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Pharmacokinetic studies on 5-aminolevulinic acid-induced protoporphyrin IX accumulation in tumours and normal tissues

Helën Heyerdahl a,b,*, Ingrid Wang c,d, David L. Liu c, Roger Berg e, Stefan Andersson-Engels e, Qian Peng b,f, Johan Moan b, Sune Svanberg e, Katarina Svanberg c,d

aDepartment of Surgical Oncology, The Norwegian Radium Hospital, Montebello, N-0310 Oslo, Norway
bDepartment of Biophysics, Institute for Cancer Research, Montebello, N-0310 Oslo, Norway
cLund University Medical Laser Centre, Lund University Hospital, S-221 85 Lund, Sweden
dDepartment of Oncology, Lund University Hospital, S-221 85 Lund, Sweden
eDepartment of Physics, Lund University, P.O. Box 118, S-221 00 Lund, Sweden
fDepartment of Pathology, The Norwegian Radium Hospital, Montebello, N-0310 Oslo, Norway

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Abstract

Laser-induced fluorescence (LIF) for in vivo point monitoring and fluorescence microscopy incorporating a CCD camera were used to study the fluorescence distribution of 5-aminolevulinic acid (ALA)-induced protoporphyrin IX (PpIX) in tumours. Fluorescence in a chemically-induced adenocarcinoma in the liver of rats and in an aggressive basal cell carcinoma in a patient were studied after intravenous injection of ALA at a dose of 30 mg/kg body weight. The LIF technique demonstrated slightly more ALA-induced PpIX fluorescence in the tumour than in the surrounding normal liver and abdominal muscle of rats. The visible parts of the human basal cell carcinoma exhibited strong ALA-induced fluorescence, while this fluorescence was much weaker in the necrotic areas of the tumour and in the surrounding normal skin. © 1997 Elsevier Science Ireland Ltd. All rights reserved

Keywords: Laser-induced fluorescence; Fluorescence diagnostics; Fluorescence microscopy; Basal cell carcinoma; 5-Aminolevulinic acid (ALA); Protoporphyrin IX build-up

1. Introduction

Practically all cells in the body require energy for metabolism. Most nucleated cells utilise haem-containing enzymes in their energy production. Thus, one may assume that they have the capability for biosynthesis of haem. Protoporphyrin IX (PpIX) is the second-last intermediate in this biosynthesis. PpIX is also an extremely efficient photosensitiser and is fortunately only rarely (i.e. in some cases of porphyria diseases) found to accumulate in the body. Thus, haem biosynthesis must be tightly regulated. It is known that haem [1] regulates the synthesis of its precursor, 5-aminolevulinic acid (ALA). By administering exogenous ALA the regulation mechanism is by-passed and PpIX is found to accumulate in cells as well as in tissues [2-5]. This is the basis for the use of ALA in photochemotherapy (PCT) of cancer. Most clinical reports of ALA-PCT have dealt with the topi-
cal application of ALA [6–10]. The tumour selectivity in this case is related to the fact that ALA, being a small molecule, passes readily through the abnormal keratin usually overlaying malignant skin tumours, but not through normal keratin [11]. ALA has also been given orally and even intravenously in tumour-bearing animals [5,12] and humans [10,12–15] and is reported to show tumour selectivity in terms of PpIX induction. Loh et al. [12] have reported selective fluorescence in inoperable rectal adenocarcinomas after oral administration of ALA. Grant et al. [13] and Barr et al. [15] treated patients with, respectively, oral cavity squamous cell carcinomas and high-grade dysplasia in columnar lined (Barrett’s) oesophagus with light after oral ALA administration and reported no adverse effects. Four patients at the Norwegian Radium Hospital, Oslo, with nodular basal cell carcinomas have undergone a total of nine treatment sessions of PCT after the intravenous or oral ALA administration, and, in all cases, selective tumour PpIX fluorescence was detected. This selectivity may be caused by a selective uptake/retention of ALA in the tumour, possibly related to a leaky microvasculature, poor lymphatic drainage or large extraacellular fluid space, a low ferrochelatase activity in the tumour [16–18] or a high tumour level of porphobilinogen deaminase [19].

In contrast to Photofrin, ALA has not been reported to cause long-term photosensitivity of skin [13]. This may be due to the fact that ALA-induced porphyrins are rapidly eliminated from the skin within 24 h after systemic administration of ALA [5,20]. A thorough knowledge of the kinetics of fluorescence generation after all pathways of administration is needed for the optimal application of ALA in tumour fluorescence diagnostics and phototherapy. The present paper presents the kinetic studies of the PpIX build-up in a chemically-induced adenocarcinoma in the liver of rats as well as in a patient with an aggressive basal cell carcinoma after i.v. injection of ALA.

2. Materials and methods

2.1. Chemicals

5-Aminolevulinic acid hydrochloride (Porphyrin Products, Logan, UT, USA) was freshly dissolved in isotonic saline before use. The solution was prepared with a concentration of 8 g/l for the rats. For the patient a solution of 2.25 g ALA dissolved in isotonic saline to a volume of 10 ml was prepared by the hospital pharmacy less than 1 h before injection. This is the total amount of ALA required for a clinical dose of 30 mg/kg body weight.

2.2. Animals and tumour model

Eleven inbred Wistar/Furth (W/Fu) rats, weighing 180–200 g, were utilised for tumour induction. The cell line of the original tumour was chemically induced by 1,2-dimethylhydrazine [21]. Viable tumour cells (3 x 10^6) were inoculated into two sites in the liver, the subcapsular region of the left lateral and the median lobes for each animal [22]. All 11 rats developed one tumour in each liver lobe with a mean diameter of 8 ± 2 mm, 8 days after the inoculation. Four rats received ALA (30 mg/kg body weight) through the femoral vein, the other seven through the portal vein immediately before the first of several recordings in the fluorescence investigation. In the case of portal vein injection, four of the rats had the perfusion to the liver temporarily stopped through

![405nm exc.](image)

Fig. 1. Laser-induced fluorescence (LIF) spectra recorded in vivo 30 min. after i.v. injection of ALA at a dose of 30 mg/kg b.w. in a chemically-induced adenocarcinoma located in the subcapsular region of the liver, normal liver and the abdominal wall muscle. The dual-peaked fluorescence with its maximum intensity at about 635 nm is specific for the ALA-induced protoporphyrin IX, that was built up in the tissue following the ALA administration. The fluorescence in the blue green wavelength region is emitted from the endogenous tissue chromophores. Tumour tissue exhibits the lowest autofluorescence intensity.
Fig. 2. (a) The protoporphyrin IX build-up within 1 h after injection in the femoral vein of ALA at a dose of 30 mg/kg b.w. in a chemically-induced adenocarcinoma located in the subcapsular region of the median and left liver lobes and normal rat liver. A total of four animals were injected. The background-free fluorescence intensity peaking at about 635 nm is expressed in terms of an internal reference compensating for variation in light and detection equipment. Each point in the diagram represents the mean value with standard deviations of measurements from the four animals. (b) The protoporphyrin IX build-up within 1 h after injection in the portal vein of ALA at a dose of 30 mg/kg b.w. in a chemically-induced adenocarcinoma located in the subcapsular region of the median and left liver lobes and normal rat liver. A total of seven animals were injected, out of which four had the perfusion to the liver temporarily stopped through clamping of the portal triad from the time of injection and 5 min after. The other three had the injection into the portal vein without changes in the liver perfusion. The background-free fluorescence intensity peaking at about 635 nm is expressed in terms of an internal reference compensating for variation in light and detection equipment. Each point in the diagram represents the mean value with standard deviations of measurements from 5-7 animals. No difference was seen in the PpIX build-up after these two ways of administering the ALA through the portal vein.
2.3. Patient

A male patient with an aggressive basal cell carcinoma was injected intravenously with ALA at a dose of 30 mg/kg body weight (b.w.) over a 15-min period to make the pain associated with the injection of the acidic solution acceptable. The ALA was delivered as a bolus dose during intravenous infusion of a 5% glucose solution. Eight tumour samples (containing surrounding normal skin) were removed 4.5 h after the ALA injection. The patient was informed of the study and possible cutaneous photosensitivity and was asked to avoid bright lights indoors as well as outdoors for at least 48 h. An informed consent was obtained from the patient.

2.4. Laser-induced fluorescence (LIF)

The PpIX fluorescence build up was monitored following laser excitation at different time intervals utilising laser-induced fluorescence (LIF) in the tumour, liver and abdominal wall muscle of rats as well as in the human basal cell carcinoma and the surrounding normal skin. A fibre-optical fluorosensor described by Andersson-Engels et al. [23, 24] was employed. As an excitation source a nitrogen-pumped dye laser was used, emitting light at 405 nm at a repetition rate of 10 Hz. The full fluorescence spectrum was captured within the wavelength region 450–750 nm. The free standing fluorescence emission peak at 635 nm was evaluated.

Fig. 3. LIF spectra recorded in vivo in a patient with an aggressive basal cell carcinoma (diameter about 150 mm and depth about 15 mm) before and at 11 different time intervals after completion of i.v. injection of 30 mg/kg b.w. of ALA. The spectra were recorded from the same tumour site on one side of the tumour crater. The tumour spectra start to show a sign of the ALA-induced protoporphyrin IX fluorescence signature already 10 min after injection.

clamping of the portal triad for 5 min from the time of injection. The other three had the injection into the portal vein without changes in the liver perfusion.

Fig. 4. (a) Evaluated data of tumour scans recorded in vivo in the patient at different time intervals after the i.v. ALA injection. The scan was placed along a line through the whole tumour crater with two normal skin spots on both sides 1 cm each from the tumour crater border. The protoporphyrin IX fluorescence intensity is expressed as the background-free fluorescence in terms of an internal reference as described in Fig. 2. The protoporphyrin-related fluorescence is seen already at 10 min after the injection and reaches its maximum at about 85 min in this aggressive basal cell carcinoma. Necrotic tumour tissue is characterised by a lower protoporphyrin IX fluorescence intensity as compared to viable tumour tissue. (b) Evaluated data from the tumour scans recorded in vivo as presented in (a). The background-free fluorescence intensity for different tissue types located in the tumour scans is shown as a function of time after the ALA injection.
2.5. Preparation of samples and fluorescence microscopy

The biopsies were prepared from both tumour and surrounding normal skin tissues. The samples were immediately immersed in liquid nitrogen and stored in a freezer at −80°C. The tissue blocks were mounted in medium (Tissue Tek II embedding compound, BDH, Poole, UK), and the tissue sections were cut with a cryostat microtome to a thickness of 8 μm and mounted on clean glass slides. The fluorescence images of the sections were directly made by means of a fluorescence microscope equipped with a CCD camera. The same frozen sections were subsequently stained with H and E staining for histological identification. Fluorescence microscopy was carried out using an Axioplan microscope (Zeiss, Germany) with a 100 W mercury lamp. The fluorescence images were recorded by a highly light-sensitive thermo-electrically cooled charge coupled device camera (CCD; resolution 385 × 578) (Astromed CCD 3200, Cambridge, UK) and hard-copied on a video printer (Sony multiscan video printer UP-930). The filter combination used for detection of the ALA-induced PpIX fluorescence consisted of a 390–440 nm excitation filter, a 460 nm beam splitter and a >600 nm emission filter.

3. Results

Three laser-induced fluorescence emission spectra recorded from tumour, normal liver tissue and abdominal muscle of rats are shown in Fig. 1. The spectra were recorded 30 min after i.v. injection of 30 mg/kg ALA. The kinetics of PpIX formation in the tumour and liver tissue after i.v. injection of ALA are shown in Fig. 2a and for portal vein injection in Fig. 2b. Fig. 3 shows 12 LIF emission spectra recorded in vivo in the patient with the basal cell carcinoma. The spectra show a sign of ALA-induced PpIX fluorescence already 10 min after the completion of the injection. The PpIX-related fluorescence peak is constant from 40 min post-injection and to 200 min, the last time point measured. Fig. 4a shows the tumour scans obtained at different times after completed ALA injection. The scans were placed along a line crossing the whole tumour including necrotic and viable tumour tissues and also normal skin on both sides. In Fig. 4b the kinetics of the Protoporphyrin IX fluorescence build-up in three different tissue areas is shown. Fig. 5 shows a fluorescence microscopy image and a corresponding transmission microphotograph of a section from the same human BCC. A strong ALA-induced PpIX fluorescence is shown in the tumour, whereas much weaker fluorescence was seen in the surrounding normal skin and dermis.

4. Discussion

When hydrophilic as well as hydrophobic photosensitisers are given i.v. to tumour bearing animals, the highest concentration of sensitiser is usually found in the liver [25]. Also, when ALA is given i.p. to mice with CaD2 mammary carcinoma significantly more PpIX is found in the liver than in the tumour [5].
This corresponds well with the findings in the present study, as shown in Fig. 1.

The kinetics of PpIX formation after i.v. injection of ALA are similar in the tumour and liver tissue (Fig. 2). These kinetics are in agreement with those found by others for oral administration [18]. Also, for portal vein injection of ALA, PpIX is formed in the tissue on the same time scale and in similar quantities, indicating that ALA is homogeneously distributed in the blood at an earlier stage than when PpIX accumulation in the tissues starts. However, it should be noted in the case of portal vein injection, that the lag-phase before PpIX starts to accumulate is slightly longer for the abdominal wall muscle than for tumour and liver tissue, i.e. 30 min. versus 10–15 min. (Fig. 2).

In the case of the human basal cell carcinoma, the ALA-induced PpIX fluorescence was clearly seen in the tumour tissue by fluorescence microscopy (Fig. 5). Moreover, it should be noted that very little PpIX was detected from normal skin outside the tumour (Figs. 3 and 4). As expected, necrotic areas of the tumour show very little PpIX-related fluorescence (Fig. 4a). The kinetics of PpIX generation is similar to that found by others for oral administration of ALA [12–14]. The retention time of PpIX (or ALA) appears to be longer in the tumour than in the adjacent normal tissue (Fig. 4b). This may be caused by factors such as a poor lymphatic drainage or a low ferrochelatase activity in the tumour tissue.

The present work indicates that upon i.v. ALA injection high concentrations of PpIX are found in tumours. The present results do not provide any information about the possible effects of human hypoxia on the activity of ALA, nor on the retention of PpIX. However, the ratio of the concentration of PpIX between human basal cell carcinoma and the surrounding normal tissue after i.v. injection of ALA is about 6 and therefore highly favourable for selective tumour photochemotherapy.

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