use of solid phase radioimmunoassay (Ausria II and Ausab, Abbott Diagnostic Division).

Liver membrane antibody (LMA) and anti-nuclear antibody (ANA) were determined as previously described (2).

## RESULTS

The number of immunoglobulin-bearing cells in each liver section was compared with the histological findings and whether or not MTX was given, as depicted in Table I. More immunoglobulin-bearing cells were seen among the patients with non-specific reactive hepatitis and cirrhosis than among those with normal liver histology. The number of immunoglobulin-bearing cells in the liver sections from patients with histological abnormalities other than hepatitis or cirrhosis was no different from the number in patients with normal liver histology. The number of immunoglobulin-bearing cells did not vary between the group of patients with lymphocytic infiltrates in the liver biopsies and the group with no infiltrates.

Results of comparisons between the duration of the disease and liver abnormalities as determined by histology, immunoglobulin-bearing cells, serum immunoglobulin levels, transaminase and alkaline phosphatase levels are presented in Table II. No correlations were found between the disease duration and any of these parameters.

HBsAg was not found in any of the patients, while anti-Hbs was present in one patient. LMA was not found in any of the sera. Two female patients aged 49 and 76 years had antinuclear antibodies in high titres. The younger of the two had hepatitis, and steatosis was found in the liver biopsy of the other patient.

## DISCUSSION

The few patients in this study with hepatitis and cirrhosis had a greater number of fluorescent cells in the liver than any others group of patients, as opposed to previously published findings (1).

MTX treatment of psoriatics with normal or moderately abnormal liver histology did not influence the number or type of immunoglobulin-bearing cells in the liver. The lack of correlation between lymphocytic infiltrates in the liver biopsies and MTX therapy also indicates that liver damage associated with MTX therapy of psoriasis might be due to other than immunological mechanisms. This in addition to the fact that there was no difference in the number of immunoglobulin-bearing cells among those patients with normal and those with moderately abnormal liver histology suggests that the determination of the number of immunoglobulin-bearing cells is not a useful procedure in detecting liver damage associated with MTX therapy.

## ACKNOWLEDGEMENT

This work was supported by grants from the Ebba Celinder Foundation.

## REFERENCES

1. Aldershvile, J., Dietrichson, O., Hardt, F. & Nielsen, J. O.: Circulating T and B lymphocytes and immunoglobulin containing cells in the liver in chronic active liver disease. Acta Pathol Microbiol Scand [C] *85:* 26, 1977.
2. Manthorpe, R., Permin, H. & Tage-Jensen, U.: Autoantibodies in Sjögren's syndrome. Scand J Rheumatol *8:* 168, 1979.
3. Nyfors, A.: Methotrexate therapy of psoriasis. Effect and side effects with particular reference to hepatic changes. A survey. Dan Med Bull *27:* 74, 1980.
4. Shapiro, H. A., Trowbridge, J. O., Lee, J. C. & Maibach, H. I.: Liver disease in psoriatics—an effect of methotrexate therapy? Arch Dermatol *110:* 547, 1974.
5. Shuster, S., Watson, A. J. & Marks, J.: Small intestine in psoriasis. Br Med J *iii:* 458, 1967.
6. Zachariae, H., Grunnet, E. & Søgaard, H.: Liver biopsy in methotrexate-treated psoriatics—a re-evaluation. Acta Dermatovener (Stockholm) *55:* 291, 1975.

# Affinity of $^3$H-8-MOP to Human Chromosomes and Cells

Hans Christian Wulf and Erik Niebuhr

*Department of Dermatology, The Finsen Institute, and University Institute of Medical Genetics, Copenhagen, Denmark*

Received March 21, 1980

*Abstract.* Human leukocytes and fibroblasts were cultured in medium containing $^3$H-8-methoxypsoralen ($10^{-6}$ M and $3.3\times10^{-6}$ M). At different stages during the culturing period the cells were exposed to ultraviolet light (1, 2, and 10 J/cm$^2$). After the culture period the slides for chromosome analysis and smears of the cells were prepared. Autoradiography of these slides could not dem-

Table 1. *Irradiation programme for all test series*

| No. | Time of UV irrad. | Change of medium after UV |
|---|---|---|
| 1 | – | – |
| 2 | Just before addition of colcemid, 2 h before preparation of cells | – |
| 3 | Just before cell preparation | – |
| 4 | 48 h before cell preparation | + (8-MOP in the medium) |
| 5 | 48 h before cell preparation | + (no 8-MOP in the medium) |

onstrate any $^3$H-8-MOP bound in the chromosomes or cell structures.

*Key words:* 8-Methoxypsoralen; UVA; Autoradiography; Chromosome analysis

It is generally agreed that 8-methoxypsoralen (8-MOP) intercalates in DNA in darkness, but binds co-valently between DNA strains under the influence of longwave ultraviolet light (UVA) (2, 3, 5).

After incubation of fibroblasts in media containing $^3$H-8-MOP, a slightly enhanced binding of radioactivity has been observed in the nucleus following UVA irradiation (1).

In view of this phenomenon we decided to test whether it was possible to trace radioactivity in metaphase chromosomes after incubation of leukocytes and fibroblasts derived from human skin in $^3$H-8-MOP alone and in combination with UVA irradiation.

## METHOD

Human leukocytes from the buffy coat of 10 ml peripheral heparinized blood (separated by gravity for 1½ h) were cultured for 72 h at 37°C. As well as the leukocytes, fibroblasts derived from human skin were incubated in culture medium (Parker 199 with 15% foetal calf serum) containing $^3$H-8-MOP (specific activity 293 mCi/mmol) and ethanol 0.24% to keep $^3$H-8-MOP in solution; 3 test series were performed:

1) Leukocytes cultured in $10^{-6}$ M $^3$H-8-MOP, light dose 10 J/cm$^2$;

2) Leukocytes and fibroblasts cultured in $10^{-6}$ M $^3$H-8-MOP and light dose 1 and 2 J/cm$^2$ respectively;

3) As 2) but with $3.3\times10^{-6}$ M $^3$H-8-MOP in the medium.

The cultures were irradiated in flat glass bottles according to the scheme shown in Table 1. Ten J/cm$^2$ was given in a PUVA 4000 apparatus for patient treatment. One J/cm$^2$ was obtained at a distance of 23 cm from 2×20 W General Electric blacklight (F 20 T 12-BL) in 30 min. Two J/cm$^2$ was given during the corresponding time period by altering the distance to the tubes.

Two hours before cell preparation, Colcemid® was added to halt cells in metaphase. Afterwards each culture was divided into two parts, one for chromosome preparation, and the other for a smear of the cells. Samples of medium from the tubes were allowed to dry on the slides.

Cells for chromosome analysis were hypotonized with KCl 75 mmol/l and fixed in methanol/glacial acetic acid twice before slides were made. Cells for the smears were rinsed in Gurr buffer, pH 6.8, to remove free $^3$H-8-MOP. The smears were given a fast dip in the fixative and allowed to air-dry. To minimize the risk of extracting not only the free $^3$H-8-MOP, cold 8-MOP was added to the buffer, KCl, and fixative in one series of experiments.

All slides were covered with film emulsion by dipping them in Ilford K2. They were then stored in the dark and examined for grains after 14 days to 6 months of exposure.

Mitoses were found by phase contrast microscopy and counting of silver grains was performed by light microscopy.

## RESULTS

Neither incubation with $^3$H-8-MOP alone nor in combination with UVA gave any silver grains in cells or chromosomes. Large numbers of small grains were seen in the film emulsion on slides prepared from the medium.

## DISCUSSION

Bredberg et al. (1) have cultured fibroblasts in $^3$H-8-MOP containing medium. The concentrations were $16\times10^{-6}$ M (3.4 μg/ml) and $38\times10^{-6}$ M (8.1 μg/ml). Grains were seen in both the cytoplasma and nucleus in equal amounts, but after 6 J/cm$^2$ the number of grains increased in the nucleus. The number of grains was dependent on the concentration of $^3$H-8-MOP.

Extrapolating from these figures to the 8-MOP concentrations $10^{-6}$ M seen in human serum/plasma 2 h after ingestion (4, 7, 8), one should not expect any grains to be found; this is accentuated by our lower specific activity, but partly compensated by our longer film exposure time.

We chose $^3$H-8-MOP concentrations of $10^{-6}$ M and $3.3\times10^{-6}$ M, as these correlate better with in vivo conditions. Under these conditions we were unable to find any grains in either cells or chromosomes, even when great care was taken not to extract loose bound $^3$H-8-MOP. This must be seen in relation to the large quantities of grains found in control slides of media even after only 14 days of film exposure.

The preparation technique involves procedures with compounds in which 8-MOP is easily soluble. This could lead to the extraction of intercalated 8-MOP, but presumably not of co-valently bound $^3$H-8-MOP in DNA after the UVA exposure. Since no grains are observed in the smears, extraction is not likely to be of influence. Furthermore, addition of cold 8-MOP to the liquids used for washing cells made no difference.

We checked that $^3$H-8-MOP was stable during the culturing period by examining the amount of $^3H_2O$ formed. Radioactivity from tritiated water was responsible for <5% of the total radioactivity and from this fact it can be deduced that $^3$H-8-MOP is stable.

Irradiation at the beginning of the culturing period could lead to cell death with consequent loss of these cells.

The lack of grains in mitoses, irradiated just prior to preparation, might be explained by the inability of DNA to react with $^3$H-8-MOP in prophase and metaphase. However, this explanation is not very plausible, since no grains were seen, either in the nucleus or in other dividing stages.

It must be appreciated that the formation of a silver grain (0.25 $\mu$m in diameter) (6) needs about 20 disintegrations to occur in the film emulsion used (6). With this sensitivity we could not detect any binding of $^3$H-8-MOP to DNA or other cell structures when using 8-MOP concentrations and light intensities corresponding to the doses given to patients.

## REFERENCES


1. Bredberg, A., Lambert, B., Swanbeck, G. & Thyresson-Hök, M.: The binding of 8-methoxypsoralen to nuclear DNA of UVA irradiated human fibroblasts *in vitro*. Acta Dermatovener (Stockholm) *57*: 389, 1977.
2. Chandra, P., Rodighiero, G., Balikcioglu, S. & Biswas, R. K.: Nucleic acid modification by Furocoumarins and light: Some biomedical implications. *In* Photochemotherapy, Basis, Technique and Side Effects (ed. E. G. Jung), 25 pp. F. K. Schattauer Verlag, Stuttgart, 1976.
3. Dall'Acqua, F., Marciani, S., Ciavatta, L. & Rodighiero, G.: Formation of inter-strand cross-linkings in the photoreactions between Furocoumarins and DNA. Z. Naturforsch *26 b*: 561, 1971.
4. Fincham, N., Greaves, M. W., Hensby, C. N. & Vella Briffa, D.: The quantitative analysis of 8-MOP in human plasma by HPLC-UV. Br J Pharmacol *63*: 373 P, 1978.
5. Pathak, M. A., Krämer, D. M. & Fitzpatrick, T. B.: Photobiology and photochemistry of Furocoumarins (psoralens). *In* Sunlight and Man, (ed. T. B. Fitzpatrick), 335 pp. University of Tokyo Press, Tokyo, 1974.
6. Rogers, A. W.: Techniques of Autoradiography, 10 pp. Elsevier Publishing Company, Amsterdam, London, New York, 1969.
7. Steiner, I., Prey, T., Gschnait, F., Washüttl, J. & Greiter, F.: Serum levels of 8-methoxypsoralen 2 hours after oral administration. Acta Dermatovener (Stockholm) *58*: 185, 1978.
8. Wilkinson, D. I. & Farber, E. M.: Gas-liquid chromatographic determination of 8-methoxypsoralen in serum. *In* Psoriasis. Proceedings of the second International Symposium (ed. E. M. Farber & A. J. Cox), 480 pp, 1976.


# Allergenicity of Trimethylol Propane Triacrylate in Ultraviolet Curing Inks in the Guinea Pig


Bert Björkner

*Department of Occupational Dermatology University Hospital, Lund and Department of Dermatology General Hospital, Malmö, Sweden*


Received February 28, 1980


*Abstract.* Trimethylol propane triacrylate (TMPTA) is a multifunctional acrylate commonly used in ultraviolet (UV) curing inks. Six men developed dermatitis when working with such UV curing inks in printing plants and all had positive patch test to TMPTA. The sensitizing capacity of TMPTA as determined by the "Guinea pig maximization test" (GPM) showed it to be a strong allergen. Cross-reaction with penta-erythritol triacrylate (PETA) occurs, but not with trimethylol propane trimethacrylate (TMPTMA).



*Key words:* Sensitizing capacity; Ultraviolet curing inks; Cross-reaction; Trimethylol propane triacrylate; Penta-erythritol triacrylate; Trimethylol propane trimethacrylate


The resins used in UV-cured inks are acrylated prepolymers diluted with multifunctional acrylates. The multifunctional acrylates have a dual role in UV-curable coatings or inks. One is to serve as cross-linking sites for the reactive base prepolymer and the other is to function as diluents for the usually more viscous base resins (12). In general, acrylic prepolymers and monomers are relatively transparent and consequently unable to initiate a fast