Journal of Chromatography, 273 (1983) 202—206
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 1537

Note

Detection of acridine in human urine after topical coal-tar treatment

M. ČERNÍKOVÁ*, H. DUBSKÝ and J. HORÁČEK

Laboratory of Dermatological Biochemistry and Institute of Forensic Medicine, Faculty of Medicine, J.E. Purkyně's University, Brno (Czechoslovakia)

Even at the present day coal-tar remains one of the basic dermatologic therapeutic agents, especially in the treatment of psoriasis and some types of eczema. Coal-tar used in therapy results from the pyrolytic decomposition of coal under thermodynamic conditions which enable the formation of a gamut of substances. Estimates of the number of compounds in tar are of the order of magnitude of thousands, of which about 550 substances have been identified. Besides various hydrocarbons coal-tar contains a high number of compounds with organically bound sulphur, nitrogen, and oxygen, mostly in the form of heterocycles [1].

Considering these facts it is understandable that the mechanisms of the therapeutic action of coal-tar have not yet been elucidated, especially when even the basic information about the penetration and resorption of this complex mixture of substances in the human skin is lacking.

In an endeavour to contribute to the solution of these problems and thus to a more rational exploitation of coal-tar as a therapeutic agent, we investigated resorption from coal-tar in dermatologic patients after local tar therapy by analyzing their urine.

MATERIALS AND METHODS

Material

For investigating the tar resorption we selected 28 patients (two females and 26 males), who required coal-tar treatment on an area larger than two-thirds of the body surface. In the treatment 10% and 20% tar paste was used; this means that in one application approximately 1—6 g of coal-tar was spread on the patient's skin.

0378-4347/83/0000—0000/$03.00

Sample preparation [2, 3]

Urine analysis was carried out always before and after the treatment, in some cases even during the treatment.

For basic orientation thin-layer chromatographic (TLC) analysis 200 ml of morning urine were taken, boiled with 50 ml of concentrated HCl, and extracted with ca. 100 ml of chloroform. The extract was then concentrated by a stream of nitrogen at 40° C to a final volume of 1 ml. A 2-μl aliquot was used for TLC.

For acridine identification organic bases were separated after transformation to sulphates by treating 100 ml of urine with 150 ml of 20% sulphuric acid. The reaction mixture was extracted by 100 ml of chloroform. Sulphates of basic substances remained in the aqueous phase, which was separated and made alkaline with 150 ml of 26% ammonium hydroxide. The released bases were then extracted into 100 ml of chloroform and the extract was concentrated to 1 ml.

Thin-layer chromatography

The urine extracts were separated on the commercial silica gel thin-layer plates Silufol (Silufol^{R}, 20 × 20 cm) and Merck (DC-Fertigplatten Kieselgel 60, 20 × 20 cm). Several solvent systems were tested. The best results were obtained with a mixture of acetic acid—benzene—diethyl ether—methanol (18:120:60:1, v/v) in the first direction, and a mixture of butanol—ammonium hydroxide (26%) (4:1) in the other direction. For detecting the polycyclic substances on chromatograms we exploited in most cases their own fluorescence in UV light (254 and 360 nm), or fluorescence quenching in the same region. Functional groups were detected by means of standard colour reactions [4].

Gas chromatography

Gas chromatography of the fractions eluted from the chromatograms was performed with a Pye GCV apparatus using two column packings. The sample volume was 5 μl.

Packing A. 5% OV-1 on Diatomite CQ (0.125—0.16 mm), column 150 cm × 4 mm I.D. Flow-rates: nitrogen 25 ml/min, air 250 ml/min, hydrogen 30 ml/min. The column temperature was 200° C, the detector temperature 240° C, the sampling temperature 300° C.

Packing B. 3% OV-17 on Diatomite CQ (0.125—0.16 mm), column 200 cm × 2 mm I.D. Gas flow-rates and temperatures as for packing A.

Gas chromatography—mass spectrometry

Gas chromatography—mass spectrometry was carried out with a Ribermag GC-MS R 1010 computer in the biochemical laboratories of the Medical Faculty in Dijon (France). A capillary column coated with a non-polar phase (Silar 5 CP) was used for the separation. The column bypassed the separator and led directly into the ionization source. Samples were introduced by means of a glass fibre. The temperature of the evaporation space was 250° C, the column temperature was 140° C.

RESULTS AND DISCUSSION

The basic information on polyaromatic and heterocyclic substances excreted in urine was obtained from TLC runs. This analysis was carried out for all patients. In all cases chromatograms of urine samples taken before and after tar application were compared. We thus found that in all cases investigated several fluorescing spots appeared on chromatograms of samples taken after the external application of tar, which did not occur in urine prior to the tar application (Fig. 1). The most marked one was a spot with an intense blue–green fluorescence with R_F values of 0.05 and 0.76 in the first and second direction, respectively.

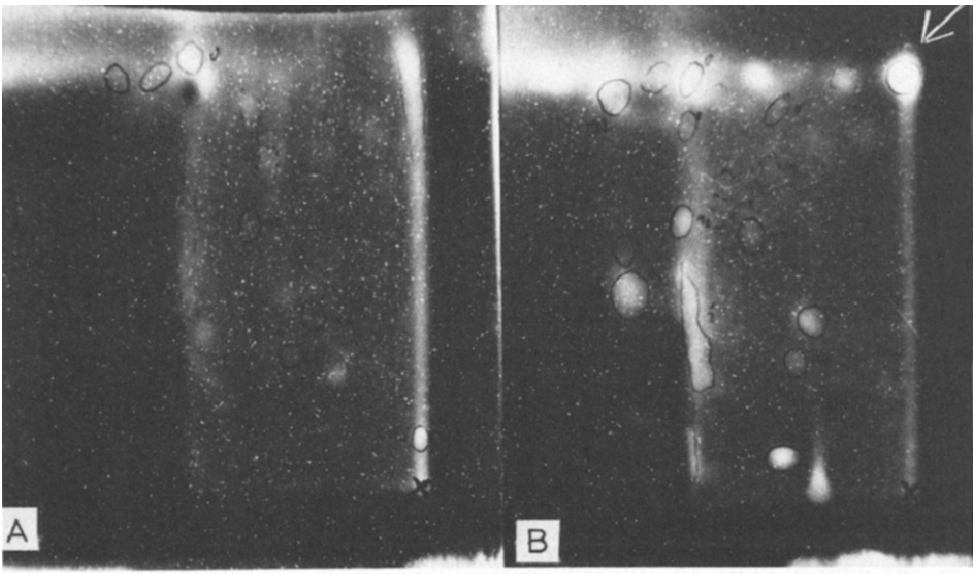

Fig. 1. Thin-layer chromatography of urine extracts: (A) before the coal-tar application, (B) after the coal-tar application. x = origin; the arrow indicates the unknown, blue–green fluorescing substance.

After comparing this unknown substance with several standards of corresponding properties it became apparent that the strongly fluorescing spot contained with high probability one or several derivatives of quinoline, isoquinoline, or acridine. Furthermore, it appeared that the found properties and chromatographic behaviour of acridine were fully identical with those of the unknown substance.

Further identification of the substance was then performed by means of gas chromatography. Organic bases isolated from both coal-tar and urine of the patients were separated on thin layers, eluted, and subjected to further separation by gas chromatography. By comparing these results with the properties of pure acridine we found that the retention times of acridine in both column packings were identical with those of the peak detected in eluates from the chromatograms, namely 2.6 and 4.9 min. The presence of acridine in urine was thus demonstrated (Fig. 2).

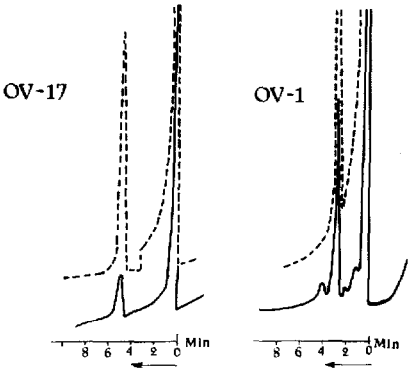

Fig. 2. Gas chromatography of acridine from urine. (——), Urine extract; (— — —), acridine standard (10^{-4} mol/l). For further details see the text.

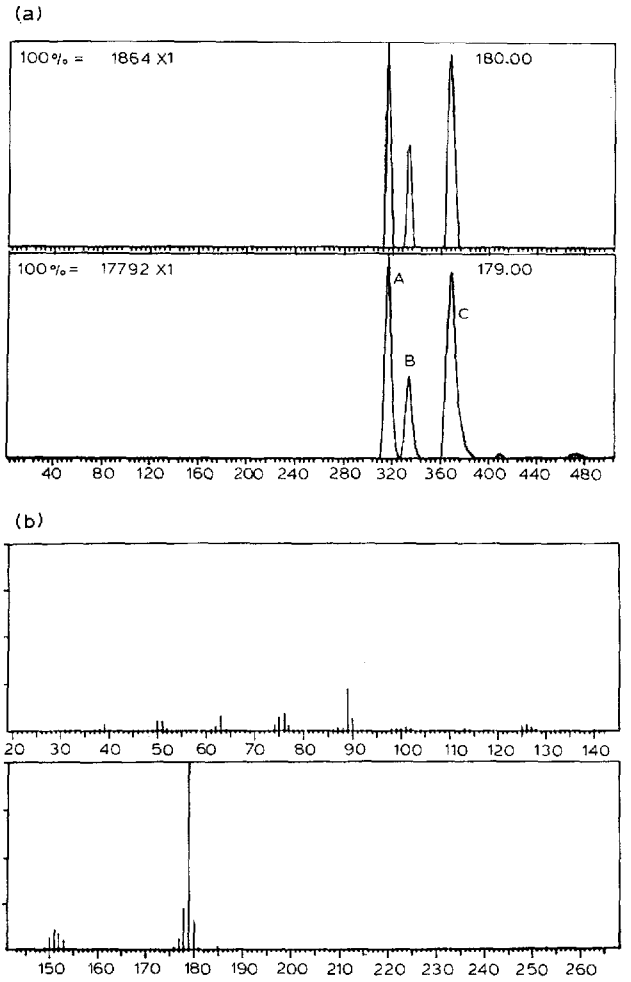

Fig. 3. Fragmentogram and mass spectrum. (a) Fragmentogram of urine extract containing acridine. (b) Mass spectrum of standard acridine, which is identical with fraction B in Fig. 3a.

Clinical verification of the demonstration of acridine in urine after percutaneous resorption was performed by a test with a paste containing acridine instead of tar. According to the data on the acridine content of coal-tar [1, 3], we prepared magistraliter a paste containing 0.12 g of pure acridine in 100 g of the paste. This corresponds to the reported 0.6% of acridine in tar if 20% tar paste is considered. The acridine paste was applied to a patient in the usual way and the excretion of acridine in urine was investigated. The acridine spot was observed on chromatograms since the first day of the application of the acridine paste, as well as after the use of coal-tar. This experiment yielded another proof for the resorption of acridine from coal-tar.

Mass spectra of standard acridine and acridine isolated from urine confirmed the identity of the two compared substances having the same retention time (Fig. 3). (The main fragment ion was *m/e* 179, the next were *m/e* 180 and 178, etc.) This finding was also in accord with data in the computer library; at the same time the computer evaluated the probability of the sought agreement by an index DI. In the given case DI was 0.279, which indicated a very high probability.

The presence of acridine in urine after the coal-tar application was convincingly demonstrated by the mass spectrographic analysis.

Acridine is present in coal-tar as one of several basic nitrogen-containing compounds. According to its content it is ranked in second place behind carbazole (coal-tar contains 1.5% of carbazole, 0.6% of acridine, 0.3% of quinoline, 0.02% of pyridine, etc.) [5].

The detection of acridine in urine is the first proof of the resorption of a coal-tar component through the skin and the investigation of this process can contribute to an improvement of coal-tar therapy by selecting the effective and relatively less toxic components of this extraordinarily complex mixture of substances.

ACKNOWLEDGEMENT

The authors are much obliged to Prof. Prudent Padieu from the Laboratoire de Biochimie in Dijon for having enabled them to carry out the analysis on the chromatograph Ribermag GS-MS R1010.

REFERENCES

1 M. Janík and J. Vymětal, Chem. Listy, 67 (1973) 561.
2 A. Dierichs and R. Kubička, Fenoly a Zásady z Uhlí, SNTL, Prague, 1956.
3 J. Vymětal, Chem. Listy, 68 (1974) 1234.
4 I.M. Hais and K. Macek (Editors), Paper Chromatography, Academic Press, London, 3rd ed., 1963.
5 M. Janík, M. Kaloč and J. Vymětal, Sb. Ved. Pr. Vys. Sk. Banske Ostrave, 24 (1978) 133.