Cannabinoide für therapeutische Verwendung in der Atherosklerose

Summary

Atherosclerosis remains the primary cause of heart disease and stroke that causes about 50% of all deaths in Western countries. The identification of promising novel anti-atherosclerotic therapies is therefore of great interest and represents a continued challenge to the medical community.

Cannabinoids, such as Δ⁹-tetrahydrocannabinol (THC), the major psychoactive compound of marijuana, their synthetic analogs and endogenous cannabinoid ligands, produce their biological effects by interacting with specific receptors. In a mouse model of atherosclerosis, we have recently shown that THC inhibits disease progression through pleiotropic effects on inflammatory cells. Blocking of cannabinoid receptor CB₂, the main cannabinoid receptor expressed on immune cells, abolished the observed effects. The potential therapeutic benefit is in conflict with the known health risks of marijuana use, as THC also binds to and activates neuronal CB₁ cannabinoid receptors. Besides its well known neurobehavioral effects, THC also mediates cardiovascular effects such as vasodilation and hypotension. The development of novel cannabinoid receptor ligands that selectively target CB₂ receptors and are devoid of adverse effects might overcome this problem. In addition, pharmacological modulation of the endocannabinoid system might also offer a new therapeutic strategy in the treatment of atherosclerosis. Several reports demonstrating an implication of the endocannabinoid system in different inflammatory conditions support this hypothesis.

Key words: atherosclerosis; chronic inflammation; cannabinoids

Zusammenfassung

Atherosklerose (Arteriosklerose) stellt nach wie vor die Hauptursache für Herzkrankungen und Schlaganfall dar und ist für etwa 50% aller Todesfälle in der westlichen Gesellschaft verantwortlich. Ein großes Interesse für die Medizin besteht daher in der Entwicklung neuer anti-atherosklerotischer Therapien.


Schlüsselwörter: Atherosclerosis; chronische Entzündung; Cannabinoid
Introduction

The discovery of membrane receptors that bind the psychoactive compound of marijuana, Δ9-tetrahydrocannabinol (THC) and their endogenous ligands has led to the description of the endocannabinoid system [1–5]. Within the last years, a whole signaling system has been identified, composed of the two known receptors, endogenous ligands and enzymes for ligand biosynthesis and inactivation [6]. All endocannabinoids identified so far are derivatives of long-chain polyunsaturated fatty acids and exhibit varying selectivity for the two cannabinoid receptors [7]. In the past few years, many different regulatory actions have been attributed to endocannabinoids, and their involvement in several pathophysiological conditions is subject of ongoing investigations. Consequently, the endocannabinoid system represents an attractive target for drug developing pharmaceutical companies.

Cannabinoid receptors

Both cannabinoid receptors are G protein-coupled receptors that modulate second messengers and signaling components such as adenylate cyclase [8], mitogen-activated protein kinases [9] or members of the NF-κB family [10, 11]. The tissue distribution of the two receptors is likely to account for the well-known psychotropic and peripheral effects of THC. Cannabinoid receptor 1 (CB₁) is expressed predominantly in the central and peripheral nervous system, while cannabinoid receptor 2 (CB₂) is present on immune cells [12]. Thus, CB₂ receptors may have physiological importance in immune response, inflammation and chronic pain [13]. So far, the presence and function of CB₂ receptors in central nervous system (CNS) neurons were controversial. However, a recent study demonstrates the expression of functional CB₂ receptors on brainstem neurons [14]. Substantial evidence further suggests the presence of the endocannabinoid system in liver, pancreas and adipocyte tissue, indicating its regulatory role in metabolic functions [15–20]. A recent study demonstrating endocannabinoid signaling in gingival tissue and receptor upregulation in response to inflammatory stimulation further indicates a modulatory function of the endocannabinoid system in periodontal inflammation [21]. In addition, CB₂ receptors have been implicated in bone mass regulation [22–24].

Moreover, there is emerging evidence suggesting that some cannabinoid effects are not mediated by either CB₁ or CB₂ receptors, which implicates additional receptors involved in these actions [25]. These include the transient receptor potential channels of type V₁ (TRPV₁), also known as vanilloid VR₁ receptors [26] as well as peroxisome proliferator-activated receptor (PPAR) gamma [27, 28].

The fact that CB₁ and CB₂ are differentially expressed depending on the cell differentiation and activation status may represent a major mechanism by which the endocannabinoid system is involved in immune functions. Indeed, stimuli such as phytohemagglutinin (PHA), lipopolysaccharide (LPS), phorbol myristate acetate (PMA), cytokines or mitogenic antibodies have been reported to regulate the expression of CB₁ and CB₂ [13].

Cannabinoids and immunomodulation

The development of selective agonists, antagonists, and transgenic mice lacking CB₁ and CB₂ receptors has contributed to broaden our current understanding of cannabinoid biology. As a consequence, the capacity of cannabinoids to regulate immune function is now well established. In vitro, THC treatment of human immune cells inhibits secretion of proinflammatory cytokines and chemokines and triggers the differentiation into a Th2 phenotype [29, 30]. As demonstrated, a CB₂-specific antagonist abrogates the majority of these immunomodulatory effects [30]. Moreover, THC-mediated inhibition of T helper cell activation is absent in CB₂-deficient mice, supporting the hypothesis that the immunomodulatory effects of cannabinoids are CB₂-dependent [31].

Cannabidiol, the major non-psychotropic constituent of the Cannabis sativa plant, has been reported to ameliorate chronic inflammation in murine collagen-induced arthritis, a mouse model of rheumatoid arthritis, by inhibiting antigen-specific lymphocyte proliferation and IFN-γ secretion [32]. Several reports described beneficial effects of cannabinoids in experimental animal models of multiple sclerosis. These effects not only affected tonic control of spasticity, but also inflammatory responses in the spinal cord [33, 34]. Interestingly, two studies employing selective inhibitors of endocannabinoid cellular uptake demonstrated improved motor function and diminished inflammatory responses in a mouse model of multiple sclerosis [35, 36]. By preventing the uptake and thus degradation of...
endocannabinoids, the inhibitors enhance their half-life in vivo. In both studies, the authors observed a decreased expression of major histocompatibility complex (MHC) class II antigen, nitric oxide synthase and proinflammatory cytokine expression.

**Effects of THC on atherosclerosis**

Encouraged by the vast number of studies demonstrating immunomodulatory properties of cannabinoids, we recently tested the anti-atherosclerotic potential of THC in a murine model [37]. In our study, we used the apolipoprotein E knock out (ApoE−/−) mouse model. These mice rapidly develop hypercholesterolemia and atherosclerotic lesions when fed a high cholesterol diet for only a few weeks. We found that THC inhibited progression of established atherosclerotic lesions (fig. 1). This was associated with reduced proliferation and IFN-γ secretion of lymphoid cells as well as reduced macrophage infiltration into atherosclerotic lesions. Moreover, we detected CB2 receptor expression within human and mouse atherosclerotic lesions (fig. 2). In vitro, we observed that THC inhibited macrophage chemotaxis in response to MCP-1 and reduced expression of the chemokine receptor CCR2. Importantly, these effects were blocked by a specific CB2 receptor antagonist [38]. It is particularly noteworthy that the observed in vitro and in vivo effects of THC were dose-dependent. The dose dependency showed a U-shaped curve, where both higher and lower doses were inactive. The effective dose was lower than the dose usually associated with psychotropic effects of THC. However, it is difficult to translate our findings obtained in the used apolipoprotein E knock out (ApoE−/−) mouse model of atherosclerosis to humans. We found very low nanomolar concentrations in blood serum of THC-treated mice, which might be a consequence of local THC storage within fat tissue, as cannabinoids are known to be very lipophilic. Indeed, several animal experiments have demonstrated that the instant uptake and unlimited storage of THC by neutral fat limits the molecular concentration of the drug present in plasma [39–41]. The hypercholesterolemic ApoE−/− mouse model is characterised by a strong accumulation of fat tissue, especially within the vessel wall. Thus, THC might be stored within atherosclerotic lesions, resulting in high local concentrations at inflammatory sites. Additional experiments are warranted to clarify whether local accumulation of THC contributes to the anti-atherosclerotic effect, and whether similar THC concentrations may be effective in humans.

**Potential role of endocannabinoids in atherosclerosis**

Today, it remains unclear whether receptor signaling via endocannabinoids plays a modulatory role in chronic inflammation ongoing during atherogenesis. Several reports demonstrating an implication of the endocannabinoid system in different inflammatory conditions support this hypothesis. For example, a recent study demonstrates that CB2 receptors mediate intrinsic protective signals that counteract proinflammatory responses in a mouse model of colonic inflammation [42]. A different report provides evidence for the involvement of CB2 receptor signaling in cutaneous inflammation [43]. Furthermore, endocannabinoid signaling has been implicated in periodontal inflammation, as both cannabinoid receptors CB1 and CB2 were upregulated under pathological conditions [21]. Finally, two studies have shown that pharmacological modulation of the endocannabinoid system to increase the half-life of endocannabinoids might have a therapeutic potential for the treatment of multiple sclerosis [35, 36].

To clarify the role of the endocannabinoid system during atherosclerosis, additional
studies employing selective CB₁ and CB₂ receptor antagonists or cannabinoid receptor deficient mice are warranted.

**Cardiovascular effects of cannabinoids**

Although cannabinoids may be of therapeutic use for the treatment of atherosclerosis, these effects are in conflict with the known adverse effects associated with marijuana consumption. Indeed, the bioactive constituents of the marijuana plant and their synthetic and endogenous analogs cause not only neurobehavioral, but also cardiovascular effects such as vasodilation and hypotension [44–47]. However, targeting the endocannabinoid system may also offer novel therapeutic strategies in the treatment of hypertension [48]. Recently published clinical trial reports to test the effectiveness of the CB₁ receptor blocker rimonabant as an antiobesity drug have shown that it also had a significant effect on lipid parameters and several other cardiovascular risk factors [49, 50].

*Figure 2*
The cannabinoid receptor CB₂ is expressed in human and mouse atherosclerotic plaques. Representative cryosections of human coronary atherosclerotic lesion (A), normal carotid artery from wild-type mouse (B), aortic arch atherosclerotic lesion from apoE⁺⁻ mouse (C, D), aortic root atherosclerotic lesion from apoE⁺⁻ mouse (E, F). Sections were immunolabeled with an anti-CB₂ receptor antibody (A, B, C, E), or with secondary antibody only (D, F).
Underlying mechanisms of the cardiovascular cannabinoid actions may involve not only activation of cannabinoid receptors on peripheral nerves, but also signaling via receptors located in the vascular wall [45]. The presence of CB receptors on vascular smooth muscle and endothelial cells as well as experiments performed with isolated arteries provide evidence for this hypothesis [44, 51–53].

Non-psychoactive cannabinoid receptor ligands for therapeutic use

Besides the risk of unwanted cardiovascular effects, a broad acceptance of cannabinoids as therapeutic agents is hampered by the fact that they exhibit psychotropic effects. Therefore, particular research interest is focusing on the development and characterisation of either synthetic or plant derived cannabinoids with therapeutic value that are non-psychoactive [54]. Several non-psychoactive synthetic cannabinoids with anti-inflammatory properties have recently been developed from plant cannabinoids. Their anti-inflammatory properties suggest that CB ligands may serve as novel immunomodulatory agents in the treatment of immune disorders such as atherosclerosis. However, little is known about the molecular mode of action of these compounds and requires further investigation.

Conclusion

A growing body of evidence suggests a broad therapeutic potential of cannabinoids for a variety of conditions. Nevertheless, the medical use has been very limited in the past, mainly due to the psychotropic effects associated with marijuana use. Now that non-psychoactive cannabinoids become available, it is essential to investigate in more detail the pharmacological and biological activities of these drugs to identify the most powerful and selective agents for therapeutic use. In particular, several newly described synthetic CB ligands with immunomodulatory properties may serve as novel therapeutic agents in the treatment of immune disorders such as atherosclerosis. The recent demonstration that THC mediates anti-atherosclerotic effects in a mouse model via CB receptor-dependent mechanisms suggests that CB activation may attenuate atherosclerosis progression.

References

Cannabinoid receptors regulate Ca(2+) signals and insulin secretion in pancreatic beta-cells. Cell Calcium 2006;39: 155-62.

21 Nakajima Y, Furuschi Y, Biswas KK, Hashiguchi T, Kawa
hara K, Yama i, et al. Endocannabinoid, anandamide in gingival tissue regulates the periodontal inflammation throu

22 Idris AI, van ’t Hof RJ, Greig IR, Ridge SA, Baker D, Ross


25 Begg M, Pacher P, Batkai S, Osei-Hyiaman D, Offertaler L, Fo

26 Zygmunt PM, Petersson J, Andersson DA, Chuang H, Sor
gard M, De Marzo V, et al. Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. Na
ture 1999;400:452–7.


31 Buckley NE, McCoy KL, Mezei E, Bonner T, Zimmer A, Felder CC, et al. Immunomodulation by cannabinoids is abs

32 Mullaiti AM, Gallily R, Sumariwalla PF, Malik AS, An


