Antinflammatory Effect of *Cannabis extracts* with High Content in Δ^9-tetrahydrocannabivarin (THCV) or Cannabidivarin (CBDV) in Murine Macrophages

B. Romano[1,5], P. Orlando[2,5], E. Pagano[1,5], R. Capasso[1,5], M.G. Cascio[3], R. Pertwee[3], V. Di Marzo[4,5], A.A. Izzo[1,5], F. Borrelli[1,5]

[1]Dept. of Pharmacy, University of Naples Federico II, Naples, Italy
[2]Institute of Protein Biochemistry, National Research Council, Naples, Italy; National Institute of Optics, National Research Council, Pozzuoli, Italy
[3]School of Medical Sciences, Institute of Medical Sciences, University of Aberdeen, Aberdeen, Scotland, UK
[4]Institute of Protein Biochemistry, National Research Council, Naples, Italy; Institute of Biomolecular Chemistry, National Research Council, Pozzuoli, Italy
[5]Endocannabinoid Research Group, Italy

Background/aim: Historical and scientific evidence suggests that *Cannabis* has immunomodulatory and anti-inflammatory effects. Recent progress in plant biotechnology has made possible the cultivation of *Cannabis* chemotypes rich in specific phytocannabinoids, from which standardized extracts, containing known amounts of phytocannabinoids, may be obtained. Here, we have investigated the effect of two standardized *Cannabis sativa* extracts with high content of Δ^9-tetrahydrocannabivarin (THCV) or cannabidivarin (CBDV), here named THCV BDS and CBDV BDS respectively, and their main pure components, *i.e.* THCV and CBDV, on nitrite production in murine macrophages. The possible interaction with cannabinoid receptors as well as possible changes in TRP channel expression were also evaluated.

Methods: Murine peritoneal macrophages were activated *in vitro* by LPS. Nitrite levels were measured using a fluorimetric assay; THCV BDS and CBDV BDS binding was assessed by the ability to displace [^{3}H]CP55940 from human cannabinoid CB_1 and CB_2 receptors; inducible nitric oxide (iNOS) and cyclooxygenase-2 (COX-2) protein expression were analysed by western blot analysis; interleukin 1β (IL-1β) were measured by ELISA kit; cannabinoid receptors as well as transient receptor potential (TRP) channels mRNA expression were analysed by quantitative RT-PCR.

Results: LPS increased nitrite production, which was associated with up-regulation of iNOS and COX-2 protein expression and IL-1β levels**.** THCV BDS and CBDV BDS (as well as pure THCV and pure CBDV) reduced LPS-stimulated nitrite levels, the effect being mediated by CB_1 receptors (in the case of CBDV BDS), CB_2 receptors (in the case of THCV BDS and pure THCV) and independent from cannabinoid receptors (in the case of CBDV). In binding assays, THCV BDS and pure THCV showed a similar affinity for both CB_1 and CB_2 receptors; CBDV BDS showed a greater affinity for both cannabinoid receptors than pure CBDV.

Pure THCV and CBDV reduced the LPS-induced up-regulation of iNOS and COX-2. Furthermore, pure THCV (but not CBDV) reduced IL-1β levels. Quantitative RT-PCR analysis in macrophages revealed the expression of CB_1, CB_2, TRPV2 and TRPV4 mRNA. LPS up-regulated CB_1, down-regulated CB_2 and left unchanged TRPV2 and TRPV4. Pure THCV and pure CBDV counteracted LPS-induced up-regulation of CB_1 receptor, without affecting CB_2, TRPV2 or TRPV4 expression.

Conclusions: *Cannabis* extracts with high content in THCV and CBDV inhibit, with different mechanisms, nitrite production in macrophages. These results may have some clinical relevance for the possible clinical use of *Cannabis*-based medicines in inflammatory diseases.