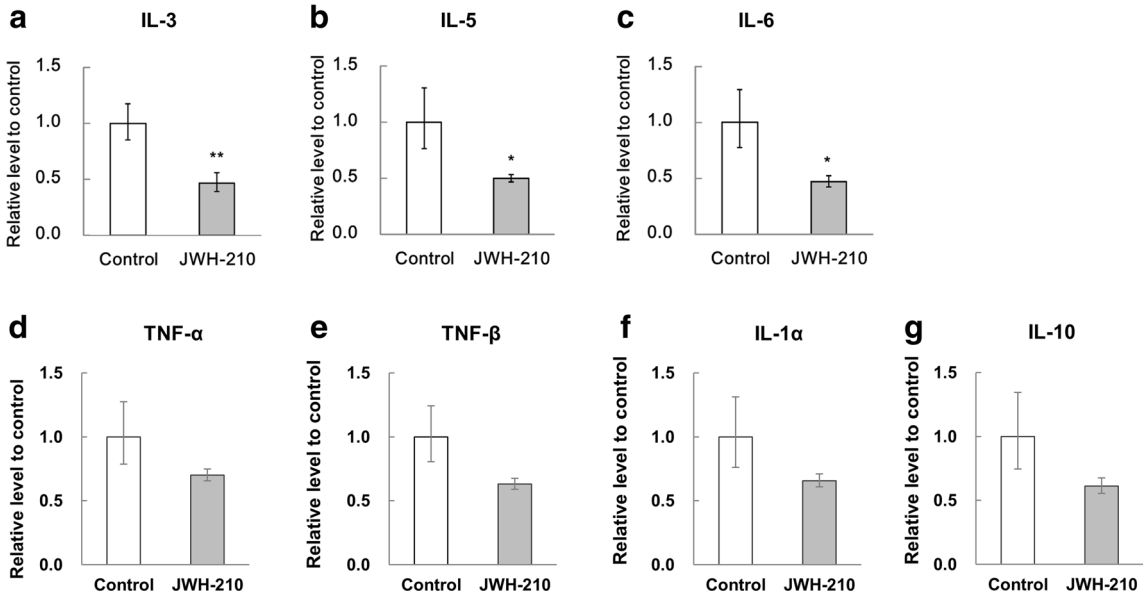

Fig. 6 Effects of JWH-210 on mRNA levels of cytokines. JWH-210 reduced mRNA levels of (**a**) IL-3, (**b**) IL-5, and (**c**) IL-6 in splenocytes. On the other hand, mRNA levels of (**d**) TNFα, (**e**) TNFβ, (**f**) IL-1α, and (**g**) IL-10 were not changed significantly. Data are shown as the means ± S.E. (n = 6). *p < 0.05, **p < 0.01 vs vehicle-treated control group (Student's t test)

suggest that JWH-210 may have an effect on the immune system via the regulation of T-cell in spleen.

JWH-210 reduced the mRNA level of cytokines in spleen

To clarify the effects of JWH-210 on the cytokine levels and T-cell marker levels in spleen, qPCR assay was performed. As Fig. 6a–c showed, JWH-210 significantly reduced IL-3, IL-5, and IL-6 mRNA levels. Otherwise, decreasing trends in TNF-α, TNF-β, IL-1α, and IL-10 mRNA levels were observed but not significantly (Fig. 6d–g). These results suggest that JWH-210 may have an effect on the immune system via the regulation of levels of cytokine associated with T-cell.

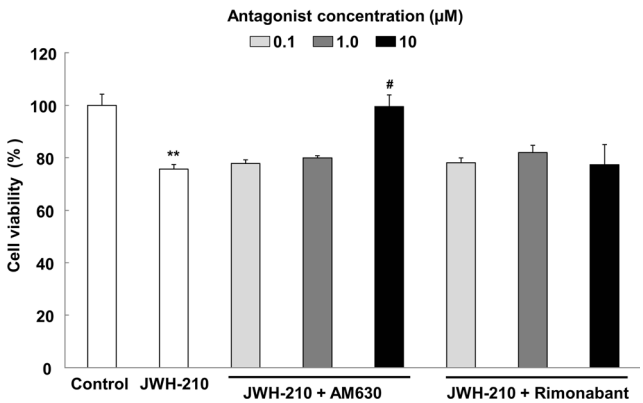

Fig. 7 Effects of cannabinoid receptor antagonists on the JWH-210-induced decrement of cell viability in splenocytes. Cell viability was measured after treatment of JWH-210 (10 μM) and antagonists in splenocytes isolated from naïve mice at indicated concentrations. Data are shown as the means ± S.E. (n = 12). **p < 0.01 and #p < 0.05 vs. vehicle-treated control and JWH-210-treated groups, respectively (one-way ANOVA, Bonferroni's)

AM630 rescued JWH-210-induced decrement in cell viability of splenocytes

In order to examine the role of CB_1 and CB_2 receptors in the decrement of cell viability, JWH-210 (10 μM) was treated in the presence of various concentrations of CB_1 and CB_2 receptor antagonists (0.1, 1, and 10 μM) for 4 h simultaneously. As Fig. 7 showed, the JWH-210-induced decrement of cell viability was rescued by AM630 (CB_2 antagonist) co-treatment at 10 μM, while rimonabant (CB_1 receptor antagonist) had no effects. These results suggested that CB_2 receptors may play a role in the decrement of cell viability induced by JWH-210.

Discussion

Synthetic cannabinoids are one of most abused novel psychoactive substances. A little information of toxicity and pharmacological activity of synthetic cannabinoids may mislead people to abuse substances without concerning health risks. We have previously reported that JWH series induced conditioned place preferences in parallel with CB_1 receptor binding affinity (Cha et al. 2014). However, cannabinoids increase susceptibility to various infectious agents in human and animal (Friedman et al. 2003), while the effects of synthetic cannabinoids on the immune system are not fully elucidated yet. In the

present study, we demonstrated that JWH-210 has severe harmful effects on the immune system. JWH-210 administration (10 mg/kg, 3 days, i.p) resulted in decrease of weight of spleen and thymus, whereas a single acute injection (1 and 10 mg/kg, i.p.) and chronic injection (0.1 mg/kg, 5 days, i.p.) did not (Supplementary Fig. 3). JWH-030 administration had no effects on lymphoid organ weights. In addition, JWH-210 reduced cell viability in vivo and ex vivo. Cell viability of primary cultured splenocytes from JWH-210-treated mice was reduced compared to the control group, and JWH-210 treatment also decreased cell viability of primary cultured splenocytes and thymocytes from naive mice. However, the cytotoxicity was not observed in primary cultured splenocytes from JWH-030-treated mice. These differences may be associated with the binding affinity of JWH-210 (Ki; 0.69 nM) and JWH-030 (Ki; 320 nM) to CB_2 receptors, which mediate immunotoxicity (Showalter et al. 1996; Huffman et al. 2005; Rieder et al. 2010). JWH-210 has effects on cell viability at 1 μM in the present study, and JWH-210 could be detected in the abusers' serum/plasma sample up to the concentration of about 0.5 μM (Karinen et al. 2015); therefore, the toxicity of JWH-210 on the immune system was needed to be further evaluated. Immune cells express high levels of CB_2 receptors mediating cannabinoid anti-inflammatory effects, immunomodulation, and immunosuppression (McKallip et al. 2002a, b; Yao and Mackie 2009; Rieder et al. 2010). We further studied the role of the cannabinoid receptors in JHW-210-induced cytotoxicity. AM630, a CB_2 receptor antagonist rescued JWH-210-induced decrement in cell viability of splenocytes; however, rimonabant, a CB_1 antagonist, did not. These results suggest that CB_2 receptor may play a role in the synthetic cannabinoid-induced harmful effects on the immune system. In addition, we demonstrated that JWH-210 reduced the mRNA expression levels of T-cell activators, such as, Cd3ε, Cd3γ, Cd74 p41, and Cd74 p31. Cd3ε and Cd3γ form the T-cell receptor-CD3 complex and play an essential role in T-cell development as well as immune response (Gagnon et al. 2012; Brazin et al. 2014). Cd74 is a nonpolymorphic type II integral membrane protein and is thought to function mainly as an MHC class II chaperone and generates two different isoforms, p31 and p41 (Starlets et al. 2006). Furthermore, the mRNA expression levels of cytokines including IL-3, IL-5, and IL-6 were reduced in the splenocytes of JWH-210 treated mice. However, there were no effects on the expression levels of TNFα, TNFβ, IL-1α, and IL-10. The qRT-PCR can determine accurately immune cell density and cytokine gene profile (Vremec et al. 2000; Mocellin et al. 2003; Tanaka et al. 2004). It has been reported that cannabinoids suppress T-cell proliferation and cytokine production in mice spleen cells (Robinson et al. 2013, 2015). These results suggest that JWH-210 may suppress T-cell density and cytokine production through the regulation of expression levels of T-cell activators.

In conclusion, our present study indicates that synthetic cannabinoids have harmful effects on the immune system. JWH-210 induced significant reductions in lymphoid organ weights, cell viability, and T-cell activator levels. Furthermore, JWH-210 inhibited the production of cytokines, which may be associated with immunosuppressive effects in drug abusers.

Acknowledgments This study was funded by the Ministry of Food and Drug Safety, Republic of Korea (grant number 15181MFDS482).

Compliance with ethical standards

Ethical approval All experimental procedures were approved by the Animal Ethics Committee, National Institute of Food and Drug Safety Evaluation and complied with the Guide for the Care and Use of Laboratory Animals (National Research Council (NRC) 1996).

Conflict of interest The authors declare that they have no conflicts of interest.

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