Albumin storage in neoplastic astroglial elements of gangliogliomas

Appendix

Supplementary methods

Patients and surgical specimens

Patients with GG (n=10), DNT (n=5) and FCD IIb (n=10) had a history of drug-refractory epilepsy and surgical removal of the epileptogenic focus was deemed essential to achieve seizure control according to established guidelines.[1] Patients with DA (n=5) and RCCM (n=6) underwent surgery due to oncological indication. All procedures were carried out in accordance with the declaration of Helsinki.

Tissue processing and standard examinations

All material was fixed with formaldehyde overnight, embedded into paraffin, cut into 4 µm sections and mounted on slides (HistoBond®+, Marienfelde Germany). Neuropathological analysis of GG, DNT, FCD IIb, DA and RCCM comprised at least H&E- and Prussian blue staining as well as immunohistochemistry for glial fibrillary acidic protein (GFAP, DakoCytomation). Additional specific examinations were done for GG (CD 34, DakoCytomation; synaptophysin, DakoCytomation), FCD IIb (MAP2, SigmaAldrich; NeuN, Chemicon), RCCM (Lu5, Bachem) and DNT (NeuN, Chemicon; S100, DakoCytomation). Macroscopic and histopathologic examinations were performed by experienced neuropathologists (AJB, PN). FCD IIb was diagnosed according to the ILAE consensus classification proposal for FCD.[2]

Double-immunohistochemistry

For double-immunohistochemistry slides were deparaffinised in xylene, rinsed in ethanol and incubated in citrate buffer (10mM, pH 6.0). After washing in phosphate-buffered saline (PBS), brain sections were blocked for 2h at 37C° with a blocking solution containing fetal calf serum (FCS) 1:10, normal goat serum (NGS) 1:100 and PBS, to inhibit nonspecific antibody binding. To identify albumin containing

astrocytes, slides were incubated with anti-albumin antibody 1:4000 (polyclonal rabbit; DakoCytomation), monoclonal GFAP-antibody 1:400 (monoclonal mouse; Sigma-Aldrich), FCS 1:10 and PBS at 4C° overnight. Sections were incubated with secondary antibodies, Cy3 mouse 1:400 (Jackson ImmunoResearch) and FITC rabbit 1:400 (Jackson ImmunoResearch) and 4`,6-diamidino-2-phenylindole 1:100 (DAPI, Sigma-Aldrich, 5mg/ml), which labels cell nuclei, FCS 1:10 and PBS. Afterwards sections were stored in a dark place for 2 hours, washed with PBS, and coverslipped with fluorescein mounting medium (Vectashield hard set, VectorLaboratories). Additional fluorescent double-immunohistochemistry for CD34/albumin and CD34/GLUT-1 were performed in all entities according to the staining protocol (see above) using anti-CD34 class II antibody 1:30 (monoclonal mouse; DakoCytomation) and anti-GLUT-1 antibody 1:100 (polyclonal rabbit; Abcam).

Image analysis

Microscope images of representative lesional and perilesional regions were acquired for each patient using a Zeiss Imager.Z1 Apotome microscope with 20x Plan Apochromat (NA 0.8) and 40x Plan NeoFLUAR (NA 0.75), AxioCam MRm and the AxioVision Software. Images of the vascular regions were observed with a Zeiss Axio Observer.A1 using the 20x Plan Apochromat, a 40x LD Plan-Neofluar (NA 0.6) and a Jenoptik ProgRes CFcool CCD camera (Jena, Germany). Pictures were stored in their original formats and final images for figures were prepared in Adobe Photoshop: levels and brightness/contrast of images were minimally and evenly adjusted over the entire micrograph.

References

1. Schramm J, Clusmann H. The surgery of epilepsy. *Neurosurgery* 2008; **62**: 463-81

2. Blumcke I, Thom M, Aronica E, Armstrong DD, Vinters HV, Palmini A, et al. The clinicopathologic spectrum of focal cortical dysplasias: a consensus classification proposed by an ad hoc Task Force of the ILAE Diagnostic Methods Commission. *Epilepsia* 2011; **52**: 158-74