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Enhanced Capillary Amyloid Angiopathy-Associated Pathology in Tg-SwDI Mice With Deleted Nitric Oxide Synthase 2

William E. Van Nostrand, PhD; Feng Xu, MD; Annemieke J.M. Rozemuller, MD, PhD

Background and Purpose—Cerebral amyloid angiopathy Type 1 is characterized by amyloid β protein deposition along cerebral capillaries and is accompanied by perivascular neuroinflammation and accumulation of phospho-tau protein. Tg-SwDI mice recapitulate capillary amyloid deposition and associated neuroinflammation but lack accumulation of perivascular phospho-tau protein.

Methods—Tg-SwDI mice were bred onto a nitric oxide synthase 2 gene knockout background and aged for 1 year. Brains were harvested and analyzed using immunohistochemical and quantitative stereological methods to determine the extent of capillary amyloid deposition, perivascular activated microglia, and cell-specific accumulation of phospho-tau protein. Similar methods were also used to compare Tg-SwDI/NOS2−/− and human cerebral amyloid angiopathy Type 1 brain tissues.

Results—The absence of nitric oxide synthase 2 gene had no effect on the regional pattern or frequency of capillary cerebral amyloid angiopathy or the numbers of perivascular activated microglia in Tg-SwDI mice. On the other hand, Tg-SwDI/NOS2−/− mice accumulated phospho-tau protein in perivascular neurons and activated microglia. Tg-SwDI/NOS2−/− mice exhibited a very similar distribution of capillary amyloid, activated microglia, and perivascular phospho-tau protein as seen in human cerebral amyloid angiopathy Type 1.

Conclusions—These findings indicate that Tg-SwDI/NOS2−/− mice more fully recapitulate the pathological changes observed with capillary amyloid in human cerebral amyloid angiopathy Type 1. (Stroke. 2010;41[suppl 1]:S135-S138.)

Key Words: capillary ■ cerebral amyloid angiopathy ■ nitric oxide synthase 2 ■ phospho-tau ■ transgenic mice

Cerebral amyloid angiopathy (CAA) is a condition characterized by accumulation of fibrillar amyloid β protein deposits in blood vessels of the brain of patients with Alzheimer disease and related disorders.1 Amyloid β is a 4 kDa peptide that is derived from its parent protein, the amyloid β protein precursor (AβPP), through the sequential processing by β- and γ-secretase enzymes.2 CAA presents as 2 subtypes: the more common CAA Type 2 affects arterioles and arteries of the cortex and leptomeninges, whereas the less common CAA Type 1 affects cerebral capillaries with or without larger cerebral vessel involvement.3 Capillary fibrillar amyloid deposition in CAA Type 1 is accompanied by a localized robust neuroinflammatory response and perivascular phospho-tau protein accumulation that is not commonly associated with the affected larger vessels in CAA Type 2. Previously, we generated a unique transgenic mouse, known as Tg-SwDI, that expresses in brain human AβPP harboring tandem Dutch (E22Q) and Iowa (D23N) amyloid β mutations associated with familial forms of CAA.4 Tg-SwDI mice were shown to develop early-onset and regionally extensive capillary amyloid with associated neuroinflammation reminiscent of CAA Type 1.4,5 Nitric oxide synthase 2 (NOS2), the inducible form of nitric oxide synthase, is 1 of 3 isoforms of nitric oxide synthase that are responsible for generating nitric oxide.6 Nitric oxide has been reported to possess both cytotoxic and cytoprotective activities depending on its concentration and cellular targets.7 Recently, we showed that breeding Tg-SwDI mice onto a NOS2 deletion background (NOS2−/−) resulted in more severe capillary CAA-related pathology, including perivascular accumulation of phospho-tau protein, neuronal loss, and greater behavioral impairments.8 In the present study, we determined if the absence of NOS2 affected the frequency of capillary CAA and associated microglial activation in Tg-SwDI mice. We also evaluated what cell types exhibit perivascular phospho-tau accumulation in Tg-SwDI/NOS2−/− mice and how these pathologies directly compare with human capillary CAA Type 1.

Methods

Transgenic Mice

All work with animals followed National Institutes of Health guidelines and was approved by the Stony Brook University Insti-
tutional Animal Care and Use Committee. The generation and characterization of Tg-SwDI and Tg-SwDI/NOS2<sup>2/2</sup> mice was performed in our laboratory as previously described. For the experiments, mice were aged to 12 months before euthanasia and collection of brain tissue for analysis.

**Immunohistochemical Analysis**

Immunostainings of mouse and human brain tissues were performed on deparaffinized sections or fresh-frozen sections as described. The following antibodies were used: rabbit polyclonal antibody to collagen Type IV for identification of blood vessels (1:100; Research Diagnostics Inc, Flanders, NJ); monoclonal antibody AT8 for detecting phospho-tau protein (1:250; Thermo Fisher Scientific); monoclonal antibody NeuN for identification of neurons (1:500; Chemicon); monoclonal antibody to glial fibrillary acidic protein for identification of astrocytes (1:1000; Chemicon); and monoclonal antibody to Major Histocompatibility Complex Class II for identification of activated microglia (1:200; BD Pharmingen, San Jose, Calif). Thioflavin-S staining for fibrillar amyloid was performed as described.

**Quantitative Analysis of Regional Capillary CAA and Activated Microglia**

The percentage of thioflavin-S-labeled blood vessels and immunostained-activated microglia in the regions of the thalamus and frontotemporal cortex was, respectively, quantified on the same set of systematically sampled thioflavin-S-stained sections using the Stereologer software system (Systems Planning and Analysis) as described.

**Results**

Immunohistochemical analysis to identify capillary fibrillar amyloid and the frequency of these deposits was measured using stereological methods. As shown in Figure 1, a similar pattern of capillary amyloid deposition in Tg-SwDI and Tg-SwDI/NOS2<sup>2/2</sup> mice was observed (Figures 1A and 1B, respectively) with no quantitative difference in the regional frequency of affected capillaries (Figure 1C). Similarly, a common pattern of capillary-associated activated microglia was observed in Tg-SwDI and Tg-SwDI/NOS2<sup>2/2</sup> mice (Figures 1D and 1E, respectively), again with no difference in the regional densities of these neuroinflammatory cells (Figure 1F). These findings indicate that the absence of NOS2 does not affect capillary CAA and associated activated microglia in Tg-SwDI mice.

**Figure 1.** Quantitation of brain capillary amyloid frequency and associated activated microglia in Tg-SwDI mice and Tg-SwDI/NOS2<sup>2/2</sup> mice. Brain sections from 12-month-old Tg-SwDI mice (A and D) or Tg-SwDI/NOS2<sup>2/2</sup> (B and E) mice were immunolabeled for collagen Type IV to identify capillaries (red) and stained with thioflavin S (green) to identify fibrillar amyloid (A and B) or immunolabeled for MHC II (brown) to identify activated microglia (D and E). Scale bar=100 μm. Quantitative stereological measurement of capillary amyloid frequency (C) and activated microglia densities (F) in thalamus and cortex of Tg-SwDI mice (gray bars) and Tg-SwDI/NOS2<sup>2/2</sup> mice (black bars). Data shown are the mean±SD (n=6 mice for each group).

**Figure 2.** Immunolabeling for hyperphosphorylated tau colocalizes with perivascular neurons and activated microglia in Tg-SwDI/ NOS2<sup>2/2</sup> mice. Brain sections from 12-month-old Tg-SwDI mice or Tg-SwDI/NOS2<sup>2/2</sup> mice were immunolabeled for hyperphosphorylated tau using AT8 antibody (green). Neurons were identified using NeuN antibody (A to D), astrocytes using GFAP antibody (E to H), and activated microglia using the MHC II antibody (I to L). Scale bars=20 μm.
Previously we reported that perivascular AT8-positive phospho-tau protein accumulates in Tg-SwDI/NOS2<sup>−/−</sup> mice. However, the specific cell types that accumulated phospho-tau were not thoroughly determined. Therefore, brain sections from Tg-SwDI and Tg-SwDI/NOS2<sup>−/−</sup> mice were double-immunolabeled for AT8 phospho-tau and markers specific for neurons, astrocytes, or activated microglia. As shown in Figure 2, AT8 phospho-tau labeling was only observed in Tg-SwDI/NOS2<sup>−/−</sup> mice. Colocalization of AT8 phospho-tau labeling was observed in cortical neurons and some thalamic neurons (Figures 2C and 2D, respectively). In contrast, no colocalization of AT8 phospho-tau was observed with astrocytes (Figure 2G–2H). However, strong colocalization of AT8 phospho-tau was observed in some cortical but many thalamic activated microglia that are tightly associated with capillary amyloid deposits (Figures 2K and 2L, respectively). These results indicate that AT8 phospho-tau is present in some perivascular neurons and many activated microglia in regions of capillary amyloid deposition in Tg-SwDI/NOS2<sup>−/−</sup> mice.

Capillary amyloid and associated pathology observed in Tg-SwDI/NOS2<sup>−/−</sup> mice were compared with human CAA Type 1 brain tissues using immuno- and histochemical stainings. Thioflavin S staining revealed a very similar pattern of capillary amyloid accumulation in Tg-SwDI/NOS2<sup>−/−</sup> mice and human CAA type-1 (Figures 3A and 3D, respectively). Similarly, brain sections from 12-month-old Tg-SwDI/NOS2<sup>−/−</sup> mice (C) and a case of human CAA Type 1 (F) were immunolabeled for hyperphosphorylated tau using AT8 antibody (red) and stained with thioflavin S (green) to identify fibrillar amyloid. Scale bars=20 μm.

**Discussion**

In the present study, we show that Tg-SwDI/NOS2<sup>−/−</sup> mice exhibit many of the pathological features of capillary amyloid found in human CAA Type 1. The absence of NOS2 does not affect the regional distribution or frequency of capillary amyloid deposition nor the levels of associated activated microglia in Tg-SwDI mice. However, we previously reported that in the absence of NOS2, the Tg-SwDI mice show additional pathological features, including perivascular phospho-tau, neuronal loss, and greater behavioral deficits. Here we further investigated which types of perivascular cells exhibit AT8-positive phospho-tau accumulation. Although some cortical neurons showed labeling for phospho-tau, we found strong labeling of perivascular activated microglia, particularly in the thalamic region that is affected with a high frequency of capillary amyloid. Although expression of phospho-tau is largely expected in neurons in neurodegenerative conditions, in mice, activated microglia have been reported to express and accumulate tau protein after episodes of ischemic injury. Whether this finding of microglial phospho-tau presentation is restricted to Tg-SwDI/NOS2<sup>−/−</sup> mice or also observed in human cases of CAA Type 1 remains to be further evaluated. In any case, the findings presented in this study suggest that Tg-SwDI/NOS2<sup>−/−</sup> mice recapitulate many of the pathological features of human CAA Type 1 and provide a valuable in vivo model to further investigate this condition.

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**Disclosures**

None.
References


