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Note

New procedure for the high-performance liquid chromatographic determination of amiodarone and desethylamiodarone with solid-phase extraction of rat plasma and tissue samples

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Knowledge of the distribution and elimination of highly lipophilic substances with long terminal half-lives is an important basis for understanding the pharmacodynamic and toxicological properties of these drugs. The antiarrhythmic drug, amiodarone, is a very lipophilic, basic drug [1], which accumulates in different organs and tissues, e.g. in adipose tissue, in long-term therapy [2]. However no animal data are available as yet on the long-term distribution kinetics of amiodarone.

A number of high-performance liquid chromatographic (HPLC) methods for the determination of amiodarone (A) and its major lipophilic metabolite, desethylamiodarone (DEA), in tissues and/or plasma have been published in recent years [3–18]. Most have been developed for therapeutic monitoring, and thus take into consideration plasma or serum only. Tissues have been included in a small number of these published methods [5,7,9,15,18]; solid-phase extraction in two cases only [17,18].

In preliminary experiments based on published reports, we obtained relatively low extraction recoveries of A, DEA and of the internal standard L8040 (I.S.) from plasma and tissue samples. Furthermore, co-extracted endogenous contaminants interfered with the peaks of the investigated compounds. In this paper we present a new procedure for liquid extraction and HPLC analysis of A and DEA, in combination with a solid-phase extraction for clean-up of plasma and tissue extracts. It has provided improved recoveries and accuracy of the peak quantification.

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EXPERIMENTAL

Chemicals

A, DEA, the I.S. [3,5-dibromo-4-[3-(dipropylamino)propoxy]phenyl](2-ethylbenzo[B]thiene-3-yl) methanone (L8040) and ^{14}C-labelled amiodarone (ring-labelled, 50 mCi/mmol) were generously given by Sanofi (Montpellier, France). Methanol of HPLC quality was purchased from Merck (Darmstadt, F.R.G.). All other chemicals used were of analytical quality.

Animal experiments

Male rats of the Sprague–Dawley-derived strain SIV-Z (Tierzucht-Institut, Zurich, Switzerland) of 250 g body mass were given single bolus ^{14}C-labelled A (50 mg/kg). The drug was administered intravenously into the femoral vein under light ether anesthesia. After various times the animals were killed by decapitation and immediately dissected. Samples were stored at $-20°C$.

Radioactivity determination

Radioactivities were determined in rat tissue homogenates, plasma and their extracts. The recoveries of total radioactivity in the extracts could be used to characterize the analytical recoveries of A and DEA. For the determination of the radioactivity, 0.2 ml of plasma, tissue homogenates or extracts were mixed with 0.8 ml of water and 10.0 ml of a scintillation mixture (Kontrogel, Kontron, Switzerland). The radioactivity was determined on a Kontron MR 2000 liquid scintillation counter using external standardization for quench correction.

HPLC instrumentation and calibration

A Kontron liquid chromatograph was used, equipped with two pumps, a gradient programmer, a variable-wavelength detector LC 730 set at 240 nm and a Model 7125 Rheodyne injection valve. The separation was performed on a 3-μm Spherisorb ODS2 column (15 cm $\times$ 4.0 mm I.D.; Phase Separations, Queensferry, U.K.) connected to a guard column (4.0 cm $\times$ 4.6 mm I.D.) packed with 30-μm Perisorb RP-18, both purchased from Merck (Darmstadt, F.R.G.). The mobile phase consisted of methanol containing 1.5% (v/v) water and 0.7% ammonia (33%) according to Plomp et al. [7]. The flow-rate was 1.3 ml/min. The peak areas were computed by a Hewlett-Packard Model 3380A integrator. Calibration curves were obtained using methanolic standard solutions containing 1, 3, 5, 7.5 and 10 μg/ml each of A and DEA. A 100-μl volume of these solutions was mixed with 50 μl of I.S. solution (20 μg/ml) and 100 μl of water, and processed on a disposable RP-8 column as described below. For the determination of the calibration function, peak-area ratios (A and DEA to I.S.) were plotted against the standard concentrations. In the observed concentration range, the relationship was linear with correlation coefficients better than 0.999. The sensitivity was less than 4 ng for both A and DEA in standard solutions.

Assay procedure

A mixture of 0.5 ml of heparinized plasma and 2.0 ml of acetonitrile was sonicated for 20 s. After centrifugation for 10 min (2000 *g*) the clear supernatant was collected and the pellet was reextracted twice with 1.0 ml of acetonitrile. The volume of the combined acetonitrile extracts was adjusted to 5.0 ml. For the solid-phase extraction, these plasma extracts were diluted with 2.5 ml of 0.2 *M* ammonia, and 50 μl of I.S. solution (20 μg/ml) were added.

Adipose tissue samples (1–2 g) were homogenized in hexane (tissue concentration 20%, w/v) with a tissue grinder and centrifuged. To 250 μl of the clear hexane extract 750 μl of hexane and 50 μl of I.S. solution (20 μg/ml) were added and extracted with 2.0 ml of 20% (v/v) 1 *M* hydrochloric acid in methanol for 30 min in a horizontal shaker. After centrifugation the hexane phase was discarded and the methanolic fraction was diluted with 1.0 ml of 0.2 *M* ammonia. This solution was further processed using the solid-phase extraction method described below.

With all other tissues, samples of 1–2 g were homogenized in methanol containing 1% (v/v) of 1 *M* hydrochloric acid. The homogenates (20%, w/v) were centrifuged (2000 *g*, 10 min) and the clear supernatants collected. The pellets were re-extracted twice with 2.0 ml of methanol–hydrochloric acid by shaking the suspensions for 10 min. The volumes of the combined extracts were adjusted to a homogenate concentration of 10% (w/v). Prior to the solid-phase extraction, 250–500 μl of the methanolic extracts were mixed with 50 μl of I.S. solution (20 μg/ml) and 250–500 μl of 0.2 *M* ammonia.

Solid-phase extraction

The extraction procedure was performed with disposable reversed-phase columns (RP-8, LD) pruchased from J.T. Baker (Phillipsburg, NJ, U.S.A.). For column conditioning and elution two methanolic solutions were used. Solution I contained 1% (v/v) 1 *M* hydrochloric acid and solution II contained 20% (v/v) water. The solid phase was conditioned by flushing the column with 4.5 ml of solution I and then 1.5 ml of solution II. After this, the sample (0.1–10.0 ml) was passed through the column under vaccuum, and the column was rinsed with 0.25 ml of solution II. A, DEA and I.S. were eluted with 0.7 ml of solution I, and the eluate was collected in a reaction tube. For the HPLC analysis the solvent was evaporated under nitrogen, the residue was redissolved in 100–300 μl of mobile phase, and 20 μl were injected into the liquid chromatograph.

RESULTS

Extraction recoveries

In plasma and tissue samples of rats treated with ^{14}C-labelled A, the recoveries of the radioactivity in the extracts were routinely determined. In plasma samples obtained 1 h after intravenous drug administration, the recovery of total drug after one extraction step was 70.9%. After one and two re-extractions of the pellets, the recovery increased to 86.2 and 92.1%, respectively. More than 98% of the radioactivity could be detected in tissue extracts after three extraction steps.

Solid-phase extraction

The solid-phase extraction procedure was designed for a quantitative adsorption of the compounds on the reversed-phase material. With a water content of 30–50% (v/v) in the methanolic sample solution, no losses of the compounds occurred even when the sample volumes were increased to 10.0 ml. Furthermore, the procedure was shown to be independent of the amount and kind of biological material in the sample. Various volumes (0.5–2.0 ml) of spiked liver extracts (concentrations of A and DEA were 0.250 and 0.125 μg/ml, respectively) were mixed with water and 50 μl of the I.S. solution, and worked up as described. Compound-to-I.S. ratios were normalized to the smallest volume processed, and no significant changes of peak-area ratios could be found.

After solid-phase extraction the columns were flushed with a small volume of methanol–water. This step led to a reduction of peak interferences at the beginning of the chromatograms. With 0.7 ml of methanol containing 1% 1 *M* hydrochloric acid all of the adsorbed A, DEA and I.S. could be eluted from the modified silica phase. The addition of hydrochloric acid allowed the elution volume to be reduced.

Following the elution step, the columns were rinsed with a larger volume of acidified methanol and a solution of 20% (v/v) of water in methanol. This treatment allowed the columns to be used up to twenty times with constant performance.

High-performance liquid chromatography

The HPLC conditions mentioned above led to a baseline separation of A, DEA and I.S. in less than 8 min (Fig. 1). With the solid-phase procedure described, interference from other sample constituents could be substantially reduced for plasma and tissue samples (Fig. 1).

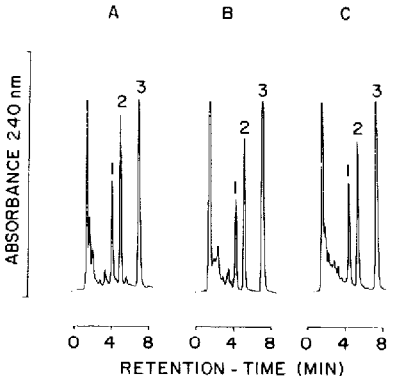

Fig. 1. Chromatograms of samples from rat plasma (A), adipose tissue (B) and muscle (C). Blank plasma was spiked with 1.0 μg/ml A and 0.5 μg/ml DEA, and the tissue samples with 20 μg/g A and 10 μg/g DEA. The samples were redissolved in 200 μl of mobile phase after the solid-phase extraction. The injection volume was 20 μl. Peaks: 1 = DEA 2 = A; 3 = I.S.

TABLE I

REPRODUCIBILITY OF THE HPLC DETERMINATION OF AMIODARONE IN PLASMA AND TISSUE SAMPLES

Amiodarone was injected intravenously (50 mg/kg), and the rat was killed 1 h after administration. Samples were analysed on three different days, and the coefficients of variation were computed.

Sample	Mean amiodarone concentration (μg/ml or μg/g)	Coefficient of variation (%)	
		Within-day ($n=4$)	Day-to-day ($n=3$)
Plasma	7.8	1.8	4.8
Lung	319	3.3	3.2
Liver	303	2.2	1.5
Heart	152	2.3	4.1
Kidney	144	2.8	4.9
Muscle (psoas)	24.6	2.1	5.4
Brain	22.1	3.4	3.1
Adipose tissue (epididymal fat)	18.3	2.6	5.4

Reproducibility

Plasma and tissue samples of rats killed 1 h after intravenous administration of 50 mg/kg A were used to test the reproducibility of the solid-phase extraction method. Plasma and tissue extracts were processed on three different days and the coefficients of variation were computed. The concentrations of A in the extracts were between 0.5 and 30 μg/ml. Within-day variations were found to be between 1.8 and 3.4%, and day-to-day variations between 1.5 and 5.4% (Table I).

CONCLUSIONS

Combination of protein precipiation and liquid extraction led to almost quantitative recoveries of A, DEA and I.S. from plasma and tissue samples. Chromatographic interferences of co-extraced sample constituents could be substantially reduced by further purification of the extracts, using disposable reversed-phase minicolumns. Hydrophilic impurities were removed by this solid-phase extraction procedure, which resulted in an improvement of peak quantification. The solid-phase extraction technique proved to be independent of sample volume and the variety of the biological starting material. As a consequence, calibration curves derived from aqueous standard solutions were used for A and DEA quantification in plasma and tissue samples. Very low sample concentrations could be assessed by increasing the volumes processed by solid-phase extraction.

The described has been successfully used for a study (to be published) on the distribution and elimination kinetics of A and DEA in rats on a much longer time-scale than has been done so far. In plasma, the drug and metabolite concentrations were determined in the range 0.030–30.0 μg/ml and in tissues in the range 0.1–500 μg/g. Compared with other recently published methods where

solid-phase extraction is used for the preparation of plasma and myocardial tissue samples, our method allows the reliable quantification of A and DEA in other tissue samples, including adipose tissues.

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REFERENCES

1 P. Châtelain and R. Laurel, Sanofi Report RS200840424/ECl, 1984.
2 L. Harris and R. Roncucci (Editors), Amiodarone, Medsi, Paris, 1986.
3 R. Flanagan, G. Storey and D. Holt, J. Chromatogr., 187 (1980) 391.
4 L. Lesko, A. Marion, A. Canada and C. Haffajee, J. Pharm. Sci., 70 (1981) 1366.
5 E. Riva, M. Gerna, P. Neyroz, R. Urso, L. Bartosek and A . Guaitani, J. Cardiovasc. Pharmacol., 4 (1982) 270.
6 M. Anastasiou-Nana, G. Levis and S. Moulopoulos, J. Clin.Pharmacol. Ther. Toxicol., 20 (1982) 524.
7 T. Plomp, M. Engels, E. Robles de Medina and R. Maes, J. Chromatogr., 273 (1983) 379.
8 N. Mostow, D. Noon, C. Myers, L. Rakita and J. Blumer, J. Chromatogr., 277 (1983) 229.
9 G. Storey, P. Adams, R. Campbell and D. Holt, J. Clin. Pathol., 36 (1983) 785.
10 L. Duranti, M. Caracciolo and G. Oriani, J. Chromatogr., 277 (1983) 401.
11 J. Shipe, Clin. Chem., 30 (1984) 1259.
12 A. Hutchings, B.P. Spragg and P.A. Routledge, J. Chromatogr., 382 (1986) 389.
13 M. Bliss, M. Mayersohn and P. Nolan, J. Chromatogr., 381 (1986) 179.
14 S. Lam, J. Chromatogr., 381 (1986) 175.
15 R. Kannan, S. Miller, V. Perez and B.N. Singh, J. Chromatogr., 385 (1987) 225.
16 K. Ress, H.M. Liebich, B. Krämer, O. Ickrath, T. Risler and L. Seipel, J. Chromatogr., 417 (1987) 465.
17 P.T. Pollak, S.G. Carruthers and D.J. Freeman, Clin. Chem., 32 (1986) 890.
18 J.A. Menius, Jr., D.J. Schumacher, E.A. Hull-Ryde, C.Y. Leung, R.G. Cummings and J.E. Lowe, J. Liq. Chromatogr., 10 (1987) 2625.