Cannabis and Cannabinoid Research
Volume 4, Number 3, 2019

DOI: 10.1089/can.2019.29014.dpi

EDITORIAL

Waiting for the Entourage

Daniele Piomelli*

THE READERS OF *Cannabis and Cannabinoid Research* need no introduction to the "entourage effect." The phrase expresses the idea that chemical constituents of cannabis act in concert to modulate the effects of Δ^9-tetrahydrocannabinol (THC) and to influence the overall pharmacological properties of the plant.[a] In the words of Ethan Russo, a key proponent of the concept:

> Is cannabis merely a crude vehicle for delivery of THC? Might it rather display herbal synergy, encompassing potentiation of activity by active or inactive components, antagonism, summation, pharmacokinetic and metabolic interactions?[3]

These are interesting and valid questions, which Russo rephrased in the hypothesis that "selective breeding of cannabis chemotypes rich in ameliorative phytocannabinoid and terpenoid content offer complementary pharmacological activities that may strengthen and broaden clinical applications and improve the therapeutic index of cannabis extracts containing THC, or other base phytocannabinoids."[3]

A research program aimed at testing this hypothesis is feasible, at least in principle. Functional interactions between two or more biologically active agents are common in pharmacology and toxicology, and there are well-established ways of investigating them. For example, a graphic method called isobolographic analysis allows one to determine whether two compounds exert additive, synergistic (i.e., super-additive), or antagonistic effects when used in combination.[4,5] This method has been successfully applied to study, for example, interactions between THC and the non-psychoactive phytocannabinoid, cannabichromene,[6] as well as interactions between THC and individual opiate analgesics (reviewed in Nielsen et al.[7]). Similarly, methods to examine functional interactions among multiple drugs have been long available[8] and may be adapted to cannabis studies.

Researchers are starting to take up the challenge. Two articles in the current issue of *Cannabis and Cannabinoid Research* address the question of whether terpenoid constituents of cannabis might modify either positively or negatively the plant's pharmacological properties. Marina Santiago and her collaborators (at Macquarie University and the University of Sydney, Australia) focused their attention on six relatively abundant terpenes (α- and β-pinene, β-caryophyllene, linalool, limonene, and β-myrcene), asking whether these compounds might affect cannabinoid CB_1 or CB_2 receptor signaling via membrane potassium channels (Santiago et al., 2019). Using a mouse pituitary tumor cell line modified to express the human CB_1 or CB_2 receptor, these authors found no evidence that individual or combined terpenes hyperpolarize cells (a response mediated by potassium channel activation) or alter the ability of a synthetic cannabinoid agonist to do so. The title of their article unequivocally states their conclusion: "Absence of entourage: terpenoids commonly found in *Cannabis sativa* do not modulate the functional activity of Δ^9-THC at human CB_1 and CB_2 receptors."

In the second study, Hannah Harris and her colleagues at the University of Mississippi examined whether terpenes might contribute to the analgesic properties of cannabis by comparing, in mice, the effects of THC to those of a total cannabis extract or of two post-distillation fractions enriched in either volatile terpenes (including those investigated by Santiago and collaborators) or THC and other phytocannabinois (Harris et al., 2019). The results show that pure THC, the cannabinoid-rich fraction, and the total extract containing both cannabinoids and terpenes all elicited comparable reductions of nociceptive responses in the hot plate, tail flick, and abdominal writhing

Departments of Anatomy and Neurobiology, Biological Chemistry and Pharmacology, University of California, Irvine, Irvine, California.

*Address correspondence to: Daniele Piomelli, PhD, Departments of Anatomy and Neurobiology, Biological Chemistry and Pharmacology, University of California, Irvine, Gillespie Neuroscience Research Facility, Room 3101, Irvine, CA 92697-1275, E-mail: piomelli@uci.edu

[a] The phrase "entourage effect" was first introduced to describe putative pharmacological interactions between the endocannabinoid 2-arachidonoyl-*sn*-glycerol (2-AG) and other fatty acid glycerol esters produced in mammalian tissues.[1] Support for this hypothesis remains inconclusive.[2]

tests. In contrast, no such effect was seen with the fraction containing only volatile terpenes.

Despite these negative findings, it would be premature to discount the existence of pharmacodynamic or pharmacokinetic interactions among cannabis constituents. There are indeed several reasons why the search for such interactions should be continued. First, some properties of THC may well be modulated by other phytocannabinoids such as cannabidiol (CBD; reviewed in Boggs et al.[9]) or Δ^9-tetrahydrocannabivarin (reviewed in Morales et al.[10]). For example, the laboratory of Ken Mackie at Indiana University has shown that coadministration of CBD may counter the cognitive and emotional impairments caused in male mice by adolescent exposure to THC.[11] Second, a vast scientific literature stretching back to the early 20th century has demonstrated that terpenoids—including those found in cannabis—exert robust and diverse pharmacological effects in animals and humans (reviewed in Maffei et al.[12]). A case in point is β-caryophyllene, which has been shown to inhibit lipopolysaccharide-stimulated expression of pro-inflammatory cytokines in human whole blood and to attenuate carrageenan-induced edema in mice via direct activation of CB_2 receptors.[13] Lastly, and perhaps most intriguingly, the existence of an "entourage effect" might help to explain variable personal experiences with different cannabis varieties. To cite Russo again: "They [cannabis varieties] smell different. They taste different. They have different effects" (quoted in Chen[14]).

The last point should give us pause, however, and not only because personal experiences and data are rarely interchangeable. What should be of immediate concern to us as scientists is how a vocal sector of the cannabis industry interprets the "entourage effect" and attributes to it an altogether magical meaning. Here is a telling example (which I prefer to leave anonymous) from many that can be found online:

> "The Entourage Effect is Powerful. The compounds [cannabinoids and terpenoids] are beneficial on their own, but together they create a stronger, more effective product. The only way to truly unlock the full potential of the cannabis plant is to experience the entourage effect with balanced ratios of terpenes, CBD, and THC. That's why here at … we like to use the whole-plant by including THC, CBD, and terpenes in all of our products. Nothing truly compares to the full therapeutic power of the entourage effect."

This would be funny if it wasn't a transparent marketing ploy to peddle snake oil to naïve consumers with potential negative consequences to their health. Correcting these misconceptions is important and requires a concerted societal effort involving regulatory authorities, the media, and the scientific community. As an active part of that community, *Cannabis and Cannabinoid Research* will continue to do its part by publishing high-quality studies aimed at exploring the pharmacological interplay among different cannabis constituents.

References

1. Ben-Shabat S, Fride E, Sheskin T, et al. An entourage effect: inactive endogenous fatty acid glycerol esters enhance 2-arachidonoyl-glycerol cannabinoid activity. Eur J Pharmacol. 1998;353:23–31.
2. Murataeva N, Dhopeshwarkar A, Yin D, et al. Where's my entourage? The curious case of 2-oleoylglycerol, 2-linolenoylglycerol, and 2-palmitoylglycerol. Pharmacol Res. 2016;110:173–180.
3. Russo EB. Taming THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects. Br J Pharmacol. 2011; 163:1344–1364.
4. Berenbaum MC. What is synergy? Pharmacol Rev. 1989;41:93–141.
5. Tallarida RJ, Porreca F, Cowan A. Statistical analysis of drug–drug and site–site interactions with isobolograms. Life Sci. 1989;45:947–961.
6. DeLong GT, Wolf CE, Poklis A, et al. Pharmacological evaluation of the natural constituent of Cannabis sativa, cannabichromene and its modulation by Δ^9-tetrahydrocannabinol. Drug Alcohol Depend. 2010; 112:126–133.
7. Nielsen S, Sabioni P, Trigo JM, et al. Opioid-sparing effect of cannabinoids: a systematic review and meta-analysis. Neuropsychopharmacology. 2017;42:1752–1765.
8. Berenbaum MC. A method for testing for synergy with any number of agents. J Infect Dis. 1978;137:122–130.
9. Boggs DL, Nguyen JD, Morgenson D, et al. Clinical and preclinical evidence for functional interactions of cannabidiol and Δ^9-tetrahydrocannabinol. Neuropsychopharmacology 2018;43: 142–154.
10. Morales P, Hurst DP, Reggio PH. Molecular targets of the phytocannabinoids: a complex picture. In: Kinghorn AD, Falk H, Gibbons S, Kobayashi J, eds. Phytocannabinoids, unraveling the complex chemistry and pharmacology *of Cannabis sativa*. Springer: Cham, Switzerland, 2017.
11. Murphy M, Mills S, Winstone J, et al. Chronic adolescent Δ^9-tetrahydrocannabinol treatment of male mice leads to long-Term cognitive and behavioral dysfunction, which are prevented by concurrent cannabidiol treatment. Cannabis Cannabinoid Res. 2017;2:235–246.
12. Maffei ME, Gertsch J, Appendino G. Plant volatiles: production, function and pharmacology. Nat Prod Rep. 2011;28:1359–1380.
13. Gertsch J, Leonti M, Raduner S, et al. Beta-caryophyllene is a dietary cannabinoid. Proc Natl Acad Sci U S A. 2008;105:9099–9104.
14. Chen A. Some of the parts: is marijuana's "entourage effect" scientifically valid? Sci Am., April 20, 2017.

Cite this article as: Piomelli D (2019) Waiting for the entourage, *Cannabis and Cannabinoid Research* 4:3, 137–138, DOI: 10.1089/can.2019.29014.dpi.

Abbreviations Used

CBD = cannabidiol
THC = Δ^9-tetrahydrocannabinol