Persistent accumulation of cyclooxygenase-1–expressing microglial cells and macrophages and transient upregulation by endothelium in human brain injury

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Object. Secondary damage after central nervous system (CNS) injury is driven in part by oxidative stress and CNS inflammation and is substantially mediated by cyclooxygenases (COXs). To date, the rapidly inducible COX-2 isoform has been primarily linked to inflammatory processes, whereas expression of COX-1 is confined to physiological functions. The authors report the differential localization of COX-1 in human traumatic brain injury (TBI).

Methods. Differential cellular COX-1 protein expression profiles were analyzed following TBI in 31 patients and compared with neuropathologically unaltered control brains by using immunohistochemistry.

In these patients with TBI, a significant increase of COX-1 protein expression by vessel endothelial and smooth-muscle cells and CD68+ microglia/macrophages was observed to be strictly confined to the lesion. Accumulation of COX-1+ microglia/macrophages in the lesion was already evident 6 hours postinjury, reaching maximal levels after several weeks and remaining elevated at submaximal levels for several months after injury. Furthermore, COX-1+ cell clusters were located in the Virchow–Robin space during the leukocyte infiltration period from Days 4 to 8 after TBI. Double-labeling experiments confirmed coexpression of COX-1 by CD68+ microglia/macrophages. The numbers of COX-1+ vessel endothelial and smooth-muscle cells increased from Day 1, remaining at submaximal levels for months after injury.

Conclusions. The prolonged accumulation of COX-1+ microglia/macrophages that were restricted to perilesional areas affected by the acute inflammatory response points to a role of COX-1 in secondary injury. The authors have identified localized, accumulated COX-1 expression as a potential pharmacological target following TBI. Their results challenge the current paradigms of a selective COX-2 role in the postinjury inflammatory response.

Key Words • prostaglandin • oxidative stress • inflammation • tissue viability

Secondary brain damage results from a complex sequence of pathophysiological events that evolve over time and space.1,17 After TBI the intracellular influx of the second messenger Ca2+ activates phospholipase A2 and COXs. The ensuing lipid peroxidation induces cell membrane damage and release of toxic prostanoids and is supported by generation of reactive O2 species. The reactive O2 species overwhelm endogenous scavenging mechanisms and inflict downstream injury executed by apoptosis and inflammation.8,12,28

Prostanoids are major contributors to the intrinsic inflammatory CNS response (prostaglandins such as PGE2, PGD2, PGI2; thromboxanes such as thromboxane A2; and prostacyclins such as PGI2). These are synthesized by the enzyme prostaglandin endoperoxide synthase (also known as COX), which is the rate-limiting enzyme.8,20 Furthermore, COX has peroxidase activity and can induce formation of O2 radicals and dopamine quinones independent of its metabolism of arachidonic acid.13

Several isoforms of COX are known: the constitutively expressed COX-1, the rapidly inducible COX-2, and a recently proposed isoform hypothetically called COX-3,20,51 referred to as a status of prolonged COX-2 expression exerting paradoxical antiinflammatory properties. Although striking differences have been observed in the structure and regulation of the COX-1 and COX-2 genes, their protein structure and enzymatic function are remarkably similar.8,12,28

The current understanding of intrinsic tissue mechanisms after TBI has identified COX-2 metabolites as major neurotoxicity mediators8,12,35,42 with tightly regulated expression induced by pathogenic stimuli. The role of activated microglia and the likewise proinflammatory COX-1 with identical enzymatic function after human TBI remains enigmatic.13,20,26,46 To provide a pathophysiological basis for the involvement of COX-1 in human TBI, we have analyzed COX-1 expression in brain-injured patients and in neuropathologically unaltered control brains.

Materials and Methods

Pathological Tissue

Tissue from the brains of 31 patients with a clinical history and
Accumulation of cyclooxygenase-1–expressing cells in brain injury

Immunohistochemical Studies

After formaldehyde fixation and paraffin embedding, rehydrated 2-μm sections were boiled seven times for 5 minutes in citrate buffer (2.1 g sodium citrate/L, pH 6) in a 600-W microwave oven. Endogenous peroxidase was inhibited with 1% H2O2 in methanol (1:10) for 15 minutes. Sections were incubated with 10% normal porcine serum to block nonspecific binding of immunoglobinulins. Monospecific polyclonal antibodies directed against COX-1 were diluted 1:400 in 1% BSA/TBS (0.025 M Tris, 0.15 M NaCl) and incubated for 1 hour at room temperature. Specific binding of antibodies was detected with a secondary biotinylated swine anti–rabbit immunoglobulin G FAb, antibody fragment (1:400) for 30 minutes, followed by incubation with a peroxidase-conjugated streptavidin–biotin complex. The enzyme was visualized with diaminobenzidine as a chromogen. Double-Labeling Experiments

In double-labeling experiments, we first labeled a cell-type or activation-specific antigen by using the avidin–biotin complex procedure in combination with alkaline phosphatase conjugates. Specific antigens were labeled with mAbs against GFAP (1:100) to detect astrocytes, or against CD68 for microglia/macrophage identification. Activated microglia/macrophages were detected with antibodies directed against human leukocyte antigen-DR, -DP, and -DQ (1:100) or MR-8 (1:100). The number of perivascular cells in the Virchow–Robin space was determined using mAbs against CD14 (1:100) to identify both resident perivascular microglia/macrophages and nonresident infiltrating peripheral blood cells. Furthermore, to characterize the cellular proliferative response, we used a PCNA mAb (1:100). In addition, we used mAbs (1:100) against CD31 for

TABLE 1
Clinical and autopsy data in patients who suffered TBI*

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>Sex</th>
<th>Survival</th>
<th>Trauma</th>
<th>Lesion Site</th>
<th>Cause of Death</th>
<th>Additional Clinical &amp; Autopsy Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 64, M</td>
<td>&lt;6 hrs</td>
<td>MVA</td>
<td>lt &amp; rt frontoparietal</td>
<td>disruption of brainstem</td>
<td>CF, BSF, enterorrhesis</td>
<td></td>
</tr>
<tr>
<td>2 57, M</td>
<td>&lt;6 hrs</td>
<td>MVA</td>
<td>lt &amp; rt frontotemporal</td>
<td>multiple trauma</td>
<td>CF, BSF, SAH, hepatothoraxis</td>
<td></td>
</tr>
<tr>
<td>3 49, M</td>
<td>&lt;6 hrs</td>
<td>fall</td>
<td>frontobasal-parietal</td>
<td>asystole</td>
<td>DAI</td>
<td></td>
</tr>
<tr>
<td>4 93, F</td>
<td>&lt;6 hrs</td>
<td>MVA</td>
<td>lt occipital</td>
<td>multiple trauma</td>
<td>CF</td>
<td></td>
</tr>
<tr>
<td>5 20, M</td>
<td>&lt;6 hrs</td>
<td>fall</td>
<td>lt temporalobasal</td>
<td>multiple trauma</td>
<td>vertebral fracture, concealed hemorrhage, tonsillar herniation</td>
<td></td>
</tr>
<tr>
<td>6 27, M</td>
<td>&lt;6 hrs</td>
<td>fall</td>
<td>rt parietooccipital</td>
<td>severe TBI</td>
<td>air embolism, res</td>
<td></td>
</tr>
<tr>
<td>7 63, F</td>
<td>&lt;6 hrs</td>
<td>MVA</td>
<td>lt &amp; rt frontoparietal</td>
<td>multiple trauma</td>
<td>BSF, SDH, SAH, hemorrhagic pleurorhea</td>
<td></td>
</tr>
<tr>
<td>8 27, F</td>
<td>&lt;6 hrs</td>
<td>MVA</td>
<td>lt &amp; rt frontal</td>
<td>herniation</td>
<td>SDH, trep, DAI</td>
<td></td>
</tr>
<tr>
<td>9 36, M</td>
<td>&lt;6 hrs</td>
<td>fall</td>
<td>lt frontobasal</td>
<td>herniation</td>
<td>SAH</td>
<td></td>
</tr>
<tr>
<td>10 43, M</td>
<td>&lt;6 hrs</td>
<td>fall</td>
<td>lt &amp; rt frontotemporal</td>
<td>herniation</td>
<td>chronic alcoholism</td>
<td></td>
</tr>
<tr>
<td>11 63, M</td>
<td>6–23 hrs</td>
<td>fall</td>
<td>lt frontal pole</td>
<td>severe brain edema</td>
<td>EDH</td>
<td></td>
</tr>
<tr>
<td>12 46, M</td>
<td>6–23 hrs</td>
<td>fall</td>
<td>rt frontobasal</td>
<td>severe brain edema</td>
<td>CF, BSF, EDH, tonsillar herniation</td>
<td></td>
</tr>
<tr>
<td>13 26, F</td>
<td>6–23 hrs</td>
<td>MVA</td>
<td>lt frontobasal</td>
<td>hemorrhagic shock</td>
<td>multiple trauma</td>
<td></td>
</tr>
<tr>
<td>14 28, M</td>
<td>6–23 hrs</td>
<td>MVA</td>
<td>lt frontotemporal</td>
<td>hemorrhagic shock</td>
<td>SAH, DAI, anoxic encephalopathy</td>
<td></td>
</tr>
<tr>
<td>15 44, M</td>
<td>6–23 hrs</td>
<td>fall</td>
<td>lt frontobasal</td>
<td>severe TBI</td>
<td>EDH, SDH, trep</td>
<td></td>
</tr>
<tr>
<td>16 52, M</td>
<td>6–23 hrs</td>
<td>fall</td>
<td>rt frontal</td>
<td>herniation</td>
<td>SDH</td>
<td></td>
</tr>
<tr>
<td>17 48, F</td>
<td>1–2.5 days</td>
<td>fall</td>
<td>lt &amp; rt frontal</td>
<td>herniation</td>
<td>SDH, DAI</td>
<td></td>
</tr>
<tr>
<td>18 87, F</td>
<td>1–2.5 days</td>
<td>MVA</td>
<td>lt &amp; rt frontal pole</td>
<td>severe TBI</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td>19 64, M</td>
<td>1–2.5 days</td>
<td>fall</td>
<td>rt parietooccipital</td>
<td>severe brain edema</td>
<td>CF, BSF, SAH, DAI, trep, liver cirrhosis</td>
<td></td>
</tr>
<tr>
<td>20 55, M</td>
<td>1–2.5 days</td>
<td>fall</td>
<td>lt &amp; rt frontotemporal</td>
<td>severe brain edema</td>
<td>CF, SDH, trep</td>
<td></td>
</tr>
<tr>
<td>21 84, F</td>
<td>6 hrs</td>
<td>fall</td>
<td>lt frontobasal</td>
<td>multiple trauma</td>
<td>diffuse focal hemorrhages in white matter</td>
<td></td>
</tr>
<tr>
<td>22 89, F</td>
<td>6 hrs</td>
<td>MV A</td>
<td>lt &amp; rt frontotemporal</td>
<td>hemorrhagic shock</td>
<td>multiple trauma, BSF</td>
<td></td>
</tr>
<tr>
<td>23 59, M</td>
<td>4–8 days</td>
<td>MVA</td>
<td>rt temporal</td>
<td>herniation</td>
<td>multiple trauma, BSF</td>
<td></td>
</tr>
<tr>
<td>24 53, M</td>
<td>4–8 days</td>
<td>MVA</td>
<td>lt &amp; rt frontotemporal</td>
<td>anoxic encephalopathy</td>
<td>CF, BSF</td>
<td></td>
</tr>
<tr>
<td>25 30, F</td>
<td>4–8 days</td>
<td>fall</td>
<td>lt frontotemporal</td>
<td>herniation</td>
<td>pregnancy, fistula of cavernous sinus, DAI</td>
<td></td>
</tr>
<tr>
<td>26 41, M</td>
<td>6 hrs</td>
<td>MVA</td>
<td>lt &amp; rt frontotemporal</td>
<td>brainstem contusion</td>
<td>CF, BSF, DAI, multiple rip fractures, wet lung</td>
<td></td>
</tr>
<tr>
<td>27 65, M</td>
<td>6 hrs</td>
<td>MV A</td>
<td>lt &amp; rt temporal, lt frontal</td>
<td>herniation</td>
<td>EDH, SDH, DAI</td>
<td></td>
</tr>
<tr>
<td>28 73, M</td>
<td>6 hrs</td>
<td>MV A</td>
<td>rt temporobasal</td>
<td>pneumonia</td>
<td>SDH, trep, hematoma 48–72 hrs, CLL</td>
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</tr>
<tr>
<td>29 80, F</td>
<td>6 hrs</td>
<td>MVA</td>
<td>lt &amp; rt temporobasal</td>
<td>herniation</td>
<td>CF, DAI</td>
<td></td>
</tr>
<tr>
<td>30 29, F</td>
<td>6 hrs</td>
<td>fall</td>
<td>lt &amp; rt frontobasal</td>
<td>multiple organ failure</td>
<td>multiple trauma, fracture of cervical vertebral spine, SAH, trep, res, anoxic encephalopathy, ARDS</td>
<td></td>
</tr>
<tr>
<td>31 29, M</td>
<td>mos</td>
<td>fall</td>
<td>lt &amp; rt frontobasal</td>
<td>pulmonary embolism</td>
<td>infantile brain disease of uncertain origin</td>
<td></td>
</tr>
</tbody>
</table>

* ARDS = adult respiratory distress syndrome; BSF = basal skull fracture; CF = calvarial fracture; CLL = chronic lymphocytic leukemia; EDH = epidural hemorrhage; ICH = intracerebral hemorrhage; ICP = intracranial pressure; MVA = motor vehicle accident; res = resuscitated; SAH = subarachnoid hemorrhage; SDH = subdural hemorrhage; trep = trepanation.

In some patients whose immune status was altered because of immunosuppressive therapy or meningitis and/or encephalitis was excluded from this study. For controls, the results were compared with tissue obtained in corresponding areas of three normal brains described previously. In addition to patient data, hemorrhagic shock was confirmed by selective inhibition of staining after preincubation for 3 hours on ice with 20-fold excess of the COX-1 peptide, whereas adding of COX-2 peptide did not block staining (Fig. 1C and D). To identify areas of DAI we used β-APP mAbs (1:50) raised against the pre-Aβ42, fusion protein.

Double-Labeling Experiments

In double-labeling experiments, we first labeled a cell-type or activation-specific antigen by using the avidin–biotin complex procedure in combination with alkaline phosphatase conjugates. Specific antigens were labeled with mAbs against GFAP (1:100) to detect astrocytes, or against CD68 for microglia/macrophage identification. Activated microglia/macrophages were detected with antibodies directed against human leukocyte antigen-DR, -DP, and -DQ (1:100) or MR-8 (1:100). The number of perivascular cells in the Virchow–Robin space was determined using mAbs against CD14 (1:100) to identify both resident perivascular microglia/macrophages and nonresident infiltrating peripheral blood cells. Furthermore, to characterize the cellular proliferative response, we used a PCNA mAb (1:100). In addition, we used mAbs (1:100) against CD31 for

J. Neurosurg. / Volume 96 / May, 2002

893
vessel endothelial cell identification and against CD4 to study the potential immune repertoire of these cells. Briefly, slices were deparaffinized, irradiated in a microwave oven for antigen retrieval, and incubated with nonspecific porcine serum as described earlier. Antibodies were added to the slices at a dilution of 1:100 in 1% BSA/TBS. Visualization was achieved by adding biotinylated secondary antibodies (1:400) for 30 minutes and alkaline phosphatase–conjugated avidin–biotin complex diluted 1:400 in BSA/TBS for 30 minutes. Next, we developed tissue sections with Luxol fast blue salt chromogen-substrate solution, yielding a blue reaction product. Between double-labeling experiments, slices were irradiated in a microwave oven for 5 minutes in citrate buffer, then COX-1+ cells were counted in 10 high-power fields (×200 with an eyepiece grid representing 0.25 mm²). Data were calculated as the MLC per square millimeter (±SEM), MLS (% ± SEM), or MLV (% ± SEM, endothelial and/or smooth-muscle cells), respectively, and compared using the two-tailed Student t-test. Additionally, to evaluate the presence of COX-1+ cells in the perivascular Virchow–Robin space, 10 vessels in the perilesional area were counted.

Histological Staining for Myelin and Nuclei

Serial tissue sections adjacent to those used for immunohistochemical studies were stained for myelin by using Luxol fast blue to identify the area of tissue that was obviously damaged or lacked myelin. Nuclei were stained with 0.1% cresyl violet to identify intact compared with damaged areas.

Evaluation and Statistical Analysis and Methodology

The COX-1+ cells from the core of traumatic brain lesions were analyzed, including adjacent reactive perilesional tissue (border zones) or remote areas of the same tissue section (> 1.5 cm from the lesion margin), and were compared with tissue from normal control brains. The COX-1+ cells were counted in 10 high-power fields (×200 with an eyepiece grid representing 0.25 mm²). Data were calculated as the MLC per square millimeter (±SEM), MLS (% ± SEM), or MLV (% ± SEM, endothelial and/or smooth-muscle cells), respectively, and compared using the two-tailed Student t-test. Additionally, to evaluate the presence of COX-1+ cells in the perivascular Virchow–Robin space, 10 vessels in the perilesional area were counted.

Fig. 1. Photomicrographs of brain tissue sections. A: Photomicrograph of a section of control brain showing COX-1+ immunoreactivity by some microglia/macrophages, which were evenly distributed throughout the CNS. B: After TBI, accumulation of COX-1+ microglia/macrophages was confined to the lesion side, which was characterized by morphological amoeboid, activated phenotypes (arrowheads). C: With aging of the lesion, COX-1+ cells demonstrated cytoplasmic vacuoles and large round nuclei indicating morphological hallmarks of phagocytic lipid-loaded, foamy macrophages (arrowheads). D: Perivascular COX-1+ microglia/macrophages (brown, arrowheads) formed clusters in the Virchow–Robin space during the leukocyte infiltration period (blue; CD31 immunoreactivity indicates vessel endothelium). E: During the leukocyte infiltration period, a number of COX-1+ microglia/macrophages were frequently observed being intermingled within the intima and/or vessel wall leaving the vessel lumen (arrowheads). F: In some cases, perikarya and processes of large neurons also expressed COX-1 in injured and normal brains. G: Double-labeling experiments identified the majority of COX-1+ cells (brown) located at the lesion site as CD68+ microglia/macrophages (blue) (arrowheads). H: No COX-1+ cells were observed to coexpress GFAP (astrocytic) antigens. Bar = 100 μm.
Accumulation of cyclooxygenase-1–expressing cells in brain injury

counted and considered positive if a minimum of two COX-1+ cells were present. The vessels revealing COX-1+ endothelial and/or smooth-muscle cells were analyzed in the same fashion, and data were given in the same way.

Sources of Supplies and Equipment

The porcine serum was acquired from Biochrom, Berlin, Germany. The monospecific polyclonal antibodies were purchased from Santa Cruz Biotechnology, Santa Cruz, CA, as were the COX-1 and COX-2 control peptides, and the mAbs against CD14 and CD11a. The swine anti–rabbit immunoglobulin G Fab, antibody fragment was obtained from Dako, Hamburg, Germany, as was the peroxidase-conjugated streptavidin–biotin complex. The diaminobenzidine was supplied by Fluka, Neu-Ulm, Germany. The β-APP A4 mAbs and the GFAP were acquired from Boehringer Mannheim, Mannheim, Germany. The mAbs against CD68 and antibodies against human leukocyte antigens DR, DP, and DQ (MHC class II antibodies) were obtained from Dako, Glostrup, Denmark. This company also supplied the PCNA and mAbs against CD31 and CD4. The MR-8 mAb (S100A8, 8-5C2) was purchased from BMA, Augst, Switzerland.

Results

Tissue obtained in the brains of 31 patients with TBI and three control brains were evaluated for COX-1 expression by using immunohistochemical studies.

Neuropathologically Unaltered Control Brains

In control brains with no neuropathological alterations, COX-1 immunoreactivity was detected in some microglia/macrophages (Fig. 1A). These accounted for 2 to 5% of all cells and were evenly distributed throughout the CNS tissue (MLC 3 ± 0). Only single cells were detected in perivascular spaces (MLS 7 ± 7%), and only rare COX-1+ cells expressed the activation-associated MHC class II antigens. Furthermore, single neurons (< 5%) and few endothelial cells (< 1%) were labeled.

Traumatic Brain Injury

We analyzed whether the number and distribution of COX-1+ cells was altered after TBI. Injured CNS tissue spared at the lesion core was approximately 1 to 5% of the total cross-sectional area of the brain. We observed lesion-associated COX-1 expression localized to endothelial cells, smooth-muscle cells, and microglia/macrophages. The COX-1+ microglia/macrophages accumulated in injured tissue of the lesion core and in developing perilesional areas, respectively, and formed perivascular clusters in the Virchow–Robin space (Fig. 2 upper left, upper center, lower left, and lower center).

After analyzing the data with the Student t-test, we detected a significantly higher number (p < 0.0001) of COX-1–expressing microglia/macrophages at the lesion site (MLC 44 ± 0.7; Fig. 1B) compared with numbers in remote areas (MLC 15 ± 0.3) or cortical control brain tissue (MLC 5 ± 0.6; Fig. 2 upper left). In these areas of both primary and delayed secondary injury, the numbers of COX-1+ microglia/macrophages had already increased less than 6 hours postinjury (p < 0.0001, MLS 33 ± 0.8), at the 1st day (< 23 hours, MLS 48 ± 1), and 4 to 8 days postinjury (MLC 50 ± 1.6), reaching maximum levels at several weeks after TBI (MLC 58 ± 1.3; Fig. 2 lower left). Subsequently, the numbers of COX-1+ cells remained elevated for up to several months following TBI (MLC 48 ± 1.1), with the longest survival time being 5 years (60 months). Therefore, accumulation of COX-1+ microglia/macrophages occurred early after traumatic BBB disruption (< 6 hours) and cell numbers remained elevated for at least several months, in addition to the period of microglia/macrophage activation occurring at Days 3 to 5.9,49 In remote areas more than 1.5 cm distant from the lesion core demarcation, COX-1+ cell numbers increased also, although to a lesser degree (Fig. 2 upper left and lower left). The COX-1+ microglia/macrophages were characterized by morphological hallmarks of both ramified and amoeboid microglia. Activated COX-1+ microglial phenotypes were confined to the lesion core and adjacent reactive tissue (border zones, Fig. 1B), whereas remote from the lesion site we also identified a few COX-1+ microglial phenotypes with fine elongations. Amoeboid cells prevailed during the first 2 weeks after TBI. With aging of the lesions, a few parenchymal ramified COX-1+ cells were also observed. Another significant population of COX-1+ microglia/macrophages, observable from Days 4 to 8, had cytoplasmic vacuoles and large round nuclei, which are morphological hallmarks of phagocytic, lipid-loaded, foamy macrophages (Fig. 1C). These COX-1+ cells persisted in the necrotic lesion core and represented the majority of COX-1+ cells in the late phases, that is, from weeks to months post-TBI.

To correlate the expression pattern of COX-1 with different qualitative entities of TBI, we investigated its expression in distinct areas of brain injury. We analyzed areas of predominantly DAI in which there were no hemorrhages by using β-APP immunohistochemical studies, and compared these findings with those in areas of primarily hemorrhagic injury. In areas of predominantly DAI (1 hour and 3 and 4 days after axonal injury) we observed a delayed accumulation of COX-1+ microglia/macrophages from Day 3 forward, whereas in hemorrhagic regions early accumulation was evident.

After human TBI, increase in perivascular COX-1+ cell numbers (Fig. 1D) were also observed in the Virchow–Robin space (p < 0.0001, MLS 32% ± 7%), compared with control brains (MLS 7 ± 7%; Figs. 1E and 2 upper center). These perivascular areas represent the major infiltration routes for extravasating cells into the CNS. Early (< 6 hours) after traumatic BBB disruption, pronounced areas of the Virchow–Robin space (MLS 26 ± 6%) contained COX-1+ microglia/macrophages (Fig. 2 lower center). During this time period a number of COX-1+ monocytes were frequently observed intermingled within the intima and vessel wall leaving the vessel lumen (Fig. 1E). Numbers of COX-1+ cells in perivascular spaces increased further during Day 1 (MLS 37 ± 6%) reaching maximum levels from Days 4 to 8 (MLS 53 ± 13%), when more than half of the counted vessels were surrounded by COX-1+ cells. Cell numbers then declined during the ensuing weeks and months after TBI. Profound accumulation of lesion-associated COX-1+ cells in the perivascular Virchow–Robin space paralleled the leukocyte infiltration period.49,56,7,48

Also at the site of the lesion, the numbers of COX-1+ vessels, which are characterized by immunopositive endothelial and/or smooth-muscle cells, increased after TBI (MLV 44.2 ± 4%) compared with control brains (MLV 13.3 ± 3%; Fig. 2 upper right). More precisely, the numbers of COX-1+ vessels (Fig. 2 lower right) demonstrated a bimodal distribution, increasing early from less than 6
**FIG. 2.** Upper: Bar graphs showing cumulative results in all injured brains, demonstrating a strictly lesion-associated accumulation of COX-1+ cells, like parenchymal and perivascular microglia/macrophages, and endothelial and smooth-muscle cells (p < 0.0001) in the lesion core and adjacent perilesional areas (p < 0.0001), compared with peripheral regions (> 1.5 cm from the lesion demarcation) and in control brains. Lower Left: Bar graph showing the temporal COX-1 expression pattern, demonstrating a significant accumulation of COX-1+ microglia/macrophages already at less than 6 hours postinjury (p < 0.0001), which increased further during the first 24 hours, until weeks (w) later, when numbers of COX-1+ microglia/macrophages reached maximum levels post-TBI. Subsequently, submaximal enhanced levels of COX-1+ microglia/macrophages persisted up to several months (m) postinjury. Lower Center: Bar graph showing COX-1+ cells in perivascular spaces (Virchow–Robin spaces, MLS SEM). Results from spaces containing two or more COX-1+ cells are given in percent of 10 counted vessels. After accumulation during the leukocyte infiltration period (< 6 hours, reaching maximum levels from 4–8 days), the numbers of perivascular spaces containing COX-1+ cell clusters declined. Lower Right: Bar graph showing COX-1+ vessels (endothelial and/or smooth-muscle cells, MLV SEM), demonstrating a bimodal distribution, increasing early from 6 hours forward, reaching the first maximum during the 1st day, followed by a decrease at 2.5 days and a second maximum 4 to 8 days postinjury. In general, the accumulation of lesion-associated COX-1+ cells, such as microglia/macrophages in parenchyma and perivascular spaces and in vessels (endothelial cells and smooth-muscle cells), was evident up to 6 months after brain injury compared with control brain (p < 0.0001) (lower left), whereas numbers of COX-1+ perivascular clusters and vessels declined after 3 weeks to almost base levels (lower center and right). (The numbers of brains tested at each time point are as follows: 0 hours, three [control brains]; < 6 hours, 10; 6–23 hours, six; 1–2.5 days, four; 4–8 days, four; weeks, five; months, two.)
hours postinjury (MLV 40 ± 8.2%), reaching the first maximum during the 1st day (MLV 62 ± 4%), followed by a decrease until 2.5 days postinjury and reaching a second maximum at 4 to 8 days (MLV 55 ± 13%). Subsequently, the number of COX-1 vessels decreased, but remained above control levels. In general, upregulation of COX-1 protein expression, demonstrated by the accumulation of COX-1 cells, such as parenchymal microglia/macrophages, was evident for up to several months after TBI, compared with control brains (p < 0.0001; Fig. 2 lower left). The COX-1 expression by monocytes in perivascular spaces and by vessels (endothelial cells) reached transient maximum levels correlating with the leukocyte infiltration period and the BBB breakdown. After this period, the numbers of COX-1–expressing cells in perivascular spaces and in vessels (endothelial cells) were still enhanced, compared with those found in normal brains (Fig. 2 lower center and lower right). In some cases, perikarya and processes of large neurons expressed COX-1 in normal and injured brains, and this expression was not restricted to lesional or perilesional areas (Fig. 1F).

**Double-Labeling Experiments**

To characterize the cellular origin of COX-1 expression, we performed double-labeling experiments. Furthermore, neurons were distinguished from nonneuronal cells by nuclear size, shape, and the presence of a nucleolus (Fig. 1F). The majority of COX-1+ cells located in the lesion core coexpressed the CD68 antigen (> 80%; Fig. 1G). The COX-1+ cells frequently coexpressed MHC class II antigens or the monocyte activation antigen MRP-8 (50–80%). This Ca++ binding protein, which specifically binds to arachidonic acid, is closely associated with microglia activation. The COX-1+ cells coexpressing the CD68 antigen were mostly observed in territories of phagocytic activity, such as the pannecrotic lesion core, indicating a predominant phagocytic immunophenotype. In contrast, coexpression with MHC class II and MRP-8 was not restricted to areas of phagocytic activity. Occasionally, COX-1+ cells coexpressed CD4 molecules (10–30%), and frequently, COX-1+ cells coexpressed PCNA (50–60%). These proliferating cells were mostly confined to the lesion core. No COX-1+ cells were identified that coexpressed pan-T-lymphocytic (CD3), pan-B-lymphocytic (CD20), or astrocytic (GFAP) antigens (Fig. 1H).

**Discussion**

In this study we have analyzed COX-1 expression in 31 traumatically injured brains and compared them with neuropathologically unaltered control brains. We observed a significant, persistent accumulation of COX-1+ microglia/macrophages and a transient upregulation of COX-1+ endothelial cells in the necrotic core and in reactive, perinecrotic areas after TBI. The majority of COX-1+ microglia/macrophages coexpressed the activation antigen MRP-8. Furthermore, in injured brains an accumulation of CD68+ COX-1+ microglia/macrophages from the lesion was observed in pivotal perivascular regions.

To date, the counterpart to COX-1, the highly inducible COX-2, has been associated with diverse pathological conditions in nonneuronal (that is, kidney and joint cartilage) and neuronal tissue. In the CNS, COX-2 expression was confined to inflammatory,34–36 neoplastic, new lesion,37,38 degenerative,39 traumatic, fibrotic, and ischemic9,16,32,42,43 disorders. Therefore, in recent studies of CNS injury attention has been focused on the COX-2 isoform, which is expressed by vulnerable neurons, endothelial cells, and astrocytes. Nevertheless, the idea of a unique role for COX-2 as the only responsible, inducible inflammatory mediator is complicated9,53 and has been questioned.24,32

The expression of the late-inducible, differentiation-associated COX-1 isoform, which is associated with tissue homeostasis, as the source of physiologically important prostaglandins did not gain much attention, although it led to identical proinflammatory and injury-producing products. We know that COX-1 is involved in the physiological response, constituting the regulation of blood homeostasis (vasoconstriction, platelet aggregation, angiogenesis, and blood pressure),19,54–56 and differentiation of neuronal,19 stem,36 and monocytic cells.14 Only scarce data on the pathophysiological role of COX-1 are available. The expression of COX-1 induced tumorigenic transformation in immortalized endothelial cells and altered the properties related to multiple drug resistance, resulting in reduced chemotoxicity in C6 glioblastoma cells.11 Disease-associated accumulation of COX-1+ microglia was evident in amyloid plaques of AD, indicating a disease-related, localized increase of COX-1.59 Likewise, little is known about the elements involved in regulating COX-1 gene expression; COX-1 is a delayed response gene, and its transcription is inducible by basic fibroblast growth factor, by epidermal growth factor in mouse myeloblastoma (MC3T3), and by nerve growth factor in neurogenic rat pheochromocytoma cells (PC12), which are used as a model to study neurite outgrowth.59 In addition, there have been reports of induction of COX-1 during differentiation of monocytes initiated by phorbol ester.14

Surprisingly, reactive microglia, which are sensors for pathological events in the CNS25 and are essential to tissue remodeling and one of the major sources of prostaglandins in this system,37,31 are not the main source of the pathogen-associated COX-2 expression.38,42 The role of microglia in lipid oxygenation is supported, however, by their capacity to produce PLA2, COX-1, and the vasoactive PGE2, and thromboxane A2. COX-1 is inducible by basic fibroblast growth factor, by epidermal growth factor in mouse myeloblastoma (MC3T3), and by nerve growth factor in neurogenic rat pheochromocytoma cells (PC12), which are used as a model to study neurite outgrowth.59 In addition, there have been reports of induction of COX-1 during differentiation of monocytes initiated by phorbol ester.14

Because COX activity is important after CNS injury,9,34,42 we defined COX-1 expression following human TBI. It appeared that COX-1 was expressed constitutively only by a few microglia/macrophages, some endothelial cells, and rare neurons. There were significant accumulations of COX-1+ microglia/macrophages at the lesion core and in adjacent perilesional areas. These results indicate a paracrine role of COX-1 metabolites in the modification of the posttraumatic microenvironment. Because it exerted the same enzymatic activity as COX-2, our data implicate a role of COX-1 in the monocyte and macrophage-mediated secondary (bystander) damage during lipid hydrolysis, damage that is induced by converting the membrane phospholipid product, arachidonic acid. The COX-1 converts...
Based on experimental and clinical data, nonselective inflammatory drug treatment after suffering brain trauma. Patients might wish to receive long-term nonsteroidal anti-inflammatory approaches based on long-term blocking, including blocking of COX-1, might delay the persistent accumulation of infiltrating COX-1-expressing T lymphocytes and by transient local upregulation of COX-2 expression and by progressive endothelial damage and vasogenic dysfunction. This theory is supported by the bimodal accumulation of COX-1+ cells and infiltrating inflammatory cells in the Virchow–Robin space (the major infiltration route for extravasating cells into the CNS), paralleling the leukocyte infiltration period, and the persistence of COX-1+ cells in damaged tissue areas. Our results indicate a substantial proinflammatory contribution of extravasating and engrafting peripheral COX-1+ blood-derived cells. These newly acquired constituents form a long-term, potential substrate for intervention.

In addition, persistent COX-1+ cell accumulation might represent an assessable residual inflammatory risk factor linking the enhanced incidence and prevalence of AD to patients who suffer head trauma. We emphasize this because, first, head injury is an important risk factor for the development of AD; second, inflammation mediated by proinflammatory entities stimulates the production of β-APP; and, third, these inflammatory mediators were detected intracerebrally after injury. Because inflammatory mechanisms, including expression of COX-1, are considered to be involved in the pathogenesis of AD, injured patients might wish to receive long-term nonsteroidal anti-inflammatory drug treatment after suffering brain trauma. Based on experimental and clinical data, nonselective COX blocking, including blocking of COX-1, might delay the onset of disease or decrease the incidence of AD within this patient group. Thus, we have identified COX-1 as a potential substrate for nonsteroidal antiinflammatory drug action in the human brain after TBI.

Conclusions

Several lines of evidence implicate a pathophysiological role of posttraumatic COX-1 expression by activated microglia/macrophages. Activated microglia/macrophages are known to promote angiogenesis and regeneration, but also to generate toxic agents. These results indicate that a local increase in COX activity can be caused by both transient local upregulation of COX-2 expression and by the persistent accumulation of infiltrating COX-1-expressing inflammatory cells. These findings substantially challenge current paradigms of a selective role of COX-2 in posttraumatic CNS injury response. Thus, therapeutic approaches based on long-term blocking, including blocking of COX-1, might be superior to selective COX-2 blocking to suppress local synthesis of prostanooids.

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References

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