Journal of Chromatography, 564 (1991) 352–354
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 5691

Short Communication

Determination of the new anticancer agent KW 2149, 7-N-[2-((2-(γ-L-glutamylamino)ethyl)dithio)ethyl]mitomycin C, an analogue of mitomycin C

G. PATTYN, A. T. VAN OOSTEROM and E. A. DE BRUIJN*

Laboratory of Cancer Research and Clinical Oncology, University of Antwerp (S-4), Universiteitsplein 1, B-2610 Wilrijk (Belgium)

and

U. R. TJADEN

Division of Analytical Chemistry, Center for Bio-Pharmaceutical Sciences, University of Leiden, P.O. Box 9502, 2300 RA Leiden (The Netherlands)

(First received August 6th, 1990; revised manuscript received October 23rd, 1990)

ABSTRACT

The new mitomycin 7-N-[2-((2-(γ-L-glutamylamino)ethyl)dithio)ethyl]mitmycin C (KW 2149) (I) proved to be active against a wide variety of experimental tumours. In order to perform pharmacokinetic studies with the new drug in Phase I sessions, a fast and reliable method has been developed based on the data of previous assays for mitomycin C. XAD-2 was preferred for isolation of I from blood plasma. The recovery of I was 50% whereas that of mitomycin C was 85%. Optimal separation was obtained on octadecyl silica columns with methanol–water (45:55, v/v) as mobile phase, while ultraviolet absorbance detection was performed at 375 nm. The assay enabled determination of I in a plasma concentration range of 20–1000 ng/ml using porfiromycin as internal standard.

INTRODUCTION

7-N-[2-((2-γ-L-Glutamylamino)ethyl)dithio)ethyl]mitomycin C (KW 2149) (I) is a recently developed antitumour agent, which is reported to be effective against MMC-resistant murine tumours [1].

High-performance liquid chromatography (HPLC) with either UV absorbance detection or electrochemical detection of mitomycin C has been reported by several investigators and reviewed recently [2]. We have exploited HPLC, as developed for mitomycin C, to determine the new analogue in human plasma. Special attention has been paid to the possibility of the co-determination of mitomycin C, since it may be expected that mitomycin C could be formed by biotransformation/degradation of I (Fig. 1).

0378-4347/91/$03.50

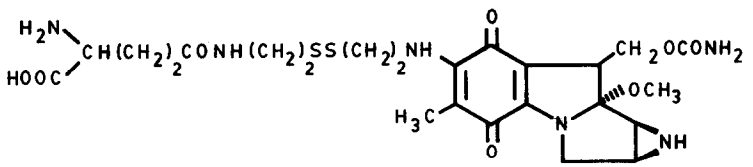

Fig. 1. Molecular structure of I: its molecular mass is 598.7.

As with mitomycin C, derivatization is not necessary because UV absorptivity of I is sufficient ($\lambda_{max} = 375$ nm, $\varepsilon_{375} = 2.28 \cdot 10^4$ mol l^{-1} cm^{-1} in water and $\lambda_{max} = 368$ nm, $\varepsilon_{368} = 2.29 \cdot 10^4$ mol l^{-1} cm^{-1} in methanol).

EXPERIMENTAL

Following the methods described for mitomycin C [2], octylsilica (Merck, LiChrospher 100 RP-8, 5 μm, 125 mm × 4.0 mm I.D.) and octadecylsilica (Beckman, Ultrasphere 5 μm, 250 mm × 4.6 mm I.D.) columns were tested, with a mobile phase of either methanol–water or acetonitrile–water. UV detection was

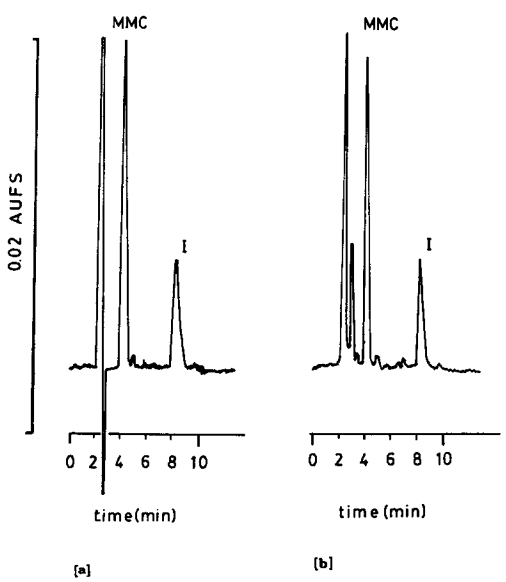

Fig. 2. (a) Chromatogram of an extract of an aqueous solution of mitomycin C (50 ng/ml) and I (200 ng/ml), obtained with methanol–water (45:55) as mobile phase on a C_{18} Ultrasphere column (5 μm particle size, 250 mm × 4.6 mm I.D.). (b) Chromatogram of an extract of human plasma spiked with I (200 ng/ml) and mitomycin C (50 ng/ml), obtained with methanol–water (45:55) as mobile phase on a C_{18} Ultrasphere column (5 μm particle size, 250 mm × 4.6 mm I.D.). XAD-2 was used for extraction.

performed at 362, 368 and 375 nm. Solid-phase extraction of plasma was carried out with Tenax (35–60 μm), C_8 (Polygosil 40–63 μm), C_{18} (Nucleosil 40–63 μm) and Amberlite XAD-2 (20–50 mesh ASTM, 0.3–0.9 mm) columns.

The optimal separation between mitomycin C and I was obtained on the octadecylsilica column with methanol–water (45:55 v/v) at a flow-rate of 1 ml/min (Fig. 2).

RESULTS AND DISCUSSION

In comparing XAD-2, Tenax, C_8 and C_{18} solid-phase extraction, the best results were obtained with XAD-2: both compounds of interest could be isolated from plasma spiked with I and mitomycin C at levels in the range 10–500 ng/ml. The calibration curves obtained in this range showed sufficient linearity, as demonstrated by the correlation coefficient of 0.9994. The recovery of mitomycin C on XAD-2 was reported earlier by us to be *ca.* 85% [3]. The recovery of I appeared to be lower: *ca.* 50%.

The resolution from plasma matrix compounds was adequate, and the linearity allowed I to be monitored in a plasma concentration range of 20–1000 ng/ml.

The within-assay precision for the analysis of I in plasma samples was determined for six samples at two different concentrations: at 1.0 μg/ml the accuracy was 105.4 ± 3.1% and at 20 ng/ml it was 94.7 ± 4.8%. The mitomycin C analogue porfiromycin can be used as an internal standard in the present system: the compound is eluted between mitomycin C and I.

Our observations demonstrate that the assays described for mitomycin C, based on HPLC with UV detection, can easily be adapted for simultaneous monitoring of I, mitomycin C and the internal standard porfiromycin.

When concentrations at the low ng/ml or even at the high pg/ml level have to be determined, *e.g.* during the monitoring of the behaviour of I following intravesical instillation, the limits of determination have to be improved. This might be possible by application of electrochemical detection. The assay described here, however, is useful for monitoring I in Phase I/II trials.

REFERENCES

1 M. Kono, Y. Saitok, M. Kasai, A. Sato, K. Shirahata, M. Morimoto and T. Ashizawa, *Chem. Pharm. Bull.*, 37 (1989) 1128.
2 U. R. Tjaden and E. A. de Bruijn, *J. Chromatogr.*, 531 (1990) 235.
3 U. R. Tjaden, J. P. Langenberg, K. Ensing, W. P. van Bennekom, E. A. de Bruijn and A. T. van Oosterom, *J. Chromatogr.*, 232 (1982) 355.