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In vitro and in vivo porphyrin accumulation by C6 glioma cells after exposure to 5-aminolevulinic acid

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Abstract

Several malignant tissues synthesize endogenous porphyrins after exposure to 5-aminolevulinic acid (5-ALA). The present experiments have been designed to elucidate whether the C6 glioma cell, a model cell for human malignant glioma, similarly synthesizes porphyrins when exposed to 5-ALA, and whether specific synthesis occurs when C6 cells are inoculated into rat brains to form a tumor. In this situation the blood–brain barrier may interfere with 5-ALA availability, and spreading of porphyrins with edema outside the tumor may occur. Flow cytometry is used to determine the course of cell volume and porphyrin fluorescence intensities in cultured C6 cells which are incubated in 1 mM 5-ALA. For the induction of experimental brain tumors, 10^4 untreated C6 cells are inoculated into the brains of rats. After 9 days animals receive 100 mg 5-ALA/kg body weight. Brains are removed after 3, 6, or 9 h and frozen coronal sections obtained for H/E staining or fluorescence spectography. Cultured C6 cells show a linear increase of protoporphyrin IX fluorescence after exposure to 5-ALA, which begins to plateau after 85 min. Marked fluorescence is also observed in solid and infiltrating experimental tumor. However, faint fluorescence also occurs in normal tissue, basal pia, choroid plexus, and, more obviously, in white-matter tracts bordering the tumor (maximal distance: 1.5 ± 0.7 mm). The observations demonstrate that C6 cells synthesize protoporphyrin IX after exposure to 5-ALA in vitro and in vivo. However, when utilizing 5-ALA for fluorescence detection or photodynamic therapy of brain tumors, attention should be paid to the possibility of protoporphyrin IX occurring outside the tumor. © 1998 Elsevier Science S.A. All rights reserved.

Keywords: Aminolevulinic acid; Malignant glioma; Fluorescence detection; Porphyrins; Photodynamic therapy

1. Introduction

The prognosis of patients suffering from malignant glioma remains poor despite presently employed multimodality regimes incorporating surgical resection and radio-, chemo- or immunotherapy [1,2]. Extending the traditional treatment armamentarium, photodynamic therapy (PDT) has been under investigation as a possible adjuvent for glioma therapy [3–8]. This mode of treatment relies on the selective uptake or retention of intravenously administered photosensitizing dyes, such as hematoporphyrin derivative, in malignant brain tissue. However, since the apparent accumulation of intravenous photosensitizing agents may partly be the product of blood–brain-barrier disruption by the tumor and extravasation of photosensititizer into tumor tissue, selectivity may be impaired by sensitizer dissipating with bulk flow into adjacent tissue traversed by edema [9,10]. Furthermore, traditional photosensitizers such as hematoporphyrin derivative may have the profound disadvantage of prolonged skin photosensitivity, sometimes lasting several weeks [11].

Recently, a novel approach has been under close scrutiny for PDT and fluorescence detection of malignant gastrointestinal, bladder, and skin tumors, in which a precursor, 5-aminolevulinic acid (5-ALA), is administered, and synthesis of endogenous porphyrins, preferentially protoporphyrin IX (PpIX), appears to be accomplished by malignant tissue in situ [12–15]. 5-ALA is a naturally occurring metabolite in the heme biosynthesis pathway. Under physiological conditions, endogenous production of 5-ALA is controlled via negative feedback inhibition by heme [16]. Excess exogenous 5-ALA circumvents feedback inhibition, leading to accumulation of highly fluorescent and photosensitizing

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