Differential metabolism of 5-ALA in patients with brain tumors

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Abstract: Protoporphyrin IX (PPIX) is known to accumulate within human brain tumors following administration of 5-aminolevulinic acid (5-ALA). It then emits red fluorescence when exposed to ultraviolet light. On this basis, 5-ALA is often utilized intraoperatively to assist with the surgical resection of malignant gliomas. However, there is little knowledge of metabolic intermediates generated during 5-ALA conversion to PPIX. We therefore conducted an investigation of both serum and urinary intermediates in patients with brain tumors (n=40) and in healthy volunteers (n=8). Study subjects were each given a 1.0 gm oral dose of 5-ALA, undergoing blood and urinary porphyrin determinations prior to and four hours after dosing. While baseline porphyrin
levels did not differ significantly between groups, urinary levels of coproporphyrins (I and III), and serum levels of uroporphyrins (I and III) at four hours were significantly higher in patients with brain tumors than in control subjects. The higher porphyrin levels corresponded with the strongest intraoperative PPIX fluorescence. Observed porphyrin elevations were subsequently attributed to efflux of metabolites after 5-ALA uptake by tumor cells. A rise in urinary coproporphyrin following 5-ALA challenge may thus have a role as a nonspecific tumor marker.

**Introduction:**

5-aminolevulinic acid (5-ALA) is a substrate of heme, synthesized from succinyl coenzyme A and glycine. It contributes to a chain of porphyrin intermediates (porphobilinogen, uroporphyrinogen and coproporphyrinogen) leading to mitochondrial generation of protoporphyrin IX (PPIX). The latter is then enzymatically converted to heme via ferrochelatase. These reactions proceed briskly in the liver and in erythrocyte precursors of even healthy individuals but are augmented in rapidly proliferating cells. With 5-ALA dosing, metabolism typically halts at porphobilinogen (PBG) due to rate-limiting PBG deaminase activity. However, the rate-limiting step in more active tumor cells shifts to ferrochelatase, so that PPIX accumulates instead; and it is this accumulation of PPIX which responds to ultraviolet light, emitting a red fluorescence.

Intraoperative fluorescence, induced by 5-ALA, enables better visualization of brain tumors, facilitating surgical resection. Unfortunately, there are no reports investigating the fate of porphyrin intermediates in the blood and urine of such patients when this therapeutic adjunct is used. For our study, we examined urinary and serum porphyrin levels in patients with brain tumors after 5-ALA administration, comparing parameters with healthy, similarly treated adult volunteers.

**Experimental and Methods:**

Forty patients with brain tumors and eight healthy adult volunteers participated in this study. Patients with tumors were given 1.0 gm of 5-ALA orally, two hours before anesthesia, and intraoperative fluorescence was performed. Blood and urine samples were collected prior to and four hours after 5-ALA dosing. The healthy volunteers were given the same 1.0 gm oral dose of 5-ALA. Blood samples were obtained before dosing, then at two, four, and six hours afterward. Urine samples were taken prior to and four hours after dosing.
For all subjects, levels of 5-ALA, coproporphyrins (I and III), and uroporphyrins (I and III) were measured.

Intraoperative fluorescence of brain tumors was achieved with a semiconductor laser device (VLD-V1 version 2 M & M Co., Ltd., Tokyo, Japan). The tumors were exposed to light of near-laser quality, with a peak wavelength of 405 nm and light output of 120 mW through optical fiber. The spectra of tumor emissions were analyzed by personal computer (Figure 1). The aim was to identify PPIX fluorescence (at peak wavelength of 636 nm) and to quantitate its relative intensity (>3000, strong [Fig 2]; <3000, weak [Fig 3]). These two parameters corresponded with direct observations—that is, a tumor with strong quantitative fluorescence also displayed strong visible red fluorescence. All urinary concentrations were adjusted for creatinine values. The results were analysed statistically using the Dunnet method.

Results

Twenty tumors were strongly fluorescent (16 malignant gliomas, one AT/RT, two meningiomas, one hemangioblastoma) and another 10 fluoresced weakly (three benign gliomas, one schwannoma, three metastatic brain tumors, three malignant lymphomas). At baseline and after 5-ALA administration, serum and urinary levels of 5-ALA, serum coproporphyrins (I and III), and urinary uroporphyrins (I and III) did not differ significantly between groups (ie, by degree of tumor fluorescence). On the other hand, there were significant differences after 5-ALA dosing between patients with strongly fluorescent tumors and healthy volunteers in terms of serum uroporphyrin I (Fig 4) and uroporphyrin III (Fig 5), and urinary coproporphyrin I (Fig 6) and coproporphyrin III (Fig 7) ($p<0.005$). After the 5-ALA administration, serum uroporphyrins (I and III) were also significantly lower in patients with weakly vs strongly fluorescent tumors ($p<0.005$), but there were no significant differences among volunteers. Furthermore, urinary coproporphyrins (I and III) were significantly higher in patients with weakly fluorescent tumors, compared with volunteers ($p<0.005$), but there was no significant difference compared with strongly fluorescent tumors.

Discussion

The clinical impact of porphyrins is underscored by disorders of synthesis. In these conditions, or so-called porphyrias, production of porphyrins and/or porphyrin precursors increase. Therefore, blood and urinary porphyrins of a healthy volunteer given 5-ALA (as a substrate of heme) would expectedly increase, as they did in our study. The reason may be that an excess of metabolic intermediates (ie, porphyrins) ultimately escapes into general circulation.

In tumor-bearing mice, blood levels of porphyrins reportedly increase. However, the blood and urinary levels of porphyrins of humans with disease,
following administration of 5-ALA, have not been similarly documented. In this study, serum uroporphyrins (I and III) and urinary coproporphyrins (I and III) after 5-ALA administration were found to significantly increase in patients with brain tumors, relative to volunteers. Unlike healthy subjects, the ongoing synthesis of heme is accelerated in the hypermetabolic cells of tumors, so that PPIX accumulates in tumor cells when 5-ALA is given. Although PPIX accumulates, porphyrin intermediates, such as coproporphyrin and uroporphyrin, are still readily synthesized. The differences in intensity of tumor fluorescence likely reflect the extent of photoactive PPIX accumulation.

Inclusive of volunteers, it was felt that variations in PPIX accumulation also accounted for differences in blood and urinary porphyrins after 5-ALA dosing. Then again, the fluorescent strength of tumors was not necessarily proportional to circulating porphyrins due to differing tumor volumes. Alternatively, other influences, such as ALA uptake by cells, mitochondrial properties, and molecules involved in PPIX metabolism (including porphobilinogen deaminase, ferrochelatase, iron content, and transferring receptor) must be considered. For example, ABCG2 and ABCB6 of the ATP-binding cassette (ABC) transporter provide for trafficking of porphyrins. ABCB6 specifically facilitates transfer of coproporphyrinogen from the cytoplasm to mitochondria. Not only is ABCG2 overexpressed in malignant brain tumors, but there are genetic ABC polymorphisms that may add to the complexity of porphyrin metabolism.

Nonetheless, the overriding factor for accumulation of PPIX is the hypermetabolism of tumor cells, which may serve as a nonspecific marker of tumor activity. In other words, a substantial rise in urinary coproporphyrins (I and III) after dosing with 5-ALA could be due to malignancy.

References

aminolevulinic acid (ALA) in cancer diagnoses and therapy. *Int Immunopharmacol.* Impress.


Separate Figure Caption List

[Figure 1]
[Figure 2]
[Figure 3]
[Figure 4]
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Figure 1] Photo of intraoperative brain tumor fluorescence using semiconductor laser device (VLD-V1 version 2: M & M Co., Ltd., Tokyo, Japan). The tumor emits red fluorescence upon exposure to a laser light with peak wavelength of 405nm and a light output of 120mW through optical fiber. Tumor emissions were analyzed by personal computer via spectrometer through a second optical fiber in parallel. PPIX-specific fluorescence and relative intensity were measured.
[Figure 2] Intraoperative photo and fluorescent wave form of strongly fluorescent glioblastoma There is surface infiltration of brain by tumor; red fluorescence is in response to a laser beam of 405nm. The wave form, with peak at 636nm was confirmed as PPIX emission. Intensity of the peak was 5000.
[Figure 3] Intraoperative view and fluorescent wave form of weakly fluorescent benign glioma. There is surface infiltration of brain by tumor (upper left); weak red fluorescence is in response to a laser beam of 405nm (upper right). The wave form, with low peak at 636nm, was confirmed as PPIX emission. Intensity of the peak was less than 1000 (lower).
[Figure 4] Comparison of the plasma uroporphyrin I levels four hours after the 5-ALA administration.
[Figure 5] Comparison of the plasma uroporphyrin III level four hours after the 5-ALA administration.
Comparison of urine coproporphyrin I level four hours after the 5-ALA administration.

Weak: Weak fluorescence tumors
Strong: Strong fluorescence tumors

$P < 0.005$
[Figure 7] Comparison of urine coproporphyrin III level four hours after the 5-ALA administration.