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DISTRIBUTION OF TRITIUM-LABELLED 8-METHOXYPSORALEN IN THE RAT, STUDIED BY WHOLE BODY AUTORADIOGRAPHY

Hans Christian Wulf[1] and James Hart[2]

[1]*Department of Dermatology, The Finsen Institute, Copenhagen, and* [2]*Department of Biology, Leo Pharmaceutical Products, Denmark*

Abstract. The distribution of ^{3}H-8-methoxypsoralen has been studied in rats by whole body autoradiography. The highest organ concentration were seen 1 hour after dosing. The most pronounced accumulation was found centrilobular in the liver, corticomedullary in the kidneys, and in the cortex of the adrenals. The concentrations in these organs were approximately six times higher than in the blood. Other organs showed concentrations similar to blood levels. Illumination with UVA (10 J/cm^2) increased the concentrations in the subcutis.

Key words: 8-MOP; Ultraviolet light; Autoradiography; Organ distribution; Rat experiments

Psoriasis has been treated for several years by using a combination of long wave ultraviolet light (UVA) and 8-methoxypsoralen (8-MOP), (PUVA treatment) (19). 8-MOP has also been used for many years in the treatment of vitiligo (12, 25).

The toxicology and mutagenicity of this compound has recently been reviewed (24), but less is known of its pharmacokinetics and organ distribution (20).

We have earlier described how 8-MOP is distributed in the rat eye (30). In the present article we describe the organ distribution of tritium-labelled 8-MOP in albino and pigmented rats using whole body autoradiography.

METHODS

Tritium-labelled 8-MOP (spec.act. 378 mCi/mmol) was obtained from the Radiochemical Centre, Amersham, England, dissolved in benzene. The 8-MOP was transferred into an aqueous solution of 20% Cremophor EL® after evaporation of the benzene with N_2.

The radiochemical purity of the solution was checked by extraction with benzene, followed by chromatography on Silica gel (benzene/acetone 9:1). Only one spot could be detected by examination of the plate under UV light (254 & 366 nm) and by autoradiography.

Five adult male brown Norwegian rats weighing about 160 g and 2 adult male Wistar rats (Wistar/Af/Han/Mol/(han 67)) weighing 130 g were used in this study. The animals were fasted overnight before dosing with ^{3}H-8-MOP.

The compound was administered orally by gastric gavage, or intravenously via the tail vein (without anaesthesia). The animals were killed at the time intervals shown in Table I.

Two of the pigmented rats were shaven without use of chemicals and irradiated with 10 J/cm^2 UVA 1 hour after administration of 8 mg/kg, and killed 2 and 4 hours after dosing.

The animals were prepared for whole body autoradiography using Ullberg's technique (28). The animals were killed by ether anaesthesia, placed on a microtome stage, embedded in an aqueous gel of carboxymethylcellulose, and frozen in a mixture of solid carbon dioxide and hexane. Sagittal sections 20 μm thick were cut onto 3M 810 tape.

Some additional sections were cut onto 3M 688 tape, fixed in buffered formalin, and the sections suspended in successive 30 ml portions of ethanol for 30 min (8-MOP is soluble in ethanol). 2 ml aliquots of the ethanol washes were taken for liquid scintillation counting, and washing was continued until less than 5% of the initial levels were obtained (10 washes).

The sections were then freeze-dried and applied in the dark to Agfa Osray M-3 film. The films were exposed at −20°C for times ranging from 20 to 260 days after which the tape and sections were removed, and the films developed. The sections were retained for comparison with the developed autoradiograms. Sections from an animal dosed with unlabelled 8-MOP were processed similarly to exclude the presence of positive or negative chemography.

Semi-quantitative measurements of the relative organ concentrations were estimated using a densitometer (5). The degree of blackening on the autoradiogram produced by the radioactivity within an organ was compared with the blackening produced by a standard tritium "staircase" exposed on the same film.

Organ concentrations were estimated relative to blood concentrations, measured within the heart.

RESULTS

Fig. 1 is an autoradiogram of a pigmented rat 1 hour after oral administration of ^{3}H-8-MOP. High con-

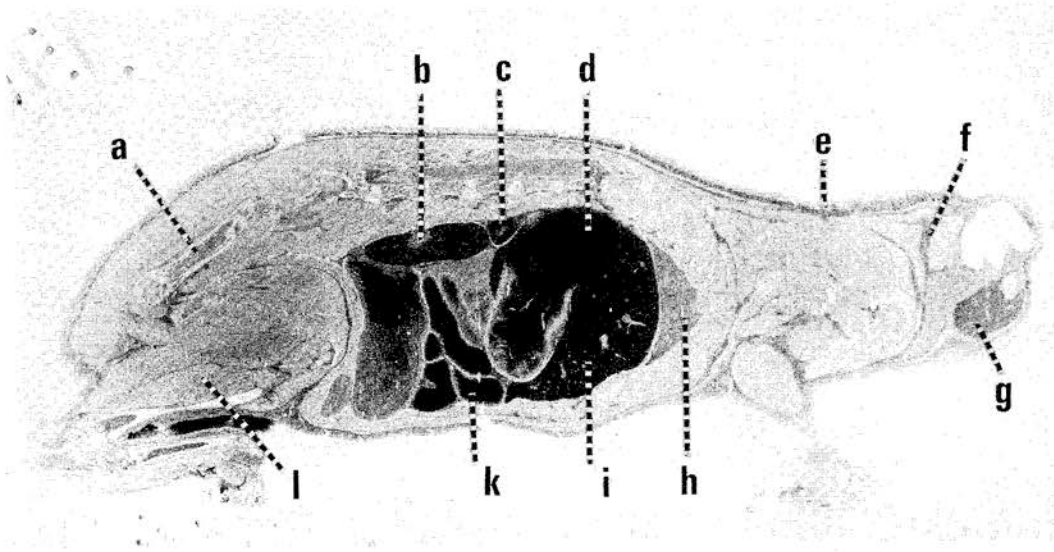

Fig. 1. Autoradiogram of a pigmented rat 2 hours after oral administration of ^{3}H-8-MOP and 1 hour after illumination with UVA-light (exposure time 274 d) (×1.2). Black grains represent radioactivity. *a*, bone marrow; *b*, kidney; *c*, adrenal; *d*, stomach; *e*, skin; *f*, connective tissue; *g*, salivary gland; *h*, lung; *i*, liver; *k*, intestine; *l*, muscle.

centrations of radioactivity can be seen in the liver, kidney, adrenal cortex, the stomach and the intestines. A qualitatively similar picture is seen in pigmented and albino rats 2 hours after administration, although the highest concentrations of radioactivity are now present in the small intestine, some have reached the caecum, and little remains in the stomach.

Four hours after oral administration, most of the radioactivity has reached the caecum.

Organ concentrations were highest in animals killed after 1 hour (Table II). UVA had no effect on the general distribution.

The distribution 1 hour after intravenous administration was very similar to the distribution after oral administration, with the exception of lower amounts of radioactivity present in the gut contents.

Accumulation within the liver was unevenly distributed. There was a centrilobular accumulation of

Table I. *Schedule of treatment*

Strain	^{3}H-8-MOP (mg/kg)	Route of administration	Killed after (hours)	UVA J/cm² 1 h after 8-MOP
Albino	3	Intravenous	1	0
Albino	3	Oral	2	0
Pigmented	3	Oral	1	0
	3	Oral	2	0
	3	Oral	4	0
Pigmented	8	Oral	2	10
	8	Oral	4	10

Table II. *Concentration of ^{3}H-8-MOP in rat organs 1, 2, and 4 h after peroral administration relative to the blood concentration 4 h after administration (=1)*

The figures in parentheses show the organ concentrations relative to the corresponding blood concentrations

Organ	1 h	2 h	4 h
Blood	4 (1)	1.5 (1)	1
Brain	2 (0.5)	0.5 (0.5)	0.5
Lung	6 (1.5)	2 (1)	1
Liver			
Centrilobular	24 (6)	12 (8)	10
Peripheral	12 (3)	6 (4)	3.5
Adrenal			
Cortex	12 (3)	6 (4)	6
Medulla	–	2 (1)	1.5
Kidney			
Cortex	16 (4)	6 (4)	3
Corticomedullary region	20 (5)	8 (6)	5
Pelvis-urine	–	18 (12)	–
Spleen	3 (0.5)	2 (1)	0.5
Testis	2 (0.5)	1.5 (1)	0.5
Muscle	3 (0.5)	0.5 (0.5)	0.5
Skin	6 (1.5)	1 (0.5)	1

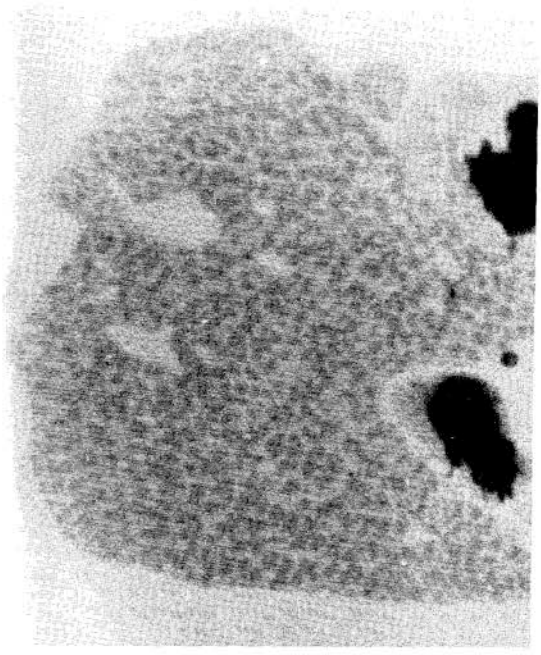

Fig. 2. Autoradiogram of the liver 2 hours after oral administration of ^{3}H-8-MOP. Note the spotted appearance. The dark spots (high concentrations) are centrilobulary arranged (×3).

radioactivity (Fig. 2), which was most evident after 4 hours. Centrilobular concentrations are estimated to be three times the concentrations in the rest of the liver, and up to 10× blood concentrations (Table II).

Sections from this animal were washed with ethanol in an attempt to extract unchanged ^{3}H-8-MOP. The centrilobular accumulation was even more evident, although it was not possible to estimate relative concentrations, since the washing procedure impaired the quality of the sections.

Some evidence of biliary excretion could be deduced from the presence of radioactivity in the intestinal contents after intravenous administration, although the apparent amounts were not great. There was no evidence of accumulation in bile ducts in the liver.

Kidney

The highest concentrations in the kidney were seen in the corticomedullary region (6× blood level) and in the pelvis (up to 12× blood concentration) (Fig. 3, Table II).

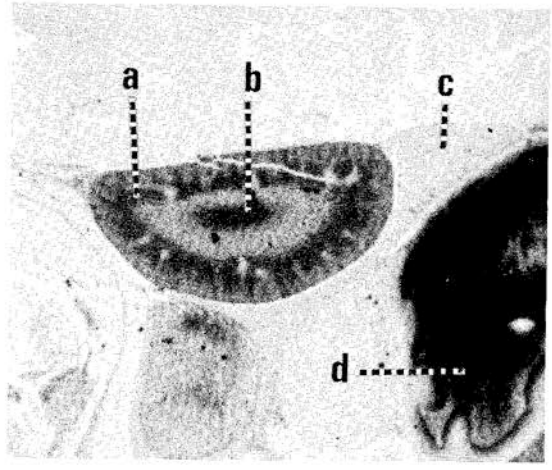

Fig. 3. Autoradiogram of the kidney 2 hours after oral administration of ^{3}H-8-MOP (×5). Black grains represent radioactivity. High concentration is seen in: *a*, corticomedullary region; *b*, pelvis; *c*, spleen; *d*, stomach.

Adrenal

Radioactivity was accumulated in the adrenal cortex (Fig. 1, Table II). Four hours after administration, cortex concentrations were six times higher than blood and roughly four times medullary concentrations. Ethanol-extracted sections retain considerable concentrations in the cortex.

Eye and associated organs

Distribution in the rat eye has been described in detail elsewhere (30). In both albino and pigmented rats low concentrations of radioactivity were seen in the cornea, and lower concentrations in the lens. High concentrations were present in the melanin layer in pigmented rats. Virtually no radioactivity was present in other structures within the eye. This distribution was not affected by illumination with 10 J/cm^{2} UVA.

Skin

Both albino and pigmented rats showed accumulation of radioactivity in the hair follicles and in connective tissue (Fig. 4*a* and 4*b*). Accumulation in the hair follicles was more evident in dorsal than in ventral skin. Distribution throughout the different skin layers was relatively even, and there was no suggestion of higher concentrations in the epidermis. There was an apparent increase in the amount of radioactivity in the subcutis after illumination with UVA (Fig. 4*b*).

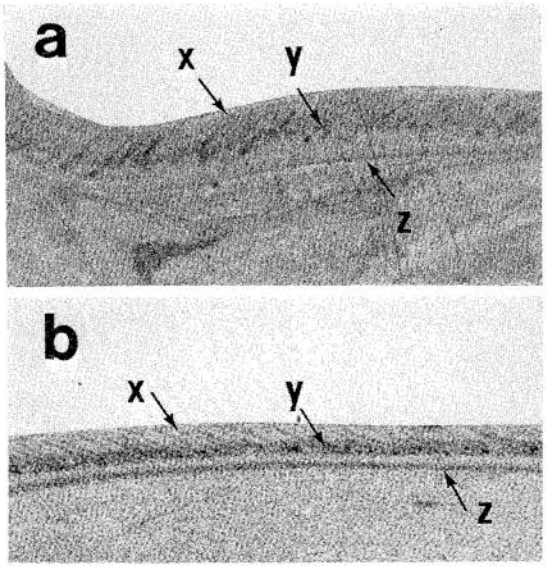

Fig. 4a. Autoradiogram of rat skin 2 hours after oral administration of 8-MOP (×6). Equal distribution can be seen through the skin. Black grains in the subcutis correspond to higher concentrations around the hair follicles.
Fig. 4b Autoradiogram of pigmented rat skin 2 hours after oral administration of ^{3}H-8-MOP and 1 hour after UVA illumination (×6). Note the increased concentration in subcutis compared with 4*a*. The highest concentrations are seen around the hair follicles. *x*, epidermis–dermis; *y*, subcutis; *z*, connective tissue.

Other organs

Radioactivity was relatively evenly distributed throughout the remaining organs. Concentrations in the brain, muscles and testes were half that of blood (Table II). The concentrations in bone marrow, spleen, pancreas, lymph glands, salivary glands and fat were close to the blood level.

DISCUSSION

8-MOP has been used for many years in the treatment of vitiligo, and in combination with UVA in the treatment of psoriasis (PUVA). 8-MOP alone is usually described as atoxic (17, 23), but dose-dependant inhibition of cell division has been described (31). If this is applicable in vivo, then a study of the distribution of 8-MOP will show which organs contain the highest concentrations and might therefore be most at risk.

Pathak et al. (21) have reported that the distribution of 8-MOP was very similar in different organs, but another article (20) describes particularly high concentrations in mouse liver, skin and blood, and very low concentrations in other organs.

We have examined the distribution in the rat up to 4 hours after oral administration of 8-MOP by whole body autoradiography. It is not possible to distinguish between 8-MOP and any possible metabolites containing the tritium label when using this technique.

The total radioactivity present in the rat organs has been estimated semiquantitatively (Table II). Results of a quantitative study will be reported shortly. The findings in the various organs and the possible toxicological implications are discussed below.

Liver

Accumulation within the liver is of particular interest, since 8-MOP is metabolised in the liver with the formation of hydroxides or glucuronide conjugates (20, 21). The relatively high concentrations (up to 10 times blood level) seen in this study emphasize the importance of this organ.

Increase in se-ASAT and se-ALAT have been described in individual patients treated with PUVA (6, 26, 29). These increases were seen only in patients with daily alcohol consumption. However, Juhlin (14) has described increased serum liver enzyme concentrations in 2 patients who were not exposed to any other liver toxic compounds. The numbers of cases reported a few, and Labby et al. (15) found no liver toxic effects after administering psoralen to healthy students. High se-ASAT levels (present before PUVA therapy was started) have been reported to fall when alcohol consumption was reduced during treatment (27).

Melski et al. (19) found no increase in mean se-ASAT levels in a group of 1308 patients treated with PUVA, but they do not discuss the possibility that individual cases may have reacted as the patients described above.

An increase in se-ASAT was found in geese treated with PUVA, but not when psoralen is given alone (10). The author suggests that this could be due to the formation of a toxin during PUVA treatment, although none has been demonstrated. We have seen no effect of UVA on the distribution of radioactivity in our animals. If any toxin is produced, then it must either be present in very small amounts, or its formation must involve the loss of the tritium atom.

The uneven distribution of radioactivity within the liver could suggest a localized binding of 8-MOP or its metabolites. Fixing of sections in formalin, followed by extensive washing in ethanol, would be expected to remove both any free 8-MOP as well as any free polar metabolites. Whilst there was some loss of radioactivity from the liver, the same centrilobular accumulation was still present in these sections. A similar distribution has been seen for non-extractable chloroform metabolites in the liver (1) as well as for other liver toxic compounds. Carbon tetrachloride also shows centrilobular accumulation (2) and is known to produce centrilobular necrosis.

Zachariae et al. (32) carried out a preliminary study on liver biopsies taken from patients before and after 1 year of treatment with PUVA. The histological change seen was a possible slight fibrosis in occasional cases.

All these reports would seem to suggest that 8-MOP can have a slight liver toxic effect, which may be additive in the presence of other compounds. No serious cases of liver toxicity have been described, and the incidence of liver complications must be low.

Biliary excretion of 8-MOP was examined after administration of 8-MOP intravenously. Rats have no gall bladder, and it is difficult to estimate biliary excretion from intestinal concentrations after oral administration. Relatively little radioactivity was seen in the intestinal contents after intravenous administration, and there was no sign of high radioactive concentrations in bile ducts within the liver. Biliary excretion is unlikely to be a major route of elimination.

Kidney

The highest radioactive concentrations within the kidney were found in the corticomedullary region, and in the urine in the pelvis. 8-MOP is excreted predominantly either hydroxylated or as glucuronides (20). The urine concentrations (12 times blood level, Table II) can be compared with the result of Pathak et al. (21) of 80% excretion in the first 8 hours after administration, and 90% after 12 hours. In man, only 60% is excreted in the urine in 24 hours (18), and this suggests the possibility of accumulation.

In a clinical study on 1308 PUVA-treated patients (19), an increase in the mean serum creatinine level was seen after treatment, although this was not significant at the 5% level ($p<0.1$).

Adrenal

There is considerable accumulation of radioactivity in the adrenal cortex, which cannot be removed by washing in buffered formalin and ethanol. The accumulation was similar in pigmented and albino rats. No accumulation was seen in the hypophysis. It is difficult to evaluate the significance of this accumulation. No changes in 17-keto or 17-ketogenic steroid excretion have been seen in PUVA-treated patients, or after administration of psoralen alone (11).

Skin

There are a number of differences between human and rat skin which are important when relating these results in rats to man. Rat epidermis is proportionately thinner than that of man (33), and this means that more UVA can penetrate the deeper layers, when the animals are shaved. Melanocytes are present in both pigmented and albino animals, though only the former contain melanin (16). Unlike man, the melanocytes are mostly concentrated around the hair follicles, and only few are present in the skin as such (22). Pigment formation is largely complete within the first 3 weeks of life, and tyrosinase activities in adult pigmented rat skin are as low as in albinos (4). Rats have no sweat glands but sebaceous glands are present around the hair follicles.

The distribution of radioactivity in the skin of both albino and pigmented rats is very similar. Skin concentrations are comparable to blood levels, but higher concentrations are present in the loose connective tissue layers under the skin and in the hair follicles, particularly in dorsal regions.

The lack of difference between albino and pigmented rats is surprising in view of the melanin binding found in the rat eye (30). It is possible that the compound has an affinity for the melanocytes around the hair follicles, irrespective of their pigmentation. Pigment is often seen to spread from around hair follicles in patients treated for vitiligo (13, 25). Accumulation in other parts of the hair follicle cannot be excluded. The resolution of whole body sections is inadequate to distinguish microscopic details.

UVA treatment produces an apparent increase in concentrations in the subcutis. It is possible that

this is a result of stronger binding, resulting from the cross-linking of 8-MOP and DNA after UVA (3, 7, 8). This cross-linking is thought to be responsible for the reduced cell turnover in psoriasis and for the photosensitizing effect (8). The subcutis accumulation is seen after a high, single 8-MOP and light dose. The dose is so high that patients can only tolerate this after intensive therapy with increased pigmentation of the skin.

Testis

Concentrations in the testis were half those of blood. Whilst no effect on motility or the number of abnormal spermatozoa is seen in man after PUVA treatment (Dahl, personal communication), massive changes have been seen in toads and in rats (9), with decreased spermatogenesis, thickening of the tunica albuginea and increased cholesterol in the Leydig cells.

Other organs

Concentrations within other organs were very similar to blood levels. Side effects of PUVA treatment such as nausea, headache and dizziness are seen after treatment with many other compounds, and a study of the distribution of 8-MOP is not likely to contribute to an understanding of these side effects.

CONCLUSION

The results show that in the rat three organs, the liver, the kidney, and adrenals are exposed to high concentrations of 8-MOP after oral administration.

Whilst there may be differences of distribution between man and rat, it would seem advisable to check patients treated with PUVA for side effects produced in these organs, as is already the case for oculotoxic effects.

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H. Chr. Wulf, M.D.
Dermatologisk afdeling
Finseninstitutet
Strandboulevarden 49
D-2100 Copenhagen