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A Method for Detecting 8-Methoxypsoralen in the Ocular Lens

Abstract. *The use of 8-methoxypsoralen for treating psoriasis could prove hazardous if this photosensitizing agent enters the ocular lens. Phosphorescence spectra of intact rat lenses reveal concentrations of 8-methoxypsoralen on the order of 10^{-5}M after intraperitoneal injection of 8-methoxypsoralen. There is evidence that this drug can function as a photosensitizing agent, enhancing ultraviolet-induced changes within the lens.*

Ambient ultraviolet radiation has been implicated as a factor in the increased fluorescence and pigmentation that develops in the nucleus of the aging human lens, and in the pathogenesis of the senile nuclear (brown) cataract (*1–3*). There is also the possibility that ultraviolet-induced lenticular change may be enhanced in the presence of photosensitizing drugs within this organ (*4–6*). The photochemical reactions of furocoumarin derivatives, particularly 8-methoxypsoralen (8-MOP) have recently received widespread attention (*7, 8*).

Since the introduction of psoralens as a method for treating vitiligo in 1947 (*9*) and since their recent use for treating psoriasis (*10*), there has been sporadic interest concerning the potential relation between psoralens and cataract formation. Some reports have suggested the experimental production of cataracts in animals given very large doses of 8-MOP and exposed to long-wave ultraviolet radiation (*4*). However, the presence of 8-MOP has never been demonstrated in the lenses of experimental animals or in human lenses.

The introduction and increasing use of 8-MOP as a major therapeutic drug for psoriasis (*10*) could prove to be a significant hazard if this drug enters the lens, and an even more significant hazard if it accumulates with repeated dosage. There is some evidence that 8-MOP and other photosensitizing agents may bind to specific macromolecules such as DNA and certain proteins, thereby enhancing their photosensitizing action (*7, 11*). It should be noted that, because the lens is completely encapsulated and never sheds any of its cells, the possibility exists that 8-MOP may not only enter the lens but also may become bound to one or more of the macromolecules within the lens and thus accumulate with repeated therapy. Hence, 8-MOP could then accumulate and exert its photosensitizing effect over a long period of time.

It is generally accepted that the photochemical action of psoralen in the skin involves cyclo addition to pyrimidine bases in DNA and light-induced interstrand cross-linking of DNA molecules (*7, 11, 12*). A similar mechanism has been proposed for damage by 8-MOP to the photosensitized lens (*6*). We now describe a method for detecting 8-MOP in the lenses of experimental rats and demonstrate that this drug does enter the lens when administered in doses approximating those given to patients with psoriasis.

We used our own laboratory bred strain of Sprague-Dawley rats, which were put in the dark after 4 weeks of age and maintained in these conditions throughout the experimental period. These animals were given a single (0.1 ml) intraperitoneal injection of 8-MOP in dimethyl sulfoxide (DMSO) (4 to 6 mg per kilogram of body weight) or a recrystallized suspension (0.2 ml) of 8-MOP in

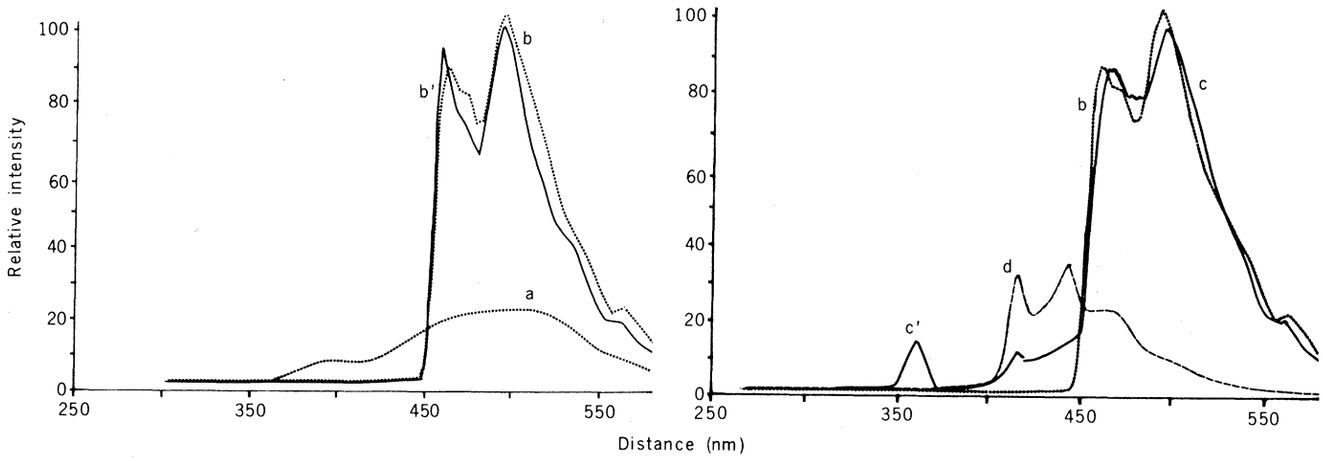

Fig. 1 (left). Fluorescence spectrum (a, ·····) of 8-MOP at 25°C. Instrument sensitivity 10×. Phosphorescence spectra of $10^{-3}M$ 8-MOP in ethylene glycol (b, ·····) and in ethanol (b′, ———) at 77°K. Instrument sensitivity 1×. Fig. 2 (right). Phosphorescence spectra of $10^{-3}M$ 8-MOP in ethylene glycol (b, ·····) and whole rat lens showing 8-MOP (c, ———) and tryptophan (d, -------) phosphorescence at 77°K. Excitation peak at 360 nm (c′). The instrument sensitivity for 8-MOP phosphorescence in lens (c) was 100× as compared to 1× sensitivity for $10^{-3}M$ 8-MOP in ethylene glycol (b). The lens tryptophan phosphorescence (d) was also obtained with 1× sensitivity and 290 nm excitation.

physiological saline. The control rats (also kept in the dark) were given a single dose (0.1 ml) of DMSO or saline alone. The animals were killed 2½ hours after injection, and the lenses were immediately removed, gently rinsed with physiological saline, and analyzed by fluorescence and phosphorescence spectroscopy.

The apparatus used for fluorescence analyses was arranged as follows. Light from a xenon arc lamp (1000 watts) passed through a quartz condensing lens, a 10-cm water filter, a Bausch & Lomb high-intensity ultraviolet grating monochromator, and a second quartz lens before impinging on the rat lens. The excitation monochromator slits were set as follows: entrance 1.7 mm, exit 1.0 mm, and in some experiments a 365-nm or a 295-nm interference filter was interposed between the monochromator and the sample. Emitted light was detected at right angles to the excitation beam, after it passed through a grating monochromator (bandpass, about 50 Å), by an RCA 1P-28 photomultiplier tube, the output being recorded on an X-Y recorder.

Phosphorescence spectra were recorded with the same apparatus, except that the sample was maintained at 77°K by immersing the sample tube in a Dewar flask filled with liquid nitrogen during the experiment. In such experiments, fluorescence and scattered light were eliminated by a rotating shutter. Typical spectra and equipment for both the fluorescence and phosphorescence have been described (*2, 3, 13*).

The fluorescence spectra were measured at room temperature in quartz cuvettes with the lens immersed in Ringer solution. The Ringer solution was removed before the lens was frozen to 77°K for the phosphorescence spectra. The 8-MOP was obtained from Sigma Chemical Company and fluorescence and phosphorescence spectra were measured for $10^{-3}M$ solutions of 8-MOP in ethylene glycol or ethanol. (8-MOP is very insoluble in water, and in order to obtain $10^{-3}M$ concentration it was necessary to use organic solvents.) 8-MOP has ultraviolet absorption peaks at 248 and 305 nm, a relatively weak fluorescence emission at approximately 520 to 530 nm (at room temperature), and a strong phosphorescence emission at 14° and 77°K (*7*) (Fig. 1).

In order to detect 8-MOP in the lens by phosphorescence spectroscopy, it is important that these lenses not contain any significant amounts of fluorescent material emitting in the 440 nm region (*2*). We have noted that lenses with such fluorescent material give an ill-defined phosphorescence emission that lies in approximately the same region as that of 8-MOP and interferes with detection of the latter phosphorescence. This problem can be overcome by the use of rats that have been kept in the dark after being weaned (4 weeks of age). The lenses from such animals do not display fluorescence at 440 nm, whereas littermates kept in ambient light develop this fluorescence as they age.

Phosphorescence studies of whole rat lenses showed the characteristic tryptophan emission spectrum when excited at 290 nm and an emission characteristic of 8-MOP when excited at 360 nm (Fig. 2). The 8-MOP phosphorescence emission from the whole lens (Fig. 2) was obtained with the use of an instrument with sensitivity 100 times greater than that used for the corresponding spectrum from $10^{-3}M$ 8-MOP in ethylene glycol (Fig. 2). Our instrument gain is linear within 10 percent, and the excitation beam diameter is small compared to the sample size. Thus, the sample volume illuminated in the lenses and in the standard solutions was nearly the same. From these data we can estimate (within a factor of 2 to 3) that the concentration of 8-MOP in the lenses of our experimental animals was on the order of $10^{-5}M$. If there are chemical components in the lens capable of quenching 8-MOP phosphorescence, the phosphorescence intensity would be reduced, and the true concentration of 8-MOP would necessarily be even higher than the value of $10^{-5}M$, which we estimated after the animals were given a single intraperitoneal injection. When rats were given ten times the dosage of 8-MOP intraperitoneally, there was a corresponding tenfold increase in the intensity of the 8-MOP phosphorescence emission. The control lenses that received DMSO alone showed no fluorescence emission in the 440 nm region and no detectable phosphorescence when excited at 360 nm. In order to determine whether 8-MOP had penetrated beyond the lens periphery, the capsules were removed from several lenses after phosphorescence spectroscopy and the measurement was repeated. The intensity of 8-MOP phosphorescence in these samples was 70 to 80 percent of that observed in the intact lens, an indication that most of the 8-MOP was present in the nuclear and inner cortical regions.

We have observed that, when 8-MOP injected rats are subjected to ultraviolet radiation (in vivo), there is a loss of the characteristic 8-MOP phosphorescence emission as compared with lenses from rats kept in the dark. These results indicate that 8-MOP undergoes photochemical changes within the lens; however, the nature of these changes remains to be elucidated. We have also noted that there is an increase in the intensity of fluorescence emission at 440 nm in lenses from rats injected with 8-MOP and exposed to ambient light for 1 or 2 days. An even greater enhancement of fluorescence emission at 440 nm was observed when the animals were subjected to ultraviolet radiation for 1 or 2 days after injection with 8-MOP. The above results are similar to our in vitro lens irradiation experiments with 8-MOP photosensitization (*5*) and indicate that (i) 8-MOP functions as a photosensitizer in the lens in vivo and (ii) is capable of enhancing ultraviolet-induced lenticular changes.

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