The AAPS Journal 2005; 7 (1) Article 15 (http://www.aapsj.org).

Ajulemic Acid (IP-751): Synthesis, Proof of Principle, Toxicity Studies, and Clinical Trials

Submitted: October 27, 2004; Accepted: December 12, 2004; Published: June 29, 2005.

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ABSTRACT

Ajulemic acid (CT-3, IP-751, 1',1'-dimethylheptyl-Δ^8-tetrahydrocannabinol-11-oic acid) (AJA) has a cannabinoid-derived structure; however, there is no evidence that it produces psychotropic actions when given at therapeutic doses. In a variety of animal assays, AJA shows efficacy in models for pain and inflammation. Furthermore, in the rat adjuvant arthritis model, it displayed a remarkable action in preventing the destruction of inflamed joints. A phase-2 human trial with chronic, neuropathic pain patients suggested that AJA could become a useful drug for treating this condition. Its low toxicity, particularly its lack of ulcerogenicity, further suggests that it will have a highly favorable therapeutic index and may replace some of the current anti-inflammatory/analgesic medications. Studies to date indicate a unique mechanism of action for AJA that may explain its lack of adverse side effects.

KEYWORDS: ajulemic acid, analgesia, anti-inflammatory, cannabinoid, IP-751

BACKGROUND

A long-standing goal both in university and industrial laboratories has been to design a cannabinoid-derived drug that would show analgesic efficacy and have a low potential for abuse. The efforts were rationalized in large measure by the long history of the use of *Cannabis* preparations for the treatment of pain, inflammation, and a host of other medical needs. Literally hundreds of compounds have been synthesized and tested; however, few have reached the stage of human testing, and only one, nabilone, is currently used albeit in limited applications. Tetrahydrocannabinol (THC), the principal psychoactive component of *Cannabis,* is available as an oral medication (Marinol) for use as an anti-emetic and as an appetite stimulant for AIDS patients. However, its potential for abuse has discouraged its acceptance and use by physicians for a wider range of therapeutic applications. GW Pharmaceuticals has announced that mixtures of THC and cannabidiol (CBD) are currently awaiting approval in the United Kingdom and Canada for use in relieving pain and spasticity in patients with multiple sclerosis.

Ajulemic acid (AJA), which has a cannabinoid-derived structure (Figure 1), is being developed to achieve the goal of providing a synthetic *Cannabis*-derived drug that will have a low potential for abuse.[1-4] The rationale used in designing the structure of AJA was based on several reports in the scientific literature relating to the metabolic transformations of THC.[5-9] As early as 1972, it was reported that a major route of metabolism for THC involves its stepwise oxidation to a series of carboxylic acid derivatives.[7] Unlike THC, the acids showed little activity in several studies of psychotropic responses in both animal models and in humans.[10] Thus, it was concluded that the acid metabolites are ''inactive,'' and further studies of their potential pharmacological properties were not initiated.

This perception has changed following a series of reports showing that the acids do possess biological actions and that these could be exploited for therapeutic applications.[5,11-14] An advantage of cannabinoids as a class of drugs is their relative safety, especially when compared with analgesics such as the opiates and other narcotics. A downside of the acid metabolites is their low potency in the animal models; however, this deficiency has been successfully resolved with the discovery of AJA.[15] The recent completion of 2 studies in humans confirmed AJA's low abuse potential over the expected range of therapeutic doses.[16]

MANUFACTURING

Stereospecific syntheses of THC and its analogs follow a general scheme, and this has been applied to the production of AJA (Scheme 1). In this procedure an alkylated resorcinol is condensed with an appropriate, chirally pure, terpene such as (+)-p-mentha-2,8-diene-1-ol (2), cis-chrysanthenol (5), cis-verbinol (6), or (+)-trans-2-carene oxide (7). The substituted resorcinol portion of the molecule is made by the reaction of 1,6-dimethoxyphenol with 1,1-dimethylheptanol in the presence of methane sulfonic acid. The product

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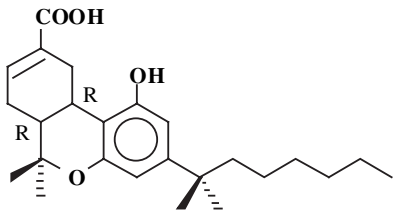


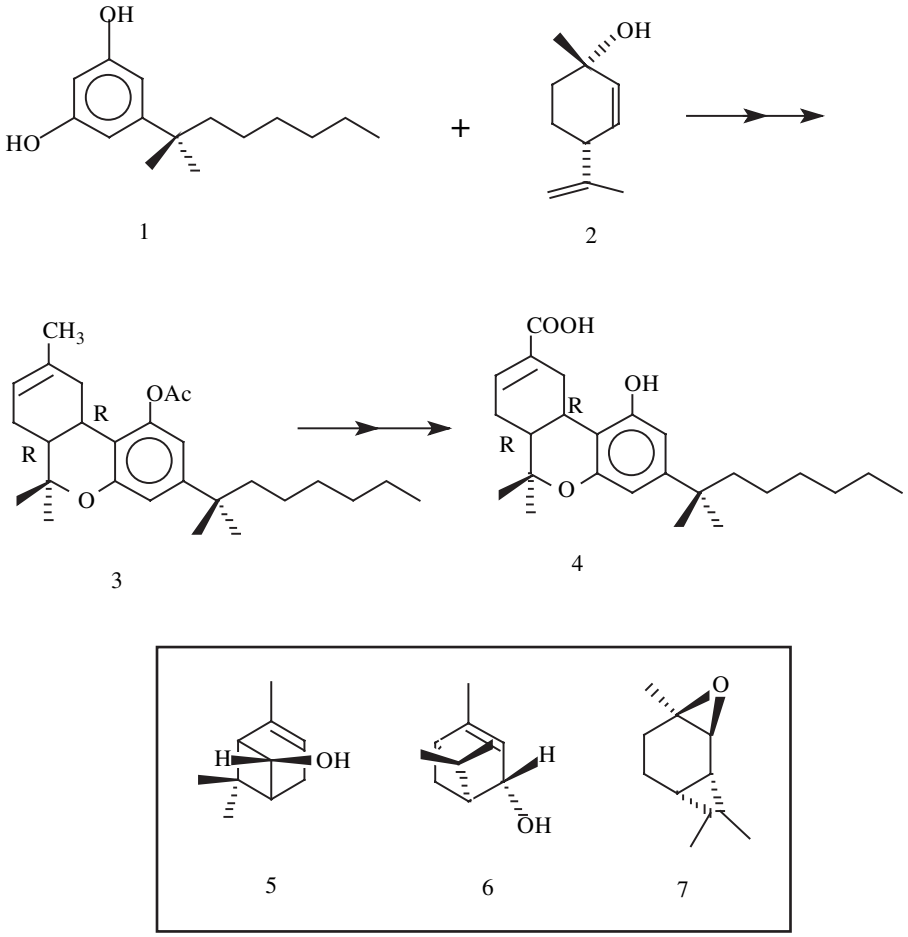
Figure 1. Structure and physical properties. Ajulemic acid, $C_{25}H_{36}O_4$, is a white crystalline solid (molecular weight 400.55, melting point 96°C to 99°C) that is soluble in most organic solvents except hexane and is soluble in aqueous buffers above pH 8. It is also known as IP-751, CT-3, and 1', 1'-dimethylheptyl-Δ^8-tetrahydrocannabinol-11-oic acid.

is then esterified with diethyl phosphite and triethylamine with cooling to yield the diethyl phosphate derivative. Reduction with lithium in liquid ammonia produces 1-(1',1'-dimethylheptyl) 3,5-dimethoxybenzene (1) that is used in the next step. This involves a condensation of the p-mentha-2,8-diene-1-ol (2) shown in Scheme 1 with dimethylheptyl resorcinol (1) catalyzed by p-toluenesulfonic acid to give the dimethylheptyl analog of Δ^8 — THC. Following conversion of the cannabinoid to the acetate (3), the allylic methyl group is oxidized to an aldehyde using selenium dioxide. Further oxidation to a carboxylic acid is accomplished by the use of sodium chlorite. Finally, free AJA (4) is obtained by saponification of the acetyl group with sodium carbonate in aqueous methanol.

PRECLINICAL STUDIES

Significant progress in defining a mechanism of action for AJA has been made. On the one hand, modest binding to the known cannabinoid receptors, CB1 and CB2 has been reported,[17,18] and effects by receptor specific antagonists as well as stereospecificity has been observed[15,19] suggesting a possible role for these receptors in the actions of AJA. On the other hand, the fact that AJA does not produce psychoactivity,[16] a process that is believed to require the activation of CB1, appears to be a contradiction. A possible explanation is that AJA, in addition to activating the receptor, selectively antagonizes a downstream event that is required for psychotropic activity but is not needed for anti-inflammatory actions. In general, the lack of data on the molecular events leading to cannabinoid-induced psychoactivity makes questionable any speculations on this subject.

Data have been obtained on several biochemical effects of AJA that may have relevance for its anti-inflammatory



Scheme 1

actions. Cannabinoid receptor binding in intact cell models causes the release of free arachidonic acid suggesting the activation of one or more phospholipases.[20] Recent findings show that AJA can likewise stimulate the release of arachidonic acid in human fibroblast-like synovial (FLS) cells.[21] The available data suggest complex effects by AJA on COX-2 activity,[22] and the expression levels of COX-2 mRNA.[21] All of these effects seem to depend very much on the model studied and the conditions of the experiment. AJA also inhibits 5-lipoxygenase (D. Morgan, unpublished data, February 22, 1993) but not COX-1[22] in agreement with its lack of ulcerogenicity[23] and its inability to prevent platelet aggregation.[24] Effects on specific cytokine levels and on the activation of nuclear factor-κB (NF-κB) have also been observed,[25] and it was reported that AJA binds directly and specifically to peroxisome proliferator-activated receptor-γ (PPAR-γ),[26] a pharmacologically important member of the nuclear receptor super family. Functional assays indicated that AJA initiates the transcriptional activity of both human and mouse PPAR-γ at pharmacological concentrations. Activation of PPAR-γ by AJA requires the AF-2 helix of the receptor, suggesting that AJA activates PPAR-γ through its ligand-dependent AF-2 region. Consistent with this, AJA binding enables PPAR-γ to recruit nuclear receptor coactivators. It was also found that AJA inhibits interleukin-8 promoter activity in a PPAR-γ-dependent manner, suggesting a link between the anti-inflammatory action of AJA and the activation of PPAR-γ. Finally, it was found that AJA treatment induces differentiation of 3T3 L1 fibroblasts into adipocytes, a process that is known to be mediated by PPAR-γ. Together, these data indicate that PPAR-γ is a molecular target for AJA under certain conditions, thus providing a possible mechanism for the anti-inflammatory activity of AJA, and perhaps other cannabinoid acids as well. These studies also suggest several possible additional therapeutic actions for AJA through the activation of PPAR-γ in multiple signaling pathways (eg, lipid metabolism, glucose homeostasis, cell differentiation).

AJA has been studied in a variety of preclinical in vivo models for anti-inflammatory activity, where it shows higher potencies than other cannabinoids.[13,19] As an example, orally administered AJA reduced the induction of paw edema in mice injected with arachidonic acid with an ED-50 of 0.02 mg/kg. A similar effect was seen when edema was induced by orally administered platelet-activating factor, where an ED-50 of 0.05 mg/kg was found.[13] A somewhat lower potency was seen with carrageenan-induced edema, where AJA inhibited the response at a higher dose showing an ED-50 of 2.2 mg/kg IV (Roche Center for Biological Research, Palo Alto, CA, unpublished data, June 16, 1997). In a different model, the migration of leukocytes into a subcutaneous air pouch following injection of tumor necrosis factor-α(TNFα) and interleukin-1β(IL1β) was markedly reduced at doses of 0.1 and 0.2 mg/kg.[22] AJA, at a dose of 0.2 mg/kg administered orally, reduced the effects of inflammation in an adjuvant-induced arthritis model in rats.[22] A dramatic effect was seen when histological examination of randomly selected specimens from this study was done, revealing a remarkable joint sparing effect in the AJA-treated animals when compared with vehicle/adjuvant treated controls (20% vs 80% ankylosis) (Figure 2).

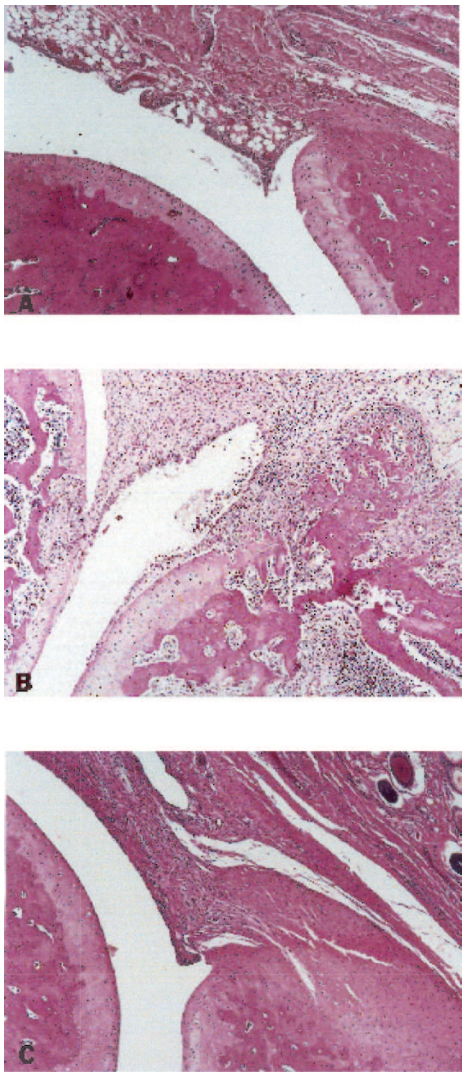


Figure 2. AJA prevents bone damage in the rat adjuvant arthritis model. Histopathalogic findings: hind paw tibiotarsal joints at site of attachment to the anterior aspect of the tibia (hematoxylin and eosin stained, original magnification × 40); (A) Normal rat joint (no Freund's complete adjuvant [FCA]); (B) Joint from vehicle/FCA-treated rat on day 35. Synovitis with pannus formation is seen—exostosis seen at joint margin; (C) Joint from AJA/FCA-treated rat on day 35. Neither active pannus nor cartilage or bone damage is seen; Rx: AJA in safflower oil (0.1 mg/kg/day) given by mouth 3 times weekly. Adapted from Stebulis et al.[21]

IL1β and TNFα are mediators of inflammation and joint tissue injury in patients with rheumatoid arthritis (RA). This prompted Zurier et al[27] to study human monocyte IL1β and TNFα responses after the addition of AJA to cells in vitro. Peripheral blood monocytes (PBM) and synovial fluid monocytes (SFM) were isolated from healthy subjects and patients with inflammatory arthritis, respectively, treated with AJA (0-30 μM) in vitro, and then stimulated with lipopolysaccharide. Cells were harvested for mRNA, and supernatants were collected for cytokine assay. Addition of AJA to PBM and SFM in vitro reduced both steady-state levels of IL1β mRNA and secretion of IL1β in a concentration-dependent manner. AJA did not influence TNFα gene expression in or secretion from PBM. Reduction of IL1β by AJA would contribute to the explanation of the joint sparing effects of AJA in the animal model of arthritis.[22]

Activation of T cells in the synovium can result in joint tissue injury in patients with RA. Bidinger et al[25] investigated the possibility that AJA would suppress human T-cell growth in vitro. T cells were isolated from peripheral blood of healthy volunteers and stimulated to proliferate with monoclonal antibodies to CD3 and CD4. They observed that T-cell proliferation was suppressed by AJA in a dose-dependent manner. Decreases in cell numbers also occurred following the addition of AJA to unstimulated cells. The involvement of apoptosis was detected by DNA fragmentation, caspase-3 activity, and microscopy, and AJA induced apoptosis of T cells in a dose- and time-dependent manner.[25] Apoptosis preceded loss of cell viability as measured by trypan blue dye exclusion, confirming that cell loss was due to programmed cell death rather than necrosis. T cells in the synovium of RA patients are resistant to apoptosis, further suggesting that a drug such as AJA may be a useful therapeutic agent for patients with RA.

The analgesic properties of AJA have been reported in a variety of animal models by several laboratories. Burstein et al[15,24] observed an antinociceptive action for AJA in the mouse hot plate assay at 55°C. Dajani et al[23] confirmed this finding and, under their conditions, reported an ED-50 of 6.7 mg/kg intragastrically that was equipotent to that observed with morphine. They also found a somewhat longer duration of activity for AJA when compared with morphine. In addition, they reported data using the tail clip assay in which an ED-50 of 4.4 mg/kg was determined. Using the paraphenylquinone (PPQ) writhing assay, Burstein et al[24] found activity for AJA with an ED-50 of 1.24 mg/kg. They also reported inhibition in both the first and second phases of the mouse formalin test, indicating both centrally and peripherally mediated analgesia for AJA.

Walker et al[28] reported that allodynia induced by paw injection of platelet activating factor (PAF) in rats was completely reversed following the administration of 5 mg/kg of AJA. Higher doses resulted in analgesia as measured by increased tolerance to mechanical pressure. No effect on motor function (rotarod assay) was seen under the conditions of the assay. Analgesia was observed in a similar model following paw injection of either carrageenan or complete Freund's adjuvant (Atlantic Pharmaceuticals, New York, NY, unpublished data, July 15, 1998).

In a seemingly different model, Recht et al[19] demonstrated that AJA is highly effective in inhibiting the proliferation of several types of cancer cells. The effect on normal cells was lower and, in all cases, cell growth resumed upon withdrawal of the AJA. The involvement PPAR-γ was suggested by changes in lipid metabolism and prostaglandin synthesis that occurred concurrently; however, there is no direct evidence for this mechanism. In the same study, a modest but significant in vivo antitumor effect was seen in a subcutaneous mouse model at a dose of 0.2 mg/kg administered orally 3 times weekly. Cancer and inflammation are sometimes considered to be analogous processes, suggesting that AJA may act by similar mechanisms in reducing the course of these 2 conditions.

SIDE EFFECTS

The use of many of the traditional anti-inflammatory agents such as aspirin and ibuprofen is limited by the formation of gastrointestinal ulcers attributed to their inhibition of COX-1 activity, which is required for gastric mucosal protection. For this reason, AJA was carefully examined[23] to detect any possible occurrence of ulcerogenicity. When given acutely to rats in doses up to 1000 mg/kg, no evidence for ulcer formation was seen. Chronic ig administration of up to 30 mg/kg likewise resulted in no ulcer formation, whereas the indomethacin control rats showed extensive formation of ulcers. This finding may be due to AJA's lack of inhibition of COX-1 as evidenced by its weak effect on human platelet aggregation.[24]

AJA's relationship to THC and its potent analgesic properties prompted a study for possible induction of opiate-like physical dependence in a 14-day rat study. None of the typical opiate withdrawal effects such as writhing, diarrhea, and wet dog shakes were observed (Atlantic, unpublished data, July 15, 1998), indicating that AJA has a low dependence liability. There were no effects on renal, cardiovascular, or gastrointestinal function and no signs of respiratory depression. Lethal doses were estimated following single doses in mice (600 mg/kg) and in rats (400 mg/kg). AJA was well tolerated in a 14-day study at doses up to 50 mg/kg. Three different standard tests for mutagenic potential gave negative findings, indicating a lack of carcinogenicity.

CLINICAL TRIALS

A phase 1, single-center, double-blind, randomized, placebo-controlled study of AJA was completed five years ago (Atlantic, unpublished data, May 8, 2000). The purpose of the study was to determine the safety, tolerability, and pharmacokinetics of a single oral dose of AJA in healthy adult male volunteers. A total of 32 subjects were given doses ranging from 0 to 10 mg and monitored for 24 hours following treatment. Pharmacokinetic measurements using mass spectrometry revealed that AJA is rapidly absorbed following oral administration and is eliminated with a terminal half-life of approximately 3 hours. The area under the curve (AUC) and C_{max} values showed a linear relationship when compared over the dose range of 1 to 10 mg/subject. Data from clinical laboratory tests, cardiovascular measurements, and tests for psychoactivity were also obtained. The latter consisted of a 12-item, yes/no questionnaire developed by the Addition Research Center at the National Institute on Drug Abuse, which is commonly referred to as the Addiction Research Center Inventory-Marijuana (ARCI-M) scale. It is designed to detect the full range of subjective responses experienced by marijuana users and has been validated by subjects following marijuana smoking. The subjects in the AJA study were told that they would receive a synthetic derivative of THC.

The data from all dose levels showed that AJA is safe and well tolerated. The scores obtained with ARCI-M scale showed no significant differences between placebo and AJA-treated volunteers. This finding is in agreement with the reported preclinical studies in rodents[5,15] and supports the conclusion that AJA does not produce a marijuana-like ''high'' at doses within the expected therapeutic range. This conclusion has recently been contested[29]; however, the basis for the assertion resides in one single-dose experiment in the mouse done at a level many-fold higher than the therapeutic dose. Burstein and Zurier have published a detailed reply to this claim pointing out the weaknesses of the arguments.[30]

The findings from a phase-2 trial to determine the efficacy of AJA for the treatment of chronic, intractable, neuropathic pain have been reported by Karst et al.[16] In a randomized, placebo-controlled, double-blind crossover trial, 21 patients (8 women and 13 men) with a mean age of 51 years who had a clinical presentation and examination consistent with chronic neuropathic pain with hyperalgesia (n = 21) and allodynia (n = 7) were recruited. The subjects were randomized into 2 7-day treatment groups in a crossover design. Two daily doses of AJA (4 10-mg capsules per day) or identical placebo capsules were given during the first 4 days, and 8 capsules per day were given in 2 daily doses in the following 3 days. After a washout and baseline period of 1 week each, patients crossed over to the second 7-day treatment period. The visual analog scale (VAS) and verbal rating scale scores for pain were used to determine the effect of AJA. The Trail-Making Test and the ARCI-M scale were used to detect possible cannabimimetic activity. The mean differences over time for the verbal analogic score (VAS) values in the AJA-placebo sequence measured 3 hours after intake of AJA differed significantly in favor of analgesia from those in the placebo-AJA sequence. Eight hours after intake of the drug, the pain scale differences between groups were less marked. There were no significant differences with respect to vital signs, blood tests, electrocardiogram, Trail-Making Test, and ARCI-M scale. In this preliminary study, AJA was effective in reducing chronic neuropathic pain compared with placebo, and no major adverse effects were observed.

In summary, the studies done with AJA demonstrate that naturally occurring cannabinoids can provide useful template molecules for the synthesis of analogs with potential anti-inflammatory activity. These could be expected to show a high therapeutic index since cannabinoids generally have minimal toxicity. It is also tempting to speculate that stable analogs of the endogenous cannabinoids such as anandamide may be discovered and will also exhibit high therapeutic indices.

ACKNOWLEDGMENTS

This publication was made possible by grants DA12178 and DA13691 from National Institute on Drug Abuse, National Institutes of Health, Bethesda, MD. Its contents are solely the responsibility of the author and do not necessarily represent the official views of the National Institute on Drug Abuse.

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