

TOPICAL VERSUS SYSTEMIC 5-AMINOLEVULINIC ACID ADMINISTRATION FOR PHOTODYNAMIC THERAPY OF THE COLON IN B10.RBP MICE

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ABSTRACT

5-aminolevulinic acid (5-ALA) is an interesting photosensitizing substance for photodynamic therapy (PDT), successfully applied topically for urological malignancy. In gastroenterology it has proven efficacy for treatment of some GI neoplasms after systemic administration. This study was aimed at investigating the possibility of topical 5-ALA administration also for the PDT of gut cancer in a mice model. 5-ALA solution at different concentrations (5%, 1.5%, and 0.5%) was instilled in the colon of mice, which was later removed and examined by fluorescence microscopy. The results of fluorescence studies were compared with those obtained in a control group treated with 5-ALA given systemically. Satisfactory epithelial fluorescence levels and good selectivity between gut layers was obtained after intracolonic 5-ALA instillation. However, mean fluorescence intensity was higher after systemic drug application. Our results suggest that 5-ALA may probably be used topically for the PDT of some gut neoplasms. © 1999 Society of Photo-Optical Instrumentation Engineers. [S1083-3668(99)00803-5]

Keywords 5-aminolevulinic acid; photodynamic therapy; gut cancer; B10.RBP mice.

1 INTRODUCTION

It has already been shown in many publications that photodynamic therapy (PDT) offers a novel approach to the therapy of different neoplasms, where radical surgery is not possible, and radiotherapy and chemotherapy are of no proven benefit. Tumor necrosis occurs via a nonthermal photochemical reaction, using photosensitizing compounds, which are preferably retained in the tumor tissues. Systemic administration of the photosensitizer is followed by exposure of laser light at an appropriate wavelength (matched to an absorption peak of the sensitizer used), resulting in the generation of cytotoxic singlet oxygen. An important disadvantage of PDT with some currently used photosensitizers (haematoporphyrin derivatives) is a prolonged skin photosensitivity lasting for about 4–6 weeks.¹ Recently, the possibility of using 5-aminolevulinic acid (5-ALA) as a sensitizing agent for the PDT arouses interest. It is a naturally occurring precursor in the biosynthetic chain for haem synthesis. Upon exogenous administration, 5-ALA is metabolized to protoporphyrin IX (PpIX), which then accumulates in different tissues. Differences in PpIX ac-

cumulation between tumor and normal tissues as well as differences between mucosa and muscle layers of gastrointestinal tract give rise to the possibility of using 5-ALA for PDT in clinical practice. Interesting results have already been obtained for the treatment of different neoplasms using topical 5-ALA administration.² The main advantages of using 5-ALA systemically as a photosensitizing agent lie in the rapid elimination of 5-ALA from the body.³ Photosensitization lasts not longer than 48 h. However, the PDT effects after oral route in esophageal, duodenal, and colorectal tumors are superficial. In contrast, the results of topical 5-ALA administration in urinary bladder tumors are more promising.^{4,5} We think that 5-ALA solution given as enema might be a convenient mode of topical administration in patients with some colorectal pathology.

The aim of this study was to investigate the photosensitization of the layers of the colon wall in the B10.RBP model after intracolonic instillation of 5-ALA solution as an enema, mimicking topical drug administration and to compare it with an intraperitoneal 5-ALA administration.

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2 MATERIALS AND METHODS

2.1 ANIMALS

Male B10.RBP mice weighing ~15–18 g were used in all experiments. The animals were kept separately in plastic cages, provided free access to water and food. Food was withdrawn 30 h prior to the intracolonic 5-ALA instillation in order to obtain a feces-free colon. Further, a special metal net was placed at the bottom of each cage, rendering it impossible for the animals to eat their own feces. For intracolonic instillation of 5-ALA and PDT animals were immobilized with ketamine intraperitoneal (ip) injection (100 mg/kg body weight).

2.2 SUBSTANCE

5-aminolevulinic acid was generously provided by DUSA (Canada). For systemic administration, 5-ALA was dissolved in phosphate buffered saline (PBS) at a concentration of 40 mg/ml, as previously described.⁶ For the purpose of intracolonic administration the stock solution of 5-ALA in saline (pH = 2.5) at a concentration of 5% was freshly prepared for each treatment regimen and diluted to the appropriate concentrations, following the results of Chang et al., who showed chemical instability of 5-ALA on aqueous solution in the neutral to basic pH range.

2.3 PHOTOSENSITIZATION STUDY

Two different routes of 5-ALA administration were used for mice photosensitization: (a) systemic, in which 5-ALA was injected intraperitoneally at a total dose of 200 mg/kg body weight; (b) as enema, in which 1 ml of 5-ALA solution at a concentration of 5%, 1.5%, or 0.5% was instilled into the colon of the animal by a balloon catheter. Each group consisted of three animals. Animals were killed 0.5, 2, and 4 h following either systemic or 5-ALA colonic instillation. The colon was subsequently dissected; its lumen washed with PBS and a short segment of proximal, mid, and distal colon removed. All specimens were immediately frozen in a bath of isopentane precooled in liquid nitrogen and then stored in liquid nitrogen. Fluorescence microscopy studies as described in detail elsewhere⁶ were performed. Briefly, 10- μ m-thick sections were cut with a Cryocut E microtome (Reichert). An inverted microscope (IMT-2, Olympus) with epifluorescence and phase-contrast attachments and with a slow-scan, cooled, charged-coupled device camera was used to obtain fluorescence images of the selected area of the section. A 10 \times objective was used throughout to give images of 880 \times 550 μ m dimensions. Fluorescence was excited using an 8 mW helium neon laser (632.8 nm wavelength) and detected in the range 660–710 nm using a combination of bandpass and longpass filters. The fluorescence signal was processed by an IBM computer generating false color-coded images. Digital quantification

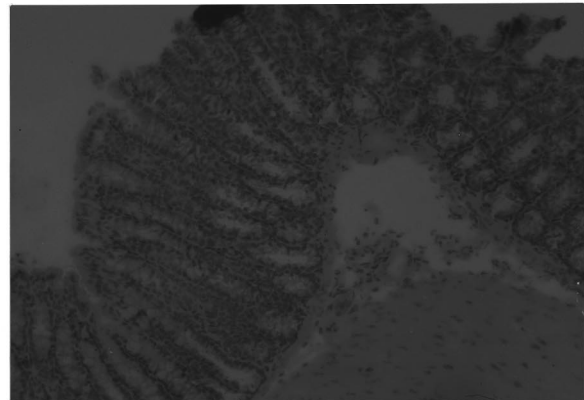
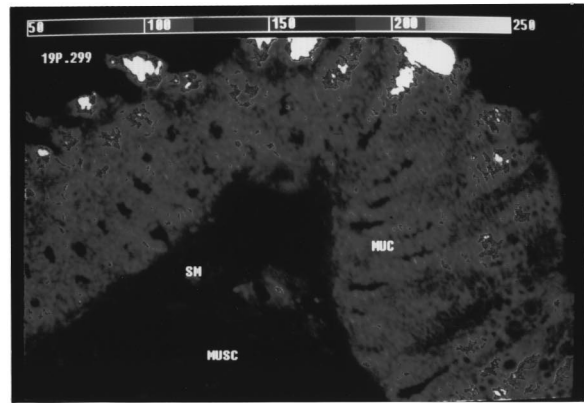


Fig. 1 Above, a computer processed fluorescence microscopy image of colon wall 4 h after instillation of 0.5% 5-ALA. Color scale depicts signal in counts per pixel (white is high fluorescence, black low). Below, the same specimen stained with hematoxylin-eosin (10 \times).

of the fluorescence intensity from the areas of interest was measured in arbitrary units of counts per pixel. After fluorescence images were obtained, the same sections were fixed in formalin and stained with hematoxylin and eosin for comparative light microscopy analysis, which allowed precise identification of the fluorescing structures. Unsensitized tissues from the control group were also examined to quantify the autofluorescence intensity levels.

2.4 STATISTICS

For comparison of mean [\pm standard deviation] fluorescence intensities in different layers of the colon wall two-tailed Student's *t*-test was applied, $p < 0.05$ being considered statistically significant.

3 RESULTS

The examples of fluorescence and light microscopy images of a colon wall after intracolonic or systemic 5-ALA administration are shown in Figures 1 and 2. Fluorescence measurements for each specific tissue layer (colonic mucosa, submucosa, and muscle)

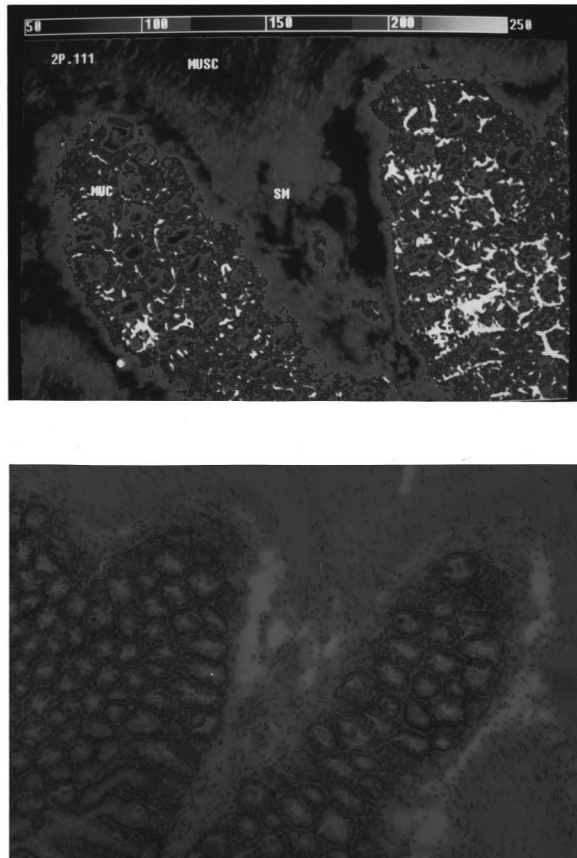


Fig. 2 Above, a computer processed fluorescence microscopy image of colon wall 2 h after intraperitoneal 5-ALA administration. Color scale depicts signal in counts per pixel (white is high fluorescence, black low). Below, the same specimen stained with hematoxylin-eosin (10 \times).

were averaged over several representative areas taken from two to three animals for each 5-ALA administration, with correction for background autofluorescence.

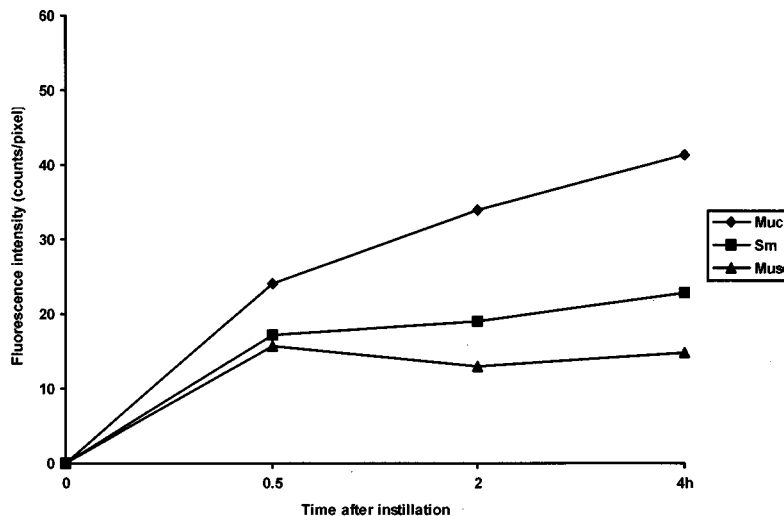


Fig. 3 Plot of colon wall fluorescence activity against time after instillation of 5% ALA. Each value is average of measurements from several areas in three mice.

At 5-ALA concentrations of 5% and 1.5% given as enema, the fluorescence intensity in colonic wall layers was similar in configuration. The fluorescence intensity was much higher in the mucosa than in the submucosa and muscle layer. Following intracolonic instillation of 5% 5-ALA solution (total dose of 2500 mg/kg body weight at pH 2.5), fluorescence in the mucosa increased in time up to 4 h after administration, and subsequently leveled off (Figure 3). With 1.5% solution (total dose 750 mg/kg body weight at pH 2.5), the highest fluorescence intensity in the mucosa was again seen 4 h after administration (Figure 4), likewise for the lowest concentration of 0.5% (total dose 250 mg/kg body weight at pH 2.5) (Figure 5). In contrast, the maximal fluorescence intensity after intraperitoneal 5-ALA administration was seen at 2 h (Figure 6). Statistically significant differences in the mean fluorescence intensity between the layers of the colon wall for the different time-points and 5-ALA concentrations were noted. At 0.5 h significant difference between mucosa and submucosa was seen after intracolonic 5% 5-ALA solution instillation (24.1 ± 10.3 vs 17.1 ± 6.9 , $p = 0.04$) and intraperitoneal administration (30.5 ± 9.4 vs 18.1 ± 7.1 , $p = 0.0007$). Moreover, at this time point mean mucosal fluorescence intensity was similar for the groups given 5-ALA as enema. In these animals 4 h after instillation the highest fluorescence intensity in the mucosa, expressed as mean signal per pixel, was seen in the group given 1.5% ALA solution, the difference comparing to 5% and 0.5% of the group being statistically significant. The highest fluorescence intensity ratio between mucosa and muscularis propria (8.5:1) was observed at 2 h after 5-ALA given intraperitoneally. With the 1.5% 5-ALA solution, a peak mucosa-to-muscle layer fluorescence ratio of 8.2:1 and mucosa-to-submucosa ratio of 6.8:1 was achieved 2 hours after instillation. Al-

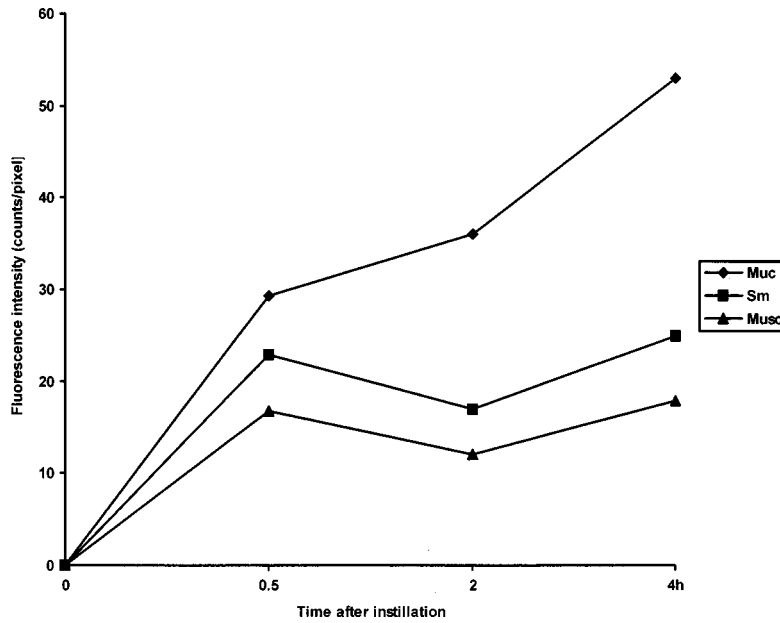


Fig. 4 Plot of colon wall fluorescence activity against time after instillation of 1.5% ALA. Each value is average of measurements from several areas in three mice.

though, as shown above, the absolute mucosal fluorescence at this time was not the maximum, it was the time showing the best selectivity between mucosa and both the submucosa and muscle layer.

4 DISCUSSION

The role of PDT using 5-aminolevulinic acid as photosensitizing agent for the treatment of gastrointestinal tract diseases still remains to be defined. Our study aimed at investigating whether topical 5-ALA administration can be used as a reliable mode of obtaining gut mucosa photosensitiza-

tion. It has already been shown that 5-ALA may be administered orally in order to sensitize gut epithelium. However, the PDT effects after oral route in esophageal, duodenal, and colorectal tumors are superficial. In this study we have tried to adopt a model of 5-ALA administration successfully tested in the management of the carcinoma *in situ* of the urinary bladder.

The finding of increased fluorescence in the colonic epithelium after 5-ALA instillation may be explained by good permeability of this epithelium to the drug. Intestinal permeation by foreign agents

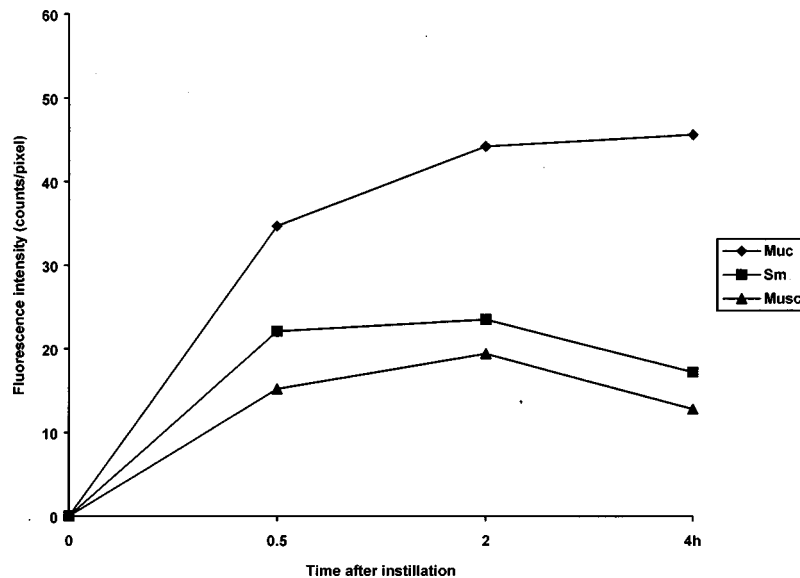


Fig. 5 Plot of colon wall fluorescence activity against time after instillation of 0.5% ALA. Each value is average of measurements from several areas in three mice.

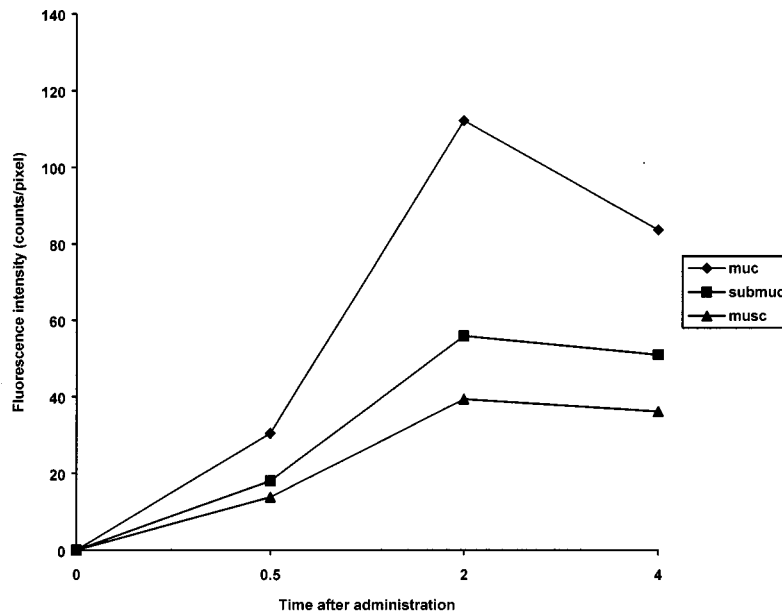


Fig. 6 Plot of colon wall fluorescence activity against time after intraperitoneal ALA administration. Each value is average of measurements from several areas in three mice.

via a noninflamed bowel wall can take place by different mechanisms, such as passive diffusion, persorption, micropinocytosis, or active transport. If specific carrier mechanisms are lacking, the most important factors for permeation of the intestinal mucosa are lipid solubility and molecular size. Hydrophilic molecules permeate the intestinal mucosa according to their molecular weight. The biochemical properties of 5-ALA (low molecular weight, high water solubility) match some of these criteria.

The unbuffered 5-ALA solution used in this study has a *pH* of 2.5, which is completely unphysiological to the colon. At this *pH* the solution is certainly more stable chemically, but obviously the mucosa might have been damaged as a result of prolonged contact with the strong acid. However, histopathological specimens from the colon do not show substantial damage resulting from instillation of such acidic solutions (Figures 1 and 2). Only some specimens from mice in which 5% 5-ALA solution was instilled show superficial damage to the epithelium. Clearly, it would be appropriate in the future to apply 5-ALA solution with higher *pH*.

The relative speeds of 5-ALA absorption into the colonic mucosa and conversion to PpIX once in the epithelium and other layers of the colon wall are unknown, but it seems likely that the duration of instillation and 5-ALA solution concentration would be of importance here. In our experiment, following intracolonic instillation of 5% and 1.5% 5-ALA solutions, photosensitization of the colon epithelium was highest at 4 h, which is later than after intraperitoneal administration (2 h). On the other hand, colon wall fluorescence intensity

against time after a 0.5% 5-ALA solution enema was comparable to the curve obtained for intraperitoneal administration. However, the absolute fluorescence levels in the epithelium were significantly higher when 5-ALA was administered systemically.

The high selectivity of PpIX accumulation between the gut epithelium and other layers is crucial for safe and selective destruction of lesions confined to the mucosa without damaging the lamina propria and the muscle layer. In our study, as the 5-ALA concentration declined from 5% to 1.5% and to 0.5%, the change of fluorescence intensity was not linear. However, the epithelium remained the layer with the highest level and ratios as high as 8.2:1 (epithelium/muscle) and 6.8:1 (epithelium/submucosa) could be obtained with the 1.5% solution. Interestingly, the former ratio is comparable (8.5:1) and the latter much better (2.4:1) to that seen after ip 5-ALA administration.

Our results suggest that intracolonic instillation of 5-ALA might be a notable option to other routes because it might lead to less skin photosensitization and maintains substantial fluorescent material (most likely PpIX) selectivity between the colonic mucosa and other layers. Furthermore, this route may avoid the liver metabolism, inevitable after oral administration.³

Concluding, topical administration of 5-ALA as an enema in the large bowel gives satisfactory photosensitization and good selectivity between gut layers. Topical mode of 5-ALA sensitization can probably be used to obtain the PDT effect, but this requires further studies.

5 SUMMARY

5.1 BACKGROUND AND AIM

5-ALA is a new photosensitizing agent for PDT. In clinical setting it is usually administered orally. Urologists successfully apply it by intravesical instillation. Topical administration in the gastrointestinal tract has not been studied yet. The aim of this study was to investigate the biodistribution of fluorescent material in the colon wall of B10.RBP mice after topical (as enema) administration of 5-ALA.

5.2 MATERIAL AND METHODS

A group of three mice each had a 5-ALA enema of about 1 ml in volume to fill the colon completely at concentrations of 0.5%, 2.5% and 5% (pH=2.5). The control groups consisting of three animals each had the 5-ALA administered ip at a dose of 200 mg/kg. The colon was removed 0.5, 2, and 4 h later for measurement of the photosensitization of the layers of the colon wall by fluorescence microscopy.

5.3 RESULTS

The highest fluorescence levels following topical 5-ALA administration was detected in the mucosa after 1.5% solution at 4 h (64 counts/pixel). The highest value following ip administration was observed in the mucosa at 2 h (143 counts/pixel). The highest fluorescence intensity ratio between mucosa and muscularis propria was observed after ip ALA administration at 2 h (8, 5:1) and between mucosa and submucosa (8:1) was noted after a 2.5% ALA enema at 2 h. However, mean fluorescence

activity in the colon obtained after intraperitoneal administration was significantly higher than after topical administration.

5.4 CONCLUSION

Topical administration of 5-ALA as an enema in the large bowel gives epithelial photosensitization and good selectivity between gut layers. Topical mode of 5-ALA sensitization can probably be used to obtain a PDT effect, but this requires further studies.

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