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Note

Picogram level determination of medetomidine in dog serum by capillary gas chromatography with negative ion chemical ionization mass spectrometry

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Medetomidine, 4-[1-(2,3-dimethylphenyl)ethyl]-1*H*-imidazole hydrochloride, was recently introduced as an analgesic sedative for dogs and cats. Medetomidine is more selective as a full agonist for central α_2-adrenoceptors than clonidine and is available as both an active (dex) and an inactive (levo) isomer [1]. Dexmedetomidine is under investigation in humans for use as premedication before anaesthesia and surgery. The pharmacokinetics of medetomidine administered as a single dose has been studied in rat, dog and cat with tritium-labelled drug [2]. Further pharmacokinetic studies have not been performed owing to the lack of a suitable (non-radioactive) analysis method. This paper describes a new gas chromatographic–mass spectrometric (GC–MS) assay for the determination of medetomidine in serum.

EXPERIMENTAL

Materials

Dexmedetomidine hydrochloride and the internal standard detomidine hydrochloride, 4-[1-(2,3-dimethylphenyl)methyl]-1*H*-imidazole hydrochloride, in pure crystalline form were obtained from the synthesis laboratories of Farmos (Oulu, Finland). 2,3,4,5,6-Pentafluorobenzoyl chloride (PFBCl) was from Fluka (Buchs, Switzerland). Other reagents were of analytical grade.

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Sample preparation

Extractive benzoylation was modified from Delbeke and Debackere [3]. To a 10-ml PTFE-lined screw-capped round-bottomed centrifuge tube were added 1 ml of 1 *M* sodium carbonate, 1 ml of serum, 500 pg of detomidine dissolved in 30 μl of water, 7 ml of *n*-hexane and 5 μl of PFBCl. Calibration samples were spiked with the following concentrations of dexmedetomidine: 0, 50, 100, 300, 500 and 700 pg/ml. The contents were mixed at ambient temperature for 30 min and centrifuged for 5 min at 1300 *g*. Next, 6 ml of the *n*-hexane layer were transferred to another 10-ml tube and evaporated until just dry under a gentle stream of air at 50°C. The residue was dissolved in 200 μl of toluene, and an aliquot (1 μl) was injected into the gas chromatograph. The column was washed by injecting 1 μl of toluene before each sample.

GC–MS conditions

GC–MS measurements were made with a Finnigan MAT TSQ 70 mass spectrometer equipped with an HP 5890A gas chromatograph. The GC column was a Hewlett-Packard fused-silica capillary column (25 m × 0.20 mm I.D.) packed with cross-linked 5% phenyl methyl silicone (HP Ultra 2). The film thickness was 0.11 μm. Helium was used as the carrier gas at a linear flow-rate of 35 cm/s. The GC oven was programmed as follows: 1 min at 90°C, 35°C/min up to

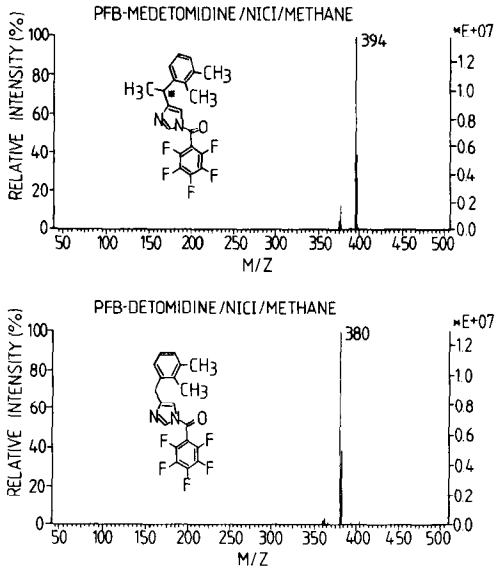

Fig. 1. Negative ion chemical ionization mass spectra and molecular structures of PFB derivatives of medetomidine and detomidine. The asymmetric carbon atom of medetomidine is marked with an asterisk.

220°C, 10°C/min up to 250°C, 35°C/min up to 275°C and 1.6 min at 275°C. The injector and transfer line temperatures were 275°C.

The MS operating mode was negative ion chemical ionization (NICI) selected-ion monitoring (SIM). Methane was used as the reagent gas. The ion source pressure reading given by a digital pressure display panel was 4.9 Torr. The source and manifold temperatures were 150 and 70°C, respectively. The electron energy was 70 eV. The emission current and electron multiplier voltage were 200 μA and 1.6 kV. Pentafluorobenzoyl derivatives (M^-) of detomidine (m/z 380.1) and dexmedetomidine (m/z 394.1) were the ions selected for detection. The peak-area ratio was calculated. The molecular structures and NICI mass spectra of the compounds can be seen in Fig. 1.

In vivo study

Dexmedetomidine (2 μg/kg) was administered into the left-side thigh muscles of a male beagle dog weighing 11.9 kg. From the evening before drug dosing until 4 h post dosing no food was available. Water intake was not restricted. Blood samples (5 ml) were drawn from the right front leg at 0, 0.167, 0.333, 0.75, 1, 1.5, 2, 3, 4 and 6 h post administration. Serum was separated and frozen for storage at −20°C until analysis.

RESULTS AND DISCUSSION

Chromatographic separation and MS detection

Taking into account the results of preliminary pharmacokinetic studies [2], subnanogram detection would be highly desirable at least for the future analysis of human samples. The physicochemical properties of medetomidine, however, are not in favour of a low detection limit. In high-performance liquid chromatography, for example, the molar absorptivity (ca. 10 000) of medetomidine does not allow for sensitive detection. Furthermore, its absorption maximum is in the low UV region below 220 nm, where most other molecules would absorb and cause interferences. Medetomidine also lacks native fluorescence.

Medetomidine and detomidine injected as free bases have the tailing peak shapes typical of nitrogen-containing compounds in a fused-silica capillary column. Masking the imidazole nitrogen by an appropriate derivatization would greatly reduce tailing, and so different derivatives were examined. The N,O-bis(trimethyl)trifluoroacetamide (BSTFA) derivatives of imidazoles in acetonitrile were made, and the resulting silyl derivatives gave better peak shapes. However, good sensitivity could not be achieved with positive ion chemical ionization (PICI).

Suitable halogen substituents could be introduced to enhance detectability by electron-capture detection (ECD) or by NICI-MS [4–6]. Imidazole reacts easily with a variety of derivatizing agents but, being a good leaving group, it also easily transfers the reactive moiety to another molecule. Consequently

heptafluorobutyryl and trifluoroacetyl derivatives of imidazoles are unstable. Trichloromethyl chloroformate derivatives give excellent chromatographic properties but the sensitivity is less than satisfactory, because the background in GC–ECD is too high for subnanogram analysis. Owing to the unfavourable isotope ratio of the halogen (Cl) in this molecule, optimal sensitivity in GC–NICI-MS cannot be achieved.

PFB derivatives of medetomidine and detomidine show good chromatographic properties and adequate sensitivity, except for the high reagent noise level in ECD. The sensitivity achieved in GC–ECD (as detection limit) is 1–2 ng/ml. Analysis of PFB derivatives by electron impact led to extensive fragmentation. Molecular ions were observed in low abundances. Under PICI conditions simple mass spectra were observed, but the detection sensitivity was not sufficient.

NICI-SIM effectively lowers the noise level. It follows that femtograms of the PFB derivatives can be detected, as shown by the results. At serum level this transforms to a detection limit of ca. 10 pg/ml. At present this is far beyond the limit of any other method of medetomidine quantitation. Since detomidine is used as the internal standard the method can be easily modified to detect this large animal sedative (Domosedan®, Farmos) in serum.

Quantitative analysis

The calibration curve obtained by assaying spiked serum samples was linear in the measured range 50–700 pg/ml. The linear regression equation was $y=0.0895+0.00310x$ ($r^2=0.9855$). The lower limit of quantification was 50 pg/ml (spiked serum), although it was possible to get a distinguishable signal down to 10 pg/ml (pure standards). The recovery from serum was determined by ten-fold assay of 100 and 500 pg/ml spiked serum concentrations. Extraction yields were 77.8 ± 2.1 and $88.7\pm8.0\%$, respectively. The precision of the method was defined as the relative standard deviation of the amounts found by ten-fold assay of 50 and 500 pg/ml concentrations (Table I). The accuracy of the method was defined as the deviation between the amount added to blank serum and the amount found (Table I).

TABLE I

PRECISION AND ACCURACY OF THE METHOD ($n=10$)

Concentration added (pg/ml)	Concentration found (mean ± S.D.) (pg/ml)	Precision (%)	Accuracy (%)
50	47.0± 9.0	19.1	−6.0
500	490.8±31.4	6.4	−1.8

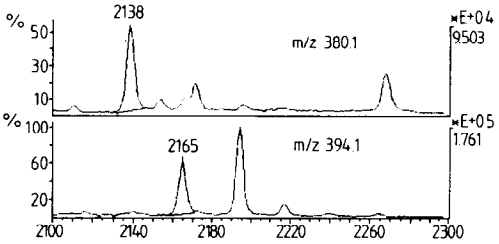

Fig. 2. Selected-ion chromatograms of extracted serum of a dog 2 h after a single dose of 2 μg/kg dexmedetomidine. Scan 2138, internal standard; scan 2165, dexmedetomidine.

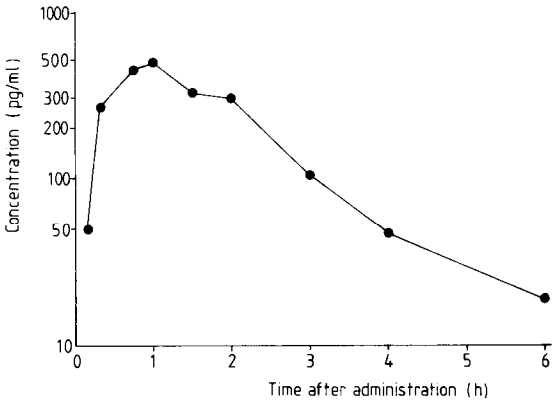

Fig. 3. Concentration–time curve of dexmedetomidine in dog serum after intramuscular administration of 2 μg/kg.

Application

Mass chromatograms of a beagle dog serum extract 2 h after a single 2 μg/kg intramuscular dose of dexmedetomidine can be seen in Fig. 2. The retention time of internal standard is 7.1 min, and that of dexmedetomidine 7.2 min. The serum concentration–time curve for dexmedetomidine is shown in Fig. 3. The peak concentration (495 pg/ml) was observed at 1 h; the half-life of absorption was 9 min and the half-life of elimination was 55 min. These results are comparable with the data obtained with radiolabelled medetomidine [2].

CONCLUSION

The GC–MS method proved to be both selective and sensitive. Low picogram concentrations of dexmedetomidine can be detected in serum.

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