0090-9556/85/1306-0646$02.00/0
DRUG METABOLISM AND DISPOSITION
Vol. 13, No. 6
Printed in U.S.A.

TISSUE UPTAKE AND METABOLISM OF AMIODARONE AFTER CHRONIC ADMINISTRATION IN RABBITS

R. KANNAN, S. MILLER, AND B. N. SINGH

Department of Medicine, UCLA School of Medicine, and Department of Cardiology, Wadsworth VA Medical Center

(Received February 11, 1985; accepted August 6, 1985)

ABSTRACT:

This study was designed to determine serum and tissue concentrations of amiodarone and its metabolite desethylamiodarone after chronic amiodarone administration in rabbits. Rabbits were administered 20 mg/kg amiodarone for 6 weeks. Serum, liver, kidney, heart, lung, spleen, bile, adipose tissue, and muscle were collected upon sacrifice. Amiodarone and desethylamiodarone concentrations were determined in serum and tissues by an HPLC procedure standardized in our laboratory. Amiodarone concentrations were the highest in fat tissue followed by lung, liver, and muscle, with the lowest concentration in serum and only traces in the brain. Desethylamiodarone concentrations in liver, lung, and kidney approached those of the parent drug while the metabolite was present in negligible amounts in fat tissue and in brain. Bile from amiodarone-treated rabbits showed the presence of two more new metabolites which have not been characterized. Desethylamiodarone/amiodarone ratios in serum and tissues after chronic administration in rabbits were lower than in man. Nevertheless, differential accumulation of amiodarone and desethylamiodarone may be relevant to efficacy and toxicity studies.

Amiodarone, a benzofuran derivative (fig. 1), is an effective antiarrhythmic drug, particularly against ventricular and supraventricular arrhythmias (1, 2). Amiodarone has a slow onset and offset of action consistent with its unusual pharmacokinetic property of a long elimination half-life ranging from 13–155 days (3, 4). During long term therapy, serum concentrations of desethylamiodarone, the major metabolite of amiodarone (fig. 1), could approach that of the parent drug (3–5). Data on the metabolism and tissue accumulation of amiodarone during chronic treatment are scarce. Riva *et al.* (6) reported serum and tissue levels of amiodarone after a single iv administration in rats. Holt *et al.* (4), Maggioni *et al.* (7), and Debbas *et al.* (8) determined the tissue concentrations of amiodarone and its metabolite in post-mortem and biopsy samples from patients who were treated with amiodarone chronically. They found that amiodarone, being lipophilic, accumulated extensively in fat tissue followed by liver, lung, and kidney. Liver and kidney also contained large amounts of desethylamiodarone.

In our previous work, we have used the rabbit as an experimental model for studying the pharmacokinetics (9) and electrophysiology (10) of amiodarone. We showed that the elimination of amiodarone and the metabolite from serum followed a biexponential function after a single iv administration with elimination half-lives of approximately 3 hr. There was a rapid uptake of both compounds by the rabbit myocardium. Myocardial concentrations were several-fold higher than those of serum and declined in parallel to those of serum as a function of time (9). Neither the drug nor the metabolite produced significant changes in electrocardiograms after acute administration, in contrast to chronic treatment in which a significant prolongation of action potential of cardiac fibers was shown (10). In an effort to correlate the efficacy and toxicity of amiodarone with its tissue accumulation, we have, in the present study, investigated the tissue uptake and metabolism of amiodarone during chronic administration in the rabbit.

This work was supported by grants from the Medical Research Service of the Veterans Administration and the American Heart Association, the Greater Los Angeles affiliate. Part of the work from this manuscript was presented at the meeting of the Federation of American Societies for Experimental Biology in April 1985.

Send reprint requests to: Ramaswamy Kannan, Ph.D., Cardiovascular Research Laboratory, Building 115, Room 312, VA Wadsworth Medical Center, Los Angeles, CA 90073.

Materials and Methods

Animal Experiments. Male New Zealand White rabbits with initial mean body weight of 3.12 ± 0.19 kg were divided into two groups of six rabbits each. One group was administered amiodarone (20 mg/kg body weight) ip as a 5% aqueous solution for 6 weeks. The other group was administered the same amount of 0.9% saline and served as the control group. The administration protocol consisted of injection of either the drug or saline as a single dose ip around 9 a.m. Monday to Thursday, double the dose on Friday, and no injections on the weekends. Body weights were monitored twice a week. Mean body weights of amiodarone-treated rabbits (2.83 kg) were significantly lower ($p < 0.01$) than those of untreated controls (3.41 kg).

On the day of the experiment, venous blood (2 ml) was collected from rabbits kept in restraining cages under light anesthesia (Nembutal, 30 mg/kg body weight) for measurement of trough drug concentrations. Collection of blood for serum drug levels and sacrifice of rabbits were done on Tuesday, Wednesday, and Thursday because of the dosing schedule described above (11). The animals were sacrificed by administration of an overdose of Nembutal (50 mg/kg) within 30 sec. The following whole organs were dissected; liver, lung, heart, spleen, kidney, and brain. Bile was collected in a graduated tube and its volume was recorded. The tissues were blotted dry and weighed. Portions of inguinal adipose tissue as well as muscle from the lower back of the animal were also removed. All tissues were kept frozen at −20°C until analysis.

Chemicals. Amiodarone hydrochloride powder, desethylamiodarone hydrochloride, and L8040, a brominated analog of amiodarone, were obtained from Labaz Inc., Belgium. Fenethazine hydrochloride was obtained from Roune Poulenc (Parris, France). HPLC grade methanol, water, and chloroform were obtained from J. T. Baker Chemical Company.

Drug Analysis. Serum levels of amiodarone and desethylamiodarone

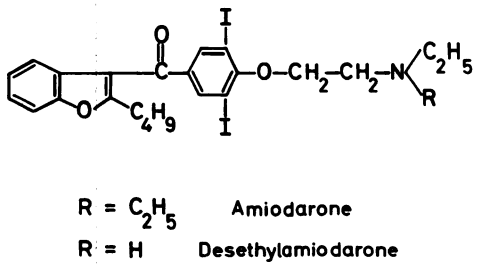

FIG. 1. *Chemical structure of amiodarone and its major metabolite desethylamiodarone.*

were determined by high performance liquid chromatography (12). Briefly, to 0.4 ml serum in polypropylene tubes (12 × 75 mm), 40 μl 2 N NaH_2PO_4 buffer at pH 4.5, and 0.5 μg (10 μl) fenethazine internal standard in methanol were added. This was followed by 0.75 ml methyl-*t*-butyl ether, and the contents were vortexed 10 sec and shaken horizontally for 20 min. After centrifugation at 2500 rpm for 10 min, the upper ether phase was transferred to culture tubes (6 × 50 mm). The aqueous phase was extracted once again with 0.75 ml ether and the ether phase after centrifugation was combined with the first extract and dried under N_2. The residue was reconstituted in 25 μl methanol for HPLC analysis.

For determining tissue concentrations of amiodarone and desethylamiodarone, 20–25 mg of tissue (wet weight) was minced and homogenized with 1 ml methanol containing 0.5 μg L8040 in a hand homogenizer. The homogenizer was washed with an additional 1 ml methanol. The combined extract was dried under N_2 and reconstituted in 0.4 ml normal rabbit serum. Further steps for drug extraction are the same as outlined above for serum. The fat tissue, because of its high lipid and drug concentrations, was processed slightly differently; 10 mg of tissue was homogenized with 1 ml chloroform/methanol (2:1 v/v) containing 0.5 μg fenethazine as internal standard. The combined homogenate from the above and an additional 1 ml of chloroform-methanol wash was dried under nitrogen. Normal rabbit serum (0.4 ml) was added and the drug was extracted with *t*-butyl ether as described for serum samples. Fenethazine was chosen as the internal standard because of an interference peak in adipose tissue samples with a retention time close to that of L8040, the internal standard used for analysis of other tissues.

The validity of the present tissue extraction procedure was compared in independent experiments with another published method of Latini *et al.* (13) in which aliquots of methanolic tissue homogenates were first evaporated to dryness, redissolved in 2 ml pH 5.4 phosphate buffer, and extracted twice with hexane. The dried residue from hexane extracts was redissolved in methanol and analyzed by HPLC according to Latini *et al.* (13). The recoveries of amiodarone and desethylamiodarone by the two methods did not differ significantly from each other and ranged from 89–116% (mean of four determinations).

Chromatographic Conditions. A Waters liquid chromatograph equipped with a M 6000A pump, a M450 variable wavelength ultraviolet detector set at 240 nm, and an Altex 5 μ particle size Ultrasphere Si column (25 cm × 4.6 mm i.d.) was used for analysis. The mobile phase consisted of methanol containing 0.07% perchloric acid adjusted to pH 4.0 with 0.1 N methanolic sodium hydroxide. The flow rate and chart speed were 1.8 ml/min and 0.5 cm/min, respectively. A Hewlett-Packard integrator (model 3380A) was used to determine drug concentration from the peak areas of amiodarone and that of the internal standard.

Results

Standardization of Amiodarone and Desethylamiodarone Analysis. Fig. 2 shows the standard curves for amiodarone and desethylamiodarone added to control rabbit sera. The curves were obtained by combining serum concentration data from four determinations in each case. The curves were linear from 0.025–6 μg/ml for amiodarone and desethylamiodarone. The slope, intercept, and correlation coefficient were 1.128, 0.202, and 0.984 for amiodarone and 1.087, 0.555, and 0.990 for desethylamiodarone.

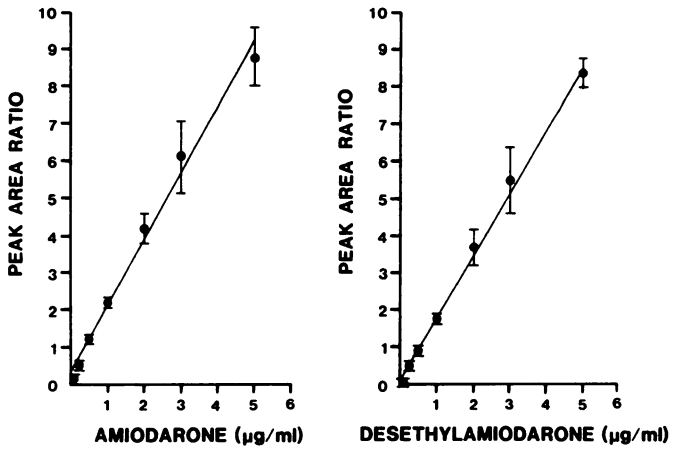

FIG. 2. *Standard curve for the analysis of amiodarone (left) and desethylamiodarone (right) added to normal rabbit serum.*

The *abscissa* represents amount of amiodarone added and the *ordinate* the peak area ratio of drug to internal standard in HPLC analysis. Each point represents mean ± SE from 4 determinations.

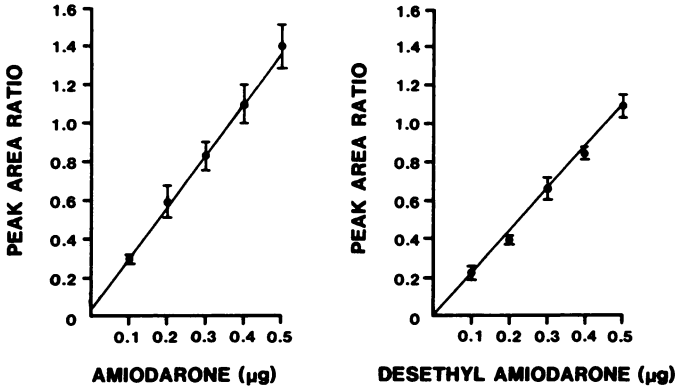

FIG. 3. *Standard curve for the analysis of amiodarone added to liver tissue from control rabbits.*

The slope, intercept, and correlation coefficient for amiodarone and desethylamiodarone were 1.282, −0.021, and 0.984 and 1.093, −0.032, and 0.986, respectively.

The above serum data were obtained using fenethazine as internal standard, according to the original report by Storey *et al.* (12). When the above procedure was adapted to tissue analysis we found that fenethazine was not suited as an internal standard due to its long retention time and the appearance of interference peaks in tissue homogenates closer to fenethazine peak. Therefore, we used a brominated analog of amiodarone, L8040, which has also been used by other workers (12–14).

Standard curves were prepared for each tissue from control rabbits from extracts of 25 mg wet weight to which varying amounts of amiodarone and desethylamiodarone and a known amount of internal standard in methanol were added. The amounts of amiodarone and desethylamiodarone added ranged from 0.1–0.5 μg for most of the tissues and a wider range of 0.5–6.0 μg for serum. These ranges were determined by preliminary analysis of tissues from drug-treated animals to estimate approximate levels of amiodarone and desethylamiodarone. Fig. 3 shows the standard curves for amiodarone and desethylamiodarone in liver tissue. Similar standard curves were obtained for

other tissues examined (data not shown). A linear correlation existed for all tissues between the amount of amiodarone and desethylamiodarone added and the amount found by HPLC. Standard curves were prepared only for amiodarone in adipose tissue; negligible quantities of desethylamiodarone were present in adipose tissue samples from amiodarone-treated animals. No attempt was made to quantitate the metabolite in adipose tissue samples. Fig. 4 shows a representative chromatogram for ventricular muscle from a amiodarone-treated rabbit. The retention times of desethylamiodarone, L8040, and amiodarone were 5.07, 5.88, and 8.60 min, respectively. HPLC chromatograms from extracts of other tissues were qualitatively similar to that of the ventricle except for that of fat which had a broad solvent front (due presumably to excess lipid) and only traces of desethylamiodarone. Extracts from bile also showed two additional peaks in the chromatogram (fig. 5), one eluting before desethylamiodarone and the other between L8040 and amiodarone. No attempts were made to fully characterize the two new metabolites.

Tissue Concentrations of Amiodarone and Desethylamiodarone. Table 1 lists the serum and tissue concentrations of amiodarone and desethylamiodarone in rabbits administered amiodarone for 6 weeks. Although body weights of drug-treated animals were significantly lower, organ wet weights of amiodarone-treated animals were not different from those of controls except for liver which showed a significant decrease ($p < 0.01$) from a mean of 3.85% body weight in controls to 2.75% in amiodarone-treated rabbits.

Fat tissue had the highest concentration of amiodarone per unit tissue weight followed by lung, liver, and muscle. The concentrations of amiodarone and desethylamiodarone in the ventricle were 6.90 and 3.12 μg/g, respectively. The lowest concentration of amiodarone was found in serum. Muscle, spleen, kidney, and bile had concentrations in the intermediate range. Liver and lung had high amounts of desethylamiodarone and the amiodarone/desethylamiodarone ratio in these tissues was close to unity whereas less vascularized tissues such as fat had insignificant amounts of the metabolite. The serum amiodarone/desethylamiodarone ratio was close to 4 which is higher than that in humans. Both the drug and the metabolite were present in negligible quantities in brain and could not be quantitated by our present assay method.

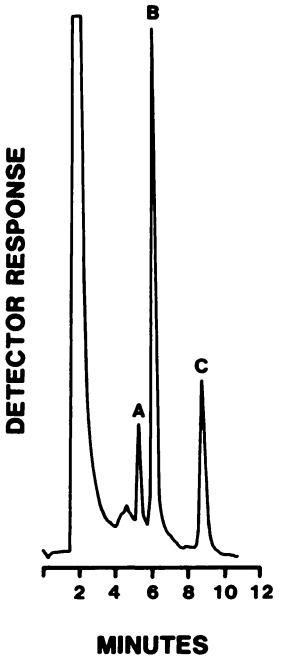

FIG. 4. *Chromatogram showing resolution of amiodarone, desethylamiodarone, and L-8040 internal standard in the extract of ventricular tissue homogenate from a rabbit treated with amiodarone.*

Peaks A, B, and *C* represent desethylamiodarone, L8040, and amiodarone, respectively. The *small broad peak* preceding peak A is an impurity.

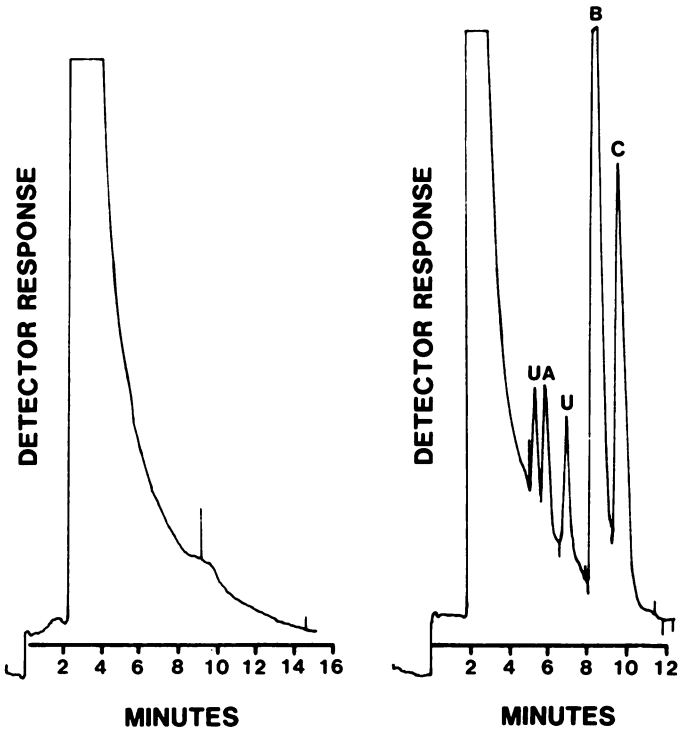

FIG. 5. *HPLC chromatogram of bile extracts from control rabbit (left) and from a rabbit treated with amiodarone chronically (right).*

U denotes an unknown peak (see legend to Fig. 4 for other denotions).

TABLE 1

Amiodarone and desethylamiodarone concentrations in serum and tissues after chronic amiodarone administration to rabbits

All values are means from 5–6 rabbits except for bile ($n = 3$).

Tissue	Amiodarone	Desethylamiodarone	Mean Amiodarone/ Desethylamiodarone Ratio (Range)
	μg/g	μg/g	
Fat	55.98 ± 8.88	Trace	
Lung	15.89 ± 4.41	23.03 ± 6.85	1.5 (0.6–3.2)
Liver	17.92 ± 7.43	18.56 ± 8.96	1.1 (0.3–1.5)
Muscle	14.11 ± 5.28	2.67 ± 0.73	4.6 (2.5–7.7)
Spleen	12.36 ± 4.20	10.49 ± 1.41	1.1 (0.1–1.9)
Kidney	10.79 ± 1.69	6.81 ± 1.08	1.6 (0.7–2.6)
Bile[a]	7.05 ± 2.68	5.28 ± 1.17	1.2 (0.9–1.7)
Ventricle	6.90 ± 1.00	3.12 ± 0.43	2.4 (1.6–4.7)
Serum[a]	1.42 ± 0.45	0.42 ± 0.20	4.0 (1.6–6.7)
Brain	Trace	Trace	

[a] Values are expressed as μg/ml.

Fig. 6 shows the tissue/serum ratio of amiodarone and desethylamiodarone in chronically treated rabbits. Fat had the highest tissue/serum amiodarone ratio of 88 followed by muscle, lung, and kidney. The ratio for the myocardium was 12, only slightly

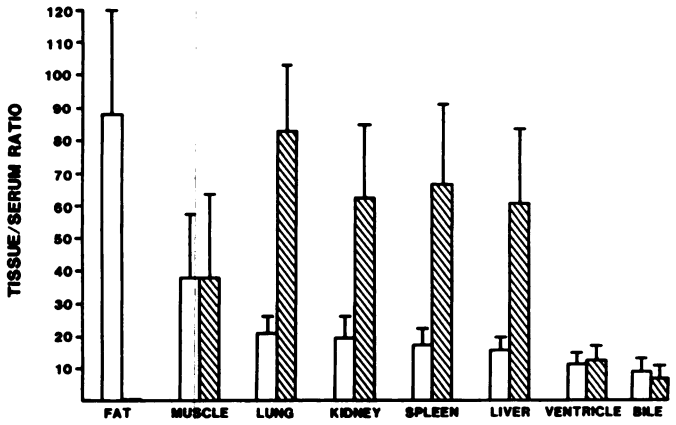

FIG. 6. *Tissue/serum ratio of amiodarone (open bars) and desethylamiodarone (hatched bars) in rabbits chronically treated with amiodarone.*

Values represent mean ± SE from 5–6 rabbits for each tissue except bile ($n = 3$).

higher than that of bile which had the lowest ratio of the tissues analyzed.

The tissue/serum ratio of desethylamiodarone showed a different pattern. Lung, spleen, kidney, and liver all had high ratios, with the highest ratio being that of lung (83.1). Ventricle and bile had the smallest tissue/serum ratios. Brain and adipose tissue had insignificant amounts of the metabolite, and therefore their ratios could not be determined with precision.

Discussion

We have standardized a liquid chromatographic method for the measurement of amiodarone and desethylamiodarone in tissues. Using this method we have studied the tissue accumulation of amiodarone and its metabolite *in vivo* after chronic administration. Previous work showed that in this model protracted amiodarone administration caused a significant prolongation of action potential duration in cardiac fibers (10, 11). Thus, the electrophysiological effects of chronic amiodarone treatment in the rabbit resemble those in man. In this study, in an effort to understand the metabolism of amiodarone and to develop a model to correlate tissue drug accumulation to efficacy and toxicity of amiodarone, we have determined the tissue levels of amiodarone and its major metabolite in several tissues in the rabbit.

Standardization of an HPLC procedure for determining tissue concentrations of the drug and metabolite gave linear curves for all the tissues in the range 0.1–0.5 μg for amiodarone and desethylamiodarone. As mentioned under "Materials and Methods," L8040 was found to be a better internal standard for tissue analysis except for adipose tissue for which we used fenethazine as internal standard. Modifications in the processing of tissue and the chromatographic conditions resulted in improved sensitivity, faster analysis, and simultaneous quantitation of both amiodarone and desethylamiodarone.

Adipose tissue had the highest concentration of amiodarone after chronic administration followed by lung, liver, and kidney. Although the concentration in the myocardium (ventricle) was about 5 times higher than that in serum, its amount was the lowest among the tissues studied. This finding is similar to postmortem tissues in humans (4, 14). The concentration of desethylamiodarone was the highest in liver, lung, and kidney which is again similar to that reported in man (8, 14). However, several aspects of the rabbit tissue distribution data differ from those in man. The serum concentrations of amiodarone and desethylamiodarone are lower and the metabolite/amiodarone ratio is significantly lower than in man not only in serum but also in several tissues. Only negligible quantities of amiodarone and desethylamiodarone were found in the brain, in contrast to available clinical data. Myocardial concentrations in the rabbit were also lower than those found in post-mortem myocardial tissue.

Desethylamiodarone is the principal metabolite of amiodarone characterized so far in man and in other animal species. In the present study we found evidence for the existence of two new metabolites in the bile of rabbits chronically treated with amiodarone. They were present in significant quantities in the bile. The chemical structures of the two metabolites are not known at the present time. In a recent review, Latini *et al.* (15) referred to their recent work (16) on the characterization of a new metabolite of amiodarone as di-*N*-desethylamiodarone in the myocardium of chronically treated dogs. It is possible that one of the metabolites isolated from the bile in the present study is di-*N*-desethylamiodarone; however, the structure of the other metabolite remains to be determined.

Although this study was not designed to evaluate the toxicity of amiodarone, the high concentrations of amiodarone and particularly the metabolite in the lung and in the liver may play a role in the well known pulmonary and hepatotoxicity of amiodarone in man reported by several workers (17–21). It is likely that amiodarone is bound strongly to these tissues. Thus, tissue binding studies of amiodarone may prove to be important in understanding the mechanism of toxicity of amiodarone. Such studies will also help in understanding the interaction of amiodarone with various other drugs that has been documented in the literature (22). However, because of the sluggish metabolism of amiodarone in the rabbit as compared to man the rabbit may not serve as a useful model for this purpose.

References

1. K. Nademanee, J. A. Hendrickson, D. S. Cannom, B. N. Goldreyer, and B. N. Singh: Control of refractory life-threatening ventricular tachyarrhythmias by amiodarone. *Am Heart J.* **101**, 759–768 (1981).
2. K. Nademanee, B. N. Singh, J. A. Hendrickson, V. Intarachot, D. S. Cannom, B. Lopez, G. Feld, J. Weiss, and W. Stevenson: Amiodarone in refractory life-threatening ventricular arrhythmias. *Ann. Intern. Med.* **98**, 577–582 (1983).
3. R. Kannan, K. Nademanee, J. A. Hendrickson, H. J. Rostami, and B. N. Singh: Amiodarone kinetics after oral doses. *Clin. Pharmacol. Ther.* **31**, 438–444 (1982).
4. D. W. Holt, G. T. Tucker, P. R. Jackson, and G. C. A. Storey: Amiodarone pharmacokinetics. *Am. Heart J.* **106**, 840–846 (1983).
5. L. Harris, W. J. McKenna, E. Rowland, G. C. A. Storey, D. M. Krikler, and D. W. Holt: Plasma amiodarone and desethylamiodarone levels in chronic therapy. *Circulation* **64**, (Suppl. IV) 263 (1981).
6. E. Riva, M. Gerna, P. Neyroz, R. Urso, I. Bartosek, and I. Guaitani: Pharmacokinetics of amiodarone in rats. *J. Cardiovasc. Pharmacol.* **4**, 270–275 (1982).
7. A. P. Maggioni, A. Maggi, A. Volpi, V. D'Aranno, G. Tognoni, and P. Giani: Amiodarone distribution in human tissues after sudden death during Holter recording. *Am. J. Cardiol.* **52**, 217–218 (1983).
8. N. M. G. Debbas, C. D. Cailar, A. Sassine, J. Derancourt, J. G. Denaille, and P. Puech: Determination of cardiac and plasma drug

levels during long-term amiodarone therapy. *Eur. J. Clin. Invest.* **13**, 123–127 (1983).

9. R. Kannan, N. Ikeda, R. Wagner, M. Drachenberg, and B. N. Singh: Serum and myocardial kinetics of amiodarone and its deethylmetabolite after intravenous administration in rabbits. *J. Pharm. Sci.* **73**, 1208–1211 (1984).
10. B. N. Singh, K. Nademanee, N. Ikeda, and R. Kannan: Pharmacology and electrophysiology of amiodarone: experimental and clinical correlations. In "New Aspects in the Medical Treatment of Tachyarrhythmias: Role of Amiodarone" (G. Breithardt and F. Loogen, eds.), pp. 46–56. Urban and Schwarzenberg, München, 1984.
11. N. Ikeda, K. Nademanee, R. Kannan, and B. N. Singh: Electrophysiologic effects of amiodarone: experimental and clinical observation relative to serum and tissue drug concentrations. *Am. Heart J.* **108**, 890–898 (1984).
12. G. C. A. Storey, D. W. Holt, P. Holt, and R. V. L. Curry: High performance liquid chromatographic measurement of amiodarone and its desethylmetabolite. Methodology and preliminary observations. *Ther. Drug Monit.* **4**, 385–388 (1982).
13. R. Latini, S. Connolly, and R. E. Kates: Myocardial disposition of amiodarone in the dog. *J. Pharmacol. Exp. Ther.* **224**, 603–608 (1983).
14. G. C. A. Storey, P. C. Adams, R. W. F. Campbell, and D. W. Holt: High performance liquid chromatographic measurement of amiodarone and desethylamiodarone in small tissue samples after enzymatic digestion. *J. Clin Pathol.* **36**, 785–789 (1983).
15. R. Latini, G. Tognoni, and R. E. Kates: Clinical pharmacokinetics of amiodarone. *Clin. Pharmacokinet.* **9**, 136–156 (1984).
16. R. Latini, R. Reginato, A. L. Burlingame, and R. E. Kates: HPLC isolation and FAB-MS identification of di-*N*-desethylamiodarone, a new metabolite of amiodarone in the dog. *Biomed. Mass. Spectrom.* **11**, 466–471 (1984).
17. S. M. Sobol and L. Rakita: Pneumonitis and pulmonary fibrosis associated with amiodarone treatment. A possible complication of a new antiarrhythmic drug. *Circulation* **65**, 819–824 (1982).
18. L. Harris, W. J. McKenna, E. Rowland, D. W. Holt, G. C. A. Storey, and D. M. Krikler: Side effects of long-term amiodarone therapy. *Circulation* **67**, 45–51 (1983).
19. F. E. Marchlinski, T. S. Gansler, H. L. Waxman, and M. E. Josephson: Amiodarone pulmonary toxicity. *Ann. Intern. Med.* **97**, 839–845 (1982).
20. J. B. Simon, P. N. Monday, J. F. Brien, and P. W. Armstrong: Amiodarone hepatotoxicity simulating alcoholic liver disease. *N. Engl. J. Med.* **311**, 167–172 (1984).
21. S. Poucell, J. Ireton, P. Valencia Mayoral, E. Downar, L. Larratt, J. Patterson, L. Blendis, and M. J. Phillips: Amiodarone-associated phospholipidosis and fibrosis of the liver: light, immunohistochemical and electron microscopic studies. *Gastroenterology* **86**, 926–936 (1984).
22. F. I. Marcus: Drug interactions with amiodarone. *Am Heart J.* **106**, 924–929 (1983).