Characterization of a New Photosensitizer (13²-Hydroxy- Bacteriopheophorbide-a Methylester) for Future Treatment of Ovarian Carcinoma (An Experimental Study)

M. S. Ismail

Department of Obstetrics and Gynecology, King Fahd University Hospital, King Faisel University, Al Khobar, KSA.

Abstract

The photosensitizer 13²-hydroxy bacteriopheophorbide-a methyl ester (13² OH- BPME) is characterized by a high absorption coefficient at the far red wavelength 750 nm and a good singlet oxygen quantum yield.

Methods & Results:

The pharmacokinetics of 13²-OH- BPME were studied in ovarian carcinoma on mice after iv administration of 7.8 µmole/kg body weight at different incubation intervals. The accumulated dye was chemically extracted from selected tissues and the concentrations were measured by absorption spectroscopy. The parenchymatous organs (liver, spleen and kidney) showed maximum 13²- OH- BPME concentrations after 2 hours incubation (liver, spleen), and 4 hours post injection (kidney). A high uptake was detected in the lung with maximum concentration at 2 hours. The malignant tissue accumulated high 13²- OH- BPME concentrations between 2-12 hours post injection with peaking at 8 hours. The 13²- OH- BPME concentrations in muscle tissue, representing the normal tumour surroundings, and in the skin were very low.

Conclusion

The results of our study suggest that PDT using 13²-OH-BPME could be effective at 8h post injection, where the tumour 13²- OH-BPME uptake is maximum and the muscle and skin uptake will be minimum.

Keywords

13²-OH- BPME, pharmacokinetics, ovarian carcinoma, spectroscopy

Introduction

Although hematoporphyrin derivative (HPD), as "first generation" photosensitizer, has been used in experimental PDT of tumours in animal and human for several years, it could not be considered an ideal photosensitizer and its drawbacks are well documented [(1)]. Therefore some "natural" photosensitizers like derivatives of chlorophyll, a (pheophorbid-a, pyropheophorbide-a, chlorine-6) and bacteriochlorophyll-a as "second generation" photosensitizers have been under investigations because of their advantageous photoproperties including relatively high photostability, high oxygen quantum yields and the good absorption capacities in the spectral range above 630 nm together with simple and inexpensive preparation. The development of photosensitizers with strong absorption around 700 nm offer the advantages of the optimum light penetration through the tissue at these wavelengths [(2)]. In this context, the investigated 13²-hydroxy-bacteriopheophorbide-a methyl ester (13²-OH-BPME) seems to be one of the promising compounds of the class of bacteriochlorophyll-a derivative. The photodynamic potential of of 13²-OH-BPME has been extensively described on the photophysical[(3)], as well as the cellular level [(4)].

Correspondence Address: Associate Prof. Dr. M. Samy Ismail, samyismael@hotmail.com.

The present paper deals with the different tissue pharmacokinetics biodistribution of 13²-OH-BPME using the method of absorption spectroscopy in order to define the optimum time for effective PDT according to the incubation time of the sensitizer.

Material & methods

Chemicals

The potential photosensitizer 13²-OH BPME was prepared according to Moser & his colleuges[4]. It is dissolved in Dulbeccos phosphate buffered saline (PBS), [Biochrom Serored, Berlin, Germany], with 1% Tween 80 [Sigma chemical, Germany] and sterilized by filtration before in vivo administration.

Animals and tumour model

B6D2F1 mice bearing ovarian carcinoma inoculated subcutaneously in the flank (0.1 ml tumour cell suspension) were used as animal model. After a steady tumour growth over one week, the tumour showed an average diameter of about 1 cm. Every experimental group consisted of 3 animals for each time interval.

Pharmacokinetic studies

13²-OH-BPME was administered iv at a dose of 5 mg/kg body weight, the incubation periods in darkness were 2h, 4h, 8h, 12h, 24h, 48h and 168h, then the animals were sacrificed and the tissues of different organs were stored in the gas phase of liquid nitrogen. The 13²-OH-BPME recovery was studied in tumour, lung, liver, spleen, kidney, muscle and skin.

Chemical extraction and spectroscopic evaluation

Tissue samples were thawed, weighed wet, refrozen and thoroughly homogenized in 1-2 ml Methanol/Acetone (1:1). The homogenates were centrifuged at 800-1000 for 10 min. The supernatants were centrifuged again at 1800-2000. Absorption spectra of the extracts were analysed spectrophotometrically (Perkin Elmer UV/Vis-Lambda 2) in the spectral range of 200-1000 nm. Concentrations were calculated using the absorption coefficient of λ (748 nm) = 15800 l./M-1 cm-1

Results

The data presented in Table 1 showed rapid accumulation of 13²-OH-BPME in parenchymatous organs. Relatively high accumulations rates were detected in the lung and maintained for 12h after administration of the sensitizer. The 13²-OH-BPME concentrations measured in the tumour revealed its maximum value after 8h post injection. A long time retention until 168h post injection was not detected in any of the investigated tissues since the concentrations was below the minimal detectable concentration of 0.023 nmol/g tissue. The muscle tissue which represented the surrounding healthy tissues for the tumour presented very low concentrations all through the incubation time. The concentrations in the skin showed its maximum concentration at 4h and its minimum concentration was at 8h post injection period. After 48h examination time no 13²-OH-BPME was detected in the skin. The maximum tumour-/ muscle ratio of 31 and tumour-/ skin ratio of 23 were detected at 8h after administration of the sensitizer.

Organ	Time						
	2h	4h	8h	12h	24h	48h	168h
Liver	20.2 ± 2.6	10 ± 1.3	8 ± 1.3	6.6 ± 1.3	1.3 ± 0.1	1.6 ± 1.9	<0.023
Spleen	4.9 ± 0.8	2.5 ± 0.16	2.4 ± 1.7	2.1 ± 0.3	<0.023	<0.023	<0.023
Lung	42 ± 17	21.5 ± 1.4	25.8 ± 6.8	22.7 ± 1.3	5.98 ± 3.7	0.7 ± 0.18	<0.023
Kidney	9.7 ± 1.2	11.5 ± 3.0	4.5 ± 0.7	2.5 ± 1.3	2.0 ± 0.8	0.4 ± 0.5	<0.023
Muscle	0.7 ± 0.8	0.3 ± 0.2	0.3 ± 0.3	0.2 ± 0.2	<0.023	0.05 ± 0.08	<0.023
Skin	0.6 ± 0.5	2.2 ± 1.7	0.4 ± 0.07	0.8 ± 0.1	0.7 ± 0.2	0.6 ± 0.2	<0.023
Tumour	5.4 ± 3.2	3.4 ± 1.2	9.5 ± 8.2	5.4 ± 1.8	1.6 ± 0.9	1.8 ± 0.4	<0.023

Table 1: 13²-OH BPME concentrations from different tissues of mice bearing ovarian carcinoma . The minimal detectable concentration was 0.023 nmol/g tissue. Mean values [nmol/g tissue] ± s.d -1 are given.

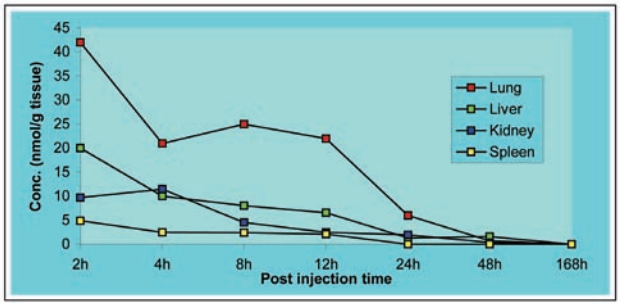

Fig. 1: 13²-OH-BPME concentrations in lung, liver, spleen and kidney at different post injection times.

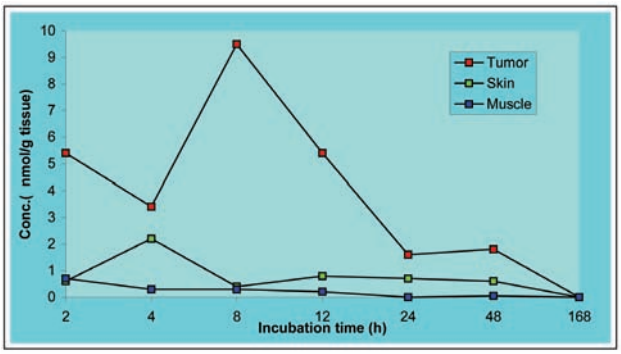

Fig. 2: 13²-OH-BPME concentrations in tumour, muscle and skin at different post injection times.

Discussion

The preferential uptake of the photosensitizer by the neoplastic tissue is an crucial parameter in PDT process. The pharmacokinetic of photosensitizer is a specific feature. PDT of course will be more effective if the light energy is applied when the concentration of the photosensitizer in the tumour tissue is higher than in the adjacent normal tissue [3-12]. Thus, it is important to quantify the photosensitizer concentration in normal and neoplastic tissues [12] in order to evaluate the biodistribution patterns, as well as the elimination rates in various tissues and organs [4], to improve the PDT planning.

The parenchymatous organs and the lung, unlike other normal tissues, accumulated very high concentrations of the photosensitizer. The data presented in this study show a high uptake of OH-BPME in liver, spleen, lung and kidney. These values remained high until 12 hours incubation time and decreased dramatically later on until 48 hours incubation time, after 48 hours OH-BPME cannot be detected except in the liver where a very minimal concentration detected. Also, muscle and skin show a low uptake during the whole incubation period. Considerable concentrations in the tumour tissue were recorded in this study with a maximum value of 9.5 nmol/g at 8 hours incubation time. After 48 hours no retention in the tumour tissue could be detected. A high tumour / muscle ratio of 31 and tumour / skin ratio of 23 after 8 hours were recorded. These high ratios resulted from high 13² OH-BPME uptake by the tumour compared with muscle and skin tissues. These high ratios might be an advantage of 13² OH-BPME in its application in the PDT procedure, as the muscle represents the normal surrounding tissues to the inoculated tumour and this high ratio during light application will leads to minimal surrounding tissue destruction. Also the high tumour/ skin ratio offers an advantage of minimising skin photosensitization side effects after therapy. In relation to the pheophorbide-a photosensitizer group, Yano et al., have recorded a pheophorbide-a tumour/ muscle ratio of 100 after 4 hours post injection [13]. Iwai and Kimura [12], compared the pharmacokinetics of pheophorbid-a and pheophorbide dimmer in mice bearing FM3A tumours and they recorded the time of maximum pheophorbide dimmer concentration between 9-12 hours and between 18-24 hours when pheoforbid-a was used. They also reported tumour-/ muscle ratios of more than 20 at this corresponding time for the both sensitizers. Röder et al [3]., investigated the pharmacokinetics of 13² OH- BPME in mice bearing Lewis lung carcinoma using the fluorescence spectroscopy as photosensitizer detection method and she suggested that PDT using 13² OH- BPME could be effective post sensitizer administration time at 12h [3]. Chan et al. [2] reported tumour / muscle- skin ratios for aluminium sulfonated phthalocyanines varying from 10 to 2. Richter et al. [5] reported a benzoporphyrin derivatives tumour/ muscle ratio of 4 and Reddi et al. [14], reported a ratio of 7.5 with zinc phthalocyanines between 18-24h post sensitizer administration.

Because of the high 13² OH-BPME tumour accumulation and the high tumour/ muscle-skin ratios recorded in the present study and in comparison with other studies, 13² OH-BPME might be considered a good candidate for its application in PDT.

Acknowledgements

Many thanks for Dr. C. Dressler for technical help in the present study and for professor H. P. Berlin. This study is a part of the program "Photodynamische Lasertherapie" granted by the Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie of the Federal Republic of Germany.

References

1. Bown S, Tralau C, Coleridge S, et al. Photodynamic therapy with porphyrin and phthalocyanine sensitisation: Quantitative studies in normal rat liver. *Br J Cancer* 1986; 54: 43-52.

2. Chan W, Marshall J, Svenson J. Effect of sulfonation on the cell and tissue distribution of the photosensitizer aluminium phthalocyanine. *Cancer Res.* 1990; 50: 4533-4538.

3. Röder B, Derssler C, Hagemann R, et al. On the pharmacokinetics of 13²-hxdroxy-bacteriopheophorbide-a methyl ester studied by fluorescence spectroscopy on Lewis lung carcinoma bearing mice. *SPIE* 1994; 2078: 427-437.

4. Moser J, Herchenbach B, Evenschor K, et al. Biotechnology of bacteriopheoforbides, naturally occurring 2nd. generation photosensitizers. *Laser in Med Sci* 1992; 7: 272.

5. Richter A. Characterisation of benzoporphyrin derivatives, a new photosensitizer *SPIE* 1988; 997: 132-138.

6. Basil J, Berlien H.-P. Basics of photodynamic therapy: In Applied Laser Medicine; 252 -288; Berlien H.-P. and Muller G., Springer Publischer, Berlin, Germany. 2003.

7. Ismail M S, and Phillip C. Laser in Gynaecology. In Applied Laser Medicine 2003; 346 -378; Berlien H.-P. and Muller G., Springer Publischer, Berlin, Germany.

8. Hornung R, Fehr M K, Walt H, et al. PEGm-THPC-mediated photodynamic effects on normal rat tissues. *Photochemistery and Photobiology*, 2000; 72 (5): 696 – 670.

9. Schlosser V, Koechli RO, Cattaneo R, et al. Phptodynamic effects in vitro in fresh gynaecological tumours analyzed with bioluminescence method. *Clinical and Laboratory medicine* 1999; 37 (2): 115 120.

10. Whaitcre CM, Feyes DK, Satoh T. et al. : Photodynamic therapy with phthalocyanine photosensetiser of SW 480 human colon cancer xenografts in athymic mice. *Cli Cancer Res* 2000; 6: 2021 – 2027.

11. Ahmed N and Mokhtar M: Mechanism of photodynamic therapy induced cell death. *Methods enzymology* 2000; 312: 342 – 385.

12. Iwai K and Kimura S. Efficiency of pheophorbide-dimmer in photodynamic therapy of mouse tumour. *J Clin Biochem Nutr* 1988; 5: 145-149.

13. Yano T, Uozumi T, Kawamoto K, et al. Photodynamic therapy for rat pituitary tumour in vitro and in vivo using pheoforbide-a and white light. *Laser in surgery and medicine* 1991; 11: 174-182.

14. Reddi E, Castro G, Biolo R, et al. Pharmacokinetic studies with zinc II phthalocyanine in tumour-bearing mice. *Br J Cancer* 1987; 56: 597-600.