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Short Communication

Analytical toxicological studies in cases of suicide by injection of Tanax, a veterinary euthanasia agent

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Introduction

Cases of suicide and homicide by administration of overdoses of curare-like substances are seldom encountered [1–6]. It appears that only one fatal case has been reported involving the use of a veterinary product, this being a mixture of pentobarbital and amobarbital [7]. The present report describes analytical toxicological studies on three cases of suicide involving Tanax, a proprietary combination of a narcotic and a curare-like substance.

Tanax has been marketed by Hoechst since 1961 for veterinary purposes only [8, 9] and contains: 20% m/m *N*-2-ethyl-2-(3-methoxyphenyl)-butyl-4-hydroxybutanamide (embutramide), 5% m/m 4,4'-methylene bis-*N,N,N*-trimethylcyclohexanaminium di-iodide (mebezonium iodide) and 0.5% m/m *p*-butylaminobenzoyl-dimethylamino-ethanol chloride. There is no literature on pharmacokinetic or metabolic data of the two active components of Tanax, so far as the authors are aware. The problem of isolating quaternary ammonium compounds from biological materials has been investigated [10–14], but there are some difficulties, primarily arising from the low solubility of these compounds in most of the common organic solvents. The present method is based on the isolation of mebezonium iodide from biological materials or from drugs by thin-layer chromatography, followed by elution from the silica gel and quantitative estimation by UV-spectrophotometry. The second coformulation constituent, embutramide, is extracted from an acidic aqueous solution by an organic solvent, and quantitatively estimated by UV-spectrophotometry.

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Experimental

Reagents and equipment

Embutramide and mebezonium iodide pure substances were obtained from Hoechst A.G. (Frankfurt-am-Main, FRG) and used without further purification. All inorganic and organic chemicals were of analytical reagent grade.

The qualitative TLC procedure employed standard solutions, comprising aqueous solutions of mebezonium iodide (0.001 mg/ml) and embutramide (0.004 mg/ml); silica gel plates (0.25 mm × 20 cm × 20 cm) obtained from Merck AG (Darmstadt, FRG); and iodoplatinate reagent, prepared by dissolving 0.25 g of platinic chloride and 5 g of potassium iodide in water and diluting to 100 ml with water.

The quantitative UV procedure for embutramide required: calibration standards prepared by spiking blank human whole blood or urine with an appropriate aqueous solution of embutramide to produce 2.5, 5, 10 and 20 μg/ml; and a Perkin Elmer 124 double-beam UV-visible spectrophotometer (spectral band-width 1 nm) with 1 cm silica cells. The quantitative TLC–UV procedure for mebezonium iodide required calibration standards prepared by spiking blank human whole blood or blank urine with an appropriate aqueous solution of mebezonium iodide to produce 2.5, 5 and 10 μg/ml; the same spectrophotometer was employed.

Methods

Qualitative TLC procedure. To 5 ml of urine or blood under investigation, and to 5 ml of blank urine or blood, a 2 ml aliquot of 20% v/v aqueous trichloroacetic acid was added then mixed and centrifuged; 100 μl of each supernatant was spotted on to the TLC plate, together with 10, 15 and 20 μl of each standard solution. After developing the plate in chloroform–ethanol (80:20 v/v) for 10 cm, the plates were dried and sprayed with the iodoplatinate reagent. The R_f values of embutramide and mebezonium iodide were 0.72 and 0.00 respectively and the corresponding spot colours were brown and violet.

Quantitative UV-assay for embutramide. 5.00 ml of blood or urine under investigation, 5.00 ml of each calibration standard and 5.00 ml of blank urine or blood were acidified with 1 ml of 1M HCl and extracted three times with 20 ml of ether by vortex-mixing for 2 min; after centrifugation at 3000 rpm, the aqueous layer was aspirated and discarded. The ether layer was evaporated to dryness and the residue was reconstituted with 5 ml of 0.1N H_2SO_4, and transferred to a 1 cm UV-cell. The UV spectra of the calibration standards (Fig. 1) were measured against the appropriate blank. The absorbance was measured at the maximum near 271 nm. The regression equation for the calibration curve obtained was: $y = 0.042\,x + 0.075$ (correlation coefficient $r = 0.988$). All samples and standards were extracted and analysed five times and the results evaluated statistically as in Table 1. The relative standard deviation (RSD) varied from 1.6 to 6% for the cases examined.

TLC–UV method for mebezonium iodide. To 5.00 ml of urine or blood under investigation, to 5.00 ml of each calibration standard and to 5.00 ml of blank urine or blood, 2.00 ml of 20% v/v aqueous trichloroacetic acid was added and the mixture centrifuged; 100 μl of each supernatant was spotted on to the TLC plates and after developing as described above, the plates were dried. The silica gel layer at the starting point, corresponding to the mebezonium iodide, was scraped off and the mebezonium iodide recovered by extracting with 5.00 ml of distilled water and centrifuging. The

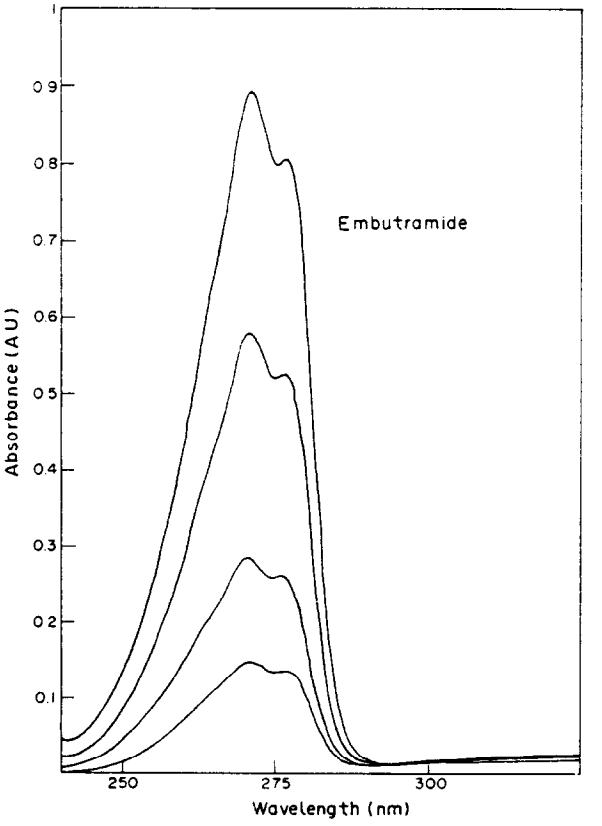

Figure 1
UV spectra of embutramide calibration standards.

Table 1
Embutramide and mebezonium iodide levels in biological specimens

Cases*	Urine (μg/ml; RSD†)		Blood (μg/ml; RSD†)	
	Embutramide	Mebezonium iodide	Embutramide	Mebezonium iodide
R.C.	2.01 (4.0)	0.80 (15.0)	3.00 (6.0)	4.50 (2.7)
B.L.	6.33 (2.2)	1.95 (8.2)	15.48 (1.0)	6.03 (2.5)
D.C.S.	4.49 (2.5)	1.82 (9.3)	12.06 (1.6)	7.48 (2.5)

* Full description in text.
† Relative standard deviation (%); $n = 5$.

aqueous supernatant was transferred to a 1 cm UV-cell; the blank supernatant solution was used as reference and the absorbance was then measured at the maximum near 225 nm. Figure 2 shows the spectra of the calibration standards. The calibration curve regression statistics were: $y = 0.065\,x + 0.19$ ($r = 0.989$). This procedure was applied to the samples under investigation and to the standards five times and the results statistically evaluated. The reproducibility observed is shown in Table 1; the range of

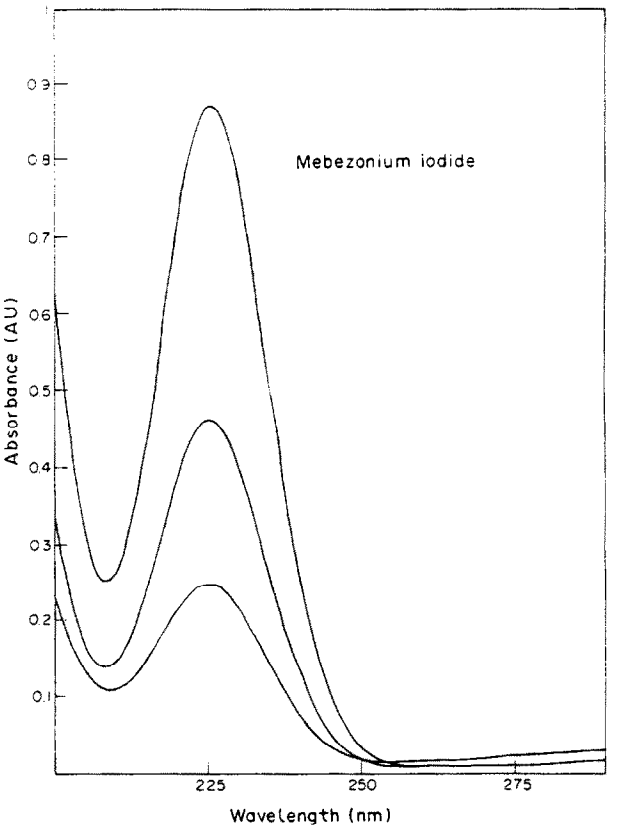

Figure 2
UV spectra of mebezonium iodide calibration standards.

RSD values was narrower for blood samples (1–6%) than for urine (2.2–15%). The recoveries from spiked whole blood samples were 98.5% for embutramide and 90.2% for mebezonium iodide at 5 μg/ml.

Case Histories

(i) R.C., an 80 year-old male veterinary surgeon, with a history of ascending myasthenia, was found in the garden of a nursing-home with a number of i.m. injection marks evident on both legs; a syringe and an empty bottle of Tanax were found near the corpse.

(ii) B.L., a 43 year-old male academic was discovered in bed with a syringe inserted into the vein of his right arm. The syringe was connected via a lift-and-force pump to a bottle of Tanax.

(iii) D.C.S., a 52 year-old male financier was given an injection of Tanax he had himself previously prepared, by a nurse unaware of the true contents of the syringe; 14 ml of Tanax still remained in the syringe when the man lost consciousness and died.

Discussion

The three cases of poisoning from Tanax reported here are particularly interesting

because curare-like substances are rarely encountered in cases of suicide or homicide. In each of the cases described, the blood and urine concentrations of the two components of Tanax are indicative of fatal poisoning.

The method for quantitation of mebezonium iodide used in this study employs ultraviolet spectrophotometry, after separating the embutramide and biological impurities by TLC from mebezonium iodide, which does not elute up the plate. The advantage of the combination of TLC with UV-spectrophotometry is that extraction can be avoided, a point of considerable interest in the detection of quaternary ammonium compounds, in view of the difficulty involved in their isolation from biological materials. The proposed methods represent a new and convenient approach to the rapid analysis of these drugs in analytical toxicology.

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