Review Article
Artigo de Revisão

Cytokine Inhibitors and Pain Control

Inibidores de Citocinas e Controle da Dor

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ABSTRACT

The authors describe the evidences supporting the role of cytokines in experimental pain, discussing possible approaches for pain control using cytokine-targeting therapies.

Keywords: *hyperalgesia, inflammatory pain, cytokines, nociception, analgesia.*

RESUMO

Os autores fazem uma revisão sobre evidências que demonstram o papel de citocinas em modelos experimentais de dor, discutindo possíveis terapias com alvo em citocinas para controle da dor.

Palavras-chave: hiperalgesia, dor inflamatória, citocinas, nocicepção, analgesia.

PAIN

The present review describes the evidences supporting the role of cytokines in experimental pain, discussing possible approaches for pain control using cytokine-targeting therapies such as soluble receptors, receptor antagonists, neutralizing antibodies, and drugs that inhibit cytokine production.

Pain is a nociceptive sensation which perception causes unpleasant emotions. It is accepted that sensitization of the primary sensory neurons is essential to inflammatory pain. Nonetheless, this sensitization of the nociceptor was, for a long time, regarded as a result of the excitatory action of various inflammatory mediators (a "soup of inflammatory mediators") released in the site of the inflamed or damaged tissues[1]. However, this hypothesis was challenged by the discovery of the non-steroidal anti-inflammatory drugs mechanism of action by Vane's group[2] and the demonstration that, in man[3] and in animals[3], eicosanoids do not cause overt pain but sensitize the pain receptors.

Likewise, other inflammatory mediators like endothelin[4,5], sympathetic amines[6,7], substance P[8-10], NGF[11,12] as well as bradykinin[13] also possess the same nociceptor sensitizing property. These mediators act directly on neuronal receptors, triggering molecular mechanisms that ultimately facilitate the electrical activity of the neuronal membrane. Although our views of the molecular mechanisms of nociceptor sensitization are not complete, there is general agreement that the G-protein coupled receptor stimulation by the inflammatory mediators activates the enzyme adenylate cyclase with production of cAMP. This substance, in turn, triggers the activation of a group of protein kinases (PKA and PKC), which in turn lead to phosphorylation of ion channels in the membrane. As a result, there is a facilitation of the inward sodium current by TTX resistant Na^+ channels, facilitation of inward Ca^{++} currents and inhibition of the outward K^+ currents. This is probably the basic peripheral mechanism of hyperalgesia, when a previously slight or ineffective thermal, mechanical or chemical stimulation becomes painful.

We refer to the mediators that directly act in the primary nociceptor as "final mediators". These mediators contrast with those "intermediate" mediators that are released during inflammation by resident and migrating cells or by plasma and act to stimulate the release of the final mediators. Researchers that investigate the inflammatory process may be aware that although the inflammatory signs and symptoms are similar, the resident and migrating cells, and the "intermediate" and "final" mediators may vary depending on time, tissue and the type of inflammatory stimuli. In general, single timepoint measurements of mediators in

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exudates or inflamed tissues give a distorted picture of the evolution of the pathological process and even suggest a disorganized soup of cells and mediators. In fact, sequential release of inflammatory mediators or cellular events observed after challenge by inflammatory stimuli will be observed only with sequential temporal determination. Most of the time, it is this temporal and physio-pathological hierarchy that allows the researcher to discover the site of action of old drugs or to propose targets for new drug development. The cascade of hypernociceptive cytokines described in this review is one of these physio-pathological hierarchic processes that our lab helped to discover in the last two decades[14]. Cytokines in general act as intermediate mediators, releasing other cytokines as well as final mediators. In some instances however, cytokines may act as a final hyperalgesic mediator and have been described as being involved in the development of neuronal damage, contributing to the symptom described in man as allodynia. In allodynia ("allo" = different) the perceived pain is different than that expected by the type of stimulus applied (touch, for example, may produce an intense pricking pain). Contrasting, in hyperalgesia, the perceived "quality" of the pain induced by the triggering stimulus (e.g. mechanical or thermal) can be anticipated by the patient. In the present review we use the term "hypernociception" to describe any increase in sensitivity of the primary nociceptor neuron in animal models, considering that the terms allodynia and hyperalgesia have been developed for use in clinical practice rather than for experimental studies.

PRO-INFLAMMATORY CYTOKINES AND PERIPHERAL INFLAMMATORY PAIN

Cytokines are defined as proteins produced and released in a coordinated sequence by cells in response to a variety of inflammatory stimuli, such as parasites, viruses, bacteria and their products, or in response to other cytokines[15,16]. In general, cytokines constitute a link between cellular injuries or immunological recognition of non-self and the local or systemic signs of inflammation[17-20].

Although chemokines are considered cytokines (chemotactic cytokines), they belong to a particular group of cytokines with specific chemical and functional properties. They are usually smaller than the other cytokines (8–10 kDa) and direct the recruitment of leukocytes from the blood stream to the extravascular tissues by acting on receptors that are differentially expressed on leukocyte subsets. Today, besides their role in leukocyte recruitment during inflammation, other functions have been attributed to the chemokines such as angiogenesis, modulation of the immune response and fever[21].

In inflammation, the resident cells such as dendritic cells, macrophages, mast cells and lymphocytes are tissue cells that release cytokines after recognizing the inflammatory stimuli. These cytokines play an essential role in the development of inflammatory pain as well as other inflammatory events, such as leukocyte migration. The principal cytokines described as participating in the development of inflammatory pain were Interleukin (IL)-1β, Tumor Necrosis Factor (TNF)-α, IL-6, and the chemokines IL-8, Chemokine-Induced Neutrophil Chemoattractant (CINC)-1 and Keratinocyte-derived Chemokine (KC)[14,22-30]. Recently, it has been demonstrated that IL-15, IL-18 and IL-12 also induce inflammatory hypernociception[19,31-33]. The role of these cytokines and chemokines as well as their interactions is discussed below.

CASCADE OF CYTOKINES MEDIATES INFLAMMATORY HYPERNOCICEPTION

Following the discovery of cytokines, many efforts were applied to characterizing their function. In the late 1980s, the participation of cytokines in the induction of inflammatory signals such as the recruitment of white blood cells, acute phase protein release, fever, and the increase of permeability of blood vessels[34,35] had already been described. With the community's new understanding of these cytokine properties, IL-1β entered the inflammatory pain scene.

Different cell types including macrophages, monocytes and glial cells produce IL-1β. In turn, this cytokine induces the production of other inflammatory mediators[36]. The demonstration that IL-1β stimulates the production of prostaglandins[37,38] and that prostaglandins ultimately sensitize the nociceptor[39,40], prompted the investigation of the IL-1β hypernociceptive role and mechanism. In a seminal finding, it was demonstrated the IL-1β importance to the genesis of inflammatory hypernociception in experimental animals[22]. The intraplantar injection of IL-1β produces a severe mechanical hypernociception, which depends on prostanoid production since the local pre-treatment with indomethacin (COX inhibitor) blocked its effects. Moreover, it was observed that IL-1β via prostaglandins production mediates inflammatory hypernociception induced by local (rat paw) administration of carrageenin (Cg) or LPS[22]. In fact, the Cg- or LPS-induced mechanical hypernociception is partially (50%) inhibited by previous

local treatment with antibodies against IL-1β[24]. This partial inhibition suggests that another hypernociceptive pathway besides the IL-1β/prostanoids is involved in the mechanical inflammatory hypernociception induced by Cg. As mentioned earlier, it seems that sympathetic amines account for the other component of inflammatory pain, at least in this experimental model.

Studying the role of chemokines in inflammatory hypernociception, it was found that whereas IL-1β induces the release of prostanoids, the neutrophil chemoattractant chemokine IL-8/CXCL8 (human chemokines) or CINC-1/CXCL1 (rat chemokines) mediates the participation of sympathetic components of inflammatory hypernociception. In fact, the IL-8/CXCL8 or CINC-1/CXCL-induced mechanical hypernociception is inhibited by β–adrenergic receptor antagonists, but not by COX inhibitors. Moreover, antiserum anti-IL-8/CXCL8 also partially inhibited (50%) the Cg-induced hypernociception in rats[23]. The CXC chemokines hypernociceptive mechanism seems to depend on the tissue evaluated. For instance, in contrast with the hypernociception induced by the administration of IL-8 in cutaneous hindpaw tissue, in rat knee joints IL-8 induces mechanical hypernociception that is prevented by IL-1ra[41].

Following these observations, another pro-inflammatory cytokine was in evidence. TNF-α was considered as a key molecule in the initiation of the inflammatory process. Generally, it is the first cytokine released in response to inflammatory stimuli such as a bacterial infection[42-44]. Moreover, the possible involvement of TNF-α in the initiation of inflammatory hypernociception was raised since TNF-α stimulates the release of IL-1β and CXC chemokines.

Once again, our group was the first to demonstrate the hypernociceptive effect and mechanisms of TNF-α in the hind paw of rats. The TNF-α hypernociception was partially inhibited by indomethacin (COX inhibitor) and atenolol (β-adrenoreceptor antagonist), and abolished by the co-treatment with these drugs, suggesting that prostanoids and sympathetic amines mediate the TNF-α-induced hypernociception. Furthermore, treatment with anti-IL-1β or anti-IL-8/CINC-1 antiserum partially inhibited TNF-α-induced hypernociception, and the combination of both antisera completely abolished TNF-α effects, suggesting that IL-1β and IL-8/CINC-1 also mediate TNF-α-induced hypernociception. In addition, the mechanical hypernociceptive effects of IL-1β and IL-8/CINC-1 are inhibited by indomethacin and atenolol, respectively. These data suggest that TNF-α induces hypernociception in rats via two independent and parallel pathways: 1. TNF-α – IL-1β Prostanoids; 2. TNF-α – CINC-1 sympathetic amines (Figure 1). Moreover, Cg- or LPS-induced hypernociception is abolished by the pretreatment with anti-TNF-α antiserum. Thus, TNF-α plays a pivotal role in the Cg and LPS induction of hypernociception in rats acting via the two parallel pathways described above[24,45].

In the same manner as TNF-α, IL-1β and IL-8/CINC, IL-6 also produces mechanical hypernociception in rats, which was inhibited by indomethacin, anti-IL-1β antiserum or IL-1ra. Regarding the cytokine cascade underlying the IL-6-induced inflammatory hypernociception, it was demonstrated that antiserum against IL-6 inhibits TNF-α-induced mechanical hypernociception in rats[24]. Thus, it seems that TNF-α, IL-6 and IL-1β sequentially precede the release of prostanoids to induce hypernociception in rats.

These experiments strongly supported our above-mentioned hypothesis that in rats there is a cascade of cytokines constituting a link between injuries and the release of primary hypernociceptive mediators. This concept allows us to understand why the inhibition of one (IL-1β or TNF-α) or several (glucocorticoids) cytokines inhibits hypernociception induction. The clinical success of anti-TNF-α in rheumatoid arthritis also exemplifies this concept[51], which opposes the idea that inflammatory hyperalgesia results from a "soup of inflammatory mediators".

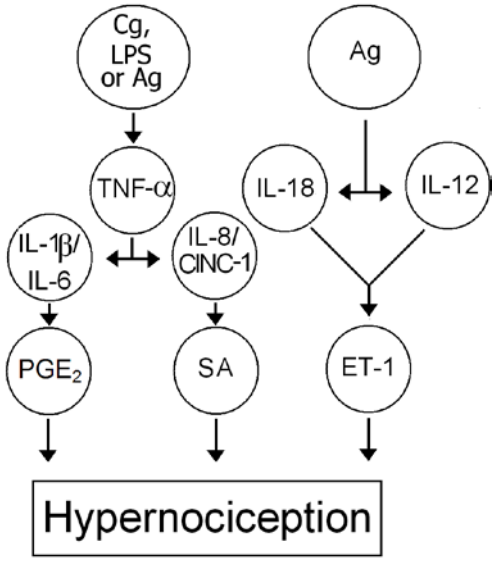

Figure 1 – Inflammatory stimulus-induced cytokine cascades mediate mechanical hypernociception in rats. The scheme represents the coordinated release of mediators triggered by the different inflammatory stimuli. Ag (antigen), Cg (carrageenin), CINC-1 (cytokine-induced neutrophil chemoattractant-1), ET-1 (endothelin-1), IL (Interleukin), LPS (lipopolysaccharide), PGE_2 (prostaglandin E_2), SA (sympathetic amines)[4,22-24,29,31,32,45-50].

Other groups also find a sequential role of cytokines in different hypernociceptive models in rats. For instance, in a model of peripheral sensitization, the intraperitoneal administration of lithium chloride or LPS sensitizes the rat to the tail flick test. It has been hypothesized that this sensitization is mediated by sequential release of TNF-α and IL-1β that activates the subdiaphragmatic vagal afferents[25]. In a different model, TNF-α trigger IL-1β mediated thermal and mechanical hypernociception induced by intraplantar administration of complete Freund's adjuvant (CFA)[29]. In this model, IL-1β induces the release of nerve growth factor (NGF), rather than stimulating prostanoid production. In fact, antibodies against NGF inhibited the hypernociception induced by CFA and IL-1β. In addition, IL-1ra inhibited CFA- and IL-1β-, but not NGF-induced mechanical and thermal hypernociception. Therefore, suggesting the following sequential release of these mediators: CFA – TNF-α – IL-1β – NGF nociceptor sensitization[26,29].

The relevance of a biological experimental concept depends on its confirmation in more than one model and animal species. In this regard, our hypothesis was further supported by recent data showing that mechanical inflammatory hypernociception in mice is also mediated by a peripheral cascade of cytokines[30]. In mice, the Cg inflammatory stimulus induces the release of TNF-α and KC/CXCL1 that trigger subsequent release of IL-1β followed by prostanoid production. KC/CXCL1 also stimulates the sympathetic component of inflammatory hypernociception (Figure 2).

In addition to experimental models, the contribution of TNF-α to inflammatory pain has also been clinically suggested. For instance, TNF-α was associated with musculoskeletal pain syndromes and pain states associated with nucleus pulposus herniation[52,53]. Furthermore, the pain associated with mandibular movement and tenderness (allodynia) on posterior palpation of the temporomandibular joint has been related to the level of TNF-α in the synovial fluid[54].

Thus, accumulating evidences in the literature suggests a role of cytokines as intermediary hypernociceptive mediators, mostly in the peripheral tissues, supporting that these molecules are likely a therapeutic target for controlling inflammatory pain states. In this regarding, our group developed several peptides based on the amino acid sequence structure of IL-1β. Among them, the tripeptide KD(P)T consistently inhibited IL-1β and inflammatory mechanical hypernociception. Recently, a selective IL-1 receptor antagonist that differs from naturally occurring IL-1ra by the presence of a methionine group named Anakinra (Amgen Inc.) was developed. Anakinra was tested in models of inflammatory diseases and is under clinical evaluation[55,56].

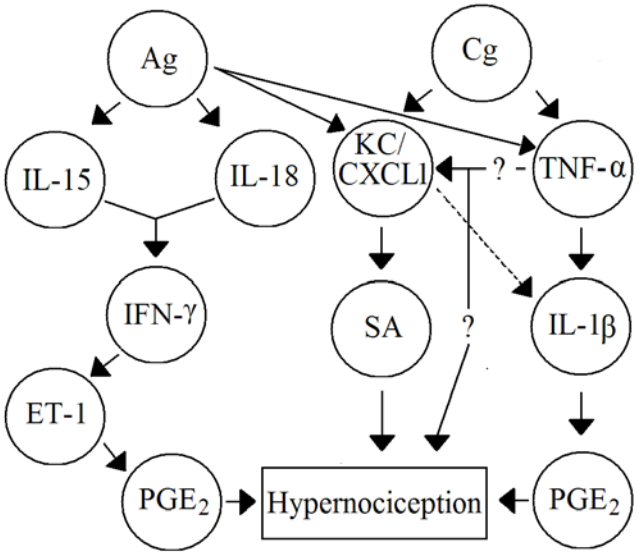

Figure 2 – Cascades of cytokines mediate mechanical hypernociception induced by non-immune and immune inflammation in mice. Ag (antigen), Cg (carrageenin), ET-1 (endothelin-1), IFN-γ (Interferon-γ), IL (Interleukin), KC/CXCL1 (Keratinocyte-derived chemokine), PGE_2 (prostaglandin E_2), SA (sympathetic amines), TNF-α (Tumour necrosis factor-α)[19,30,33].

Besides IL-1β, TNF has also become a target for the treatment of inflammatory diseases including arthritis. Drugs such as infliximab (chimeric anti-TNF-α antibody), etanercept (p75 TNF-α receptor / immunoglobulin G fusion protein), and recently, adalimumab (fully humanized monoclonal anti-TNF-α antibody) are available. These anti-TNF-α therapies were shown to be effective in different diseases that are generally associated with pain such as uveitis[57], skin and joint manifestations of psoriasis[58], and mainly in rheumatoid arthritis[59-62].

Despite the great amount of data concerning the nociceptive role of TNF-α and the beneficial effect of anti-TNF drugs on inflammatory diseases as mentioned above, there are few studies suggesting that these drugs inhibit hypernociception induction. Etanercept as well as anti-TNF antibodies reduced experimental inflammation and neuropathy-induced hypernociception[63-65] and infliximab therapy significantly reduced pain scores in rheumatoid arthritis[51,66]. It is noteworthy that chronic anti-TNF therapies may present side effects, including in some cases heart failure and also immune suppression, which may lead to serious infections such as tuberculosis[67,68].

Although specific cytokine inhibitors (e.g., anankira, etanercept, infliximab) are available, the high cost of these the-

rapies must be considered. Taking this into account, some already-known drugs emerge as an alternative for inhibition of cytokines. In this sense, we investigated the potential of pentoxifylline and thalidomide for reducing inflammatory hypernociception. In fact, thalidomide and pentoxifylline reduced hypernociception associated with inflammation by a mechanism dependent on the inhibition of cytokine production, mainly at the peripheral site. Therefore, the cost-benefit relationship of pentoxifylline and thalidomide prompt the use of these drugs or drugs with similar mechanisms of action to control inflammatory pain.

ROLE OF IL-15 AND IL-18 IN TH-1 IMMUNE INFLAMMATION-INDUCED HYPERNOCICEPTION

Th1 immune inflammatory responses are involved in the development of different diseases including rheumatoid arthritis, myocarditis, encephalomyelitis, diabetes, and lupus[69-72]. Indeed, these inflammatory diseases are frequently accompanied by hyperalgesia. Thus, the development of inflammatory nociception models that mimic these diseases is of great importance, because they would allow rational studies of the pathophysiology of those conditions and, therefore, better and more specific therapies. In this regard, models such as the antigen-induced inflammatory hypernociception in previously immunized animals resemble the immune inflammation triggered by Th1 responses[19].

It has been demonstrated that cytokines (TNF-α, IL-1β, IL-8/CINC-1 and IL-6) also play an important role in hypernociception induced by an immune challenge. In fact, specific anti-rat TNF-α, anti-rat IL-1β and anti-human IL-8 antiserum or IL-1ra decreased the antigen-induced hypernociception, and the levels of these cytokines were elevated in the paw after the administration of the antigen in immunized rats and mice[73](Figures 1 and 2). Besides the role of these well-known hyperalgesic cytokines described above, other cytokines (IL-15, IL-18) seem important during hypernociception resulting from Th1 immune inflammatory responses.

IL-18 and IL-15 share a role in the development of RA, in the driving of Th1 responses, and in the induction of interferon-gamma (IFN-γ) production[69-71,74]. Supporting these evidences, IL-15 and IL-18-targeting therapies ameliorate collagen-induced arthritis, which is a classical model of RA[75-77]. Based on this experimental finding, it was performed a clinical trial (phase I-II) using a monoclonal anti-IL-15 antibody for RA treatment. This approach presented significant beneficial effect on RA severity in RA patients[78].

Taking this evidence into account, we have recently reported that IL-15 and IL-18 mediate Th1-like-induced inflammatory hypernociception via the sequential release of IFN-γ, endothelin-1 (ET-1) and PGE_2. The hypernociception induced by IL-15 and IL-18 is independent of each other, suggesting that they are acting synergistically[19,33]. Therefore, IL-15 and IL-18 targeting therapies might also be of importance for down-regulating adaptive inflammation-induced hypernociception, probably with lower incidence of side effects such as propensity to infections[67,68].

Besides IL-15 and IL-18, IL-12 is also an important cytokine for arthritis development[70,79]. Its pronociceptive effect was demonstrated in rats. IL-12 injection induces mechanical hypernociception, which is mediated by ET-1 action on ET_B receptors, and is not dependent of prostanoids, sympathetic amines or leukotrienes in rats[32]. This effect is important since human volunteers reported pain in the site of IL-12 injection during clinical trials for cancer treatment or vaccine adjuvant[32].

CYTOKINES AND DIRECT NOCICEPTORS SENSITIZATION

Despite the evidences that the hypernociceptive effects of cytokines are indirect as described above, it has been reported that sensory neurons express cytokine receptors. Therefore, these cytokines might also directly sensitize the nociceptor during inflammation. In effect, some research groups demonstrated that cytokines such as TNF-α evoke action potentials in nociceptive neurons when applied topically to peripheral axons in vivo, and increases the sensitivity to mechanical and chemical stimuli[27,80,81]. Investigating the TNF mechanism to provoke this direct effect in neurons, Jin & Gereau[82] investigated the effect of this cytokine on TTX-R Na+ activity. It was demonstrated that TNF-α acting on TNFR1 enhances TTX-R Na+ currents in primary afferent nociceptive neurons by activating the neuronal MAP kinase (p38) pathway.

The direct effect of IL-1β, IL-6 and chemokines has also been demonstrated. For instance, IL-1β and IL-6 together with their soluble receptor (sIL-6R) are able to sensitize the sensory neurons to heat[83,84]. The IL-6 effect was due to the activation of Janus tyrosine kinase and the PKC intracellular signaling pathway[83,84]. Moreover, CCL3/MIP-1α sensitizes DRG neurons to capsaicin or

anandamide by Gi-protein-, phospholipase C-, and PKC-dependent mechanism in vitro[85].

Nevertheless, it is important to be careful in the interpretation of these results and other possibilities should be considered: 1) although the cytokines are acting directly on sensory neurons, they could produce secondary mediators, such as prostanoids, which finally sensitized the nociceptors. Actually, Nicol et al. [27], demonstrated: 1) that TNF-α enhances the sensitivity of cultured neurons to capsaicin in a COX products-dependent manner; 2) that cytokines act on cells other than sensory neurons, such as dorsal root ganglion satellite cells, that in turn produce direct sensitizing mediators; and 3) the relevance of the direct effect of cytokines to the genesis of inflammatory hypernociception. In this context, Parada et al.[86] showed that the effective attenuation of TNFR1 expression in peripheral sensory neurons by intrathecal treatment with antisense oligodeoxynucleotides to this receptor did not alter either TNF-α- or Cg-induced acute mechanical hypernociception, when injected in rat paws. Therefore, it seems that TNF-acting on sensory neuron membrane TNFR1 is not necessary to the onset of peripheral acute inflammatory hypernociception. Thus, similar experiments have to be performed with IL-6 and IL-1β to determine the importance of their direct effect on the sensitive neuron for the genesis of inflammatory hypernociception.

CHRONIC INFLAMMATORY PAIN MEDIATED BY CYTOKINES

The contribution of cytokines to the genesis of acute inflammatory pain has been extensively demonstrated, as mentioned above. However, its role in chronic inflammatory pain states is still unclear. One problem is that there are few experimental models of chronic inflammatory pain. Although some acute inflammatory models could become chronic such as the CFA-induced inflammation, it is still an unsolved acute inflammation. The clinically important chronic pain cases are when the lesion is already resolved but the pain persists.

In an attempt to study these cases we developed an experimental model to investigate the mechanism of the phenomenon mentioned above. Actually, a persistent hypernociceptive state can be induced by successive daily injections of PGE_2 or dopamine. After 14 daily injections of the stimulus (PGE_2 or dopamine), the sensitivity of the nociceptor does not return to its basal level but, instead, reaches a plateau, which persists for more than thirty days[87]. Other studies have reported that a persistent hypernociception can also be triggered by the persistent presence of stimuli[88].

In line with the PGE_2- or dopamine-induced persistent hypernociception, it was demonstrated that repeated intraplantar injections of IL-1β, IL-8 or TNF-α also cause persistent mechanical hypernociception that is prevented by daily treatment with indomethacin and atenolol. In fact, the daily treatment with indomethacin or atenolol inhibits 50% of the persistent hypernociception induced by TNF-α, and the combination of indomethacin and atenolol blocked the onset of the process. These results suggest that, as in the case of acute hypernociception, the persistent hypernociception induced by IL-1β and IL-8 is due to the endogenous release of eicosanoids and sympathetic amines, respectively. Moreover, both mediators play a role in the development of the persistent hypernociception induced by TNF-α[89].

In addition to the data generated using the model of persistent hypernociception described above, the possible role of TNF-α in "chronic pain" was further supported by the results obtained using hypernociceptive priming. In this animal model, the injection of Cg induces an inflammatory hypernociception lasting hours to days, which produces a "primed" state lasting several weeks. During this time, injection of PGE_2 induces hypernociception that is markedly enhanced and prolonged compared to PGE_2-induced hypernociception in normal "unprimed" rats[90].

Studying the genesis of the priming state, Parada et al.[86] showed that intrathecal administration of ODN antisense to TNFR1, which reduced the TNFR1 mRNA in sensory neurons, but not in the peripheral tissue, attenuates either Cg- or TNF-α-induced priming without affecting the acute hypernociception. The mechanism by which TNF-α induces chronic hypernociceptive priming is not completely clear, but it has been suggested that this cytokine induces an increase of PKCε levels in the nociceptor[91]. Moreover, the modulation of TTX-R Na+ currents via p38 activation could also be responsible for amplifying the latter effect of PGE2 on this channel activity during chronic priming.

PHARMACOLOGICAL INHIBITION OF INFLAMMATORY HYPERNOCICEPTION BY DRUGS THAT BLOCK THE RELEASE OF CYTOKINES

In addition to the drugs that block cytokines action such as soluble receptors, neutralizing antibodies and receptor antagonists as described in the previous sections

(1.1.-1.6.), there are additional approaches to inhibit cytokines effects. The inhibition of cytokines production is an efficient therapy for preventing hypernociception. Therefore, the following sections (2.1.- 2.3.) will focus in drugs that act by this mechanism.

GLUCOCORTICOIDS

The main mechanism described for glucocorticoids is related to the modulation of nuclear factor κB (NFκB). This transcription factor was identified in the nuclei of mature B lymphocytes as a transcription factor that binds to a 10 bp DNA element in the kappa immunoglobulin light-chain enhancer[92]. The NF-κB is a key player in controlling both innate and adaptive immunity. NF-κB is present in the cytoplasm in association with inhibitory proteins that are known as inhibitors of NF-κB (IκBs). After activation by a large number of inducers, the IκB proteins become phosphorylated, ubiquitylated and, subsequently, degraded by the proteasome. The degradation of IκB allows the translocation of NF-κB proteins to the nucleus and bind their cognate DNA binding sites to regulate the transcription of a large number of genes, including pro-inflammatory cytokines, chemokines, adhesion molecules, matrix metalloproteinases (MMPs), COX-2 and inducible nitric oxide synthase (iNOS)[93].

Furthermore, there are accumulating evidence that NF-κB activity is also regulated by the direct modification of NF-κB proteins through phosphorylation and, perhaps, acetylation. Several investigators have shown that IL-1 and TNF induce NF-κB phosphorylation and activation by pathways that are distinct from those leading to IκB degradation and NF-κB nuclear translocation[93].

A main mechanism by which transcription factors regulate gene expression is to bring histone acetyltransferases (HATs) and histone deacetylases (HDACs) to target sites to modify histone acetylation. Histoneacetylation status influences the folding and functional state of the chromatin fibre and modulates the accessibility of DNA to the transcriptional machinery for gene expression. Previous studies have shown that NF-κB interacts with HATs, to positively regulate gene expression, and with HDACs to negatively regulate transcription[93].

It is well recognized that steroid hormones are important inhibitor of NF-κB. The mechanism of action of glucocorticoids (GC) involves the binding of GC with the GC receptor (GR) in the cell cytoplasm. The complex GC-GR can directly bind to the NF-κB, inhibiting its action. In addition, the complex GC-GR can migrate to the cell nucleus and bind to GC-responsive genes (GREs), inhibiting the production of pro-nociceptive cytokines (TNF-α and IL-1β) and affecting their transport. In addition the complex GC-GR increases the IκB synthesis, which in turn binds to NF-κB, inhibiting its translocation to the nucleus[94].

Taking into account the evidence in the literature, we investigated whether glucocorticoids have antinociceptive effects. It was found that the hypernociceptive responses to intraplantar injections of carrageenin, bradykinin, TNF-α, IL-1β, and IL-6 were inhibited by pretreatment with dexamethasone. However, the hypernociceptive responses to IL-8, PGE_2 and dopamine were not affected, which is in agreement with the cascade of cytokines described in rats. Dexamethasone also inhibited TNF-α release by a murine macrophage cell-line stimulated with LPS. Furthermore, there is evidence that the antinociceptive effects of dexamethasone are, at least in part, mediated by annexin-1 (previously designated lipocortin-1). The lipocortin-1-mediated glucocorticoids antinociceptive effect was demonstrated by Ferreira et al.[95], using the rat paw constant pressure test. The inhibition of hypernociceptive responses to injections of bradykinin and IL-1β by dexamethasone was reversed by antiserum anti-lipocortin-1, injected subcutaneously, 24h and 1h before hypernociceptive substances. This was supported by the finding that the hypernociceptive responses to injections of bradykinin, TNF-α and IL-1β, but not responses to PGE_2, were inhibited by pretreatment with lipocortin-$1_{(2\text{-}26)}$. Further supporting the lipocortin-1-mediated dexamethasone effects, lipocortin-$1_{(2\text{-}26)}$ partially inhibited TNF-α release by a murine macrophage cell-line stimulated with LPS, and antiserum anti-lipocortin-1 partially reversed the inhibition by dexamethasone. Therefore, these data are consistent with a partial role for endogenous lipocortin-1 in mediating the antinociceptive effect of dexamethasone. It is probable that GC-GREs-induced inhibition of NF-κB activity mediates the other component of the dexamethasone antinociceptive activity.

In agreement with the proposed mechanisms of action of glucocorticosteroids, dexamethasone inhibits not only the expression of inflammatory mediators, but also the expression of receptors such as the B_1 (bradykinin receptor), which are upregulated during the hypernociceptive response to formalin[96] and Cg. Additionally, both thermal and mechanical hypernociception induced by sciatic nerve ligation is inhibited by betamethasone[97].

Furthermore, there is evidence that corticosteroid modulation (pituitary-adrenal axis) may have a role in regulating the stress-induced analgesia, and this may implicate the interactions of the corticosteroids with pain-inhibiting systems[98]. Both exogenous (pharmacological) and endogenous (physiological) glucocorticoids suppress the hypernociception and the up-regulation of spinal preprodynorphin mRNA induced by sustained inflammation provoked by CFA. The results also suggest that spinal preprodynorphin mRNA suppression may partially underlie the inhibition of the CFA-induced hypernociception[99].

Besides the glucocorticosteroids, there are also other inhibitors of NF-κB action, especially IκB kinase inhibitors (IKK). The IKK inhibitors prevent the ubiquination of IκB, the first step in the activation of NF-κB. Thus, it would be expected that inhibition of IKK activity may prevent injury-, infection-, or stress-induced upregulation of various proinflammatory genes and may thereby reduce hypernociception and inflammation. In cell culture experiments, the IKK inhibitor S1627 inhibited IL-1β-stimulated nuclear translocation and DNA-binding of NF-κB. The effects of S1627 on hypernociception were also evaluated[100]. S1627 reversed thermal and mechanical hypernociception, and the inflammatory paw edema in the zymosan-induced paw inflammation model. S1627 also significantly reduced tactile and thermal hypernociception in the CCI model of neuropathic pain. However, the drug had no effect on acute inflammatory nociception induced by formalin and did not affect responses to heat and tactile stimuli in naive animals. In agreement with this S1627 prevented the zymosan-induced nuclear translocation of NF-κB in the spinal cord and the upregulation of NF-κB-responsive genes including COX-2, TNF-α and IL-1β.

THALIDOMIDE

Thalidomide (α-N-phthalylglutamic-acid-amine) was developed as an anti-emetic drug to be used mainly during pregnancy. However, the use of thalidomide is restricted due to its teratogenic effects. It is the drug of choice for the treatment of erythema nodosum leprosum, an acute inflammatory complication occurring in 30% of lepromatous leprosy patients, usually in association with initiation of multidrug therapy[101]. The research on thalidomide mechanisms of action demonstrated that it selectively inhibits TNF-α production by human alveolar macrophages[102] and human monocytes stimulated by E. coli or Mycobacterium leprae products[103,104]. In fact, thalidomide inhibits TNF-α production by enhancing its mRNA degradation[102]. Furthermore, thalidomide does not affect the production of other cytokines by LPS-stimulated monocytes[103].

The specific and effective inhibitory action of thalidomide on TNF-α production ensures the demonstration of its effectiveness for the treatment of different diseases such as graft-versus-host disease[105], oral aphthous ulcers in patients with human immunodeficiency virus infection[106], refractory rheumatoid arthritis[107], and neutrophil migration to the synovial cavity during immune inflammation[108].

Considering the evidence on the potential therapeutic use of thalidomide in diseases associated with TNF-α, we investigated whether it would have analgesic effects. It was found that thalidomide prevents the Cg-induced mechanical hypernociception in rats and acetic acid induced-abdominal contortions in mice. Nevertheless, it did not affect the mechanical hypernociceptive effect of TNF-α or PGE_2. These results reinforce the idea that the inhibition of inflammatory hypernociception by thalidomide may be associated with the selective inhibition of TNF-α mRNA expression[48,104]. Moreover, the analgesic effect of thalidomide is peripheral and indirect, neither affecting edema nor inducing antihypernociceptive cytokines such as IL-4 and IL-10. The potential therapeutic use of thalidomide to control pain has been also demonstrated on the hypernociception induced by CCI in rats[109] and carrageenin-induced chronic hypernociceptive priming, in which phenomena TNF-α has a pivotal role[86]. Thus, there is consistent evidence for the potential usefulness of thalidomide in the management of inflammatory pain associated with TNF-α.

PENTOXIFYLLINE

Pentoxifylline was originally developed for the treatment of vascular diseases, and its pharmacokinetics and pharmacodynamics ensure its safety. Similar to thalidomide, the main mechanism of action of pentoxifylline is the reduction of TNF-α production by inhibiting mRNA expression by more than 50%[110-112]. Such an effect has been detected in vivo in patients with systemic inflammatory manifestation of erythema leprosum nodosum[112] and in vitro in peripheral blood mononuclear cells[110,112]. Furthermore, pentoxifylline concentration-dependently inhibited IL-2 and IFN-γ concomitantly with the inhibition of TNF-α in peripheral blood mononuclear cells[113]. Nevertheless, the inhibition of IL-1β[104,114,115] and IL-6[112,116] remains controversial. In vivo, pentoxifylline did not affect the levels of other

cytokines such as IL-10[112], and in vitro, pentoxifylline may augment IL-10 production in unstimulated whole blood cells or inhibit its production[117]. Thus, it is possible that the modulation/increase of IL-10 production by pentoxifylline may also account for its anti-hypernociceptive effect depending on the experimental model.

Because TNF-α and other cytokines have a crucial role in the development of different inflammatory diseases, pentoxifylline is a potential drug for the treatment of glomerulonephritis[118], leprae[112], streptozotocin-induced diabetes mellitus[119], and sepsis[116,120].

Concerning inflammatory pain, it has been demonstrated in humans that the pre-treatment with pentoxifylline diminished the opioid requirement in the early post-cholecystectomy operative period, as well as TNF-α and IL-1β serum levels. In contrast, a recent study using the same procedure described above demonstrated that the post-treatment with pentoxifylline did not modify the serum levels of TNF-α and IL-6 or the opioid requirement[121]. These data suggest the importance of the pharmacokinetics of pentoxifylline for its action.

Furthermore, besides the demonstrations of the effectiveness of pentoxifylline in humans, there is consistent evidence of its antinociceptive activity in experimental models of hypernociception. In rats, pentoxifylline inhibits mechanical hypernociception administered pre or post-injury[122]. Pentoxifylline is also efficacious in different models of inflammatory nociception such as the writhing response induced by acetic acid and zymosan, zymosan-induced articular hypernociception in rats, and carrageenin-, bradykinin- and TNF-α-induced mechanical hypernociception. However, pentoxifylline did not inhibit iloprost (PGI_2 analogue), IL-1β or PGE_2 effects, suggesting that the mechanism of action underlying this drug's effect is upstream of IL-1β release. No central effects of pentoxifylline were detected. Thus, pentoxifylline could be useful for the treatment of inflammatory pain[50].

Besides the inhibition of TNF-α and other cytokines, it has been reported that pentoxifylline inhibits in vivo the endotoxin-induced NF-κB activation in intestine of rats during sepsis. Pentoxifylline may downregulate NF-κB expression via PKC inhibition[123]. Although there is no direct evidence that such a mechanism accounts for pentoxifylline's antinociceptive actions, it is probably an additional beneficial effect since the NF-κB activation ultimately leads to pro-inflammatory cytokine production[124]. Moreover, as pentoxifylline unspecifically inhibits PKC and PKA[123] this may also contribute to the control of pain since there is clearly evidence for the participation of PKCε in hypernociceptive priming[86,91] and PKA in hypernociception induction[125,126].

It is important to mention that although specific cytokine inhibitors (anankira, etanercept, infliximab) are available, the high cost of these therapies must be considered. Taking this into account, the cost-benefit relationship of pentoxifylline and thalidomide prompt the use of these drugs.

CONCLUSIONS

This review reinforces the crucial role of cytokines and chemokines mediating inflammatory pain in the majority of the described experimental nociceptive models. Its central goal was to show the concept that cytokine/chemokine cascades link the inflammatory stimuli and the release of the final mediators (such as prostaglandins, sympathetic amines) ultimately responsible for the nociceptor sensitization. In addition, cytokines/chemokines may also directly sensitize the nociceptors. Thus, substances that block the synthesis or action of the nociceptive cytokines/chemokines could be useful for inflammatory pain treatment.

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