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Note

High-performance liquid chromatography determination of Nocodazole® in liposomes and L 1210 cells

C. BRASSINNE*
Service de Médecine et Laboratoire d'Investigation Clinique Henri Tagnon, Institut Jules Bordet, 1 rue Héger-Bordet, 1000 Brussels (Belgium)
and
R. VANHAELEN-FASTRÉ and M. VANHAELEN
Institut de Pharmacie, Université Libre de Bruxelles, Campus Plaine, B 205-4, 1050 Brussels (Belgium)
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Nocodazole®, methyl [5-(2-thienylcarbonyl)-1H-benzimidazol-2-yl]carbamate (R 17934; NSC 238159), is a well known water-insoluble antimitotic drug active in mouse leukaemia[1,2]. Recently, we have described its entrapping in liposomes of various lipid compositions and demonstrated the changes induced in L 1210 cells by these liposomes[3].

This paper describes a rapid and efficient extraction procedure that allows the recovery of the drug from liposome preparations or cell pellets; Nocodazole is determined by reversed-phase high-performance liquid chromatography (HPLC) after purification of the crude extracts by means of semi-preparative thin-layer chromatography (TLC).

EXPERIMENTAL

Reagents

Nocodazole was purchased from Aldrich Europe (Janssen Pharmaceutica, Beerse, Belgium). Triton X-100 was obtained from Sigma (St. Louis, MO, U.S.A.). Whatman No. 1 PS paper was supplied by H. Reeve Angel (Clifton, NJ, U.S.A.). Silica gel PF 254 was obtained from E. Merck (Darmstadt, G.F.R.).

Extraction procedure

A 1-ml sample (liposome preparation or cell pellet suspension) was disrupted by addition of 0.5 ml 2% (v/v) Triton X-100 in water, and diluted in 2 ml 50 m*M* Tris–150 m*M* NaCl buffer. Nocodazole was twice extracted with 50 ml chloroform; the organic phases were separated, filtered on Whatman No. 1 PS paper and evaporated to dryness under vacuum at 35–40°C.

Semi-preparative TLC

TLC was performed on 20 × 20 cm plates coated with silica gel PF 254 (thickness 0.4 mm). The plates were prewashed with chloroform–methanol (50:50

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v/v) during 12 h and reactivated by heating at 120°C for 30 min. The dry extract was solubilized ultrasonically in 1 ml anhydrous tetrahydrofuran and quantitatively applied 1.5 cm from the lower edge of a plate. The plate was developed with chloroform–methanol (90:10 v/v) to a distance of 14 cm from the point of application. The band of Nocodazole was detected under UV light at 254 nm, scraped off and eluted with 8 ml chloroform–methanol (50:50 v/v); the eluate was then evaporated to dryness.

HPLC

A Waters Assoc. chromatograph was equipped with a Model 6000 A solvent delivery system, a Model U6K injector and a Model 440 absorbance detector operating at 254 nm. The stainless-steel column (30 cm × 3.9 mm I.D.) prepacked with μBondapak C_{18} (mean particle size 10 μm) was also supplied by Waters Assoc. (Milford, MA, U.S.A.). The column was preceded by an on-line stainless-steel precolumn (5 cm × 3.9 mm I.D.) packed with Vydac™-201 RP (particle size 30–44 μm; Macherey, Nagel & Co., Düren, G.F.R.).

The prepurified samples and the Nocodazole standards were solubilized in tetrahydrofuran containing 10% Tris–NaCl buffer at pH 7.4.

RESULTS AND DISCUSSION

The best extraction recovery was obtained with the chloroform extraction, both for the liposome preparations and the cell pellets.

TLC conditions were essential to separate Nocodazole (R_F = 0.57) from lipids and cellular materials, which might otherwise become strongly fixed to the octadecyl silica gel and interfere with the separation.

As shown in Fig. 1, the HPLC elution profiles of Nocodazole standard (a), Nocodazole extracted from liposomes (b) or cell pellet (c) were similar and no interference was observed at the retention time of Nocodazole. All samples were analyzed

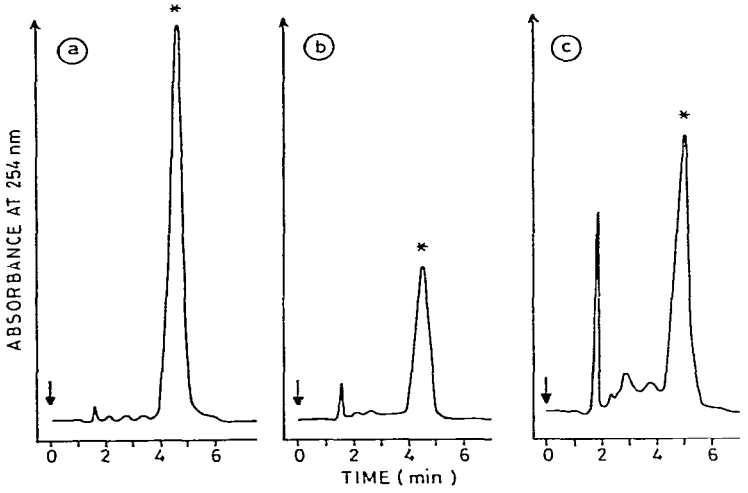

Fig. 1. High-performance liquid chromatograms of Nocodazole (*). a, Standard; b, liposome extract; c, cell pellet extract. Column: μBondapak C_{18} (10 μm). Mobile phase: ethanol–water (45:55 v/v); flow-rate 1.4 ml/min. Sensitivity: 0.005 a.u.f.s. Chart speed: 1 cm/min. Sample size: 25 μl.

in duplicate and the drug concentration was calculated from the Nocodazole peak area. The relationship between the peak area and the amount of Nocodazole present in the sample was linear. The mean recovery of pure Nocodazole within the concentration range of 0.1–500 μg was 95% under these experimental conditions. Moreover, the high sensitivity of the method shows its usefulness for the determination of Nocodazole in biological materials after *in vivo* administration of the free or liposome-entrapped drug[4].

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REFERENCES

1 M. J. De Brabander, R. M. L. Van De Veire, F. E. M. Aerts, M. Borgers and P. A. J. Janssen, *Cancer Res.*, 36 (1978) 905.
2 M. De Brabander, G. Geuens, R. Van De Veire, F. Thone, E. Aerts, L. Desplanter, J. De Cree and M. Borgers, *Eur. J. Cancer*, 13 (1977) 511.
3 J. Frühling, W. Penasse, G. Laurent, Ch. Brassinne, J. Hildebrand, M. Vanhaelen, R. Vanhaelen-Fastré, M. Deleers and J. M. Ruysschaert, *Eur. J. Cancer*, 16 (1980) 1409.
4 C. Laduron, G. Atassi, J. Hildebrand, J. M. Ruysschaert and C. Brassinne, submitted for publication.