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EDITORIAL

The curative effect of a cannabinoid 2 receptor agonist on functional failure and disruptive inflammation caused by intestinal ischemia and reperfusion

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This editorial refers to the article 'The curative effect of a cannabinoid 2 receptor agonist on functional failure and disruptive inflammation caused by intestinal ischemia and reperfusion' by Sait Bayram et al. published in Fundamental and Clinical Pharmacology.

As we learn more about the endocannabinoid system (ECS), our understanding and grasp of the system's ubiquitous presence is expanding. In light of this, there is also a growing body of evidence for the therapeutic potential of ECS modulation in a range of clinical situations. Strategies include manipulation of the cannabinoid 1 (CB1) receptor, mostly in terms of Central Nervous System (CNS) processes, and activation of the cannabinoid 2 (CB2) receptor as an anti-inflammatory target. We know that the production of endocannabinoids is increased during inflammatory states, in particular by activated immune cells [1]. Several lines of evidence confirm an overall anti-inflammatory effect following activation of the CB2 receptor, with actions through the inhibition of cytokine and chemokine production, induction of apoptosis, as well as through inhibition of cell proliferation and activation of regulatory T cells [2]. This immunomodulatory action is, however, both complex and situation-dependent. Nevertheless, there is promising pre-clinical evidence in various models, such as those of neuroinflammation, sepsis, arthritis, and vascular inflammation [3,4].

During ischemia/reperfusion (I/R) injury, initial tissue damage is based on the duration of ischemic insult. Subsequent reperfusion leads to further damage via various mechanisms, which may be greater in magnitude than damage from the initial insult [5]. Mitochondrial perturbations, which occur during ischemia, are followed by a range of inflammatory mechanisms that are triggered by reperfusion. These include the release of pro-inflammatory cytokines, activation of complement, the recruitment of immune cells, and the generation of reactive oxygen species [6]. Specifically, in the case of the intestine, I/R injury is seen commonly in the setting of certain surgical procedures including abdominal aortic aneurysm repair, cardiopulmonary bypass, and intestinal transplantation, in addition to conditions such as strangulated hernias [7]. In fact, the intestine is likely one of the most ischemia-sensitive tissues [6]. Considering that the mortality rate in patients with acute mesenteric ischemia exceeds 60% [8], and that a subset of these patients will have had restoration of perfusion (and thus potentially experience an I/R injury), a clinical need exists in addressing potential strategies aimed at minimizing damage and promoting gastrointestinal function.

One such potential therapeutic strategy would aim to minimize damaging inflammation which occurs secondary to reperfusion. No ECS (CB2)-targeted strategies are currently used in the setting of intestinal ischemia and so this would represent a novel therapeutic approach. Further, additional benefit may be achieved through strategies that function to increase intestinal motility and improve intestinal barrier function.

When considering CB2 receptor-based immunomodulatory strategies, timing of administration becomes a major consideration. Timing may optimize drug efficacy in some situations, while in others may be an important safety consideration. For example, in conditions involving a waxing and waning immune status, such as sepsis, poorly timed therapy could lead to worsening of periods of immunosuppression. It is also important to note that situations exist where direct damage from inflammatory processes must be limited; however, at the same time, some immune function must be preserved to manage infection and mediate tissue repair.

In the setting of acute intestinal ischemia, CB2 agonists could likely be administered at first recognition of the ischemia or thereafter. Theoretically, if the drug was administered a significant time before restoration of blood flow, serum levels may decline past therapeutic concentrations by the time that perfusion is restored to the intestine. At the same time, this strategy may act as a pre-treatment which accesses circulating immune cells which could prime them toward a less robust response once perfusion is restored. Another consideration is that not all cases of intestinal ischemia develop acutely and may not involve specifically an I/R injury, but rather an extended period of ischemia secondary to decreased (but not absent) perfusion. Modulation of the CB2 receptor is non-psychotropic and is not associated with the known CNS effects of cannabinoids and *Cannabis* (which instead act in part via activation of central CB1 receptors). Regarding safety, it is worth highlighting that the administration of CB2 agonists as proposed in the case of I/R injury would likely be short-term.

Bayram et al. [9] conducted a study in male Wistar rats subjected to an intestinal ischemia/reperfusion (I/R) injury via a 30-min occlusion of the superior mesenteric artery followed by perfusion for 150 min. The animals were treated with an intravenous infusion (5 min prior to arterial occlusion) of the CB2 receptor agonist, AM-1241, at three doses, or the CB2 receptor agonist, AM-1241, at high dose along with a CB2 receptor antagonist (JTE-907), in addition to sham and vehicle treatments. Muscular activity in the terminal ileum was assessed and significantly decreased following I/R injury (vs. sham), with high-dose CB2 receptor agonist treatment leading to significant restoration of contractility (vs. vehicle). Levels of the oxidative stress marker malondialdehyde and the neutrophil activity marker myeloperoxidase were significantly higher in I/R injury groups (vs. sham), and treatment with high-dose CB2 agonist led to a significant decrease in levels of these markers (vs. vehicle). Conversely, I/R injury led to depletion of the antioxidant glutathione (vs. sham), and treatment with high-dose CB2 agonist led to a significant restoration of glutathione levels (vs. vehicle). All effects of high-dose CB2 receptor agonist were reversed with co-administration of the CB2 receptor antagonist.

The authors acknowledge that conflicting evidence exists regarding their selected CB2 receptor agonist used in the current study (AM-1241), with some studies indicating action as a CB2 receptor inverse agonist in rats. In our opinion, selection of cannabinoid receptor modulators should aim to use well-characterized compounds with documented binding profiles specific to the species of interest, or risks weakening arguments drawn from experimental results. As an example, the most widely used CB2-selective agonist is JWH 133, and similarly for AM630 or SR144528 as receptor antagonists [10]. Given this, the current study may have benefited from addition of a CB2 receptor inverse agonist group, as well as validation of findings through addition of a well-characterized CB2 receptor agonist group.

The paper by Bayram et al. assessed markers of intestinal function and of oxidative stress response observed in I/R injury. Future studies in this model could benefit from a broader assay of the drug's immunomodulatory actions. Specifically, a wider net could be cast for cytokines/chemokines and cellular adhesion molecules, in addition to adding histological/immunohistochemical analysis and in vivo imaging techniques. Imaging techniques such as intravital microscopy can enable real-time quantification of leukocyte adherence to the endothelium of intestinal vessels, in addition to shedding light on microcirculatory function. Of note, some limitations exist with models of intestinal I/R. Kalogeris et al. highlight that establishing the 'point of no return' for damage due to ischemic insult is more difficult with intestinal ischemia than for other organs [5]. Further, it has been described that occlusion of the superior mesenteric artery, as performed in the study by Bayram et al., leads to a gradient of ischemia along the length of the gut, though the distal small intestine (sampled by Bayram et al.) is most affected by ischemia [11]. Further establishing this intestinal I/R model could benefit from quantification of CB2 receptor expression, such as through quantitative PCR of the receptor's mRNA, as previously described in intestinal inflammatory models [12].

Taken together, the data presented by Bayram et al. support a role for CB2 receptor activation as a potential therapeutic strategy in the setting of I/R injury of the intestine. This is in line with previous studies examining the role of the CB2 receptor in I/R injury in organs such as the liver [13] and heart [14], which demonstrate decreases in various parameters of inflammation and conclude that this pharmacologic strategy is likely protective. In the clinical realm, the ECS is a relatively untapped target with considerable potential. There is strong evidence to support targetting the CB2 receptor as an anti-inflammatory strategy, and it stands to reason that this may be a logical approach in the setting of intestinal I/R injury due to the considerable inflammatory component.

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