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Low dose 5-aminolevulinic acid: Implications in spectroscopic measurements during brain tumor surgery

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Summary
Background: Using 5-aminolevulinic acid (ALA) as an intraoperative fluorescence contrast has been proven to improve the resection of glioblastoma and contribute to prolonged patient survival. ALA accumulates as protoporphyrin IX (PpIX) in the tumor cells and is administered in an advised dose of 20 mg/kg body weight (b.w.) for brain tumor resection using fluorescence surgical microscopes. PpIX fluorescence availability and intensities of a four folds lower ALA dose (5 mg/kg b.w.) has been investigated in glioblastomas and skin using a spectroscopy system adapted for surgical guidance.
Methods: A total of 30 adult patients diagnosed with high grade gliomas were included in the analysis. ALA was orally administered in doses of 5 mg/kg b.w. ($n = 15$) dissolved in orange juice or 20 mg/kg b.w. ($n = 15$) dissolved in water. A fluorescence spectroscopy system with a handheld fiber-optical probe was used for performing the quantitative fluorescence measurements.
Results: The binominal comparison of the diagnostic performance parameters showed no significant statistical difference ($p > 0.05$). The median fluorescence values in tumor were 2—3 times higher for the high ALA dose group. No PpIX was detected in the skin of the patients in the low dose group (0/4) while PpIX was detected in the skin of the majority of the patients in the high ALA dose group (13/14).
Conclusions: Application of 5 mg/kg ALA was evaluated as equally reliable as the higher dose regarding the diagnostic performance when guidance was performed using a spectroscopic system. Moreover, no PpIX was detected in the skin of the patients.

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Introduction

Application of 5-aminolevulinic acid (5-ALA or ALA) for detection of highly malignant brain tumors has gained increasing popularity among the neurosurgeons in the recent years following extensive studies by Stummer et al. on the effectiveness of the method to increase gross total resection of the glioblastomas, an infiltrative and highly malignant brain tumor [1,2]. Accordingly, the gross total resection of glioblastoma was reported to have increased from 44% to 78% using fluorescence guidance leading to 5 months longer survival [2]. The principles of fluorescence guidance is based on optical excitation and detection of Protoporphyrin IX (PpIX), a product of ALA which accumulates in the tumor cells due to the broken blood brain barrier and the altered enzyme levels. PpIX re-emits a distinguished fluorescence peak at $\lambda = 635$ nm in the visible optical region when excited with light of appropriate wavelength (λ). The commonly applied excitation wavelength for diagnostic purposes is blue light ($\lambda = 405$ nm), which in addition to PpIX excites the native tissue fluorophores. The exhibited fluorescence has a shoulder peak at about $\lambda = 500-530$ nm and is referred to as autofluorescence. Detection of PpIX during operation is conventionally performed using surgical microscopes with added fluorescence detection modalities [3,4]. The fluorescence microscopes are designed to pass the fluorescence in the visible optical region above the excitation spectrum to the operator [5] thus the fluorescence observed by the operator is expected to be directly correlated to the PpIX fluorescence.

Together with the surgical microscope oral administration of an ALA dose of 20 mg/kg b.w.; i.e., 1.5 g of 5-aminolevulinic acid HCL dissolved in 50 ml water is recommended [6,7]. From the investigated doses of 0.2, 2 and 20 mg/kg, the latter dose was chosen according to the detection threshold of the microscope as the 0.2 and 2 mg/kg b.w. doses did not show a distinguished visible fluorescence [6]. The tumor resection using the microscope is based on removing the sites with strong fluorescence and leaving the weak fluorescence sites both of which are based on the visual observation of the surgeon. Fiber-optic probe based spectroscopy is an alternative to the surgical microscope for detecting fluorescence objectively [8–12]. The probe is beneficial when the microscope fails to show any fluorescence specifically at the end of tumor removal for examining the resection cavity. The probe is moreover able to examine the tumor extents in depth or be used for stereotactic biopsy together with a stereotactic frame. The combination of the two microscopy and spectroscopy techniques however appears more beneficial for guidance during open brain tumor surgery. As the detection sensitivity of the probe is higher than the microscope [11,13] application of ALA at a lower dose [14] may be possible when spectroscopic detection techniques are used during open biopsy, stereotactic biopsy or even open brain surgery. In a previous study initial results for tissue discrimination using low ALA dose has been published [15]. The aim of the present study was to investigate the clinical relevant implications of applying a four times lower ALA dose (5 mg/kg) than the recommended for the microscope and to compare the fluorescence signal intensities and the diagnostic performance for each dose. The studies were performed using a fluorescence spectroscopy system and a handheld fiber optic probe described previously [9].

Materials and methods

Patients and surgical procedure

Fifteen patients, aged 47–73 (62 ± 8) years, six females, nine males operated on 2007–2011 and one case in 2013 were given a dose of 5 (5.1 ± 0.3) mg/kg ALA (Local pharmacy) dissolved in orange juice. One of the patients who was later included in the study received Gliolan® (Medac GmBH, Hamburg, Germany) at 5 mg/kg dose dissolved in water instead as that is the recommended solvent for clinical administration of Gliolan. Fifteen other patients, aged 40–78 (63 ± 12) years, eight females and seven males operated on 2012–2014 were given one package of Gliolan® dissolved in water according to instructions provided by the company. One package of Gliolan® equaled an approximate dose of 20 (19.9 ± 4.3, range: 13–30) mg/kg b.w. ALA. All the patients included were pathologically diagnosed with high grade tumors glioblastoma multiforme (GBM) and oligodendroglioma grade III (OD III), in the low dose group (14 GBM, 1 mixed OD III and GBM) and in the high dose group (13 GBM and two OD III). Studies were approved by the local ethics committee (No: M139-07, T110-08, 2010/70-32, 2012/333-32) and written informed consent was received from all of the patients in the study. Twenty-nine patients were operated in Linköping University Hospital and one patient in the Umeå University Hospital. A total of six surgeons after initial training participated in performing the measurements. The surgical procedure was performed according to the normal routine of the clinic. Optical measurements on the brain tumor were done during the operation and in the resection cavity. Biopsy samples with an approximate diameter of 1–2 mm were taken from the suspected malignant tissue after the optical measurements. The biopsies were sent for pathological examination postoperatively either fresh or formalin fixated. The diagnosis was based on representative 4 μm thick sample cuts.

Spectroscopy system and measurements

Measurements were performed using a fluorescence spectroscopy system connected to a fiber optic probe disinfected and sterilized prior to the operation. The system was designed and adapted for neurosurgical applications as we have described in a previous publication [9]. The probe consisted of a central fiber connected to the excitation light ($\lambda = 405$ nm) and several surrounding fibers connected to a 2048 element charge coupled device (CCD) based spectrometer (EPP 2000, StellarNet, Inc., Tampa, FL, USA) with maximum 8192 photon counts expressed in arbitrary units (a.u.). Measurement settings were 10 mW laser power and 0.4 s pulse length (4 mJ) for all the patients except for three patients in the 5 mg/kg ALA dose group included early on in the study where the laser power was 5 mW instead. These patients were omitted from the quantitative analysis of data but were included in Table 1 for the binominal comparison.

As the skin photosensitivity is the major adverse effect reported [16–18], fluorescence intensities in the skin of the