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Ajulemic acid: A novel cannabinoid produces analgesia without a "high"

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Abstract

A long-standing goal in cannabinoid research has been the discovery of potent synthetic analogs of the natural substances that might be developed as clinically useful drugs. This requires, among other things, that they be free of the psychotropic effects that characterize the recreational use of *Cannabis*. An important driving force for this goal is the long history of the use of *Cannabis* as a medicinal agent especially in the treatment of pain and inflammation. While few compounds appear to have these properties, ajulemic acid (AJA), also known as CT-3 and IP-751, is a potential candidate that could achieve this goal. Its chemical structure was derived from that of the major metabolite of Δ^9-THC, the principal psychotropic constituent of *Cannabis*. In preclinical studies it displayed many of the properties of non-steroidal anti-inflammatory drugs (NSAIDs); however, it seems to be free of undesirable side effects. The initial short-term trials in healthy human subjects, as well as in patients with chronic neuropathic pain, demonstrated a complete absence of psychotropic actions. Moreover, it proved to be more effective than placebo in reducing this type of pain as measured by the visual analog scale. Unlike the narcotic analgesics, signs of dependency were not observed after withdrawal of the drug at the end of the one-week treatment period. Data on its mechanism of action are not yet complete; however, the activation of PPAR-γ, and regulation of eicosanoid and cytokine production, appear to be important for its potential therapeutic effects.

Keywords: Ajulemic acid; Cannabinoid; Pain; Inflammation

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[1] Owner of the composition-of-matter patent on ajulemic acid.

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Introduction

The psychotropic principal of *Cannabis*, tetrahydrocannabinol (THC), is a highly lipophyllic molecule whose structure is shown in Fig. 1. THC is metabolized rapidly in the body to a number of oxygenated products. However, the most important route in the human involves oxidation of the allylic methyl group (Burstein et al., 1972), as is outlined in Fig. 1. Oxidation can occur elsewhere in the molecule, notably on the side-chain, leading to polyfunctional metabolites. A remarkable change in the biological activities of these substances accompanies the course of metabolism. While monohydroxy THC and its aldehyde product (not shown) have pharmacological profiles similar to THC, the terminal carboxy metabolite has no psychotropic effect in humans (Perez-Reyes, 1985), and does not produce behavioral responses typical of THC in laboratory animals (Burstein et al., 1988). In fact, the metabolite, THC-11 oic acid (Fig. 1), antagonizes the cataleptic effect of THC in mice by an undetermined mechanism (Burstein, 1987).

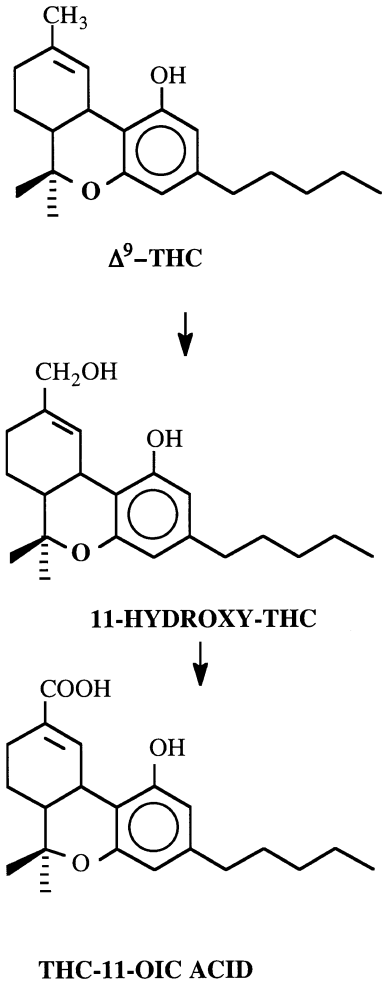
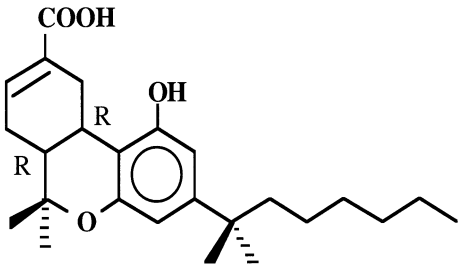


Fig. 1. The major pathway of metabolism for THC.



AJULEMIC ACID (CT-3)

Fig. 2. Structure of ajulemic acid (AJA). Also known as CT-3 and IP-751. DMH-11 C is dimethylheptyl-THC-11-oic acid.

It has long been known that modification of the pentyl side chain of THC can produce molecules with altered potencies (Loev et al., 1973). In particular, increasing the chain length to seven carbons and introducing branching close to the aromatic ring can lead to compounds with potencies that are 50–100 times greater than THC. This strategy was employed in designing the structure of ajulemic acid (AJA), the dimethyl heptyl (DMH) analog of THC-11-oic acid (Fig. 2). An initial study supported the expectation for its increased potency as an analgesic/anti-inflammatory agent in several models (Burstein et al., 1992). Three recent reviews describe many of the early studies on THC-11-oic acid as well as AJA (Burstein, 2002, 1999, 2000).

Ajulemic acid: preclinical studies

AJA shows potent anti-inflammatory activity (Zurier et al., 1998) and, in addition, is a powerful analgesic agent (Burstein et al., 1992, 1998; Dajani et al., 1999). In a variety of preclinical pain models, including the formalin assay, the PPQ writhing test, the hot plate assay and the tail clip assay, ED-50 values of 1–10 mg/kg were reported. In a direct comparison with morphine, it was found to be equipotent (Dajani et al., 1999). It also was effective in abolishing allodynia in a PAF-induced paw pain model in rats (J.M. Walker, personal communication).

As reported for THC-11-oic acid (Burstein, 1987), AJA did not produce a cataleptic response in the mouse as measured by the ring test. Moreover, AJA significantly antagonized THC induced catalepsy in the same model, in a dose related manner. The mechanism for this observed antagonism remains a matter for speculation. However, since THC induced catalepsy is thought to be mediated by the CB_1 receptor, it seems more likely that antagonism by the acids may involve inhibition of a downstream process following CB_1 receptor-ligand interaction given the low affinity (K_i = 480.6 nM in rat brain synaptosomal membranes) of AJA (Rhee et al., 1997).

The paw edema assay in rodents has been used extensively to evaluate molecules as potential anti-inflammatory agents. Most NSAIDs show potent inhibition of induction of edema following injection of substances such as carrageenin, arachidonic acid, and PAF with subtle differences being attached to the significance in the use of each. We have applied this assay to the study on AJA (Burstein et al., 1992) and an example of the data obtained and shown in Fig. 3 suggests a potent anti-inflammatory capability. The enantiomeric (S, S) form of AJA was also subjected to this test and was found to be considerably

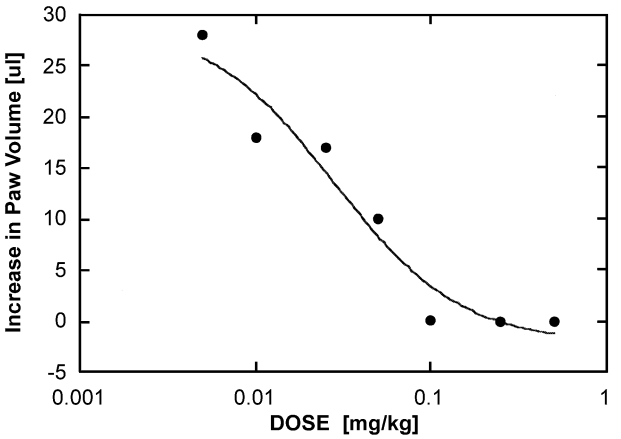


Fig. 3. Inhibition of arachidonic acid induced paw edema in mice by ajulemic acid. The data shown were obtained using the conditions described in Burstein et al., 1992. Briefly, arachidonic acid (1.0 mg in 25 μl of saline) was injected s.c. into the hind paw 30 min after the AJA given p.o. Data were analyzed by ANOVA for significance. P = 0.006 (N = 5).

less active by at least an order of magnitude. This high degree of stereospecificity indicates the possibility of receptor mediation in this effect of AJA. As mentioned above, the binding affinity of AJA for either CB_1 (K_i = 480.6 nM in rat brain synaptosomal membranes) or CB_2 (K_i = 170.5 nM in COS cells) is lower than would be expected from its in vivo potency; hence, the existence of another receptor for AJA is postulated.

Further examples of the anti-inflammatory actions of AJA on both chronic (rat adjuvant induced arthritis) and acute (mouse subcutaneous air pouch) models were demonstrated (Zurier et al., 1998). Most noteworthy is the ability of AJA to prevent joint tissue injury, which represents an important goal in the treatment of RA.

Initial pre-clinical data on HU-320 (DMH-CBD-11-oic acid), a close analog of AJA, have been reported by Sumariwalla et al. (Sumariwalla et al., 2004). This compound was synthesized following the same rationale that led to AJA, namely, introduction of a carboxy group and elaboration of the side chain to a dimethylheptyl group; however, cannabidiol (CBD) was the template rather than THC. The report indicates anti-inflammatory action in several animal models; however, no analgesia was seen. A single dose (20 mg/kg) comparison of HU-320 with AJA and THC was shown in which AJA produced catalepsy and hypothermia values similar to THC. It should be noted, however, that 20 mg/kg is 200 times the therapeutic dose for AJA in the rat adjuvant arthritis model (Zurier et al., 1998) and 20 times the analgesic dose in the rat tail clip assay (Dajani et al., 1999). This poses a question about the relevance of the comparison published by Sumariwalla et al. with reference to their assertion that AJA would be psychoactive in humans since, apparently, it is not (vide infra).

Mechanism studies

The anti-inflammatory actions of the cannabinoid acids were discovered following the observation that they could inhibit agonist-induced PGE_2 production by intact WI-38 human lung fibroblast cells in

culture (Burstein et al., 1986). Subsequent studies (Zurier et al., 1998) suggested that AJA was a cyclooxygenase-2 (COX-2) selective inhibitor that produces this response in these models by some yet-to-be-defined mechanism. Ongoing studies suggest that possible AJA-sensitive mediators that would modulate COX-2 mediated synthesis are either peroxisome proliferator-activated receptor-γ (PPAR-γ), or, nuclear factor-κB (NF-κB).

COX-2 is an inducible enzyme that catalyzes the conversion of arachidonic acid into prostaglandins such as PGE_2 that, in turn, can promote an inflammatory response. Its levels are rapidly and robustly elevated in response to a host of pro inflammatory stimuli. A number of drugs for the treatment of inflammation (e.g. celecoxib and reficoxib) are believed to act as direct inhibitors of COX-2 activity. However, few agents are known that reduce inflammation by modulating the levels of COX-2. The PGJ series of prostaglandins, in contrast, are considered to be anti-inflammatory and their levels were seen to rise after AJA treatment (Stebulis et al., unpublished data). In the case of AJA, the available data do not fully support or refute either mechanism for the observed changes in prostaglandin levels.

Consistent with their low psychotropic activity, the cannabinoid acids exhibit only weak binding to either of the cannabinoid receptors CB_1 or CB_2 (Compton et al., 1993; Rhee et al., 1997; Yamamoto et al., 1998). A striking decrease in potency is apparent across the board when the synthetic metabolite analog AJA (DMH-THC-11-oic acid) is compared with the THC analog DMH-THC both for binding and inhibition of adenylate cyclase. Moreover, THC itself generally shows greater receptor mediated activity than AJA with one minor exception: binding to CB_1 in transfected cells. However, the finding that the CB_1 antagonist SR141716A does not alter the biological effects of AJA (Recht et al., 2001) suggests that CB_1 binding is not an important factor. Thus, at therapeutic doses, it appears that AJA acts at a site (or sites) other than CB_1 or CB_2.

Very recent findings suggest a possible candidate for a cannabinoid acid receptor, namely, PPAR-γ (Liu et al., 2003). The data reported demonstrate that AJA activates this nuclear receptor that in turn regulates cellular processes such as the expression of cytokines, lipid metabolism and glucose homeostasis. AJA also has a moderate binding affinity for PPAR-γ (Liu et al., 2003), that has been shown to have a large hydrophobic ligand-binding domain. The endogenous ligands for PPAR-γ include several eicosanoids, suggesting a possible indirect activation mechanism for AJA since it is known that AJA, like many other cannabinoids, stimulates arachidonic acid release (Burstein, unpublished data). Several drugs known as thiazolidenediones, currently in use for the treatment of Type 2 diabetes, are PPAR-γ ligands (examples are rosiglitazone and pioglitazone), suggesting a possible use for AJA.

Although a variety of mediators contribute to inflammatory responses, it seems clear that the actions of interleukin-1β (IL-1β) and tumor necrosis factor α (TNFα) are central to progression of joint tissue injury in patients with rheumatoid arthritis (RA). Blockade of TNFα with neutralizing antibodies or soluble receptors, and blockade of IL-1β action by administration of interleukin-1 receptor antagonist (IL-1ra) reduce joint swelling and pain of patients with RA. Indeed, IL-1β is a potential target of therapeutic intervention in many conditions characterized by inflammation and altered immune responses. This prompted the investigation of monocyte IL-1β responses after addition of AJA to human cells in vitro (Zurier et al., 2003). The data in Fig. 4 show that AJA suppresses IL-1β production by activated human peripheral blood monocytes. In contrast, in a similar model, AJA had little effect on TNFα (Zurier et al., 2003). The differential effects of AJA on IL-1β and TNFα may help explain the results of an animal study (Zurier et al., 1998) in which AJA reduces clinical inflammation (joint redness and swelling) only modestly, but almost completely prevents joint cartilage and bone damage. Studies of

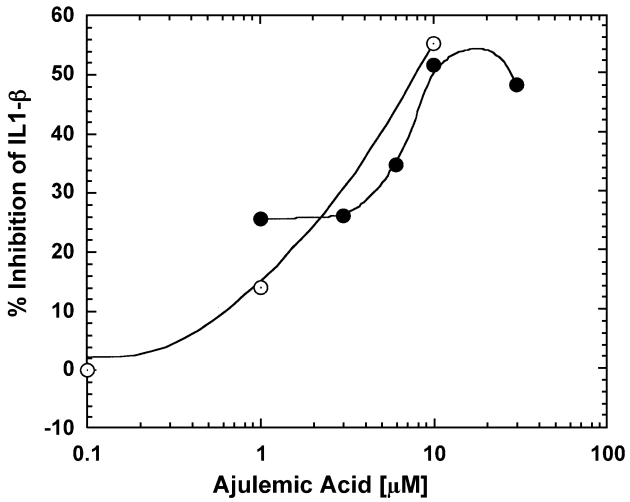


Fig. 4. AJA suppression of IL1-β release from stimulated human PBMC. Cells (2×10^6/ml) from healthy volunteers were incubated with AJA at the indicated concentrations for 60 min, then stimulated for 4 hr with LPS (10 ng/ml). Supernatant IL1-β was measured by an ELISA; triplicate determinations were made on each sample. Values shown represent the ratio of AJA–LPS treated cells to LPS only treated cells. The LPS only control cells gave a value of 4132 ± 193 pg/ml of IL1-β. All treated samples significantly lower than the control by ANOVA ($p = 0.0001$). Each curve (open circles and closed circles) represents data from a different donor. (Bidinger et al., 2003).

cytokines in animal models of arthritis are instructive in that regard. In the animal studies, blockade or elimination of IL-1β does not prevent joint swelling, but it is effective in reducing joint cartilage degradation and bone erosion, events which lead to crippling in patients with RA. In contrast, blockade or elimination of TNFα prevents joint swelling but not cartilage and bone damage (Joosten et al., 1999).

T lymphocyte activation and proliferation are central to the initiation of joint tissue injury in patients with chronic inflammatory diseases (Panayi et al., 1992), and T cells can influence monocyte activation. Thus, IL-2 secreted by activated T cells is a powerful activator of human monocytes (Espinoza-Delgado et al., 1995) whereas other cytokines secreted by T cells suppress monocyte activation. In turn, IL-1β is necessary for optimal proliferation of T cells. An investigation of the effect of AJA on proliferation of isolated T lymphocytes in vitro found that AJA suppressed T cell growth in a dose-dependent manner (Bidinger et al., 2003).

Formation of DNA fragments, that represent 180 base pair cuts between nucleosomes, is characteristic of apoptosis (Nakajima et al., 1995) and precedes loss of cell membrane integrity. In a series of experiments, exposure of T cells to 10 μM AJA for 6 hr increased DNA fragmentation 2.4 fold more than cells not treated with AJA ($p = 0.038$ vs. untreated cells) (Bidinger et al., 2003). The range of the increase in fragmentation in AJA treated cells (1.3 to 5.6 fold greater than untreated cells) is likely due to the different sensitivity to apoptosis of cells from different donors. When DNA fragmentation was compared with cell viability, the former preceded the latter when their time courses were compared.

A family of pro enzymes, the cysteine proteases (caspases), are activated during apoptosis (Nagata, 1997). Caspase-3 activity is essential to the process (Thornberry and Lazebnik, 1998). A 4 hr incubation of cells with AJA increased caspase-3 activity in a concentration-dependent manner (Bidinger et al., 2003) AJA (10 μM) induced a greater than 9 fold increase in caspase-3 activity after 6 hr incubation with

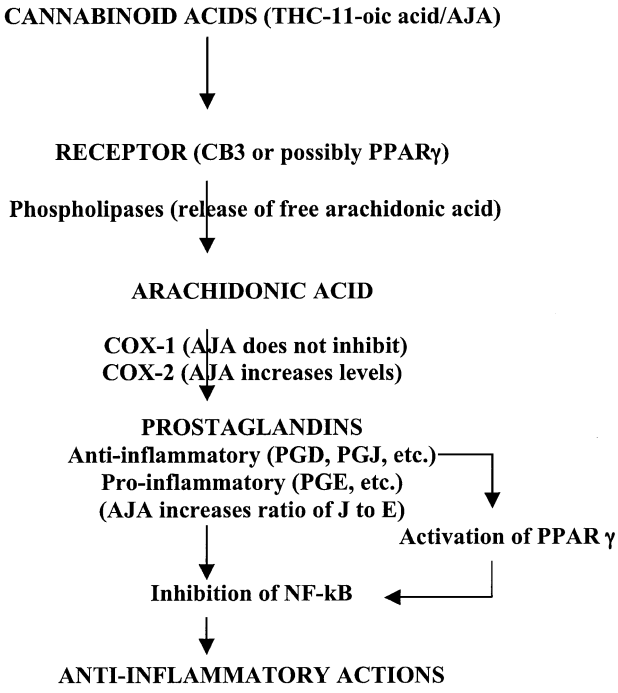


Fig. 5. A putative mechanism for the anti-inflammatory/analgesic actions of AJA. The potency and stereospecificity of AJA in several models suggests that a high-affinity binding site may be involved in its actions. AJA promotes the release of free arachidonic acid presumably by the activation of one or more phospholipases making available substrate for enhanced eicosanoid production (Burstein, unpublished data). While AJA modulates cellular levels of COX-2, it does not appear to have any effect on PG synthesis by COX-1. This allows for the possibility of increased ratios of the anti-inflammatory (cyclopentenone) PGs to the pro-inflammatory PGs and this is what has been observed. This, in turn, can inhibit the actions of the pro inflammatory transcription factor, NF-κB either directly or through activation of PPAR γ. AJA appears to be an effective inhibitor of NF-κB (Zurier et al., unpublished data). A number of genes involved in the process of inflammation could thus be down regulated as a consequence.

T cells. The increase in caspase-3 activity was equivalent to that induced by TNFα and camptothecin, known inducers of apoptosis.

The findings discussed in this section are summarized in a putative mechanism of action for the anti-inflammatory actions of AJA that is shown in Fig. 5.

What is the site of action for ajulemic acid?

This is, of course, a question of great interest and efforts are continuing to provide data that may help to elucidate the nature of the cellular targets of AJA. The COX enzymes and PPAR-γ are possible candidates whose mediation may contribute to the pharmacological profile of AJA. The classical receptor discovery approach based on the detection of a high-affinity, ligand-binding site cannot be applied since a high specific activity radiolabelled derivative of AJA is not currently available. The preparation of such a substance is not a trivial task. Moreover, the use of existing labeled ligands is unlikely to lead to the

discovery of a unique AJA binding site. In order to answer this question, a ligand will have to be made that does not bind to either CB_1 or CB_2 but shows high affinity for a putative AJA receptor.

Clinical findings

Recently, in a randomized, double blind, placebo controlled clinical trial (RCT) investigating AJA against placebo in 21 patients suffering from chronic neuropathic pain due to traumatic lesions of peripheral or central nerves (mean duration of pain 11.5 years), the absence of psychoactive properties was confirmed although the daily doses given were as high as 80 mg and some of the patients combined AJA with other central acting compounds (Karst et al., 2003). Impairment of cognition and subjective drug effects were determined by part B of the Trail Making Test (TMT) and the Addiction Research Center Inventory-Marijuana (ARCI-M). Tiredness and dry mouth were reported significantly more often when AJA was administered but no major psychological or physical events were observed, the latter was measured by regularly recording vital signs, weight, temperature, electrocardiography findings, hematological and blood chemistry studies. In addition, signs of dependency were not observed after withdrawal of the drug at the end of the one-week treatment. AJA given in daily doses of 40 and 80 mg was significantly more effective than placebo in reducing chronic neuropathic pain measured by the visual analog scale (VAS) with greater effects at 3 hours after intake than at 8 hours (Fig. 6). These findings correspond to the pharmacokinetic data shown in 6 human volunteers after a single oral

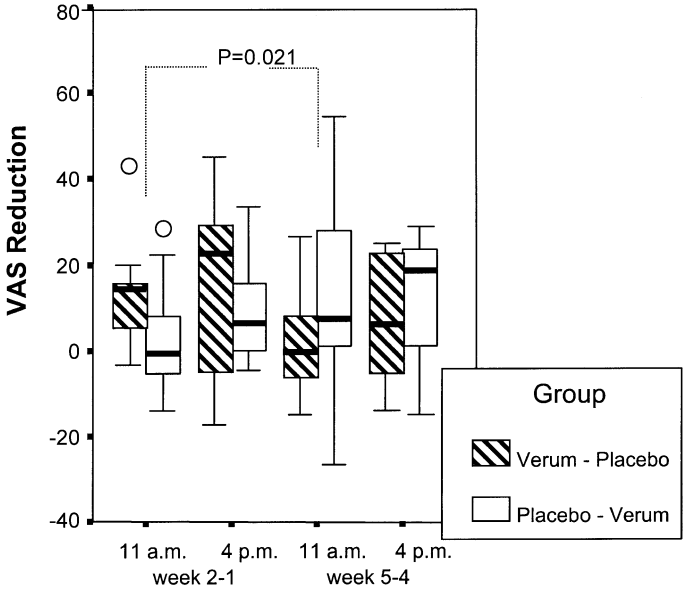


Fig. 6. Effect of AJA on reduction (week 2-week 1; week 5-week 4) of the visual analog scores (VAS), range 0–100, in a placebo-controlled crossover study of 21 patients with chronic neuropathic pain. The Verum-Placebo group (hatched boxes) received AJA in week 2, placebo in week 5. The Placebo-Verum group (white boxes) received AJA in week 5, placebo in week 2. Washout time in week 3. Medians (black bars), inter quartile ranges (boxes), whole ranges (t-hairs), and extreme values (circles) are indicated. For differences over time, the reduction in the VAS scores was significantly different (P = 0.02) in the 11 a.m. measurements. (Karst et al., 2003).

administration of AJA. The time to highest plasma concentration (t_{max}) was reached in most participants 1 or 2 hours after absorption from the empty gastrointestinal tract and declined thereafter with apparent half-lives of 6 hours. Thus far, the preliminary clinical data suggest AJA is a safe and efficacious agent in the treatment of chronic neuropathic pain.

Many unanswered questions surrounding the actions of AJA remain. These concern both the scope and the mechanism of action, and current efforts are being made to address these points. The reported activation of PPAR-γ by AJA opens the possibility of therapeutic uses in addition to treatment of pain and inflammation. In fact, PPAR-γ may eventually be shown to be the most important target site of this unique cannabinoid.

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References

Bidinger, B., Torres, R., Rossetti, R.G., Brown, L., Beltre, R., Burstein, S., Lian, J.B., Stein, G.S., Zurier, R.B., 2003. Ajulemic acid, a nonpsychoactive cannabinoid acid, induces apoptosis in human T lymphocytes. Clinical Immunology 108, 95–102.

Burstein, S., Rosenfeld, J., Wittstruck, T., 1972. Isolation and characterization of two major urinary metabolites of 1-tetrahydrocannabinol. Science 176, 422–423.

Burstein, S., Hunter, S.A., Latham, V., Renzulli, L., 1986. Prostaglandins and cannabis-XVI. Antagonism of delta 1-tetrahydrocannabinol action by its metabolites. Biochemical Pharmacology 35, 2553–2558.

Burstein, S., 2002. Therapeutic Potential of Ajulemic Acid (CT-3). In: Grotenhermen, F. (Ed.), Cannabis and Cannabinoids. Haworth press, New York, pp. 381–388.

Burstein, S.H., 1987. Inhibitory and stimulatory effects of cannabinoids on eicosanoid synthesis. NIDA Research Monograph 79, 158–172.

Burstein, S.H., Hull, K., Hunter, S.A., Latham, V., 1988. Cannabinoids and pain responses: a possible role for prostaglandins. FASEB Journal 2, 3022–3026.

Burstein, S.H., Audette, C.A., Breuer, A., Devane, W.A., Colodner, S., Doyle, S.A., Mechoulam, R., 1992. Synthetic nonpsychotropic cannabinoids with potent antiinflammatory, analgesic, and leukocyte antiadhesion activities. Journal of Medicinal Chemistry 35, 3135–3141.

Burstein, S.H., Friderichs, E., Kogel, B., Schneider, J., Selve, N., 1998. Analgesic effects of 1′,1′ dimethylheptyl-delta8-THC-11-oic acid (CT3) in mice. Life Sciences 63, 161–168.

Burstein, S.H., 1999. The cannabinoid acids: nonpsychoactive derivatives with therapeutic potential. Pharmacology and Therapeutics 82, 87–96.

Burstein, S.H., 2000. Ajulemic acid (CT3): a potent analog of the acid metabolites of THC. Current Pharmaceutical Design 6, 1339–1345.

Compton, D.R., Rice, K.C., De Costa, B.R., Razdan, R.K., Melvin, L.S., Johnson, M.R., Martin, B.R., 1993. Cannabinoid structure-activity relationships: correlation of receptor binding and in vivo activities. Journal of Pharmacology and Experimental Therapeutics 265, 218–226.

Dajani, E.Z., Larsen, K.R., Taylor, J., Dajani, N.E., Shahwan, T.G., Neeleman, S.D., Taylor, M.S., Dayton, M.T., Mir, G.N., 1999. 1′,1′-Dimethylheptyl-delta-8-tetrahydrocannabinol-11-oic acid: a novel, orally effective cannabinoid with analgesic and anti-inflammatory properties. Journal of Pharmacology and Experimental Therapeutics 291, 31–38.

Espinoza-Delgado, I., Bosco, M.C., Musso, T., Gusella, G.L., Longo, D.L., Varesio, L., 1995. Interleukin-2 and human monocyte activation. Journal of Leukocyte Biology 57, 13–19.

Joosten, L.A., Helsen, M.M., Saxne, T., van De Loo, F.A., Heinegard, D., van Den Berg, W.B., 1999. IL-1 alpha beta blockade prevents cartilage and bone destruction in murine type II collagen-induced arthritis, whereas TNF-alpha blockade only ameliorates joint inflammation. Journal of Immunology 163, 5049–5055.

Karst, M., Salim, K., Burstein, S., Conrad, I., Hoy, L., Schneider, U., 2003. Analgesic effect of the synthetic cannabinoid CT-3 on chronic neuropathic pain: a randomized controlled trial. JAMA 290, 1757–1762.

Liu, J., Li, H., Burstein, S.H., Zurier, R.B., Chen, J.D., 2003. Activation and binding of peroxisome proliferator-activated receptor gamma by synthetic cannabinoid ajulemic acid. Molecular Pharmacology 63, 983–992.

Loev, B., Bender, P.E., Dowalo, F., Macko, E., Fowler, P.J., 1973. Cannabinoids. Structure-activity studies related to 1,2-dimethylheptyl derivatives. Journal of Medicinal Chemistry 16, 1200–1206.

Nagata, S., 1997. Apoptosis by death factor. Cell 88, 355–365.

Nakajima, T., Aono, H., Hasunuma, T., Yamamoto, K., Shirai, T., Hirohata, K., Nishioka, K., 1995. Apoptosis and functional Fas antigen in rheumatoid arthritis synoviocytes. Arthritis and Rheumatism 38, 485–491.

Panayi, G.S., Lanchbury, J.S., Kingsley, G.H., 1992. The importance of the T cell in initiating and maintaining the chronic synovitis of rheumatoid arthritis. Arthritis and Rheumatism 35, 729–735.

Perez-Reyes, M., 1985. Pharmacodynamics of certain drugs of abuse, 287–310.

Recht, L.D., Salmonsen, R., Rosetti, R., Jang, T., Pipia, G., Kubiatowski, T., Karim, P., Ross, A.H., Zurier, R., Litofsky, N.S., Burstein, S., 2001. Antitumor effects of ajulemic acid (CT3), a synthetic non-psychoactive cannabinoid. Biochemical Pharmacology 62, 755–763.

Rhee, M.H., Vogel, Z., Barg, J., Bayewitch, M., Levy, R., Hanus, L., Breuer, A., Mechoulam, R., 1997. Cannabinol derivatives: binding to cannabinoid receptors and inhibition of adenylylcyclase. Journal of Medicinal Chemistry 40, 3228–3233.

Sumariwalla, P.F., Gallily, R., Tchilibon, S., Fride, E., Mechoulam, R., Feldmann, M., 2004. A novel synthetic, nonpsychoactive cannabinoid acid (HU-320) with antiinflammatory properties in murine collagen-induced arthritis. Arthritis and Rheumatism 50, 985–998.

Thornberry, N.A., Lazebnik, Y., 1998. Caspases: enemies within. Science 281, 1312–1316.

Yamamoto, I., Kimura, T., Kamei, A., Yoshida, H., Watanabe, K., Ho, I.K., Yoshimura, H., 1998. Competitive inhibition of delta8-tetrahydrocannabinol and its active metabolites for cannabinoid receptor binding. Biological and Pharmaceutical Bulletin 21, 408–410.

Zurier, R.B., Rossetti, R.G., Lane, J.H., Goldberg, J.M., Hunter, S.A., Burstein, S.H., 1998. Dimethylheptyl-THC-11 oic acid: a nonpsychoactive antiinflammatory agent with a cannabinoid template structure. Arthritis and Rheumatism 41, 163–170.

Zurier, R.B., Rossetti, R.G., Burstein, S.H., Bidinger, B., 2003. Suppression of human monocyte interleukin-1beta production by ajulemic acid, a nonpsychoactive cannabinoid. Biochemical Pharmacology 65, 649–655.