**N-acetyl-aspartate: In-vitro expression in oligodendrocytes; implications for proton-MRS signal in vivo.**

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**Introduction**

N-acetyl-aspartate (NAA) is a major brain metabolite and its presence is used increasingly in clinical and experimental MRS studies as a putative neuronal marker. A reduction in NAA levels as assessed by in vivo proton MRS has been suggested to be indicative of neuronal viability. However, temporal observations of brain pathologies such as multiple sclerosis (MS), mitochondrial encephalopathy with lactic acidosis and stroke-like episodes (MELAS; 1) and hypothyroidism (2) have shown reversibility in NAA levels, possibly reflecting recovery of neuronal function. Knowledge of the cellular localisation of NAA is critical in interpreting these findings.

The assumption that NAA is specific to neurons is based on previous immunohistochemical studies on whole brain using NAA specific antibodies. The neuronal localisation was further substantiated by cell culture experiments by Urenjak et al. (who also observed its presence in the O-2A [oligodendrocyte-type-2 astrocyte] progenitors and immature oligodendrocytes, but not in the mature oligodendrocytes (3). More recently, studies on oligodendrocyte biology have revealed the requirement for trophic factors to promote the generation, maturation and survival of oligodendrocytes in vitro (5). In the present study we have used this new information to implement a more pertinent cell cultivation procedure and demonstrate that mature oligodendrocytes can express NAA in vitro.

**Materials and Methods**

Cells. O-2A progenitor cells were cultivated as described previously (4). O-2A progenitors were allowed to spontaneously differentiate into oligodendrocytes by withdrawal of growth factors. Mature oligodendrocytes were then cultured under two different conditions. One set was supplemented with CNTF, while the other set of oligodendrocytes was cultured in media alone (4). The purity, survival and differentiation were verified by observing the cells' morphologies and antigenic phenotype.

MRS: Perchloric acid extracts were prepared for 1H-NMR spectroscopy operating at 400Hz (Varian Unity Inova). Trimethylsilylpropionate was added to each sample as a concentration/chemical shift standard for 1H-NMR. The samples were subjected to further analysis for NAA by HPLC.

**Results**

We find that 1H-NMR spectroscopic and HPLC analysis demonstrated the presence of NAA in O-2A progenitors, as previously reported in the literature (15.1 ± 2.9 [mean ± SD] nmol/mg protein). However, NAA was also seen in both preparations of mature oligodendrocytes, with higher levels in the CNTF treated population (9.5 ± 1.5 in the untreated cells and 15.5 ± 1.8 in the CNTF treated oligodendrocytes [mean ± SD] nmol/mg protein). The levels of NAA seen in mature oligodendrocytes (in the presence of the trophic factor-CNTF) were comparable to those seen in a variety of neuronal cultures; cerebellar granule neurons 12.3 ± 2.6 (3); cortical neurons 34.5 ± 3.7 and dorsal root ganglion neurons 15.6 ± 1.9 (5).

**Conclusions**

This study demonstrates the presence of NAA in cultured mature oligodendrocytes and contributes new information to the debate on the distribution of NAA in the adult brain. This also raises the question; do the NAA changes observed in clinical in vivo proton MRS studies reflect neuronal loss alone, especially in brain pathologies of white matter tracts that inevitably have an oligodendrocyte component? However, the function/s of NAA still remains obscure, and until this ambiguity is resolved, the utility of NAA as a diagnostic tool in certain brain disorders will require further work.

**References**