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Amiodarone and desethylamiodarone tissue uptake in rats chronically treated with amiodarone is non-linear with the dose

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Four groups of rats were given amiodarone chronically at 25, 37·5, 50, 75 mg kg^{-1}/12 h for 3 weeks; on day 21 the animals were killed and blood, plasma, heart, lung, liver and fat were collected and assayed for amiodarone and desethylamiodarone. Amiodarone plasma concentrations ranged from 0·74 to 4·68 μg mL^{-1} and desethylamiodarone from 0·08 to 2·05 μg mL^{-1}. Plasma, blood and tissue concentrations of amiodarone and desethylamiodarone increased significantly with the dose. Blood/plasma and tissue/plasma partition ratios of amiodarone and desethylamiodarone increased significantly at the higher doses. Blood/plasma ratio was a good predictor of tissue/plasma ratios of amiodarone and its metabolite, except in fat. Total phospholipid concentrations in lung were correlated with amiodarone and desethylamiodarone concentrations in plasma, blood and lung.

Amiodarone, a drug effective on several kinds of arrhythmias, has a pharmacokinetic profile that is not yet well understood (Latini et al 1984). Its main features are an extremely long plasma elimination half-life and extensive accumulation in tissues observed in animals (Riva et al 1982; Latini et al 1983) and in man (Maggioni et al 1983; Adams et al 1985).

It has been shown that some electrophysiological effects of amiodarone in animals (Latini et al 1983; Connolly et al 1984) and man (Escoubet et al 1985; Barbieri et al 1986) are better described by its tissue (myocardium) concentrations, which seem to equilibrate with plasma relatively slowly. It might be of interest, therefore, to clarify the relationship between plasma and tissue concentrations of this drug under different dosage regimens. Tissue distribution of a drug can change as a function of the dose and time after administration, as has already been shown on theoretical grounds (Øie et al 1980) and experimentally (Gonzalez et al 1975; Guentert & Øie 1980; Harashima et al 1985). One reason for this peculiar behaviour is saturable, concentration-dependent binding of the drug to plasma and/or tissue proteins. If this were true for amiodarone, the apparent discrepancy often reported between plasma concentrations of the drug and its effects (therapeutic and toxic) could be partly explained (Latini et al 1984).

We studied tissue distribution of amiodarone and desethylamiodarone, its active metabolite, in rats chronically treated with amiodarone at various doses (25 to 75 mg kg^{-1}/12 h). Amiodarone and desethylamiodarone were also measured in blood since it has been reported that red blood cell concentrations of amiodarone and desethylamiodarone during chronic treatment in man predict side effects better than plasma levels (Heger et al 1984).

A well known biochemical effect of amiodarone in the rat, the increase in total lung phospholipids (Mazue et al 1984), was related to drug and metabolite concentrations.

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MATERIALS AND METHODS

Male CD/COBS rats, 250–275 g, were given different doses of amiodarone suspended in a 0·4% aqueous solution of carboxymethylcellulose by gavage. Amiodarone doses were 25, 37·5, 50, 75 mg kg^{-1} per 12 h and 5–9 rats per group were used; 10 control pair-fed rats received only the vehicle. Treatment was given for 3 weeks and on days 7 and 14, just before the evening dose (trough concentrations), blood was sampled from 3 rats in each treatment group, from a tail vein, under light ether anaesthesia, for amiodarone and desethylamiodarone assay (Latini et al 1983). At the end of the 3-week treatment, rats were killed by exsanguination through the carotid arteries; blood and tissues were collected as previously described (Riva et al 1982). Plasma, whole blood, heart, lung, liver and epididymal fat were kept at −20 °C until assayed for amiodarone and desethylamiodarone (Latini et al 1983).

Body weight and food consumption were recorded regularly throughout the treatment as a basis for

adjusting the amiodarone dose and pair-feeding; micro-haematocrit was measured at the end of the study. Total phospholipids in lung homogenates expressed as mg of phospholipid phosphorus/100 g of tissue (Svanborg & Svennerholm 1961) and total plasma proteins were determined (Lowry et al 1951).

Dose groups were compared by one-way analysis of variance followed by a Duncan test for multiple comparison on a HP-85 desk computer (Rocchetti & Recchia 1982). Linear correlations were performed with SPSS program (Hull & Nie 1981) running on a DEC VAX 750 (WMS).

RESULTS

Trough blood concentrations of amiodarone did not change from the first week to the end of treatment (week 3). Desethylamiodarone concentrations, however, rose at all doses from week 1 to week 2, thereafter remaining the same.

Amiodarone trough plasma concentrations at week 3 ranged from 0·74 to 4·68 $\mu g\ mL^{-1}$ and desethylamiodarone from 0·08 to 2·05 $\mu g\ mL^{-1}$ in all groups of rats (Table 1).

Plasma, blood and tissue concentrations of amiodarone and desethylamiodarone increased significantly ($P < 0·01$) with the dose (Table 1).

Amiodarone tissue distribution

Blood/plasma and tissue/plasma partition ratios were not constant at all doses, but tended to be higher at higher doses (Table 2). Blood/plasma and tissue/plasma ratios with the 75 mg kg^{-1} dose, and lung/plasma and fat/plasma ratios with the 50 mg kg^{-1} dose were significantly higher ($P \leq 0·05$) than after 25 mg kg^{-1}. Lung/plasma ratios of amiodarone showed the most striking increases.

To show this trend better, the tissue/plasma ratios were correlated with plasma concentrations. Except for adipose tissue ($P = 0·02$), the correlation was highly significant in all cases ($P < 0·01$) (Fig. 1). The correlations were still significant when the rat with a plasma concentration of amiodarone of 4·68 $\mu g\ mL^{-1}$ was omitted.

Tissue/plasma concentration ratios were also plotted against blood/plasma ratios: highly significant r values ($P < 0·01$) were found for heart, lung and liver, but not for fat ($P < 0·05$) (Fig. 2).

Desethylamiodarone tissue distribution

Blood/plasma and tissue/plasma concentration ratios for desethylamiodarone were higher than for amiodarone in all tissues except fat, where they were much lower (Table 2). Tissue/plasma concentration ratios with 75 mg kg^{-1} were significantly higher ($P \leq 0·05$) than those at the lower doses for heart, lung and liver, but not for blood and fat (Table 2). Correlations between tissue/plasma ratios and plasma desethylamiodarone concentrations were statistically significant for liver, lung and fat ($P < 0·01$); as for amiodarone, they were all positive, with the exception of fat, where the slope was negative and significantly different from zero ($P < 0·01$).

The correlations between desethylamiodarone tissue/plasma ratios and blood/plasma ratios tended to be closer than for plasma alone and were statistically significant ($P < 0·01$) for heart, lung and liver, but not for fat.

Biochemical effect

Amiodarone treatment significantly ($P \leq 0·05$) raised the total phospholipid concentration in lung only at 50 and 75 mg kg^{-1} doses; lung concentrations

Table 1. Amiodarone (A) and desethylamiodarone (DEA) plasma, blood and tissue concentrations (mean ± s.e.m.) in rats treated for 3 weeks with different oral doses of amiodarone.

	A and DEA concentrations ($\mu g\ mL^{-1}$ or $\mu g\ g^{-1}$ wet tissue)											
	Plasma		Blood		Heart		Lung		Liver		Fat	
Dose*	A	DEA	A	DEA	A	DEA	A	DEA	A	DEA	A	DEA
25	0·94 ±	0·13 ±	0·80 ±	0·30 ±	22 ±	12 ±	84 ±	63 ±	33 ±	18 ±	272 ±	28 ±
(n = 5)	0·11	0·03	0·12	0·07	2	2	10	13	4	5	19	3
37·5	1·78 ±	0·28 ±	1·70 ±	0·70 ±	36 ±	28 ±	273 ±	251 ±	89 ±	52 ±	630 ±	57 ±
(n = 5)	0·09	0·05	0·16	0·31	9	5	58	86	13	12	63	6
50	2·24 ±	0·51 ±	2·43 ±	1·34 ±	65 ±	47 ±	711 ±	950 ±	118 ±	95 ±	941 ±	75 ±
(n = 9)	0.21	0.07	0.20	0.25	7	8	177	242	14	17	61	12
75	3·14 ±	1·00 ±	4·24 ±	2·64 ±	122 ±	151 ±	1181 ±	2033 ±	331 ±	498 ±	1453 ±	128 ±
(n = 4)	1·49	0·36	1·18	0·42	39	72	50	224	10	259	257	55
n = 23												

* mg kg^{-1}/12 h.

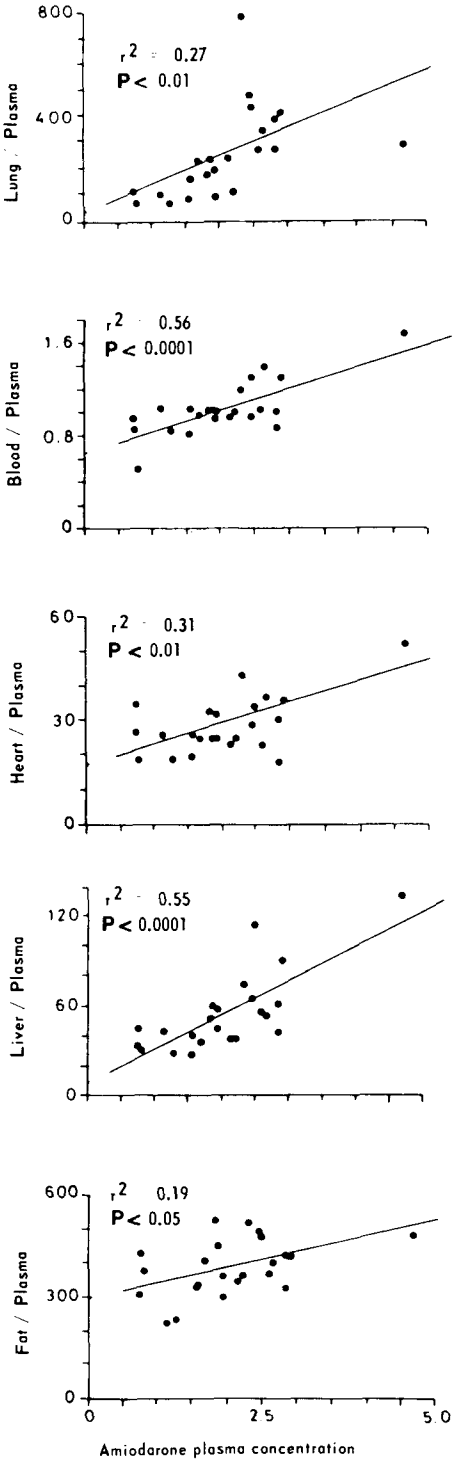

FIG. 1. Correlations between amiodarone plasma concentrations (μg mL^{-1}) and tissue/plasma ratios in 23 rats given different oral doses of the drug for 3 weeks.

of phospholipid phosphorus of rats treated with the lowest dose of amiodarone, 25 mg kg^{-1}/12 h, were not significantly different from those of pair-fed rats, being, respectively, 66 ± 2·4 and 74 ± 3·0 mg/100 g. Lung phospholipid concentrations were correlated with plasma, blood and lung amiodarone concentration (Fig. 3); similar correlations were found for desethylamiodarone.

Total plasma protein concentrations were similar in treated, pair-fed and control groups.

DISCUSSION

Amiodarone and desethylamiodarone kinetics were studied in rats given multiple doses of amiodarone to maintain plasma concentrations of the drug in the range of those found in patients (Latini et al 1984).

Rats have a much shorter plasma half-life of elimination of amiodarone, 8·5 h (Riva et al 1982), than man where it is around 30 days (Latini et al 1984). That is why a twice-a-day regimen was necessary to ensure some accumulation of the drug in the rat body. Even if man and rat have different plasma half-lives, their tissue/plasma ratios of amiodarone and desethylamiodarone are similar; in fact, Barbieri et al (1986) found atrium/plasma ratios, in biopsy samples from patients treated for 1 to 4 weeks, of 33·4 ± 6 for amiodarone and of 78·2 ± 17 for its metabolite.

Trough plasma concentrations of amiodarone at 3 weeks were linearly correlated with the dose of amiodarone; this probably means that amiodarone plasma clearance is constant within the concentration range studied. Blood clearance decreases with increasing doses, due to the parallel increase in blood/plasma ratio (Table 2), suggesting that attraction of red blood cells for amiodarone represents a restrictive binding form. At the lowest dose regimen (25 mg kg^{-1}/12 h) the average amiodarone blood concentration at steady-state, calculated from a blood clearance figure of 40 mL min^{-1} kg^{-1} (Riva et al 1982), and 100% bioavailability, is 0·87 μg mL^{-1}, which is close to the experimental value of 0·80 μg mL^{-1}. This indicates that oral bioavailability of amiodarone, at the lowest dose, when probably its disposition is linear, was close to one.

The most interesting aspect of amiodarone kinetics observed in this study was the non-linear tissue uptake of the drug. This was particularly evident in the lung, followed by blood and liver; desethylamiodarone followed more or less the same pattern. In other words, tissue/plasma concentration ratios of amiodarone and desethylamiodarone increase with the plasma concentration of the compounds (Fig. 1).

Table 2. Amiodarone (A) and desethylamiodarone (DEA) tissue/plasma concentration ratios (mean ± s.e.m.) in rats treated for 3 weeks with different oral doses of amiodarone.

	Tissue/plasma concentration ratio									
	Blood		Heart		Lung		Liver		Fat	
Dose*	A	DEA	A	DEA	A	DEA	A	DEA	A	DEA
25	0·84 ±	2·26 ±	23 ±	88 ±	91 ±	485 ±	35 ±	138 ±	308 ±	233 ±
(n = 5)	0·09	0·21	3	10	10	61	3	8	40	29
37·5	0·95 ±	2·66 ±	25 ±	107 ±	151 ±	1000 ±	50 ±	181 ±	352 ±	221 ±
(n = 5)	0·04	0·55	2	15	29	240	6	27	26	33
50	1·03 ±	2·48 ±	24 ±	91 ±	309 ±	1710 ±	49 ±	165 ±	405 ±	168 ±
(n = 9)	0·05	0·28	4	6	88	281	4	17	24	18
75	1·29 ±	3·12 ±	37 ±	137 ±	397 ±	2494 ±	100 ±	362 ±	463 ±	171 ±
(n = 4)	0·14	0·47	5	15	42	460	15	130	16	19
n = 23										

* mg kg^{-1}/12 h.

Two hypotheses, which do not exclude each other, can be offered to explain this behaviour.

The first is that the binding of amiodarone and desethylamiodarone to plasma proteins is saturable so the free fraction of the compounds becomes larger as their total concentration rises; more drug diffuses into tissues and the partition ratio increases. This is also observed in red blood cells whose uptake of drugs is often dependent on plasma protein binding (Kurata & Wilkinson 1974; Bower 1982; Harashima et al 1985). The presence of albumin in the extracellular fluid (Jusko & Gretch 1976) should not greatly influence the amiodarone tissue/plasma ratio, whose high values indicate that binding to cellular structures other than extracellular albumin is the major determinant of tissue distribution of amiodarone and desethylamiodarone. Direct measurement of plasma protein binding of the drug would probably throw some light on the mechanism. Unfortunately, there are no simple and reliable methods yet for measuring amiodarone protein binding, due both to its low solubility in water mediums and to its binding to dialysis membranes.

The second hypothesis takes into account the increase in phospholipids induced by amiodarone in lung and liver, known as 'phospholipidosis' (Lull-

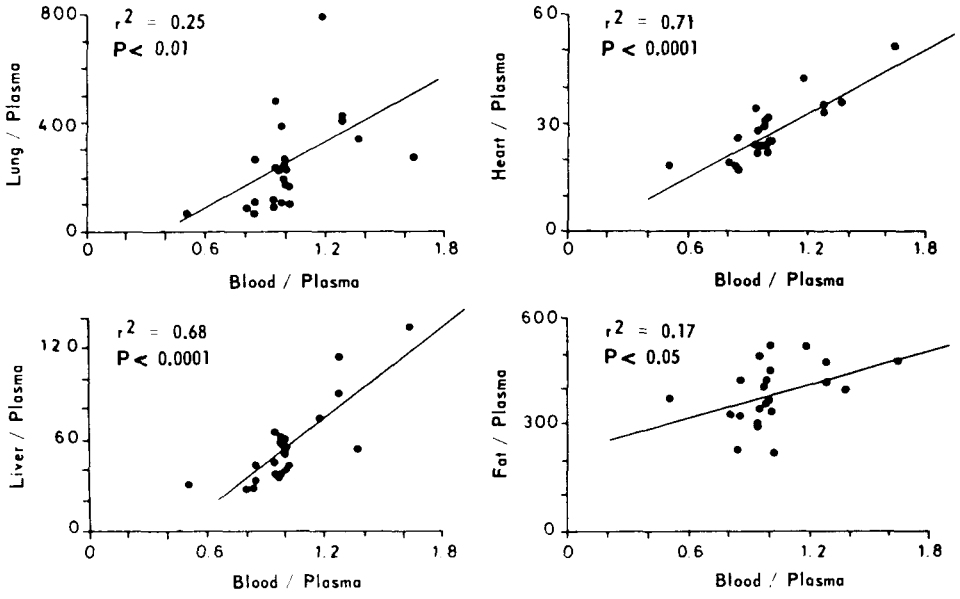

FIG. 2. Correlations between amiodarone blood/plasma and tissue/plasma ratios in 23 rats given different oral doses of the drug for 3 weeks.

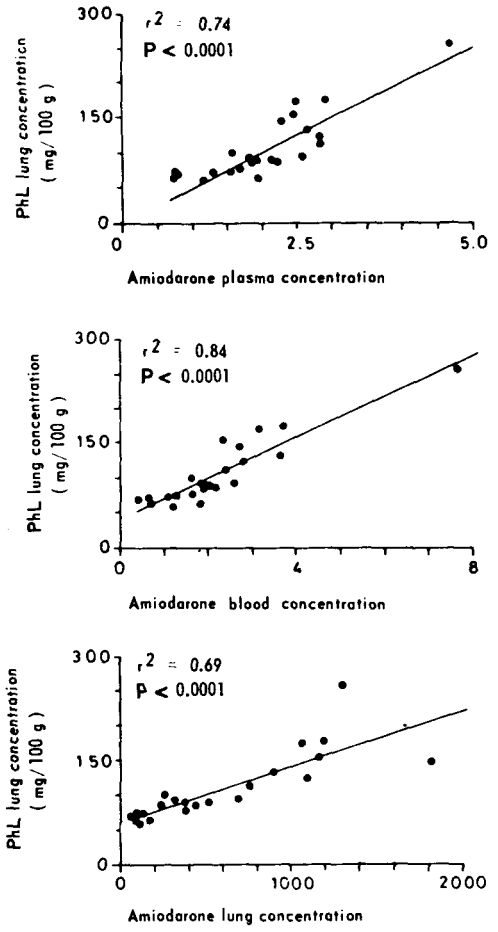

FIG. 3. Correlations between amiodarone concentrations in plasma ($\mu g\,mL^{-1}$), blood, lung ($\mu g\,mL^{-1}$), and lung ($\mu g\,g^{-1}$) total phospholipid phosphorus (PhL) concentrations in 23 rats given different oral doses of amiodarone for 3 weeks.

mann et al 1978), which might increase the solubility of the drug and its metabolite in these tissues (Fichtl et al 1980), on account of the well known affinity of amiodarone, as all amphiphilic molecules, for phospholipids (Lullmann et al 1978). This hypothesis does not explain the similar pattern of increase in blood/, heart/ and fat/plasma ratios; in these tissues phospholipid content rises—if at all—much less than in liver and lung.

Our results show a clear correlation between amiodarone and desethylamiodarone plasma or lung concentrations and lung phospholipid levels. We cannot exclude from our data that time of exposure is an important determinant of the biochemical alteration observed. No conclusion can be drawn about the relative roles of amiodarone and desethylamiodarone in the increase of lung phospholipids since both amiodarone and desethylamiodarone correlate with lung phospholipid concentrations, but are also correlated with each other. Preliminary unpublished results of our group show that rats treated with desethylamiodarone alone also develop phospholipidosis.

Heger et al (1984) showed a correlation between red blood cell concentrations of amiodarone and desethylamiodarone and side effects in patients chronically treated with amiodarone; they hypothesized that red blood cell uptake of amiodarone and desethylamiodarone was comparable with tissue uptake. We have studied blood/plasma ratios of amiodarone and desethylamiodarone in our animals in relation to tissue uptake. Since interindividual variability in haematocrit values was negligible, blood concentrations well-represented red blood cell concentrations in all cases. Blood/plasma ratios correlated well with tissue/plasma ratios for heart, lung and liver, indicating that red blood cell uptake of amiodarone and desethylamiodarone does, in fact, in some way reflect tissue uptake (Heger et al 1984). Fat behaved differently, since blood/plasma ratios could not predict its concentrations of amiodarone and desethylamiodarone; however, this can be understood, considering the different composition of this tissue.

In conclusion, this study shows that the relation between amiodarone distribution in tissues and the dose of the plasma concentration is non-linear, but we cannot explain why.

Amiodarone and desethylamiodarone concentrations are closely correlated with the increase in lung phospholipid content.

Red blood cell/plasma partition of amiodarone and desethylamiodarone was related to the tissue/plasma concentration ratio. The possible clinical significance of this experimental finding merits further research in the light of what has already been reported by others (Heger et al 1984).

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