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Mechanisms of Digoxin–Amiodarone Interaction in the Rat[1] (42713)

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Abstract. Amiodarone and digoxin are often used in combination and clinical experience suggests that amiodarone may increase serum digoxin levels and toxicity. We have investigated the influence of amiodarone on digoxin pharmacokinetics and tissue distribution in the rat.

Forty-nine rats were injected with 10 mg/kg amiodarone sc three times a day for 7 days, while 49 others were injected with saline only. On the eighth day, all the rats received 0.5 mg/kg digoxin ip; 4, 5, 6, 7, 8, 10, and 12 hr later, groups of 7 amiodarone-pretreated and control animals were sacrificed, and plasma, heart, liver, muscle, brain, and kidney digoxin concentrations measured by radioimmunoassay. Data were analyzed by two-way ANOVA, with group comparisons using the Waller–Duncan multiple comparison procedure. Digoxin levels were significantly higher in the plasma, heart, muscle, and kidney of the amiodarone-pretreated rats at most points of measurement ($P < 0.05$) whereas liver digoxin levels were elevated at 8, 10, and 12 hr. Kidney/plasma, heart/plasma, muscle/plasma, and especially liver/plasma ratios in the control groups significantly exceeded the values found in the amiodarone-pretreated group at most time points. Concentrations of digoxin in brain were not changed. This suggests that the volume of distribution is significantly altered in the amiodarone-pretreated group. Amiodarone increases plasma digoxin levels in rats as it does in humans, but the mechanism is unclear.

Spironolactone, quinidine, and verapamil have all been found to increase serum digoxin levels when used in combination with digoxin in humans (1–3) and in various animal species including the rat (4, 5). More recently, the antiarrhythmic amiodarone, which is sometimes administered when digoxin or other drugs fail to normalize heart rhythm (6), has been shown to interact with digoxin in humans with significant side effects (7, 8). The adverse effects include gastrointestinal, central nervous system, and cardiovascular effects (8) and probably are attributable to increases in serum digoxin concentrations. Such increases have been shown in several studies of concomitant digoxin–amiodarone therapy (8–13).

The mechanism whereby amiodarone increases plasma levels of digoxin in humans has not yet been completely determined. Koren *et al.* (14) measured digoxin uptake in isolated rat heart, kidney, and muscle slices. They found that amiodarone inhibited renal digoxin uptake *in vitro* and they suggested that this may be a partial explanation for the elevated plasma levels of digoxin *in vivo* in the rat and even in humans treated with both drugs. They also showed that in rats on chronic digoxin treatment simultaneous administration of amiodarone led to a 10-fold increase in serum digoxin concentration with no change in serum creatinine concentration. Venkatesh *et al.* (15) performed a similar study in rats and demonstrated a 3- to 6-fold increase in serum digoxin levels. They also measured digoxin levels in myocardium, brain, and skeletal muscle, and found a decreased tissue–serum digoxin ratio in all three. Furthermore, Harrison and Gibaldi (16) estimated that renal clearance accounted for only about 15% of total clearance in the rat. It appears that the mechanism of the amiodarone-induced alteration in pharmacokinetics in the rat (and humans) is not completely understood, and so we have studied in more detail the pharmacokinetics and tissue distribution of digoxin in rats. Our results confirm previous data in

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showing increased serum and heart digoxin levels with a lower heart/plasma ratio. We have also extended these findings by showing that kidney/plasma and liver/plasma digoxin ratios were significantly decreased. We have restricted our analysis to the elimination phase of digoxin as determined by Harrison and Gibaldi for this species (16).

Methods and Materials. Ninety-eight male Charles River rats (Haifa, Israel) weighing between 250 and 350 g were housed under standard laboratory conditions and allowed food and water *ad lib.* Forty-nine rats received 10 mg/kg amiodarone dissolved in saline sc, 3 times a day for 7 days. Forty-nine control rats received the same volume of saline per kilogram without amiodarone. On the eighth day, all animals were injected with 0.5 mg/kg digoxin (Lanoxin) ip; 4, 5, 6, 7, 8, 10, and 12 hr later, groups of 7 amiodarone-pretreated and control animals were sacrificed. Blood samples were immediately centrifuged and the plasma quickly frozen. The heart, liver, kidney, brain, and section of diaphragmatic muscle were removed from each animal, cleaned of fat and fibrous tissue, and also frozen. Later these organs were weighed, sliced, and homogenized.

Digoxin was extracted using chloroform and ethanol (2:1). Plasma digoxin and tissue levels were measured by radioimmunoassay (17) as modified by Gorodischer *et al.* (18). Two extractions with chloroform/ethanol extracted essentially 100% of the digoxin. The cross-reactivity of the antibody with digitoxin was <3%. The variation between repeat determinations of digoxin was <10%. Tissue/plasma ratios were calculated in both groups at all points of measurement.

Statistics. The tissue/plasma ratios were ranked before analysis. The ranked tissue/plasma ratio data and the unranked tissue concentration data were analyzed by a two-way analysis of variance (ANOVA) using the SPSS-X statistical software package on a DEC Microvax computer. The statistical significance of the ranked (nonparametric) data was determined using an F table with infinite degrees of freedom (19). The statistical significance of the unranked tissue concentration data was calculated by the computer program. Comparisons between groups were done using the Waller–Duncan multiple comparison procedure (20). The least-squares difference (LSD) was calculated from the mean square residual (MSE) as derived from the ANOVA:

$$\mathrm{LSD} = 2\sqrt{\mathrm{MSE}\left(\frac{1}{n_1}+\frac{1}{n_2}\right)},$$

where n_1 and n_2 are the sample sizes of the groups compared. If the difference of the group means $(\bar{X}_1 - \bar{X}_2)$ is greater than LSD, then the difference is significant at the 0.05 level.

Results. In saline-treated control rats, plasma digoxin concentrations fell from a value of 37.6 ± 4.9 (SEM) ng/ml at 4 hr to 3.14 ± 0.90 ng/ml 12 hr after a single ip dose of 0.5 mg/kg digoxin (Fig. 1). The corresponding values for amiodarone-pretreated rats were 79.1 ± 9.4 and 13.4 ± 1.7 ng/ml, respectively. The kinetics of disappearance of digoxin for both treatments appeared similar, but no estimates of $t_{1/2}$ were possible because serial samples were not taken. The plasma digoxin concentrations in amiodarone-treated rats were significantly higher at all time points except 10 and 12 hr ($P < 0.05$).

Digoxin concentrations in kidney, heart, muscle, and liver (ng/g) were higher than

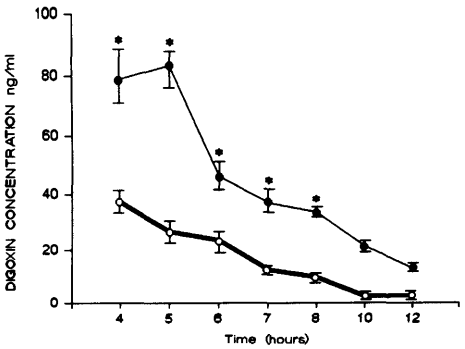

FIG. 1. Effect of amiodarone on digoxin concentrations in plasma. Groups of seven rats were injected with amiodarone (10 mg/kg sc) or saline 3 times per day for 7 days. On the eighth day, they were injected with digoxin (0.5 mg/kg ip) and sacrificed at the times indicated. Open circles: saline-pretreated rats. Closed circles: amiodarone-pretreated rats. *$P < 0.05$ for amiodarone vs saline group. Each point represents the mean (±SEM) of groups of seven rats. The same symbols apply to Figs. 2–9.

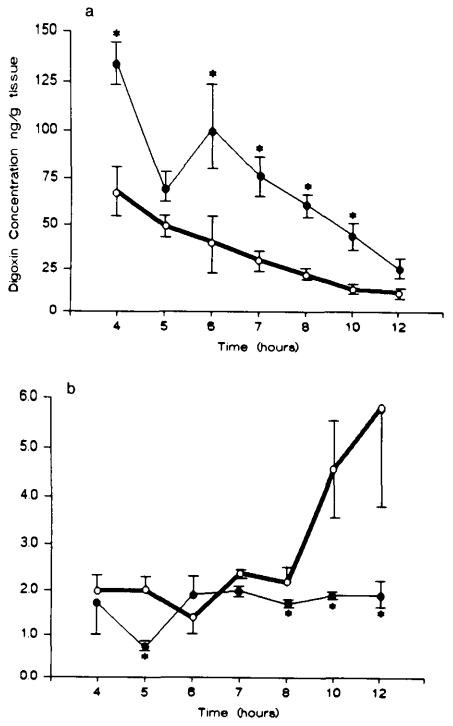

FIG. 2. (a) Effect of amiodarone on digoxin concentrations in kidney. (b) Effect of amiodarone on kidney/plasma concentration ratios of digoxin in rats.

plasma levels in both controls and amiodarone-pretreated rats at most time points (Figs. 2a–5a). The rate of disappearance of digoxin from kidney followed closely the disappearance from plasma in both groups (Fig. 2a). However, concentrations in heart tissue were almost constant from 4 to 8 hr post-digoxin before declining (Fig. 3a). Heart concentrations were higher in treated rats at all times except 4, 8, and 12 hr ($P < 0.05$). In control animals, liver concentrations were highest at 4 hr, slightly lower from 5 to 8 hr, and then decreased (Fig. 4a). In amiodarone-pretreated rats, liver digoxin concentrations were almost constant from 4 to 10 hr, then fell slightly at 12 hr (Fig. 4a). Digoxin concentrations in skeletal muscle (diaphragm) also appeared to disappear in a similar kinetic fashion as plasma concentrations (Fig. 5a). However, again muscle concentrations were higher after amiodarone treatment except at 12 hr. Concentrations of digoxin in brain were also measured, but the amounts were very small and no difference between control and treated groups was found (data not shown).

Even though actual tissue concentrations of digoxin were higher in amiodarone-pretreated rats, the tissue/plasma concentration ratios were lower at most time points (Figs. 2b–5b). It should be noted that the heart/plasma ratio at 8 hr appears anomalously high, and may reflect an analytical error of unknown origin. A difference between amiodarone- and saline-pretreated rats is especially evident in liver, where the ratio was 9.03 ± 2.36 and 19.1 ± 1.42, respectively, at 12 hr ($P < 0.05$; Fig. 4b).

Discussion. Diuretics or antiarrhythmic drugs are often used in combination with digitalis preparations in the treatment of congestive heart failure in children and adults, and several of these drugs have been found to increase serum digoxin levels. A partial list of these drugs includes the diuretic, spironolactone (1), and the antiarrhythmics quinidine (2, 3), verapamil (21), and amiodarone (8–13). It is important to

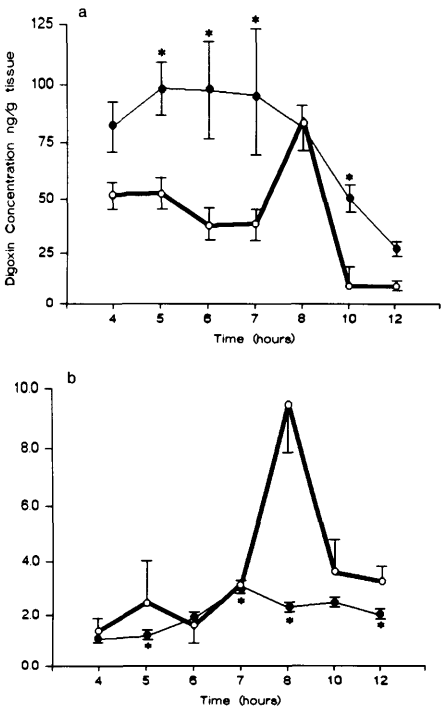

FIG. 3. (a) Effect of amiodarone on digoxin concentrations in heart. (b) Effect of amiodarone on heart/plasma concentration ratios of digoxin in rats.

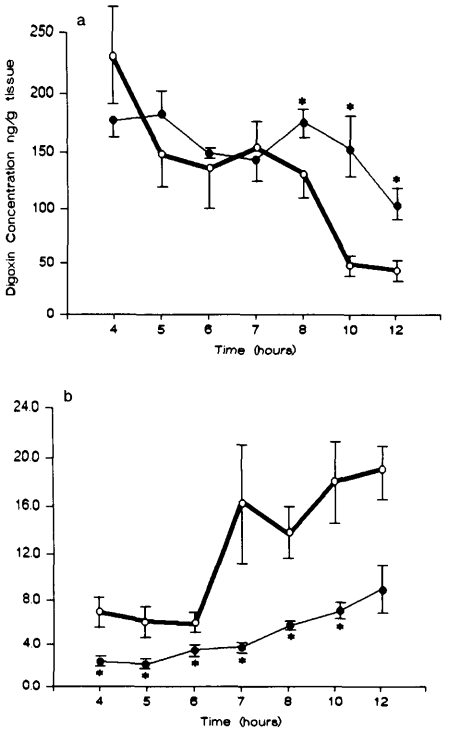

FIG. 4. (a) Effect of amiodarone on digoxin concentrations in liver. (b) Effect of amiodarone on liver/plasma concentration ratios of digoxin in rats.

understand the mechanisms for these drug-induced digoxin elevations in order to alter therapy, if necessary, and perhaps be able to predict other, currently unsuspected, interactions. The mechanisms involved in the interaction between amiodarone and digoxin are still unclear.

It is well known that the therapeutic index for digoxin is very small; i.e., the difference between doses which produce clinically beneficial effects and those which produce toxicity is very narrow. One of the important side effects of digoxin is the appearance of supraventricular and ventricular arrhythmias, particularly at high plasma concentrations (22). Adult rats also develop ventricular and supraventricular tachyarrhythmias after digoxin administration, but the doses required are in the range of 10–40 mg/kg (4, 23–25). Despite the high doses required and differences in elimination kinetics, the similarity in arrhythmias observed in rats and humans suggests that the rat may be a good species (16) to study certain aspects of digoxin toxicity. One of the most likely reasons why increased digoxin toxicity is seen after prolonged amiodarone treatment could be that amiodarone alters the pharmacokinetics and/or tissue distribution of digoxin, leading to higher concentrations in target organs such as the heart.

We have studied the effect of chronic amiodarone treatment on the tissue distribution and pharmacokinetics of digoxin in rats given a single ip dose of 0.5 mg/kg digoxin. At this dose, little or no effect on rat electrocardiograms (ECGs) would be expected because at 10 mg/kg sc, digoxin alone produced only a 30% increase in the PR interval and no other ECG changes in adult rats (24). We gave digoxin ip. This route of administration is generally considered to give faster drug absorption than po, although perhaps slower than sc. In fact, the time of onset and the doses required to produce arrhythmias in adult rats are very similar after ip or sc ad-

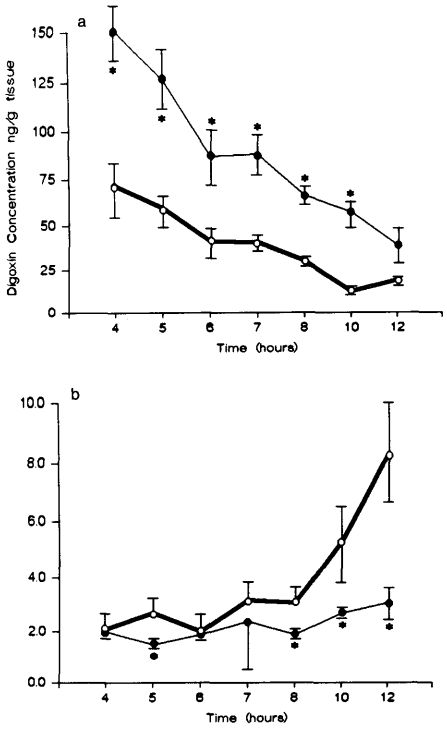

FIG. 5. (a) Effect of amiodarone on digoxin concentrations in muscle. (b) Effect of amiodarone on muscle/plasma concentration ratios of digoxin in rats.

ministration. Digoxin produced arrhythmias in 100% of female rats after an average of 64 min when given 40 mg/kg sc (25) and after 79 min in male rats administered 20 mg/kg ip (4). This argues that our use of the ip route in this study is valid.

We measured endogenous digoxin-like immunoreactive substances (EDIS) in liver and plasma. We found that in control rats the amount of EDIS was equivalent to about 0.1 ng/g in liver and 0.1 ng/ml in plasma by our RIA technique, substantially less than that reported by Valdes *et al.* (26). They found values (ng/g protein) of 39.5 for adrenal, 5.2 for liver, 1.1 for kidney, and 0.0 for heart, lung, and brain. Rat serum levels were 1.0 ng/g protein. Assuming protein contents of 100 mg/g tissue or 100 mg/ml serum, this corresponds to 400, 52, 11, 0, 0, 0, and 10 ng/g tissue or ng/ml serum, respectively. The source of the serum EDIS was speculated to be the adrenal glands. In our rats receiving only digoxin, the measured values in kidney, muscle, heart, liver, and plasma were higher than the values quoted above at most time points up to 7–8 hr and substantially above the amounts of EDIS we found in control rats. However, the tissues from the animals pretreated with amiodarone always had higher levels of digoxin immunoreactivity. It is conceivable that amiodarone specifically acted upon the adrenals to enhance the release of EDIS, but the EDIS would have to be taken up by all the tissues (e.g., heart, liver, kidney) to account for the elevation seen here. Verification of this would require actual separation of EDIS from digoxin (e.g., by HPLC) before RIA, a capability we did not have. In any event, such an effect of amiodarone would have required a tremendous release of EDIS in light of the negligible amounts we found in control animals.

Fenster *et al.* (10) studied the pharmacokinetics of digoxin in humans after 3 weeks of oral amiodarone, 400 mg daily. Systemic clearance was reduced 26%, renal clearance by 20%, and nonrenal clearance by 32%. In 10 normal volunteers treated with digoxin only, they found that renal clearance was about 45% of systemic clearance, and nonrenal clearance was about 55% in humans. Renal excretion is the predominant mode of digoxin elimination in most reported studies. However, in some patients, especially adult subjects, digoxin is metabolized to an appreciable extent to reduction products such as dihydrodigoxin and dihydrodigoxigenin (27, 28). In approximately 10% of individuals (adults) receiving digoxin, such metabolism accounts for 30 to 40% of the total excretion of digoxin and its metabolites. This nonrenal clearance of digoxin is a less frequent occurrence in children (29, 30). Therefore, the results of Fenster *et al.* (10) are in contrast with most previous reports. Harrison and Gibaldi (16) estimated renal clearance was about 15% in rats, and nonrenal clearance about 85%. Thus, in both species, clearance by nonrenal routes occurs, but with variable distribution between the two routes. We can not be sure from our data whether renal or nonrenal clearances or both were affected, since we did not collect urine or feces. However, the data are consistent with the idea that amiodarone changes the volume of distribution of digoxin.

Other investigators have studied the digoxin–amiodarone interaction in the rat. Koren *et al.* (14) gave rats digoxin sc at a dose of 0.1 mg/kg/day for 14 days, with amiodarone also being administered at a dose of 10 mg/kg sc three times daily for Days 7–13. Serum digoxin levels rose from 0.67 ng/ml on Day 7 (preamiodarone) to 11.22 ng/ml on Day 14 (postamiodarone). The measurements were made 24 hr after the previous digoxin dose. In view of the fact that we used a digoxin dose of 0.5 mg/kg ip (i.e., 5× higher), our data coincide well with theirs. We observed a plasma–digoxin level of 3.14 ± 0.90 ng/ml in controls and 13.4 ± 1.72 ng/ml in treated rats 12 hr after digoxin. In experiments *in vitro* with kidney slices, Koren *et al.* (14) also showed that amiodarone inhibited digoxin uptake. They suggested that inhibition of renal tubular uptake of digoxin and/or secretion was an important factor in the amiodarone-induced elevations in serum digoxin.

Venkatesh *et al.* (15) used a similar experimental design, except that they gave rats 0.25 mg/kg/day of digoxin orally for 23 days and either zero, 66 or 132 mg/kg/day amiodarone orally from Days 6 to 23. They also analyzed tissue levels of digoxin in myocardium, skeletal muscle, and brain. The serum di-

goxin levels in controls, 0.88 ± 0.36 ng/ml, were similar to those of Koren *et al.* (14) but somewhat less than ours. Even though they used higher doses of amiodarone, serum digoxin concentrations were only elevated 3× (low dose group) and 6× (high dose group). It is unclear at what time the rats were sacrificed with respect to the drug dosing, but oral (instead of parenteral) dosing may have contributed to the relatively small elevation. More importantly, they found increases in tissue levels of digoxin, with greater increases in skeletal muscle and brain. Although their tissue/plasma ratios were relatively higher than ours, they found a 42% decrease in this ratio in myocardial tissue after the low dose amiodarone, almost the same as the 43% decrease we observed at 12 hr. Venkatesh *et al.* (15) suggested that their data were consistent with the fact that central nervous system and neuromuscular toxicities are more prominent than myocardial toxicity when amiodarone is given after digoxin. They also suggested that the decreased binding of digoxin by tissues resulted in elevated serum levels, but they did not measure binding directly, nor did we. However, they do not explain the reason(s) for decreased clearance of digoxin after amiodarone treatment.

Most digoxin is found in the GI tract, followed by skeletal muscle, and liver, after iv administration to rats (16). We did not measure digoxin in the GI tract. Digoxin levels in diaphragms were measured as representative of skeletal muscle. If there was a large displacement from the gut, this would have led to increases in plasma and tissue (non-GI) concentrations if clearance was not increased. In contrast to Vankatesh *et al.* (15), we found no significant changes in brain concentrations.

We have found that amiodarone pretreatment increases plasma levels of digoxin in the rat, in confirmation of the rat and human studies mentioned above. Furthermore, we have shown that both renal/plasma and especially liver/plasma digoxin concentration ratios are significantly decreased in the rat during the elimination phase. This could mean that both decreased renal and hepatic clearances may contribute to the increased plasma digoxin levels, which is in accord with the human data of Fenster *et al.* (10) cited above. On the other hand, decreased tissue/plasma ratios for heart and liver are consistent with an increase in the volume of distribution for digoxin. Displacement of digoxin from tissue-binding sites, as suggested by Venkatesh *et al.* (15) may be a factor. As a corollary, this suggests that the rat may be a good model to study certain aspects of this interaction.

In view of the fact that the interaction between amiodarone and digoxin may be more acute in children than adults (9), it may be worthwhile to repeat these studies in young rats.

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1. Waldorff S, Andersen JD, Heeboll-Nielsen N, Nielsen OG, Motke E, Sorenson U, Steiness E. Spironolactone induced changes in digoxin kinetics. Clin Pharmacol Ther **24**:162–167, 1983.
2. Ejvinsson G. Effect of quinidine on plasma concentration of digoxin. Brit Med J **282**:279–280, 1978.
3. Hager WD, Fenster P, Mayersohn M, Perrier D, Graves P, Marcus FI, Goldman S. Digoxin-quinidine interaction. N Engl J Med **300**:1238–1241, 1979.
4. Kaplanski J, Weinhouse E, Topaz M, Genchik G. Verapamil and digoxin: Interaction in the rat. Res Commun Chem Pathol Pharmacol **42**:377–388, 1983.
5. Weinhouse E, Kaplanski J, Genchik G. Plasma and tissue levels of digoxin in the rat following pretreatment with verapamil. Res Commun Chem Pathol Pharmacol **47**:469–472, 1985.
6. Wheeler PJ, Puritz R, Ingram DV, Chamberlain DA. Amiodarone: Treatment of refractory supraventricular and ventricular arrhythmia. Postgrad Med J **55**:1–9, 1979.
7. McGovern B, Garan H, Kelly E, Ruskin JN. Adverse reactions during treatment with amiodarone hydrochloride. Brit Med J **287**:175–180, 1983.
8. Nademanee K, Kannan R, Hendrickson J, Ookhtens M, Kay I, Singh BN. Amiodarone-digoxin interaction: clinical significance, time course of development, potential pharmacokinetic mechanisms and therapeutic implications. J Amer Coll Cardiol **4**:111–116, 1984.
9. Koren G, Hesslein PS, MacLeod SM. Digoxin toxicity associated with amiodarone therapy in children. J Pediatr **104**:467–470, 1984.
10. Fenster PE, White NW, Hanson CD. Pharmacoki-

netic evaluation of the digoxin-amiodarone interaction. J Amer Coll Cardiol **5**:108–112, 1985.
11. Koren G, Parker R. Interpretation of excessive serum concentrations of digoxin in children. Amer J Cardiol **55**:1210–1214, 1985.
12. Koren G, Parker R. Interaction between digoxin and commonly coadministered drugs in children. Pediatrics **75**:1032–1037, 1985.
13. Moysey JO, Jaggarao NSV, Grundy EN, Chamberlain DA. Amiodarone increases plasma digoxin concentration (abstract). Brit Med J **282**:272, 1981.
14. Koren G, Soldin S, MacLeod SM. Digoxin-amiodarone interaction: *in vivo* and *in vitro* studies in rats. Canad J Physiol Pharmacol **61**:1483–1486, 1983.
15. Venkatesh N, Al-Sarraf L, Kannan R, Singh BN. Tissue-serum correlates of digoxin-amiodarone pharmacokinetic interaction in rats? Evidence for selective tissue accumulation and reduced tissue binding. J Pharmaceut Sci **74**:1067–1070, 1985.
16. Harrison LI, Gibaldi M. Physiologically based pharmacokinetic model for digoxin distribution and elimination in the rat. J Pharm Sci **66**:1138–1142, 1977.
17. Smith JW, Butler VP, Haher E. Determination of therapeutic and toxic serum digoxin concentration by radioimmunoassay. N Engl J Med **281**:1212–1216, 1969.
18. Gorodischer R, Jusko WJ, Yaffe SJ. Tissue and erythrocyte distribution of digoxin in infants. Clin Pharmacol Ther **19**:256–263, 1976.
19. Conover WJ, Iman RL. Rank transformation as a bridge between parametric and nonparametric statistics. Amer Stat **35**:124–129, 1981.
20. Miller RG Jr. Simultaneous Statistical Inference, 2nd ed. New York, Springer-Verlag, 1981.
21. Pederson KE, Dorph-Pederson A, Avidt S, Klitgaard NE, Nielsen-Kudsk F. Digoxin-verapamil interaction, a single dose pharmacokinetic study. Clin Pharmacol Ther **30**:311–317, 1981.
22. Halkin H, Radomsky M, Blieden L, Frand M, Millman P, Boichis H. Steady-state serum digoxin concentration in relation to digitalis toxicity in neonates and infants. Pediatrics **61**:184–188, 1978.
23. Weinhouse E, Kaplanski J, Warszawski D, Danon A, Gorodischer R. Cardiac toxicity of digoxin in newborn and adult rats. Pediatr Pharmacol **1**:97–103, 1980.
24. Weinhouse E, Kaplanski J, Martin O, Danon A. Quantitative electrocardiographic effects of digoxin in newborn and adult rats. Pediatr Pharmacol **2**:245–253, 1982.
25. Kaplanski J, Weinhouse E, Martin O. Modification of digoxin induced arrhythmogenicity in adult rats following atropine administration. Res Commun Chem Pathol Pharmacol **39**:173–176, 1983.
26. Valdes R, Becker S, Siegfried BA. Measurement of endogenous digoxin-like immunoactive factors in animal and human tissues (abstract). Clin Chem **31**:1015, 1985.
27. Lindenbaum J, Rund DG, Butler VP, Tse-Eng D, Saha JR. Inactivation of digoxin by the gut flora: reversal by antibiotic therapy. N Engl J Med **305**:789–794, 1981.
28. Antman EM, Smith TW. Digitalis toxicity. Mod Concepts Cardiovasc Dis **55**:26–30, 1986.
29. Wettrell G, Andersson KE. Clinical pharmacokinetics of digoxin in infants. Clin Pharmacokinet **2**:17–31, 1977.
30. Weinhouse E, Gootman N, Camacho JA, Diamant S, Kaplanski J, Danon A. Digoxin toxicity in childhood: emphasis on recent advances. Pediatr Rev Commun **1**:67–87, 1987.

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