PHENOTYPIC CHARACTERISATION OF NITRATED ASTROCYTES FOLLOWING POSTNATAL EXCITOTOXIC DAMAGE

L. Acarin, H. Peluffo*, B. González and B. Castellano
Histologia, Facultat de Medicina, Univ. Autonoma Barcelona, Bellaterra, Spain

Nitric oxide (NO) and oxygen radicals have been implicated in the pathogenesis of acute CNS injury for their ability to form the potent oxidant peroxynitrite. Peroxynitrite induces lipid peroxydation, DNA damage, energy depletion and protein nitration, and is commonly demonstrated by nitrotyrosine detection. Nitrated astrocytes are found in several pathologies and are mediators of cell death in vitro. In order to characterise the phenotypic identity of nitrated astrocytes we have used an in vivo model of postnatal excitotoxic cortical damage and confocal microscopy. We have correlated astroglial nitrotyrosine immunoreactivity with the expression of the cytoskeletal proteins GFAP and vimentin, the antioxidant proteins metallothionein I-II (MT I-II) and heat shock protein 27 (HSP27), the NO-producing enzyme iNOS, and the apoptotic marker caspase-3. Neuronal degeneration in the excitotoxically-damaged cortex was accompanied by strong astroglial nitration. Scattered non-hypertrophic nitrated astrocytes expressed iNOS only during the first 24 hours post lesion (PL). However, most nitrated astrocytes lacked iNOS labeling and were highly hypertrophic and located in the adjacent white matter at 1-3 days PL and in the glial scar from 5 days PL. Noteworthy, not all hypertrophic astrocytes were nitrated, but those showing nitrotyrosine were the most hypertophic and showed the strongest GFAP labeling. Interestingly, only nitrated astrocytes showed vimentin expression, and nitrotyrosine labeling colocalised intracellularly with this cytoskeletal protein. With regard to the antioxidant proteins studied, astroglial nitration correlated with MT I-II expression, but not with HSP27. Finally, hypertrophic nitrated astrocytes generally displayed cleaved caspase-3. In conclusion, hypertrophic nitrated astrocytes overexpress the cytoskeletal proteins GFAP and vimentin, a putative target for tyrosine nitration, express the antioxidant protein MT I-II which may provide cell protection against oxidative stress, but may finally undergo apoptosis, as they show caspase-3 activation.

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