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Comparison of digoxin-induced cardiac toxicity in resistant and sensitive species

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We have examined the species-specific arrhythmogenic effects of digoxin in the rat (resistant species) and in the guinea-pig (sensitive species). 26 adult rats and 23 adult guinea-pigs were anaesthetized with pentobarbitone and injected subcutaneously with varying doses of digoxin. Electrocardiograms were monitored continuously for 4½ h following digoxin administration. The arrhythmogenic dose (AD50) and lethal dose 50 under anaesthesia (LD50) were determined using the method of Litchfield & Wilcoxin. AD50 in rats was 13·0 ± 1·0 mg kg^{-1} (mean ± s.d.) compared with 0·60 ± 0·04 ($P < 0·01$) in the guinea-pig and LD50 was 30·0 ± 1·9 mg kg^{-1} in the rat compared with 0·60 ± 0·04 ($P < 0·01$) in the guinea-pig. The onset of arrhythmias was not dose-dependent in the rat but was clearly so in the guinea-pig; for example 102 ± 15 min (mean ± s.e.m.) following 0·5 mg kg^{-1}, and 43 ± 2 min following 1 mg kg^{-1}. In the rat the onset of arrhythmias was 54·0 ± 11·5 min. Supraventricular arrhythmias (paroxysmal atrial tachycardia) appeared in 73% of rats compared with only 18% of guinea-pigs whereas ventricular arrhythmias (ventricular tachycardia), multiple premature ventricular contractions and or multifocal PVC's occurred in 100% of guinea-pigs compared with only 32% of rats. All guinea-pigs that developed arrhythmias died whereas several rats recovered from supraventricular tachycardias. In conclusion, the guinea-pig is much more sensitive to digoxin toxicity than the rat, develops arrhythmias at much lower doses and these arrhythmias are much more likely to be ventricular in origin and cause fatality.

In addition to its positive inotropic effect on cardiac muscle, particularly diseased cardiac muscle, digoxin possesses antiarrhythmic properties particularly in the case of supraventricular tachycardias (Nadas & Fyler 1972). However, in toxic doses digoxin can also cause troublesome and even fatal arrhythmias in man (Joubert 1976) and in animals (Kelliher & Roberts 1976; Weinhouse et al 1980, 1982).

Traditionally, animal species have been defined as either sensitive or relatively resistant to the cardiac glycosides (Allen & Schwartz 1969). The rat has been described as a resistant species (Boor et al 1976; Franke & Joshi 1967; Fricke et al 1975); whereas, the guinea-pig is considered a sensitive species (Allen & Schwartz 1969; Stephen et al 1976). These definitions have been primarily based upon kinetic studies including myocardial uptake and subcellular distribution studies. Rat hearts demonstrate markedly lower digoxin uptake when compared with guinea-pig hearts under the same perfusion conditions and in addition microsomal binding is greater in the case of guinea-pig myocardial cells (Dutta et al 1968).

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In the present study we have attempted to broaden and sharpen the species criteria for digoxin sensitivity using the LD50 and arrhythmogenic dose50 (AD50) as well as the types of arrhythmia and have described two reliable models for the characterization of cardiotonic action.

MATERIALS AND METHODS

Thirty-one adult female Charles-Rivers rats and 28 adult female guinea-pigs were lightly anaesthetized with sodium pentobarbitone (Nembutal) subcutaneously (s.c.), 30 mg kg^{-1}, and connected to continuous electrocardiographic monitoring by the use of limb-lead needle electrodes (Electronics for medicine, VR6). Rectal temperature was maintained near 37 °C by the use of an overhead lamp.

One hour after anaesthesia 5 rats (group I) and 5 guinea-pigs (group V) were injected with propylene glycol s.c. and electrocardiograms (e.c.g.) were recorded for an additional 4½ h. Six rats (group II) received 10 mg kg^{-1} digoxin (Sigma Chemical Co. St. Louis, Missouri) dissolved in propylene glycol, s.c., 6 rats (group III) received 20 mg kg^{-1} digoxin

and 14 rats (group IV) received 40 mg kg^{-1} digoxin. Eight guinea-pigs (group VI) received 0·5 mg kg^{-1} digoxin, 8 guinea-pigs (group VII) received 1 mg kg^{-1} digoxin and 7 guinea-pigs (group VIII) received 4 mg kg^{-1} digoxin. The volume of propylene glycol injected in all animals was constant, 4 mg kg^{-1}. E.c.g. recordings were made in all cases either until the death of an animal or for 4½ h after digoxin administration.

The arrhythmogenic dose 50 (AD50) and lethal dose50 (LD50) were determined for rats and guinea-pigs by the method of Litchfield & Wilcoxin (1949). The time of onset of arrhythmias, and types of arrhythmias were recorded and compared for each dose in each species and between both species. The results of the time of onset of arrhythmias were analysed by analysis of variance and Duncan's multiple range test.

RESULTS

All animals receiving propylene glycol only were alive and well at the end of each experiment and did not develop any e.c.g. changes or arrhythmias.

As can be seen from Table 1, the AD50 in rats is more than 20 times greater than that in guinea-pigs and the LD50 is 50 times greater in rats than in guinea-pigs. In rats, the LD50 is more than twice the AD50 ($P < 0·01$) whereas in guinea-pigs LD50 and AD50 are identical.

Table 1. AD50 and LD50 values in rats and guinea-pigs after digoxin (mean ± s.d.).

Species	AD50 (mg kg^{-1})	LD50 (mg kg^{-1})
Rat (n=26)	13·0 ± 1·0	30·0 ± 1·9**
Guinea-pig (n=23)	0.60 ± 0.04*	0.60 ± 0.04*

* Significant difference ($P < 0·01$) v rat.
** Significant difference ($P < 0·01$) v AD50.

When the time of onset of arrhythmias (Table 2) was compared in rats for the various doses of digoxin, no significant change was found between the groups, i.e. either arrhythmias appeared or did not, but those that occurred were fairly constant in time of onset but more frequent with increasing dosage. However, when compared in guinea-pigs, the time of onset of arrhythmias was clearly dose-dependent, with significantly and progressively earlier time of onset with increasing doses.

Paroxysmal atrial tachycardia (supraventricular tachyarrhythmia) was observed in 73% of rats which produced arrhythmias but in only 18% of guinea-pigs ($P < 0·01$). Whereas, 100% of guinea-pigs developing arrhythmias presented with either multiple and or multifocal premature ventricular beats (82%) or ventricular tachycardia (65%), only 32% of rats developed ventricular arrhythmias of any sort ($P < 0·01$). In addition all guinea-pigs producing arrhythmias died as a result of their arrhythmias (ventricular) whereas many rats recovered even after prolonged arrhythmias (especially supraventricular). Atrioventricular dissociation was observed in twice as many guinea-pigs (65%) as rats (32%) ($P < 0·01$) and in both species was almost always followed by death. Two to one heart block was found equally in both species.

Table 2. Onset of arrhythmias in rats and guinea-pigs after digoxin (mean ± s.e.m.).

Species	Dose (mg kg^{-1})	Onset of arrhythmias (min)
Rat	10, 20, 40 (n=6, 6, 14)	54 ± 11·5
Guinea-pig	0·5 (n=8)	102 ± 15
	1·0 (n=8)	43 ± 2[a]
	4·0 (n=7)	21 ± 3[ab]

[a] Significant difference ($P < 0·01$) vs 0·5.
[b] Significant difference ($P < 0·01$) vs 1·0.

Table 3. Types of arrhythmias observed in rats and guinea pigs after digoxin.

Species and types of arrhythmias	Total arrhythmias %	(No.)
Rat (22/26)		
Paroxysmal atrial tachycardia	73	(16)
2:1 heart block	32	(7)
Atrioventricular dissociation	32	(7)
Ventricular tachycardia	32	(7)
Guinea-pig (17/23)		
Multiple PVC's and/or multifocal PVC's	82 } 100	(17)
Ventricular tachycardia	65 }	
Atrioventricular dissociation	65	(11)
2:1 heart block	29	(5)
Paroxysmal atrial tachycardia	18	(3)

Fig. 1a shows an example of paroxysmal atrial tachycardia more characteristic of rats after digoxin, and Fig. 1b shows two examples of ventricular tachycardia more characteristic of guinea-pigs after digoxin toxicity. Fig. 1c shows an example of multiple and multifocal premature ventricular beats characteristic of digoxin toxicity in guinea-pigs.

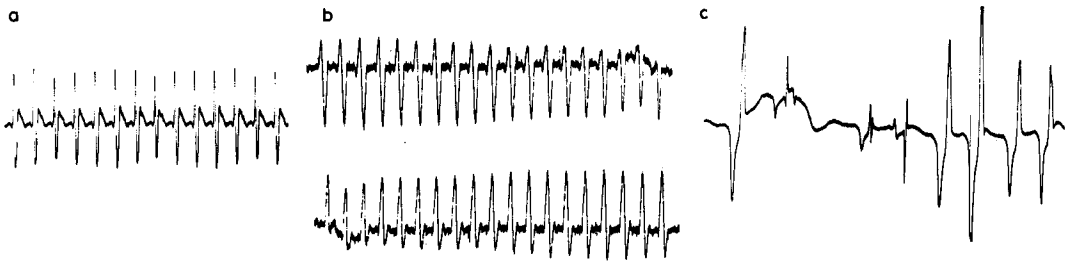

FIG. 1. Representative arrhythmias appearing in the rat and guinea-pig after digoxin. Paroxysmal atrial tachycardia in a rat receiving 40 mg kg^{-1} of digoxin s.c. (a); two examples of ventricular tachycardia in guinea-pigs receiving 4 mg kg^{-1} of digoxin s.c. (b); multiple and multifocal premature ventricular beats in a guinea-pig receiving 1 mg kg^{-1} of digoxin s.c. (c).

DISCUSSION

Several authors have claimed that the rat is relatively digitalis-resistant and electrocardiographically insensitive to cardiac glycosides (Franke et al 1967; Fricke et al 1975; and Boor et al 1976). We have already demonstrated that when high enough doses are given to newborn and adult rats, marked toxicity is observed especially in the newborn. Compared with most species, however, very high doses are needed to produce quantitative e.c.g. effects (Weinhouse et al 1982) or arrhythmias (Weinhouse et al 1980). However, the rat is clearly not 'electrocardiographically insensitive to cardiac glycosides' and certainly not to digoxin. On the other hand man (Halkin et al 1978), rabbits (Kelliher & Roberts 1976), guinea-pigs (Allen & Schwartz 1969; Stephen et al 1976) and other species are sensitive to the various cardiac glycosides. Several studies have been carried out to explain this difference in sensitivity, particularly in rats and guinea-pigs, at cellular and biochemical levels (Dutta & Marks 1966; Dutta et al 1968; Allen & Schwartz 1969; Stephen et al 1976). Myocardial uptake is much greater in guinea-pigs compared with rats under identical perfusion conditions and microsomal binding is also greater in the guinea-pig myocardial cells (Dutta et al 1968). Furthermore, digoxin is mainly metabolized in the liver in rats compared with primarily renal excretion in most other species (Gorodischer 1980). Hence, definite differences in pharmacokinetics exist in the rat when compared with the guinea-pig. However, it is not our present purpose to expand upon the reasons for species differences in digoxin sensitivity but rather to sharpen the criteria for considering one species sensitive and another resistant, based upon several criteria not emphasized in the past.

On the basis of this study we would define a digoxin-sensitive species as one in which (i) toxicity may be observed following relatively low doses of digoxin, (ii) there are low AD50 and low LD50 values, (iii) ventricular arrhythmias are the predominant type of arrhythmias observed, and (iv) these arrhythmias are likely to cause fatality. The guinea-pig fulfils these criteria in so far as 0·5 mg kg^{-1} of digoxin can already produce e.c.g. effects including ventricular and fatal arrhythmias, and the AD50 is 20 times lower than in the rat, while the LD50 is 50 times lower than in the rat. The very high sensitivity is further exaggerated by the fact that the AD50 and LD50 are identical in the guinea-pig. On the other hand the rat fulfils the following criteria and may be considered an example of digoxin-resistant species: (i) very high doses of digoxin are needed to demonstrate any e.c.g. effects, (ii) AD50 and LD50 are high values, (iii) supraventricular arrhythmias (paroxysmal atrial tachycardia) are the predominant type of arrhythmias observed and (iv) these arrhythmias are often reversible and not fatal. At least 10 mg kg^{-1} of digoxin is needed to produce arrhythmias in the rat compared with only 0·5 mg kg^{-1} of digoxin in the guinea-pig. Furthermore in rats, in contrast to guinea-pigs, the LD50 is more than twice as high as the AD50. The predominant type of arrhythmia in rats is paroxysmal atrial tachycardia and many of these rats recover during the duration of the experiment.

Perhaps, a fifth criterion may be added to each list. We have already shown that in the rat, the so-called resistant species, the newborns are much more sensitive to digoxin toxicity than the adults and in several respects resemble so-called adult 'sensitive' species. LD50 and AD50 value are much lower in newborn rats compared with adults and arrhythmias are more lethal (Weinhouse et al 1980, 1982). In contrast, in the guinea-pig, the sensitive species, Wollenberger et al (1953) found that the intravenous lethal dose of ouabain per kg weight decreased by about 25% between birth and maturity. Therefore it

may be true that in resistant species the newborns are more sensitive in contrast to sensitive species where the adults are more sensitive.

In conclusion we have found that the rat may serve as a reliable model for studying supraventricular tachyarryhthmias and the guinea-pig as a model for studying ventricular tachyarrhythmias. Furthermore, the guinea-pig is much more sensitive to digoxin toxicity than the rat, develops arrhythmias at much lower doses and these arrhythmias are much more likely to be ventricular in origin and lethal.

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