

Effect of quinolinic acid on gene expression in human astrocytes: Implications for Alzheimer's disease

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Abstract. Activated microglia and astrocytes play a key role in the neuroinflammatory response during Alzheimer's disease (AD). The kynurenine pathway (KP) of tryptophan degradation is activated and production of the excitotoxin quinolinic acid (QUIN) by monocytic cells is increased. We studied here the effects of QUIN in pathophysiological concentrations on the expression of genes including IL-1 β , IL-6, S100 β , Glutamate synthetase (GS) glial fibrillary acidic protein (GFAP) that are commonly associated with astrocytes in the development of neuroinflammation in AD. We found that IL-6, S100 β and GS genes were constitutively expressed in human adult astrocytes (HAA) and only with TNF α , but not QUIN, IL-6 and S100 β expression were increased compared with controls in HAA. IL-1 β expression was increased by IFN- γ , TNF α and QUIN in HAA. These preliminary results suggest that QUIN's role in astroglial inflammatory response is mediated by increase of IL-1 β expression. Therefore, QUIN is likely to play a role in astroglial inflammatory response that may contribute to the pathogenesis of AD. © 2007 Published by Elsevier B.V.

Keywords: Alzheimer's disease; Inflammation; Quinolinic acid; Human adult astrocytes; Kynurenine pathway; IL1 β ; IL-6; S100 β ; GFAP; GS; TNF α ; IFN γ

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1. Introduction

Alzheimer's disease (AD) is a progressive degenerative disease that affects cognition, behaviour, and the ability to perform activities of daily living [1]. AD is associated with the dysfunction and degeneration of select populations of vulnerable neurons in the hippocampus and other cortical brain regions [2]. There is increasing evidence that quinolinic acid (QUIN) is involved in the pathogenesis of AD [14, #6558]. QUIN is the end-metabolite of the kynurenine pathway (KP), which is involved in the catabolism of L-tryptophan. However, this pathway is different between species and even between cell types. For example, both human macrophages and microglia have complete KP whereas human astrocytes lack one central enzyme and are unable to produce QUIN [3].

To date, little is known about the role of QUIN on astrocytes, which make up the brain's largest resident cells. Studies have found that QUIN could contribute and amplify brain inflammation by increasing production of chemokines and chemokine receptors by astrocytes [4]. However, that may not be the sole effect on astrocytes. There is also evidence that QUIN can increase GFAP, an astrocyte specific protein [5]. The effect of QUIN on other astrocyte specific proteins are unknown, particularly those associated with AD. Glutamate synthetase (GS) and S100 β are two such proteins. GS is an important enzyme for glutamate–glutamine cycle and was found to be reduced in the vicinity of astrocyte endfeet and perisynaptic regions of neurons. This reduction in GS may implicate the astrocyte in glutamate excitotoxicity that may lead to AD [6]. S100 β is a neurotrophic cytokine at normal concentrations but when overexpressed, S100 β can lead to neuronal death [7]. IL-1 β is a major driving force in AD pathogenesis and has been known to be overproduced in neuritic plaques found in AD. Moreover, it has been discovered that IL-1 β can activate astrocytes and up-regulate S100 β production [8]. Astrocytes are a major inducible source of IL-6 which can be a neurotrophic cytokine. However, overexpression could lead to neuroinflammation adding to the pathophysiology of AD. Many factors particularly, IL-1 β and TNF α have been shown to induce IL-6 expression in rodent astrocytes [9,10]. Thus, it would be useful to investigate the effect of QUIN on IL-1 β and IL-6 expression in human adult astrocytes. Since IFN- γ can increase the production of QUIN via the KP [11] and TNF α can induce IL-6 expression in astrocytes, these cytokines will be included in this study in order to simulate inflammation.

2. Materials and method

2.1. Preparation of purified primary cultures of human adult astrocytes (HAA)

Human adult brain tissues were obtained from the Neurosurgery Department of St. Vincent's Hospital from patients who had brain surgery following informed consent. AA were prepared using a previously described method by Guillemain et al. [12].

2.2. Stimulation of purified cultures of human astrocytes

Equal numbers of astrocytes were seeded into 6-well plates in 2 ml of RPMI 1640 medium. The cells were left to grow until 90% confluence. Then, astrocytes were treated

Table 1

Simplified deduction of each gene expression observed in human adult astrocytes after 24 hour with respect to housekeeping gene, GAPDH. ('+' represents up-regulation and '-' represents down-regulation)

HAA	Control	IFN γ	TNF α	Q350	Q500	Q1200
GFAP	+	+	-	+	+	+
IL-1 β	+	++	+++	++	++	++
S100 β	+	+	++	+	+	+
IL-6	+	+	++	+	+	+
GS	+	+	+	+	+	+
GAPDH	+	+	+	+	+	+

with IFN- γ (100 IU/ml), TNF α (100 IU/ml), and QUIN (350, 500 and 1200 nM) and incubated for 6, 12, 24, 48 and 72 hours in 37 °C, 5% CO₂. Untreated strocytes were used as negative controls.

2.3. RNA extraction, end-point (ep) PCR

The RNA of each sample was extracted using the Invitrogen Trizol protocol. The RNA of each sample was quantified at A260/280 wavelength using a spectrometer. ~1 μ g of RNA was used to synthesize cDNA using the Theroscript X (Invitrogen) enzyme following the manufacturer's instructions. Next, ep-PCR was carried out with conditions of 95 °C denaturation, 60 °C annealing, and 72 °C extension temperatures.

3. Results

The above table represents the quantification of GFAP, IL-1 β , S100 β , IL-6 and GS in comparison to their respective controls (Table 1). The experiment has been done in triplicate and results of each sample including controls were averaged relative to GAPDH values. Therefore to compare each sample with respect to control after normalizing with GAPDH, each control is always set as 1. IL-1 β expression increased substantially compared with the control by TNF α . An increase of IL-1 β expression was observed in all QUIN samples and IFN γ treated samples. GFAP expression was down regulated by TNF α compared with the control while all the QUIN samples did not have any significant effect on GFAP expression in the HAA. Only TNF α demonstrated a slight increase in S100 β and IL-6 expression compared with controls while GS expression remains the same in all samples in comparison to the controls.

4. Discussion

This study showed that QUIN's effect on astroglial gene expression is limited mostly to IL-1 β . QUIN did not have any significant effect on IL-6, GS, GFAP and S100 β expression. Lawrence et al. [13] suggested that IL-1 β 's neurotoxicity is increased in the presence of other insults including excitatory amino acid receptor over-activation. Our data provide an additional mechanism for QUIN associated neurotoxicity. It is already known that QUIN itself is an excitotoxin and that secondarily, it can lead to accumulation of excitotoxic concentrations

of glutamate through its ability to inhibit glutamate uptake by astrocytes [14]. We showed here that a part of QUIN's neurotoxicity might be associated with its capacity to increase IL-1 β per se increasing the neurotoxicity mediated by IL-1 β . TNF α induced a very significant increase in IL-1 β expression in HAA. This result is consistent with the data observed by Churchill et al. [15]. IL-1 β expression was increased in IFN- γ and QUIN samples compared with untreated HAA. Moreover, we also found the same pattern of IL-6 and S100 β expression in HAA. It has been previously shown that IL-6 expression in astrocytes can be enhanced by IL-1 β and/or TNF α [16] and that TNF α can increase IL-6 expression only in HAA. The results imply that IL-6 and S100 β genes in HAA are prone to respond to TNF α stimulation but not IFN α stimulation. However, the combined effects of TNF α and IFN- γ on IL-6 and S100 β during inflammation cannot be ruled out. Glutamine synthetase (GS) is constitutively expressed in HAA. Robinson et al. [17] showed that the astrocytic expression of GS is diminished in AD and HIV-encephalitis [18]. From our data, QUIN did not have any effect on the expression of GS in HAA implying that other factors are involved in the regulation of GS expression in astrocytes. Additionally, IFN- γ and lipopolysaccharide (LPS) combinations have been previously shown to decrease GS expression in astrocytes via the NO/iNOS pathway [19]. These studies lead us to believe that other factors such as pro-inflammatory cytokines or metabolites related to AD or other brain inflammation disorders need to be present in order to have an effect on GS expression and activity. Increase in GFAP expression is considered a marker for astrogliosis, glial reactive injury, and even neurodegeneration [20]. However, our results show that QUIN did not have any significant effect of GFAP expression. This is probably due to the duration it takes for QUIN to act on GFAP expression; 24 hours of stimulation may be too short to observe any significant changes. Therefore, a timeline study of GFAP expression would be useful. A decrease in GFAP may be associated with overall reduction in synaptic capabilities of neurons [21]. Our results demonstrated that stimulation with TNF α resulted in down regulation of GFAP, implying cellular damage leading to cell death. This may be due to the simultaneous effect of TNF α on the overproduction S100 β which is known to lead to apoptosis [22]. Apoptotic cells are known to process and release IL-1 β which can lead to further cell damage [23]. However, up-regulation of IL-6 by TNF α in HAA could be either damaging or neuroprotective following chronic or acute neuropathological insults, respectively [24]. Further experiments will investigate exact membranous and intracellular mechanisms of QUIN activation on astrocytes. Understanding these pathways will provide new potential therapeutic targets to limit neuroinflammation in AD and other brain diseases.

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