The Cannabinoids: Looking Back and Ahead

Raphael Mechoulam

Boston, 2016
"….modulating endocannabinoid activity may have therapeutic potential in almost all diseases affecting humans, including obesity/metabolic syndrome, diabetes and diabetic complications, neurodegenerative, inflammatory, cardiovascular, liver, gastrointestinal, skin diseases, pain, psychiatric disorders, cachexia, cancer, chemotherapy-induced nausea and vomiting, among many others."

Pacher and Kunos review, FEBS, 2013
Gan-zi-gun-nu – the drug that takes away the mind
Azallu – hand of ghost, poison of all limbs (neurological diseases?)
Qunnabu – used in religious rites
Pliny, the Elder (79 AD): The roots boiled in water ease cramped joints, gout too and similar violent pain.

Dioscorides (90 AD): The sodden root when placed on inflammations soothes them, eliminates edema and disperses obdurate matter above inflamed joints.
For the relief of certain kinds of pain, I believe, there is no more useful medicine than Cannabis within our reach.

J. Russell Reynolds, Archives of Medicine, Vol 2, 154, 1859
Δ⁹-tetrahydrocannabinol (Δ⁹-THC) (Gaoni and Mechoulam, 1964)

Cannabidiol (CBD) (Mechoulam and Shvo, 1963)

Cannabigerol (CBG) (Gaoni and Mechoulam, 1964)

Cannabinol (CBN) (Adams et al., 1940)

Cannabichromene (CBC) (Claussen et al., 1966; Mechoulam and Gaoni, 1966)

Cannabicyclol (CBL) (Crombie et al., 1968)

Representative natural cannabinoids

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cannabidiol (CBD)

Mechoulam and Shvo: Tetrahedron 19, 2073 (1963)

Δ⁹-tetrahydrocannabinol (Δ⁹-THC)

Gaoni and Mechoulam: J.Amer.Chem.Soc. 86, 1646 (1964)
Brain regions in which cannabinoid receptors are abundant

Basal ganglia
  - Substantia nigra pars reticulata
  - Enteropeduncular nucleus
  - Globus pallidus
  - Putamen
Cerebellum
Hippocampus
Cerebral cortex, especially cingulate, frontal, and parietal regions
Intrabulbar anterior commissure
Nucleus accumbens

Movement control
Body-movement coordination
Learning and memory, stress
Higher cognitive function
Link between cerebral hemispheres
Reward pathway
\text{HU-210} \xrightarrow{\text{H}_2} \text{\textsuperscript{3}H-HU-243}

\text{* = tris(triphenylphosphine)rhodium}
anandamide

Δ⁹-tetrahydrocannabinol (Δ⁹-THC)

2-arachidonoyl glycerol (2-AG)

The endocannabinoid system

CB1 and CB2 receptors

Anandamide; 2-arachidonoyl glycerol (2-AG)

N-acyl-aminoacids (or N-acyl-ethanolamides)

Enzymes: synthesis of endocannabinoids
hydrolysis of endocannabinoids

THC and Cannabidiol (CBD)
What do endocannabinoids do?

“Relax, eat, sleep, forget and protect”

Di Marzo, 1998
Physiological systems and conditions affected by cannabinoids (a partial list)

- Anxiety
- Appetite/feeding
- Blood pressure
- Bone formation
- Cerebral blood flow
- Digestive system
- Emesis and nausea
- Immune system
- Inflammation
- Memory
- Mood
- Movement
- Neuroprotection
- Pain
- Reproduction
- Stress
Neuroprotection
Levels of 2-AG in mouse brain after CHI

Anova with Tukey post-test: P<0.0001, F=36.01

- ★★★ - P<0.001 vs. control
- ★★ - P<0.01 vs. control
- ★ - P<0.05 vs. control

Nature 413, 527 (2001)
2-AG Reduces Infarct Volume 24 h After CHI

Unpaired t-test, P = 0.03
Actions through the CB1 receptor
Prevention of side effects of cancer chemotherapy
Appetite and feeding
Physical growth during treatment with $\Delta^9$-THC and CB$_1$ antagonist (SR141716A)

Control
THC
SR141716A
THC + SR141716A

12 (out of 13) pups dead

all pups died
Mean change in weight from baseline in patients with AIDS treated with dronabinol (THC) and placebo (Beal et al., 1995)
Preliminary, Open-Label, Pilot Study of Add-On Oral \( \Delta^9 \)-Tetrahydrocannabinol in Chronic Post-Traumatic Stress Disorder

Roitman P., Mechoulam R., Cooper-Kazaz R., Shalev A.
Methods  Ten outpatients with chronic PTSD, on stable medication, received 5 mg Δ⁹-THC twice a day as add-on treatment for 3 weeks.

Results  Orally absorbable Δ⁹-THC was safe and well tolerated by patients with chronic PTSD.

There were mild adverse effects in three patients, none of which led to treatment discontinuation. The intervention caused a statistically significant improvement in global symptom severity, sleep quality, frequency of nightmares, and PTSD hyperarousal symptoms.
Actions through $\text{CB}_2$ receptor
The mammalian body has a highly developed immune system, whose main role is to guard against protein attack and reduce the damage caused. It is inconceivable that through evolution analogous biological protective systems have not been developed against non-protein attacks.
We believe that the CB$_2$ receptor is indeed part of general protective system and its stimulation leads mostly to sequences of activities of a protective nature.

This receptor works in conjunction with the immune system and with various other physiological systems.
The Most Interesting Compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>CB₂ R Binding Ki (nM)</th>
<th>CB₁R Binding Ki (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HU-308</td>
<td>14</td>
<td>&gt;10,000</td>
</tr>
<tr>
<td>HU-910</td>
<td>6</td>
<td>1410</td>
</tr>
</tbody>
</table>
HU-910 attenuates inflammation.

HU-910 lowers damage associated with hepatic reperfusion injury.

Horvath et al. 2012
Inflammation

Crohn's disease

Inflammatory bowel disease

Asthma
Actions through cannabidiol (CBD)
Anxiety
CBD produced anxiolytic-like effects in the elevated plus maze
Inflammation
Cannabidiol (i.p.), acute

Clinical score vs. Days after onset of arthritis

- Control
- 2.5 mg/kg
- 5 mg/kg
- 10 mg/kg
- 20 mg/kg

PNAS, 97, 9561-9566 (2000)
Synovium is the most critical site of cytokine production in arthritis. Synovial cells from arthritic mice spontaneously produce large amounts of TNF when cultured *in vitro*. Cells from arthritic mice which had been treated with CBD produced significantly less (50%) TNF.
Diabetes type 1
CBD (5 mg/kg/day) i.p. to female 14 week old NOD mice with latent diabetes. Administered for 4 weeks/5 days a week. Observed until 24 weeks of age. End point: glucosuria.
Histological analysis of pancreas tissue from mice treated with CBD, vehicle and untreated.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of scored islets</th>
<th>Intact islets</th>
<th>Total ruined islets</th>
<th>Full infiltrated islets</th>
<th>Partial infiltrated islets</th>
<th>Percent intact islets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>73</td>
<td>6</td>
<td>29</td>
<td>28</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Vehicle</td>
<td>94</td>
<td>12</td>
<td>30</td>
<td>43</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>CBD</td>
<td>140</td>
<td>108</td>
<td>-</td>
<td>12</td>
<td>15</td>
<td>77</td>
</tr>
</tbody>
</table>
Clinical Trials
Epilepsy

Double blind.
Drug: CBD in capsules
Patients: 15 epileptic patients, who did not benefit from known antiepileptic drugs.
Dose: 200-300 mg/day for 4.5 months.
Results: 4 patients (out of 8) remained almost completely free of seizures.
  3 patients had partial improvement
  1 patient showed no improvement
Placebo patients: only one showed improvement

Cunha, Carlini, Mechoulam, 1980
In a double-blind, anti-schizophrenia clinical trial of CBD vs amisulpride (a potent antipsychotic) both treatments led to significant clinical improvement, but CBD displayed a superior side effect profile. Moreover, CBD treatment was accompanied by a significant increase in serum anandamide levels (Leweke et al., 2012)
Graft-versus-host disease

Graft-versus-host disease (GVHD) is a complication that can occur after a bone marrow transplant in which the newly transplanted donor cells attack the transplant recipient’s body.

M. Yeshurun et al., (2014) administered CBD (300mg/day) to 46 patients with hematological malignancies for 30 days and followed them for 8 months.
Chronic GVHD (after 100 days)

<table>
<thead>
<tr>
<th>Grade</th>
<th>101 patients control</th>
<th>46 patients (with CBD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-4 grade</td>
<td>46%</td>
<td>12%</td>
</tr>
<tr>
<td>3-4 grade</td>
<td>10%</td>
<td>5%</td>
</tr>
</tbody>
</table>
New CBD-type molecules
CBD $\xrightarrow{1\text{-fluoropyridinium triflate}}$ HU-474
cannabidiol diacetate $\xrightarrow{\text{SeO}_2\text{, t-BuOOH}}$ 10-OH-CBD diacetate $\xrightarrow{\text{DAST}}$ 10-F-CBD diacetate
Brain injury

2-AG

glutamate cytokines, ROS

Vasoconstrictors (e.g. ET-1, Thromboxane)

neuronal & glial cell death

cerebral ischemia

cerebroprotection
Regulation of vasodilation
phosphatidylethanolamine

N-arachidonoylphosphatidylethanolamine

phosphatidylserine

N-arachidonoylphosphatidylserine

anandamide

arachidonoylserine
anandamide

2-arachidonoyl glycerol (2-AG)

arachidonoyl serine
Fatty Acids – Ethanol Amides

- **Arachidonoyl ethanolamide** (anandamide)
- **Palmitoyl ethanolamide** --- anti-inflammatory
- **Stearoyl ethanolamide** --- causes apoptosis
- **Oleoyl ethanolamide** --- regulates feeding
Fatty Acids – Amino Acids (FAAA)

Arachidonoyl glycine --- antinociceptive
Arachidonoyl serine --- vasodilator;
  neuroprotective
Arachidonoyl dopamine --- affects
  neurotransmission in dopaminergic neurons
Oleoyl serine --- anti-osteoporotic (also found in brain)
Palmitoyl serine --- neuroprotective
Bone Remodeling
Oleoyl Serine Stimulates Osteoblast Number

**MC3T3 E1 osteoblasts**

**Primary newborn calvarial osteoblasts**
Oleoyl Serine Rescues Ovariectomy-induced Bone Loss

Day 0  
OVX

Day 42  
OS treatment  
5 mg/Kg/day

Day 84  
Analysis

Sham OVX  
OVX/VEH  
OVX/OS

BV/TV (%)  

p = 0.065  
p = 0.017
SUMMARY

1. Endocannabinoids are involved in a large number physiological processes. THC – a plant cannabinoid – mimics their actions.
2. CBD derivatives – may lead to a wide spectrum of novel drugs. May act through DNA methylation.
3. Fatty acids – amino acids (FAAA) and derivatives may lead to better understanding of biological processes as well as to novel drugs.
4. CB2 specific agonists – may lead to a wide spectrum of novel drugs. May be part of a general protective system.
Collaboration in Israel

Jerusalem
Prof. L. Hanuš
Prof. E. Fride
Dr. W. A. Devane
Dr. A. Breuer
Dr. S. Ben-Shabat
Dr. D. Panikashvili
Dr. G. Milman
Dr. N. Kogan

Jerusalem
Prof. I. Bab
Prof. E. Shohami
Prof. R. Gallily
Prof. E. Berry
Dr. R. Durst

Rehovot
Prof. Z. Vogel

Tel Hashomer
Dr. S. Almog

Haifa
Prof. A. Mandelbaum
Collaboration abroad

Aberdeen
R. Pertwee

Richmond
B. Martin
A. H. Lichtman

Bonn
M. Karsak
A. Zimmer

Canada
L. A. Parker

Brno
A. Šulcová

Bethesda
G. Kunos
M. Spatz

Greece
C. Simeonidou

Napoli
V. Di Marzo

Rome
M. Maccarrone

Siberia
L. Maslov

London
M. Feldmann
A. M. Malfait
P. F. Sumariwalla
<table>
<thead>
<tr>
<th>Disease, samples (H: human; R: rodent; P: pig)</th>
<th>Increase in endocannabinoid levels</th>
<th>Expression of CB$_2$</th>
<th>Effects attributed to CB$_2$ stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inflammatory bowel disease, colitis, diverticulitis (R, H)</strong></td>
<td>Inflamed gut</td>
<td>Epithelial cells, infiltrating inflammatory cells, enteric nerves</td>
<td>Attenuation of inflammation and visceral sensitivity</td>
</tr>
<tr>
<td><strong>Pancreatitis (R, H)</strong></td>
<td>Inflamed pancreas</td>
<td>Pancreas</td>
<td>Attenuation of inflammation</td>
</tr>
<tr>
<td><strong>Nephropathy (R)</strong></td>
<td>Kidney</td>
<td>N.D.</td>
<td>Attenuation of inflammation (chemokine signaling and chemotaxis, inflammatory cell infiltration and endothelial activation) and oxidative/nitrosative stress</td>
</tr>
<tr>
<td><strong>Neurodegenerative/neuroinflammatory disorders (multiple sclerosis, Alzheimer’s, Parkinson’s, and Huntington’s disease) (R, H)</strong></td>
<td>Brain, spinal fluid</td>
<td>Microglia, inflammatory cells, brain lesions, neurons?</td>
<td>Attenuation of inflammation (microglia activation, secondary immune cell infiltration), facilitation of neurogenesis</td>
</tr>
<tr>
<td><strong>Pain (R)</strong></td>
<td>Site of induced chronic inflammatory pain</td>
<td>Inflammatory cells,</td>
<td>Attenuation of chronic inflammatory pain</td>
</tr>
<tr>
<td><strong>Skin disorders (R)</strong></td>
<td>Inflamed skin</td>
<td>Infiltrating immune cells</td>
<td>Context dependent anti- or pro-inflammatory effects in contact dermatitis</td>
</tr>
<tr>
<td><strong>Bone disorders (e.g. osteoporosis) (R)</strong></td>
<td></td>
<td>Osteoblasts, osteoclasts</td>
<td>Attenuates bone loss by enhances endocortical osteoblast number and function and restrains trabecular osteoclastogenesis</td>
</tr>
<tr>
<td><strong>Rheumatoid arthritis (H)</strong></td>
<td>Synovial fluid</td>
<td></td>
<td></td>
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