Concentration of $^3$H-8-methoxypsoralen and its metabolites in the rat lens and eye after a single oral administration

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Concentrations of $^3$H-8-methoxypsoralen (MOP), its lipid- and water-soluble metabolites, tritiated water, and an insoluble compound have been measured in rat serum, lens, and eye tissue by liquid scintillation, thin-layer chromatography, and other techniques. The radioactivity has been measured in albino and pigmented rats up to 1 week after medication, and $^3$H-8-MOP and metabolite concentrations have been measured in pigmented rats from 10 min to 24 hr after oral administration by stomach tube of a solution of $^3$H-8-MOP (1 mg/kg body weight). The animals were kept in the dark. The maximum $^3$H-8-MOP concentrations were seen 10 min after medication in all the organs examined. The concentrations (in μg/kg) at 10 min, 2 hr, and 24 hr, respectively, after medication were: serum, 698, 55, and 0.8; lens, 11.3, 5.2, and 1.4; other eye tissue, 393, 23, and 2.5. Water soluble metabolites might accumulate in the lens, but the absolute concentration is small. There was no accumulation of metabolites in the other organs. The considerable amount of radioactivity still present in tissues 1 week after a single medication was a result of the formation of tritiated water from the degraded $^3$H-8-MOP.

Key words: 8-methoxypsoralen, metabolites, rat eye, rat lens, pharmacokinetics

In experimental animals, treatment with large doses of 8-methoxypsoralen (MOP) and long-wave ultraviolet light (UVA) can produce cataract.1,2 Patients who are 8-MOP and UVA (PUVA)-treated for psoriasis normally protect their eyes during and after the treatment with UV-protective goggles, and there has not been any publication reporting cataract in such patients.

Autoradiographic studies in rat eyes have revealed a higher binding capacity in pigmented rats compared with that in albino rats after medication with $^3$H-8-MOP.3 Because measurements of the 8-MOP concentrations in the lens and eye have been few and metabolite concentrations have not been estimated, we decided to examine these under conditions near to the human situation. Animals were treated with 1 mg of $^3$H-8-MOP/kg and were kept in the dark.

In this study, radioactivity in lens and eye was compared in albino and pigmented rats. The pharmacokinetics of $^3$H-8-MOP in pigmented rats kept in the dark were evaluated by measuring the concentrations of $^3$H-8-MOP, its lipid- and water-soluble metabolites (LM and WM, respectively), an insoluble compound (IC), and tritiated water ($^3$H$_2$O). The same compounds were measured in serum to check earlier measurements in serum.4

Method

$^3$H-8-MOP (337 mCi/mmol) was kept in a water solution by 15% Cremophor EL. No cold 8-MOP...
was added. A more detailed description of this and the stability of the solution has been previously described.

**Measurement of radioactivity.** Ten pigmented DA rats with a mean weight of 96.4 gm and seven albino Sprague-Dawley rats with a mean weight of 96.6 gm were medicated orally with 0.5 ml of $^3$H-8-MOP solution via a stomach tube, a dose of approximately 1 mg/kg (150 $\mu$Ci per animal). The rats were killed 10 and 30 min, 1, 2, 4, 8, and 24 hr, and 2, 4, and 7 days after administration of $^3$H-8-MOP. The last albino rat was killed 24 hr after administration.

Serum was prepared from whole blood obtained by heart puncture. The eyes from both strains were dissected and the lenses were removed. The remaining eye tissue, including vitreous but without aqueous humor, was treated as a single sample. The samples (lens, $\sim$ 24 mg; eye, $\sim$ 35 mg, and serum, 60 $\mu$l) were dissolved in Soluene --350 (Ambac Industries, Inc., Morton’s Grove, Ill.), and the radioactivity was counted by liquid scintillation counting (LSC). The standard deviation of the counts was less than 1.5%. The counting efficiency for tritium was 25.6%.

**Measurement of $^3$H-8-MOP and LM.** The technique has been described in detail previously. Recovery of $^3$H-8-MOP added to serum in vitro was 95.5% ± 0.6% (mean and S.E. M., n = 4) for the whole procedure. Recovery of added $^3$H-8-MOP from the lens was 93.7% ± 1.0% (n = 4) and from the eye 92.8% ± 0.7% (n = 4). For these measurements, 40 pigmented rats were medicated orally with 1 mg of $^3$H-8-MOP/kg.

**Fig. 1.** Radioactivity (log dpm/ml) in serum from pigmented rats. The abscissa shows time after a single oral medication with 1 mg/kg. Each point is the mean of two measurements on tissue from one animal.

**Fig. 2.** Time course of the relative distribution of $^3$H-8-MOP, LM, and WM present in the radioactivity measured in serum from pigmented rats (Fig. 1). The remainder (to 100%) is largely caused by $^3$H$_2$O (not shown). Each point is the mean of five measurements on serum from five rats. Vertical bars indicate standard deviation. Where bars are not shown, the S.D. is smaller than the symbol used to indicate the point.

**Fig. 3.** Absolute concentrations of $^3$H-8-MOP in serum from pigmented rats, calculated from the curves in Figs. 1 and 2.

Measurement of IC. The radioactivity in each sample glass after removal of extraction solvents represents the IC.

Results

Serum. The radioactivity for each sample time (Fig. 1) comprises $^3$H-8-MOP, LM, WM, $^3$H$_2$O, and IC. The relative distribution between these categories is shown in Fig. 2. The proportion of $^3$H$_2$O is not shown. The value of IC was constant at 1.09% ± 0.16% (n = 40). The measured radioactivity 1 week after medication was still 21% of the value at 2 hr after medication.

The concentration time-course of $^3$H-8-MOP was calculated from the data in Figs. 1 and 2 and is shown in Fig. 3. Practically no $^3$H-8-MOP was left in serum 24 hr after medication (0.8 µg/L of serum).

Lens. Radioactivity in the lens was generally a little higher in the pigmented rats than that in the albino rats. Fig. 4 shows that the initial rise in radioactivity took place over 6 to 8 hr. The radioactivity 1 week after medication was still 35% of that 2 hr after medication. The distribution of $^3$H-8-MOP and its metabolites is shown in Fig. 5. The percentage of radioactivity in IC was independent of time-after-medication (1.73% ± 0.35%; n = 8). The maximum 8-MOP concentration (11.3 µg/kg) was seen 10 min after medication (Fig. 6).

Eye. The maximum radioactive concentrations in the eye were measured 10 min after medication. The concentration was about 50% higher in the pigmented rat than that in the albino rat, with levels at 10 min after medication as high as 125%. The concentration time-curve showed a minimum roughly 2 hr after medication when radioactivity was accumulating in the lens. One week after medication the radioactivity was 31% of that 2 hr after medication.

The relative concentrations of $^3$H-8-MOP and its metabolites are shown in Fig. 7. The amount of radioactivity in IC was independent of the time-after-medication (2.68% ± 1.00%; n = 8). The maximum $^3$H-8-MOP concentration measured was 393 µg/kg of tissue 10 min after medication.

Fig. 4. Radioactivity in the lens and the rest of the eye in pigmented and albino rats after oral administration of $^3$H-8-MOP. Each point is the mean of two measurements.

Fig. 5. Time course of the relative distribution of radioactivity in the lens of pigmented rats (Fig. 4). Apart from $^3$H-8-MOP and its WM and LM, $^3$H$_2$O is also present, which brings the total to near 100%. Each point is the result of a measurement on a pool of tissue from five animals.
Discussion

Comments on sensitivity and specificity have been given previously.4

Radioactivity is present in the animals more than 1 week after medication; the "stable" radioactivity is caused by tritiated water, which has a long biological life.

Serum. The $^3\text{H}$-8-MOP concentration with a peak 10 min after medication is practically identical to our earlier results,4 except for the very low concentrations (<10 μg of 8-MOP/L) where the values are within a factor of 2 to 3. The same factor seems to be valid for the lowest concentrations of LM and WM.

Lens. Animal experiments have shown that 8-MOP and UVA can produce cataract.1,2 When patient-related PUVA doses were given to albino guinea pigs in a long-term study, no ophthalmoscopic, slit-lamp, or histologic manifestations of ocular injury were seen.5

Autoradiography has not given any suggestion of accumulation of $^3\text{H}$-8-MOP and metabolites in the lens measured as radioactivity after removal of $^3\text{H}_2\text{O}.3$ The slow increase in radioactive concentrations in the lens during the first 8 hr (Fig. 4) seems to be at the expense of concentration in the rest of the eye, presumably by diffusion. This results in a very low maximum $^3\text{H}$-8-MOP concentration in the lens (11 μg/kg). Because the $^3\text{H}$-8-MOP concentration in the lens is much lower than that in the eye, diffusion from the lens is slow, leading to nearly equal levels for eye and lens after 24 hr. The very low $^3\text{H}$-8-MOP concentration we have found in the lens is within a factor of 2 to 3 when compared with that in serum conditions.

Fig. 5 shows that there is a risk of accumulation of WM. The risk of accumulation is, however, low in the rat if medication takes place within the normal therapeutic frequency of every second day, since the absolute concentration of WM is low.

We think that our measurements might be considered comparable to the human situation, since we found equal serum concentrations in rat and man.

Lerman and Borkman6 have found concentrations of 2160 μg of 8-MOP/kg in the rat lens 2½ hr after intraperitoneal administration of 4 to 6 mg of 8-MOP (dissolved in dimethylsulfoxide)/kg. Twenty-four hours after such treatment little or no 8-MOP could be found in the lens by fluorescence and phosphorescence spectroscopy. As in our study, these rats were kept in the dark.7 These
findings are not comparable to our measurements because of the higher dose used and the different administration route. Twelve hours after medication with 0.75 mg of 8-MOP/kg, the concentration in one human lens was about 22 μg/kg as measured by the same technique. This result is in good agreement with our measurements in rat lens.

**Eye.** Pigmented spots in retina have been seen previously in pigmented animals fed Ammi Majus seeds (8-MOP) and exposed to sunlight but not in albino animals given therapeutic doses. In our experiment the pigmented rat eyes show higher radioactive concentrations than those in the albino rat eyes. Part of the explanation may be the high concentration of radioactivity in the pigmented layer in the uvea/retina of pigmented rats, which was previously demonstrated. Even though this layer may only represent a few cells, the extremely high concentrations here may be enough to affect the mean value for the whole eye. There does not seem to be any evidence of accumulation of 3H-8-MOP or its metabolites in the eye (Figs. 6 and 7).

After treatment of rats with high doses of 8-MOP (about 8 mg/kg orally or intraperitoneally) followed by UVA, specific binding has been demonstrated in the lenses and skin.

When we UV-irradiated (~10 J/cm²) rats that had been medicated as in this investigation, the radioactivity rose by about 15% not only in the irradiated organs but also in internal organs. The proportions of the radioactivity caused by 3H-8-MOP and the metabolites were the same both in irradiated and nonirradiated organs. We have thus not been able to demonstrate a specific binding caused by UVA with low 8-MOP concentrations. A reasonable explanation for the generally higher radioactivity might be an enhanced absorption from the intestine.

Provided that PUVA-treated patients protect their eyes from UV-light during treatment, we think that this investigation with rats kept in the dark accurately reflects the human situation, taking both the medication dose and the use of protective goggles into consideration.

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**REFERENCES**