UNIVERSITY OF MIAMI
MILLER SCHOOL of MEDICINE

Management of SCI Induced Chronic Pain in rats Using Cannabidiol and β-caryophyllene

Amanda Pacheco-Spiewak, A. Eeswara, S. C. Gross, D. Ball, S. Jergova, J. Sagen

Miami Project to Cure Paralysis, University of Miami, FL

THE MIAMI PROJECT TO CURE PARALYSIS

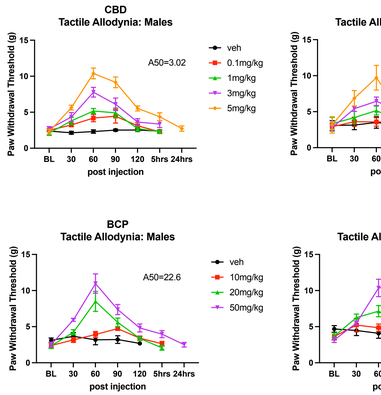
Introduction

The most frequently reported use of medical marijuana is for pain relief, but there is a paucity of preclinical studies which evaluate the effects of cannabis components in chronic pain models. Of the 80-100 cannabinoid compounds produced by the cannabis plant, Cannabidiol (CBD) and β-caryophyllene (BCP) are two components which have been suggested to have significant pain reducing properties. Spinal cord injuries (SCI) frequently result in chronic pain which can be so severe it is one's top quality of life concern. Patients with SCI have reported substantial pain relief from marijuana and medicinal extracts, yet progress in the field has been hampered by lack of solid supporting preclinical evidence. Thus, the goal of this study was to evaluate CBD and BCP individually and their potentially synergistic pain-relieving combination.

Methods

Animals: Male and female rats Sprague Dawley, 140g initially.
Spinal cord injury (SCI): 60 second clip compression at T6-T8.
Dose ratio: At least 3 doses of each agent tested to generate time course and dose-response curves and to calculate antinociceptive A50. CBD was tested at 0.1-5.0 mg/kg ip in a 3:1:16 ethanol/Tween 80/0.9% NaCl plus 2% Tween vehicle. BCP was orally administered (oral gavage) at 10-50 mg/kg po in 5% Tween in saline. Control treatments with vehicles only were used. CBD/BCP dose was estimated based on single drug A50 values with two-tailed isobolographic analyses using the numerical method (JFlashCalc software program). For males, CBD/BCP ratio 1:20 was tested for cold allodynia and 3:22 for tactile allodynia. For females, CBD/BCP ratios of 2:20 and 7:75, respectively for cold and tactile allodynia were used. **Behavior**: Von Frey test (tactile) and Acetone evaporation test (cold). Rats were tested at baseline and every 30 min for at least 2 hrs post-injection. If some analgesic effects remained, they were again tested at 5 hrs and up to 24 hrs post-drug administration.
Analysis: Plot dose-response curves, behavioral effects of drugs converted to percent maximum possible effect for each test (MPE % = (Post-drug threshold − Baseline threshold)/(Pre-injury threshold−Baseline threshold)∗100). Baseline was considered as post-SCI pre-drug threshold. A50 values (50% antinociceptive dose) are calculated from linear portions of log dose–response curves using JflashCalc Pharmacological Calculations software package (http://u.arizona.edu/~michaelo/jflashcalc.html).

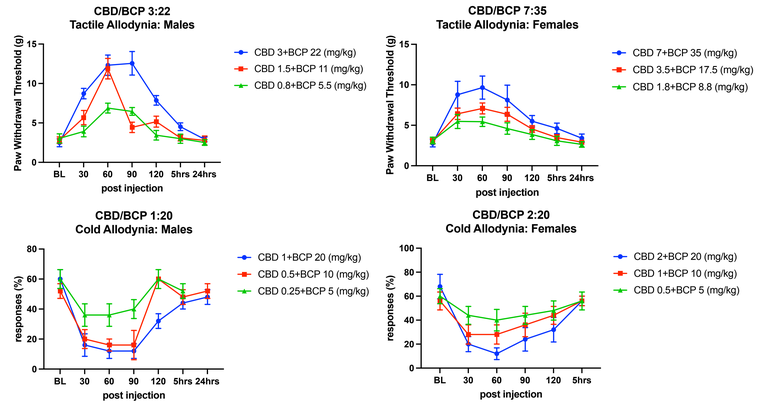
Dose Response Curve



CBD and BCP both produced dose-related reduction in hypersensitivity in both males and females. Peak effects of both drugs were at 60 min post-administration, with CBD anti-allodynic effects lasting longer in males and BCP lasting longer in females. CBD was less potent in reducing tactile allodynia than cold allodynia (higher A50s). This was particularly observed in females, with higher A50s (lower potency) for both CBD and BCP in females in reducing tactile allodynia.

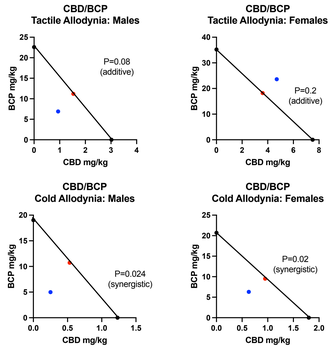
High baseline responses to acetone droplet indicative of cold allodynia were observed in all rats, and this continued in vehicle treated animals. Both CBD and BCP produced dose-related reductions in cold allodynia in male and female rats. Onset of effects was observed by 30 min. CBD effects appeared longer-lasting, with strongly reduced cold allodynia still observed at 2 hours post-administration and some residual reduced cold hypersensitivity remaining at 5 hours at the higher dose, while BCP effects peaked at 60-90 min and began to resolve by approximately 2 hours.

Dose Response Curve - Combination



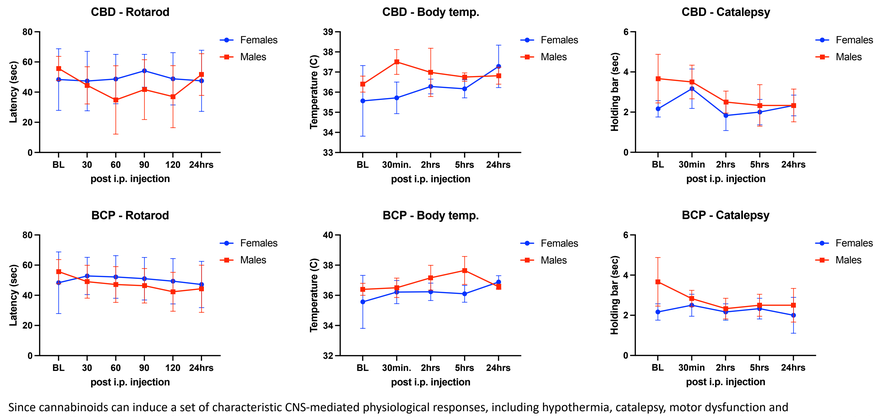
For testing the combination, the fixed dose ratio of the A50 values of the drugs is estimated based on the previously determined single drug A50 values. CBD/BCP produced dose-related reduction in hypersensitivity in both males and females. Peak effects of drug combination were at approximately 60 min post-administration for tactile and cold allodynia. Effects of the combination appear more potent in reducing cold allodynia than tactile allodynia (higher A50s) in both males and females.

Isobolographic Analyses



Isobolographic analyses for tactile and cold allodynia in males show markedly reduced combined A50 and significant synergistic effects of the combination for cold allodynia. The calculated A50s for females were similar, albeit slightly less effective for both drugs. When combined CBD/BCP was tested, reduced A50 and significant anti-allodynic synergism was indicated for cold but not tactile allodynia in females. The line indicates the theoretical line of additivity (red dot is theoretical additive of the combined A50 ratios). The blue dots below the line of additivity shows the actual combination A50, indicating synergism.

Side Effects



Since cannabinoids can induce a set of characteristic CNS-mediated physiological responses, including hypothermia, catalepsy, motor dysfunction and antinociception, this "tetrad" of behaviors is being evaluated in order to identify potential adverse effects. In preliminary studies, male and female rats were injected with CBD (i.p. 5mg/kg) or orally administered BCP (oral gavage 10mg/kg) and observed for up to 24 hours post-injection. A modest effect on rotarod in males injected with CBD was observed, but this was not statistically significant in this preliminary group. No other significant adverse effects have thus far been observed.

Results

- Both CBD and BCP administered individually reduced cold and tactile hypersensitivity in both male and female rats.
- The combination of CBD and BCP produced synergistic effects for cold allodynia in both male and female rats.
- Additive effects were observed in male rats for tactile allodynia.
- The effects of both compounds and the combination appeared less potent in females compared to males, suggesting that sex differences will need to be considered when developing cannabinoid pain-reducing strategies.
- We observed no overt side effects from combining CBD and BCP.

Supported by Consortium for Medical Marijuana Clinical Outcomes Research