Pharmacological Research 113 (2016) 500–514

Contents lists available at ScienceDirect

Pharmacological Research

journal homepage: www.elsevier.com/locate/yphrs

Novel celecoxib analogues inhibit glial production of prostaglandin E2, nitric oxide, and oxygen radicals reverting the neuroinflammatory responses induced by misfolded prion protein fragment 90-231 or lipopolysaccharide

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ARTICLE INFO

Article history:
Received 3 August 2016
Received in revised form 5 September 2016
Accepted 12 September 2016
Available online 22 September 2016

Chemical compounds studied in this article:
Celecoxib (PubChem CID: 2662)
Lipopolysaccharide (PubChem CID: 11970143)
Nitric oxide (PubChem CID: 145068)
Prostaglandin E2 (PubChem CID: 5280360)

Keywords:
Microglia
Neuroinflammation
Prion diseases
Nitric oxide
PGE_2
Non-steroidal antinflammatory drugs

ABSTRACT

We tested the efficacy of novel cyclooxygenase 2 (COX-2) inhibitors in counteracting glia-driven neuroinflammation induced by the amyloidogenic prion protein fragment PrP90-231 or lipopolysaccharide (LPS). In search for molecules with higher efficacy than celecoxib, we focused our study on its 2,3-diaryl-1,3-thiazolidin-4-one analogues. As experimental models, we used the immortalized microglial cell line N9, rat purified microglial primary cultures, and mixed cultures of astrocytes and microglia. Microglia activation in response to PrP90-231 or LPS was characterized by growth arrest, morphology changes and the production of reactive oxygen species (ROS). Moreover, PrP90-231 treatment caused the overexpression of the inducible nitric oxide synthase (iNOS) and COX-2, with the consequent nitric oxide (NO), and prostaglandin E_2 (PGE_2) accumulation. These effects were challenged by different celecoxib analogues, among which Q22 (3-[4-(sulfamoyl)phenyl]-2-(4-tolyl)thiazolidin-4-one) inhibited microglia activation more efficiently than celecoxib, lowering both iNOS and COX-2 activity and reducing ROS release. During neurodegenerative diseases, neuroinflammation induced by amyloidogenic peptides causes the activation of both astrocytes and microglia with these cell populations mutually regulating each other. Thus the effects of PrP90-231 and LPS were also studied on mixed glial cultures containing astrocytes and microglia. PrP90-231 treatment elicited different responses in the co-cultures induced astrocyte proliferation and microglia growth arrest, resulting in a differential ability to release proinflammatory molecules with the production of NO and ROS mainly attributable on microglia, while COX-2 expression was induced also in astrocytes. Q22 effects on both NO and PGE_2 secretion were more significant in the mixed glial cultures than in purified microglia, demonstrating Q22 ability to revert the functional interaction between astrocytes and microglia. These results demonstrate that Q22 is a powerful drug able to revert glial neuroinflammatory responses and might represent a lead to explore the chemical space around celecoxib frameworks to design even more effective agents, paving the way to novel approaches to contrast the neuroinflammation-dependent toxicity.

1. Introduction

Neuroinflammation in the central nervous system (CNS) is an important mechanism of defence against traumatic injury and environmental toxins, but it may also represent a major contributor to neuronal damage during neurological and neurodegenerative diseases including, Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), HIV dementia, and transmissible spongiform encephalopathies (TSEs or prion diseases) [1]. Microglia are CNS resident immune cells that comprise approximately 10–12% of glial cells in the brain and predominate in the grey matter. Microglia exists in a surveillance state characterized by ramified morphology but can be readily converted into an activated state in response to stress stimuli, pathogens,

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http://dx.doi.org/10.1016/j.phrs.2016.09.010
1043-6618/

protein aggregates, or particulate matter from dying neurons; activated microglia act as phagocytes and antigen-presenting cells, and release pro- and anti-inflammatory cytokines [2]. Although microglia forms the first line of protection for neural parenchyma, its uncontrolled activation may be toxic to neurons because of the release of reactive oxygen (H_2O_2, or O_2^-) and nitrogen (nitric oxide, NO) species, inflammatory cytokines (IL-1β, TNF-α, IL-6), and prostaglandins (PGE_2) [3–5]. Brains of AD patients or animals in which Aβ deposits and plaques were experimentally induced, are characterized by the presence of reactive astrocytes and microglial cells that cluster around β-amyloid deposits, and show a significant increase in oxidative stress markers, suggesting that the release of reactive oxygen and nitrogen species from astrocytes and/or microglia may contribute to neuronal degeneration [6,7].

Among the mediators that sustain neuroinflammation, PGE_2 contributes to neuronal loss in AD and other proteinopathies, included prion diseases [8]. AD patients show increased concentration of PGE_2 in the cerebrospinal fluid and upregulated cyclooxygenase-2 (COX-2) in the brain; moreover, in mouse models of AD the deletion of PGE_2 receptors result in neuroprotective effects [9,10]. Thus, being the activity of COXs the rate-limiting step in the conversion of arachidonic acid into prostaglandins, it was hypothesized that the neuronal injury induced by glial activation in AD and prion diseases can be reduced by non-steroidal antinflammatory drugs (NSAIDs) treatment [11,12]. This possibility is also supported by epidemiological studies showing that classical NSAIDs prevent or retard AD development [13–15].

Prion diseases are neurodegenerative disorders affecting humans and animals, histopathologically characterized by spongiform degeneration of grey matter, neuronal loss and gliosis [16]. They recognize as pathogenic event the misfolding of a glycoprotein, called prion protein (PrP), from a "cellular" form (PrP^C) into a protease-resistant and aggregation-prone counterpart named, according to the prototypical form of prion disease, prion protein "scrapie" (PrP^{Sc}) [17]. The peculiarity of PrP^{Sc} is the infectivity granted by a self-propagating process that initiates with PrP^{Sc}–PrP^C interaction-refolding, in which PrP^{Sc} acts as template to drive in infected hosts the misfolding of PrP^C to produce newly-formed PrP^{Sc} [17]. Beside this distinctive trait, prion diseases are proteinophaties that share with AD the brain deposition of misfolded protein aggregates. In particular, PrP^{Sc} insolubility and resistance to proteolysis favours its aggregation as oligomers, fibrils, and amyloid plaques that play a causal role in the neurodegeneration [18]. Indeed, according to the "gain of toxicity" hypothesis, neuronal loss during prion diseases is caused by folding intermediates generated along the conversion of PrP^C into PrP^{Sc}, before the formation of "mature" fibrils and their deposition in plaques [18]. Immuno-histochemical analysis of *post-mortem* brain tissues from CJD-affected humans and scrapie from experimentally-ill animals demonstrated the presence of a marked microglial response in spatial and temporal association with PrP^{Sc} amyloid deposits [19–21], suggesting a causal relationship among PrP^{Sc} deposition, glial reaction and neuronal death. In prion-affected brains, insoluble PrP^{Sc} is detected either as full length protein or as C-terminal fragments, which originate from partial proteolysis of its N-terminal portion [22]. These fragments, often found in proximity of spongiotic areas, retain the ability to aggregate as amyloid. Obtained as recombinant polypeptide, the C-terminal PrP fragment encompassing amino acids 90-231 of the human sequence (PrP90-231), has been successfully used to investigate the molecular mechanisms involved in PrP^{Sc} infectivity and neurotoxicity [23,24]. Exploiting the intrinsic plasticity of PrP90-231 fragment, we developed a model to study the neurotoxic activity of PrP^{Sc} [25]: this peptide, although non-toxic when folded as α-helix, acquires a gain-of-function when refolded into a β-sheet conformation, causing neuronal death and glial activation, inducing astrocytes and microglia to release NO and a complex array of cyto-/chemokines [26–33].

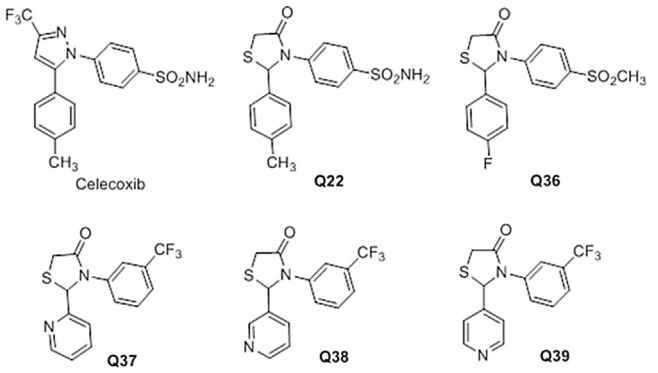

Fig. 1. Chemical structures of celecoxib and of the 2,3-diaryl-1,3-thiazolidin-4-one derivatives tested in this study.

To study the possible role of NSAIDs in preventing amyloid-driven neuroinflammation, we recently demonstrated that PrP90-231 induces PGE_2 release from microglia through the activation of COX-2, since this effect was selectively inhibited by celecoxib but not by ketoprofen, a prevalent COX-1 blocker [31].

Among the inflammatory agents that elicit neuroinflammatory responses, bacterial endotoxin lipopolysaccharide (LPS) has been widely used to model the molecular and cellular events following during neuroinflammation [34,35]. LPS experimental use contributed to the demonstration that neuronal death during CNS amyloidosis can be favored by peripheral inflammation [36]. Moreover, the neuroinflammatory potential of amyloidogenic peptides has often been evaluated in comparison with LPS to qualitatively and quantitatively detect differences between these stimuli [37].

In the present work, we searched for novel compounds able to inhibit with higher potency or efficacy than reference drugs, glial cell activation induced by neuroinflammatory stimuli, such as PrP90-231 and LPS. We tested a panel of 2,3-diaryl-1,3-thiazolidin-4-one derivatives (Fig. 1) structurally related to celecoxib and previously found to possess anti-inflammatory activity in animal models of chemical-induced oedema and hyperalgesia [38] as far as their ability to inhibit glial activation with higher efficacy than celecoxib. In particular, since the main glial populations, microglia and astrocytes, *in vivo* interact with each other to modulate their activity, we used a fully characterized cell culture model of mixed glial cell cultures in comparison with purified microglia. We identified two of these compounds, namely Q22 (3-[4-(sulfamoyl)phenyl]-2-(4-tolyl)thiazolidin-4-one) and, to a lower extent, Q36 (2-(4-fluorophenyl)-3-[4-(methylsulfonyl)phenyl]thiazolidin-4-one) that were able to revert PrP90-231- and LPS-induced microglia and astrocyte activation, reducing the release of neurotoxic factors involved in the inflammatory cascade such as PGE_2, NO and ROS.

2. Materials and methods

2.1. Chemicals

2,3-Diaryl-1,3-thiazolidin-4-one derivatives (**Q22:** 3-[4-(sulfamoyl)phenyl]-2-(4-tolyl)thiazolidin-4-one; **Q36:** 2-(4-fluorophenyl)-3-[4-(methylsul-fonyl)phenyl]thiazol-idin-4-one; **Q37:** 2-(pyridin-2-yl)-3-[3-(trifluoromethyl)phenyl]thiazolidin-4-one; **Q38:** 2-(pyridin-3-yl)-3-[3-(trifluoromethyl)phenyl]thiazolidin-4-one; **Q39:** 2-(pyridin-4-yl)-3-[3-(trifluoromethyl)phenyl]-thiazolidin-4-one) (Fig. 1) were synthesized as previously described [38].