CANNABIDIOL IS A SUBTYPE-SPECIFIC INHIBITOR OF INFLAMMATORY LIPOXYGENASE ENZYMES.

Aidan J Hampson

AIMS

- This study examines cannabidiol as an inhibitor of 5-, and 15-LO's, both at the enzyme level and in whole human blood cells. The nature of cannabidiol inhibition is also be examined.
- The consequences of cannabidiol's selective LO inhibition is illustrated. It is demonstrated that non-inflammatory 12-LO products, which are unaffected by cannabidiol, can markedly protect cultured neurons from glutamate toxicity

Introduction

Over the last twenty years, various reports have suggested that cannabinoids may affect immune cell function and inflammation by altering eicosanoid production. In particular, reports have suggested that cannabinoids inhibit lipoxygenases (LO's), which produce potent inflammatory mediators (1) associated with many pathologies including; arthritis, asthma, atherosclerosis (2).

Unfortunately, the existing knowledge base concerning the anti-inflammatory effects of cannabinoids is somewhat imperfect. Some studies have indicated that cannabinoids inhibit lipoxygenases (LO's; 3,4), although others have reported no inhibition (5) and still other reports describe cannabinoids as activators of LO dependent pathways (6,7).

Furthermore, while cannabinoids may inhibit LO's, they also cause release of LO substrates through phospholipase A_2 activation.

Most LO inhibitors work through an antioxidant action in the enzyme active site. A recent report (8) indicated that cannabinoids such as THC and cannabidiol are powerful antioxidants, which may explain their alleged effects as LO inhibitors. This same report indicated that, due to these antioxidant properties, non-psychoactive cannabinoids such as cannabidiol appear to be good (*in vitro*) neuroprotectants.

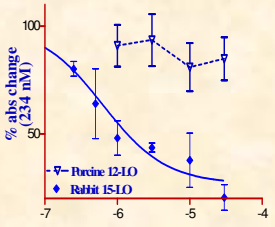
Inflammation and oedema are substantial components of many neuropathologies including Stroke, head trauma and possibly AIDS dementia. It is possible therefore, that some of the neuroprotective properties of cannabidiol can be accounted for through its effects on eicosanoid production.

Conclusions

The confusion created by apparently conflicting earlier reports, reflects the specificity of cannabidiol and other cannabinoids as lipoxygenase inhibitors.

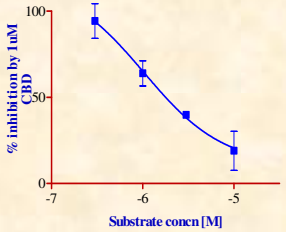
1. Cannabidiol competitively inhibits 5- and 15-type enzymes, but has no effect on 12-LO activity.
2. Cannabinoids stimulate on phospholipase A_2 activity, which, when combined with blockade of inflammatory LO's, results in cannabidiol potentiating 12-LO product formation.
3. 12-HETE, the product of 12-LO is neuroprotective *in vitro* in both AMPAr and NMDAr dependent glutamate toxicity assays.
4. 12-LO is known to be activated following ischaemic damage, (10) suggesting that part of the protective effect of cannabidiol may be due to its enhancing effect on 12-HETE formation.

Cannabidiol inhibits 15-LO but not 12-LO



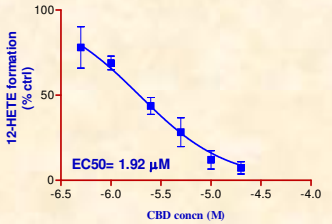
Rate of LO product was examined by uv Spectrocopy. Absorption at 234 – 238 nM represents formation of a conjugated diene, an indicator of LO activity. EC50= 550nM, Substrate = 10μM linoleic acid

Cannabidiol inhibition is *competitive*



CBD inhibition decreases as substrate concentration increases. Furthermore, HPLC analysis indicated CBD is not a substrate for 15-LO

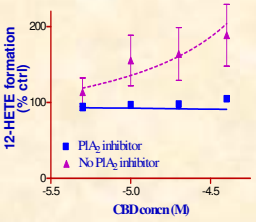
Effect of CBD on 5-LO activity (RBL-2H3)



5-LO is a major inflammatory enzyme.
RBL-2H3 cells were incubated with 10μM arachidonic acid and CBD. Samples were analysed by rp-HPLC and peak areas calculated

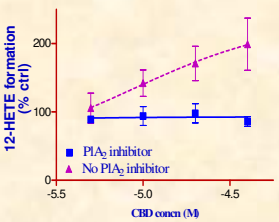
6

Effect of CBD on 12-HETE formation (12-LO product) in human leukocytes

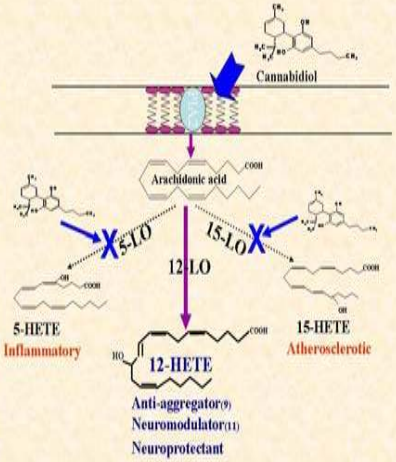


Human leukocytes incubated with arachidonic acid ± CBD and Phospholipase A_2 inhibitor (80 μM manoalide). CBD increases 12-HETE formation by activating PIA_2 while having no effect on 12-LO.

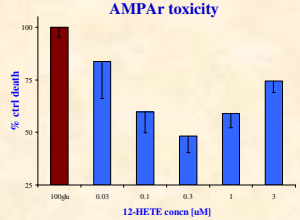
Effect of CBD on human platelet 12-LO



Platelet LO is not related to leukocyte-LO. Leukocyte 12-LO is closely related to 15-LO. However, CBD inhibits 15-LO but not 12-LO's. Analysis was performed by HPLC

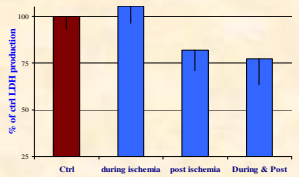


12-HETE protects neurons from AMPAr toxicity



Cortical neurons were exposed to glutamate (plus MK-801 and cyclothiazide) for 20 hours ±12-HETE

12-HETE protects cortical neurons from NMDAr toxicity



(0.5ug / ml) 12-HETE was applied both during glutamate insult and afterwards ("reperfusion" phase)

References

1. Samuelsson B: *Adv Exp Med Biol* **1997** 433:1
2. Steinhilber D: Curr Med Chem **1999** 6:71
3. Burstein SH et al: *J Pharmacol Exp Ther* **1989** 251: 531
4. Evans TA et al: *Biochem Pharmacol* **1987** 36: 2035
5. White HL; Tansik RL: *Prostaglandins Med* **1980** 4:409
6. Audette CA; Burstein SH:*Life Sci* **1990** 47: 753
7. Diaz S et al: *J Pharmacol Exp Ther* **1994** 268: 1289
8. Hampson AJ et al: *Proc Natl Acad Sci U S A* **1998** 95: 8268
9. Yamamoto S et al: *Prog Lipid Res* **1997** 36:23
10. Murphy E et al: *Circ Res* **1995** 76:457
11. Piomelli D; GreenGard P *Trends Pharmacol Sci* **1990** 11:367