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Impact of indomethacin on neuroinflammation and hippocampal neurogenesis in aged mice

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HIGHLIGHTS

- Effect of indomethacin on age-related neuroinflammation and neurogenesis was investigated in 24-month-old mice.
- Transcript expression of inflammatory mediators was upregulated in aged brains.
- Indomethacin decreased hippocampal COX-1 and COX-2 transcript expression.
- Gfap and Iba1 transcript expression were reduced by indomethacin in the aged hippocampus.
- Neurogenesis was unaffected by indomethacin.

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ABSTRACT

Age-induced neuroinflammation could be a contributing factor to the restricted neurogenesis in aged mice. Indomethacin, a common non-steroidal anti-inflammatory drug, has been demonstrated to partially restore neurogenesis under pathophysiological inflammation-associated conditions in adult C57BL/6 mice. This study investigated whether indomethacin is able to decrease age-related neuroinflammation in the hippocampus (24-month-old mice) and thereby stimulate neurogenesis. During hippocampal aging, the transcript expression of pro-inflammatory cytokines (Tnfα, Il-1α, Il-1β), the chemokine Mip-1α, and markers for activated astrocytes (Gfap, Lcn2, but not Vim and Serpina3n) and microglia (Iba1, F4/80, Cd68, Cd86) significantly increased. Treatment with indomethacin significantly decreased COX-1 and COX-2 transcript expression. Of the age-related inflammatory mediators, only Gfap and Iba1 were affected by indomethacin treatment in the hippocampus, with a significantly reduced transcript expression being detected for both markers. Neurogenesis was unaffected by indomethacin. Thus, our data reveal that administration of indomethacin to aged mice is not able to effectively decrease neuroinflammation and promote neurogenesis.

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1. Introduction

Aging dramatically reduces basal neurogenesis in the hippocampus [11,13,21]. This has been proposed as a possible explanation for cognitive impairments in aged organisms [6]. The age-related decline in neurogenesis results from a reduced proliferation of neural stem cells as well as from a delayed maturation of newborn neurons [11,13,21]. As a consequence of

Abbreviations: BrdU, bromodeoxyuridine; Cd68, cluster of differentiation 68; Cd86, cluster of differentiation 86; COX, cyclooxygenase; CTR, control; DCX, doublecortin; Gfap, glial fibrillary acidic protein; Iba1, ionized calcium-binding adaptor molecule 1; Il-1α, interleukin 1α; Il-1β, interleukin 1β; INDO, indomethacin; Lcn2, lipocalin 2; Mip-1α, macrophage inflammatory protein 1α; qPCR, quantitative polymerase chain reaction; Serpina3n, serine (or cysteine) peptidase inhibitor, clade A, member 3N; Tnfα, tumor necrosis factor α; Vim, vimentin.

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aging, the secretion of inflammatory mediators increases in the brain where activated microglia and astrocytes release cytokines and chemokines [22]. While it is accepted that experimentally induced cerebral inflammation (e.g. by lipopolysaccharide or irradiation) inhibits adult hippocampal neurogenesis [2,7,16], it is still unclear to what extent the age-related increased inflammatory status is responsible for the suppressed neurogenesis in the aged brain.

To investigate the effect of an anti-inflammatory treatment on neurogenesis in aged brains, we utilized the common non-steroidal anti-inflammatory drug indomethacin, which is a non-selective inhibitor of the prostanoid-forming enzymes cyclooxygenase-1/2 (COX-1 and COX-2) [3]. Indomethacin has previously been shown to be effective in different experimental models of cerebral inflammation as well as in models of Alzheimer's disease [16,17]. This study investigated the age-related expression of various inflammatory mediators (pro-inflammatory cytokines, chemokines, markers for activated microglia and astrocytes) proposed to modify hippocampal neurogenesis. Based on these data, the effect of a transient administration of indomethacin on hippocampal neuroinflammation and neurogenesis in non-pathological aged mice was assessed. We hypothesized that the application of indomethacin would attenuate the inflammatory processes in aged brains and thereby function as a potential stimulator of hippocampal neurogenesis.

2. Materials and methods

2.1. Experimental design

Indomethacin was applied for 11 weeks to 22-month-old mice (INDO). As in previous studies [20], indomethacin (Sigma) was dissolved in absolute ethanol (10 mg/ml stock solution), and diluted in drinking water to a final concentration of 10 μg/ml. To analyze hippocampal neurogenesis, mice received two daily intraperitoneal injections of bromodeoxyuridine (BrdU) (50 mg/kg body weight) over 6 days during the 6th week of indomethacin application ($n = 5$). Age-matched controls ($n = 10$) and adult 5-month-old mice without indomethacin treatment ($n = 8$) also received injections of BrdU. For quantitative polymerase chain reaction (qPCR) studies, mice were housed under the same conditions without receiving BrdU injections (24 months, INDO ($n = 5$); 24 months, control (CTR) ($n = 7$); 5 months, CTR ($n = 10$)). All animal procedures were performed on male C57BL/6 mice and approved by the local government (Thueringer Landesamt für Lebensmittelsicherheit und Verbraucherschutz (TLLV), Germany) and conformed to international guidelines on the ethical use of animals.

Table 1
Specific primers for qPCR.

Gene	Primer sequence (5′–3′)	GenBank accession number
Cd68	fw: TTCTGCTGTGGAAATGCAAG rv: GAGAAACATGGCCCGAAGT	NM_009853.1
Cd86	fw: AGCAACTAATGCTGAAAGCACA rv: CGGAATAGTCTATAGCCCTCCA	NM_019388.3
COX-1	fw: ACTATGGGGTTGAGGCACTG rv: GCCACATGCAGAACATGATAG	NM_008969.3
COX-2	fw: GGGTGTGAAGGGAAATAAGGA rv: GGGCAAAGAATGCAAACATC	NM_011198.3
F4/80	fw: ACTATTGGGAGCTACTTCTGCACT rv: TAGGAGCCTGGTACATTGGTG	NM_010130.4
Gfap	fw: AGAAAGGTTGAATCGCTGGA rv: GCCACTGCCTCGTATTGAGT	NM_010277.3
Lcn2	fw: TGGAAGAACCAAGGAGCTGT rv: GGTGGGGACAGAGAAGATGA	NM_008491.1
Serpina3n	fw: TGCGAAACTGTACCCTCTGA rv: TCATTTGGGGTTGGCTATCT	NM_009252.2
Vimentin	fw: TGAAGGAAGAGATGGCTCGT rv: GGTGTCAACCAGAGGAAGTGA	X56397.1

2.2. Immunohistochemistry

Mice were deeply anesthetized and transcardially perfused with 4% paraformaldehyde. Every 6th free-floating section was used for immunohistochemistry, as previously described in detail [14]. Slices were treated with a rat primary antibody against BrdU (1:500, AbD Serotec), followed by a biotinylated secondary antibody (donkey anti-rat, 1:500, Dianova Inc.).

For triple-labeling immunofluorescence, every 6th section was incubated with the following primary antibodies: rat anti-BrdU (as above), goat anti-doublecortin (DCX) (1:80, Santa Cruz Biotechnology), and mouse anti-NeuN (1:500, Chemicon). Slices were further processed with secondary antibodies – Rhodamine anti-rat (1:500, Dianova Inc.), Alexa 488 anti-goat (1:500, Molecular Probes), and Cy5 anti-mouse (1:500, Dianova Inc.) – as previously described [14], followed by incubation in copper(II) sulfate (Sigma) to mask the age-related accumulation of lipofuscin.

$BrdU^+$ cells were quantified throughout the subgranular and granular cell layer of the entire rostro-caudal extent of the dentate gyrus using an Axioplan 2 microscope (Carl Zeiss). The resulting numbers were multiplied by a factor of six to attain the estimated total number of $BrdU^+$ cells.

For phenotyping of $BrdU^+$ cells, random fields of dentate gyrus containing $BrdU^+$ cells were selected and z-stacks were scanned by confocal laser microscopy (LSM510, Carl Zeiss). Phenotypes of 30 $BrdU^+$ cells per dentate gyrus were determined in adult mice.

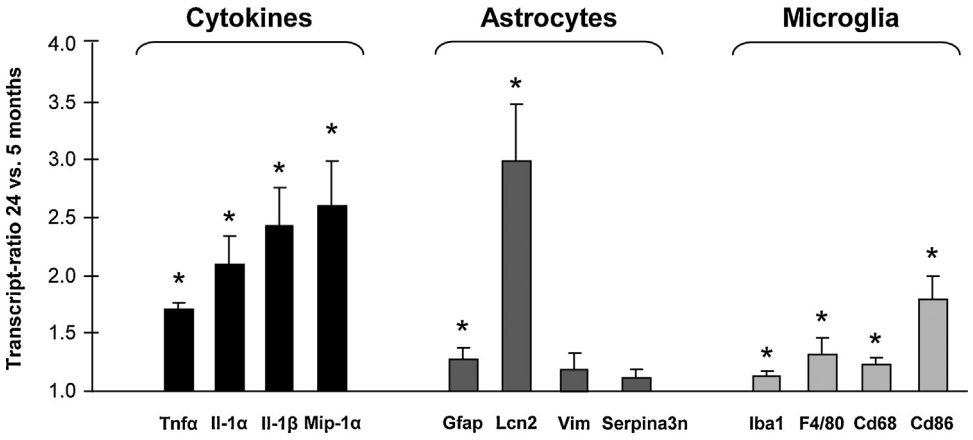

Fig. 1. Age-related transcript expression of inflammatory mediators. All investigated genes were significantly upregulated in the hippocampus during aging, except vimentin and Serpina3n. Transcript expression is presented as ratio of 24- vs. 5-month-old mice ± s.e.m. Mann–Whitney-*U* test, * $p \leq 0.05$, $n = 7$–10.

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