Quantifying psoralen in tissues by fluorescence: dosimetry for PUVA to block restenosis.

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ABSTRACT

PUVA therapy may prove effective in preventing restenosis of vessels following balloon angioplasty to open vessels narrowed by atherosclerosis. The technique relies on the ability of PUVA (psoralen administration followed by ultraviolet A irradiation) to cause crosslinks and monoadducts that prevent cellular proliferation without causing cell death. Such PUVA treatment has been successful in controlling the cutaneous cell proliferation of psoriasis.

The efficacy of PUVA treatment depends on the drug concentration and the light dose. The amount of light delivered is easily modified to adapt to variations in the drug concentration if the drug levels in the vessel wall are known.

This paper demonstrates the feasibility of assaying psoralen levels in (1) tissues and (2) in serum samples using psoralen fluorescence as an indictor.

KEY WORDS: atherosclerosis, angioplasty, restenosis, psoralen, PUVA, fluorescence

1. INTRODUCTION

The concept of assaying psoralen levels using fluorescence was tested in two systems.

First, the ability to detect psoralen in tissues such as the media of a vessel wall was tested by a simple model: the subcutaneous muscle layer of the rat. Ultimately, the amount of psoralen in a tissue is the important parameter. Is it possible to measure psoralen fluorescence in vessel wall using the same optical fiber inserted for delivery of the UVA radiation which crosslinks the psoralen for therapeutic action?

Second, the ability to detect psoralen in serum samples was tested. The equilibration of psoralen between serum and tissues is thought to be rapid. Therefore, perhaps a convenient means of immediately assaying serum levels of psoralen would be useful during the PUVA therapy.

2. METHODS

Experiment 1: *in vitro* test in rat

Full-thickness skin samples were freshly obtained from the leg of a sacrificed rat (hairless *fuzzy* rat). The samples included the subcutaneous muscle layer. Each sample was cut into two identical pieces. One sample was placed in 81% phosphate buffered saline, 19% EtOH. The other was placed in same 81:19 saline/EtOH + 20 μg/ml psoralen. The above solutions were a simple 5-fold dilution of the stock obtained solution (100 μg/ml psoralen, 8MOP, in 95% EtOH). Tissues were soaked for about 1.5 hr.

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To assay the uptake of psoralen, the subcutaneous muscle layer was excited by 337 nm using a pulsed nitrogen laser which elicited fluorescence emission. The fluorescence was collected by the optical fiber catheter and detected by a spectral analyzer in the 400-800 nm range.

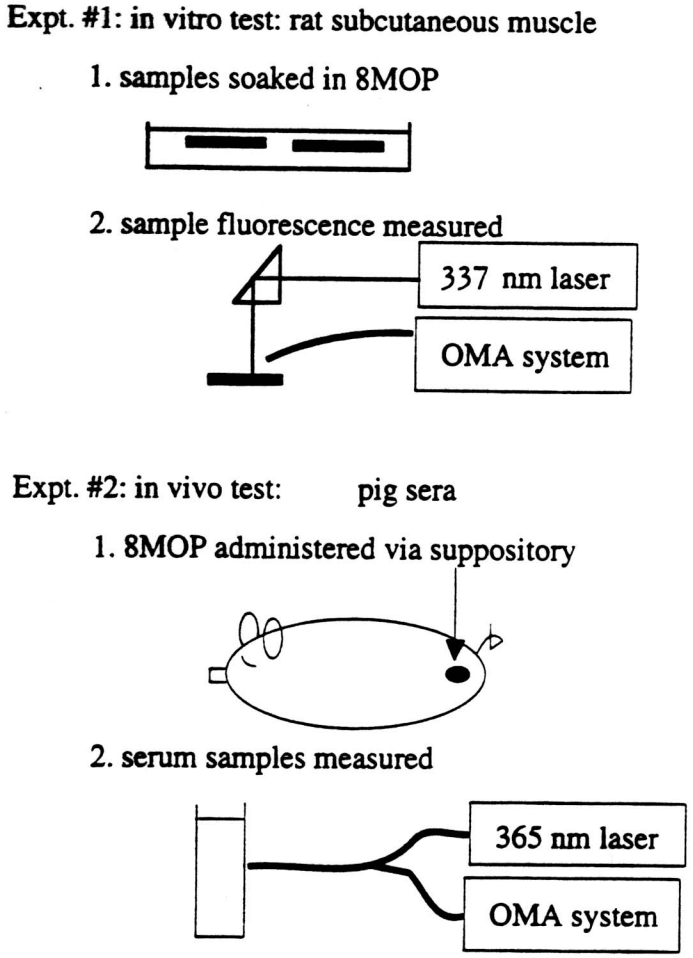

Figure 1: Experimental protocol. (A) In vitro test in rat subcutaneous muscle samples soaked in 8MOP solution. (B) In vitro test in sera obtained from pigs administered 8MOP via a rectal suppository.

Experiment 2: in vivo test in pig

Psoralen was delivered to a 27.2-kg pig by rectal suppository at a dose of 11 mg/kg (30 ml dose of 5 mg 8MOP per ml of polypropylene glycol, for a total of 300 mg 8MOP). Blood samples were drawn just prior to administration as a control, immediately after administration, and at 15, 30, 60, 90, and 120 min thereafter. The blood samples were allowed to clot, then were centrifuged to yield serum samples which were frozen for later analysis.

Later after thawing, 1-ml aliquots of the serum samples were diluted in water 1:2 to yield a 1/3 dilution. The diluted serum was placed in a quartz cuvette. The optical fiber fluorimeter delivered 337 nm light and collected fluorescence in the 400-800 nm range.

Additional testing involved additions of known amounts of 8MOP (stock solution was 5 mg/ml in polypropylene glycol) to either water or pig serum controls obtained prior to 8MOP administration.

Optical fiber fluorimeter

The fluorimeter system consists of a central fiber for delivery of the excitation light and 7 surrounding fibers for collection of fluorescence emission. The output spectra was dispersed by a spectrograph, detected by an Optical Multichannel Analyzer (OMA), and acquired via a GPIB port by a Macintosh computer.

Experiment 1 involved direct excitation of the samples by 337 nm excitation delivered via a 90° turning prism. The fluorescence was collected by the 7-fiber catheter of the fluorimeter system, with the catheter placed about 3 cm from the sample at ~30° off normal.

Experiment 2 involved delivery of 365-nm excitation light (308-nm XeCl excimer laser-pumped dye laser) via the central optical fiber of the catheter and collection of fluorescence emission with the 7-fiber catheter. The fiber was placed against the side of the quartz cuvette which held the serum sample.

3. RESULTS

Experiment 1

Figure 2A shows the original spectra (mean of 3 spectra) for samples ± 8MOP exposure. The autofluorescence of the tissue had a peak at about 460 nm. The 8MOP fluorescence spectrum was observable relative to the background autofluorescence of the tissue. Figure 2B shows the difference spectrum due to addition of 8MOP. The peak fluorescence was at about 500 nm.

Experiment 2

To illustrate the psoralen fluorescence, 0.6 μg/ml 8MOP was added to a 1/3 dilution of control pig serum (no prior 8MOP exposure). Figure 3A shows the original spectra for the sera ± 8MOP. Figure 3B shows the difference spectrum and again the peak fluorescence was at about 500 nm.

Figure 4 shows the calibration of the flourescence response. Known amounts of 8MOP were added to either water or to control pig sera. The fluorescence response was quite linear. The calibration curve (boxes) for the pig sera was used to interpret the experimental measurements of pig sera obtained at different time points following 8MOP administration.

Figure 5 shows the time course of 8MOP in the pig serum. The level of psoralen rose very rapidly after administration of the suppository and remained rather constant for at least 2 hr.

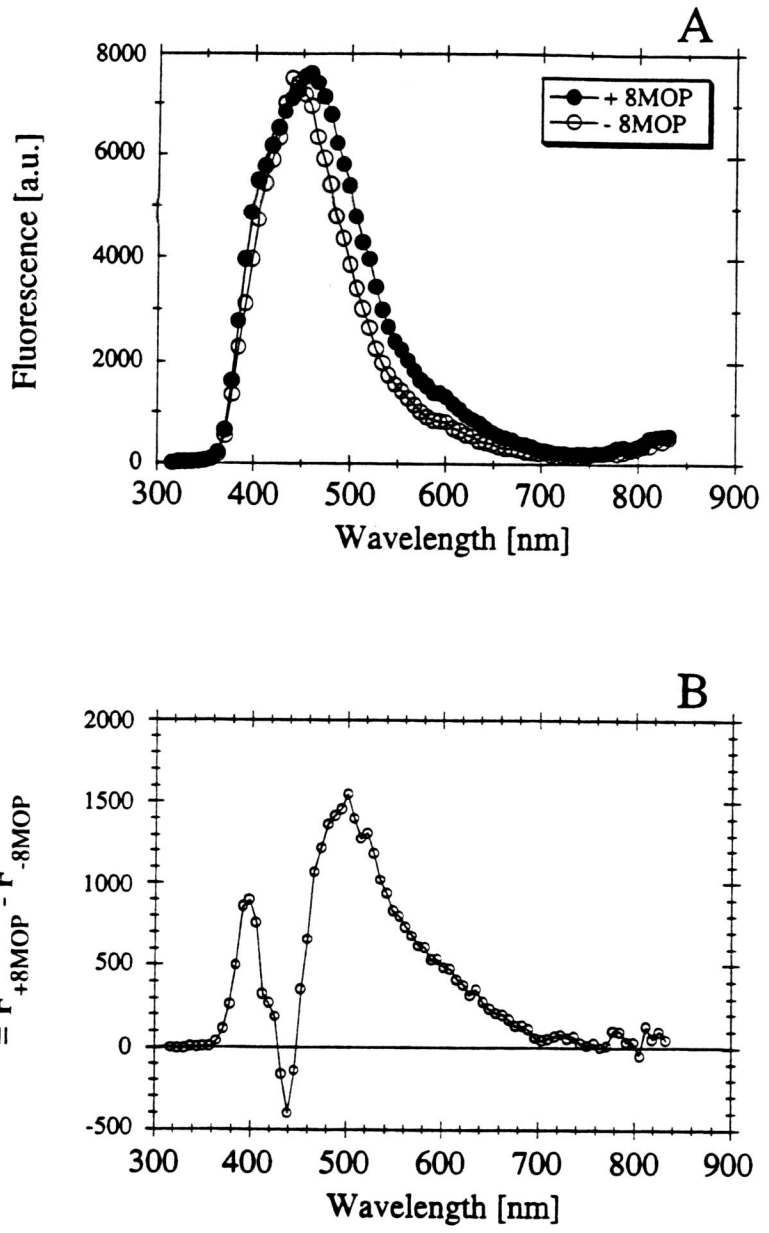

Figure 2: In vitro test on rat subcutaneous muscle layer. (A) Fluorescence spectra ± 8MOP. (B) Difference spectrum. (337-nm excitation)

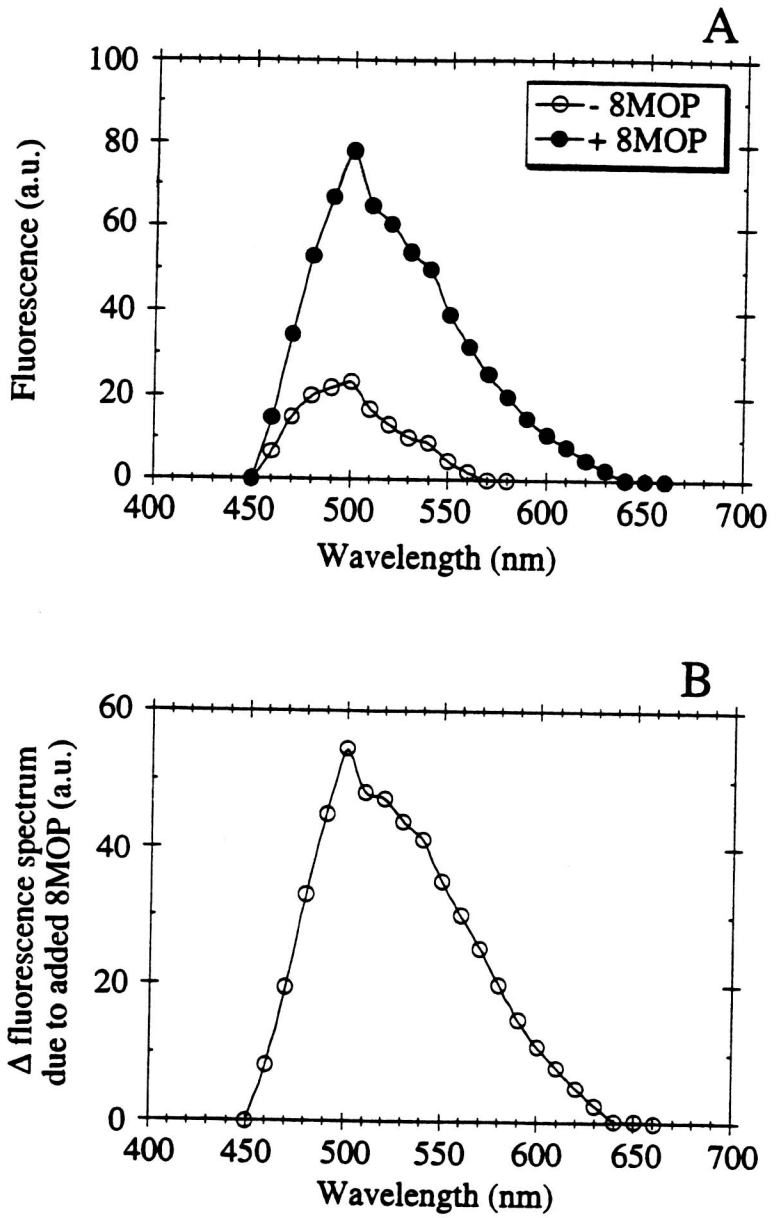

Figure 3: Incremental fluorescence spectrum of 8MOP in pig serum. (A) Serum autofluorescence ± 0.6 µg/ml 8MOP (serum:water = 1:3). (B) Difference spectrum. (365-nm excitation)

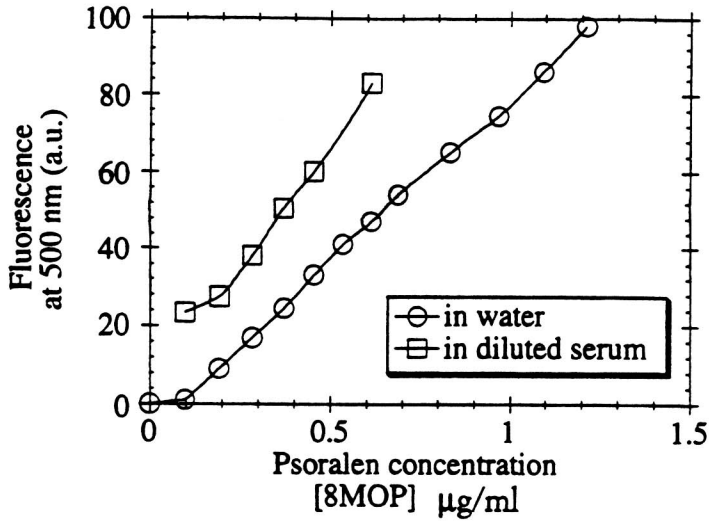

Fig. 4: Calibration curve. Assay for 8MOP was based on fluorescence peak at 500 nm (365-nm excitation). Additions of 8MOP to a water solution and a 1/3 dilution of pig serum were tested. Serum calibration curve [boxes] served as calibration curve for interpretation of pig serum experiment.

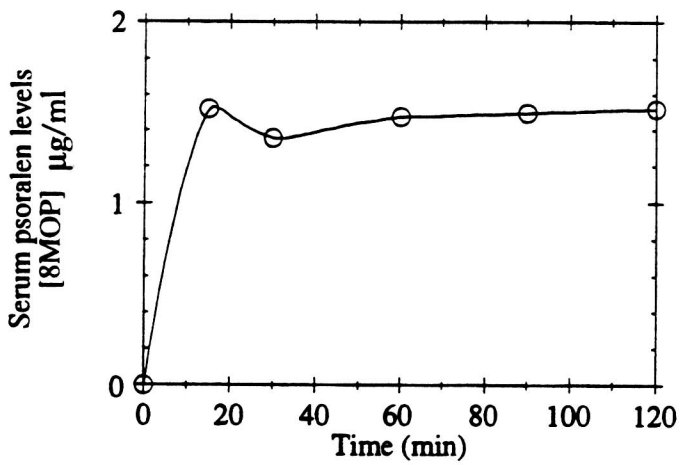

Fig. 5: Time course of 8MOP is pig serum following suppository delivery. The serum levels quickly rose to a stable value that persisted at least 2 hr.

4. CONCLUSION

The optical fiber fluorimeter provides a rapid convenient means of assaying the 8MOP levels in the serum.

(1) Futher work should be conducted on the calibration of the fluorescence response. We used 8MOP in a stock solution of polypropylene glycol (PPG) and did notice slight effects of the PPG on the fluorescence spectra, observed as a slight shift in the peak. The sensitivity of the assay

to the condition of the serum probably can be lessened by using a buffered solution rather than water to dilute the serum sample.

(2) The role of light scattering deserves more attention. The serum can appear more or less turbid, depending on its state. The choice of a standard 1-cm-thick cuvette versus a thin-walled cuvetted (eg., 1-mm thickness) can affect the geometry of excitation delivery and emission collection, which can influence the shape of the spectra.

Despite the above considerations, the assay appeared to be relatively trouble free. The technique promises a simple rapid assay of psoralen levels in serum samples.

The ability to measure psoralen levels directly in the vessel wall using the same optical fiber used for UVA irradiation appears to be feasible.

5. ACKNOWLEDGEMENTS

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