Protective Effects of Pharmaceutically Manufactured Cannabidiol in a Mouse Model of Acute Pericarditis

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BACKGROUND

Pericarditis results from an intense inflammatory reaction of the pericardium. This reaction involves a stereotypical response associated with **NLRP3 inflammasome activation**, an intracellular sensor of injury and stress, and triggers an inflammatory process that leads to the release of proinflammatory cytokines, such as interleukin-1β.

Given the scarce knowledge of its pathophysiology, there are limited treatment options for acute pericarditis, mostly consisting of NSAID and colchicine.

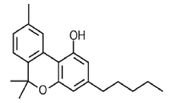

Figure 1. CBD molecule

Cannabidiol (CBD) is a small, lipophilic, non-psychoactive compound of Cannabis sativa, that has a good safety profile and few side effects. Several in vitro and in vivo studies have shown that it possesses anti-inflammatory properties.

Although CBD has been suggested to be useful in treating a variety of heart diseases, the clinical efficacy of CBD treatment in pericarditis is still unknown.

HYPOTHESIS:

CBD reduces the NLRP3 inflammasome activity and blunts pericardial inflammation in a mouse model of acute pericarditis.

METHODS

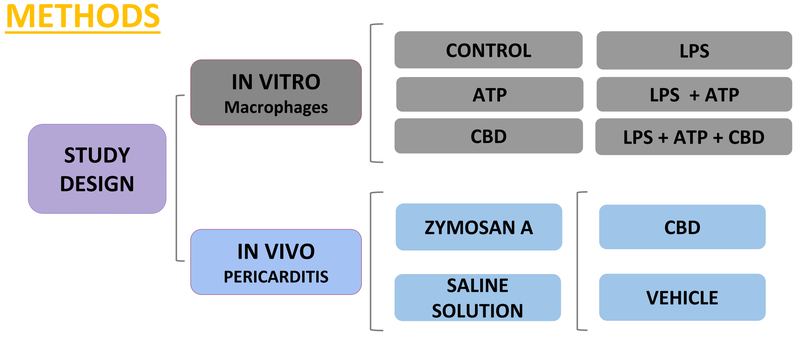

Figure 2. Study design

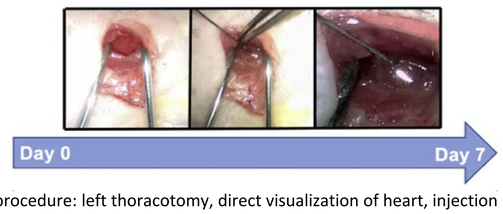

Figure 3. Surgical procedure: left thoracotomy, direct visualization of heart, injection into pericardial sac

IN VITRO: J774.1 macrophages were stimulated with LPS (1µg/ml for 6 hours), ATP (5 mM for 30 min), or LPS+ATP, with or without CBD 10µM. IL-1β and IL-6 were measured in the cell culture media using specific ELISA assays. The IL-1β measurement was then repeated with 10, 1, 0.1, and 0.01 µM CBD.

RNA was extracted from cultured cells through affinity columns and converted to complementary DNA, and then a Real-time polymerase chain reaction was performed.

IN VIVO: Acute pericarditis was induced in 10-week-old male mice, under isoflurane anesthesia (1,5-3,0 % O_2), by injecting 50 µl of Zymosan A (Zym, 1 mg/ 50 µl), which activates NLRP3, into the pericardial sac. Control mice received an equal volume of NaCl 0.9%. Mice were randomized to intraperitoneal daily injections of CBD (10 or 1 mg/kg, 0.1 ml), or an equal volume of vehicle (Veh, 1:1:18 of EtOH: Cremophor: H_2O). Pericarditis severity was assessed by the presence of effusion at echocardiography and pericardial thickening at pathology.

RESULTS

IN VITRO: LPS+ATP increased IL-1β concentration (449.1±48.1 pg/ml) vs control (6.4±0.1 pg/ml, p<0.0001). CBD 10 µM treatment reduced IL-1β concentration (118.7±40.2 pg/ml, p<0.0001).

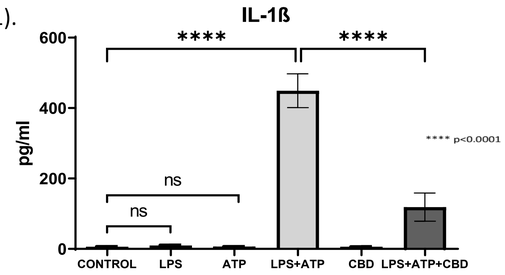

Figure 4. ELISA assay testing IL-1β concentration in vitro.

The expression of IL-6 and the NLRP3 inflammasome components is regulated by the NF-kB pathway and LPS, but IL-6 release is independent of the NLRP3 inflammasome. To test whether CBD has a broader anti-inflammatory effect, we measured the level of IL-6 in the culture media of cells treated with LPS. LPS alone significantly increased IL-6 concentration. This effect was abolished by CBD treatment, suggesting an anti-inflammatory activity beyond the specific inhibition of NLRP3.

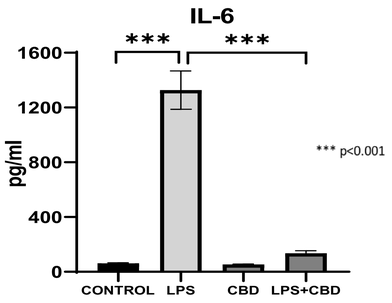

Figure 5. IL-6 ELISA ASSAY of cell culture media of cultured macrophages.

In accordance with the results in figure 5, the transcription of NLRP3 and IL-1β was increased after in vitro treatment with LPS and reduced by the treatment with CBD, indicating an indirect inhibition of the inflammasome pathway.

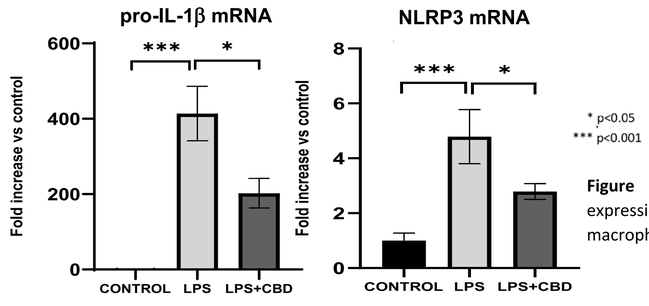

Figure 6. mRNA expression in cultured macrophages.

A dose-response reduction in IL-1β was seen with CBD treatment.

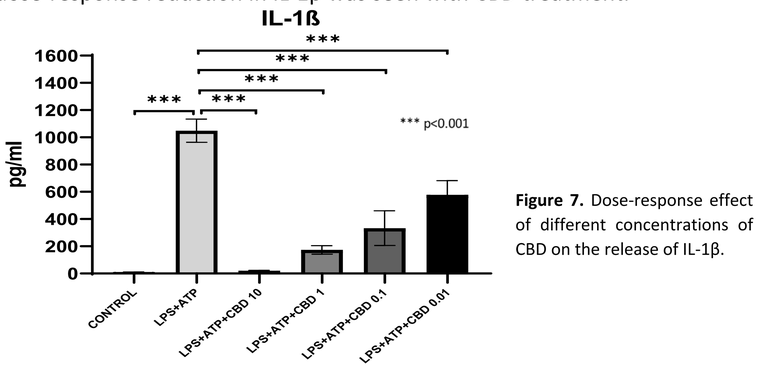

Figure 7. Dose-response effect of different concentrations of CBD on the release of IL-1β.

IN VIVO: Seven days after surgery, when compared to Zym+Veh mice, CBD 10 mg/kg treatment reduced pericardial effusion (0.12±0.01 mm vs 0.26±0.05, p<0.01) and pericardial thickness (3.6±0.6 µm vs 6.5±1.1, p<0.05). A lower dose of CBD tested (1 mg/kg) was equally effective. There was no statistically significant difference between Zym+CBD and NaCl+Veh for any parameter.

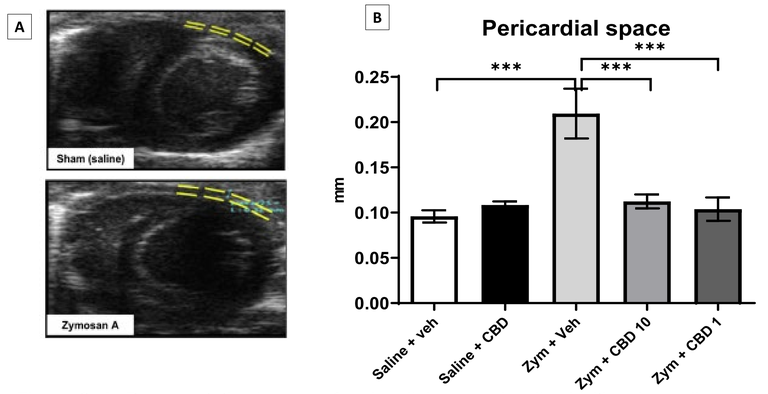

Figure 8. A. Representative images of pericardial space visualized with echocardiography in the mouse model. **B.** Average measurement of the pericardial space in the pericarditis animal model. *** p<0.001

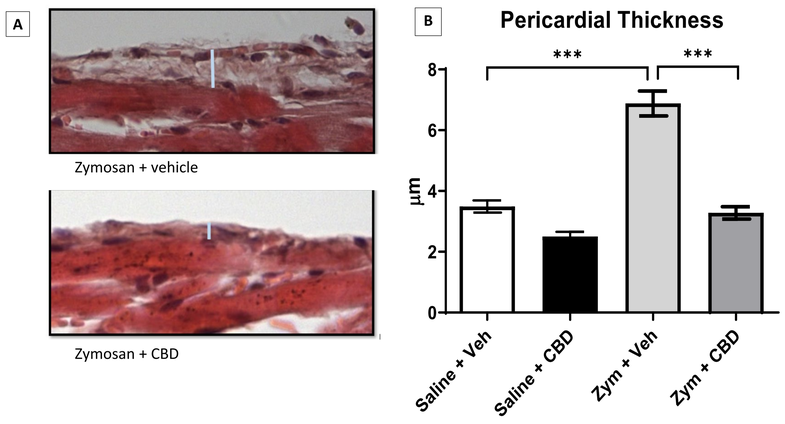

Figure 9. A. Representative images of the pericardial thickness of mice heart sections stained with hematoxylin and eosin. **B.** Average measurements of the pericardial thickness in pericarditis animal model. *** p<0.001

CONCLUSIONS

Cannabidiol suppresses IL-1β and IL-6 secretion by macrophages in vitro and significantly reduces pericardial effusion and thickness in a mouse model of acute pericarditis.

Cannabidiol may represent a novel strategy for treating pericarditis, its complications, and preventing its recurrence.

DISCLOSURES

Dr. Hamer and Mr. Bolton are employees and shareholders in Cardiol Therapeutics Inc. Dr. Toldo received research grant funding from Cardiol Therapeutics Inc. (synthetic cannabidiol), Kiniksa (Interleukin-1 inhibitor), Takeda, Olatec, and Serpin Pharma. He has served as a paid scientific advisor to Cardiol Therapeutics Inc. Dr. Abbate has served as a paid scientific advisor for GSK, Kiniksa, Merck, Novartis, Olatec, Serpin Pharma, and Swedish Orphan Biovitrum.