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BRAIN RESEARCH

Research Report

Novel molecular imaging of cell death in experimental cerebral stroke

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ABSTRACT

Cell death is the basic neuropathological substrate in cerebral ischemia, and its non-invasive imaging may improve diagnosis and treatment for stroke patients. *ApoSense* is a novel family of low-molecular weight compounds for detection and imaging of cell death in vivo. We now report on imaging of cell death and monitoring of efficacy of neuroprotective treatment in vivo by intravenous administration of the *ApoSense* compound DDC (didansylcystine), in experimental stroke in rodents. Rats and mice were subjected to a short-term (2 h) or permanent occlusion of the middle cerebral artery (MCA) and injected with DDC or ^{3}H-labeled DDC. Fluorescent and autoradiographic studies, respectively, were performed ex vivo, comprising assessment of DDC uptake in the infarct region, in correlation with tissue histopathology. Neuroprotection was induced by a caspase inhibitor (Q-VD-OPH), and its effect was monitored by DDC. Following its intravenous administration, DDC accumulated selectively in injured neurons within the region of infarct. Caspase inhibition exerted a 45% reduction in infarct volume, which was well reported by DDC. This is the first report on a small molecule probe for detection in vivo of cell death in cerebral stroke. DDC may potentially assist in addressing the current "neuroimaging/neurohistology gap", for molecular assessment of the extent of stroke-related cell death.

1. Introduction

Ischemic stroke is a major cause of morbidity and mortality. Cerebral cell death is the neuropathological substrate of cerebral stroke, and its extent is a key factor determining disease course and prognosis for the stroke patient. Modulation of the stroke-related neuronal cell loss is the ultimate target for the novel stroke therapies of thrombolysis or neuroprotection (Wahlgren and Ahmed, 2004). There is a need in clinical practice for imaging agents that upon their systemic administration, will allow assessment of this fundamental disease-related *cellular* process in the *clinical set-up*. Often there is only a rough correlation between clinical symptoms and signs and the extent of underlying cerebral cell death. Current imaging methods, such as computerized tomography (CT scan) or magnetic resonance imaging (MRI) have certain limitations in reporting on events occurring at the cellular level (Rivers and Wardlaw, 2005). The term

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neuroimaging/neurohistology gap (Heckl et al., 2004) describes this current situation, calling for improvements in neuroimaging tools, in order to address the underlying histopathological cellular process. Molecular imaging is a rapidly developing field, aimed at addressing this need.

Imaging of apoptosis in stroke has been attempted with radio-labeled annexin V, a 36 kDa protein that binds to phosphatidylserine (PS), exposed on the cell surface of the apoptotic cell. However, annexin V in stroke imaging was found to underscore the infarct region, while showing multiple bilateral foci of increased signal, extending beyond the regions damaged by the ischemic insult (Blankenberg et al., 2006; Lorberboym et al., 2006; Mari et al., 2004). This questionable selectivity and specificity of annexin V for imaging of cell death in stroke may be attributed, at least in part, to its being a relatively large protein, with limited brain access and slow blood clearance. In addition, this probe binds in early apoptosis only on the cell surface, without intracellular uptake and accumulation (Boersma et al., 2005), thus potentially reducing signal/noise ratio.

ApoSense is a novel family of low-molecular weight compounds, designed to address the challenge of apoptosis imaging in vivo. It comprises amphipathic compounds that do not cross the plasma membrane of intact viable cells, but perform selective passage through the membrane of apoptotic cells and accumulation in the cytoplasm from the early cellular stages of the death process. We have previously shown imaging of cell death, both in vitro and in vivo, by two members of the *ApoSense* family: DDC (*N*,*N*'-didansyl-L-cystine, MW=707) (Damianovich et al., 2006), and NST-732 [(5-dimethylamino)-1-napththalene-sulfonyl-α-ethyl-fluoroalanine, MW=368) (Aloya et al., 2006). Upon intravenous systemic administration in vivo in animal models of disease-related apoptosis, these compounds have been shown to target specifically the cells undergoing cell death, with marked intracellular accumulation.

We now report that administration of DDC intravenously in vivo leads to selective targeting and accumulation of the compound in cells undergoing cell death in experimental stroke in rodents, thus allowing imaging of the process and also reporting on the effect of neuroprotection exerted by caspase inhibition. Fluorescent and autoradiographic studies ex vivo are used in the present study to demonstrate this performance. Based on these results, radio-labeling of DDC and analogues with a positron emission tomography (PET) radio-isotope (e.g., ^{11}C or ^{18}F) is now being pursued, towards non-invasive, clinical imaging of cell death in stroke with this small-molecule probe.

2. Results

Fluorescent whole-organ imaging of the rat brain, performed 24 h after the short-term MCA occlusion revealed a large and distinct region of increased uptake of DDC. The region corresponded to the area supplied by the MCA, and comprised two distinct parts: a densely marked area, reflecting the core of the infarct, and a peripheral area that was clearly marked, but with relatively less signal intensity (Fig. 1).

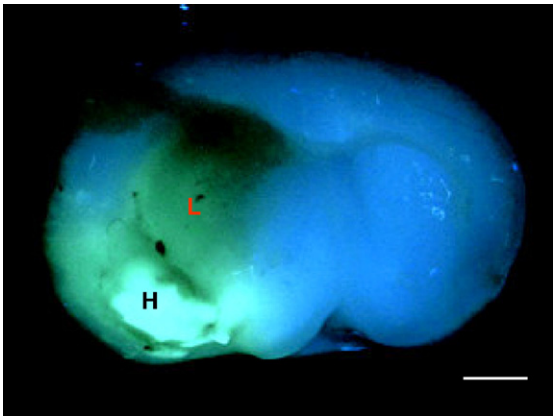

Fig. 1 – Whole-brain fluorescent imaging with DDC, 24 h after short-term MCA occlusion and 2 h after intravenous administration of the compound, in a rat. A region of high signal intensity (H) within the infarct was surrounded by lower signal intensity (L) region. Scale bar=0.28 mm.

A microscopic view of the infarct region (Fig. 2) revealed that the fluorescent signal observed in the whole-brain imaging originated from cellular uptake and accumulation of DDC within individual cells in the infarct region. While a high density of labeled cells was observed at the infarct core, creating quite a uniform appearance (Fig. 2A), the peripheral region was characterized by a lower density of labeled cells, with a mosaic of DDC-labeled cells and DDC-excluding cells, creating together a "starry sky" appearance of the slide on fluorescent microscopy (Fig. 2B). Respectively, overall signal intensity in the region of the infarct core was markedly higher than that of the periphery (Figs. 2A versus B), in correlation with the respective differential enhanced signal intensity observed in the whole-organ imaging (Fig. 1). Some of the cells manifesting DDC uptake had characteristic neuronal outline of polygonal shapes, with occasional detection of the DDC-loaded neurites, extending from the cell bodies (Fig. 2B).

In order to evaluate whether the cells manifesting DDC uptake were cells undergoing cell death, sequential slides were examined for correlation between cellular DDC uptake and (i) characteristic apoptotic morphology of cell shrinkage and plasma acidification as assessed by H&E staining; and (ii) apoptotic nuclear fragmentation, assessed by TUNEL nuclear staining. Fig. 3 exemplifies the correlation between DDC uptake after intravenous administration and H&E staining in the hippocampus region, known to be relatively sensitive to ischemia (Guegan and Sola, 2000). Selective uptake of DDC was observed in a specific population of cells (Fig. 3A). H&E staining of a consecutive slide revealed that this population of DDC-positive cells was characterized by cytoplasmic acidification and cell shrinkage, characteristic of cell death (Fig. 3B). A correlation was observed between DDC cellular uptake (Fig. 4A) and positive TUNEL staining (Fig. 4B), indicating apoptotic nuclear fragmentation in the DDC-positive cells. Staining with NeuN-specific antibody further confirmed neuronal identity (Fig. 5A) of cortical cells manifesting DDC uptake (Fig. 5B) in the region of infarct. Taken together, these results confirm uptake of DDC into