Cancer Chemother Pharmacol (2006) 58: 478–486
DOI 10.1007/s00280-006-0186-9

ORIGINAL ARTICLE

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# Distribution of 5-aminolevulinic acid derivatives and induced porphyrin kinetics in mice tissues

Received: 30 September 2005 / Accepted: 30 December 2005 / Published online: 17 February 2006


**Abstract** *Purpose*: Porphyrins synthesised from 5-aminolevulinic acid (ALA) have been successfully used for the photodiagnosis and photodynamic treatment of cancer. To find a more efficient pro-photosensitiser, we synthesised two ALA esters: *R*,*S*-ALA-2-(hydroxymethyl)tetrahydropyranyl ester (THP-ALA) and ALA-Undecanoyl ester (Und-ALA). *Methods*: In mice bearing a subcutaneous mammary adenocarcinoma, we studied the distribution of the porphyrins formed from these esters in tissues after systemic administration, to establish if these esters are retained in any specific tissue, which could potentially be targeted for photodynamic treatment with ALA derivatives. We also investigated the topical use of these esters. *Results*: After systemic administration, tumour and skin overlying tumour porphyrin levels were lower from the ALA esters than from ALA. Other tissues such as liver, colon, kidney, skin and spleen also accumulated less porphyrins from the esters, showing that there is no specific retention of the esters in these tissues. However, the brain was the only organ that synthesised more porphyrins from THP-ALA than from ALA. The kinetics of porphyrin synthesis from ALA esters is comparable to those from ALA in almost all tissues, showing that esterases activities are not limiting the availability of the hydrolysed ALA. Both THP-ALA and Und-ALA, applied topically on the skin over the tumour, exhibited higher selectivity than ALA for the site of application, whereas the amount of tumour porphyrin was the same from ALA and THP-ALA but lower from Und-ALA. *Conclusions*: THP-ALA may be useful for the treatment of brain tumours after systemic administration, whereas THP-ALA and Und-ALA may be used more suitable for the treatment of superficial tumours due to their higher selectivity.

**Keywords** Photodynamic therapy · Aminolevulinic acid · ALA esters · ALA derivatives · In vivo distribution

**Abbreviations** ALA: 5-Aminolevulinic acid · He-ALA: ALA-hexyl ester · i.p.: Intraperitoneal · PBS: Phosphate buffered saline · PDT: Photodynamic therapy · P: Apparent partition coefficient between octanol and PBS · PpIX: Protoporphyrin IX · SOT: Skin overlying the tumour · THP-ALA: *R*,*S*-ALA-2-(hydroxymethyl)tetrahydropyranyl ester · Und-ALA: ALA-Undecanoyl ester

## Introduction

Photodynamic therapy (PDT) of cancer is based on the administration of a photosensitising compound with tumour-localising properties, and subsequent irradiation with light of an appropriate wavelength leading to selective damage to the treated tissue [1].

5-Aminolevulinic acid (ALA) has been successfully used as a tool for the photodiagnosis [2] and photodynamic treatment of neoplastic tissue [3]. While ALA itself is neither fluorescent nor a photosensitiser, it can induce the biochemical formation of Protoporphyrin IX (PpIX). Two molecules of ALA are converted into porphobilinogen, and then five enzymes, three cytosolic and two mitochondrial, lead to the formation of PpIX, which is a very efficient photosensitiser.

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The ALA is hydrophilic and does not easily penetrate through intact skin, nodular skin lesions, the stomach and the intestinal walls and in general through cell membranes [4, 5]. Ester derivatives of ALA are more lipophilic and are expected to penetrate better through lipid membranes and into tissues. Esterification of ALA with aliphatic linear and cyclic alcohols was found to reduce the amount of ALA required for photosensitisation [6].

In a mammary adenocarcinoma cell line, ALA-hexyl ester (He-ALA) which is the most well-studied ALA ester, induced the maximal amount of porphyrin synthesis employing a concentration 60-fold lower than ALA [7]. However, systemic administration of He-ALA to mice, induced in all tissues except the brain, a lower porphyrin synthesis than ALA [8]. Topical administration of He-ALA also failed to improve porphyrin levels. The main advantage of the use of ALA derivatives was found to be confinement of porphyrins formed in the site of application [9, 10].

To find a better pro-drug we synthesised and studied in vitro in a mammary adenocarcinoma cell line, two different ALA derivatives: *R*,*S*-ALA-2-(hydroxymethyl)tetrahydropyranyl ester (THP-ALA) and the highly lipophilic ALA-Undecanoyl ester (Und-ALA). We found that these compounds did not improve ALA efficacy in terms of porphyrin synthesis. In addition, intraperitoneal (i.p.) injection of 0.8 mmol/kg THP-ALA and Und-ALA to tumour bearing mice, resulted in a lower porphyrin concentration in the tumour when compared to ALA, which may be due to retention of ALA derivatives either within the blood vessels in the initial phase of distribution and/or within the capillaries of the tumour [11].

The aim of this work was to study the distribution of the porphyrins formed from the ALA esters THP-ALA and Und-ALA in mice tissues after systemic administration, in order to establish if these esters are retained in any specific tissue so that we can identify which organ could be potentially successfully targeted by PDT with ALA derivatives. We also investigated the topical use of Und-ALA and THP-ALA in terms of selectivity and total porphyrin synthesis. In addition, we performed studies of affinity studies to establish possible correlations between this system and the in vivo models.

## Materials and methods

### Chemicals

The ALA was obtained from Sigma Chem Co. ALA derivatives were obtained as the hydrochloric acid salts.

He-ALA and Und-ALA were synthesised according to the method of Takeya [12] by reacting ALA with hexanol and undecanol, respectively, in the presence of thionyl chloride. The mixture was stirred at 70°C until ALA·HCl was completely dissolved and the reaction was confirmed by thin layer chromatography ($CH_2Cl_2$/MeOH 9:1). The solvent excess was evaporated under high vacuum. After addition of diethylether, the HCl salts of the ALA esters were allowed to crystallise at 4°C for purification. Yields ranged from 60 to 40%.

The THP-ALA was similarly prepared. The crude product was purified by flash column chromatography on silica gel eluting with $CH_2Cl_2$/MeOH mixtures. Finally, the product was recrystallised in diethylether. The yield was about 20%.

Purities of the synthesised compounds were always higher than 95%, as established by thin layer chromatography and nuclear magnetic resonance techniques.

### Animals

Male BALB/c mice 12 weeks old, weighing 20–25 g were used. They were provided with food (Purina 3, Molinos Río de la Plata) and water ad libitum. A suspension of $1.65\times10^5$ cells of the LM3 cell line (Instituto de Oncología AH Roffo) was subcutaneously injected on the flanks of male BALB/c mice. Experiments were performed at approximately day 20 after implantation. Tumours of the same uniform size (1 cm diameter) were employed. Animals received human care and were treated in accordance with guidelines established by the Animal Care and Use Committee of the Argentine Association of Specialists in Laboratory Animals (AADEALC), in full accord with the UK Guidelines for the Welfare of animals in Experimental Neoplasia [13].

### ALA administration

The hydrochloric acid salts of ALA and ALA derivatives were dissolved in saline in a final volume of 0.15 ml immediately before i.p. injection.

For topical administration, ALA and ALA derivatives were dissolved in 0.3 ml of a saline:polyethyleneglycol 4000 (1:1) solution immediately before use.

The ALA and ALA derivatives' formulations were applied on the tumour, after shaving the hair and rubbing with a smooth paintbrush for a period of 5 min, a time at which no vestiges of lotion were visible.

The area of application was denoted as skin overlying the tumour (SOT), whereas normal skin taken from the opposite flank was named distant skin.

### Tissue porphyrin extraction

After ALA or ALA derivative applications, the animals were killed. Before killing, mice were injected with heparin (0.15 ml, 1,000 IU) and after killing; they were perfused with 200 ml of sterile saline. The tissue samples were homogenised in a 4:1 solution of ethyl acetate:glacial acetic acid mixture according to Batlle [14]. Briefly, the mixtures were centrifuged for 30 min at 3,000*g*, and the supernatants were added with an equal volume of 5% HCl. Extraction with HCl was repeated until there was no detectable fluorescence in the organic layer. The aqueous

fraction was used for the determination of porphyrins. For fluorometric determination, a Shimazdu RF-510 spectrofluorometer was used, with an emission detection wavelength of 604 nm and an excitation wavelength of 406 nm, for which both hydrophilic as well as hydrophobic porphyrins are detected. PpIX (New Frontiers, USA) was employed as a reference standard.

## ALA determination in brain

Prior to these experiments, we found that THP-ALA content can be determined indistinguishably from ALA by Mauzerall and Granick [15] method according to Di Venosa et al. [16] findings.

The mice were injected with ALA or THP-ALA and after 5 min, they were killed and the brains were rinsed carefully. Trichloroacetic acid was added to deproteinise and the samples were centrifuged for 30 min at 3000*g*. Modifications of the Mauzerall and Granick [15] method were used for ALA/THP-ALA determination. A condensation reaction was developed at pH 4.8 in presence of acetyl acetone and the resulting pyrroles were quantified at 555 nm after addition of the Ehrlich reagent. Standards of ALA and THP-ALA were also condensed and employed for calculations.

## Fluorescence spectroscopy

In vivo fluorescence measurements were carried out for kinetics of PpIX formation after topical application of ALA or ALA derivatives. Fluorescence was measured using a fibre-optic probe coupled to a Perkin-Elmer LS50B spectrofluorometer. The excitation light at 407 nm was coupled into the fibre-optic probe, enabling conduction of excitation light to the skin surface and collection of the PpIX fluorescence emission. Taking into account the attenuation coefficient for skin, the 407 nm light penetrates deep enough into the skin to excite the PpIX in the epidermis and dermis [17]. The fibre tip was fitted with a rubber spacer that provided a constant fixed distance of 7 mm between the fibre and the tissue, which ensured the optimum fluorescence signal from the sample. Fluorescence intensity was measured as a function of time and expressed in arbitrary units (fluorescence units) taking the emission value at 635 nm. In addition, fluorescence emission spectra were measured to verify that the fluorescence signal corresponds to PpIX, and we found no contribution of hydrophilic porphyrins. PpIX fluorescence correlates with the concentration of PpIX in SOT [17].

## ALA and ALA derivatives affinity for tissues

In these experiments 0.5 g of tissues were homogenised in 1 M sodium acetate buffer pH 4.8 (10% w/v) and ALA or ALA derivatives were added to a final concentration of 0.125 mM. The homogenates were incubated in the presence of ALA or ALA derivatives during 30 min at room temperature. Incubation time, pH and temperature did not change the outcome of

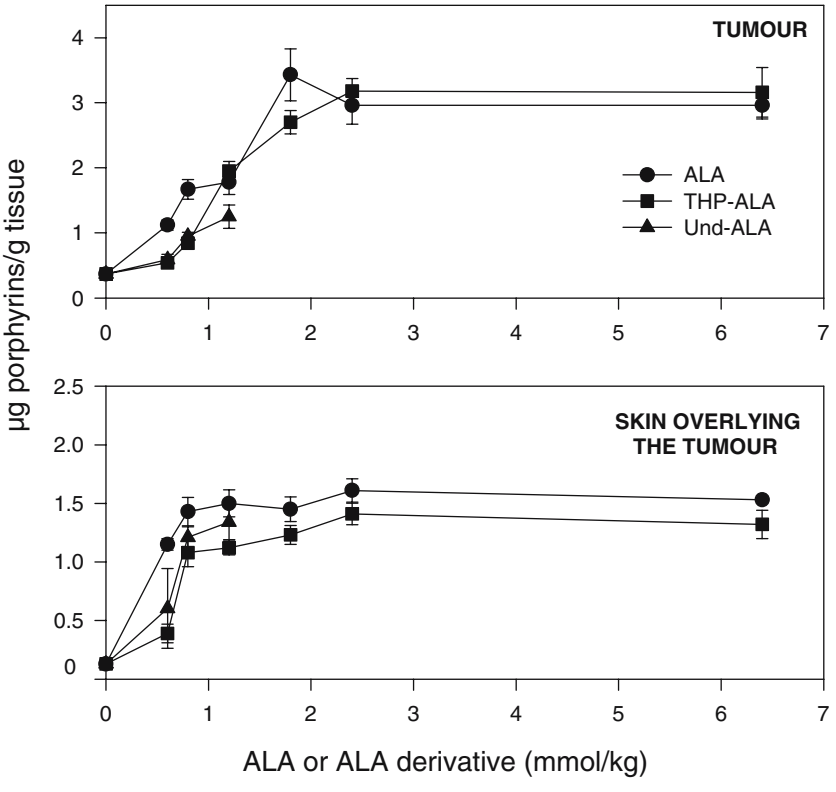


**Fig. 1** Porphyrin accumulation in tumour and skin overlying the tumour after i.p. administration of increasing ALA or ALA derivative doses. Different amounts of ALA, THP-ALA or Und-ALA were injected i.p. to mice. Three hours later, tissues were excised and porphyrins extracted as detailed in Materials and methods. Each *data point* represents the average of three determinations. *Error bars* show standard deviations

the experiments. Afterwards, trichloroacetic acid was added to precipitate proteins and then the homogenates were centrifuged for 30 min at 3,000*g*. The supernatants were kept and the precipitates were washed three times with the sodium acetate buffer, keeping the supernatants for ALA or ALA derivatives determinations. The four supernatants were condensed with acetyl acetone, and ALA or ALA derivatives were measured as described above. The percentages of recovered ALA or ALA derivatives were calculated from the condensed controls without tissue.

## Determination of partition coefficients

The apparent partition coefficients (*P*) for ALA and ALA esters were determined in two-phase octanol-buffer system [18]. Phosphate buffered saline (PBS) (10 ml of 0.1 M solution of pH 7.4) and octanol (10 ml) were saturated by stirring for 30 min. Each compound (10 $\mu$mol) was dissolved in 2 ml of aqueous phase and 2 ml of octanol. The compounds were stirred vigorously, and then the two phases were left to be separated overnight at 4°C. The absorption of the pro-drugs in both phases was determined with UV spectrometry at 270 nm.

The partition coefficients (*P*) were calculated as the ratios of the absorbance of the compounds in octanol and PBS at pH 7.4, according to

$$P = \mathrm{abs}_{\mathrm{oct}}/\mathrm{abs}_{\mathrm{PBS}} = C_{\mathrm{oct}}/C_{\mathrm{PBS}}$$

where $\mathrm{abs}_{\mathrm{oct}}$ and $\mathrm{abs}_{\mathrm{PBS}}$ represent the UV absorbance of the pro-drugs in octanol and aqueous phases, and $C_{\mathrm{oct}}$ and $C_{\mathrm{PBS}}$ represent the concentration of the pro-drugs in organic and PBS phases, respectively.

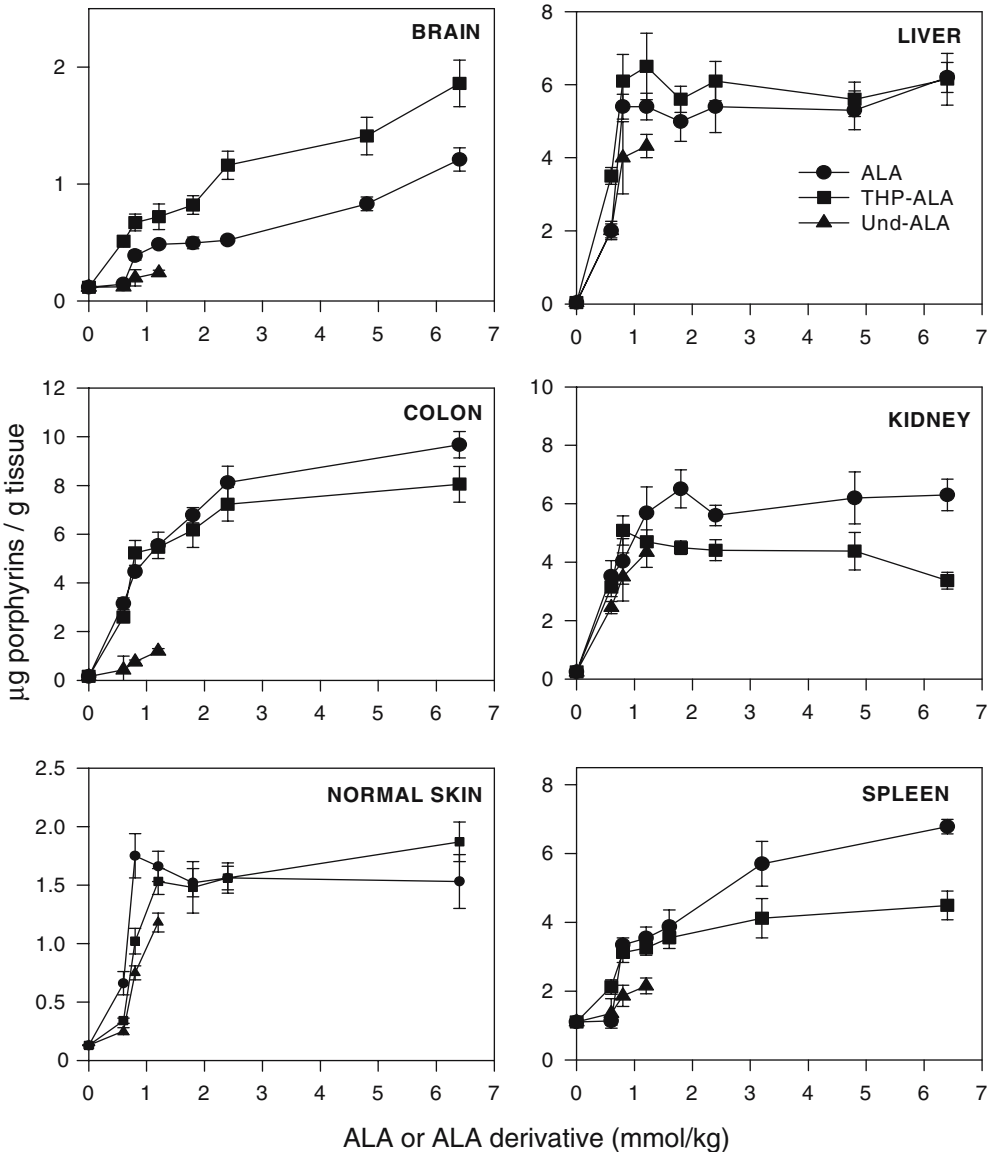


**Fig. 2** Porphyrin accumulation in brain, colon, normal skin, liver, kidney and spleen after i.p. administration of increasing ALA or ALA derivative doses. Different amounts of ALA, THP-ALA or Und-ALA were injected i.p. to mice. Three hours later, tissues were excised and porphyrins extracted as detailed in Materials and methods. Each *data point* represents the average of three determinations. *Error bars* show standard deviations

### Statistical analysis

The unpaired $t$ test was used to establish the significance of differences between groups. Differences were considered statistically significant when $P < 0.05$. Explant and affinity experiments: three independent experiments, in triplicates. In vivo experiments: three mice per group were employed.

## Results

Figure 1 shows that in tumour and SOT, chemically extracted porphyrins from i.p. THP-ALA are significantly lower compared to porphyrins produced from i.p. ALA at low concentrations (at 0.6 mmol/kg $P = 0.0009$ for tumour and $P = 0.0008$ for SOT), whereas at concentrations equal or higher than 1.2 mmol/kg the values tend to be similar (at 1.2 mmol/kg $P = 0.290$ for tumour and $P = 0.06$ for SOT). Und-ALA i.p. dose could not be further increased from 1.2 mmol/kg due to lethal toxicity to mice. However, over the whole concentration range studied, Und-ALA induced lower tumour porphyrin levels than equimolar ALA. In contrast, at low concentrations, porphyrin levels in SOT from Und-ALA are lower than from ALA (at 0.6 mmol/kg $P = 0.05$), but comparable at higher concentrations (at 1.2 mmol/kg $P = 0.215$).

The total amount of chemically extracted porphyrins from i.p. THP-ALA is comparable to that from ALA in colon, liver and normal skin, but not in kidney and spleen, where porphyrin levels from THP-ALA are significantly lower at high doses (Fig. 2). In contrast, the brain is the only tissue that synthesises significantly more porphyrins from THP-ALA than ALA. In the concentration range studied, all the tissues except for colon, brain and spleen, show similar porphyrin synthesis from i.p. Und-ALA compared to ALA.

THP-ALA and Und-ALA, as well as ALA, induce in all tissues maximum porphyrins at short incubation times; that is between 2 and 5 h after i.p. administration (Figs. 3, 4). The exception is kidney porphyrin production from Und-ALA, where porphyrin levels are sustained up to 10 h after administration.

Porphyrin kinetics after topical application of ALA and ALA derivatives in the SOT of mice and distant skin was measured non-invasively by fibre-optic measurements (Fig. 5). The three compounds exhibit maximal porphyrin synthesis between 3 and 4 h after application. However, neither THP-ALA nor Und-ALA attains the porphyrin levels induced by ALA. However, whereas porphyrin synthesis in distant skin from ALA is significant, synthesis from ALA esters remains close to basal values.

Table 1 shows the values of chemically extracted porphyrins from SOT, tumour and distant skin after topical application of ALA, THP-ALA and Und-ALA. Whereas, tumour porphyrin synthesis from THP-ALA is not significantly different from ALA, SOT porphyrins are lower from THP-ALA ($P = 0.026$). Und-ALA

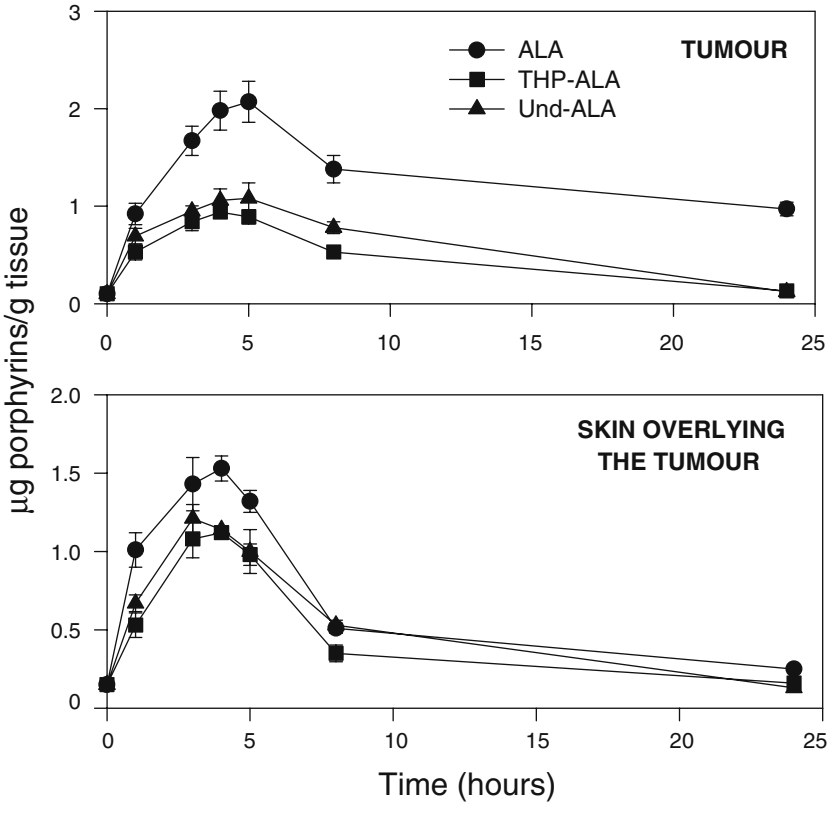


**Fig. 3** Porphyrin synthesis from ALA or ALA derivatives in tumour and skin overlying the tumour after i.p. administration as a function of time. A dose of 0.8 mmol/kg of ALA, THP-ALA or Und-ALA was injected i.p. to mice. At different times, tissues were excised and porphyrins extracted as detailed in Materials and methods. Each *data point* represents the average of three determinations. *Error bars* show standard deviations

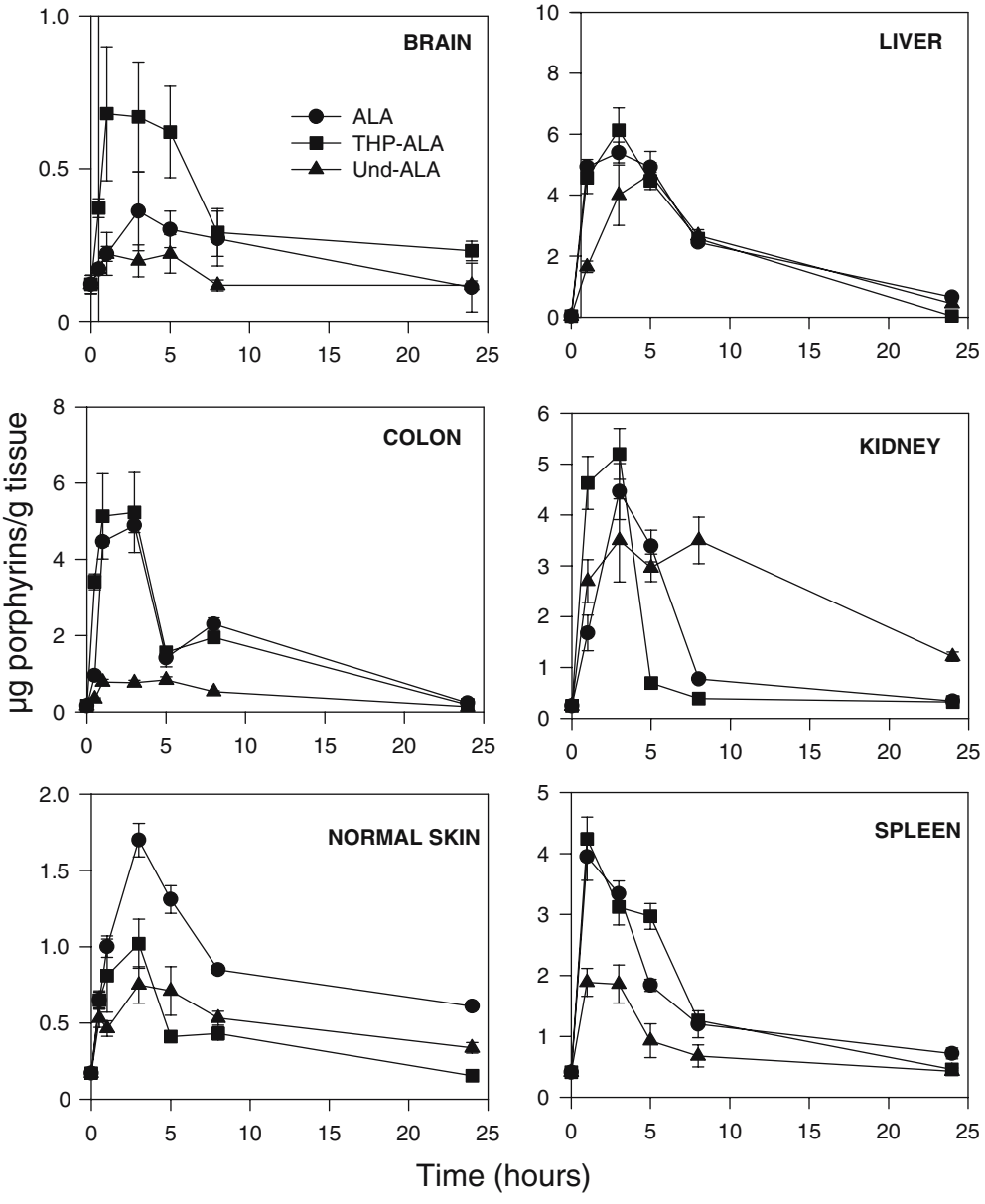


**Fig. 4** Porphyrin synthesis from ALA or ALA derivatives in accumulation in brain, colon, normal skin, liver, kidney and spleen after i.p. administration as a function of time. A dose of 0.8 mmol/kg of ALA, THP-ALA or Und-ALA was injected i.p. to mice. At different times, tissues were excised and porphyrins extracted as detailed in Materials and methods. Each *data point* represents the average of three determinations. *Error bars* show standard deviations

induces both lower tumour ($P = 0.03$) and SOT porphyrins ($P = 0.001$) compared to ALA. Distant skin accumulates $0.64 \pm 0.11$ $\mu$g porphyrins/g from ALA whereas porphyrin synthesis from THP-ALA and Und-ALA remains within basal values. Similarly, porphyrins from THP-ALA and Und-ALA were not accumulated in the rest of the organs after topical application (data not shown).

The THP-ALA accumulation in brain after 5 min of i.p. injection is significantly higher than ALA accumulation ($P < 0.005$; Table 2). However, THP-ALA accumulated is 3.5 times lower than that obtained in previous work for He-ALA [8].

Table 3 shows the affinity of ALA and ALA derivatives for different tissue homogenates. We included ALA, Und-ALA, THP-ALA and the well-studied He-ALA. It is apparent that Und-ALA displays more affinity for all tissues, specially the brain, tumour and kidney. ALA is 100% recovered from all tissues, as are THP-ALA and He-ALA except for the brain that retains 20% of these ALA esters.

In the tissues where recovery is total, almost 100% of ALA and THP-ALA is recovered in the first washing, whereas for He-ALA 70–80% is recovered in the first washing and the rest is recovered in the second and third washing (data not shown). This suggests that He-ALA affinity for tissues is higher than that of ALA and THP-ALA.

We used a fixed ratio for tissue: buffer (10% w/v). However, increasing this ratio resulted in a higher retention of Und-ALA and He-ALA in all tissues.

We have also assessed the lipophilicity of ALA and ALA esters by measuring the log $P$ values, which is the log of the apparent partition coefficients of the compounds

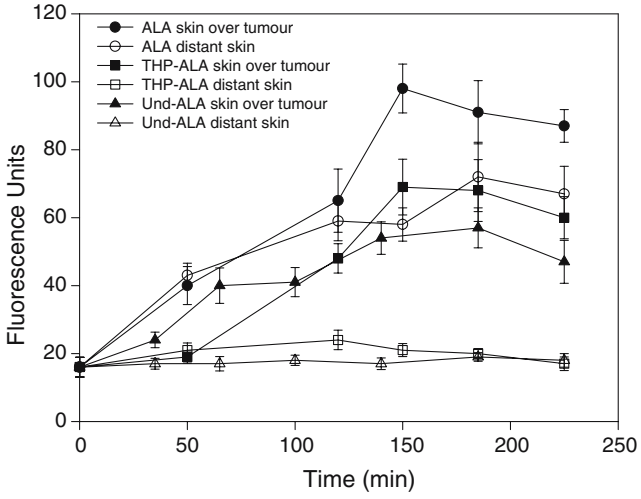


**Fig. 5** Fluorescence spectroscopy of porphyrins in skin overlying tumour and distant skin after topical ALA and ALA derivatives application. ALA or ALA derivatives were applied topically in equimolar concentrations (5 mg ALA, 7.85 mg THP-ALA and 9.55 mg Und-ALA) in the skin over the tumour. At different times after application, fluorescence was monitored by a fibre-optic probe coupled to a spectrofluorometer. Determinations were made over the tumour skin and over a distant skin. We show here the average of three mice per treatment

between octanol and PBS (Table 4). The log *P* value for ALA is negative, consistent with its hydrophilic nature, whereas the esters employed in this work are hydrophobic, with the order of increasing lipophilicity: THP-ALA < He-ALA < Und-ALA.

## Discussion

Brain porphyrins from THP-ALA are from 1.5 to 2 times higher than obtained from equimolar ALA concentrations. This might be explained by a high amount of the ALA ester crossing the blood–brain barrier. As it has been previously shown for He-ALA [8] the structure of the blood–brain barrier allows lipophilic molecules to extravasate and reach the brain, although He-ALA induced even higher brain porphyrins. According to Uehlinger et al. [18], log *P* for He-ALA is 1.838 and in this work we measured the log *P* of THP-ALA at 1.010. This means that THP-ALA although not being as lipophilic as He-ALA, may be sufficiently lipophilic to cross the blood–brain barrier. The role of lipophilicity has long been recognised as being important in central nervous system. It has been demonstrated that the most potent compounds acting on the central nervous system have a log *P* value of $2 \pm 0.5$ [19]. Und-ALA, with a log *P* of 3.262 might be too lipophilic to cross the brain barrier, and probably the molecule can be retained without entering the cells. Alternatively, Und-ALA might precipitate following systemic administration or bind to plasma proteins thus showing a limited biological activity.

It is worth to note that the high brain porphyrin content from THP-ALA does not imply that the compound will be selective for brain tumours.

With the exception of the brain, porphyrin synthesis from Und-ALA and THP-ALA was not improved in the rest of the tissues analysed. We have previously hypothesised for ALA esters that the low efficacy of tumour porphyrin synthesis after their systemic administration may be due to retention of ALA derivatives either within the blood vessels in the initial phase of distribution and/or within the capillaries of the tumour [11]. We carried out affinity studies to elucidate which tissue is responsible for the retention of ALA esters.

The affinity of ALA derivatives for tissue homogenates is higher for Und-ALA and He-ALA than for ALA and THP-ALA, that is, for the compounds having lower log *P* and are consequently more hydrophobic. However, THP-ALA only binds to the brain homogenates, showing a correlation between affinity and high porphyrin synthesis (Fig. 2) in this case but not in the rest of the tissues.

From affinity studies we can say that Und-ALA is not retained in blood vessels or tissue capillaries, but in cell

**Table 2** ALA, THP-ALA and He-ALA levels in brain

| | nmol ALA or THP-ALA/g brain |
|---|---|
| Control | $17 \pm 3$ |
| ALA | $60 \pm 5$ |
| THP-ALA | $101 \pm 12$ |
| He-ALA | $404 \pm 10$[a] |

2.4 mmol/kg of ALA, THP-ALA or He-ALA were administered i.p. to mice. After 5 min, mice were killed and ALA and ALA esters were determined as described in Materials and methods. Controls correspond to basal ALA levels of non-treated mice
[a]Taken from previous work [8]

**Table 1** Porphyrin accumulation in tumour, skin overlying the tumour and distant skin after topical application of ALA and ALA derivatives

| | Control | ALA | THP-ALA | Und-ALA |
|---|---|---|---|---|
| Tumour | $0.36 \pm 0.025$ | $1.10 \pm 0.25$ | $0.78 \pm 0.17$ | $0.59 \pm 0.11$ |
| Skin overlying tumour | $0.12 \pm 0.002$ | $1.17 \pm 0.11$ | $0.75 \pm 0.18$ | $0.46 \pm 0.06$ |
| Distant skin | $0.12 \pm 0.002$ | $0.64 \pm 0.11$ | $0.15 \pm 0.03$ | $0.13 \pm 0.04$ |

Topical application of 5 mg ALA, 7.85 mg THP-ALA and 9.55 mg Und-ALA (equimolar concentrations) were performed on the skin overlying the tumour. Porphyrins were extracted after 4 h and expressed as $\mu$g/g tissue. Control corresponds to basal porphyrin values

**Table 3** ALA and ALA derivatives affinity for tissues

| | Liver | Brain | Tumour | Kidney | Skin | SOT | Spleen | Colon |
|---|---|---|---|---|---|---|---|---|
| ALA | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| THP-ALA | 100 | 82.3 | 100 | 100 | 100 | 100 | 100 | 100 |
| Und-ALA | 47.7 | 18.2 | 35.7 | 34.4 | 66.4 | 59.0 | 45.2 | 54.3 |
| He-ALA | 100 | 83 | 100 | 100 | 100 | 100 | 100 | 100 |

ALA or ALA derivatives were incubated with tissue homogenates 30 min at room temperature as explained in Materials and methods. The results are expressed as the means of the percentages of recovered ALA or ALA derivatives and were calculated from the condensed controls without tissue ($n = 3$)

**Table 4** Log *P* values for ALA and ALA esters

| | Log *P* |
|---|---|
| ALA | −1.430 |
| THP-ALA | 1.010 |
| He-ALA | 1.838[a] |
| Und-ALA | 3.262 |

*P* is the apparent partition coefficient between octanol and PBS
[a]Taken from literature [20]

membranes or proteins in general by simple unspecific affinity. It has been previously reported [20] that long-chained ALA esters tend to remain in the cell membrane thus synthesising lower PpIX. In addition, binding to albumin, lipoproteins, endothelium or extracellular matrix of tissues is also possible [21].

The kinetics of porphyrin synthesis after i.p. administration and topical application of Und-ALA and THP-ALA to mice is quite similar to that from ALA, suggesting that once inside the body, the ALA molecules are released and that the de-esterification process does not affect the rate of buildup of tetrapyrroles. Stability studies carried out in PBS and minimal essential medium employing ionic exchange chromatography [16] show that neither Und-ALA nor THP-ALA hydrolyses spontaneously during 6 h at pH 7.4 (data not shown), confirming that the hydrolysis is entirely carried out by esterases.

Moan et al. [22] found a different kinetic of skin porphyrins from ALA methyl ester after i.p. administration. In that work porphyrin levels peaked 1 h after administration, showing that PpIX induced by ALA methyl ester seems to have a shorter lifetime in the tissues than PpIX induced by ALA, probably due to a higher stability of ALA in tissues.

It has been previously found that topical He-ALA induces porphyrins confined to the site of application [9, 10, 23]. Both THP-ALA and Und-ALA also exhibit high selectivity for the site of topical application, showing that this is a general feature for the esters of ALA. These compounds appear to be hydrolysed intracellularly at the site of application and the liberated ALA does not diffuse to distant sites.

The SOT kinetics of PpIX synthesis from topical ALA esters is similar to those obtained from ALA. However, the amount of PpIX formed is significantly lower. On the other hand, comparable tumour porphyrin levels are synthesised from ALA and THP-ALA. Probably THP-ALA diffuses easily through skin, leading to high tumour porphyrin values.

Porphyrins produced from Und-ALA in tumour are significantly lower compared with ALA after topical application. The low efficacy of He-ALA to reach tumour tissue after topical application was ascribed to stratum corneum retention of lipophilic molecules [10], and probably the same reason applies for Und-ALA, which has an even higher log *P* value, although the retained molecules are not hydrolysed to give a higher porphyrin content in SOT.

In summary, THP-ALA may be useful for the treatment of brain tumours after systemic administration, whereas both THP-ALA and Und-ALA may be useful for the treatment of superficial tumours due to higher selectivity for the site of application.

**Acknowledgements** This research was supported by CONICET (PIP 02283 and 05508/3), the Science and Technology Argentine Agency (PICT 05-9042) and Wellcome Trust (grant number 067062/Z/029/Z). A.B., H.F. and A.C. hold the posts of Superior, Associate and Assistant Researcher at the CONICET, respectively. Gabriela Di Venosa is a CONICET fellow.

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