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Inflammation in the Pathogenesis of Rheumatoid Arthritis and in Experimental Arthritis: Evaluation of Combinations of Carnosic Acid and Extract of *Rhodiola rosea* L. with Methotrexate

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Abstract

The host immune response generates the pro-inflammatory immune response as a protective measure against invading pathogens, allergens, and/or trauma. However, dysregulated and chronic inflammation may result in secondary damage to tissues and immune pathology to the host. Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease which primarily involves synovial inflammation, joint pain, immobility, and stiffness. Increased infiltration of inflammatory immune cells and fibroblast-like synoviocytes into joints, form pannus and small blood vessels that lead to synovium and cartilage destruction. In this chapter we will focus on the role of inflammatory cytokines (IL-1β, IL-6 and IL-17), chemokine monocyte chemotactic protein-1 and matrix metalloproteinase-9 in the pathogenesis of experimental arthritis in animals and in human RA. Further, we will be discussing about methotrexate’s (cornerstone of anti-rheumatic therapy) immune suppressing activity, anti-inflammatory properties of carnosic acid and extract of *Rhodiola rosea* L., and their innovative combination treatments with methotrexate in rat adjuvant arthritis.

Keywords: arthritis, IL-1β, IL-6, IL-17, monocyte chemotactic protein-1, matrix metalloproteinase-9, carnosic acid, *Rhodiola rosea* L.

1. Introduction

Inflammation is an inherent defensive mechanism against damage of tissues, infection and is quickly stopped in physiological state of organism. In chronic diseases, the inflammation continues and is able to cause substantial organ and tissue damage. A lot of evidence showed that pathological inflammatory response is closely related with different chronic diseases, particularly autoimmune ones, such as systemic lupus erythematosus, rheumatoid arthritis (RA), inflammatory bowel disease, diabetes, and gout [1–3]. Although the key feature of inflammatory dysregulation in many chronic diseases has been supported by plenty of studies,
the pathogenesis of this dysregulation in the autoimmune diseases is not well understood yet. Knowledge about the signaling and mechanism of regulation of inflammation will bring noticeable clinical benefits for the therapy of autoimmune disease.

In this chapter we will present our preliminary results from new original combination treatments of methotrexate with carnosic acid and with extract of *Rhodiola rosea* L and discuss about the role of IL-1β, IL-6 and IL-17, chemokine monocyte chemotactic protein-1 and matrix metalloproteinase-9 in the pathogenesis of experimental arthritis in animals and in human RA.

2. Cytokines involved in rheumatoid arthritis

To fully understand a complex disease like a RA, animal models are indispensable due to their ability to mimic the conditions and demonstrate the similarity to the human RA. Rodent models are essential for further knowledge of the pathogenic processes of RA in humans and therefore are important in the process of testing new and already existing drugs for their efficiency and safety. There are many animal models used for the research of RA, but each model varies in the similarities to the human RA. The most frequently used animal models are collagen-induced arthritis and adjuvant-induced arthritis models. Less often are used animal models with proteoglycan-induced arthritis and streptococcal cell wall-induced arthritis [4].

The adjuvant-induced arthritis (AIA) model has been used widely for testing novel drugs for inflammatory arthritis and for studies of the disease pathogenesis. After administering an injection with complete adjuvant, it was possible to induce polyarthritis [5]. AA is inducible in susceptible rat strains, for example, Lewis rat strain, by a single subcutaneous injection of heat-killed *Mycobacterium tuberculosis* H37Ra in oil. Following the induction, the inflammation begins in 8–10 days, the symptoms are the most apparent on the 15th or 16th day, and then undergo spontaneous recovery. Autoimmune inflammation of the paws starts with the infiltration of mononuclear cells, mostly lymphocytes, macrophages, and monocytes [6]. The severity of the RA could lead to chronic malformation of affected joints, together with ankylosis. Adjuvant-induced arthritis exhibit similar symptoms to human RA, such as joint swelling, invasion of lymphocytes, and destruction of cartilage [4]. The difference between AIA in rats and human RA seems to be in the rapid onset of the erosive polyarthritis in the AIA model. Rheumatoid Factor is not present, the disease seems to have a monophasic course. There is also an involvement of axial skeleton seen in the model of AIA, affected gastrointestinal, genitourinary tract and skin, periostitis, ankylosis, and extra-articular manifestations not typical of RA [7]. Inflamed joints of rats with AIA contain activated T-cells. T-cells infiltrating joints originate from several compartments, such as the spleen, Peyer’s patches, lymph nodes, and T-cell pool that recirculates [8]. Specific antigen heat shock protein (Hsp65) has been shown to activate the immune response, with peptide 180–186 being the responsible epitope [9]. The cytokines that are expressed in the joint during the early stages of inflammation include IL-17, IFN, and TNF-α, as well as cytokines implicated in macrophage stimulation. Increased levels of IL-4, IL-6, monocyte chemotactic protein 1 (MCP-1), and TGF-β can be observed as inflammation progresses in the joint. TNF-α, IL-1β, IL-21, and IL-17 all contribute to the pathology of this disorder [8]. The main source of the irreversible tissue damage is in an area rich in macrophages, called the pannus, which is located at the junction of the synovium lining of the joint capsule together with the cartilage and a bone. Pannus cells migrate over the cartilage and into the subchondral bone, subsequently
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causing the erosion of these tissues [10]. The activity of matrix metalloproteinases (MMPs) seems to be the reason for the irreversible destruction of the cartilage seen in RA. MMPs are enzymes produced as a response to proinflammatory cytokines as IL-1 and TNFα by activated macrophages and fibroblasts [11]. MMPs can be further divided into three main groups. Collagenase MMP-1 (interstitial) and MMP-8 (neutrophil), whose major substrates are collagen forms I, II, and III, belong to the first group. The second group consists of the gelatinase/type IV collagenases such as MMP-2, the 72kD gelatinase A, and 92-kD gelatinase B (MMP-9). The main function of these matrix metalloproteinases from the second group is to degrade gelatin and collagen type IV in the basement membrane. Group 3 consists of the stromelysins, stromelysin 1 (MMP-3), stromelysin 2 (MMP-10), and pump-1 (MMP-7). These stromelysins have activity against a range spectrum of substrates, mainly proteoglycans, fibronectin, laminin, and some collagens [11]. During arthritis, especially MMP-1 and MMP-3 play an important role in the pathophysiology of the disease, and what is worse, the destruction of the connective tissue they cause is largely irreversible [12–14]. Fibroblasts from a healthy organism produce very low levels of both enzymes [12–14]. On the other hand, during RA and osteoarthritis levels of these enzymes rapidly increase in response to various stimuli [12–14]. Potent inducers of collagenases and stromelysins could be cytokines such as IL-1α and IL-1β, epidermal growth factor (EGF), platelet-derived growth factor, and tumor necrosis factor α. Inducers of these two enzymes could also be crystals of monosodium urate monohydrate, debris phagocytosis, and formulation of multinucleated giant cells. In an environment of stimulated synovial fibroblast cells, which resembles proliferating rheumatoid synovial tissue, collagenase and stromelysin becomes major gene product of these synovial fibroblasts [14]. Patients with RA and OA also have higher levels of collagenase and stromelysin in cartilage and the synovial fluid, especially patients with RA [15, 16]. The level of enzymatic activity is increased concordantly with the severity of the disease [17]. Apart from MMPs, there are other enzymes synthesized by cells within cartilage and bone as well as infiltrating inflammatory cells. These enzymes include aspartic, serine, and cysteine endopeptidases such as cathepsin B, which are capable of cleaving and therefore destroying the main components of cartilage and bone (such as proteoglycan and collagen type I, II, IX, X, and XI) [18].

2.1 Interleukin-1β

Interleukin-1β (IL-1β) is a cytokine belonging to the same family of cytokines as IL-1α, yet they show different features and are produced by two different genes [19]. IL-1β is mainly produced by macrophages as an inactive precursor (pro-IL-1β) and then cleaved by cysteine protease caspase-1 into its mature form (IL-1β) [20]. The major distinction between IL-1β and IL-1α is that pro-IL-1β is biologically inactive, while pro-IL-1α and mature IL-1α can bind to their receptors and therefore stimulate cellular responses. Most IL-1α also stays coupled with the plasma membrane and stimulates cells by direct cell–cell interaction, which can induce its functions [21]. IL-1β is produced by blood monocytes, tissue macrophages, and dendritic cells by direct cellular contact with stimulated T-lymphocytes, a mechanism related to chronic inflammation [22]. IL-1β mRNA requires an extra signal for synthesis so transcription of IL-1β is a rate-limiting step of its synthesis. The extra signal to induce the production of IL-1β can be a microbial product or cytokines as TNF-α, IL-1α, IL-18, or IL-1β itself [23]. By binding to the same receptors as IL-1α and IL-1β, yet not inducing any consequent cellular responses, IL-1 receptor antagonist (IL-1 Ra) acts as a naturally occurring inhibitor [24]. IL-1β seems to be not present in healthy individuals, or its levels...
are hard to detect by standard assays. Such low levels are needed to be maintained due to the potency of IL-1β to induce inflammatory responses [25]. During RA, serum levels of IL-1β are higher in patients with RA compared to healthy individuals, and the concentrations of IL-1β increase during the acute phase of the disease [26].

2.2 Interleukin-6

IL-6 has been suggested to be a major player in the pathological changes during RA because of the broad spectrum of activities IL-6 participates in. IL-6 is recognized as an endogenous pyrogen [27], and also as an inducer of acute phase response genes [28]. IL-6 stimulates B- and T-cells activity and promotes proliferation of plasmablast into mature immunoglobulin-producing plasma cells [29]. IL-6 acts stimulatory on the immune system's cells, vascular endothelial cells, synovial fibroblasts, and osteoclasts upon coupling with its soluble IL-6 receptor (sIL-6Rα). Activated sIL-6Rα complex stimulates the production of a subset of chemokines by endothelial cells and subsequently upregulates the expression of adhesion molecules, resulting in direct recruitment of leukocytes to the sites of inflammation [30]. Apart from that, by having stimulatory effects on synovial fibroblast and osteoclast activation, IL-6 contributes to the formation of synovial pannus and bone resorption in inflamed joints [31, 32]. Interestingly, patients with various forms of arthritis have high levels of IL-6 in serum and synovial fluids, but on the other hand, their structural cells from joints (chondrocytes, fibroblasts, synoviocytes, and endothelial cells) lack expression of IL-6R [33]. These cells are also not responsive to IL-6 itself. The complex of IL-6 bound to its receptor might, therefore, represents the mechanism behind the action of IL-6 during arthritis. In a synovial fluid of RA patients, it has been shown that an increase in sIL-6Rα correlates with the extent of the joint destruction which coincides with more advanced stages of RA [32].

2.3 Interleukin-17

IL-17 is another cytokine possibly contributing to the pathogenesis of RA. IL-17 is produced by CD4+ CD45RO+ memory T cells in synovium during RA, upon activation with phorbolmyristate acetate/ionomycin or CD3/CD28 Abs [34, 35]. IL-17A is relatively homologous to IL-17F (~50%) with which it can form heterodimers (IL-17A/F). Activated human CD4+ T cells produce IL-17A/F heterodimers along with IL-17A and IL-17F homodimers [36]. The signaling is based on the coupling of IL-1A and IL-1F to a multimeric receptor composed of two subunits IL-17RA and IL-17RC [37]. Cytokines from the IL-17 family activate pro-inflammatory pathways through activating NF-κB or inducing signaling through MAPK and the C/EBP transcription factors. It seems IL-17A signaling intends to activate a gene expression of an innate-type inflammatory effector program that mediates potent inflammation and plays a critical role in a defense of a host [38]. It has been shown that IL-17 can trigger the production of IL-6, IL-8, GM-CSF, and also prostaglandin E2 (PGE2), a strong mediator of inflammation, in human synoviocytes [34, 35, 39]. Additionally, IL-17 showed stimulating effect on granulopoiesis in a murine model [40], on osteoclastogenesis [41], up-regulated synthesis of NO in cultured human cartilage [42], stimulated the synthesis of proinflammatory mediators as TNF-α, IL-1β, IL-10, IL-12, stromelysin, and IL-1Ra in human peripheral blood macrophages [43]. Furthermore, levels of IL-17 in synovial fluid and serum from RA patients are high in contrast to OA patients [44].
2.4 Monocyte chemoattractant protein-1

The rheumatoid synovial environment suggests a possible role for leukocyte chemoattractant molecules such as chemokines. Chemokines form a superfamily consisting of low molecular weight peptides (7–15 kDa) with conserved four-cysteine motif and consist of at least two subfamilies: first are the C-X-C (α) chemokines which all majorly attract neutrophils. Here belong IL-8, melanoma growth stimulating activity, and epithelial neutrophil-activating peptide 78. Secondly, C-C (β) chemokines are RANTES (regulated upon activation normal T cell expressed and secreted), monocyte chemoattractant protein 1 (MCP-1), and macrophage inflammatory protein 1α (MIP-1α), which chiefly recruit T cells and monocytes [45]. Many of the cells present in RA joints, such as endothelial cells, macrophages, fibroblasts, and lymphocytes can release chemokines. In the pathogenesis of RA, members of both subclasses of chemokines have been implicated. The production of MCP-1 is enhanced in human RA patients compared to osteoarthritis patients [46]. In the murine model of collagen-induced arthritis the earliest detectable levels of MIP-1α, MCP-1, and MIP-2 expression were observed 4 weeks after the initial collagen challenge [47].

2.5 Matrix metalloproteinase 9

Degradation of articular cartilage is important feature of RA and is caused by elevated activity of proteolytic enzymes [48]. In RA, synovial fibroblasts are extensively producing the matrix-degrading enzymes [49] known as matrix metalloproteinases (MMPs). MMPs are a zinc-dependent peptidases, which are degrading the components of extracellular matrix. MMPs are the key proteases associated with the degradation and invasion through anatomical barriers [50]. The MMP-9 (gelatinase B) and MMP-2 (gelatinase A), are very important in the degradation of collagen by cleaving the denatured collagen, produced by collagenases. Moreover, these MMPs degrade other substrates, such as collagen I and II [51] and aggrecan, which is abundant in cartilage [50].

MMP-9 has a posttranscriptional regulation on multiple levels. Its activity is inhibited in tissues by inhibitors of metalloproteinase (TIMP-1 to TIMP-4) with strongest binding between TIMP-1 and MMP-9 [52]. MMPs (including MMP-9) are produced and secreted in latent soluble form of enzyme, which needs activation extracelullarly. In tissues the mast cell-derived trypptase and chymase are effective activators of MMPs [53, 54]. Regulation of MMPs is situated at the level of their transcription. Expression of MMPs is modulated by different stimuli including also cytokines [55] and growth factors [56].

MMP-9 was first discovered in neutrophils [57]. MMP-9 is also present in other leukocytes including T cells, macrophages, and eosinophils [58]. MMP-9 cleaves IL-8 and increases its activity as a chemoattractant for neutrophil more than 10-fold according to acute and chronic inflammatory processes [59]. The evidence is now growing that along with the storage of serine proteases, mast cells are secreting significant amount of MMPs such as MMP-9 [60, 61]. Although there is limited evidence for the expression of MMP-9 in mast cells in rheumatoid synovium [62], its regulation in RA is poorly understood. MMP-9 expression in rheumatoid synovial mast cells is via its regulation by TNF-α and IFN-γ in cord blood-derived human mast cell and the human mast cell line-1 (HMC-1). MMP-9 is not a product which is permanently stored in mast cells, but this enzyme is secreted under inflammatory conditions. MMP-9 may help in the migration of mast cell progenitors to inflammatory sites and could also promote the local damage of tissues [63]. In RA, MMP-9 is markedly elevated in serum and joint synovial fluid and positively correlates
with disease progression and severity [64]. MMP-9 knockout mice show decreased severity of antibody-induced arthritis [65].

3. Innovative combination treatments of methotrexate with natural compounds in experimental arthritis

Current drugs for rheumatoid arthritis (RA) are: corticosteroids, disease-modifying anti-rheumatic drugs (DMARDs), non-steroidal anti-inflammatory drugs (NSAIDs), and biological response modifiers [66]. However, these antirheumatics have several adverse effects. NSAIDs are dangerous to patients due to the adverse effects such as bleeding of upper gastrointestinal tract, liver, and kidney adverse reactions [67]. Moreover, cognitive disorders, headaches, allergic reactions often force the patients to stop the treatment. This behavior is greatly limiting the use of NSAIDs. The long-term administration of corticosteroids can induce hyperplenism, hypertension, infection, osteoporosis and fractures [68]. DMARDs often cause diarrhea, rashes, vomiting, decreased white blood cell levels, and impaired kidney and liver functions [69]. Biological agents with high target specificity and less side effects are the new agents for therapy of RA [70]. However, these biological agents are expensive and not available for many patients [71]. Thus, development of novel anti-rheumatic drugs and strategies for RA therapy is a high priority. The combination treatments of low-dose methotrexate (MTX) with natural substances, which have the potential to improve the efficacy and to reduce adverse side effects of drugs, could be one possible direction in these strategies for RA therapy. Extract or phytochemical selected for combination therapy with MTX is expected to have anti-inflammatory and antioxidant activity to treat the inflammation and oxidative stress, occurring during RA development. Many chronic diseases with inflammatory pathology are abundant in elderly population. The widely administered anti-inflammatory drugs have many side effects and are expensive (biologic drugs). Alternative option are natural extracts and substances used in traditional medicine. These natural products offer a possibility to identify the bioactive compounds and for the development of new inflammatory drugs. Traditional remedies and phytochemicals are being used for the treatment of inflammatory and other disorders since ancient times [72] and with proper scientific research background can be more extensively used for treatment also in the present.

3.1 Methotrexate

MTX is still for decades a primary antirheumatic drug and the cornerstone of the RA treatment. MTX has an acceptable safety profile, efficacy, and low cost as well as many years of clinical experience make it the gold standard of RA treatment and the key drug for combination with different biological drugs [73]. MTX is usually effective in RA treatment and patients are usually administered for several years with MTX, thus information about long-term safety is very important. However, administration of MTX is in some cases limited because of its toxic adverse effects. During long treatment period by MTX, often adverse reactions occur such as mucous ulceration, cytopenia, nausea, liver damage and serious infections. Some studies showed that due to toxic manifestations, the interruption of MTX treatment in RA patients is in the range from 10–37% [74].

Despite the introduction of numerous biologic agents for the treatment of RA, low-dose MTX therapy remains still the gold standard in the RA therapy. MTX is generally the first-line drug for the treatment of RA, psoriatic arthritis, and it enhances the effect of most biologic agents in RA. Methotrexate inhibits
polyglutamates inhibit aminoimidazole-4-carboxamide ribonucleotide (AICAR) transformylase (ATIC), leading to intracellular accumulation of AICAR and increased adenosine release; adenosine binds to cell surface receptors and suppresses many inflammatory and immune reactions [75].

The activity of MTX has also been studied in monocyte cell lines. Different from fibroblast like synoviocytes and T-lymphocytes, monocytes trigger apoptosis as a response to MTX treatment. Moreover, MTX activates a dose-dependent elevation in the expression of inflammatory cytokines, such as TNF, IL-1 and IL-6, in monocyctic cell lines [76]. Adenosine (AS) via its receptors regulates monocyte activity, and hence MTX may influence monocytes indirectly by increasing AS release by other immune cells. AS binds to its A₁ receptor on peripheral blood monocytes and activates the formation of giant cells with multiple nuclei [77]. Moreover, the binding of AS to A₂₃ receptors and A₃ receptors on monocytes decreases the production and release of IL-6 and TNF and initiates the transformation of inflammatory M₁ phenotype of monocytes to anti-inflammatory M₂ phenotype.

Macrophages with M₂ phenotype have are responsible for termination of inflammation, clearing the apoptotic cells and support wound healing by secreting profibrotic and angiogenic cytokines. Adenosine, binding on A₂₃ receptors, inhibits the production of inflammatory cytokines and promotes the expression of anti-inflammatory mediators such as vascular endothelial growth factor and IL-10 [78]. A₂₃ receptor stimulation triggers a switching from an M₁ (pro-inflammatory phenotype) to a modified macrophage M₂ phenotype [79]. One way by which A₂₃ receptor binding affects macrophage function is by stimulating the expression of the NR4A - orphan nuclear receptor, which is inhibiting the activation of NFκB-dependent nuclear gene expression [80]. A₂b receptor also induces the switching from a M₁ macrophage phenotype to a M₂ phenotype [81]. Cultivating synovial fibroblasts and T cells from RA patients triggered T cell TNF-α, IL-17, and IFNγ expression, which resulted in increased fibroblast IL-6, IL8 and IL-15 expression [82]. Methotrexate inhibited the upregulation of IL-6, IL8 and IL-15 by stimulated RA synovial fibroblasts. MTX also decreased IFNγ and IL-17 expression in T cells co-cultured with RA synovial fibroblasts (Table 1).

3.2 Combination of methotrexate and carnosic acid

In our previous study, we have selected the carnosic acid for combination with methotrexate for its anti-inflammatory and antioxidative properties, to reduce the development of rat adjuvant arthritis.

3.2.1 Carnosic acid

Carnosic acid (CA) was discovered first by Linde in Salvia officinalis L. [83]. Carnosic acid (C₂₀H₂₈O₄, Figure 1), is a phenolic diterpene that belongs to the terpene class of secondary metabolites [84], is localized in rosemary leaves, more precisely in chloroplasts of trichome cells. CA and carnosol have been reported to display beneficial effects against acute and chronic inflammation, cardiovascular diseases, obesity, and cancer [85, 86], inhibition of prostaglandin synthesis [87], skin inflammation [88], inhibition of NF-κB [89], inhibition of 5-lipoxygenase [90] and antioxidant activity in vivo [91].

CA prevented cartilage degeneration though induction of hemoxetogenase-1 (HO-1) in cell culture with human chondrocytes. The results showed that CA increased enzyme levels in a dose-dependent manner. Moreover, it was able to restore HO-1 levels under IL-1β treatment, which specifically inhibits the antioxidant effects of this enzyme. CA induced HO-1 and miR-140 expression in human
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articular chondrocytes, thus cartilage degeneration was attenuated by CA treatment [92]. The activation of macrophages triggered by exogenous infection or endogenous stress stimuli is thought to be implicated in the pathogenesis of various inflammatory diseases. In a study of Wang et al. [93], authors applied an integrated approach based on unbiased proteomics and bioinformatics analysis to elucidate the anti-inflammatory property of CA. CA significantly inhibited the increase of NO and TNF-α, downregulated cyclooxygenase-2 (COX-2) protein expression and decreased the transcriptional level of inflammatory genes including NOS-2, TNF-α, COX-2, in LPS-stimulated RA W264.7 macrophages. The liquid chromatography-based assessment showed CA negatively regulated 217 proteins elicited by lipopolysaccharide (LPS), which are responsible for multiple inflammatory pathways including nuclear factor (NF)-κB, MAPK and FoxO signaling. A following analysis showed that CA effectively inhibited ERK/JNK/p38 MAPKs, IKKβ/IκB-α/NF-κB and FoxO1/3 signaling. These results illustrate the ability of CA to regulate the inflammatory signaling triggered by LPS [93].

In another study by de Oliveira [94] authors have found that activation of cell antioxidant defense is mediated via transcription factor nuclear factor erythroid 2-related factor (Nrf2). Therefore, authors investigated whether CA is able to block paraquat (PQ)-induced inflammatory alterations in SH-SY5Y neuroblastoma cells. CA reduced the PQ-induced changes on the levels of TNF-α, IL-1β, and COX-2 via

Table 1.
Immune regulatory action of low dose MTX in the RA synovial tissue (according to Miranda-Carús et al. [82]).

| Cell type               | Methotrexate action                                                                 |
|------------------------|-------------------------------------------------------------------------------------|
| Monocyte               | Inhibition of IL-1β, IL-6, and TNF-α production; downregulation of receptors FcγRI   |
|                        | and IIa; increases ROS synthesis and apoptosis                                       |
| Macrophage             | Inhibition of IL-1β, IL-6, and TNF-α production;                                     |
| Th-1 lymphocyte        | Decreases IL-2, IFN-γ and IL-17 gene expression; increases ROS synthesis and         |
|                        | apoptosis                                                                           |
| Th-2 lymphocyte        | Increases IL-4 and IL-10 gene expression                                             |
| Neutrophil             | Increases ROS synthesis                                                              |
| Synovial fibroblast    | Inhibition of IL-15, IL-6, and IL-8 expression; inhibitory effect on prostaglandin E2|
|                        | production; inhibition of COX-2 and MMP expression                                   |

ROS, reactive oxygen species; COX-2, cyclooxygenase 2; MMP, synovial matrix metalloproteinase.

Figure 1.
Chemical structure of carnosic acid.
signaling responsible for the activation of the Nrf2/HO-1 pathway. Furthermore, they observed a crosstalk between the Nrf2/HO-1 signaling pathway and the activation of the nuclear factor-κB [94]. Two Rosemary extracts and their main components - CA and carnosol affected the cell migration. Monocyte chemoattractant protein-1 (MCP-1) and matrix metalloproteinase-9 (MMP-9) were determined by Western blot and gelatin zymography, respectively, in RAW 264.7 macrophages and vascular smooth muscle cells (VSMCs). MMP-9 and MCP-1 levels were significantly diminished with methanol extract (RM), n-hexane fraction (RH), and CA in RAW 264.7 macrophages. RM, RH, CA, and carnosol suppressed TNF-α induced VSMC migration by inhibiting MMP-9 expression. Rosemary, especially its CA component, has potential anti-atherosclerotic effects related to cell migration [95].

Liu and colleagues [96] studied the anti-inflammatory activity of CA on destruction of osteoclasts, fibroblast-like synoviocytes in the collagen-induced arthritis model. Above-mentioned in vitro and in vivo experiments showed that CA inhibited the expression of pro-inflammatory cytokines such as IL-1β, IL-6, TNF-α, IL-17, IL-8 and MMP-3, and suppressed the secretion of RANKL. Moreover, authors determined that CA reduced osteoclastogenesis and resorption of the bone in vitro and had therapeutic protective activity against joint damage in vivo. Further results showed that CA inhibited RANKL-induced activations of MAPKs (JNK and p38) and NF-κB resulting in the suppressing of NFATc1 [96].

3.2.2 Effect of the combination therapy of methotrexate and carnosic acid in rat adjuvant arthritis

In this section we will present our preliminary results from combination therapy of methotrexate (MTX) and carnosic acid in rat adjuvant arthritis.

Hind paw volume (HPV) was significantly increased on days 14, 21 and 28 during the development of AA. CA in monotherapy was without a significant effect on this parameter. The administration of methotrexate in sub-therapeutic dose markedly reduced HPV on days 14 and 21, but not on day 28. The combination of MTX and CA was more effective in decreasing the HPV on days 14, 21 and 28 than MTX in monotherapy. The most effective reduction of HPV was on day 21 (Table 2).

MCP-1 is responsible for recruiting monocytes on the sites of inflammation, and it is involved in the pathogenesis of human [46] and also in experimental arthritis [47]. AA caused a significant increase in the levels of MCP-1 on days 14, 21 and 28. Neither CA nor MTX administered in monotherapy were able to significantly reduce the elevated MCP-1 levels on days 14, 21 and 28. On day 21, only the combination of MTX and CA significantly decreased the level of MCP-1 in plasma of AA animals (Table 3).

3.3 Combination of methotrexate and ethanol extract of Rhodiola rosea

*Rhodiola rosea* L. is known as an adaptogen and has been confirmed to possess protective effects against inflammatory diseases, including cardiovascular diseases, neurodegenerative diseases, diabetes, sepsis, and cancer [97]. Less is known about the anti-inflammatory activity of *Rhodiola* extract in the experimental arthritis, thus we decided to select this extract for our study in monotherapy and in combination with methotrexate.

3.3.1 Rhodiola rosea L.

In this section we will focus on the anti-inflammatory effect of *Rhodiola rosea* L. (RhR). RhR has been found to possess anti-inflammatory properties in diseases
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Pu et al. [97] have found that seven compounds (Ferulic acid, Kaempferol, Salidroside, Tyrosol, Catechin, Gallic acid and Caffeic acid phenethyl ester) isolated from RhR showed protective activity against LPS-induced sepsis in mice via decreasing TNF-α, nitric oxide and lactate dehydrogenase [97]. By many scientists, salidroside (SAL) was reported to possess protective ability in many disease models through particularly regulating different inflammatory mediators.

### Table 2.
**Effect of carnosic acid, methotrexate and their combination on hind paw swelling.**

|                    | Day 7   | Day 14  | Day 21  | Day 28  |
|--------------------|---------|---------|---------|---------|
| CO                 | 4.66 ± 1.83 | 8.14 ± 3.23 | 9.79 ± 2.27 | 12.35 ± 1.95 |
| AA                 | 6.82 ± 2.13 | 35.90 ± 5.40 | 71.79 ± 5.45 | 54.81 ± 5.56*** |
| AA-CA              | 4.73 ± 1.56 | 43.59 ± 9.70 | 72.63 ± 4.80 | 55.79 ± 5.11 |
| AA-MTX             | 8.26 ± 1.85 | 11.63 ± 2.58* | 30.47 ± 7.85*** | 34.40 ± 9.74 |
| AA-CA-MTX          | 3.84 ± 1.30 | 7.41 ± 1.53** | 8.43 ± 0.81*** | 12.33 ± 1.90*** |

CO: healthy control animals, AA: untreated arthritic animals, AA-CA: arthritic animals treated with carnosic acid, AA-MTX: arthritic animals treated with methotrexate, AA-CA-MTX: arthritic animals treated combination of methotrexate and carnosic acid.

Values are expressed as average ± standard error of mean, statistical significance was calculated using ANOVA-Tukey–Kramer post hoc test.

\*p < 0.05.
\*p < 0.01.
\*p < 0.001 vs. CO.
\"p < 0.05.
\"p < 0.01.
\"p < 0.001 vs. AA.
\*p < 0.05 vs AA-MTX.

Table 3.
**Effect of carnosic acid, methotrexate and their combination on levels of monocyte chemotactic protein-1 in blood plasma.**

| MCP-1 (pg/mL) | Day 14   | Day 21   | Day 28   |
|--------------|----------|----------|----------|
| CO           | 306.43 ± 791 | 337.27 ± 17.06 | 137.36 ± 20.61 |
| AA           | 395.68 ± 19.20 | 516.31 ± 22.00** | 183.96 ± 12.48 |
| AA-CA        | 431.30 ± 21.14 | 510.00 ± 21.92 | 174.75 ± 18.45 |
| AA-MTX       | 410.44 ± 9.75 | 491.74 ± 20.25 | 181.87 ± 25.07 |
| AA-CA-MTX    | 411.82 ± 17.71 | 429.94 ± 13.38* | 165.21 ± 13.95 |

CO: healthy control animals, AA: untreated arthritic animals, AA-CA: arthritic animals treated with carnosic acid, AA-MTX: arthritic animals treated with methotrexate, AA-CA-MTX: arthritic animals treated combination of methotrexate and carnosic acid.

Values are expressed as average ± standard error of mean, statistical significance was calculated using ANOVA-Tukey–Kramer post hoc test.

\*p < 0.05.
\*p < 0.01.
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\"p < 0.05.
\"p < 0.01.
\"p < 0.001 vs. AA.
\*p < 0.05 vs AA-MTX.

such as sepsis, endotoxemia, asthma *in vivo* and *in vitro*. Pu et al. [97] have found that seven compounds (Ferulic acid, Kaempferol, Salidroside, Tyrosol, Catechin, Gallic acid and Caffeic acid phenethyl ester) isolated from RhR showed protective activity against LPS-induced sepsis in mice via decreasing TNF-α, nitric oxide and lactate dehydrogenase [97]. By many scientists, salidroside (SAL) was reported to possess protective ability in many disease models through particularly regulating different inflammatory mediators.

SAL decreased the inflammatory injury via reducing inflammatory cytokines (IL-1β, TNFα, IL-6), small molecules (mainly nitric oxide), chemokines (monocyte chemo-attractant protein (MCP)-1 and macrophage inflammatory protein (MIP)-1α) and COX-2 in animal models, such as LPS induced endotoxemia in mice [98], LPS induced murine acute lung injury [99], ovalbumin induced asthma in
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DOI: http://dx.doi.org/10.5772/intechopen.99073  
mice [100], and ethanol triggered acute gastric ulceration [101]. Further in vitro experiment confirmed the protective effects of SAL in neuro-inflammation. In murine microglial BV2 cells treated by LPS, Lee et al. showed that the main compounds of RhR (salidroside and rosarin) reduced the production of nitric oxide and inflammatory cytokines such as IL-6, IL-1β, and TNF-α via the NF-κB and MAPK signaling pathways [102]. Another in vitro study showed that SAL may inhibit the synthesis of inflammatory mediators. Authors found that in mice macrophages (J74.1 and RAW264.7) activated by LPS, SAL pre-treatment can reduce the levels of IL-1β, TNFα, IL-6, NO and MCP-1 via NF-κB pathway [103]. Further experiment showed that the mechanism might also be associated with down regulation of STAT3 and JAK2, and with translocation of STAT3 in nucleus [99]. STAT3 belongs to STAT (Signal Transducers and Activators of Transcription) family and has a key role in inflammatory processes. Many cytokines bind to GP130, which is a IL-6-type cytokines receptor, and activate Janus kinases (JAKs), what leads to the phosphorylation of STAT3. The phosphorylated STAT3 is translocated into the nucleus and regulates the expression of different target genes including also pro-inflammatory mediators [104].  
Osteoarthritis (OA) is the most common disease, which seriously affects the daily life of the elderly. Currently, no drug therapy has been shown to explicitly block the progression of OA. The study by Gao et al. [105] showed that salidroside could significantly promote the proliferation of chondrocytes in OA rats induced by an anterior cruciate ligament transection and renew the OA-induced changes of cartilage. Salidroside increased the levels of aggrecan and collagen II and reduced the MMP-13 level. Moreover, salidroside reduced Th-17 cells and the levels of IKBa and p65, and IL-17, while elevated the count of CD4 + IL-10+ cells and IL-10. The reduction of IL-17 levels further diminished the dissociation of IKBa to p65, what resulted in the reduction of the release of VCAM-1 and TNF-α. Salidroside decreases the cartilage degradation via promoting proliferation of chondrocytes, reducing collagen fibrosis, and regulating the inflammatory processes and immune responses through NF-κB pathway in anterior cruciate ligament transection-induced OA in rats [105]. Another study involving chondrocytes by Wu et al. [106] showed that salidroside suppressed IL-1β-induced apoptosis in chondrocytes. Salidroside stimulated proliferation of chondrocytes, reduced IL-1β-triggered inflammation and apoptosis, and scavenged NO and reactive oxygen species generated by chondrocytes. Salidroside upregulated the level of B-cell lymphoma 2 protein and downregulated the level of apoptosis regulator Bax. Salidroside also inhibited the production of caspase 3/9 and suppressed the phosphorylation of phosphoinositide-3-kinases (PI3K) and protein kinase B (AKT). These results indicate that salidroside prevents osteoarthritis by its anti-inflammatory, anti-apoptotic and pro-proliferating activities by suppressing the PI3K/AKT pathway [106].  

3.3.2 Effect of the combination therapy of methotrexate and extract of Rhodiola rosea in rat adjuvant arthritis  

Hind paw volume (HPV) was significantly increased on days 14 and day 21 during the development of AA. Administration of Rhodiola rosea ethanol extract (RS) in monotherapy markedly decreased HPV on day 14, but it had no effect on HPV on day 21. MTX and the combination of MTX with RS administered in monotherapy significantly decreased the HPV on days 14 and 21 (Table 4).  

AA caused significant increase in the levels of IL-6 on days 14 and 21. Administration of MTX in monotherapy significantly decreased the plasmatic level of IL-6 only on day 14. Administration of RS in monotherapy had no effect on
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However, the combination treatment of MTX and RS significantly decreased the levels of IL-6 on both measured days (Table 5).

4. Conclusions

Animal models of rheumatoid arthritis (RA) are used widely in research on pathogenesis of inflammatory arthritis and in the testing of potential anti-arthritic agents. In this chapter we highlighted the importance of inflammatory mediators IL-1β, IL-6, IL-17, MCP-1 and MMP-9 in experimental arthritis and RA. We have demonstrated, that MTX is a therapeutic standard for human arthritis as well as for adjuvant arthritis in rats, which make this model suitable for studying the pharmacotherapy of RA. Our preliminary results with combination treatments of MTX with carnosic acid and Rhodiola rosea ethanol extract showed, that these combinations are more effective in reducing hind paw volume, and the levels of MCP-1 and IL-6 than MTX in monotherapy. Thus, natural compounds with anti-inflammatory activities could be also a perspective candidate for combination treatments with MTX to treat human autoimmune diseases.

### Table 4.
Effect of Rhodiola rosea ethanol extract, methotrexate and their combination on hind paw swelling.

|                  | Day 7     | Day 14    | Day 21    |
|------------------|-----------|-----------|-----------|
| CO               | 0.55 ± 1.05 | 7.14 ± 1.33 | 11.99 ± 1.01 |
| AA               | 3.16 ± 1.63 | 21.34 ± 3.70** | 55.38 ± 2.76** |
| AA-MTX           | 3.95 ± 0.91 | 5.40 ± 0.86*** | 14.79 ± 2.66*** |
| AA-RS            | 3.79 ± 1.88 | 8.35 ± 2.12** | 48.62 ± 5.34 |
| AA-RS-MTX        | 6.13 ± 1.66 | 7.77 ± 2.49** | 12.10 ± 4.24*** |

CO: healthy control animals, AA: untreated arthritic animals, AA-RS: arthritic animals treated with extract of Rhodiola rosea, AA-MTX: arthritic animals treated with methotrexate, AA-RS-MTX: arthritic animals treated combination of methotrexate and extract of Rhodiola rosea.

Values are expressed as average ± standard error of mean, statistical significance was calculated using ANOVA-Tukey–Kramer post hoc test.

**p < 0.001 vs. CO.

***p < 0.001 vs. AA.

### Table 5.
Effect of Rhodiola rosea ethanol extract, methotrexate and their combination on levels of IL-6 in blood plasma.

|       | IL-6 (pg/mL) | Day 14     | Day 21     |
|-------|--------------|------------|------------|
| CO    | 62.67 ± 4.30 | 51.50 ± 4.77 |
| AA    | 141.45 ± 14.66 | 88.33 ± 5.74 |
| AA-MTX| 82.30 ± 18.95* | 70.19 ± 7.12 |
| AA-RS | 148.92 ± 10.44 | 77.99 ± 5.44 |
| AA-RS-MTX | 70.05 ± 6.84* | 43.13 ± 3.05* |

CO: healthy control animals, AA: untreated arthritic animals, AA-RS: arthritic animals treated with extract of Rhodiola rosea, AA-MTX: arthritic animals treated with methotrexate, AA-RS-MTX: arthritic animals treated combination of methotrexate and extract of Rhodiola rosea.

Values are expressed as average ± standard error of mean, statistical significance was calculated using ANOVA-Tukey–Kramer post hoc test.

*p < 0.05 vs. CO.

* * *p < 0.001 vs. AA.
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Conflict of interest

Authors have no conflict of interests.

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References

[1] Chen Z, Bozec A, Ramming A, Schett G. Anti-inflammatory and immune-regulatory cytokines in rheumatoid arthritis. Nat Rev Rheumatol. 2019;15:9-17. https://doi.org/10.1038/s41584-018-0109-2.

[2] Shen HH, Yang YX, Meng X, Luo XY, Li XM, Shuai ZW, Ye DQ, Pan HF. NLRP3: A promising therapeutic target for autoimmune diseases. Autoimmun Rev. 2018;17:694-702. DOI: 10.1016/j.autrev.2018.01.020.

[3] Nikooopour E, Schwartz JA, Singh B. Therapeutic benefits of regulating inflammation in autoimmunity. Inflamm Allergy Drug Targets. 2008;7:203-210. DOI: 10.2174/187152808785748155.

[4] Choudhary N, Bhatt LK, Prabhavalkar KS. Experimental animal models for rheumatoid arthritis. Immunopharmacol Immunotoxicol. 2018;40:193-210. DOI: 10.1080/08923973.2018.1434793.

[5] Pearson CM, Waksman BH, Sharp JT. Studies of arthritis and other lesions induced in rats by injection of mycobacterial adjuvant. V. Changes affecting the skin and mucous membranes. Comparison of the experimental process with human disease. J Exp Med. 1961;113:485-510. DOI: 10.1084/jem.113.3.485.

[6] Pearson CM, Wood FD. Studies of arthritis and other lesions induced in rats by the injection of mycobacterial adjuvant. VII. Pathologic details of the arthritis and spondylitis. Am J Pathol. 1963;42:73-95.

[7] Joe B, Wilder RL. Animal models of rheumatoid arthritis. Mol Med Today. 1999;5:367-369. DOI: 10.1016/s1357-4310(99)01528-2.

[8] Roy T, Ghosh S. Animal models of rheumatoid arthritis: correlation and usefulness with human rheumatoid arthritis. Indo American Journal of Pharm Research. 2013;3:6131-6142.

[9]Billingham ME, Carney S, Butler R and Colston MJ. A mycobacterial 65-kD heat shock protein induces antigen-specific suppression of adjuvant arthritis, but is not itself arthritogenic. J Exp Med 1990;171:339-344.

[10]Allard SA, Muirden KD, Camplejohn KL, Maini RN. Chondrocyte-derived cells and matrix at the rheumatoid cartilage-pannus junction identified with monoclonal antibodies. Rheumatol Int. 1987;7:153-159. DOI: 10.1007/BF00270363.

[11]Vincenti MP, Clark IM, Brinckerhoff CE. Using inhibitors of metalloproteinases to treat arthritis. Easier said than done? Arthritis Rheum. 1994;37:1115-1126. DOI: 10.1002/art.1780370802.

[12]Harris ED Jr. Rheumatoid arthritis. Pathophysiology and implications for therapy. N Engl J Med. 1990;322:1277-1289. DOI: 10.1056/NEJM19900533221805.

[13]Werb Z. Proteases and matrix degradation. In: Kelley WN, Harris ED Jr, Ruddy S, Sledge CB, editors. Textbook of Rheumatology. 3th ed. Philadelphia: WB Saunders; 1989. p. 300-321.

[14]Brinckerhoff CE, Auble DT. Regulation of collagenase gene expression in synovial fibroblasts. Ann N Y Acad Sci. 1990;580:355-374. DOI: 10.1111/j.1749-6632.1990.tb17944.x.

[15]Walakovits LA, Moore VL, Bhardwaj N, Gallick GS, Lark MW. Detection of stromelysin and collagenase in synovial fluid from patients with rheumatoid arthritis and
posttraumatic knee injury. Arthritis Rheum. 1992;35:35-42. DOI: 10.1002/art.1780350106.

[16] Clark IM, Powell LK, Ramsey S, Hazleman BL, Cawston TE. The measurement of collagenase, tissue inhibitor of metalloproteinases (TIMP), and collagenase-TIMP complex in synovial fluids from patients with osteoarthritis and rheumatoid arthritis. Arthritis Rheum. 1993;36:372-379. DOI: 10.1002/art.1780360313.

[17] Dean DD, Martel-Pelletier J, Pelletier JP, Howell DS, Woessner JF Jr. Evidence for metalloproteinase and metalloproteinase inhibitor imbalance in human osteoarthritic cartilage. J Clin Invest. 1989;84:678-685. DOI: 10.1172/JCI114215.

[18] Buttle DJ, Saklatvala J. Lysosomal cysteine endopeptidases mediate interleukin 1-stimulated cartilage proteoglycan degradation. Biochem J. 1992;287:657-661. DOI: 10.1042/bj2870657.

[19] Dinarello CA, Ikejima T, Warner SJ, Orencole SF, Lonnemann G, Cannon JG, Libby P. Interleukin 1 induces interleukin 1. I. Induction of circulating interleukin 1 in rabbits in vivo and in human mononuclear cells in vitro. J Immunol. 1987;139:1902-1910.

[20] Black RA, Kronheim SR, Cantrell M, Deely MC, March CJ, Prickett KS, Wignall J, Conlon PJ, Cosman D, Hopp TP, et al. Generation of biologically active interleukin-1 beta by proteolytic cleavage of the inactive precursor. J Biol Chem. 1988;263:9437-9442.

[21] Niki Y, Yamada H, Kikuchi T, Toyama Y, Matsumoto H, Fujikawa K, Tada N. Membrane-associated IL-1 contributes to chronic synovitis and cartilage destruction in human IL-1 alpha transgenic mice. J Immunol. 2004;172:577-584. DOI: 10.4049/jimmunol.172.1.577.

[22] Burger D, Dayer JM. The role of human T-lymphocyte-monocyte contact in inflammation and tissue destruction. Arthritis Res. 2002;4:169-176. DOI: 10.1186/ar558.

[23] Dinarello CA. Biologic basis for interleukin-1 in disease. Blood. 1996;87:2095-2147.

[24] Eisenberg SP, Brewer MT, Verderber E, Heimdal P, Brandhuber BJ, Thompson RC. Interleukin 1 receptor antagonist is a member of the interleukin 1 gene family: evolution of a cytokine control mechanism. Proc Natl Acad Sci U S A. 1991;88:5232-5236. DOI: 10.1073/pnas.88.12.5232.

[25] Burger D, Dayer JM, Palmer G, Gabay C. Is IL-1 a good therapeutic target in the treatment of arthritis? Best Pract Res Clin Rheumatol. 2006;20:879-896. DOI: 10.1016/j.berh.2006.06.004.

[26] Altomonte L, Zoli A, Mirone L, Scolieri P, Magaró M. Serum levels of interleukin-1b, tumour necrosis factor-a and interleukin-2 in rheumatoid arthritis. Correlation with disease activity. Clin Rheumatol. 1992;11:202-205. DOI: 10.1007/BF02207957.

[27] Chai Z, Gatti S, Toniatti C, Poli V, Bartfai T. Interleukin (IL)-6 gene expression in the central nervous system is necessary for fever response to lipopolysaccharide or IL-1 beta: a study on IL-6-deficient mice. J Exp Med. 1996;183:311-316. DOI: 10.1084/jem.183.1.311.

[28] Streetz KL, Wüstefeld T, Klein C, Manns MP, Trautwein C. Mediators of inflammation and acute phase response in the liver. Cell Mol Biol (Noisy-le-grand). 2001;47:661-673.

[29] Van Snick J. Interleukin-6: an overview. Annu Rev Immunol.
[30] Romano M, Sironi M, Toniatti C, Polentarutti N, Fruscella P, Ghezzi P, Faggioni R, Luini W, van Hinsberg V, Sozzani S, Bussolino F, Poli V, Ciliberto G, Mantovani A. Role of IL-6 and its soluble receptor in induction of chemokines and leukocyte recruitment. Immunity. 1997;6:315-325. DOI: 10.1016/s1074-7613(00)80334-9.

[31] Tamura T, Udagawa N, Takahashi N, Miyaura C, Tanaka S, Yamada Y, Koishihara Y, Ohsugi Y, Kumaki K, Taga T, et al. Soluble interleukin-6 receptor triggers osteoclast formation by interleukin 6. Proc Natl Acad Sci U S A. 1993;90:11924-11928. DOI: 10.1073/pnas.90.24.11924.

[32] Mihara M, Moriya Y, Kishimoto T, Ohsugi Y. Interleukin-6 (IL-6) induces the proliferation of synovial fibroblastic cells in the presence of soluble IL-6 receptor. Br J Rheumatol. 1995;34:321-325. DOI: 10.1093/rheumatology/34.4.321.

[33] Desgeorges A, Gabay C, Silacci P, Novick D, Roux-Lombard P, Grau G, Dayer JM, Vischer T, Guerne PA. Concentrations and origins of soluble interleukin 6 receptor-alpha in serum and synovial fluid. J Rheumatol. 1997;24:1510-1516.

[34] Yao Z, Painter SL, Fanslow WC, Ulrich D, Macduff BM, Spriggs MK, Armitage RJ. Human IL-17: a novel cytokine derived from T cells. J Immunol. 1995;155:5483-5486. PMID: 7499828.

[35] Fossiez F, Djossou O, Chomarat P, Flores-Romo L, Ait-Yahia S, Maat C, Pin JJ, Garrone P, Garcia E, Saenland S, Blanchard D, Gaillard C, Das Mahapatra B, Rouvier E, Golstein P, Banchereau J, Lebecque S. T cell interleukin-17 induces stromal cells to produce proinflammatory and hematopoietic cytokines. J Exp Med. 1996;183:2593-2603. DOI: 10.1084/jem.183.6.2593.

[36] Wright JF, Guo Y, Quazi A, Luxenberg DP, Bennett F, Ross JF, QiY, Whitters MJ, Tomkinson KN, Dunussi-Joannopoulos K, Carreno BM, Collins M, Wolfman NM. Identification of an interleukin 17F/17A heterodimer in activated human CD4+ T cells. J Biol Chem. 2007;282:13447-13555. DOI: 10.1074/jbc.M700499200.

[37] Gaffen SL. Structure and signalling in the IL-17 receptor family. Nat Rev Immunol. 2009;9:556-567. DOI: 10.1038/nri2586.

[38] Shen F, Gaffen SL. Structure-function relationships in the IL-17 receptor: implications for signal transduction and therapy. Cytokine. 2008;41:92-104. DOI: 10.1016/j.cyto.2007.11.013.

[39] Yao Z, Fanslow WC, Seldin MF, Rousseau AM, Painter SL, Comeau MR, Cohen JI, Spriggs MK. Herpesvirus Saimiri encodes a new cytokine, IL-17, which binds to a novel cytokine receptor. Immunity. 1995;3:811-821. DOI: 10.1016/1074-7613(95)90070-5.

[40] Schwarzenberger P, La Russa V, Miller A, Ye P, Huang W, Zieske A, Nelson S, Bagby GJ, Stoltz D, Mynatt RL, Spriggs M, Kolls JK. IL-17 stimulates granulopoiesis in mice: use of an alternate, novel gene therapy-derived method for in vivo evaluation of cytokines. J Immunol. 1998;161:6383-6389.

[41] Kotake S, Udagawa N, Takahashi N, Matsuzaki K, Itoh K, Ishiyama S, Saito S, Inoue K, Kamatani N, Gillespie MT, Martin TJ, Suda T. IL-17 in synovial fluids from patients with rheumatoid arthritis is a potent stimulator of osteoclastogenesis. J Clin Invest. 1999;103:1345-1352. DOI: 10.1172/JCI5703.
[42] Attur MG, Patel RN, Abramson SB, Amin AR. Interleukin-17 up-regulation of nitric oxide production in human osteoarthritis cartilage. Arthritis Rheum. 1997;40:1050-1053. DOI: 10.1002/art.1780400609.

[43] Jovanovic DV, Di Battista JA, Martel-Pelletier J, Jolicoeur FC, He Y, Zhang M, Mineau F, Pelletier JP. IL-17 stimulates the production and expression of proinflammatory cytokines, IL-beta and TNF-alpha, by human macrophages. J Immunol. 1998;160:3513-3521.

[44] Ziolkowska M, Koc A, Luszczyskiewicz G, Ksiezykowska-Pietrzak K, Klimczak E, Chwalinska-Sadowska H, Maslinski W. High levels of IL-17 in rheumatoid arthritis patients: IL-17 triggers in vitro IL-17 production via cyclosporin A-sensitive mechanism. J Immunol. 2000;164:2832-2838. DOI: 10.4049/jimmunol.164.5.2832.

[45] Taub DD, Oppenheim JJ. Review of the chemokine meeting the Third International Symposium of Chemotactic Cytokines. Cytokine. 1993;5:175-179. DOI: 10.1016/1043-4666(93)90001-l.

[46] Koch AE, Kunkel SL, Harlow LA, Johnson B, Evanooff HL, Haines GK, Burdick MD, Pope RM, Strieter RM. Enhanced production of monocyte chemoattractant protein-1 in rheumatoid arthritis. J Clin Invest. 1992;90:772-779. DOI: 10.1172/JCI119590.

[47] Kasama T, Strieter RM, Lukacs NW, Lincoln PM, Burdick MD, Kunkel SL. Interleukin-10 expression and chemokine regulation during the evolution of murine type II collagen-induced arthritis. J Clin Invest. 1995;95:2868-2876. DOI: 10.1172/JCI117993.

[48] Tchetverikov I, Lard LR, DeGroot J, Verzijl N, TeKoppele JM, Breedveld FC, Huizinga TW, Hanemaaijer R. Matrix metalloproteinases-3, -8, -9 as markers of disease activity and joint damage progression in early rheumatoid arthritis. Ann Rheum Dis. 2003;62:1094-1099. DOI: 10.1136/ard.62.11.1094.

[49] Xue M, March L, Sambrook PN, Jackson CJ. Differential regulation of matrix metalloproteinase 2 and matrix metalloproteinase 9 by activated protein C: relevance to inflammation in rheumatoid arthritis. Arthritis Rheum. 2007;56:2864-74.

[50] Nagase H, Visse R, Murphy G. Structure and function of matrix metalloproteinases and TIMPs. Cardiovasc Res. 2006;62:562-73. DOI: 10.1016/j.cardiores.2005.12.002.

[51] van den Steen PE, Grillet B, Opdenakker G. Gelatinase B participates in collagen II degradation and releases glycosylated remnant epitopes in rheumatoid arthritis, Adv Exp Med Biol. 2005;564:45-55.

[52] Brew K, Dinakarpandian D, Nagase H. Tissue inhibitors of metalloproteinases: evolution, structure and function. Biochim Biophys Acta. 2000;1477:267-283. DOI: 10.1016/s0167-4838(99)00279-4.

[53] Lohi J, Harvima I, Keski-Oja J. Pericellular substrates of human mast cell tryptase: 72,000 dalton gelatinase and fibronectin. J Cell Biochem. 1992;50:337-349. DOI: 10.1002/jcb.240500402.

[54] Fang KC, Raymond WW, Lazarus SC, Caughey GH. Dog mastocytoma cells secrete a 92-kD gelatinase activated extracellularly by mast cell chymase. J Clin Invest. 1996;97:1589-1596. DOI: 10.1172/JCI118583.

[55] Ries C, Petrides PE. Cytokine regulation of matrix metalloproteinase activity and its regulatory dysfunction
Inflammation

in disease. Biol Chem Hoppe Seyler. 1995;376:345-355.

[56] Vaday GG, Schor H, Rahat MA, Lahat N, Lider O. Transforming growth factor-betas suppress tumor necrosis factor alpha-induced matrix metalloproteinase-9 expression in monocytes. J Leukoc Biol. 2001;69:613-621.

[57] Sopata I, Dancewicz AM. Presence of a gelatin-specific protease and its latent form in human leucocytes. Biochim Biophys Acta. 1974;370:510-523. DOI: 10.1016/0005-2744(74)90112-0.

[58] Okada S, Kita H, George TJ, Gleich GJ, Leiferman KM. Migration of eosinophils through basement membrane components in vitro: role of matrix metalloproteinase-9. Am J Respir Cell Mol Biol. 1997;17:519-528. DOI: 10.1165/ajrccmb.17.4.2877.

[59] Yu Q, Stamenkovic I. Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-beta and promotes tumor invasion and angiogenesis. Genes Dev. 2000;14:163-176.

[60] Tanaka, A., K. Aria, Y. Kitamura, H. Matsuda. Matrix metalloproteinase-9 production, a newly identified function of mast cell progenitors, is downregulated by c-kit receptor activation. Blood.1999;94:2390-2395.

[61] Baram D, Vaday GG, Salamon P, Drucker I, Hershkoviz R, Mekori YA. Human mast cells release metalloproteinase-9 on contact with activated T cells: juxtaocular regulation by TNF-a. J. Immunol. 2001;167:4008-4016.

[62] Kanbe N, Tanaka A, Kanbe M, Itakura A, Kurosawa M, Matsuda H. Human mast cells produce matrix metalloproteinase 9. Eur J Immunol. 1999;29:2645-2649. DOI: 10.1002/ (SICI)1521-4141(199908)29:08<2645::AID-IMMU2645>3.0.CO;2-1.

[63] Di Girolamo N, Indoh I, Jackson N, Wakefield D, McNeil HP, Yan W, Geczcy C, Arm JF, Tedla N. Human mast cell-derived gelatinase B (matrix metalloproteinase-9) is regulated by inflammatory cytokines: role in cell migration. J Immunol. 2006;177:2638-2650. DOI: 10.4049/jimmunol.177.4.2638.

[64] Kim KS, Choi HM, Lee YA, Choi IA, Lee SH, Hong SJ, Yang HI, Yoo MC. Expression levels and association of gelatinases MMP-2 and MMP-9 and collagenases MMP-1 and MMP-13 with VEGF in synovial fluid of patients with arthritis. Rheumatol Int. 2011;31:543-547. DOI: 10.1007/s00296-010-1592-1.

[65] Itoh T, Matsuda H, Tanioka M, Kuwabara K, Itohara S, Suzuki R. The role of matrix metalloproteinase-2 and matrix metalloproteinase-9 in antibody-induced arthritis. J Immunol. 2002;169:2643-2647. DOI: 10.4049/jimmunol.169.5.2643.

[66] Koenders MI, van den Berg WB. Novel therapeutic targets in rheumatoid arthritis. Trends Pharmacol Sci. 2015;36:189-195. DOI: 10.1016/j.tips.2015.02.001.

[67] Laporte JR, Ibáñez L, Vidal X, Vendrell L, Leone R. Upper gastrointestinal bleeding associated with the use of NSAIDs: newer versus older agents. Drug Saf. 2004;27:411-420. DOI: 10.2165/00002018-20042706-00005.

[68] Satyanarayanasetty D, Pawar K, Nadig P, Haran A. Multiple Adverse Effects of Systemic Corticosteroids: A Case Report. J Clin Diagn Res. 2015;9:F001-2. DOI: 10.7860/JCDR/2015/12110.5939.

[69] Gilani ST, Khan DA, Khan FA, Ahmed M. Adverse effects of low dose
methotrexate in rheumatoid arthritis patients. J Coll Physicians Surg Pak. 2012;22:101-104.

[70] Shire MG, Muller GW. TNF-α inhibitors and rheumatoid arthritis. Expert Opin. Ther. Patents. 2005;8:531-544.

[71] Lekander I, Borgström F, Lysholm J, van Vollenhoven RF, Lindblad S, Geborek P, Kobelt G. The cost-effectiveness of TNF-inhibitors for the treatment of rheumatoid arthritis in Swedish clinical practice. Eur J Health Econ. 2013;14:863-873. DOI: 10.1007/s10198-012-0431-6.

[72] Gautam R, Jachak SM. Recent developments in anti-inflammatory natural products. Med Res Rev. 2009;29:767-820. DOI: 10.1002/med.20156.

[73] Shinde CG, Venkatesh MP, Kumar TM, Shivakumar HG. Methotrexate: a gold standard for treatment of rheumatoid arthritis. J Pain Palliat Care Pharmacother. 2014;28:351-358.

[74] Salliot C, van der Heijde D. Long-term safety of methotrexate monotherapy in patients with rheumatoid arthritis: a systematic literature research. Ann Rheum Dis. 2009;68:1100-1104. DOI: 10.1136/ard.2008.093690.

[75] Cronstein BN, Aune TM. Methotrexate and its mechanisms of action in inflammatory arthritis. Nat Rev Rheumatol. 2020;16:145-154. DOI: 10.1038/s41584-020-0373-9.

[76] Olsen NJ, Spurlock CF 3rd, Aune TM. Methotrexate induces production of IL-1 and IL-6 in the monocyctic cell line U937. Arthritis Res Ther. 2014;16:R17. DOI: 10.1186/ar4444.

[77] Merrill JT, Shen C, Schreibman D, Coffey D, Zakharenko O, Fisher R, Lahita RG, Salmon J, Cronstein BN. Adenosine A1 receptor promotion of multinucleated giant cell formation by human monocytes: a mechanism for methotrexate-induced nodulosis in rheumatoid arthritis. Arthritis Rheum. 1997;40:1308-1315. DOI: 10.1002/1529-0131(199707)40:7<1308::AID-ART16>3.0.CO;2-M.

[78] Williams AJ, Cronstein BN. The effect of A(2A) adenosine receptor activation on C-C chemokine receptor 7 expression in human THP1 macrophages during inflammation. Inflammation. 2012;35:614-622. DOI: 10.1007/s10753-011-9353-1.

[79] Leibovich SJ, Chen JF, Pinhal-Enfield G, Belem PC, Elson G, Rosania A, Ramanathan M, Montesinos C, Jacobson M, Schwarzschild MA, Fink JS, Cronstein B. Synergistic up-regulation of vascular endothelial growth factor expression in murine macrophages by adenosine A(2A) receptor agonists and endotoxin. Am J Pathol. 2002;160:2231-2244. DOI: 10.1016/S0002-9440(10)61170-4.

[80] Murphy EP, Crean D. Molecular Interactions between NR4A Orphan Nuclear Receptors and NF-κB Are Required for Appropriate Inflammatory Responses and Immune Cell Homeostasis. Biomolecules. 2015;5:1302-1318. DOI: 10.3390/biom5031302.

[81] Koscsó B, Csóka B, Kókai E, Németh ZH, Pacher P, Virág L, Leibovich SJ, Haskó G. Adenosine augments IL-10-induced STAT3 signaling in M2c macrophages. J Leukoc Biol. 2013;94:1309-1315. DOI: 10.1189/jlb.0113043.

[82] Miranda-Carús ME, Balsa A, Benito-Miguel M, Pérez de Ayala C, Martín-Mola E. IL-15 and the initiation of cell contact-dependent synovial fibroblast-T lymphocyte cross-talk in...
rheumatoid arthritis: effect of methotrexate. J Immunol. 2004;173:1463-1476. DOI: 10.4049/jimmunol.173.2.1463.

[83] Linde H. Ein neues Diterpen aus Salvia officinalis L. und eine Notiz zur Konstitution von Pikrosalvin. HCA. 1964;47:1234-1239. https://DOI.org/10.1002/hlca.19640470517.

[84] Hill RA, Connolly JD. Triterpenoids. Nat Prod Rep. 2013;30:1028-1065. DOI: 10.1039/c3np70032a.

[85] Visanji JM, Thompson DG, Padfield PJ. Induction of G2/M phase cell cycle arrest by carnosol and carnosic acid is associated with alteration of cyclin A and cyclin B1 levels. Cancer Lett. 2006;231:130-136.

[86] Chen JH, Ou HP, Lin CY, Lin FJ, Wu CR, Chang SW, Tsai CW. Carnosic acid prevents 6-hydroxydopamine-induced cell death in SH-SYSY cells via mediation of glutathione synthesis. Chem Res Toxicol. 2012;25:1893-1901. DOI: 10.1021/tr300171u.

[87] Bauer J, Kuehnl S, Rollinger JM, Scherer O, Northoff H, Stuppern H, Werz O, Koebeler A. Carnosol and carnosic acids from Salvia officinalis inhibit microsomal prostaglandin E2 synthase-1. J Pharmocol Exp Ther. 2012;342:169-176.

[88] Oh J, Yu T, Choi SJ, Yang Y, Baek HS, An SA, Kwon LK, Kim J, Rho HS, Shin SS, Choi WS, Hong S, Cho JY. Syk/Src pathway-targeted inhibition of skin inflammatory responses by carnosic acid. Mediators Inflamm. 2012;2012:781375.

[89] Lian KC, Chuang JJ, Hsieh CW, Wung BS, Huang GD, Jian TY, Sun YW. Dual mechanisms of NF-kappaB inhibition in carnosol-treated endothelial cells. Toxicol Appl Pharmacol. 2010; 245:21-35. DOI: 10.1016/j.taap.2010.01.003.

[90] Poeckel D, Greiner C, Verhoff M, Rau O, Tausch L, Hörnig C, Steinhilber D, Schubert-Zsilavecz M, Werz O. Carnosic acid and carnosol potentely inhibit human 5-lipooxygenase and suppress pro-inflammatory responses of stimulated human polymorphonuclear leukocytes. Biochem Pharmacol. 2008;76:91-97. DOI: 10.1016/j.bcp.2008.04.013.

[91] Hall ED, Wang JA, Miller DM, Cebak JE, Hill RL. Newer pharmacological approaches for antioxidant neuroprotection in traumatic brain injury. Neuropharmacology. 2019;145(Pt B):247-258. DOI: 10.1016/j.neuropharm.2018.08.005.

[92] Takada T, Miyaki S, Ishitobi H, Hirai Y, Nakasa T, Igarashi K, Lotz MK, Ochi M. Bach1 deficiency reduces severity of osteoarthritis through upregulation of heme oxygenase-1. Arthritis Res Ther. 2015;17:285. DOI: 10.1186/s13075-015-0792-1.

[93] Wang LC, Wei WH, Zhang XW, Liu D, Zeng KW, Tu PF. An Integrated Proteomics and Bioinformatics Approach Reveals the Anti-inflammatory Mechanism of Carnosic Acid. Front Pharmacol. 2018;9:370.

[94] de Oliveira MR, de Souza ICC, Fürstenau CR. Carnosic Acid Induces Anti-Inflammatory Effects in Paraquat-Treated SH-SYSY Cells Through a Mechanism Involving a Crosstalk Between the Nrf2/HO-1 Axis and NF-kB. Mol Neurobiol. 2018;55:890-897. DOI: 10.1007/s12035-017-0389-6.

[95] Chae IG, Yu MH, Im NK, Jung YT, Lee J, Chun KS, Lee IS. Effect of Rosemarinus officinalis L. on MMP-9, MCP-1 levels, and cell migration in RAW 264.7 and smooth muscle cells. J Med Food. 2012;15:879-886. DOI: 10.1089/jmf.2012.2162.

[96] Liu M, Zhou X, Zhou L, Liu Z, Yuan J, Cheng J, Zhao J, Wu L, Li H,
Inflammation in the Pathogenesis of Rheumatoid Arthritis and in Experimental Arthritis...

DOI: http://dx.doi.org/10.5772/intechopen.99073

Qiu H, Xu J. Carnosic acid inhibits inflammation response and joint destruction on osteoclasts, fibroblast-like synoviocytes, and collagen-induced arthritis rats. J Cell Physiol. 2018;233:6291-6303. DOI: 10.1002/jcp.26517.

Pu WL, Zhang MY, Bai RY, Sun LK, Li WH, Yu YL, Zhang Y, Song L, Wang ZX, Peng YF, Shi H, Zhou K, Li TX. Anti-inflammatory effects of Rhodiola rosea L.: A review. Biomed Pharmacother. 2020;121:109552. DOI: 10.1016/j.biopha.2019.109552.

Guan S, Feng H, Song B, Guo W, Xiong Y, Huang G, Zhong W, Huo M, Chen N, Lu J, Deng X. Salidroside attenuates LPS-induced pro-inflammatory cytokine responses and improves survival in murine endotoxemia. Int Immunopharmacol. 2011;11:2194-2209. DOI: 10.1016/j.intimp.2011.09.018.

Qi Z, Qi S, Ling L, Lv J, Feng Z. Salidroside attenuates inflammatory response via suppressing JAK2-STAT3 pathway activation and preventing STAT3 transfer into nucleus. Int Immunopharmacol. 2016;35:265-271. DOI: 10.1016/j.intimp.2016.04.004.

Wang J, Jin RG, Xiao L, Wang QJ, Yan TH. Anti-asthma effects of synthetic salidroside through regulation of Th1/Th2 balance. Chin J Nat Med. 2014;12:500-4. DOI: 10.1016/S1875-5364(14)60078-9.

Chang X, Luo F, Jiang W, Zhu L, Gao J, He H, Wei T, Gong S, Yan T. Protective activity of salidroside against ethanol-induced gastric ulcer via the MAPK/NF-κB pathway in vivo and in vitro. Int Immunopharmacol. 2015;28:604-615. DOI: 10.1016/j.intimp.2015.07.031.

Lee Y, Jung JC, Jang S, Kim J, Ali Z, Khan IA, Oh S. Anti-Inflammatory and Neuroprotective Effects of Constituents Isolated from Rhodiola rosea. Evid Based Complement Alternat Med. 2013;2013:514049. DOI: 10.1155/2013/514049.

Huang Q, Hu XL. Effects of salidroside on the secretion of inflammatory mediators induced by lipopolysaccharide in murine macrophage cell line J774.1. Sheng Li Xue Bao. 2017;69:41-46.

Hillmer EJ, Zhang H, Li HS, Watowich SS. STAT3 signaling in immunity. Cytokine Growth Factor Rev. 2016;31:1-15. DOI: 10.1016/j.cytogfr.2016.05.001.

Gao H, Peng L, Li C, Ji Q, Li P. Salidroside Alleviates Cartilage Degeneration Through NF-κB Pathway in Osteoarthritis Rats. Drug Des Devel Ther. 2020;14:1445-1454. DOI: 10.2147/DDDT.S242862.

Wu M, Hu R, Wang J, An Y, Lu L, Long C, Yan L. Salidroside Suppresses IL-1β-Induced Apoptosis in Chondrocytes via Phosphatidylinositol 3-Kinases (PI3K)/Akt Signaling Inhibition. Med Sci Monit. 2019;25:5833-5840.