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Research Report

Cleaved caspase-3 expression after experimental stroke exhibits different phenotypes and is predominantly non-apoptotic

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ABSTRACT

Cleaved caspase-3 (CC3) is well known as an executioner protease of apoptosis following brain ischemia. However, an increasing body of evidence suggests several non-apoptotic functions of CC3. To improve our understanding of the relation between cell death-related and non-adverse effects of postischemic caspase-3 activation, we examined the spatiotemporal distribution and identity of CC3-positive cells at days 2, 3 and 4 after permanent middle cerebral artery occlusion in rats. The lacking colocalization of CC3 and TUNEL staining indicated, that CC3 expression was predominantly non-apoptotic. Nuclear CC3 expression was frequently found to be colocalized with GFAP-positive astrocytes within the tissue adjacent to the infarct, whereas cytoplasmatic CC3 expression occurred solely in the lesion. Multiple fluorescence labeling revealed costaining of cytoplasmatic CC3 with markers directed against astrocytes, macrophages/microglia and supposedly pericytes. Our findings suggest that CC3 expression was predominantly associated with cellular responses to stroke such as reactive astrogliosis and the infiltration of macrophages.

1. Introduction

Ischemic stroke is one of the most common causes of death worldwide; however, its causal treatment, thrombolysis, can still be applied to less than a tenth of patients (Huang et al., 2006). This considerable limitation is mainly attributed to a short therapeutic time window of currently 4.5 h after stroke onset (Lees et al., 2010). Hence, it is mandatory to develop new treatment strategies beyond the paradigm 'time is brain.' An increasing body of evidence indicates that diverse pathophysiological mechanisms cause a delayed brain damage after stroke, which may be a target for novel therapeutic approaches (Endres et al., 2008). Apoptosis plays a major role in delayed cell death after stroke (Broughton et al., 2009) and it was shown recently that apoptosis occurred even 4 days after stroke, caused by a subpopulation of infiltrating T-lymphocytes (Shichita et al., 2009). It is known that caspases play an important role during initiation and progress of apoptosis after cerebral ischemia (Broughton et al., 2009; Le et al., 2002) and especially caspase-3 is a common surrogate marker in preclinical stroke studies. However, there is increasing evidence for several non-apoptotic functions of caspases, such as proliferation, differentiation and

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cell cycle regulation (Schwerk and Schulze-Osthoff, 2003). The role of caspase-3 in the postischemic brain is hence characterized by the antithesis of initiating delayed neuronal cell death and mediating neuroprotection and CNS remodeling (McLaughlin, 2004). The objective of this study was to improve the understanding of the spatiotemporal caspase-3 expression after focal cerebral ischemia using multi-labeling immunohistochemistry.

2. Results

The permanent occlusion of the middle cerebral artery caused a circumscribed lesion within the right cortex; this area was clearly definable by a complete loss of MAP-2-immunoreactive neuropil (Fig. 1D). Cleaved caspase-3 (CC3) expression was found widespread in the affected hemisphere; however, the distribution and phenotypes displayed considerable spatiotemporal differences.

We asked first whether the different forms of CC3 expression were in fact associated with apoptosis. To answer this question we compared the distribution patterns of CC3-containing cells and of TUNEL-positive nuclei. We found numerous TUNEL-stained cells with condensed and fragmented nuclei (Fig. 1A) within the core of the ischemic lesion (Fig. 1E, region III); however, this area was almost completely free of CC3 expression. In contrast, areas with cytoplasmatic (Fig. 1E, region II) or nuclear (Fig. 1E, region I) CC3 expression were mostly devoid of TUNEL-positive cells (Fig. 1B). The colocalization of CC3 and TUNEL was a rare event, occurring primarily within the edge zone of TUNEL expression (Fig. 1E, between region II and III). A colocalization of CC3 and TUNEL was thereby characterized by cytoplasmatic CC3-immunoreactivity and nuclear TUNEL staining (Fig. 1C).

Expression of CC3 within the tissue that exhibits MAP-2-positive neuropil was characterized solely by nuclear immunoreactivity at any time point investigated (Fig. 2D–F). These CC3-immunoreactive nuclei were observed mainly within an approximately 1 mm broad area adjacent to the lesion border (Fig. 1E, region I). The amount of CC3-positive nuclei increased significantly from 48 h to 96 h after the onset of ischemia (Fig. 2A), and most of them were colocalized with the astrocytic marker GFAP (Fig. 2D–F). Even though a quantification of cells coexpressing GFAP and CC3 was unfeasible due to the complex GFAP-containing structures, we found that the GFAP expression correlated significantly with the nuclear CC3 expression (Fig. 2B–C). Furthermore, we did not observe any colocalization of MAP-2-positive neurons and CC3.

In addition to the nuclear CC3 expression within the MAP-2-positive infarct border, we found multi-faceted cytoplasmatic CC3 expression within the edge of the MAP-2-negative lesion

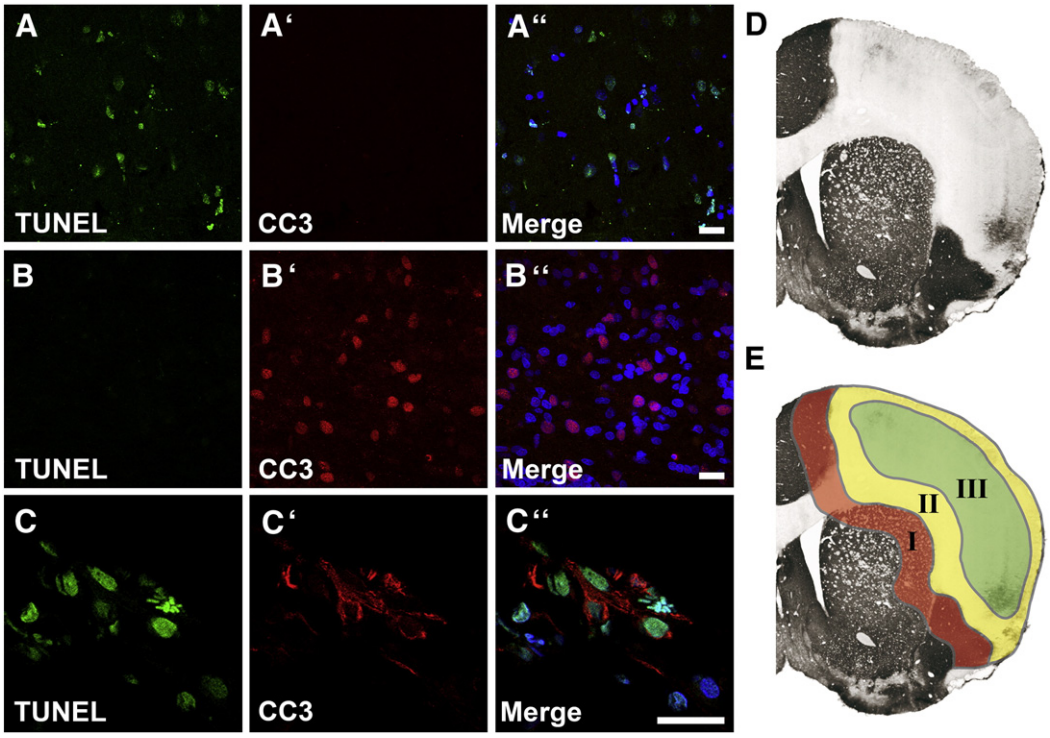

Fig. 1 – Immunohistochemical examination of apoptosis using double staining for TUNEL and cleaved caspase 3 (CC3). Within the infarct core (E, region III), many nuclei were positive for TUNEL (A) without costaining of CC3 (A'). The TUNEL-positive nuclei were condensed and fragmented in terms of an apoptotic phenotype (A"). Numerous TUNEL-negative (B) and CC3-positive nuclei (B') were found in the tissue adjacent to the lesion (E, region I). The intermediate area (E, region II) was characterized by exclusively cytoplasmatic CC3 expression (please refer to Fig. 3) that was rarely associated with TUNEL-positive nuclei (C–C"). The border between region I and II was characterized by a change of the cellular compartment expressing CC3, e.g., from the nucleus to the cytosol, and by a threshold of MAP-2 staining (D). Images are superpositions of 10 single confocal images (Z: 1 μm each). Scale bar = 20 μm.