Chapter 13

Porphyrin Synthesis from 5-Aminolevulinic Acid in Patients with Glioma

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1. Introduction

The molecule 5-aminolevulinic acid (5-ALA) is a substrate for a heme synthesized in cells using succinyl CoA and glycine. Initially, 5-ALA is converted to porphobilinogen (PBG), and the metabolism progresses by the action of PBG deaminase, and its product is incorporated into the mitochondria via uroporphyrinogen and coproporphyrinogen by an enzymatic reaction and transformed into protoporphyrin IX (PPIX). This PPIX is then converted to a heme by the action of ferrochelatase. These metabolic reactions are conducted in the liver and erythroblasts of normal human subjects [16]. It is thought that a larger amount of 5-ALA is incorporated by rapidly proliferating tumor cells as compared to normal cells, due to the active synthesis of heme [5, 20]. In the metabolic pathway of 5-ALA, it is reported that the rate limiting enzyme in normal cells is different from that in tumor cells [16]. PBG deaminase is the rate limiting enzyme in normal cells [3]. Large amounts of uroporphyrinogen, coproporphyrinogen, and PPIX are produced in tumor cells compared to normal cells because metabolism after the PBG is promoted by high PBG deaminase activity and low ferrochelatase activity [15]. The PPIX accumulates in tumor cells and produces a red fluorescent response to ultraviolet light. Intraoperative photodynamic diagnosis using this method is used for patients with glioma [21, 24, 26]. There are numerous reports concerning the PPIX concentration in the tumor tissue; however, reports concerning plasma titers and urinary excretion of uroporphyrinogen and coproporphyrinogen after 5-ALA administration are rare [25]. In this study, 5-ALA was administered to healthy adult volunteers, and changes in serum and urinary porphyrins were measured. Urinary excretion of porphyrins in volunteers and patients with brain tumors after the 5-ALA administration were compared and examined.

2. Materials and Methods

Healthy adult volunteers (n = 8) and 58 patients with glioma (12 benign gliomas (WHO grade II) and 46 glioblastomas) who were resected by intraoperative fluorescence diagnosis using 5-ALA were enrolled in the study. Eight adult volunteers were given 1 gram of 5-ALA orally, and blood sampling was done before administration, 2 hours, 4 hours, and 6 hours later. Blood samples were analyzed for plasma titers of 5-ALA, PPIX, coproporphyrin I (CPI), coproporphyrin III (CPIII), uroporphyrin I (UPI), and uroporphyrin III (UPIII). Urine collection was done before and 4 hours after 5-ALA administration, and the urinary excretion of 5-ALA, CPI, CPIII, UPI, and UPIII were measured. Fifty-eight patients with glioma were given 1 gram of 5-ALA orally 2 hours before anesthesia, and blood and urine samples were collected from these patients 4 hours later for analyses of plasma titers and urinary excretion of 5-ALA, PPIX, CPI, CPIII, UPI, and UPIII. Intraoperative fluorescence diagnosis for 58 patients with glioma was performed using a semiconductor laser device (VLD-V1 version 2: M & M Co., Ltd., Tokyo, Japan). The brain tumor was exposed to a laser light that had a peak wavelength of 405 nm and a light output of 120 mW by placing the optical fiber as close as possible. The spectra of the response light from tumors were analyzed by a personal computer for a fluorescent identification of PPIX and the measurement of the fluorescent objective strength. When fluorescence from PPIX was observed, a waveform with a peak at 636 nm was observed. When the intensity of the peak at 636 nm was ≥3000, the tumor was defined as a strong fluorescence tumor. In contrast, when the intensity of the peak at 636 nm was < 3000, the tumor was defined as a weak fluorescence tumor. Two groups distributed based on these spectra were examined for macroscopic findings; i.e., fluorescence for the strong PPIX group was observed macroscopically in all of the strong fluorescence tumors. When there was not a waveform peak at 636 nm, and the fluorescence of PPIX was not observed for a tumor, the tumor was considered to be a non-fluorescence tumor. The total urinary excretion products were adjusted for creatinine value. Testing for significant differences was done using analysis of variance (analysis of variance; ANOVA) or the PLSD (protected least significant difference) method of Fisher as a post-hoc test.

3. Results

The plasma 5-ALA concentration in volunteers reached a peak 2 hours after 5-ALA administration (Figure 1). The plasma titer peaks of protoporphyrin IX, CPI, and CPIII in volunteers were reached 2 hours after administration of 5-ALA. The plasma titer peaks of UPI and UPIII in volunteers were reached 4 hours after 5-ALA administration (Figure 2). The plasma titer and urinary excretion of CPI, CPIII, UPI, UPIII, and 5-ALA were not significantly different in volunteers and glioma patients prior to 5-ALA administration (data not shown). Urinary excretion CPI and CPIII 4 hours after 5-ALA administration were significantly higher in patients with glioma than in volunteers (Figures 3, 4) ($p < 0.0001$). Urinary excretion of UPI and UPIII 4 hours after 5-ALA administration in volunteers and glioma patients were not significantly different (Figures 5, 6). Urinary excretion of CPI, CPIII, UPI, UPIII, and 5-

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ALA 4 hours after 5-ALA administration in patients with benign glioma was not significantly different compared to patients with glioblastoma. All of the glioblastomas were strong fluorescence tumors. Benign gliomas were comprised of 3 strong fluorescence tumors, 5 weak fluorescence tumors, and 4 non-fluorescence tumors. Urinary excretion of CPI, CPIII, UPI, UPIII, and 5-ALA 4 hours after 5-ALA administration was not significantly different when comparing strong fluorescence tumors, weak fluorescence tumors, and non-fluorescence tumors in benign glioma patients (data not shown).

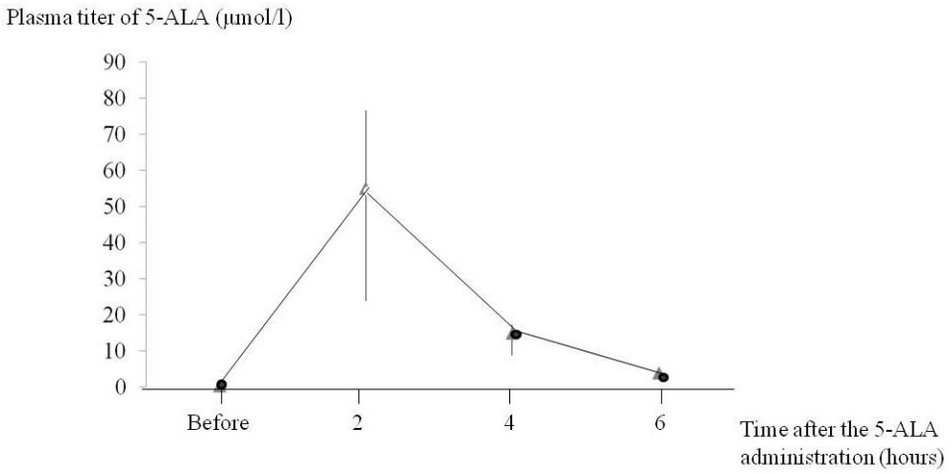

Figure 1. Graph shows the plasma titer of 5-ALA when 5-ALA was administered to volunteers. The peak concentration is reached 2 hours after administration of 5-ALA, and the concentration is ≤ 50% of the peak level 4 hours later. The concentration is ≤ 10% of the peak level 6 hours later.

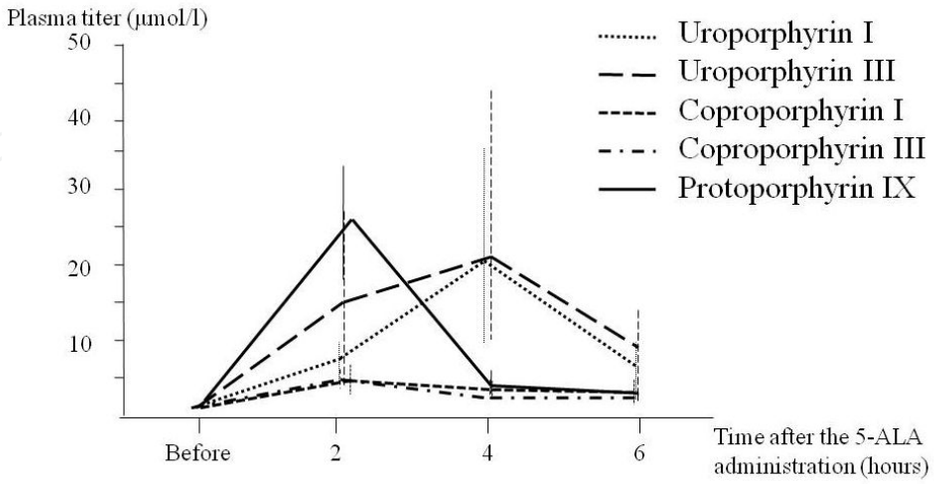

Figure 2. Graph shows various plasma titers of porphyrins when 5-ALA was administered to volunteers. The plasma titers of protoporphyrin IX, CPI, and CPIII reached maximal levels 2 hours following 5-ALA administration. The plasma titers of UPI and UPIII peaked at 4 hours after administration of 5-ALA.

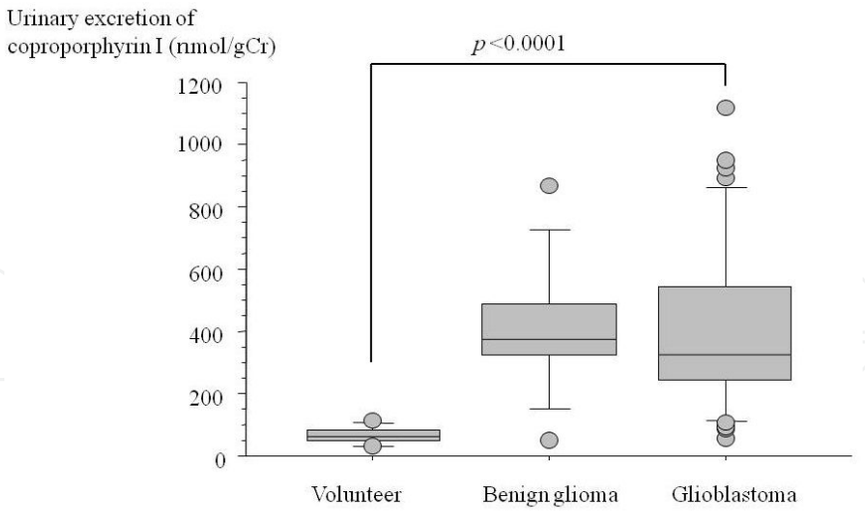

Figure 3. Graph shows urinary excretion of CPI 4 hours following administration of 5-ALA to normal volunteers, and patients with benign gliomas and glioblastomas. Urinary excretion of CPI in patients with benign gliomas and glioblastomas was significantly higher than in normal volunteers.

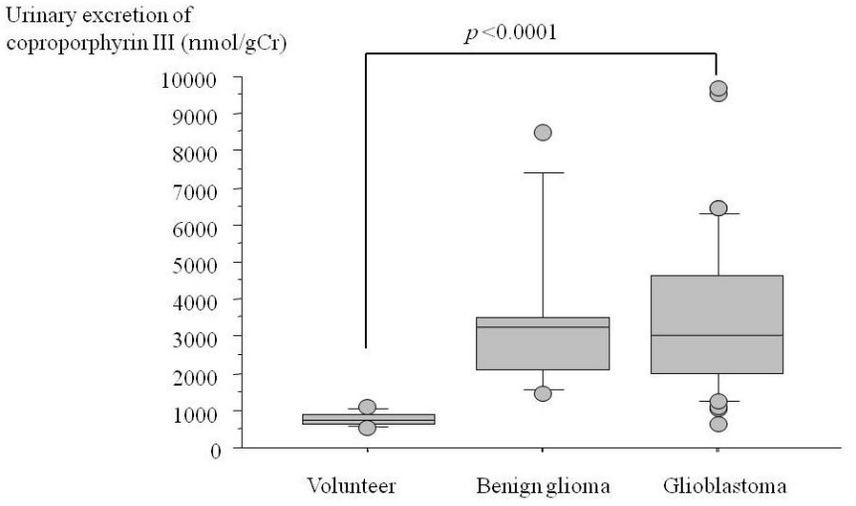

Figure 4. Graph shows urinary excretion of CPIII 4 hours after 5-ALA was administered to normal volunteers, and patients with benign gliomas and glioblastomas. Urinary excretion of CPIII in patients with benign gliomas and glioblastomas was significantly higher than in normal volunteers.

4. Discussion

It was reported that plasma titers and urinary excretion of porphyrins were increased when tumor-bearing mice were administered 5-ALA [8]. Also, it was reported that the plasma titers and urinary excretion of these porphyrins were increased when 5-ALA was administered to adult mice without malignant tumors [14]. The reason why these porphyrins

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increase in healthy volunteers after 5-ALA administration might be that those porphyrins leak as the intermediate product after being metabolized by the erythroblasts and liver [14]. When healthy volunteers were administered 5-ALA in our study, the plasma titers of 5-ALA, CPI, CPIII, UPI, UPIII, and PPIX reached maximum levels at 2-4 hours following 5-ALA administration. Therefore, it was thought that most of these porphyrins were done being excreted in the urine 4 hours after 5-ALA administration.

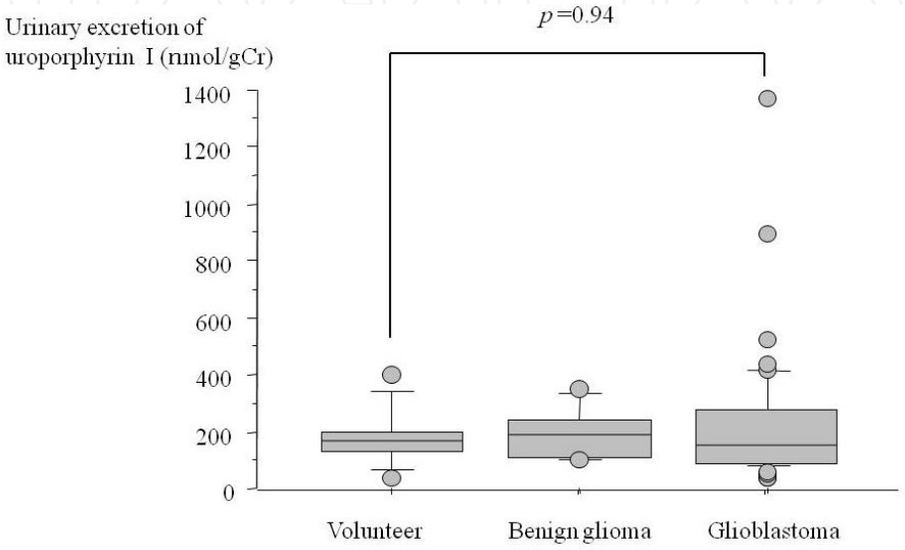

Figure 5. Graph shows urinary excretion of UPI 4 hours after 5-ALA was administered to normal volunteers, and patients with benign gliomas and glioblastomas. The urinary excretion of UPI showed no significant differences among subjects who were normal volunteers, and patients with benign gliomas and glioblastomas.

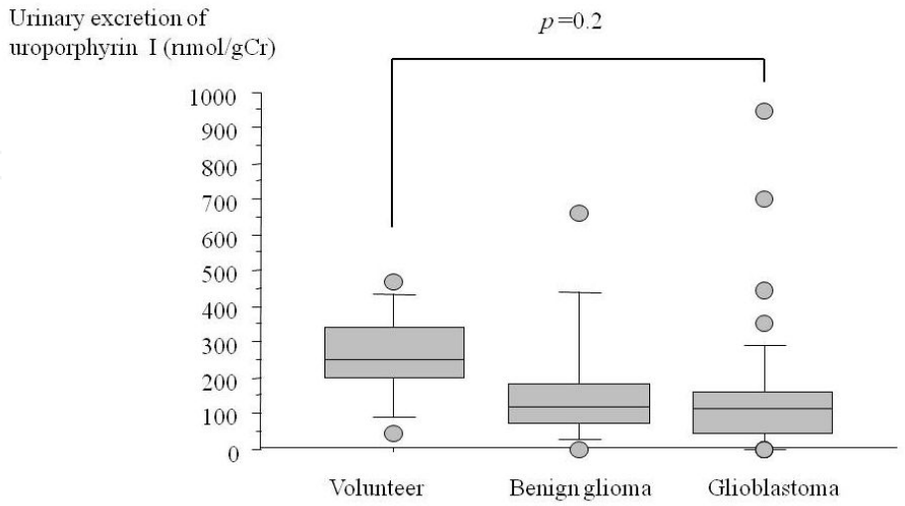

Figure 6. Graph shows urinary excretion of UPIII 4 hours after 5-ALA was administered to normal volunteers and patients with benign gliomas and the glioblastomas. The urinary excretion of UPIII showed no significant differences among subjects who were normal volunteers, and patients with benign gliomas and glioblastomas

If 5-ALA were not administered, there would be no difference in the metabolism of these porphyrins in volunteers and glioma patients, because plasma titers and urinary excretion of CPI, CPIII, UPI, UPIII, and 5-ALA were not significantly different in volunteers and glioma patients prior to 5-ALA administration. Also, there would not be the specific metabolic process in tumor cells for converting 5-ALA to uroporphyrin, because urinary excretion of UPI and UPIII was not different when comparing volunteers, patients with benign glioma, and patients with glioblastoma. However, the urinary excretion of CPI and CPIII 4 hours after 5-ALA administration significantly increased in patients with glioma as compared to volunteers. Therefore, it was thought that high levels of CPI and CPIII were being produced in tumor cells as compared to normal cells. Heme is rapidly synthesized in cells with accelerated metabolism, such as tumor cells, and 5-ALA is incorporated into tumor cells and metabolized. Consequently, large quantities of CPI and CPIII are produced as the metabolites, and it was thought that urinary excretion increased [11]. In the healthy adults without a tumor, PBG deaminase becomes the rate limiting enzyme even if 5-ALA is incorporated into cells, and extensive metabolism occurs prior to production of PBG. Therefore, CPI and CPIII, which are down-stream metabolites, will not be produced in large quantities [3].

It is known that ATP-binding cassette (ABC) transporters, such as ABCG2 and ABCB6, are associated with trafficking of these porphyrins. ABCG2 is expressed mainly in the cell membrane and serves as the active transporter for anticancer drugs present outside of cells [6]. ABCG2 drains excessive levels of porphyrin outside of cells while performing this function [13]. ABCB6 transports coproporphyrinogen from the cytoplasm into the mitochondria, and both. ABCG2 and ABCB6 are overexpressed in cancer cells [6, 12, 23]. ABCG2 is overexpressed in brain tumors [1, 9], and its presence is more common in malignant tumors [9]. CPI and CPIII that are produced in the cytoplasm of tumor cells are actively transported by ABCG2 overexpressed in brain tumor cells. This was considered the cause of higher urinary excretion of CPI and CPIII in patients with a brain tumor compared to normal volunteers. Similarly, it is reported that urinary excretion of porphyrins increases even in cancers other than brain tumors [7]. Therefore, these porphyrins may be used as a nonspecific marker in screening for tumors [25]. In other words, presence of hypermetabolic tumor cells may be suggested if urinary excretion measures of CPI and CPIII are elevated in a person orally administered orally 5-ALA. In such a case, it may be useful to investigate the whole body for the presence of a tumor.

The urinary excretion of CPI and CPIII were not different in benign glioma and glioblastoma patients, and the metabolism of 5-ALA to produce CPI and CPIII should not differ between benign gliomas and glioblastomas. Many of the benign gliomas were weak fluorescence or nonfluorescence tumors, whereas all glioblastomas were strong fluorescence tumors. In other words, high levels of PPIX had accumulated in the glioblastomas, while only low levels of PPIX accumulated in benign gliomas. Metabolic processes involved in the pathway leading from CPI and CP III to PPIX will be different in benign gliomas and glioblastomas. These differences will be due differences in the activity of ABCB6 which transports CPI and CPIII in mitochondria, or coproporphyrinogen oxidase, which converts CPI and CPIII into PPIX. Concerning these ABC transporters, it is reported in accordance with genetic polymorphism that the functions and levels of expression are different [23]. Therefore, the intracellular PPIX

concentration will be higher for a tumor having higher levels of ABCB6 [12]. Also, the glioblastomas will have much higher levels of PPIX than benign gliomas, because the presence of coproporphyrinogen oxidase is common in a malignant tumor [22].

However, differences in PPIX accumulation (fluorescent strength) are not necessarily proportional to urinary excretion of these porphyrins after 5-ALA administration. The volume of a tumor (the total amount of PPIX) is one factor affecting results. The factors related to differences in accumulation of protoporphyrin IX have been investigated in various ways, and various factors such as ALA uptake by cells [2, 19], mitochondrial properties [18], molecules involved in PpIX metabolism including porphobilinogen deaminase [4], ferrochelatase [15], iron content [10], transferring receptor [17], and mRNA levels of the coproporphyrinogen oxidase (CPOX) gene [13] are thought to be associated with protoporphyrin IX accumulation. Due to the involvement of these multiple factors, differences in the accumulation of PPIX will occur [24].

5. Conclusion

Following administration of 5-ALA, patients with a brain tumor showed higher urinary excretion of CPI and CPIII than did healthy volunteers. This was due to the active production of porphyrins in metabolically active tumor cells. The urinary excretion of CPI and CPIII following administration of 5-ALAcould possibly be used as a screening assay for the presence or absence of a tumor. Differences in the presence of the ABC transporter may contribute to metabolic differences of the porphyrins in benign gliomas and glioblastomas.

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