Research Article

Pharmacokinetics of Photogem Using Fluorescence Spectroscopy in Dimethylhydrazine-Induced Murine Colorectal Carcinoma

Raduan Hage,[1,2] Juliana Ferreira,[2] Vanderlei Salvador Bagnato,[3] José Dirceu Vollet-Filho,[3] and Hélio Plapler[1]

[1] *Departamento de Cirurgia, Universidade Federal de São Paulo (UNIFESP), Rua Botucatu 740, 04023-900 São Paulo, SP, Brazil*

[2] *Instituto de Pesquisa e Desenvolvimento—IP&D, Universidade do Vale do Paraíba (UNIVAP), Avenida Shishima Hifumi 2911, 12244-000 São José dos Campos, SP, Brazil*

[3] *Instituto de Física de São Carlos, Universidade de São Paulo, Avenida Trabalhador São Carlense 400, 13566-590 São Carlos, SP, Brazil*

Correspondence should be addressed to Raduan Hage, raduan.dcir@epm.br

This study aimed to investigate the pharmacokinetics of a hematoporphyrin derivative in colonic tumors induced by dimethylhydrazine and adjacent normal colon in Wistar rats using an *in vivo* fluorescence spectroscopy technique. In conventional clinical application of photodynamic therapy, the interval between photosensitizer (PS) administration and lesion illumination is often standardized without taking into account variations due to the type or localization of the tumor and intrinsic differences in the microcirculation and vascular permeability of each target organ. The analysis of the fluorescence spectra was based on the intensity of porphyrin emission band centered at around 620 nm in normal colon and colon tumors. The photosensitizer fluorescence intensity rapidly grew for carcinoma and normal colon, reaching the maximum values 1 and 3 hours after PS injection, respectively. Data presented here allow us to verify that the best compromise between selectivity and drug concentration for colon carcinoma in rats took place in the interval between 1 to 4 h after PS injection.

1. Introduction

Photodynamic therapy (PDT) is an established modality for the treatment against some types of cancers and other nononcologic diseases. PDT involves the interaction of the photosensitizer with a visible light of a specific wavelength and oxygen. The therapeutic effect occurs during the absorption of light, energy by the photosensitizer leading to excited state, generating highly reactive oxygen species, which destroys cellular substrates and leads to cell death. The effects of PDT depend on the simultaneous presence of the PS, excitation light and oxygen [1, 2]. Although oxidative damage is one important component contributing to tumor therapy outcome with PDT, the effects on tumor vasculature interlinked with elicited host response also play a critical role [3].

The efficiency of PDT is directly related to several factors inherent to the target tissue (cellular and vascular structure of the tumor), photosensitizer (selectivity, solubility in lipid, and cellular placement), and parameters of irradiation, such as wavelength (nm), energy doses (J/cm^2), and intensity (W/cm^2) [4].

Selectivity of treatment is imparted by a combination of factors, including accumulation of photosensitizer (PS) by the target lesion and the time interval between the PS administration and lesion illumination [5–7]. In conventional clinical application of PDT, the interval between photosensitizer administration and lesion illumination is often standardized, without taking into account variations due to the type or localization of the tumor. In this form, tumors in the skin, liver, kidneys, intestine, and other organs are considered similar concerning PS accumulation or even its selectivity. Beside the intrinsic differences of each organ, there are still differences own to each type of tumor, associated to structural differences in the microcirculation and vascular permeability. The standardization of interval

between photosensitizer application and illumination for all tumor types is not appropriated due to the physiological dependence of the vascularization, mainly when a systemic administration of PS is considered [6].

Most of the experimental studies use the same interval between the administration of the FS and the lesion illumination taking into account only the PS used. Perhaps this is one of the reasons why different results are presented in the literature. In order to improve PDT, the study of temporal evolution of photosensitizer distribution should be done before planning PDT experiments involving treatment of tumor lesions [6].

Fluorescence spectroscopy has been performed extensively in animal models to evaluate the uptake of photosensitizing agents for photodynamic therapy [6, 8]. The method of *in vivo* fluorescence spectroscopy is a rapid and useful technique that may be readily applied to obtain information related to the pharmacokinetic behavior of fluorescent drugs [9, 10]. Although the absolute concentration of sensitizer is difficult to be established, fluorescent monitoring is suitable to follow the time variation for the accumulation of a specific PS after injection using the fluorescence peaks associated with this photosensitizer in several tissues [6]. One of the advantages of this technique over other types of fluorescence methods, such as fluorescence microscopy [5, 11, 12] and radiolabeling technique [13, 14], is the fact that the sacrifice of the animals for the measurements is not necessary, thus reducing the number of animals sacrificed to obtain a given amount of information [6, 15]. It also allows simultaneous multiple measurements of a site with optic fiber bundle delivery system and a charge-coupled device. Furthermore, it permits easy access to internal hollow organs by endoscopy [15].

In this study PS uptake measurements were carried out to evaluate the maximal tissue accumulation of the PS (Photogem) in dimethylhydrazine- (DMH-) induced colonic tumors and adjacent normal colon in Wistar rats using an *in vivo* fluorescence spectroscopy technique. This study shall provide information that can be used in future PDT investigations in this experimental animal colon tumor model.

2. Materials and Methods

2.1. Animals. The whole experiment was carried out in accordance with the guidelines of the Committee of Ethics in Research of Universidade Federal de São Paulo for animal care (Protocol number: 1033/05). The male Wistar rats, 180 g weight, received subcutaneous injections of 1,2-dimethylhydrazine (DMH-Aldrich Chemical Co., Milwaukee, WI, USA) on the dose of 65 mg/kg at weekly interval for five weeks for tumor induction. The animals were kept under adequate conditions and observed for 16 to 18 weeks, the time required for the development of intestinal tumors [16].

2.2. Photosensitizer. The PS chosen for this study is Photogem. This is a hematoporphyrin derivative, made by Photogem LLC Co. (Moscow, Russia). It is usually described

as chemically similar to Photofrin. Both Photogem and Photofrin are mixtures of monomers, dimers, and oligomers of hematoporphyrin derivatives and first-generation PSs. The 5 mg/mL stock solutions of Photogem in 20 mM phosphate-buffered saline, pH 7.4, were stored in the dark at 4°C [17–22].

2.3. Fluorescence System. Fluorescence collection was obtained using a commercial system (Spectral-Fluorescence System for Diagnosis of Malignant Tumors, "Spectr-Cluster," v. 2.05 m, Cluster Ltd., Moscow, Russia) composed of a Y-type optical fiber which delivers the laser light through a central fiber and collects the fluorescence from a target tissue using six fibers distributed around central one (integrant part of the system, by Cluster Ltd., Moscow, Russia) and a spectrometer (also built into the commercial system, by Cluster Ltd., Moscow, Russia) to collect fluorescence in the visible to near infrared range (540–850 nm).

The excitation light source was the 532 nm line of Nd:YAG laser with 10 mW of power, which is low enough to avoid thermal effects in the target tissue. This wavelength was chosen for fluorescence spectroscopy since it represents good cost-benefit commitment between light penetration in tissue and fluorescence excitation of porphyrins (although longer wavelengths would allow deeper evaluation, PS excitation would not be observed for those regions). The fluorescent light was filtered with standard optical filter placed at the spectrometer entrance, avoiding the influence of the fluorescence excitation. Figure 1 shows a schematic of the design, illustrating the components of the system.

2.4. Fluorescence Measurements. The animal was anesthetized with xylazine (5 mg/kg), ketamine (75 mg/kg), and butorphanol (1 mg/kg) injected intraperitoneal (i.p.). A midline laparotomy was performed, the site of the tumor was identified by palpation, and a longitudinal colostomy was carried out to 2 cm of distance of the opposite the tumor base. Photogem was administered at a dose of 2 mg/kg intravenously on the left caudal vein. The optical fiber was perpendicularly positioned on the surface, touching the tissue (Figure 2), in which fluorescence was evaluated at regular time intervals. For all the kinetics measurements, time $t = 0$ corresponds to the end of the injection. After the spectral acquisition of each point, the optical fiber tip was cleaned with physiological solution of 0.9% sodium chloride. Animals presented 4 colon tumors (2 tumors/animal), for each one, and fluorescence spectra were collected at three different positions. Each data point is the average of three individual measurements in close regions of the lesion or the normal tissue investigated. Such averaging is relevant to avoid eventual laser intensity fluctuations. The fluorescence from 540 to 800 nm was recorded in real time, and the specific emission bands were used to quantify the amount of PS present in the observed tissue. All the spectra were normalized using the maximum amplitude at the excitation wavelength, observed as a peak in the fluorescence graphs. Data collection and treatment for normal tissue fluorescence was performed likewise.
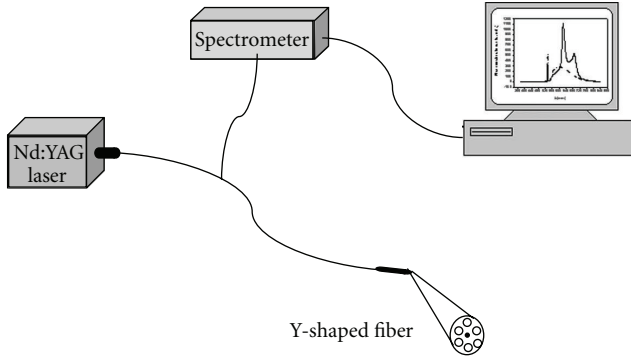
FIGURE 1: Schematic representation for the experimental setup.

FIGURE 2: Fluorescence spectra acquisition in dimethylhydrazine-induced murine colorectal carcinoma.
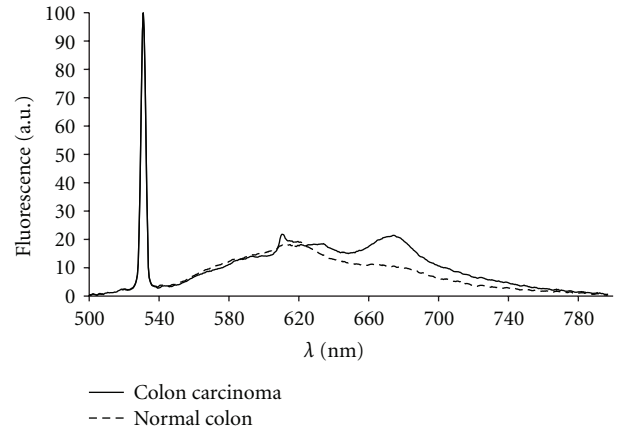

FIGURE 3: Typical fluorescence emission spectra from colon carcinoma and normal colon.

TABLE 1: The relative concentration of Photogem in colon carcinoma and normal colon according to fluorescence intensity at 622 nm as a function of time after PS administration is presented. Data is shown as the respective values and standard deviation (SD).

| Time (h) | Fluorescence signal ± SD | |
	Colon carcinoma	Normal colon
1	51.76 ± 7.65	28.29 ± 9.12
2	46.42 ± 11.51	34.36 ± 3.55
3	45.53 ± 5.11	36.46 ± 14.40
4	45.62 ± 3.24	33.70 ± 7.58
5	33.74 ± 4.91	29.59 ± 5.22
6	28.07 ± 7.84	27.95 ± 13.46
7	25.61 ± 5.16	25.92 ± 4.35
8	24.97 ± 7.49	23.77 ± 5.42

2.5. Histological Analysis. After fluorescence measurements, samples of the normal colon and colon carcinoma were collected and placed in 10% neutral buffered formalin for routine histology preparation and hematoxylin and eosin (H&E) staining for histological examination.

3. Results

Results in Figure 3 show typical autofluorescence (i.e., from nonsensitized tissue) spectra from colon carcinoma and normal colon. Two fluorescence peaks are observable, one at around 620 nm and another at around 680 nm, mainly in the colon carcinoma spectrum.

Figure 4 shows a temporal sequence of plots with colon carcinoma and normal colon spectrum obtained in different times after the PS administration. The presented spectra were normalized to the peak observed at excitation wavelength (as described), and the tissue autofluorescence previous to injection (as shown in Figure 3) was subtracted from the collected fluorescence signal during pharmacokinetics. Knowledge of the autofluorescence spectrum before injection allowed for subtraction of this component to obtain the fluorescence spectrum of PS at several time intervals after injection [15]. Table 1 shows the relative concentration of Photogem in colon carcinoma and normal colon according to fluorescence intensity at 622 nm as a function of time after PS administration.

In order to determine the time for accumulation in minutes ($T_{acc(min)}$) and for elimination (T_{elim}) of the photosensitizer, a simple exponential law for each regime was considered. Origin (version 8.0) software was used to fit experimental data. Fitting was performed using the equation

$$C = C_0 \left(1 - e^{-t/T_{acc}} \right) \tag{1}$$

for accumulation and

$$C = C_0 e^{-t/T_{elim}} \tag{2}$$

for elimination ((1) and (2)) in which C represents the concentration-related fluorescence signal, C_0 represents the initial concentration-related fluorescence signal, t represents time, and τ represents the accumulation (T_{acc}) or elimination (T_{elim}) rates. T_{acc} and T_{elim} were both obtained by the best fit of equations (Table 2). First of all we observe a selectivity effects; colon carcinoma has always an uptake higher than normal tissue, based in the best fitting (Figure 5).

In this study, *selectivity* is assumed to be defined as the PS fluorescence intensity of the colon carcinoma (FI_{carc}) plus the
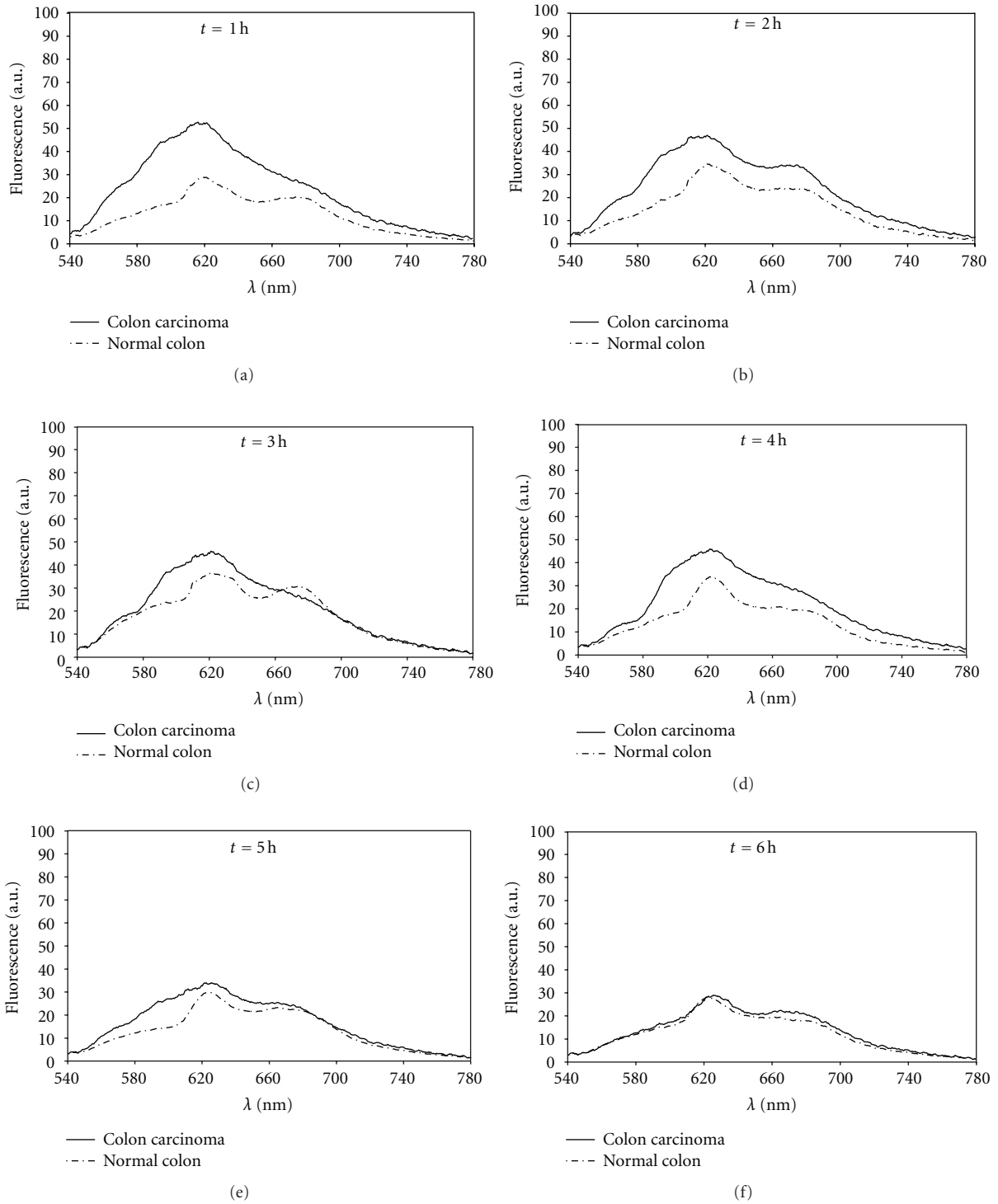
FIGURE 4: Temporal sequence of colon carcinoma and normal colon fluorescence spectra after intravenous injection of Photogem ((a) = 1 h; (b) = 2 h; (c) = 3 h; (d) = 4 h; (e) = 5 h; (f) = 6 h).

PS fluorescence intensity of normal colon (FI_{normal}), divided by FI_{normal}:

$$S = \frac{(FI_{carc} + FI_{normal})}{FI_{normal}}. \tag{3}$$

This parameter represents the ability to differentiate tumor tissue from normal tissue using fluorescence spectroscopy information. Selectivity values are shown in Table 3 and Figure 6.
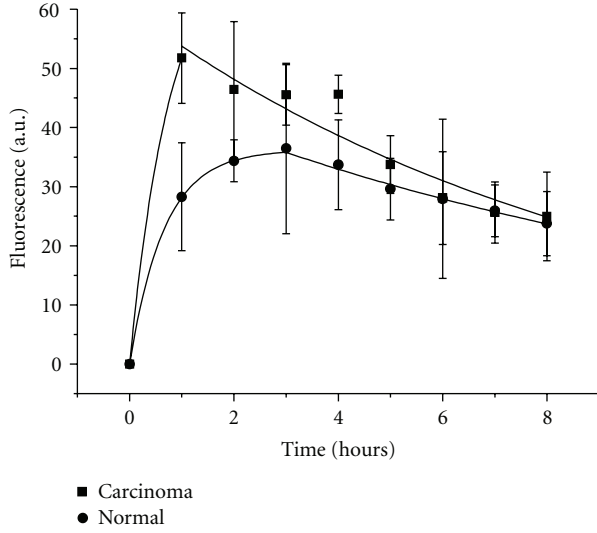
FIGURE 5: Fitting of the accumulative evolution as well as the elimination time evolution with the equations presented in the text.
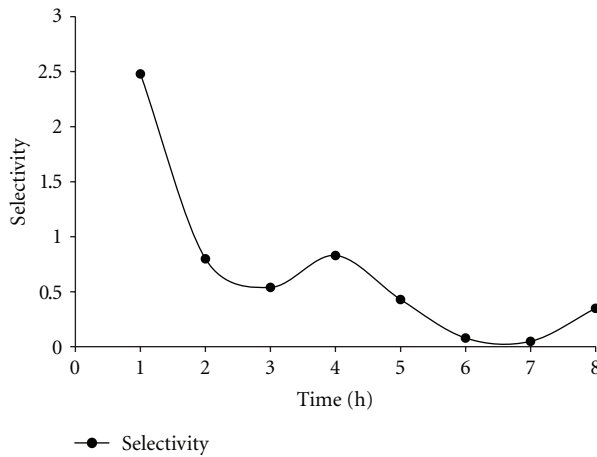

FIGURE 6: Fitting of the selectivity ratio between tumor and normal tissue.

TABLE 2: Time for accumulation (T_{acc}) and elimination (T_{elim}) of the photosensitizer obtained for the normal and colon carcinoma, presented both in minutes.

	$T_{acc(min)}$	T_{elim}
Normal colon	40 min	720 min
Colon carcinoma	47 min	540 min

TABLE 3: Selectivity values as a function of time.

Time (h)	Selectivity
1	2.48
2	0.80
3	0.54
4	0.83
5	0.43
6	0.08
7	0.05
8	0.35

4. Discussion

Fluorescence intensity of photosensitizer as a function of time after intravenous injection is reported for an experimental animal colon tumor model using *in vivo* fluorescence spectroscopy technique.

Fluorescence collection was used in this study as an evaluator of the PS concentration in tissue, since it has been observed in the literature that there is a correlation between PS fluorescence and concentration [19]. Presently, one is not able to obtain the exact amount of PS concentration from fluorescence spectroscopy measurements. Hence, it is assumed that the normalized fluorescence spectra can give one information about the actual PS concentration *in vivo*. Therefore, in this study, the information related to concentration is completely based on the fluorescence collection.

This study does not take into account variations on fluorescence collection due to tumor dynamics and/or growth rate, because the technique here used is not able to take those variations into account properly (it is not the main issue of the study, anyway, since it aims to an evaluation that can be easily performed in preclinical and clinical trials).

The fluorescence peaks observed in Figure 3 (which was collected before the PS administration) can be attributed to the presence of endogenous porphyrins, possibly related to either an altered metabolism of the cancer tissue or microbial synthesis [8, 23]. After photosensitizer injection the appearance of the emission bands associated with the presence of porphyrin is evident in all spectra, especially in the band centered around 620 nm, one of main fluorescence band of the Photogem [6, 24]. Therefore, the accumulation of Photogem was evaluated by the difference at the main peak at 622 nm. The amplitude of these emission peaks can be associated with relative porphyrin quantities present in the tissue, which are related to the fluorescence spectrum of the photosensitizer previous to injection. It cannot be, however, associated with absolute concentration, due to fluorescence distortions observed for porphyrins in tissue, which make difficult to extract information that can be associated with accurate concentration values [6].

Considering the additional amplitude of photosensitized tissue fluorescence as being proportional to the tissue concentration of photosensitizer, we observe that for tumor tissue the time of maximum accumulation is around 1 h and for the normal tissue 3 h (Table 1). Since the time variation of the photosensitizer fluorescence is the compromise between accumulation and clearance, we will consider that, initially, this compromise is dominated by the accumulation of photosensitizer and the long times behavior as dominated by elimination (Figure 5).

After determining the time of accumulation and elimination of PS for both carcinoma and normal colon, one could notice that both the accumulation and elimination time intervals of PS were reached faster in colon carcinoma than

in adjacent normal tissue, although the initial time of accumulation (T_{acc}) was similar (Table 2) for both. During the photosensitization of a tissue, there are many mechanisms taking place, which determine the observed times. The mechanism by which the PS is localized preferentially in cancerous tissue is still not well understood, but it is believed to be associated with the tumor characteristics including an increased number of low-density lipoprotein receptors, the presence of macrophages, low pH, large interstitial space, leaky vasculature, large amounts of newly synthesized collagen, and high lipid content [24, 25]. Additionally, tumors usually present a density of vessels that is higher than normal tissue. This fact contributes to an increase in the drug uptake rate, resulting in a faster accumulation in the tumor. Meanwhile, the considerable high amounts of immature collagen fibers that are present on the tumor allow the higher accumulation quantity. The photosensitizer is also predominantly retained in tumor stroma that is the main component of the collagen fibers [12, 25]. For this reason, it is believed that tumors can accumulate a higher amount of sensitizer, as observed through the presented measurements. The observation of almost twice more PS on the tumor than on the normal tissue after one hour is in clear agreement with this argument. On the other hand, the PS was eliminated slower from normal tissue than from carcinoma tissue, suggesting that the vascular network and the lymphatic system surrounding the tumor are the determinant factors of the elimination time [12, 25].

This result follows the same behavior described by Melo et al. [6] that observed the faster accumulation of Photogem in the liver (30 min) and kidney (40 min), while a slower one has been observed for skin, indicating that the clearance time between PS administration and performance of PDT is intimately correlated with the tissue being treated.

We verify that selectivity seems to be a monotonic function of time: a decrease is always observed (Table 3 and Figure 6). This behavior is an indication that the main contribution to selectivity arises from the accumulation mechanisms more than from elimination mechanisms.

The interval between photosensitizer administration and illumination is based on the time at which there is maximal difference between PS uptake in target tissue and surrounding normal tissue.

The initial interest in PDT was based on the selective retention of PS in tumors promoting selective tumor destruction [26]. Haematoporphyrin derivatives accumulation in a tissue is probably due to host factors, that is, vascular permeability and lymphatic clearance, coupled with its ability to dissociate from serum protein-binding sites and bind to cellular sites [24, 25].

In terms of measurement of PS concentration *in vivo*, there is clear evidence that the variation of drug concentration among subjects, and even the variation within tumor, can be very large [10]. Another important observation is the fact that Photogem is not a chemically pure compound but a mixture of purified fractions of hematoporphyrin derivate, oligomeric and monomeric porphyrins, and when in solution it tends to dimerize, and this type of reaction may perhaps contribute to a variance in fluorescence signal [6]. The

Photogem fluorescence intensity rapidly grew for both colon carcinoma and normal colon reaching the maximum values 1 and 3 hours after PS injection, respectively.

Another important point to be considered in the analysis is that tissue absorption of the excitation and fluorescence light is difficult to be avoided, since the excitation and the very fluorescence of the PS molecules are affected by the surrounding medium. Even using lesion autofluorescence previous to sensitization to account for those modifications, it would not avoid completely this effect, because the PS molecule own emission is modified in tissue. So, we have used only the fluorescence collected from the sensitized lesion, and such consideration may have an influence on the fluctuation observed for PS concentration on the collected data.

For Photofrin the maximal tumor selectivity ratio (2 : 1) between tumor and normal tissue has been found between 1 and 2 hours after intravenous injection for early-stage squamous cell carcinomas of the oral cavity, the upper aerodigestive tract, the esophagus, and the tracheobronchial tree [15], which is in accordance with the data from our present study for colon carcinoma. The maximum selectivity about 2.5 : 1 was reached after 1 hour of the Photogem injection (Table 3 and Figure 6).

The photosensitizer is also predominantly retained in tumor stroma, with no detectable difference between tumor mucosa and normal mucosa [26]. Ideally, the photosensitiser should be selectively retained within the tumor to be destroyed with minimal retention in the surrounding normal tissues. Such high selectivity, however, is not attained with currently used photosensitizers (haematoporphyrin derivative and its purified versions), and therefore damage to normal tissues has to be considered, although this is acceptable provided that safe healing occurs [26].

In order to eradicate a tumor, all malignant cells must be destroyed, mainly those in the area where the tumor is invading normal tissue. Apparently, the photosensitizer selectivity for tumor tissue, in this region, does not seem to offer many therapeutic benefits. The results may support evidence that, during PDT treatments, it would be necessary to use treatment parameters that kill at least some normal tissue to obtain complete eradication of a tumor [12].

The results reported in the present study show that the method of *in vivo* fluorescence spectroscopy showed to be an useful technique that may be readily applied to obtain information related to the pharmacokinetic behavior of fluorescent drugs in dimethylhydrazine- (DMH-) induced colonic tumors and adjacent normal colon, according to other studies that evaluated, by the same technique, the kinetics of HpD in organs such as kidney, liver [6], and skin [9].

Many investigators only compare the photosensitizer uptake in subcutaneously transplanted tumors with that in surrounding tissues (muscle and skin), and such results have little relevance to clinical practice for tumors of organs such as the colon [12]. In the present study, the PS uptake was carried out in an animal model of colon cancer therefore with the similar microenvironment is found in clinical practice.

In the studied interval, the drug concentration between tumor and normal tissue assumes a selectivity which is still

decreasing with time. Above 4 h, it seems that selectivity becomes independent of time. Also for the selected interval, the drug concentration on the tumor compared to the normal tissue is always above 70% higher, which has been considered appropriate to perform PDT.

As a final concern on the validity of the assumptions here made, it should be emphasized that this is an initial study. Evidence observed here is not conclusive, but allows estimating a good interval to the use of haematoporphyrin derivatives in rats for the treatment of colon carcinoma. Besides, improving fluorescence techniques with biochemical analyses might allow better understanding on the PS dynamics in normal and abnormal tissue. Performing those trials shall be the emphasis of future studies.

5. Conclusion

Pharmacokinetics monitoring has the determination of better time windows for the performance of PDT treatment as a main purpose. Data presented here is initial, and studies using larger experimental groups and evaluating biochemical information resulting form kinetics. However, this initial study evidences a best compromise between selectivity and drug concentration for colon carcinoma in rats and that this takes place during the interval between 1 to 4 h after PS injection.

Conflict of Interests

No competing financial interests exist.

Acknowledgments

References

[1] T. J. Dougherty, C. J. Gomer, B. W. Henderson et al., "Photodynamic therapy," *Journal of the National Cancer Institute*, vol. 90, no. 12, pp. 889–905, 1998.

[2] S. Pervaiz and M. Olivo, "Art and science of photodynamic therapy," *Clinical and Experimental Pharmacology and Physiology*, vol. 33, no. 5-6, pp. 551–556, 2006.

[3] M. Korbelik and I. Cecic, "Complement activation cascade and its regulation: relevance for the response of solid tumors to photodynamic therapy," *Journal of Photochemistry and Photobiology B*, vol. 93, no. 1, pp. 53–59, 2008.

[4] B. C. Wilson and M. S. Patterson, "The physics, biophysics and technology of photodynamic therapy," *Physics in Medicine and Biology*, vol. 53, no. 9, pp. R61–R109, 2008.

[5] S. A. Pahernik, M. Dellia, F. Berr, A. Tannapfel, C. H. Wittekind, and A. E. Goetz, "Distribution and pharrnacokinetics of Photofrin® in human bile duct cancer," *Journal of Photochemistry and Photobiology B*, vol. 47, pp. 58–62, 1998.

[6] C. A. S. Melo, C. Kurachi, C. Grecco, C. H. Sibata, O. Castro-e-Silva, and V. S. Bagnato, "Pharmacokinetics of Photogem using fluorescence monitoring in Wistar rats," *Journal of Photochemistry and Photobiology B*, vol. 73, no. 3, pp. 183–188, 2004.

[7] J. Stukavec, V. Duchac, L. Horak, and P. Pouckova, "Photodynamic therapy of human colorectal carcinoma cell line," *Photomedicine and Laser Surgery*, vol. 27, no. 1, pp. 107–110, 2009.

[8] N. Ramanujam, "Fluorescence spectroscopy of neoplastic and non-neoplastic tissues," *Neoplasia*, vol. 2, no. 1-2, pp. 89–117, 2000.

[9] J. C. Kennedy, P. Nadeau, Z. J. Petryka, R. H. Pottier, and G. Weagle, "Clearance times of porphyrin derivatives from mice as measured by in vivo fluorescence spectroscopy," *Photochemistry and Photobiology*, vol. 55, no. 5, pp. 729–734, 1992.

[10] C. Sheng, B. W. Pogue, E. Wang, J. E. Hutchins, and P. J. Hoopes, "Assessment of photosensitizer dosimetry and tissue damage assay for photodynamic therapy in advanced-stage tumors," *Photochemistry and Photobiology*, vol. 79, no. 6, pp. 520–525, 2004.

[11] M. V. Agrez, R. E. Wharen, and R. E. Anderson, "Hematoporphyrin derivative: quantitative uptake in dimethylhydrazine-induced murine colorectal carcinoma," *Journal of Surgical Oncology*, vol. 24, no. 3, pp. 173–176, 1983.

[12] H. Barr, P. Chatlani, C. J. Tralau, A. J. MacRobert, P. B. Boulos, and S. G. Bown, "Local eradication of rat colon cancer with photodynamic therapy: correlation of distribution of photosensitiser with biological effects in normal and tumour tissue," *Gut*, vol. 32, no. 5, pp. 517–523, 1991.

[13] R. B. Veenhuizen and F. A. Stewart, "The importance of fluence rate in photodynamic therapy: is there a parallel with ionizing radiation dose-rate effects?" *Radiotherapy and Oncology*, vol. 37, no. 2, pp. 131–135, 1995.

[14] P. Cramers, M. Ruevekamp, H. Oppelaar, O. Dalesio, P. Baas, and F. A. Stewart, "Foscan® uptake and tissue distribution in relation to photodynamic efficacy," *British Journal of Cancer*, vol. 88, no. 2, pp. 283–290, 2003.

[15] D. R. Braichotte, G. A. Wagnieres, R. Bays, P. Monnier, and H. E. Van den Bergh, "Clinical pharmacokinetic studies of photofrin by fluorescence spectroscopy in the oral cavity, the esophagus, and the bronchi," *Cancer*, vol. 75, no. 11, pp. 2768–2778, 1995.

[16] L. L. S. Laranjeira, M. O. Taha, A. Ferme, R. Lemos, and H. Plapler, "Localização de lesões tumorais induzidas pela 1,2-dimetilhidrazina e seu grau de atipia no cólon de ratos," *Acta Cirúrgica Brasileira*, vol. 13, no. 3, 1998.

[17] P. Company, Photogem, in Russian Pharmacopoeia. Ministry of Health of Russian Federation, 1999.

[18] A. F. Mironov, A. N. Nizhnik, and A. Y. Nockel, "Hematoporphyrin derivatives: an oligomeric composition study," *Journal of Photochemistry and Photobiology B*, vol. 4, no. 3, pp. 297–306, 1990.

[19] J. D. Vollet-Filho, P. F. C. Menezes, L. T. Moriyama et al., "Possibility for a full optical determination of photodynamic therapy outcome," *Journal of Applied Physics*, vol. 105, no. 10, Article ID 102038, 2009.

[20] J. Ferreira, C. Kurachi, C. A. S. Melo, L. T. Moriyama, S. Zucoloto, and V. S. Bagnato, "Necrosis Characteristics of Photodynamics Therapy in Normal Rat Liver," *Laser Physics*, vol. 14, no. 2, pp. 209–212, 2004.

[21] J. Ferreira, L. T. Moriyama, C. Kurachi et al., "Experimental determination of threshold dose in photodynamic therapy in normal rat liver," *Laser Physics Letters*, vol. 4, no. 6, pp. 469–475, 2007.

[22] K. T. Moesta, B. Ebert, T. Handke et al., "Protoporphyrin IX occurs naturally in colorectal cancers and their metastases," *Cancer Research*, vol. 61, no. 3, pp. 991–999, 2001.

[23] V. Kulvietis, E. Zakarevicius, J. Lapienis, G. Grazieliene, V. Zalgeviciene, and R. Rotomskis, "Accumulation of exogenous sensitizers in rat brain," *Acta Medica Lituanica*, vol. 14, pp. 219–224, 2007.

[24] P. J. Bugelski, C. W. Porter, and T. J. Dougherty, "Autoradiographic distribution of hematoporphyrin derivative in normal and tumor tissue of the mouse," *Cancer Research*, vol. 41, no. 11 I, pp. 4606–4612, 1981.

[25] H. Barr, S. G. Bown, N. Krasner, and P. B. Boulos, "Photodynamic therapy for colorectal disease," *International Journal of Colorectal Disease*, vol. 4, no. 1, pp. 15–19, 1989.

[26] S. G. Bown, "Photodynamic therapy to scientists and clinicians—one world or two?" *Journal of Photochemistry and Photobiology B*, vol. 6, no. 1-2, pp. 1–12, 1990.