Early increased density of cyclooxygenase-2 (COX-2) immunoreactive neurons in Down syndrome

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DOI: https://doi.org/10.5114/fn.2017.68582

Abstract

Neuroinflammation is one of the hallmarks of Alzheimer's disease. One of the enzymes involved in neuroinflammation, even in early stages of the disease, is COX-2, an inducible cyclooxygenase responsible for the generation of eicosanoids and for the generation of free radicals. Individuals with Down syndrome develop Alzheimer’s disease early in life. Previous studies pointed to the possible overexpression of COX-2 and correlated it to brain regions affected by the disease. We analysed the COX-2 expression levels in individuals with Down syndrome and in young, adult and old mice of the Ts65Dn mouse model for Down syndrome. We have observed an overexpression of COX-2 in both, Down syndrome individuals and mice. Importantly, mice already presented an overexpression of COX-2 at postnatal day 30, before neurodegeneration begins; which suggests that neuroinflammation may underlie the posterior neurodegeneration observed in individuals with Down syndrome and in Ts65Dn mice and could be a factor for the premature appearance of Alzheimer’s disease.

Key words: Ts65Dn, Alzheimer’s disease, neuroinflammation, microglia.

Introduction

Down syndrome (DS) is the most common chromosomal aneuploidy [26]. Trisomy of chromosome 21 induces a phenotype with two hallmarks: intellectual disability and early development of Alzheimer’s disease (AD) [20].

Alzheimer’s disease development may be related to the presence of the amyloid precursor protein (APP) and the S100 genes on chromosome 21 [1,11]. Alzheimer’s disease has been widely associated with neuroinflammation, a process that may be responsible for neuronal death observed in patients. One of the key enzymes at the top of the neuroinflammatory cascade is the COX family (cyclooxygenases) which comprises two members: COX-1 that is expressed under basal conditions and COX-2, an inducible isoform, although expressed weakly under basal conditions. Several studies have analysed the expression of COX-2 in AD brains showing contradictory effects, probably due to the different disease stages in which the studies were performed [5,35].

The Ts65Dn mouse is a DS model which is segmentally trisomic for a portion of mouse chromosome 16 and orthologous to the long arm of the human chromosome 21. This segment contains approximately 140 genes, many of which are highly conserved between mice and humans [32]. Ts65Dn mice repro-
duce many of the alterations observed in DS, including the cholinergic degeneration present in AD.

In this study, we aim to characterize COX-2 expression in the human temporal cortex in DS and in the Ts65Dn mouse model with age.

Material and methods

Experimental mice were generated by repeated backcrossing of Ts65Dn females to C57/6Ei C3H/HeSnJ (B6EiC3) F1 hybrid males. The parental generation was obtained from the research colony of Jackson Laboratory. Euploid littermates of Ts65Dn mice served as controls. We used a total of 18 trisomic and 18 euploid mice in three groups: young (1 month), adult (3-4 months) and old (12-14 months). The genotypic characterization was established by qRT-PCR using SYBR Green PCR master mix (Applied Biosystems). The amount of each gene was quantified by the ABI PRISM 7700 (Applied Biosystems). The genes analysed were APP (3 copies) and Apo-B (2 copies) [13,18]. Animal experimentation was conducted in accordance with Directive 2010/63/EU of the European Parliament and Council of 22 September 2010 on the protection of animals used for scientific purposes and was approved by the Committee of Bioethics of the University of Valencia. Every effort was made to minimize the number of animals used and their suffering.

Animals were transcardially perfused under deep anaesthesia (cholal hydrate 4%, 1 ml/100 gr bw) using 4% paraformaldehyde in phosphate buffer. Brains were cryoprotected using 30% sucrose. Fifty microns thick sections (6 subseries) were collected from each brain using a sliding freezing microtome.

Human samples were obtained from the BIOBANC HCB – IDIBAPS (Barcelona, Spain). Temporal cortex human brain tissue had been fixed (24 h, paraformaldehyde in buffered solution), cryoprotected (sucrose 30%), stored at −80°C and cut (8-10 µm) with a cryostat. We tested 5 controls (average age 55 years old, 31-78, PMI 11 h, 7-17 h) and 5 individuals with DS (average age 53.6 years old, 36-67, PMI 11.5, 6-18 h).

Single and double immunofluorescence

Tissue was processed “free-floating” (mouse brain sections) or on slides (human sections) for immunofluorescence as follows. Sections were incubated with citrate buffer (0.01 M, pH 6.0) for 1 minute at 100°C. After this, sections were treated for 1 h with 5% normal donkey serum (NDS) (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) in PBS with 0.2% Triton-X100 (Sigma-Aldrich, St. Louis, MO, USA) and incubated overnight at room temperature either with only polyclonal goat IgG anti-COX2 (1 : 500, Santa Cruz) antibody or with a mix of COX-2 antibody and one of the following antibodies: monoclonal mouse IgG anti-NeuN (1 : 100, Chemicon), monoclonal mouse IgG anti-Iba1 (1 : 1000, Chemicon), polyclonal rabbit IgG anti-GFAP (1 : 1000, Sigma-Aldrich) or monoclonal mouse IgG anti-RIP (1 : 500, DSHB). Secondary antibodies were Alexa 488 donkey anti-goat IgG (1 : 200 Molecular Probes) and one of the following: Alexa 555 donkey anti-mouse IgG (1 : 200 Molecular Probes) or Alexa 555 donkey anti-rabbit IgG (1 : 200 Molecular Probes). Sections were mounted using Dako fluorescent medium (Dako North America, California). The sections were analysed using a confocal microscope (Leica TSC-SPE). Stacks (z-step 1.15 μm) were analysed using ImageJ software. All studied sections passed through all procedures simultaneously. All slides were coded prior to analysis until the experiment was completed.

Quantification of COX-2 expression

We analysed (1) the number of cells expressing COX-2 (high expression and low expression cells) and (2) the intensity of expression per cell in the temporal cortex of humans and mice (euploid and trisomic) of different ages. (1) For the number of cells, we counted the immunoreactive cells in 500-μm-wide strips (20 strips per group) running perpendicular to the pial surface including all layers of the temporal cortex. After measuring the area, we calculated cellular density. (2) For the intensity of expression per cell, we measured the intensity of fluorescence emission in 50 cells per individual using ImageJ software. Means were determined for each experimental group and data were statistically analysed using SPSS (version 15). The difference between groups was analysed in humans with one way ANOVA (phenotype) and in mice with two way ANOVA (age and phenotype). Parallel Nissl-stained sections were used to locate the analysed region.

Results

COX-2-positive cells could be found in all layers of the temporal cortex, as well as in other cortical regions of the adult mouse. We observed COX-2
Fig. 1. COX-2 expression in mice and humans. **A)** Adult mouse cortex. High expression of COX-2 in small cells (arrowheads) and low expression in large cells. **B)** COX-2 in the human temporal cortex. **C)** In mice, large COX-2 expressing cells are NeuN positive (neurons, arrowheads) but small cells are not. **D)** COX-2 small cells are Iba-1 (microglia, arrowhead). GFAP-positive astrocytes (E) and RIP-positive oligodendrocytes (F) lack COX-2. Scale bar 50 μm.
Overexpression of cyclooxygenase-2 (COX-2) in Down syndrome

Overexpression of cyclooxygenase-2 (COX-2) in Down syndrome was observed in two types of cells: small cells with high expression (arrowheads, Fig. 1A) and large cells with low expression (Fig. 1A). Expression of COX-2 in the temporal cortex of humans presented a similar pattern to that of mouse; however, the population of small intensely stained cells was absent (Fig. 1B).

Phenotypic characterization of COX-2-expressing cells in the mouse temporal cortex reflected that the large COX-2-positive cells corresponded to neurons (NeuN positive, Fig. 1C) while the small COX-2 positive cells corresponded to microglia (Iba-1 positive, Fig. 1D). COX-2 was absent in astrocytes (GFAP, Fig. 1E) and oligodendrocytes (RIP, Fig. 1F).

Next, we studied COX-2 expression in DS individuals (5 controls and 5 individuals with DS) and found more COX-2-positive cells in individuals with DS (428.0 ± 29.4 cells/mm²) than in controls (331.0 ± 25.4 cells/mm², p < 0.05) (Fig. 2A). Analysis of the intensity of COX-2 expression in both, control and DS individuals, showed that it was similar in the positive cells in the two groups (Fig. 2B).

In the second part of the study we set out to study whether the increase in COX-2-positive cell number observed in humans was present in the temporal cortex of the mouse model for DS Ts65Dn and if so, to determine the time point of onset of the over-expression of this molecule. As shown above, there are two populations of cells in mice (Fig. 1B): small, high COX-2-expressing cells (microglia) and large, low COX-2-expressing cells (neurons) in young (1 month), adult (4 months) and old (12-14 months) mice. Analysis of the microglial cells expressing COX-2 (Fig. 3A) revealed a phenotype-dependent decrease (p < 0.05). Moreover, the number of microglial cells expressing COX-2 was reduced with age (young-adult p < 0.001, and adult-old p < 0.05). Young: control 122.0 ± 11.2 vs. trisomic 94.4 ± 4.1 cells/mm²; adults: control 70.3 ± 3.0 vs. trisomic 62.5 ± 7.9 cells/mm²; old: control 48.9 ± 10.6 vs. trisomic 38.4 ± 10.8 cells/mm². Analysis of the number of neurons expressing COX-2 in mice (Fig. 3B) (similar population as observed in humans) showed that the COX-2 neuronal density was not altered between age groups, but similar to humans, we found more COX-2-expressing neurons in trisomic mice (p < 0.001; Fig. 3B). Control: young 696.9 ± 20.1; adult 680.3 ± 25.2; old 669.7 ± 22.4 cells/mm²; trisomic: young 843.9 ± 25.7; adult 836.4 ± 30.5; old 824.2 ± 58.2 cells/mm². Finally we analysed the intensity of COX-2 expression in the cytoplasm of neurons (Fig. 3C) in the different groups of mice. We observed a decrease in the intensity related to age, however, similar to our findings in humans, there was no difference in the intensity of expression of COX-2 between control and trisomic mice at any age studied.

Discussion

In conclusion, we have found an increased number of COX-2 immunoreactive cells in the human DS temporal cortex as well as in the brain of the mouse model for DS (Ts65Dn) at any age examined. Our findings in humans confirm previous studies where an increased expression of COX-2 has been observed in other brain regions [24].

Fig. 2. Alterations in COX-2 expression in the human temporal cortex in DS. A) COX-2 cell density in the human temporal cortex (control vs. DS). B) Intensity of expression of COX-2 in the cytoplasm of neurons in the human temporal cortex (control vs. DS), values are represented in %, being 100 in control conditions (*p < 0.05).
In mice it has been shown previously that COX-2 is present in neurons [16,35], although some studies found it in microglia [4,34], and even astrocytes [14]. Using an antibody previously tested in humans, we and others have shown that in humans COX-2-positive cells are always neurons [22]. However, in the mouse temporal cortex we found, in addition to large COX-2-expressing NeuN-positive cells (neurons, Fig. 1C), small COX-2-expressing cells which were Iba-1-positive and therefore microglia (Fig. 1D). COX-2 was absent in astrocytes (GFAP, Fig. 1E) and oligodendrocytes (RIP, Fig. 1F). Here we show that in the Ts65Dn mouse model, the increased number of COX-2-expressing neurons in DS is independent of age. The principal observation of our study is that this alteration starts early in DS, even in young animals (1 month old). This fact opens the possibility that, given that the alteration in COX-2 is previous to any deleterious observation, perhaps the degeneration observed in DS could be related to the alterations observed in markers such as COX-2.

The mechanism underlying the overexpression of COX-2 in DS has not been elucidated. One possibility is that the higher expression of COX-2 may be related to an extra copy of S100B present in individuals with DS [4]. S100B induces the expression of NF-κB, which is responsible, in turn, for COX-2 transcription.

Overexpression of COX-2 seems to be beneficial in the short run [9]. However, a chronic expression may be deleterious for the brain. In our animals, the density of COX-2 neurons remains unaltered with age, although some studies in old animals reported an increase with age [17]. Perhaps the reduced expression observed in the cytoplasm of old neurons in control and Ts65Dn mice, could lead to an underestimation of the number of positive cells in old animals.

Control of COX-2 expression is fundamental because of the reaction products of COX-2, pro-inflammatory prostanooids and reactive oxygen species, may be cytotoxic and cause CNS injury [29]. However the clear role of COX-2 in the neuroinflammatory process is still a matter of controversy (for a review, see [31]). COX-2 shows a low expression under basal conditions, but is increased during inflammatory responses and can be induced by cytokines and tumour necrosis factor [33]. Pathol-
Overexpression of cyclooxygenase-2 (COX-2) in Down syndrome

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Abstract

Down syndrome (DS) is a genetic condition caused by the presence of an extra chromosome 21. COX-2 is an enzyme involved in the production of prostaglandins, which are involved in various physiological functions, including the regulation of inflammation. Previous studies have shown that COX-2 is overexpressed in Down syndrome cells and that this overexpression is associated with neurodegeneration and cognitive impairment. This study investigates the role of COX-2 in Down syndrome and its potential as a therapeutic target.

Keywords

Down syndrome, COX-2, Neurodegeneration, Alzheimer's disease.

Introduction

Down syndrome is a genetic condition associated with learning disabilities, congenital heart defects, and an increased risk of early onset of Alzheimer's disease (AD) and epilepsy. The overexpression of cyclooxygenase-2 (COX-2) in DS is a phenomenon that has been observed in various animal models of DS, including the Ts65Dn model. COX-2 is a key enzyme that synthesizes prostaglandins, which are involved in the regulation of inflammation and other physiological functions.

Materials and Methods

The authors used various in vitro and in vivo models to investigate the role of COX-2 in DS. These models included primary cultures of human fibroblasts and induced pluripotent stem cells (iPSCs) from individuals with DS, as well as animal models such as the Ts65Dn mouse.

Results

The study found that COX-2 overexpression in DS is associated with an increase in inflammatory cytokines and a decrease in neurogenesis. The overexpression of COX-2 leads to an increase in some inhibitory neurons, including the so-called IS cells, which inhibits inhibition observed in DS. This overactivation could be the basis of the increased expression of COX-2 and the dysregulation observed in the balance between excitation and inhibition observed in DS.

Discussion

The overexpression of COX-2 in DS is related to the high prevalence of epilepsy and Alzheimer's disease in DS. It is possible that the overexpression of COX-2 in DS is a contributing factor to the early onset of AD and the increased risk of epilepsy in DS.

Conclusion

The authors conclude that COX-2 is a potential therapeutic target for the treatment of Alzheimer's disease and epilepsy in DS. Further research is needed to understand the role of COX-2 in DS and to develop effective therapeutic strategies.

Disclosure

Authors report no conflict of interest.

References


Acknowledgments

This study was supported by the Jerome Lejeune Foundation.


