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SERUM DIGOXIN DETERMINATION IN OUTPATIENTS—NEED FOR STANDARDIZATION

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1 Blood samples were taken from 30 outpatients for serum digoxin analysis before and after 5 min – 4 h of rest in the supine position.

2 The digoxin concentration increased significantly during rest and a steady-state concentration was reached after approximately 2 h of rest.

3 The mean increase in serum digoxin after 2 h of rest was 23% with a considerable range, i.e. 0–75% (0–0.6 nmol/l).

4 On the analogy of a previous finding of a decrease in serum digoxin concentration during exercise in healthy subjects ingesting digoxin, the present results suggest that everyday physical activity affects the serum digoxin concentration. Standardized rest in the supine position prior to collecting blood samples from outpatients is therefore necessary for reliable serum digoxin determinations.

Introduction

Estimation of serum digoxin has been considered a valuable aid chiefly in two situations: suspicion of either digoxin intoxication or non-compliance.

Serum digoxin concentrations of outpatients have been reported to be significantly lower than corresponding concentrations of inpatients (Sheiner *et al.*, 1974). This phenomenon has been interpreted as poor outpatient compliance (Sheiner *et al.*, 1974). However, exercise has recently been found to increase digoxin binding in skeletal muscle with a concomitant decrease in serum digoxin concentration (Jogestrand & Sundqvist, 1981). After the exercise serum digoxin increased again. Even everyday physical activity performed by patients treated with digoxin has been found to decrease serum digoxin concentration (Jogestrand, 1981). Thus, when comparing serum digoxin concentrations measured in the outpatient department immediately after the patient's arrival and after at least 40 min rest in the supine position the concentration measured after rest was significantly higher.

The purpose of the present investigation was to study the time course of the increase in serum digoxin during standardized rest in the outpatient department and to estimate the interindividual range of the maximal increase in serum digoxin concentration.

Methods

Thirty patients (18 men and 12 women, aged 48–86, mean age 62.7 years) took part in the study, which was approved by the Ethical Committee of the Karolinska Hospital. The nature and purpose of the investigation were explained to the participants and their informed consent was obtained.

All patients had been treated with digoxin within the dose range of 0.13–0.375 mg/day for at least 1 month without any change in dosage. Twenty patients had also been treated with diuretics and seven with a β-adrenoceptor blocker. Six patients had been treated with verapamil and one patient with nifedipine. None of the patients were treated with quinidine or spironolactone.

No patient had signs of digoxin intoxication.

The investigations were made in the outpatient department 14–24 h after the last dose of digoxin. The patients arrived at the clinic between 08.00 h and 10.00 h after 1–3.5 h of everyday physical activity. A cannula was inserted into an antecubital vein and the first blood sample was taken 1–3 min after the patients had been placed in the supine position. In the first 10 patients venous blood samples were then taken after 5, 15, 30, 45, 60, 75, 90, 105 and 120 min in the supine position. In the next 20 patients blood sampling was repeated after 30, 60, 90, 120, 150, 180,

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210 and 240 min in the supine position. In all patients blood samples were also taken before rest in the supine position for analysis of electrolytes and serum creatinine.

Serum was separated simultaneously from all blood samples after completion of the investigation and the serum samples were then frozen until analysed. The serum digoxin concentration was measured by radioimmunoassay (Smith *et al.*, 1969) using a commercial ^{125}I-RIA kit (New England Nuclear, Massachusetts, USA). The coefficient of variation of duplicate analysis was 6%. The serum concentrations given are the mean values of duplicates.

Sodium, potassium, calcium and creatinine concentrations in the serum were assayed on a Technicon Autoanalyzer.

Mean values ± s.d. are given unless otherwise stated. Multiple linear regression analysis and Student's *t*-test for paired observations were used to test for statistical significance.

Results

Serum creatinine varied between 76 and 150 μmol/l. All but five patients had normal serum electrolyte concentrations. Of the patients with electrolyte disturbances one had a slight elevation of the sodium concentration, two had a calcium concentration above, and two below the reference values of the laboratory.

In the first ten patients the serum digoxin concentration before the supine rest period was 0.96 ± 0.32 nmol/l. This concentration did not change significantly after the first 30 min. After 45 min of rest, however, the serum digoxin concentration had increased significantly to 1.10 ± 0.37 nmol/l and the concentration continued to rise, reaching the highest value of 1.24 ± 0.46 nmol/l after 120 min of rest.

The following 20 patients had an initial serum digoxin concentration of 0.98 ± 0.39 nmol/l (Figure 1). The concentration after 30 min of rest was not significantly altered (Figure 1). However, after 1 h of rest the serum digoxin concentration had increased significantly and continued to do so until a levelling off after 1.5–2 h of rest (Figure 1). The digoxin concentrations after 3 and 4 h of rest were not significantly different from the concentration measured after 2 h of rest.

The mean serum digoxin concentration after 2 h of rest, 1.19 ± 0.42 nmol/l (n = 30), was 23% higher than the mean concentration before rest, 0.97 ± 0.37 nmol/l. The range of increase in serum digoxin was 0–75% (0–0.6 nmol/l).

To exclude the possibility that an *in vitro* reaction owing to the longer storage at room temperature may have caused the described change in serum digoxin concentration, the following experiment was performed. Two simultaneous blood samples were taken from 21 of the patients. Serum was immediately separated from one of the samples and frozen. The second blood sample was stored at room temperature for either 2 h (n = 10) or 4 h (n = 11) before separation and freezing. There were no statistically significant differences between the digoxin concentrations analysed after different storage intervals (Table 1).

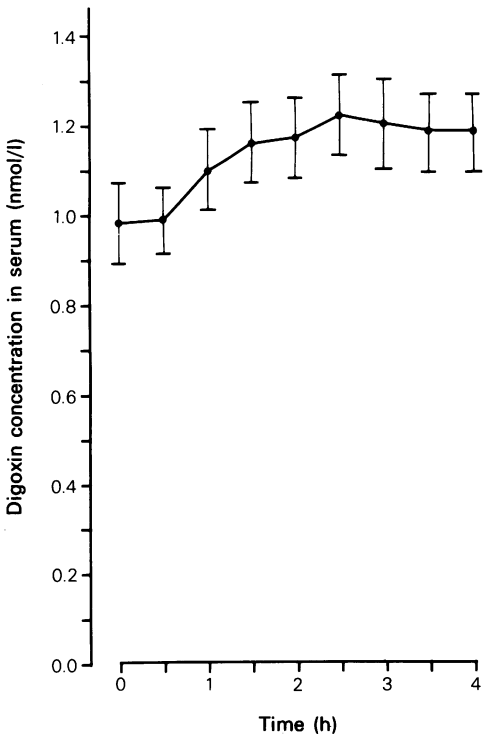

Figure 1 Digoxin concentrations in serum (mean ± s.e. mean) before and after 0.5, 1, 1.5, 2, 2.5, 3, 3.5 and 4 h of rest in the supine position (n = 20). The digoxin concentrations after 1–4 h of rest are significantly higher than the concentrations before rest ($P < 0.001$).

The relation between the assayed serum digoxin concentration (in patients not treated with verapamil or nifedipine), before and after 2 h of rest, and factors known to influence this concentration (age, sex, body weight, serum creatinine and digoxin dose) was assessed. Using multiple stepwise regression analysis a statistically significant correlation was found between serum digoxin concentration measured after 2 h of rest and sex, body weight and digoxin dose with a fraction of explained variance of 0.33 ($P < 0.05$). The corresponding correlation between serum digoxin concentration before rest and sex, body weight and dose of digoxin was not statistically significant, the fraction of explained variance being 0.17.

Table 1 Digoxin concentration (nmol/l) analysed in serum separated and frozen immediately after blood sampling and in serum stored at room temperature for 2 h (n = 10) and 4 h (n = 11), respectively, before separation and freezing.

	2 h	*4 h*
Serum separated immediately and frozen	0.99 ±0.46	0.95 ±0.41
Serum stored at room temperature before separation and freezing	1.00 ±0.50	0.99 ±0.42
	NS	NS

Discussion

The results of this investigation confirm our earlier report that rest in the supine position significantly increases serum digoxin in outpatients (Jogestrand, 1981).

The present study shows that, in outpatients performing everyday physical acitivity, a steady-state in serum digoxin is not reached until the patient has rested in the supine position for about 2 h. It is noteworthy that during the first 30 min of rest the serum digoxin concentration did not change significantly. If the increase in serum digoxin is related to and caused by decreased binding of skeletal muscle digoxin, a continuous increase in serum digoxin is to be expected. This may have been counteracted, however, during the first 30 min in the supine position by the rapid change in plasma volume that has been shown to occur during rest in the supine position after physical exercise (Kirsch *et al.*, 1968). It has been demonstrated that during rest after everyday physical activity the rise in plasma water is completed within 20–40 min (Ekelund *et al.*, 1971). Since 20–30% of serum digoxin is bound to serum proteins (for references see, for instance, Iisalo, 1977), an increase in plasma water would produce a slight decrease in serum digoxin concentration and thereby mask an increase in serum digoxin owing to decreased binding of skeletal muscle digoxin.

The increase in serum digoxin is not explained by an *in vitro* reaction owing to different periods of sample storage at room temperature. Thus, the analysed digoxin concentrations in samples stored for 2 h or 4 h at room temperature did not differ from those of the samples not stored at room temperature.

The mean increase in serum digoxin after 2 h of rest was 23%. The interindividual range of the increase in serum digoxin concentration was considerable: 0–75% (0–0.6 nmol/l), showing that the effect of everyday physical activity prior to blood sampling on the serum digoxin concentration is an unpredictable factor in outpatients. Consequently, a constant correction factor cannot be used. Standardized rest in the supine position before taking the blood sample is therefore necessary to obtain reliable values.

To illustrate the importance of standardized rest, before taking blood samples in outpatients, the relation between the assayed serum digoxin concentration, before and after 2 h of rest, and factors known to influence this concentration was assessed. The patients treated with verapamil and nifedipine were excluded since these drugs have been reported to increase serum digoxin concentration (Klein *et al.*, 1980; Belz *et al.*, 1981). A statistically significant correlation was found between serum digoxin concentration measured after 2 h of rest and sex, body weight and digoxin dose, while the corresponding correlation between serum digoxin concentration before rest and sex, body weight and dose of digoxin was not statistically significant. The difference in explained variability in serum digoxin concentration is most probably a consequence of the unpredictable effect of physical activity on the serum digoxin concentration without standardized rest in the supine position before blood sampling.

The clinical significance of the described phenomenon remains to be established. According to the recommended therapeutic range of 1.0–2.5 nmol/l (Department of Clinical Pharmacology, Karolinska Hospital) 18 (60%) of the investigated patients had subtherapeutic digoxin concentrations when the blood samples were taken before rest. After 2 h of rest 6 of these 18 patients had serum digoxin concentrations within the therapeutic range. Thus, it is most probable that the difference in serum digoxin concentrations between outpatients and inpatients reported earlier (Sheiner *et al.*, 1974) is related to differences in physical activity prior to blood sampling and not entirely due to poor patient compliance as has been suggested (Sheiner *et al.*, 1974). Further support for this assumption is provided by the results of a previous investigation of 13 digoxin-treated patients with serum digoxin analysis in the ward and 1–3 weeks later in the outpatient department (Jogestrand, 1981). The serum digoxin concentration in the outpatient department was significantly lower than that measured in the ward. However, when the patients were allowed to rest in the supine position for at least 40 min before blood sampling the digoxin concentration increased so as to become not significantly different from the concentration measured on the ward.

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