BOTANICAL ANALGESIC AND ANTI-INFLAMMATORY DRUGS

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Contents

Summary

In this chapter we highlight some of the main advances achieved in recent years in the identification of plants with antinociceptive and/or anti-inflammatory activities. We also review the main botanicals which have contributed to the development of modern analgesic and anti-inflammatory drugs. Data described in this chapter indicates that many plant-derived substances are capable of interfering with one or more biological targets implicated in both the onset and maintenance of pain and inflammation. Therefore, plant-related substances represent potential alternatives for the development

of new drugs for the treatment of inflammatory and painful diseases, especially those which present a chronic profile.

1. Introduction

Over the years, natural products have contributed enormously to the discovery of drugs for use in modern medicine. It is estimated that about 40% of all medicines on the market today have been derived directly or indirectly from natural sources, 25% being from plants, 13% from micro-organisms and 3% from animals. In this regard, some relevant examples of drugs derived from natural sources need to be cited: morphine, pilocarpine, digitalics, quinine, artemisinin, atropine, scopolamine and captopril; many drugs used in the treatment of cancer, such as vinblastine, vincristine and paclitaxel; and also a great number of antibiotics and immunosuppressive agents.
It has been shown that many plant-derived compounds present significant anti-inflammatory and/or antinociceptive effects. These molecules could represent valuable therapeutic tools for the treatment of chronic inflammatory conditions, which are normally associated with painful alterations. Understanding of the molecular and cellular mechanisms involved in inflammation and pain transmission has increased dramatically in the last few years. Recent advances in this area have enabled the identification of several potential targets involved in inflammatory and painful pathologies. This is of great interest as many inflammation-related diseases associated with ageing—such as rheumatoid arthritis, Alzheimer's disease, multiple sclerosis and atherosclerosis—remain without a satisfactory treatment.

Pivotal biological targets for anti-inflammatory or analgesic drug development include most inflammatory mediators (arachidonic acid metabolites, peptides and neuropeptides, cytokines and chemokines, proteases, excitatory amino acids, etc.), protein kinases (protein kinase C- PKC, MAP kinases – MAPK, tyrosine kinases – TKs), transcription factors (AP-1, NF-κB, CREB), and/or inducible enzymes (inducible NO synthase – iNOS and cyclooxygenase-2 – COX-2) and cytokines (IL-1β, TNF-α etc.). In this chapter we will emphasize the recent advances in the identification of plant-derived compounds able to interfere directly or indirectly with one or more targets implicated in pain and/or inflammation. Attempts have been made to highlight the most promising plant-derived compounds for the treatment of relevant inflammatory and pain disorders in the clinic.

2. Principal plants that have contributed to the development of modern analgesic and anti-inflammatory drugs

2.1. Papaver somniferum

There is compelling evidence indicating that *Papaver somniferum* L. (Papaveraceae) (popularly known as Opium Poppy or keshi) has been used as an analgesic since ancient times, possibly as long ago as 3000 BC. Despite the growing use of *P. somniferum* as a painkiller, it was only in 1905 that the German scientist Sertüner first isolated the main analgesic constituent of this plant, the alkaloid morphine. In addition to morphine, the opium latex contains many other alkaloids, including codeine, thebaine, and papaverine. However, the mechanism(s) responsible for the analgesic actions of the alkaloid

morphine remained unknown until the end of the twentieth century, when the existence of the first endogenous opioids named enkephalins, β-endorphin and dynorphin was reported. Each of the endogenous opioids is formed by the proteolysis of larger precursor molecules. There are three precursors: proenkephalin A that gives rise to the family of enkephalins; prodynorphin (or proenkephalin B) which is processed into dynorphins A and B; and α- and β-neoendorphins and propriomelanocortin that yields β-lipotropin, corticotrophin and melanotropins along with β-endorphin.

Currently, three opioid receptor subtypes are known, which were identified by molecular cloning techniques. The μ-, κ- and δ-opioid receptors are members of the G-protein coupled seven-transmembrane receptor (GPCR) superfamily, and share extensive structural homologies. More recently, the endogenous opioid nociceptin/orphanin FQ has been isolated, as well as its precursor molecule pronociceptin. This heptadecapeptide has some homology to the dynorphin family, but lacks the N-terminal tyrosine residue which is essential for activation of the classical opioid receptors. Nociceptin binds to the opioid-receptor-like 1 receptor (ORL1).

Despite tremendous efforts in the search for safe, efficacious and non-addictive opioids for the treatment of pain, especially persistent pain, morphine remains the most valuable painkiller in contemporary medicine. The opioid receptors are located at various levels in the pain transmission pathways, for example the spinal cord, the midbrain and thalamus and the peripheral sensory nerve fibres. Thus, the activation of these receptors has been associated with spinal, supraspinal and peripheral analgesia.

A great amount of recent evidence has focused on the integral links between the immune system and inflammatory pain. Several studies have highlighted an important crosstalk between the immune system and peripheral opioid antinociception. Immune cells such as T and B lymphocytes, neutrophils, eosinophils, monocytes, and macrophages can express, synthesise, and contain opioid peptides and, in addition, deliver these active opioid peptides to the inflamed tissues. In addition, opioid-containing leukocytes have been reported as being recruited to the site of inflammation and they secrete opioid peptides that bind to opioid receptor-expressing sensory nerve terminals. In turn, these endogenous opioid peptides selectively bind to the opioid receptors on sensory nerve terminals, decreasing the neuronal excitability, inhibiting the release of pro-inflammatory factors, and reducing the nociception. Both endogenous and exogenous opioids have been also documented to alter antibody responses, cell-mediated immunity, phagocytic activity, chemotaxis and respiratory burst responses of neutrophils and mononuclear phagocytes. More importantly, endogenous and exogenous opioids exert a major part of their effects on the immune response by altering the expression of a large number of cytokines and/or cytokine receptors. Thus, peripheral acting opioids can prevent and reverse the action of multiple excitatory agents at the same time, instead of blocking only a single noxious stimulus. Of interest, many plant extracts and/or plant-derived compounds have their analgesic effects mediated, at least in part, by the activation of opioid mechanisms. In this way, it is expected that the identification of peripheral opioid agonists, or modulators of endogenous opioids from natural sources might represent relevant strategies for the development of innovative analgesic and anti-inflammatory drugs.

2.2. *Salix* species

About 500 species of plants belonging to the genus *Salix*, popularly known as willow, exist worldwide. These plants have been used since early times as antipyretic agents and in traditional medicine for the management of rheumatism. However, only in 1838 was the main active principle from the willow bark, named salicin, first isolated by Leroux. Other constituents were also isolated from willow species, such as the phenolic glycosides salicortin, fragilin and tremulacin. However, a milestone in the discovery of modern non-steroidal anti-inflammatory drugs (NSAID) was the synthesis, by Dreser in 1899, of acetylsalicylic acid, which was named aspirin. To date, most NSAIDs are still widely used in clinical management of inflammatory and nociceptive disorders, including rheumatism, osteoarthritis, headache, acute and chronic pain, dental surgery, etc.

John Vane discovered the mechanism by which aspirin exerts its anti-inflammatory and analgesic effects in 1971. He demonstrated that aspirin has the ability to inhibit the activity of the enzyme cyclooxygenase (COX), responsible for the formation of prostanoids from the arachidonic acid pathway. This finding remarkably increased understanding of the mechanisms of action of NSAIDs and also the interest of the pharmaceutical companies in developing safer and more efficacious NSAIDs. In the early 1990s, several groups of investigators reported the existence of a new COX enzyme named COX-2. While COX-1 is present in a constitutive form, the COX-2 enzyme is normally, but not exclusively, expressed in an inducible manner in certain inflammatory and painful disorders. The discovery of COX-2 has enabled a great advance in the understanding of the mechanisms by which some NSAIDs exert their effects. In addition, it was also reported that the therapeutic effects of salicylic acid and aspirin are the consequences, at least in part, of their ability to inhibit COX-2 induction. Recently, new selective NSAIDs, known as COX-2 blockers, have been developed and launched on the market to treat inflammatory and pain disorders. Therefore, COX-2 represents an interesting target for the development of new analgesic and anti-inflammatory drugs. In this regard, several literature reports have indicated that many botanical extracts or compounds have the ability to inhibit the induction of COX-2 expression under inflammatory conditions.

The extracts of the bark of *Salix* species have also been used for fever, mild rheumatic complaints, and pain, including mild headache. The extract is available in various forms, including hydroalcoholic or aqueous extracts, dried, or as tinctures or solutions. The principal active ingredient is salicin, which is the prodrug of various salicylate derivatives. It has been recently reported that the willow bark extract (standardised to yield 240 mg of salicin) was able to induce a moderate analgesic effect in patients with osteoarthritis when compared with placebo, in a double-blind, randomised controlled trial. Furthermore, it has been shown in a randomised controlled study that the willow bark extract was effective for the management of musculoskeletal pain, including low back pain. The therapeutic effects of the willow bark extract were found to be similar to those observed for the selective inhibitor of COX-2, rofecoxib. In addition, an open non-randomised study demonstrated the potential economic impact of using the willow bark extract to treat low back pain, in comparison to NSAIDs. It was also shown that an extract from the bark of *S. daphnoides* Vill. was able to inhibit in a dose-dependent manner both COX-1 and COX-2 activity in whole blood samples, and to a lesser extent

the production of TNFα and IL-1β, when tested *in vitro*. On the other hand, when administered *in vivo* to three volunteers, the extract did not display any significant anti-inflammatory effect.

2.3. *Cannabis sativa*

Preparations of *Cannabis sativa* L. (Moraceae) have been used as natural therapeutics since antiquity for the treatment of several ailments. The use of marijuana for medical purposes can be traced back 5000 years. The *Cannabis* plant contains a complex mixture of substances that include at least 60 different cannabinoids, some of which have been shown to present several pharmacological activities, such as antinociceptive actions, antiemetic and anticonvulsive actions, hypothermia, hyperactivity, lowering of intraocular pressure and immunosuppressive actions. Until 1964, it was generally assumed that the active principles of *Cannabis* were an unidentified mixture of isomers of tetrahydrocannabinols (THC). In 1964, a pure compound was isolated from *C. sativa* the (-)-Δ^9-*trans*-THC. It has also been reported that another constituent isolated from *C. sativa*, cannabidiol, exerts important anti-inflammatory effects.

Pharmacological, biochemical and molecular biology studies have suggested that THC and other active cannabinoids might exert their effects by acting at specific receptor sites. To date, two cannabinoid receptors have been cloned—CB_1 and CB_2—belonging to the seven transmembrane G-protein coupled receptor. CB_1 receptors are expressed in neurones of both the central and peripheral nervous systems, whereas CB_2 receptors are found centrally and peripherally in non-neuronal tissues mainly in immune cells. Besides these two receptors, there is evidence supporting the existence of uncloned cannabinoid receptors.

The relevance of cannabinoids for the control of physiological processes and the medical interest in *C. sativa* or in its active principle, THC, have increased with the discovery of a series of arachidonic acid derivatives, collectively known as endocannabinoids. The first to be discovered at the beginning of the 1990s was anandamide (arachidonoylethanolamide), followed by 2-arachidonoylglycerol (2-AG), among others. In the past two years, noladin ether, virodhamine (O-arachidonoylethanolamine), N-archidonoydopamine (NADA) and docosatetraenylethanolamide (DEA) have been found in the CNS. Anandamide and 2-AG are able to activate both CB_1 and CB_2 receptors and, subsequently, exhibit *in vitro* and *in vivo* pharmacological properties that are very similar, although not identical, to those of THC. It is believed that the main role of the endocannabinoid system is to regulate synaptic neurotransmission. Cannabinoids have been shown to inhibit nociception in virtually every experimental paradigm in humans and animals, either via the activation of CB_1 receptors at spinal or supra-spinal levels, or by exerting CB_2-like effects in peripheral regions. This finding is consistent with the high densities of CB_1 receptors on primary afferent nociceptors (particularly in the dorsal spinal cord), whereas CB_2 receptors seem to be mainly located in the peripheral tissues. In addition, cannabinoids have been demonstrated to enhance the analgesic effects of opioids by activating the spinal κ-opioid receptors. Alternatively, it has been proposed that the effects of anandamide might be mediated through its ability to bind to the vanilloid VR1

receptor, which is present on primary afferent neurons and known to play an important role in nociceptive responses.

Cannabinoids and anandamide were shown to exert antinociceptive effects in animal models of inflammatory pain when injected directly into the spinal cord, the brain stem or the thalamus. In addition, behavioural studies have shown that cannabinoids reduce thermal and mechanical allodynia in rat models of neuropathic pain. Interestingly, noxious stimulation has been shown to induce an increase in the release of anandamide in the periaqueductal grey region of the brainstem, a key site for the modulation of nociceptive information. The antinociceptive effects of cannabinoids were found to be blocked by the CB_1 antagonist rimonabant, but the antagonist itself does not alter the basal pain thresholds, ruling out the existence of a tonic activity involving the endocannabinoid system.

Cannabis preparations have been described in Indian folk medicine as a remedy for inflammation, chronic pain and asthma. However, clinical data indicating whether marijuana has analgesic properties are weak, as the result of the restricted availability of the plant. Regarding THC, there have been some interesting clinical results. For instance, it was reported that the oral administration of THC had analgesic effects in patients suffering from advanced cancer who had pain associated with the disease, but this was accompanied by some undesirable side effects, such as sedation and mental clouding. Moreover, THC was able to induce a significant reduction in the chronic relapsing pain in patients with familiar Mediterranean fever. In addition, a randomised, double-blind, placebo-controlled study showed that the oral administration of THC (5 mg) was ineffective for postoperative pain. The authors suggested that further investigation using higher doses or the repeated administration of THC would present better results, but this could be associated with a higher incidence of side-effects. It was also demonstrated that THC did not significantly reduce pain in a randomised, placebo-controlled, double-blind crossover trial, although it did slightly improved the analgesic effects of morphine. Another randomised, placebo-controlled, double-blind crossover trial conducted with CT-3, a potent analogue of THC-11-Oic acid, showed beneficial effects for this compound in reducing neuropathic pain, with no major adverse effects. There is a wide range of possibilities for the use of cannabinoids in the treatment of pain, but more adequate and well-controlled clinical trials are still necessary to determine to what extent cannabinoids represent real promise for the development of new analgesic drugs.

During the last few years, several studies have shown that cannabinoids modulate a variety of immune cell functions in humans and animals. There is evidence that cannabinoids modulate T helper cell development, chemotaxis, and tumour development. Recent interesting and comprehensive reviews have discussed in detail some specific functions of the cannabinoid system related to the modulation of the inflammatory response. More specifically, evidence has indicated that cannabinoids present inhibitory effects on neuroinflammatory alterations associated with degenerative or neurodegenerative conditions, such as multiple sclerosis, Alzheimer's disease, HIV encephalopathy, ischemia and traumatic brain injury. In addition, double-blind, randomised controlled clinical trials have demonstrated that cannabinoids produce subjective symptomatic improvement of spasticity related to multiple sclerosis, but provide no clear evidence of efficacy. It is expected that cannabinoid agonists or

modulators of endogenous cannabinoids might represent a relevant strategy for the development of innovative analgesic and anti-inflammatory drugs.

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Biographical Sketches

João B. Calixto graduated with a degree in Biological Sciences from University of Brasilia in 1973 and received his Master Science in Pharmacology from Escola Paulista de Medicina (actually UNIFESP) in 1976 and concluded his PhD in Pharmacology in 1984 from the Faculty of Medicine of Ribeirão Preto, University of São Paulo. He is currently a full Professor of Pharmacology and was head of the Department of Pharmacology of the Federal University of Santa Catarina, Brazil and Coordinator of the Post-graduation Program of the same university. His is also a Member of the Brazilian Academy of Science and he was twice President of the Brazilian Society of Pharmacology and Experimental Therapeutics. He has more than 250 papers published in peer referee Journals; he is one of the Editors of the Brazilian Journal of Medical and Biological Sciences and he is member of Editorial Board of several international Journals. His recent interest areas of research include mechanisms of action of peptides in smooth muscles, pain, inflammation, and study of the extracts and active principles from medicinal plants.

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