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Short Communication

Chemical stability of the new antitumour agent intoplicine in infusion fluids*

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Introduction

Intoplicine (RP60475F, NSC D645008, 11-(3-dimethylaminopropylamino)-3-hydroxy-8-methyl-7H-benzo[e]pyrido[4,3-b]-indole dimethanesulphonate, Fig. 1), is an investigational, antitumour drug that belongs to a new class of cytotoxic agents which interact with both nuclear DNA modifying enzymes topoisomerases I and II [1, 2]. The cytotoxicity of intoplicine, mediated by inhibition of topoisomerases, is induced by the stabilization of the cleavable enzyme–DNA complexes which, ultimately, leads to cell death. In *in vitro* studies intoplicine was found to be a potent inhibitor of DNA, RNA and, to a lesser extent, protein synthesis. *In vivo* the drug demonstrated a broad spectrum of activity against a variety of transplantable murine tumours [3]. On the basis of these interesting laboratory results, clinical phase I studies have been initiated in the USA and Europe to find the maximum tolerable dose which can be safely administered.

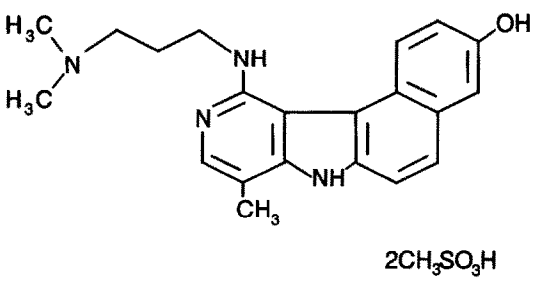

Figure 1
Structure of intoplicine dimethanesulphonate.

In the authors' hospital, patients suffering from a tumour that does not respond to other forms of chemotherapy are entered for the clinical phase I study with intoplicine. The drug is administered by intravenous infusion over 24 h.

This study was initiated with the objective of evaluating the chemical stability of intoplicine in commonly used infusion fluids at concentrations of 1 and 0.1 mg ml^{-1}, respectively. The storage conditions were chosen to mimic the conditions of storage (in a refrigerator at 4°C and in the dark) and during administration on the ward (room temperature and under normal room fluorescent light conditions in a day–night diurnal cycle). This is the first report in the literature on the chemical stability of intoplicine.

Experimental

Materials

Pharmaceutical dosage forms of intoplicine (RP60475F, lot CB05045), 100 mg (as dimethanesulphonate) 5 ml^{-1} were used. The product originated from Rhône-Poulenc Rorer, (Antony Cedex, France). The infusion fluids, 0.9% sodium chloride and 5% dextrose, were prepared in the pharmacy department of the hospital and were sterilized by autoclaving for 20 min at 120°C. The solvents were of analytical or HPLC grade and were used

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without further purification; dichloromethane was purchased from Mallinckrodt (Paris, KY, USA); 2-propanol and ammonia (25%, w/w) were purchased from Merck (Darmstadt, Germany).

Equipment

The HPLC equipment comprised a SP 8800 solvent delivery system, a SP 8880 autosampler injector (all from Spectra Physics, San Jose, CA, USA), a Model 441 UV detector (Waters, Milford, MA, USA), equipped with a fixed wavelength filter at 254 nm. A 100 × 3.0 mm i.d. glass cartridge column packed with 5-μm ChromSpher® silica gel (Chrompack BV, Bergen op Zoom, The Netherlands) was used. The eluent was dichloromethane–2-propanol–ammonia (10%, w/w) (40:40:1, v/v/v). The flow rate was 0.6 ml min^{-1}. Peak areas were computed by a SP 4270 integrator (Spectra Physics).

Methods

Five millilitres of the intoplicine formulation was added to 100 ml of either 0.9% sodium chloride and 5% dextrose to give an initial intoplicine concentration of 1 mg ml^{-1}. Solutions of 0.1 mg ml^{-1} were prepared by further dilution with 0.9% sodium chloride and 5% dextrose, respectively. The infusion fluids were prepared under aseptic conditions in a vertical laminar-airflow biological safety cabinet. The admixtures were stored in glass bottles in a refrigerator (4°C) in the dark or at room temperature (20–22°C) subjected to normal fluorescent room light in a day–night diurnal cycle (8 h in the dark and 16 h in the light). Immediately after sample preparation and at specific times thereafter (4, 8 h, 1, 2, 4 and 7 days), 100 μl aliquots were withdrawn from each container and mixed with 2-propanol (1.00 ml) in an autosampler vial. The samples were then analysed quantitatively for undegraded intoplicine using HPLC. An aliquot (10 μl) of each sample was injected onto the HPLC column. The test solutions were visually inspected daily against a light and dark background for changes in clarity and colour and for signs of precipitation. The pH of the test solutions was measured at each sampling time. An accelerated stability study was performed at 90°C in 0.1 M hydrochloric acid and in 3% (w/v) hydrogen peroxide solution; the intoplicine concentration was 1 mg ml^{-1}. Periodically, 100 μl samples were withdrawn for a period up to 48 h and were mixed with 2-propanol (1.00 ml) in an autosampler vial. An aliquot (10 μl) of each sample was injected onto the HPLC column. Quantitation of the undegraded drug was accomplished by comparing the intoplicine areas with those of freshly prepared standard solutions of intoplicine in water–2-propanol (1:10, v/v).

Results and Discussion

Calibration curves were linear ($r > 0.999$, $P < 0.0001$) within the working concentration range of 0.05–2.0 mg ml^{-1}. Relative standard deviations of 0.82 and 0.91% were determined for replicate injections ($n = 6$) of the lower and upper limits of the calibration curve, respectively. A relative standard deviation of 3.12% was determined by injection of six diluted samples. Stability tests under conditions of stress (in 0.1 M hydrochloric acid and 3% (w/v) hydrogen peroxide solution at 90°C) demonstrate that intoplicine rapidly degrades in hydrogen peroxide solution but is stable in acid. Hydrogen peroxide solutions were selected for these experiments in order to subject the drug to an oxidative reaction medium.

A typical HPLC chromatogram (Fig. 2) of a decomposition mixture of intoplicine in a 3% (w/v) hydrogen peroxide solution shows the intoplicine peak with a retention time of 6.5 min and a degradation product with a retention time of approximately 75 min. The half-life for the degradation of intoplicine in 3% (w/v) hydrogen peroxide is about 2 h (Fig. 3). The drug in 0.1 M hydrochloric acid is stable for at least 7 days at 90°C. During the first 5 h of degradation, the sum of the peak absorptivities at 254 nm for the parent drug and the degradation product is constant and then decreases, which indicates consecutive decomposition of the degradation product. This suggests that the chromophore of the first degradation product is similar to that of intoplicine; however, further degradation leads to changes in the chromophore. No other degradation products were detected. Work to elucidate the structure of the degradation product is ongoing. The experimental conditions for the stability tests of intoplicine in infusion fluids were similar to those associated with actual preparation of the infusion admixtures in the pharmacy, storage,

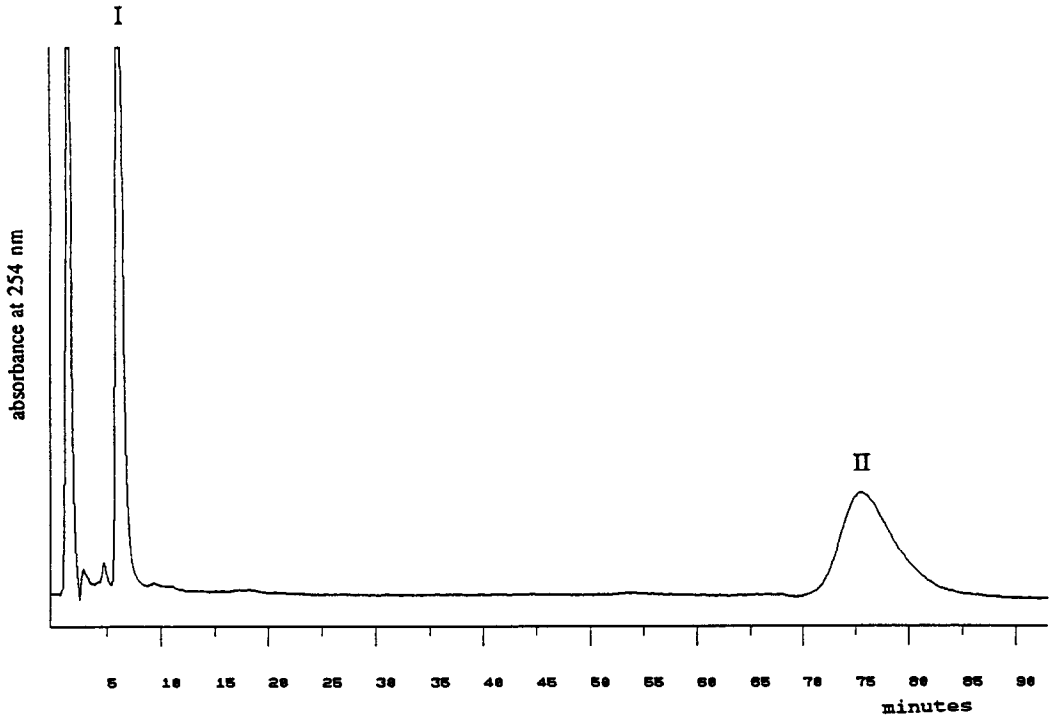

Figure 2
HPLC chromatogram of a decomposition mixture (t = 8 h at 90°C in 3% (w/v) hydrogen peroxide) of intoplicine (I) (retention time = 6.5 min). The retention time of the degradation product (II) is 75 min.

Table 1
Stability of intoplicine in infusion fluids

		Initial concentration remaining at indicated time (%)*	
	Intoplicine conc. (mg ml^{-1})	0	7 days
0.9% sodium chloride			
4°C; dark	1.0	100	99.5 ± 2.3
4°C; dark	0.1	100	101.5 ± 0.9
22°C; light–dark†	1.0	100	101.0 ± 4.7
22°C; light–dark†	0.1	100	97.9 ± 3.6
5% dextrose			
4°C; dark	1.0	100	102.5 ± 2.4
4°C; dark	0.1	100	102.0 ± 0.3
22°C; light–dark†	1.0	100	102.5 ± 5.1
22°C; light–dark†	0.1	100	99.9 ± 4.4

*Percentages are reported as mean ± SD of three determinations.
†Admixtures were subjected to normal fluorescent room light in a day–night diurnal cycle (8 h dark, 16 h light).

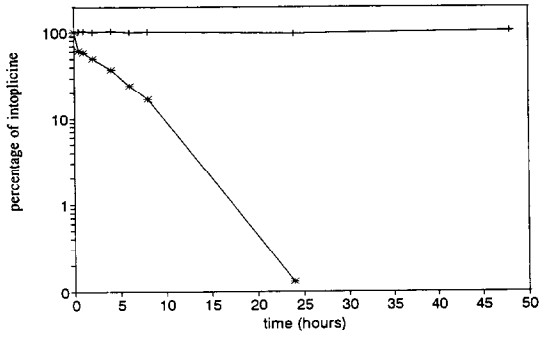

Figure 3
Stability of intoplicine (1 mg ml^{-1} at 90°C) in 0.1 M hydrochloric acid (+) and in 3% (w/v) hydrogen peroxide (*) as a function of time.

and administration by prolonged infusion on the ward.

The results of the stability tests are summarized in Table 1. The data show that intoplicine is stable for at least 7 days under the chosen experimental conditions. Furthermore, the samples remained clear without changes in colour and with no signs of precipitation. During the storage period of 7 days the pH remained constant.

Conclusions

This article reports on the chemical stability of intoplicine in two commonly used infusion fluids. The intravenous fluids were analysed using a HPLC assay. The results showed that, in terms of chemical stability, it is justified to prepare an infusion admixture with intoplicine and store it for 7 days, assuming that bacterial contamination is minimized by the use of proper aseptic technique.

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