The Role of Arachidonic Acid Metabolites (Endocannabinoids and Eicosanoids) in the Immune Processes: A Review

Yulia K. Denisenko, Elena G. Lobanova, Tatyana P. Novgorodtseva, Tatyana A. Gvozdenko, Anna V. Nazarenko

Vladivostok Branch of Far Eastern Scientific Center of Physiology and Pathology of Respiration – Research Institute of Medical Climatology and Rehabilitation Treatment, Vladivostok, Russia

Email address
denisenko.imkvl@gmail.com (Y. K. Denisenko)

Citation

Abstract
This review is devoted to the modern concepts of arachidonic acid metabolites – endocannabinoids and eicosanoids. We discussed their biosynthetic pathways, the interaction mechanisms and the role in the immune response. The results of our research on the endocannabinoid system and on the role of eicosanoids in the inflammatory process are given. We found that the synthetic ligands of cannabinoid receptors (WIN 55,212-2 and anandamide) dose-dependently inhibit the lipoxygenase pathway of the pro-inflammatory leukotriene B4 and the ability of the immune cells to express IL-2, IL-8, and TNF-α. The activities of the immune and the endocannabinoid systems have the reciprocal relationship which is characterized by the decrease in the CB2 receptor expression and the activation of immune mechanisms. The obtained results on the role of endocannabinoid system in the regulation of the immune response are contributing to the understanding of molecular and cell mechanisms of inflammation development.

1. Introduction

Recently, various signaling pathways regulating the immune response have attracted considerable attention from researchers. The special mediators – endocannabinoids and eicosanoids – play an important role in the cellular and molecular mechanisms of the immune system functioning. These mediators belong to a group of biologically active substances of lipid nature, performing a variety of functions in the body [1-6]. Eicosanoids, their enzymes and receptors are known for their role in the development of many chronic diseases [7-10]. Also, there are some limited data on participation of the endocannabinoid system in the immune processes [11-14].

The substrate for the biosynthesis of eicosanoids and endocannabinoids is formed from phospholipids (PL) containing arachidonic acid (AA) (Fig. 1) [15-17].

Fig 1. Arachidonic acid.
Moreover, endocannabinoids and eicosanoids use the same enzyme systems in metabolism [17]. Taking this into account, we can assume a potential relationship between the signal function of endocannabinoids and eicosanoids, their joint role in the immune processes. However, the attempts to analyze the functions and interactions of these signaling systems led researchers to an ambiguous conclusions. Many questions about the mechanisms of the relationship between the endocannabinoid and eicosanoid-derived second messengers and their functioning in health and disease remain open. This review is devoted to the modern concepts of arachidonic acid metabolites - endocannabinoids and eicosanoids, their biosynthetic pathways, the interaction mechanisms and the role in the immune response.

2. Eicosanoid Synthesis

The nature of eicosanoids was determined in 1957, when S. Bergström isolated two new substances from sheep prostate glands and called them prostaglandin F and prostaglandin E (PG). The term prostaglandin was firstly introduced by U. von Euler who won the Nobel Prize in Physiology or Medicine in 1970 for his work on neurotransmitters. In 1964 S. Bergström and D.A. van Dorp proved that the precursor of prostaglandins is an arachidonic acid released from phospholipids [18, 19]. In 1976, Salvador Moncada reported finding of a substance that prevents formation of blood clots and is able to dilate blood vessels. This substance was named prostaglandin I, or prostacyclin.

The eicosanoids may be regarded as a large group of molecular mediators, synthesized mainly from arachidonic acid. There are three pathways of AA oxidation: with participation of membrane-bound cyclooxygenase (COX), cytoplasmic lipoxigenases (LOX) and epoxygenases (cytochrome P450-like enzymes) [20, 9, 6, 21]. Depending upon the enzymes involved in the eicosanoid synthesis, we distinguish prostanoids (prostaglandins and thromboxanes - TX), which are metabolized by COX, and leukotrienes (LT), formed under the effect of LOX [22, 23, 24, 25] (Fig. 2).

The eicosanoid synthesis via cyclooxygenase begins with the addition of two oxygen molecules to AA and formation of unstable intermediates - endoperoxides. Further, as a result of successive reactions, the endoperoxides can be converted into
prostaglandin-I$_2$ or prostacyclin (via prostaglandin-I$_2$-synthase) or into thromboxane A$_2$ (via thromboxane-A$_2$-synthase) (Fig. 3). Two isoforms of COX are distinguished: COX-1 and COX-2. It is believed that COX-1 is a constitutive form of COX that regulates the homeostatic functions, whereas the expression of COX-2 occurs during inflammation [25]. The association of COX with an inflammatory response has led to the development of COX-selective inhibitors. Both COX isoforms are inhibited by non-steroidal anti-inflammatory drugs such as aspirin, ibuprofen and indomethacin.

![Fig 3. Cyclooxygenase and lipoxygenase in the arachidonic acid cascade.](image)

**3. Synthesis of Endocannabinoids**

Scientific interest in the cannabinoids increased in the 1960s, when ∆9-tetrahydrocannabinol (THC), a component of cannabis, was discovered [27]. In 1988, Devane with coworkers succeeded to detect in the rat brain tissue some specific binding sites for THC, the so-called cannabinoid receptor type 1 (CB$_1$) [28]. CB$_1$ was cloned by Matsuda et al. in 1990 [29]. The cannabinoid receptor type 2 (CB$_2$) was identified in 1993[30]. Subsequently, it was shown that the cannabinoid CB$_1$ receptors are localized in the structures of the peripheral and central nervous system and involved in the processes of conducting and perception of nociceptive signals. CB$_2$ receptors are expressed primarily on immune modulating cells, where they mediate an immunomodulatory effect [30-32].

The identification of the cannabinoid receptor ligands was the next stage in the study of the mechanisms of cannabinoids action. In 1992, it was shown that the ethanolamide of arachidonic acid (arachidonoyl ethanolamide) or anandamide is an endogenous ligand of the cannabinoid receptors [28] (Fig. 4a). In 1995, the second cannabinoid receptor ligand, 2-arachidonoylglycerol (2-AG), was discovered [33, 34] (Fig. 4b).

![a) anandamide.](image)
The main biosynthetic pathway for anandamide begins with N-arachidonoyl phosphatidylethanolamine metabolized by N-acyl-phosphatidylethanolamine-hydrolyzing phospholipase D (NAPE-PLD) [35] (Fig. 5).

4. The Relationship of the Metabolic Pathways in the Biosynthesis of Endocannabinoids and Eicosanoids

Previously, it was believed that the metabolisms of eicosanoids and endocannabinoids occur independently of one another. This suggested an involvement of unrelated enzyme systems. However, phospholipases initiating the biosynthesis of eicosanoids and endocannabinoids are activated by identical secondary messengers (e.g., through an increase in the level of intracellular Ca\(^{2+}\)). Very probably, this may cause simultaneous initiation of the formation of signaling molecules [37, 38, 39]. Hampson et al. were the first to demonstrate the ability of LOX-12 to use either arachidonic acid or anandamide as a substrate [40]. The anandamide metabolizes to 12-hydroxyeicosatetraenoic acid ethanolamide (12-HETE-EA) via LOX-12. Besides, LOX-12 uses both AA and anandamide as a substrate with the same efficiency. The ability to metabolize anandamide was revealed also for LOX-15 [41]. Yu et al. have shown the ability of COX-2 (but not COX-1) to oxidize fatty acids ethanolamides [42]. The experiments with carrageenan-induced paw edema in rats showed that inhibition of COX-2 results in the accumulation of anandamide. This proved participation of COX-2 in endocannabinoid oxygenation. There is evidence that the converted 2-AG and anandamide are not ligands for the
cannabinoid receptors in COX-2 reactions. Oxidized COX-2- or LOX-endocannabinoids exert biological activity via specific receptors. However, only receptors for prostanoid ligands are characterized [43]. The identification of the specific receptors for oxidized endocannabinoids is a main task in clarifying the mechanisms of their action. In future, the determination of their physiological role and functioning at various pathological conditions, and, at last, the identification of the specific receptor blockers for oxidized endocannabinoid ligands will provide new pharmacological directions and ways for therapeutic strategies. The available evidence supports the hypothesis that the oxygenation provides a mechanism for modulating the activity of the endocannabinoid system. A decrease in the level of anti-inflammatory endocannabinoids might be one of mechanisms for the pro-inflammatory action of COX-2.

The first evidence of the affinity of anandamide and cytochrome P450 was obtained in 1993 [44]. It was shown that cytochrome P450 3A and 2D6 are able to metabolize anandamide. There is no data on involvement of cytochrome P450 in degradation of 2-AG.

It is known that cannabinoids, and anandamide in particular, stimulate secretion of arachidonic acid and its metabolites [45, 46]. As noted above, anandamide hydrolysis produces free AA, which can be converted to eicosanoids in cyclooxygenase or lipoxygenase reactions (Fig. 7, path A). Furthermore, endocannabinoids themselves can be exposed to the action of COX or LOX, which also leads to the formation of eicosanoids (Fig. 7, path B) [13]. Consequently, the source of AA for the eicosanoid synthesis may be not only AA-containing phospholipids but also cannabinoids. Perhaps, both reactions play an equivalent role in the synthesis of eicosanoids.

We have studied the dose-dependent effect of the synthetic cannabinoid receptor ligands WIN 55,212-2 and anandamide on the synthesis of eicosanoids – leukotriene B₄ and thromboxane B₂ – in blood cells in vitro [47]. It has been established that the synthetic ligands of cannabinoid receptors dose-dependently inhibit the lipoxygenase pathway of the pro-inflammatory leukotriene B₄ – and thus suppress the activity of immune system. Low concentrations of WIN 55,212-2 and anandamide (0.1 and 1.0µM, respectively) had no effect on the levels of leukotriene B₄. The administration of 3.0 µM WIN 55,212-2 and anandamide reduced the levels of leukotriene B₄. The greatest inhibitory effect on the
production of pro-inflammatory eicosanoids by blood cells was detected at 10.0 µM concentration. So, the production of leukotriene B4 was reduced by 83% (p<0.001) after 10.0 µM WIN55,212-2 and by 67% (p<0.001) after 10.0 µM anandamide. Consequently, 10.0 µM WIN55,212-2 inhibit the synthesis of leukotriene B4 in blood cells at a greater extent than anandamide. However, WIN 55,212-2 and anandamide had no effect on the synthesis of thromboxane B2 by blood cells.

The obtained experimental data suggest that the synthetic cannabinoids are able to inhibit the lipoxygenase pathway of eicosanoid biosynthesis, and thus inhibit the formation of the proinflammatory mediator – leukotriene B4. Apparently, the mechanisms of thromboxane synthesis do not involve the endocannabinoid system. S. Diaz [48] demonstrated the ability of cannabinoids (Δ9-tetrahydrocannabinol) to activate the metabolism of arachidonic acid by the lipoxygenase pathway. Though this effect is realized only at sufficiently high concentrations of the endocannabinoids (32.0µM), which is not realistic to implement under physiological conditions. The controversial data on the effect of endocannabinoids on fatty acid metabolism and eicosanoid synthesis is due to different experimental doses and chemical structure of cannabinoids. In addition, the effect of the synthetic cannabinoid receptor ligands on the synthesis of proinflammatory mediators has a pronounced dose-dependent character. These studies demonstrate the ability of the endocannabinoid system to regulate the activity of the synthesis of pro-inflammatory eicosanoids.

An evident relationship of enzymatic pathways of the biosynthesis of arachidonic acid metabolites suggests their functional overlapping.

5. The Role of Arachidonic Acid Metabolites in Immune Processes

In contrast to cannabinoids, the biological effects of eicosanoids were discovered simultaneously with the establishment of their structure. It is known that the eicosanoids are secondary messengers of hydrophilic hormones. They control smooth muscle contraction (blood vessels, bronchi, uterus), are involved in the release of the products of intracellular synthesis (hormones, mucoids). Eicosanoids affect bone tissue metabolism, the peripheral nervous system, the immune system, the movement and aggregation of cells (leukocytes and thrombocytes), nociceptive processing. They can act as local bioregulators for cells synthesizing them (autocrine effect) and for neighboring cells (paracrine effect) by binding to membrane receptors. In some cases, their action is mediated by cAMP and cGMP [9, 49, 10].

Under pathological conditions, the levels of eicosanoids increase dramatically [10]. In chronic inflammatory diseases (bronchial asthma, rheumatoid arthritis, ulcerative colitis, psoriasis, eczema, etc.) the leukocytes accumulated in the focus of inflammation express great amounts of various enzymes, including phospholipase A2. This causes a release of AA, rapidly metabolizing to eicosanoids. Eicosanoids, in their turn, cause an increased vascular permeability, exudation of the liquid portion of blood into the extracellular space, and tissular edema, increased blood flow and tissue hyperemia, fever, and an increased migration of leukocytes into the focus of inflammation—all this causes even a greater release of phospholipase A2, and further maintenance of inflammation.

Eicosanoids play an important role in the development of non-specific systemic inflammatory reaction [8]. So, the leukotrienes are mediators of the allergic and inflammatory processes, and they are synthesized directly by leukocytes. An increased leukotriene synthesis occurs mainly at immediate-type allergic reaction sand begins after the binding of antigen to IgE, presented on these cells’ surface[26, 7]. The biological action of leukotriene B4 is mediated by the B-LT-receptor [7].

Prostaglandin D2 (PGD2) is the first product of the arachidonic acid oxidation by the cyclooxygenase pathway, which plays a key role in immediate type allergic reactions and inflammation [19]. It is formed primarily in mast cells. The advent of prostaglandin D2 in the serum indicates the degranulation of mast cells and the development of the early phase of immediate allergic reactions. The intradermal administration of PGD2 causes vasodilation and increased vascular permeability, which leads to the persistent redness, blister formation and the exit of leukocytes, lymphocytes and monocytes from the vascular bed [50]. The inhalation of PGD2 causes bronchoconstriction, indicating an important role of this AA metabolite in the pathogenesis of anaphylactic reactions and systemic mastocytosis.

The biological functions of endocannabinoids are also very diverse. Endocannabinoids regulate the nervous, endocrine, sexual, and immune systems; they are involved in the coordination of movements, in the maintenance of a constant body temperature, and in the formation of memory and appetite processes [51-53, 1, 3, 54-57].

The suppression of the immune response by natural cannabinoids has been shown in several studies [48, 58]. Cannabinoids reduce the overall resistance to bacterial and viral infections, lymphocyte proliferation, antibody synthesis, the activity of natural killer cells and macrophages. At the molecular level, these effects are explained by decreased synthesis of interferon, the tumor necrosis factor-α (TNF-α), and interleukin (IL-2) [12, 59]. Rockwell et al. showed that 2-AG anandamide inhibit the secretion of IL-2 inactivated T-cells. At the same time, it was shown that natural cannabinoids cause an increase rather than a decrease in the supernatant IL-1 activity and increased proliferation of B-lymphocytes [13]. All these data suggest a complex nature of modulation of the immune response by cannabinoids.

CB2 receptor ligands inhibit the signaling pathway, triggering activation of the toll-like receptor complex CD14 / TLR4 / MD2, that leads to expression of proinflammatory cytokines (IL-1β, IL-6, IL-8 and TNF-α) and induction of TH1 immune response [17].

Our in vitro studies on the effect of WIN 55,212-2 and
anandamide synthetic cannabinoid receptor ligands on cytokines synthesis by the immune competent cells [60-62] have shown that cannabinoids inhibit the ability of the immune cells to express IL-2, IL-8, and TNF-α. The revealed effects indicate the immunomodulatory ability of cannabinoids. At the same time, the question remains open: what mechanism is responsible for the regulation of the immune system, whether the immune system is controlled through a direct effect of cannabinoids on the synthesis of pro-inflammatory mediators or through an indirect impact via cannabinoid CB receptors? Because it has been reported that cannabinoids implement their many effects via a receptor-independent pathway [63].

As it was noted above, the endocannabinoid system contains receptors of two main types: CB1 receptors that are mostly expressed in the brain and spinal cord and are responsible for the motor and cognitive functions, and, secondly, the cannabinoid CB2 receptors [28, 30]. According to the literature data, it is known that the CB2 receptors are mainly localized in the membranes of the immune cells, where they mediate an immunoregulatory effect [11]. Our research has also shown that CB2 receptors are located on immune cells. But CB1 receptors have not been found on cells of the immune system. A flow cytometric analysis of the CB2 receptor in immune competent cells showed that the normal content of CB2 receptor is not below 90% [64].

To determine the role of the CB2 receptor in the regulation of the immune response, we simulated the conditions of activation of the immune system. The immune system was stimulated in vivo by intracorporeal irradiation of the blood and by enrichment of the blood with an ozone-oxygen mixture. The induction of the immune system cells in vivo by intracorporeal irradiation led to a decrease to 60% in the number of cells with the CB2 receptors [64]. The intravenous administration of an ozone-oxygen mixture resulted in reduction of the number of cells with the CB2 receptors to 70%. So, the activation of the immune system is accompanied by a decrease in the number of cells expressing the endocannabinoid CB2 receptor. A reduced expression of the CB2 receptor in immune cells can be mediated by blockade of endogenous cannabinoids synthesized de novo, triggering the limiting of the function of the cannabinoid system and thereby activating the immune system. Under normal conditions, the over expression of the cannabinoid receptor CB1 leads to the maintenance of the physiological balance in the synthesis of pro-inflammatory and anti-inflammatory mediators. The pooled results of the study suggest that blockade of the CB2 receptor expression in the development of the immune response is an important mechanism for the regulation of the inflammatory process and prove a reciprocal relationship between the activities of the immune and the endocannabinoid systems. New data on the role of the endocannabinoid system in the regulation of the immune response contribute significantly to the study of cellular and molecular mechanisms in the development of an inflammatory response.

6. Conclusion

Thus, the metabolites of arachidonic acid are involved in the regulatory mechanisms of the immune-metabolic response of the cell; they mediate the intensity of the inflammatory processes. The relationship between the metabolism of endocannabinoids and eicosanoids implies their cross-functioning and an interactive regulation. Further research in this area will enhance understanding of the cellular and molecular mechanisms of signal communication and the intersystem integration of the components of the endocannabinoid and eicosanoid systems and so would help in developing innovative technologies for control of their functions.

Abbreviations

- PL, phospholipids; AA, arachidonic acid; PG, prostaglandin; COX, cyclooxygenase; LOX, lipoxygenase; TX, thromboxane; LT, leukotriene; THC, Δ-9-tetrahydrocannabinol; CB1, cannabinoid receptor type 1; CB2, cannabinoid receptor type 2; 2-AG, 2-arachidonoylglycerol; TNF-α, tumor necrosis factor-α; IL, interleukin.

Acknowledgements

The work was done within the scientific-research program of our Institute, state registration 01200706095. The authors declare that no competing interests exist.

Authors’ Contributions

All authors have made substantial contributions to this work. YKD and EGL were responsible for the initial conception and design of the manuscript. YKD wrote the first draft of the manuscript. EGL carried out laboratory investigations. TPN contributed to drafting and final corrections of the manuscript. TPN and TAG supported literature research and participated in critical revision of the manuscript. AVN made final corrections of the manuscript. All the authors read and approved the final manuscript.

References


International Journal of Chemical and Biomedical Science 2015; 1(3): 70-78


