

THE LEVEL OF NEURONAL NITRIC OXIDE SYNTHASE IS A CRITICAL DETERMINANT OF NEURONAL DEATH FOLLOWING FETAL HYPOXIA-ISCHEMIA

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BACKGROUND: We have previously published that apparent diffusion coefficient (ADC) by MRI in fetal hypoxia-ischemia (HI) differentiates fetuses that are destined to be hypertonic postnatally from those that do not have motor deficits. We have also shown that neuronal nitric oxide synthase (nNOS) inhibitors prevent the motor deficits and perinatal deaths if given prior to HI.

OBJECTIVE: We hypothesized that nNOS is a critical determinant of neuronal death that results in hypertonia. We tested a novel methodology combining ADC prediction with high speed flow cytometric sorting of cells and quantitative RTPCR.

METHODS: Rabbit dams at E25 were subjected to 40 min uterine ischemia in the 3T magnet and serial ADC measurements obtained. ADC threshold was used to categorize fetuses destined to be hypertonic. After 30 min of reperfusion, fetal brains were removed and dissociated into single cell suspensions. Cells were stained by Rhodamine 123 and propidium iodide (PI) and sorted. Sorted cells were then assessed for nNOS gene expression by quantitative RTPCR.

RESULTS: Cells were categorized into dead, injured and healthy cells based on Rhodamine and PI staining. Dead cells (PI+ and rhodamine-) showed higher nNOS expression than injured (PI+ and rhodamine+) or healthy cells (PI- and rhodamine+) (See Figure panel A). In fetuses destined to be hypertonic, nNOS expression was even higher (see Figure panel B).

CONCLUSIONS: We conclude that nNOS is a critical determinant of cell death. We speculate that a certain threshold level of nNOS tips the fetus from a low risk to a high risk fetus for subsequent hypertonia.

