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Determination of droperidol in plasma by liquid chromatography

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Abstract

A reversed-phase high performance liquid chromatographic method is described for the determination of droperidol concentrations in plasma. Following extraction, separation of droperidol and the internal standard flurazepam was achieved with a Spherisorb Nitrile, 5 μm, S5CN 250 mm × 4.6 mm column at 200 nm. The mobile phase was phosphate buffer (0.05 M, pH 2.4), acetonitrile and ethanol (65:20:15, v/v/v). The assay was rapid, sensitive and linear over the range 2–4000 ng ml^{-1}. Precision of the assay expressed as the intra- and inter-day relative standard deviations (%RSD) did not exceed 10%. Flunitrazepam, midazolam and nitrazepam were also resolved with this technique and did not interfere with droperidol or flurazepam. Resolution of all five compounds was complete in less than 6 min. The assay was used to study the pharmacokinetics of high dose droperidol infusions during and after cardiac surgery.

Keywords: Benzodiazepines; Droperidol; Plasma; Reversed-phase chromatography

1. Introduction

Droperidol (1-{-1-[-4-(*p*-fluorophenyl)-4-oxobutyl]-1,2,3,6,-tetrahydro-4-pyridyl}-2-benzimidazoline) is a butyrophenone with neuroleptic and antiemetic properties, that may be administered in combination with volatile agents during balanced anesthesia or with potent narcotics to produce a state of neuroleptic-analgesia [1]. In many instances droperidol is administered at an empirical dose of 10 mg as a preoperative adjunct; however, at plasma concentrations >300 ng ml^{-1} droperidol has a marked antihypertensive effect via a non-specific vasodilation [2]. This effect, in conjunction with the neuroleptic properties, may make droperidol a useful component of total intravenous anesthesia in long hemodynamically-challenging procedures.

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Cardiac surgery is associated with potent stimuli manifested as episodes of prolonged hypertension and resultant tachycardia and should be an ideal candidate for titrated neuroleptic-analgesia. In order to better understand the pharmacokinetic–pharmacodynamic relationship of high dose droperidol anesthesia and its ability to obtund responses to noxious stimuli in this type of operation, a selective and rapid assay, linear over a wide range of concentrations, was required. Radiolabeling [3] and electrochemical [4] techniques are sensitive but were impractical for the present purposes. Radioimmunoassay methods [5] are also sensitive but may lack selectivity at lower concentrations and are expensive. Modified gas chromatographic procedures [6–9] are laborious due to the amount of clean-up work required.

Existing HPLC methods for the determination of droperidol utilize solid-phase extraction, complex gradient elution [10] or other time-consuming extraction methods [11]. Moreover, retention times are unacceptably long for use with isocratic systems. The method described is rapid, sensitive and makes use of a selective organic extractant (ether:heptane, 90:10, v/v). Resolution of droperidol, the internal standard (IS) flurazepam and structural analogs of the IS (Fig. 1) that may cause interference (flunitrazepam, midazolam and nitrazepam) was complete in less than 6 min.

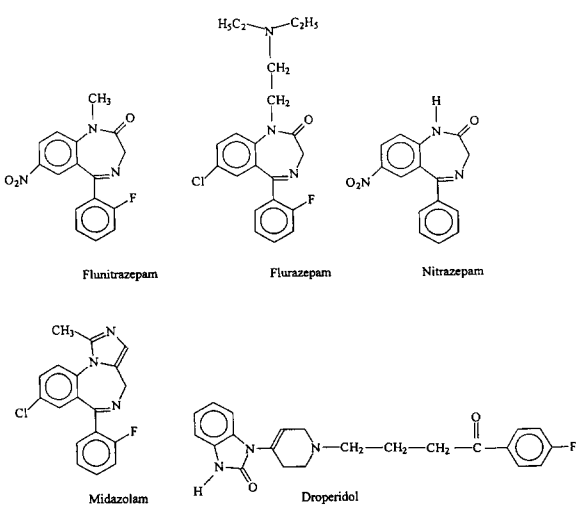

Fig. 1. Chemical structures of flunitrazepam, flurazepam, nitrazepam, midazolam and droperidol.

2. Experimental

2.1. Chemicals

Droperidol was kindly supplied by Jansenn Cilag, NZ and flurazepam, flunitrazepam, midazolam and nitrazepam were donated by Roche, NZ. Analytical-grade heptane and diethyl ether were bought from EM Science. HPLC-grade acetonitrile with 190 nm cutoff (far-UV) and ethanol were purchased from Fischer Scientific and HPLC-grade water was obtained by distillation in glass and passage through a MilliQ purification system (Millipore Corporation, Bedford, MA). All other chemicals and reagents were of analytical grade and were used as received.

2.2. Chromatographic system

A Shimadzu SPD-6AV UV–Vis detector and an LC-6A pump (Shimadzu Corporation, Analytical Instruments Plant, Kyoto, Japan) were connected with an LKB 2157-010 autosampler (Bromma, Sweden) and a Spherisorb Nitrile 5 μm S5CN column (250 mm × 4.6 mm, Phase Separations, Queensferry, UK). All chromatograms were recorded on an Hitachi D-2500 integrator (Naka Works, Hitachi Ltd., Tokyo, Japan). The mobile phase consisted of phosphate buffer (0.05 M adjusted to pH 2.4 with orthophosphoric acid), far-UV-grade acetonitrile and ethanol (65:20:15, v/v/v) and was filtered under suction (0.22 μm, Millipore, Bedford, MA) and degassed with helium before use. The flow rate was 1.4 ml min^{-1} and column effluent was monitored at 200 nm under ambient conditions (18–24°C).

2.3. Sample collection and storage

After institutional approval 17 consenting patients scheduled to undergo myocardial revascularization and/or valvular replacement surgeries entered the study. Anesthesia was induced with thiopentone and maintained with a variable rate of droperidol infusion and supplemental fentanyl. 5 ml blood samples were withdrawn via a radial artery cannula at preselected times before, during and after surgery and were stored on crushed ice

in heparinized vacutainers. Following centrifugation, plasma was either analyzed immediately or was frozen at −72°C. Spiked plasma standards prepared at the commencement of the trial were stored under similar conditions to act as quality control samples and were randomly placed into each analytical sequence.

2.4. Standard solutions

Stock solutions of droperidol and benzodiazepines were prepared in methanol (50 μg ml^{-1}). The working concentrations of droperidol (20 ng ml^{-1}–20 μg ml^{-1}) and the IS flurazepam (10 μg ml^{-1}) and further dilutions of the other benzodiazepines were made with double distilled water. The solutions were stored at 4°C and showed no significant alterations in peak heights or areas (95–105%) determined daily by direct injection throughout the course of the study. Droperidol standards for analysis were made in drug-free human plasma by dilution to the final concentrations 2, 5, 10, 20, 50, 100, 200, 500, 1000, 2000 and 4000 ng ml^{-1}.

2.5. Sample preparation

To 1 ml of plasma in a silanized centrifuge tube 200 μl of potassium hydroxide (0.5 M), 100 μl of the internal standard (flurazepam, 10 μg ml^{-1}) and 8 ml of diethyl ether:heptane (90:10, v/v) were added. The mixture was shaken horizontally at 2 Hz for 10 min and then separated by centrifugation (1000*g*, 10 min). Approximately 7.5 ml of the organic layer was transferred to a second tube and removed by rotary evaporation (600*g*, 45°C) under vacuum (Savant Instruments Inc., Farmingdale, NY). The unit was purged with oxygen-free nitrogen before the vacuum was applied. The residue was reconstituted with 200 μl of mobile phase and 100 μl was injected onto the column.

3. Results and discussion

The retention and resolution characteristics of nitrazepam, flunitrazepam, droperidol, midazolam and flurazepam under the described chromatographic conditions are shown in Fig. 2. Decreasing the concentration of the acetonitrile component of the mobile phase from 20% to 15% gave slightly better resolution and longer retention times, but was only necessary when interference was detected in a patient blank. An increase in the ethanol concentration from 10% to 15% of the mobile phase dramatically improved the peak shape of all five drugs. Further increases of the ethanolic component did not show any improvement. Decreasing the pH (2.4 to 2.2) and increasing the molarity (0.05 to 0.1 M) of the buffer component improved the peak resolution further but this benefit was outweighed by increased column wear and the risk of precipitation during analysis.

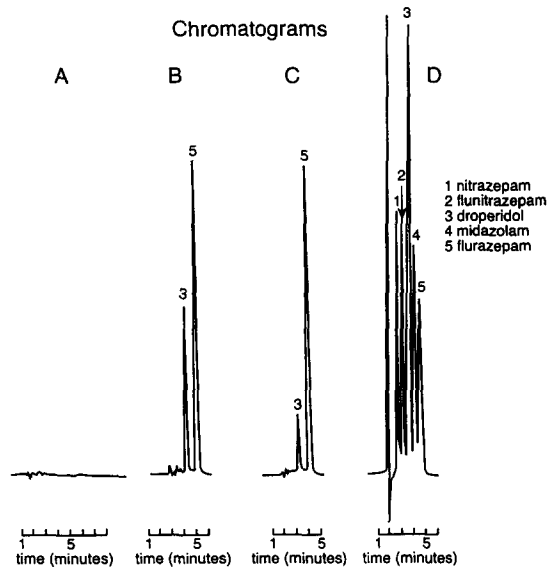

Fig. 2. Chromatograms of (A) blank plasma, (B) plasma spiked with droperidol (500 ng ml^{-1}), (C) a patient sample (droperidol concentration of 182 ng ml^{-1}), and (D) plasma spiked with concentrations of 200 ng ml^{-1} nitrazepam, 125 ng ml^{-1} flunitrazepam, 175 ng ml^{-1} droperidol, 100 ng ml^{-1} midazolam and 75 ng ml^{-1} flurazepam.

Retention times and capacity factors for droperidol and benzodiazepines were calculated according to Kirkland [12] and are shown in Table 1. From 22 calibration curves ($n = 216$) over the range 2–4000 ng ml^{-1} a high correlation ($r = 0.98$) was observed between peak-height ratios of droperidol and the IS. The equation for the rela-

Table 1
Retention times (t_R) and capacity factors (k') of droperidol and four benzodiazepines

Compound	t_R (min)	k'
Nitrazepam	2.96	0.48
Flunitrazepam	3.47	0.74
Droperidol	4.06	1.03
Midazolam	4.46	1.23
Flurazepam	4.88	1.44

tionship between peak-height ratio (y) and plasma concentration of dropreidol (x) was: $y = 0.00108x + 0.00052$. Intra- and inter-day variability of the HPLC procedure were determined by replicate analysis of plasma standards containing 5, 20, 200, 1000 and 2000 ng ml^{-1} of droperidol. The results are shown in Table 2.

The limit of quanification (2 ng ml^{-1}) was set as the lowest concentration with a relative standard deviation (RSD) of less than 10%. The limit of detection (0.5 ng ml^{-1}) was the smallest concentration capable of producing a signal-to-noise ratio greater than three. No indication of droperidol degradation on storage in frozen plasma was apparaent over the time course of the study (3 months).

Table 2
Intra- and inter-day precision and accuracy of the droperidol assay procedure

Parameter	Theoretical droperidol plasma concentration (ng ml^{-1})				
	5	20	200	1000	2000
Intra-day					
Samples (n)	5	5	5	5	5
Mean	4.7	19	189	996	2017
±SD	0.3	0.9	8.3	40.8	80.3
Precision (%RSD)	6.3	4.5	4.4	4.1	4.0
Accuracy (%)	94	95	94.5	99.6	100.9
Inter-day					
Samples (n)	10	10	10	10	10
Mean	4.7	19.2	192	987	1996
±SD	0.4	1.2	11.1	53.3	103.8
Precision (%RSD)	8.6	6.1	5.8	5.4	5.2
Accuracy (%)	94	96	96	98.7	99.8

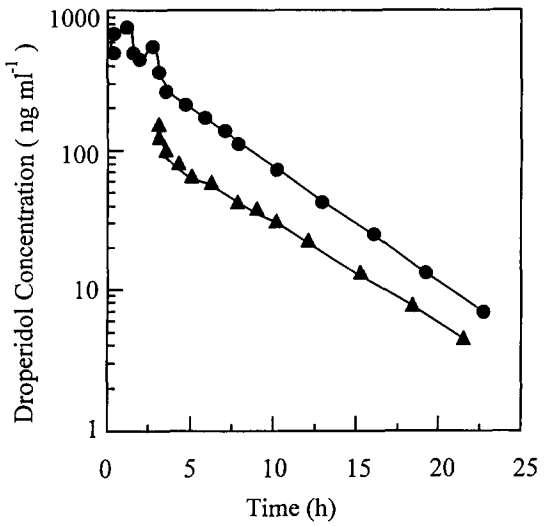

Fig. 3. Concentration–time profiles of droperidol in two subjects: (●) 425 mg intra-operatively and (▲) 10 mg post-operatively.

The extraction efficiencies of droperidol and flurazepam (92.1 ± 4.8% and 83.7 ± 5.2% respectively) were determined by comparing the areas and heights of spiked samples to known concentrations. The best ratio of ether:heptane was found to be 90:10 v/v. Increasing the amount of heptane caused an increase in the extraction efficiency of nitrazepam, which is poorly soluble in ether [13], but increased the time required to evaporate the organic layer. Decreasing the amount of heptane caused a noticeable decrease in the extraction of droperidol and greater baseline noise after the residue was reconstituted and injected.

The concentration–time profiles of two subjects of similar age, weight and health are shown in Fig. 3. The first subject received droperidol as a high dose variable rate infusion (total intra-operative dose of 425 mg) with supplemental fentanyl to maintain anesthesia. The second subject underwent similar surgery with a standard fentanyl–isoflurane technique and had a 10 mg bolus dose of droperidol administered post-operatively. The half-line of droperidol was

found to be 3.47 and 4.22 h respectively. The terminal elimination half-life of droperidol is approximately 2.5 h in subjects not undergoing cardiac surgery [1,14].

An increase in elimination half-life following cardiac procedures is due to many factors that combine to alter preoperative physiology. Changes in fluid balance, body temperature and protein binding cause alterations in enzymatic rates and regional blood flow leading to decreased clearance and wide variability in the rate and extent of drug distribution. These changes are associated with the cardio-pulmonary bypass period unique to this type of procedure and may linger for an extended time post-operatively. Another possible reason for this increased half-life may be the higher than normal plasma concentrations of droperidol that are required to both prevent hypertensive episodes and maintain a pseudo-anesthetic state of non-responsiveness in the patient. The two patients considered received 425 mg and 10 mg respectively but the droperidol half-lives were similar. This indicates that decreased elimination of droperidol following cardiac surgery does not appear to be dose-dependent.

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