that result in capping by other mechanisms, e.g. capping due to excessive die wall friction, C_i values may not be realistic.

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Photoactivity of coal tar preparations on microorganisms and DNA

FRED W. WHITEHEAD†, TAE JOO CHOI, G. H. NEIL TOWERS*, JOHN C. MITCHELL, *Department of Botany, Faculty of Science and Division of Dermatology, Faculty of Medicine, University of British Columbia, Vancouver, B.C., Canada*

Coal tar and ultraviolet light have been used in the phototherapy of psoriasis for over 50 years (Goeckerman 1925) but the mode of action remains unclear. The 'synthesis' of DNA in mouse epidermis is inhibited by this form of photochemotherapy (Stoughton et al 1978; Walter et al 1978). Moreover, the formation of DNA interstrand crosslinks similar to those formed with 8-methoxypsoralen (8-MOP) and UVA (320–400 nm) (Scott et al 1976; Song & Tapley 1979) have been reported with coal tar and UVA (Pathak & Biswas 1977). We now report that although we have demonstrated phototoxic activity of coal tar preparations against bacteria and yeasts we were unable to demonstrate the formation of DNA crosslinks with isolated calf thymus DNA.

Materials and methods

The method of Daniels was used for phototoxicity assays with bacteria and yeasts (Towers et al 1977). Briefly, agar plates are spread with living *Candida albicans*. Putative photosensitizers are dried on discs of filter paper, which are added to the plates. One set of plates is incubated in the dark and the other in longwave u.v. light. Phototoxic compounds are those which cause a halo of growth inhibition after incubation in near u.v. (300–400 nm) but not after incubation in the dark.

Calf thymus DNA (Type I, Na salt, Sigma Chemical Co., St Louis, U.S.A.) was dissolved in sodium phosphate butter (0·02 M total phosphate concentration), pH 6·5. DNA solutions were diluted to obtain an absorbance of 2·0 at 260 nm. Two ml of the solutions were mixed with the appropriate compound in plastic dishes (35 × 100 mm style) with lids. The mixtures were shaken under a bank of four Sylvania or Westinghouse black light blue fluorescent bulbs (F20T12-BLB) for 3 h at 23–29 °C. A dark control dish was wrapped in aluminium foil. The intensity of radiation at 360 nm was 2 mW cm^{-2} when measured with an IL 700 radiometer (International Light, Inc.).

Three coal tar preparations were tested for the production of crosslinks in DNA. These were a tar distillate (Doak Pharmacal Co., Inc., Westbury, N.Y.), an oil (Doak Oil, which contains 10% Doak tar distillate in mineral oil) and a coal tar preparation from Currie Products Ltd, Hamilton, Ontario, Canada. 8-MOP (Sigma Chemical Co.) was also tested. The amounts tested were: tar distillate—100 µl, oil—500 µl; coal tar—5 µl of a suspension in benzene (40 mg ml^{-1}), 8-MOP 10 µg (3·3 µl at 3 mg ml^{-1} in 95% ethanol).

The method which we used to detect crosslinks in DNA is similar to that of Cole (1970). DNA solutions were irradiated, transferred to test tubes, heated for 10 min (100 °C) and placed in an ice bath for 5 min. This procedure denatures native DNA but allows renaturation of crosslinked DNA. The DNA solutions were chromatographed on a column of 3 g of hydroxylapatite (Bio-Rad Laboratories, Richmond, CA, U.S.A.). Solutions were applied to the column at a flow rate of 1 ml min^{-1} in this order, (1) the DNA sample, (2) 8 ml of 0·02 M phosphate buffer and (3) a linear gradient (200 ml) of 0·02–0·6 M phosphate buffer. DNA with crosslinks (i.e., renatured DNA) and denatured DNA were separated by the gradient. Fractions of 70 drops (3·6 ml) were collected and their absorbance at 260 nm was measured.

Results

Phototoxicity results are presented in Table 1. The tar distillate was phototoxic for all organisms tested except *Streptococcus faecalis*. Doak oil was phototoxic to all

* Correspondence.
† Present address: B.C. Cancer Foundation Cancer Research Centre, 601 W. 10th Street, Vancouver, B.C. V5Z 1L3, Canada.

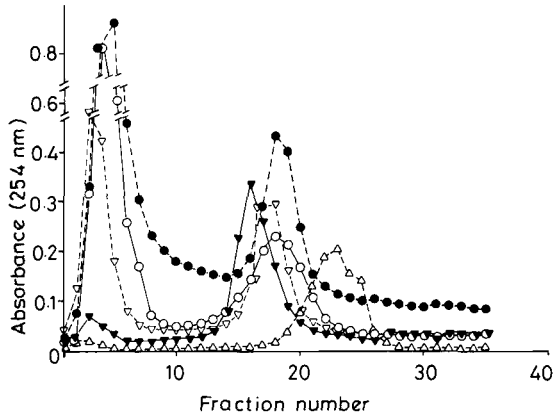

FIG. 1. Hydroxylapatite chromatography of calf thymus DNA which treated with three coal tar preparations (tar distillate (Doak Pharmacal Co), (○); Doak oil forte (●); coal tar (Currie Products Ltd), (▼)) or 8-MOP (△) and UVA. DNA which was incubated in the dark (▽) served as a control. Absorbance (260 nm) was plotted against fraction numbers. The initial peak of absorbance was due to coal tar itself.

organisms tested except *S. faecalis* and *Escherichia coli*. The difference in sensitivity of *E. coli* to the tar distillate versus Doak oil was confirmed by a second experiment. The coal tar preparation was phototoxic for three microorganisms (Table 1), although it had no phototoxicity for the fungus, *Trichoderma viride*.

Although phototoxic, the tar preparations did not induce crosslinks in calf thymus DNA (Fig. 1). After treatment with each coal tar plus UVA, the DNA was eluted from hydroxylapatite at the same position as the dark control. Thus, the DNA remained denatured (highest absorbance in fractions 16–18).

It is possible that crosslinks could be formed by coal tar after a delay, i.e., dimer formation could be a slow process after a fast photochemical reaction to form monoadducts. To test this idea, we repeated the tar distillate plus UVA experiment, storing the mixture (at 6 °C) for 16 h after irradiation, but before boiling and cooling. The idea was not validated, since the DNA did not renature (Fig. 1). As expected, irradiation in the presence of 8-MOP caused crosslinks to be formed and the DNA was renatured (highest absorbance in fraction 23).

To test the possibility that coal tar and 8-MOP compete for the same sites in DNA, we performed a competition experiment in which DNA (2 ml, A_{260} = 2) was irradiated in the presence of both 8-MOP (10 μg) and coal tar distillate (100 μl). This amount of coal tar totally suppressed the formation of crosslinks by 8-MOP and UVA.

Table 1. Inhibition of bacterial and fungal growth by coal tars in the presence of UVA-A light.

	Tar distillate (Doak Pharmacal Co.)		Doak oil forte		Coal tar (Currie Products Ltd)	
Test organism	UV	Dark	UV	Dark	UV	Dark
Bacteria						
Bacillus subtilis	+	(+)	+	(+)	+	−
Streptococcus faecalis	−	−	−	−	+*	+
Staphylococcus aureus	+	(+)	+	−	NT	NT
Staphylococcus albus	+	(+)	+*	+	NT	NT
Escherichia coli	+	−	−	−	NT	NT
Yeasts						
Candida albicans	+	(+)	+	−	NT	NT
Saccharomyces cerevisiae	+*	+	+	−	+*	+

−, no growth inhibition; +, growth inhibition; (+), weak growth inhibition (i.e. halo of inhibition ≤12 mm; NT, not tested.
* Greater inhibition was observed after UV exposure.

Discussion

The observation that coal tar and UVA appeared to cause DNA crosslink formation (Pathak & Biswas 1977) suggested a molecular action for coal tar and ultraviolet light. However, our results show that, although they are photoactive, three coal tar preparations did not crosslink DNA, either immediately or by a delayed process. Components of coal tar may intercalate in DNA. Furthermore, the phototoxicity of coal tar preparations is suggestive of a photoreaction with DNA. This photoactivity differs from the activity of 8-MOP since DNA crosslinks are not formed. The suppression of 8-MOP/DNA crosslink formation by coal tar can be explained if it is assumed that components of coal tar compete for 'sites' in the DNA helix which would be occupied by 8-MOP. However, coal tar components could also interfere at some other stage of crosslink formation by 8-MOP. One possibility is that coal tar components may cause quenching of UVA.

Certain components of coal tar, such as benz(a)-pyrene, induce aryl hydrocarbon hydroxylase in skin (Bickers & Kappas 1978). The enzyme produces epoxides which are metabolized to diol epoxides which then form adducts with DNA (Daudel et al 1975; Koreeda et al 1978). Perhaps these adducts are responsible for the result of Walter et al (1978) who showed that the 'synthesis' of mouse epidermal DNA was inhibited by coal tar. Since the inhibition was increased by UVA treatment of skin, it is possible that other components of coal tar react photochemically with DNA.

Concern has been expressed over the long-term hazard of the coal tar and ultraviolet light regimen for psoriasis patients. Perhaps the hazard will be understood once the molecular mechanism is known for coal tar plus UVA therapy. Recently it was reported that urine extracts from patients undergoing this therapy cause mutations in bacteria (Wheeler et al 1981).

In conclusion, we have shown that coal tar exhibits phototoxic effects on various microorganisms. These effects cannot be explained by the formation of crosslinks in DNA, however they might be explained by an intercalation and monovalent adduct formation. A molecular explanation for coal tar action should provide

a foundation for means of estimation of the risk/benefit ratio of its use compared to that of 8-MOP in the phototherapy of psoriasis.

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4-Substituted-2-anilinothiazolin-5-ones as substrates of α-chymotrypsin

AL K. FADEN, H. J. SMITH,* *Welsh School of Pharmacy, University of Wales Institute of Science & Technology, Cathays Park, Cardiff, U.K.*

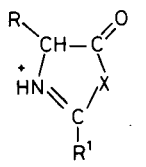
The 2-anilinothiazolin-5-one derivatives (ATZ; 1a) of leucine, alanine, valine and tryptophan are substrates of α-chymotrypsin, the order of reactivity being ATZ-Try >> ATZ-Ala ≃ ATZ-Leu >> ATZ-Val. Deacylation of the acyl enzyme gives the 5-substituted-3-phenyl-2-thioxo-4-imidazolidinone (PTH-amino acid; II) by intramolecular participation of the anilino-*N*-atom, except for ATZ-Try which may be alternatively bound at the active site since the 2-substituted-5-phenylhydantoic acid (III) is formed.

The oxazolinones, 2-phenyloxazolin-5-one (PO; Ib, R=H,) and 2-phenyl-4,4-dimethyloxazolin-5-one (PDMO; Ib, R=$(CH_3)_2$·)

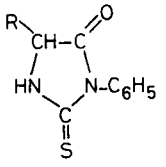
$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} \underset{\text{acyl-enzyme}}{ES^1} \xrightarrow{k_3} E + P \quad (1)$$

are substrates of α-chymotrypsin (de Jersey & Zerner 1969; de Jersey et al 1966) the reaction proceeding through the acyl-enzyme intermediate where the rate controlling step is governed by k_3 (Bender 1962) (eqn 1). The related, 4-methyl-2-phenylthiazolin-5-one (Ic) is a poor substrate of the enzyme (Coletti-Previero et al 1973) and on the premise that poor substrates which give a relatively stable acyl enzyme (low value for k_3) would be satisfactory inhibitors in vivo we have studied the reaction between several 4-substituted-2-anilinothiazolin-5-ones (ATZ-amino acids; Ia) and the enzyme.

* Correspondence.



(I)



(II)

C_6H_5·NH·CS·NH·CH(R)COOH

(III)

(Ia): $R^1 = -NH.C_6H_5$, X = S
(Ib): $R^1 = -C_6H_5$, X = O
(Ic): R = Me, $R^1 = C_6H_5$, X = S

Materials and methods

α-Chymotrypsin (BDH) was thrice recrystallized. The 5-substituted-3-phenyl-2-thioxo-4-imidazolidinones (PTH-amino acids) were purchased from Sigma and the corresponding sodium phenylhydantoates were prepared by the general method of Edman (1956). Spectra were measured in acetonitrile (12%) - phosphate buffer (0·01 M), pH 7·4. All melting points are corrected.

4-(2'-Methylpropyl)-2-anilinothiazolin-5-one hydrochloride (ATZ-leucine, I(a), R = $(CH_3)_2CH.CH_2$). This was prepared by Edman's method (1956) except that the syrup containing the sodium phenylhydantoate was repeatedly extracted with acetone and ether before