Title: The optimization of fluorescence imaging of brain tumor tissue differentiated from brain edema – in vivo kinetic study of 5-aminolevulinic acid and talapofin sodium –

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Abstract:

　　Object:　We aimed to clarify the optimal timing for the fluorescence imaging of brain tumor tissue differentiated from brain edema after the administration of photosensitizers.

　　Methods:　We have performed an in vivo study of the kinetics of 5-aminolevulinic acid (5-ALA) in comparison with talaporfin sodium using the rat brain tumor model and rat vasogenic edema model produced by cold injury. The in vivo kinetics of 5-ALA and talaporfin sodium in brain tumor model and the vasogenic edema model was determined by a fluorescence macroscope and a microplate reader.

　　Results:　The in vivo kinetic study of 5-ALA showed mild fluorescence intensity of protoporphyrin IX (PpIX) in brain tumor differentiated from vasogenic edema. The mean lesion-to-normal-brain ratio (L/N ratio) in the group of brain tumor model 2 hours after the administration of 5-ALA was 7.78±4.61, which was significantly higher (P<0.01) than that of the vasogenic edema 2 hours after the administration of 5-ALA (2.75±1.12). In vivo kinetic study of talaporfin sodium showed high fluorescence intensity and retention in brain tumor differentiated from by vasogenic edema. The mean L/N ratio of the fluorescence intensity in the group of brain tumor model 12 hours after the administration of talaporfin sodium was 23.1±11.9, which was significantly higher (P<0.01) than that of the vasogenic edema 12 hours after the administration (8.93±8.03).

　　Conclusions: The optimization of fluorescence imaging of brain tumors differentiated from brain edema is possible in the case of 5-ALA within 6 hours, and also possible in the case of talaporfin sodium beyond 12 hours.

Introduction:

　　Malignant gliomas, especially for glioblastoma, have poor prognosis in spite of the combination of surgery, radiotherapy, and chemotherapy. Even using the combination of radiotherapy (60 Gy over a period of 6 weeks) and temozolomide as a new standard chemotherapeutic agent after the brain tumor removal, the median survival is 14.6 months (1). The poor prognosis of malignant gliomas is due to the infiltrative growth and radiochemoresistance of tumors and dose limiting factor of

normal brain tissue. According to the infiltrative properties of malignant gliomas, the microscopic invading cells are present at the distance of 2-3 cm in brain tissue or even further from the main tumor mass that is usually identified by contrast enhancement area at a magnetic resonance image (MRI) (2, 3, 4).

The survival benefit which is associated with the safe maximum cytoreduction of glioma, has been shown in previous reports. Usually, removal of malignant gliomas means the removal of contrast-enhanced brain tumor tissue using a neurosurgical microscope. However, complete resection of the contrast-enhanced brain tumor was accomplished in less than 20% of patients (5, 6, 7, 8). It is very difficult to distinguish between a brain tumor and normal brain tissue at a brain-tumor interface intraoperatively. Since many years ago, fluorescence guided surgery using photosensitizer and intraoperative photodynamic therapy have been known in the field of neurosurgery (9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19). Hematoporphyrin derivatives (HpDs) are the first generation photosensitizers, which have high accumulation in cancerous tissues, including glioma tissue (14, 20, 21). However, HpDs were not widely used in the field of neurooncology, because these showed prolonged skin photosensitization, as well as retention in normal brain tissue and low penetration of the brain tissue by a laser system due to the short wave length for the excitation of HpDs (22, 23, 24). Although other porphyrins, such as benzoporphyrin (25), boronated porphyrin (26) and meta-tetra hydroxyphenyl chlorine (27), were reported to be candidate photosensitizers for photodynamic therapy, they are still under investigation and have not been clinically involved in the neurosurgical treatment. 5-aminolevulinic acid (5-ALA) is a biochemical precursor of the hemoglobin that elicits synthesis and accumulation of fluorescent porphyrins in various epithelia and cancer tissue (28). 5-ALA is a prodrug of the second generation photosensitizer and a potential photosensitizer for the intraoperative photodynamic diagnosis and the photodynamic therapy. An epoch-making result was reported by Stummer *et al.* (29). Fluorescence guided surgery using 5-ALA 3 to 4 hours prior to anesthesia comes to be beneficial for the improvement of the complete resection of a contrast-enhanced tumor and as well as for the 6-months progression free survival of the patients, as shown by the randomised controlled multicentre phase III trial (29). Moreover, other authors report favorable results of the photodynamic therapy using 5-ALA (15, 30, 31, 32).

However, the fundamental relation between intraoperative photodynamic diagnosis and its corresponding pathology still remains vague, and little is known about the distribution and the accumulation of photosensitizers in the peritumoral edematous area. Recently some clinical case reports highlighted the positive fluorescence at the

area of the radiation necrosis, degenerative tissue and the inflammation (33, 34). The optimization of the intraoperative fluorescence diagnosis of brain tumor tissue differentiated from brain edema leads not only to the improvement of resectability of the brain tumor but also to the future application of the photodynamic therapy with less harmful effect on normal neural tissue.

Therefore, we compared the fluorescence of brain tumor tissue and brain edema using 5-ALA and talaporfin sodium – the second generation photosensitizer derived from chlorine, in an in vivo experiment. We also investigated the kinetics of 5-ALA and talaporfin sodium measuring the relative fluorescence intensity in brain tumor tissue using the experimental brain tumor model and in brain edema using the vasogenic edema model, compared with the fluorescence of the ipsilateral surrounding brain tissue and the contralateral normal brain tissue in both cases.

Materials and methods:

Animal care:

Animal experiments were carried out in a humane manner after receiving approval from the Instituional Animal Experiment Committee of the Universiry of Tsukuba (08-283), and in accordance with the Regulation for Animal Experiments in our university and Fundamental Guideline for Proper Conduct of Animal Experiment and Related Activities in Academic Research Institutions under the jurisdiction of the Ministry of Education, Culture, Sports, Science and Technology of Japan.

The rat brain tumor model:

We used the rat brain tumor model produced by the inoculation of resuspended tumor cells into the frontal rat brain (20). Two weeks prior to the experiment, Wistar rats at the age of 6 weeks were used for brain tumor models. The animal holding rooms were maintained at constant temperature and humidity using 12-hour light-and-dark schedule. The Wistar rats were anesthetized firstly by the isoflurane gas, and then by the intraperitoneal administration of 4.5mg/100g body weight sodium pentobarbital. The rats were set in a stereotactic headholder and craniotomy was performed on the right frontal bone. Then, $1 \times 10^5/10\mu l$ of C6 glioma cells were inoculated into the right stria medium through the small burr hole. The stereotactic manipulator with a 27-gauge fine needle was used for tumor inoculation.

The rat vasogenic edema model:

The vasogenic edema model was used because the edema production is well standardized and resembles the peritumoral edema in many aspects (35, 36). Wistar rats at the age of 8 weeks were used for the brain vasogenic edema model. The rats were anesthetized and the craniotomy was performed in the same way as in the case of the rat brain tumor models. The dura mater was kept intact. Cold injury was produced in the right frontal cortex by applying a cylindrical copper probe (4mm in diameter) cooled to -80℃ with a mixture of dry ice and acetone. The duration of cooling was 1 minute.

Fluorescence imaging:

Two weeks after the inoculation, the whole brains were extracted from the euthanized rat tumor models or rat edema models at each time interval of 0 hour, 0.5 hour, 2 hours, 6 hours, 12 hours after the intraperitoneal injection of 100 mg/kg body weight 5-ALA (n=5 in each group) or after the intravenous injection of 5 mg/kg body weight talaporfin sodium (n=5 in each group). The concentration of the 5-ALA was 20mg/1ml 0.9 % saline, and the one of the talaporfin sodium was 5mg/1ml 0.9 % saline. The fluorescence images of the extracted whole brain and coronal tissue section with 5mm thickness were recorded using fluorescence macroscope (KEYENCE ltd., Osaka, Japan) fitted with a filter combination consisting of a 415 ± 100 nm bandpass excitation filter, a 475 nm dichroitic filter, and a 610 nm longpass filter.

Measurement of the fluorescence intensity of the samples:

The tissue samples were obtained from the coronal tissue sections at each time interval of 0 hour, 0.5 hour, 2 hours, 6 hours, 12 hours after the injection of 5-ALA (n=5 in each group) or talaporfin sodium (n=5 in each group). The coronal tissue sections were made at the same time in the experiment of the fluorescence imaging and the tissue samples corresponding to the tumor (or edema) brain tissue, the brain tissue ipsilateral to the tumor (or edema), the brain tissue contralateral to the tumor (or edema) were obtained. The samples were well homogenized with 1 ml of 100% dimethyl sulfoxide (DMSO) and the 100μl aliquots of the supernatant were put into each well of a 96-well plate. The rats were all kept in the dark room between the injection of 5-ALA or talaporfin sodium and the obtaining of the fluorescence images. The relative fluorescence intensities of the samples were measured in arbitrary units (a.u.) using a microplate reader (Bertold Japan ltd., Tokyo, Japan) with an excitation wavelength of 405/40 nm, and an emission wavelength of 630 ± 10nm in the case of protoporphyrin IX (PpIX) generated from 5-ALA, and an emission wavelength of 670 ± 10nm in the case of talaporfin sodium. The relative fluorescence intensities of the samples (a.u.) were

normalized to the relative fluorescence intensities per 1g-weight of the samples (a.u.). At the same time, the spectrums of the samples were recorded by a handheld laser system (VLD-V1 version 2; M & M Co., Ltd., Tokyo, Japan) with the peak wavelength of 405 ± 1 nm and output of 40 mW with scanning through a fiber optic cable.

We used the contralateral cortical brain tissue as a control in all the cases. The lesion-to-normal-brain ratio (L/N ratio) was calculated and examined for the tumor brain tissue and the brain tissue ipsilateral to the tumor in comparison with the contralateral cortical brain tissue. The L/N ration was also calculated and examined for the brain edema and the brain tissue ipsilateral to the brain edema, in comparison with the contralateral cortical brain tissue.

The results of relative fluorescence intensities (a.u.) were expressed as means+standard deviation of mean. The statistical difference was determined by two–sided Student's t-test. The difference with $P<0.05$ was considered significant. All the samples of the rats were kept in a dark room during the experiments.

Results:

Fluorescence imaging:

In the 5-ALA administered brain tumor models, no fluorescence was detected in the tumor tissue before the drug injection. About 2 hours after the drug injection, maximum red fluorescence was detected in and around the tumor bulk in the tumor models heterogeneously (Figure 1-a,b,c,d,e). At 6 hour time intervals, the red fluorescence decreased in comparison with that of the 2 hour intervals. By 12 hours, the red fluorescence had almost disappeared. In the 5-ALA administered vasogenic edema models, no fluorescence was detected at the area of edema before the drug injection. At the 0.5 hour time interval, slight red fluorescence was detected in the peripheral areas of the vasogenic brain edema, and had gradually increased by 2 hours. About 6 hours after the drug injection, maximum red fluorescence was detected at the area of edema sparing the central necrotic core of edema, and at the same time, red fluorescence was also detected in the white matter in the ipsilateral corpus callosum (Figure 2-a,b,c,d,e). By 12 hours, the red fluorescence had decreased moderately.

In the talaporfin sodium administered brain tumor models, no fluorescence was detected in the tumor tissue before the drug injection. 2 hours after the drug injection, slight red fluorescence was detected homogenously in the tumor bulk. About 6 hours after, maximum red fluorescence was detected not only in the tumor bulk, but also in the peritumoral areas heterogeneously in the tumor model (Figure 3-a,b,c,d,e). After 12

hours, the red fluorescence was still observed. In the talaporfin soidum administered vasogenic edema models, no fluorescence was detected in the area of edema before the drug injection. At the 0.5 hour time interval, slight red fluorescence was detected in the peripheral area of the vasogenic edema, and gradually increased by 2 hours. About 6 hours after, maximum red fluorescence was detected in the peripheral areas sparing the central necrotic core of the vasogenic edema, and at the same time, red fluorescence was also detected in the white matter in the ipsilateral corpus callosum (Figure 4-a,b,c,d,e). By 12 hours, red fluorescence had decreased moderately.

The measurement of the fluorescence intensities of the samples:

The profiles of the time distribution of endogenous PpIX generated from 5-ALA in both model types are shown in figures 1-f and 2-f. The peak fluorescence intensities of the tumor tissues were observed around 2 hours after the administration of 5-ALA, and those of the vasogenic edema models were observed beyond the period of 6 hours after the administration of 5-ALA. Also, the profiles of the time distribution of talaporfin sodium in the brain tumor model and the vasogenic edema model are shown in figures 3-f and 4-f. The peak fluorescence intensities of the tumor models were observed around 6 hours after the administration of talaporfin sodium, and those of the vasogenic edema models were observed around 2 hours after the administration of talaporfin sodium. The background spectrums recorded by the handheld spectrometer at a wavelength of 500 nm were so small and negligible, that we estimated the fluorescence intensities recorded by the microplate reader at a wave length of 670 nm in case of talaporfin sodium and at a wave length of 630 nm in case of PpIX induced from 5-ALA as relative fluorescence intensities respectively.

The mean L/N ratio of the brain tumor group using 5-ALA was 7.78 ± 4.61, which was significantly higher ($P < 0.01$) than that of the vasogenic brain edema group using 5-ALA (2.75 ± 1.12) at 6 hours as shown in figure 9. The mean L/N ratio of the brain tumor group using talaporfin sodium was 23.1 ± 11.9, which was significantly higher ($P < 0.01$) than that of the vasogenic brain edema group using talaporfin sodium (8.93 ± 8.03) at the period of 12 hours as shown in figure 10.

Discussion:

In the case of 5-ALA, the optimal timing of fluorescence imaging of brain tumors was within the period of 6 hours, because the time interval to gain the peak relative fluorescence intensity of brain tumor tissue was within the period of 6 hours

and that of brain edema was beyond the period of 6 hours. In the case of talaporfin sodium, the optimal timing of fluorescence imaging of brain tumors was beyond the period of 12 hours because the time interval to gain the peak fluorescence intensity both in brain tumor and brain edema was within 6 hours. However, the retention of the relative fluorescence intensity lasted over 12 hours only in the brain tumor, but not in the edema.

In our study we used a unique approach to assess the optimal timing of intraoperative fluorescence imaging of brain tumors by evaluating different kinetic characteristics of 5-ALA in brain tumor tissue in comparison with vasogenic edema. Moreover, we also evaluated the mean L/N ratio of the fluorescence intensity in the tumor tissue in comparison with the contralateral normal brain tissue in rat brain tumor models for the period of 12 hours, which was not previously observed in scientific publications. 5-ALA showed mild brightness in our rat brain tumor models.

The values of the tumor-to-normal-brain ratio of relative fluorescence, obtained in the present study, were close to those reported in other studies (18, 37, 39). The reported values of the tumor- to-normal brain ratio are about 10 at the period of 6 hours after the administration of 5-ALA(18, 39). For the intraoperative application 5-ALA is given to the patient orally 3 to 4 hours before the induction of anesthesia (29). In several laboratory experiments, maximum fluorescence intensity is observed around 6 hours after the administration of 5-ALA (18, 39). However, according to our data, the optimal timing of the intraoperative fluorescence imaging of a brain tumor is a little bit earlier than 6 hours after the administration of 5-ALA. The time needed to gain the peak relative fluorescence intensity using 5-ALA depends on several factors such as the use of different animals for tumor models (15, 27, 37, 38, 39), different delivery systems (18, 27, 30, 37, 38, 39, 40), different drug doses and treatment regimens including laser intensity (30, 31, 41). Olivo *et al.* (37) showed that the time to gain peak relative fluorescence was 4.5 hours after the administration of 5-ALA in rabbit brain tumor models. In most of the reported experiments (18, 37, 39) 5-ALA was administered intravenously, whilst in our study the intraperitoneal injection of 5-ALA was used. However, there is no difference in the distribution of PpIX in normal brain tissue in the subcutaneous tumor model between intravenous and intraperitoneal administration of 5-ALA (38). Concerning different drug doses and treatment regimens, in the study with microfluorometry of frozen tissue sections (30) the peak fluorescence of PpIX was observed 4 hours after the repetitive injections of 5-ALA. However, the optimal dose of 5-ALA for intracranial photodynamic therapy using the rat brain tumor model at optimal laser intensity such as fluence and fluence rate was reported as 60 mg/kg by

intraperitoneal injection, and no increase of fluorescence was observed when the dose over 60 mg/kg was used (30). In our study we used 5-ALA at the dose of 100 mg/kg, which was enough to get optimal fluorescence.

In some articles the retention of talaporfin sodium in several cancer tissues is reported (42). However, there is no precise information about kinetics of talaporfin sodium in rat brain tumor model. We observed the fluorescence of talaporfin sodium in rat brain tumor model up to 12 hours after the administration. Such a long period of the observation in our experiment can be considered as a new approach to measure the relative fluorescence intensity of rat brain tumor tissue after the administration of talaporfin sodium. Talaporfin sodium showed high fluorescence intensity and retention in rat brain tumors in our experiment. Talaporfin sodium is a new photosensitizer excited at a longer wavelength of light in comparison with 5-ALA, so it can be considered as a potential photosensitizer for the neurosurgical application. In our study the maximum tumor-to-contralateral-brain ratio of relative fluorescence 12 hours after the administration of talaporfin sodium was above 20, and the retention of this drug in brain tumor tissue lasted up to 12 hours. There is only one article reporting about the kinetics of talaporfin sodium in the brain tumor model, in which the observation of the drug retention was restricted to 4 hours, due to the use of live rats with open cranial window (43). However, the precise mechanism of the accumulation of talaporfin sodium in tumor cells still remains unknown. Further studies are needed to clarify the distribution of talaporfin sodium in brain tumor models using human glioma cell-lines.

There are some causative factors in the distribution and the accumulation of photosensitizers in the vasogenic edema model. One reason is thought to be the permeability of blood brain barrier due to the dysfunction or rupture of endothelial cells after cold injury in the vasogenic edema model (44, 46). Another reason may be the bulk flow from the edema lesion. Stummer *et al.* (24) showed that photofrin II spreads within the edema and is washed out by the edema fluid generated in the cerebral lesion, and photofrin II was detectable at a distance of 5 mm from the cold-injury lesion. Reulen *et al.* (47) indicated that the bulk flow, but not the diffusion, should be considered as the main mechanism for the spread of edema through the white matter. Our results showed that PpIX and talaporfin sodium accumulate in the ipsilateral brain and white tract fibers such as corpus callosum.

Our study represents the systematical kinetics of 5-ALA as well as talaporfin sodium in the vasogenic brain edema model. There is a clear difference between the in vivo kinetics of 5-ALA and talaporfin sodium in the rat brain vasogenic edema model according to our data. These drugs are different in metabolism (42, 48) and delivery

(39). 5-ALA itself is metabolized and converted to PpIX in rat brain (48). Because time is needed to metabolize 5-ALA to PpIX in rat brain (39, 48, 49, 50), the peak of the relative fluorescence in vasogenic edema model in this case is thought to be observed later than that of talaporfin sodium. In Habedae's *et al.* experiment (37), low fluorescence was observed at the site of cryo-lesions 6 hours after the intraperitoneal administration of 5-ALA in the rat vasogenic edema model. However, there is a difference between our vasogenic edema model and Habedae's one (37) in several aspects. The vasogenic edema models in our experiment were studied 24 hours after the cold injury, while in Habedae's experiment vasogenic edema models were studied right after the cold injury. Nag *et al.* (45) demonstrated that the permeability of the blood brain barrier in the vasogenic edema models increased for such macromolecules as serum proteins as soon as 12 hours after the cold injury, and the surrounding areas were penetrated by reactive astrocytes and macrophages as soon as 24 hours after the cold injury. In comparison with Habedae's models, the vasogenic edema models in our study seemed to possess the higher permeability for the photosensitizers due to the extent dysfunction of the endothelial cells after the cold injury. Also, the accumulation of the photosensitizers in reactive astrocytes and macrophages was observed in the surrounding areas of the vasogenic edema, which complied with the kinetics of many other porphyrin compounds (34, 51, 52). In contrast to 5-ALA, talaporfin sodium was accumulated in vasogenic edema in several hours, and washed out from the edema lesion by 12 hours in our experiment. Talaporfin sodium usually binds with the macromolecules such as serum albumin in animals. However, talaporfin sodium itself is not metabolized in rat brain (42). Thus, talaporfin sodium bound with albumin may be exudated earlier from leaky blood brain barrier of the edema lesion (45) than from tumor vessels, and washed out by the bulk flow of the edema. (47)

The fluorescence intensity of the contralateral brain tissue in the vasogenic edema model after the administration of 5-ALA was different from that observed after the administration of talaporfin sodium. In the case of 5–ALA, there was no change in the fluorescence intensity of the contralateral brain tissue in the vasogenic edema model, while in the case of talaporfin sodium the increase of the fluorescence intensity was observed in our experiment. 5-ALA is converted to the endogenous PpIX in mitochondria by the enzymatic metabolic activity (38), thus the fluorescence intensity of PpIX may not be influenced directly by the cerebral blood flow (CBF) and the bulk flow from the edema lesion. It is known, that there are no studies showing the increase of the fluorescence intensity in the contralateral brain tissue in the vasogenic edema model after the administration of 5-ALA (18, 37). In contrast to 5-ALA, talaporfin

sodium itself is not metabolized in rat brain (42), thus the fluorescence intensity in the contralateral brain tissue in the vasogenic edema model can be directly influenced by the local CBF and the bulk flow in brain edema. According to the previous reports, the CBF in the contralateral hemisphere of the rat vasogenic edema model may be partially increased (53, 54). Morever, many previous reports showed the bulk flow from the edema to the contralateral rat brain (18, 37). Therefore, the increase of the fluorescence intensity of the contralateral brain tissue in vasogenic edema model occurred after the talaporfin sodium administration.

Conclusion:

The optimization of the fluorescence imaging of brain tumor tissue differentiated from brain edema was achieved using 5-ALA and talaporfin sodium. Our results support the reliability of photodynamic diagnosis and the prospective safe application of photodynamic therapy in clinical practice.

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Legends for Figures:

Figure 1. Macroscopic view of the rat brain tumor model 2 hours after the administration of 5-aminolevulinic acid (5-ALA). Fine dotted lines and coarse dotted lines indicate the margins of the tumor bulk corresponding to those of a hematoxylin-eosin (HE) stain. Images of a) the whole brain, and b) the coronal section are provided. Fluorescence images of c) whole brain, and d) coronal section were obtained using 610 nm long path filter. The e) coronal section is stained with HE.

f) The time course distribution of the relative fluorescence intensity of the rat brain tumor, the ipsilateral cortical brain and the contralateral cortical brain is shown in arbitrary units after the administration of 5-ALA.

Figure 2. Macroscopic view of rat the vasogenic edema model 2 hours after the administration of 5-ALA. Fine dotted lines and coarse dotted lines indicate the margin of the edema corresponding to those of a hematoxylin-eosin (HE) stain. On the images a) the whole brain, and b) the coronal section are shown. Fluorescence images of c) whole brain, and d) coronal section were obtained using 610 nm long path filter. The e) coronal section is stained with HE. f) The time course distribution of the relative fluorescence intensity of the rat brain vasogenic edema, the ipsilateral cortical brain and the contralateral cortical brain is shown in arbitrary units after the administration of 5-ALA.

Figure 3.   Macroscopic view of the rat brain tumor model 6 hours after the administration of talaporfin sodium. Fine dotted lines and coarse dotted lines indicate the margin of the tumor bulk corresponding to those of a hematoxylin-eosin (HE) stain. On the images a) the whole brain, and b) the coronal section are shown. Fluorescence images of c) whole brain, and d) coronal section were obtained using 610 nm long path filter. The e) coronal section is stained with HE. f) The time course distribution of the relative fluorescence intensity of the rat brain tumor, the ipsilateral cortical brain and the contralateral cortical brain is shown in arbitrary units after the administration of talaporfin sodium.

Figure 4. Macroscopic view of the rat vasogenic edema model 6 hours after the administration of talaporfin sodium. Fine dotted lines and coarse dotted lines indicate the margin of the edema corresponding to those of a hematoxylin-eosin (HE) stain. On the images a) the whole brain, and b) the coronal section are shown. Fluorescence images of c) whole brain, and d) coronal section were obtained using 610 nm long path filter. The e) coronal section is stained with HE. f) The time course distribution of the relative fluorescence intensity of the rat brain vasogenic edema, the ipsilateral cortical brain and the contralateral cortical brain is shown in arbitrary units after the administration of talaporfin sodium.

Figure 5. The distribution of the lesion-to-normal-brain ratio in the rat tumor

model and vasogenic edema model after the administration of 5-ALA. Single, double

and triple asterisks indicate the significance obtained with t-test (p<0.01).

Figure 6. The distribution of the lesion-to-normal-brain ratio in the rat tumor model and vasogenic edema model after the administration of talaporfin sodium. Single, double and triple asterisks indicate the significance obtained with t-test (p<0.01).