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NSAIDs in neuroblastoma therapy

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Abstract

Cyclooxygenases (COX) catalyse the conversion of arachidonic acid to prostaglandins. COX-2 is upregulated in several adult epithelial cancers. In neuroblastoma it has been shown that the majority of primary tumours and cell lines express high levels of COX-2, whereas normal adrenal medullas from children do not express COX-2. Treatment of neuroblastoma cells with nonsteroidal anti-inflammatory drugs (NSAIDs), inhibitors of COX, induces caspase-dependent apoptosis via the intrinsic mitochondrial pathway. Established neuroblastoma xenografts in nude rats treated with the dual COX-1/COX-2 inhibitor, diclofenac, or the COX-2 specific inhibitor, celecoxib significantly inhibits neuroblastoma growth in vivo. In vitro, arachidonic acid and diclofenac synergistically induces neuroblastoma cell death. This effect is further pronounced when lipoxygenases is inhibited simultaneously. Proton MR-spectroscopy (^{1}H MRS) of neuroblastoma cells treated with COX-inhibitors demonstrates accumulation of polyunsaturated fatty acids and depletion of choline compounds. Thus, ^{1}H MRS, which can be performed with clinical MR-scanners, is likely to provide pharmacodynamic markers of neuroblastoma response to COX-inhibition.

Taken together, these data suggest the use of NSAIDs as a novel adjuvant therapy for children with neuroblastoma.

Keywords: Neuroblastoma; Cyclooxygenase-2; Non-steroidal anti-inflammatory drugs; Apoptosis; Therapy

1. Introduction

Arachidonic acid is released from cellular phospholipids by phospholipaseA_2 and converted to prostaglandins by two cyclooxygenase enzymes, COX-1 and COX-2 [1]. COX-1 is constitutively expressed in most tissues, whereas inflammatory stimuli, hormones and mitogens induce COX-2 expression [1,2]. Increased amount of COX-2 are found commonly in both premalignant and malignant tissues of epithelial origin in adults and has been implicated in resistance to apoptosis, promotion of cell proliferation, increased tumour invasiveness, induction of metastases and angiogenesis as well as decreased immune surveillance [2]. Non-steroidal anti-inflammatory drugs (NSAIDs) are potent

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inhibitors of cylooxygenases. Epidemiological studies show that use of NSAID is associated with a reduced risk of several adult malignancies [3]. Consistent with this, tumour formation and growth are reduced in COX-1 or COX-2 deficient animals [4–6]. Hence, numerous experimental, epidemiologic, and clinical studies suggest that NSAIDs, particularly highly selective COX-2 inhibitors, have promise as anticancer agents.

2. Expression of cyclooxygenase-2 in neuroblastoma

A majority of neuroblastoma tissues and cell lines as well as ganglioneuromas express high levels of COX-2. In our study, 27 of 28 neuroblastoma samples (96%) showed specific expression of COX-2 protein in the cytoplasm of the tumour cells. No COX-2 protein was detected in the surrounding non-malignant adrenal medulla tissues [7]. The neuroblastoma samples were from different biological subsets and at all clinical stages. Seven out of 28 tumours were MYCN-amplified whereas nine had 1p-deletions. Some of the samples derived from high-stage or MYCN-amplified tumors obtained at surgery after preoperative chemotherapy. Three stage 4S tumours were included in the analysis and all expressed COX-2. Moreover, bone marrow metastasis of a relapsed stage 4 neuroblastoma with MYCN-amplification also expressed high level of COX-2 (Fig. 1). Only one investigated neuroblastoma sample, obtained from a localised stage 1 tumour with favourable biology (triploid DNA without MYCN-amplification), did not express COX-2. Three ganglioneuromas were investigated and showed COX-2 staining in the tumour-derived differentiated ganglion cells but not in the surrounding benign stroma (Fig. 1). Non-malignant adrenals from children, ages 12–25 months, showed a weak staining for COX-2 in the cortex whereas the medulla, where the majority of primary neuroblastoma arise, was negative for COX-2 (Fig. 1).

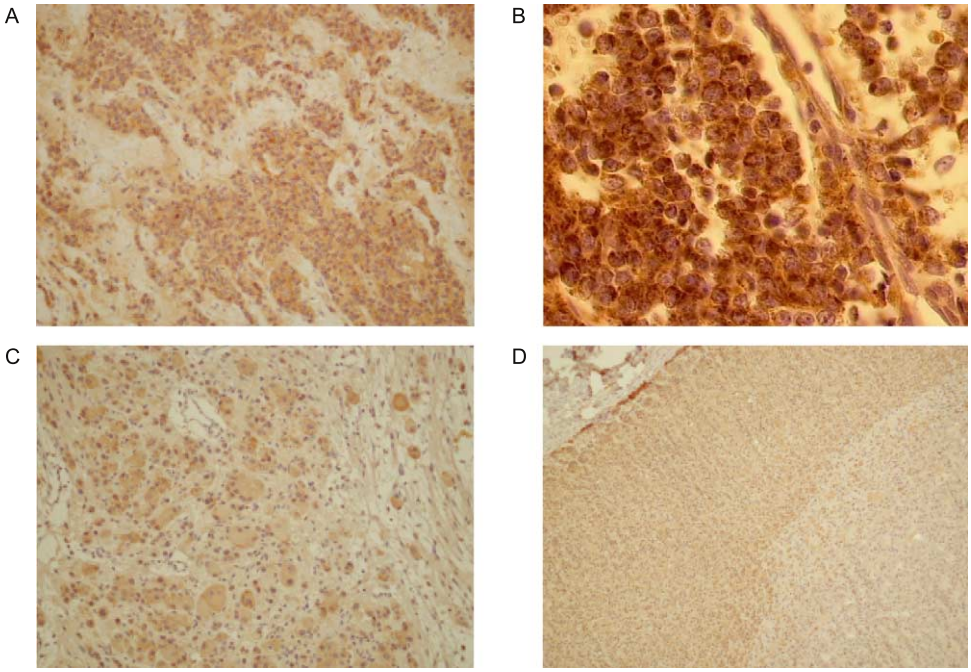

Fig. 1. COX-2 expression in primary and metastatic neuroblastoma, ganglioneuroma and non-malignant adrenal tissue. Immunohistochemistry showing specific COX-2 expression in the cytoplasm of tumour cells in a stage 4 MYCN-amplified 1p-deleted neuroblastoma from a 1½-year-old boy (A), bone marrow metastasis of a stage 4 MYCN-amplified neuroblastoma in a 2-year-old boy (B) and in differentiated ganglion cells in a benign ganglioneuroma from a 5-year-old girl (C). Non-malignant adrenal tissue, from a 2-year-old girl, shows COX-2 expression in the cortex but not in the medulla (D).