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Measurement of digitalis-glycoside levels in ocular tissues:

A way to improve postmortem diagnosis of lethal digitalis-glycoside poisoning? II. Digitoxin*

S. Ritz, P. Harding, W. Martz, H. W. Schütz, and H.-J. Kaatsch

Institut für Rechtsmedizin, Christian-Albrechts-Universität zu Kiel, Arnold-Heller-Strasse 12, W-2300 Kiel 1, Federal Republic of Germany

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Summary. Postmortem digitoxin levels in the choroid-retina and vitreous humor of patients who had undergone digitoxin therapy (therapeutic group) and in one case of suicidal digitoxin poisoning were measured and compared with levels in femoral vein blood, myocardium, kidney and liver. The results were interpreted in light of the medical history of each patient. The digitoxin level in the choroid-retina of the single case of suicidal poisoning was far higher than the choroid-retinal levels in the therapeutic group. In the latter, variation in choroid-retinal levels was comparable to that in the other tissues. In cases where the choroid-retina of the right and left eyes were examined, digitoxin levels in both eyes were essentially equal. There was no indication of significant changes in choroid-retinal levels due to postmortem diffusion of digitoxin into the vitreous body. Based on these results, determination of digitoxin levels in the choroid-retina could contribute to improving postmortem diagnosis of lethal digitoxin poisoning.

Key words: Postmortem diagnosis – Digitoxin poisoning – Ocular tissues

Zusammenfassung. Bei mit Digitoxin therapierten Klinikpatienten (therapeutisches Kollektiv) sowie in einem Fall einer suicidalen Digitoxinvergiftung wurden postmortem Digitoxinkonzentrationen in Choroidretina und Glaskörperflüssigkeit bestimmt. Die erhaltenen Werte wurden mit den Digitoxinspiegeln in Femoralvenenblut, Myocard, Niere und Leber verglichen und unter Berücksichtigung anamnestischer Daten bewertet. In dem untersuchten Intoxikationsfall lag der Choroidretinaspiegel weit über den im therapeutischen Kollektiv bestimmten Werten. Die Streuung der Choroidretinakonzentrationen war im therapeutischen Kollektiv mit den Streuungen der übrigen Gewebespiegel vergleichbar. In einigen Fällen wurden Digitoxinspiegel in der Choroidretina beider Augen bestimmt, die Werte für rechtes und linkes Auge stimmten jeweils sehr gut überein. Mit einer wesentlichen Verfälschung der Choroidretinaspiegel durch eine mögliche postmortale Diffusion des Digitoxins in den Glaskörper ist nach unseren Erfahrungen nicht zu rechnen. Unsere Untersuchungsergebnisse sprechen dafür, daß die Digitoxinkonzentration in der Choroidretina als zusätzlicher Parameter in der postmortalen Diagnostik von tödlichen Digitoxinintoxikationen genutzt werden kann.

Schlüsselwörter: Digitoxinintoxikation – Postmortale Diagnostik – Okuläre Gewebe

Introduction

Animal studies have demonstrated that digoxin and digitoxin accumulate in ocular tissues, especially in the retina, following administration [5–7, 9–11].

For *digoxin* we could confirm the results of these animal experiments through postmortem measurement of digoxin levels in human ocular tissues [15]. Our results support the thesis that measurement of digoxin levels in human choroid-retina can aid in the postmortem diagnosis of digoxin intoxication.

In order to determine whether investigation of ocular tissues is also useful in cases of suspected *digitoxin* poisoning, we measured postmortem digitoxin levels in ocular tissues (choroid-retina, vitreous body) of patients who had received in-patient digitoxin therapy and of one case of suicidal digitoxin poisoning. Digitoxin levels in the ocular tissues were compared with those in the heart, kidney, liver and serum and the results interpreted in light of the available anamnestic data.

Patients and methods

Postmortem digitoxin levels in choroid-retina, vitreous humor, serum, myocardium, kidney and liver were measured in 35 patients

* This study contains results from the dissertation of P. Harding

Correspondence to: S. Ritz

who had received in-patient digitoxin therapy prior to death (therapeutic group) and in one case of suicidal digitoxin poisoning.

The 35 patients in the *therapeutic group* had died in the University Hospital of Christian-Albrechts-University in Kiel and were autopsied in the University Institute of Pathology. The interval between death and autopsy (postmortem interval) ranged from 21 to 106 h.

Medical records and autopsy reports were evaluated in all 35 therapeutic cases.

The age of the patients (14 women, 21 men) ranged from 34 to 90 years, with a mean age of 71 years. All 35 patients were reported to have died of natural causes. In no patient was digitalis-glycoside intoxication suspected.

At least 27 of the 35 patients had received *long term therapy* with digitoxin (0.1 mg/day peroral in 16 cases; 0.07 mg/day peroral in 11 cases) up to the time of death. In 2 patients treatment with digitoxin (0.1 mg/day peroral) was discontinued 9 and 11 days before death. In 3 other patients we could not determine up to what time the digitoxin therapy had been given. Three further patients died shortly after initiation of the digitoxin therapy, before the distribution phase was complete. The length of the digitoxin-free interval (time between last digitoxin dose and death) ranged from 0.8 h to 11 days.

According to the police report on the *case of suicidal intoxication*, the 76-year-old woman was found dead in her apartment. A sharp can-opener and a lamp lay beside the body. The lamp was plugged in and the empty light socket was live. Also found in the apartment were a cup containing traces of a white powdery substance, an empty bottle for the medication "Digimerck" (digitoxin) and a suicide note. Large quantities of vomitus were found near the body. A similar substance was found in the facial area at autopsy. The middle and ring fingers of the right hand showed skin changes indicative of electrical burns. Multiple superficial slashes and stab wounds were found on the front of the throat, the inside of the left wrist, and on the left front of the thorax. The electrical burns appeared to have been caused by insertion of the two fingers into the lamp socket. The pattern and location of the superficial wounds suggest they could have been inflicted by the woman herself using the can-opener.

The main internal findings at autopsy were signs of acute cardiac failure and a gastrointestinal content containing whitish granular particles resembling the remnants of tablets and extending into the lower small intestine. Histological examination of the electrical markings did not indicate an inflammatory reaction.

Choroid-retina[1] of one eye, vitreous humor, femoral vein blood, and myocardium, kidney, and liver tissues were extracted at autopsy as outlined previously [15]. In 4 cases choroid-retinal samples were taken from both eyes.

All samples were prepared as described previously [15]. Measurements were made with a fluorescence polarization immunoassay (FPIA; TDx Measurement System for Therapeutica, TDx Digitoxin, Abbott Laboratories).

Results

1. *Therapeutic group*

The variations in digitoxin levels in body fluids and tissues for the entire therapeutic group ($n = 35$) as well as in the subgroup of 27 patients who had received *long-term digitoxin therapy* are summarized in Table 1.

The mean serum levels for both groups were within the therapeutic range (10–30 ng/ml); serum levels significantly outside the therapeutic range were not observed.

Table 1. Range of variation, mean values and standard deviations (Mv ± s) for digitoxin levels in tissues and body fluids of the entire therapeutic group ($n = 35$) and of the subgroup of 27 patients who underwent *long-term therapy* (n.d. = below the detection threshold of 1 ng/ml)

Tissues and body fluids	Digitoxin levels (ng/g wet weight or ng/ml)	
	Entire therapeutic group ($n = 35$)	Long-term therapy ($n = 27$)
Myocardium	5.7–264.8 ng/g ($n = 34$) Mv ± s: 84.0 ± 44.8 ng/g	41.6–264.8 ng/g ($n = 26$) Mv ± s: 91.6 ± 45.5 ng/g
Kidney	16.4–365.1 ng/g ($n = 35$) Mv ± s: 100.2 ± 87.9 ng/g	26.4–365.1 ng/g ($n = 27$) Mv ± s: 116.7 ± 93.4 ng/g
Liver	1.5–92.3 ng/g ($n = 34$) Mv ± s: 42.9 ± 23.0 ng/g	17.0–92.3 ng/g ($n = 26$) Mv ± s: 49.0 ± 21.0 ng/g
Serum	1.3–32.1 ng/ml ($n = 25$) Mv ± s: 18.2 ± 8.1 ng/ml	5.4–32.0 ng/ml ($n = 19$) Mv ± s: 18.4 ± 6.1 ng/ml
Choroid-retina	3.2–158.1 ng/g ($n = 34$) Mv ± s: 60.7 ± 34.9 ng/g	19.5–158.1 ng/g ($n = 26$) Mv ± s: 63.4 ± 34.2 ng/g
Vitreous humor	n.d.–6.3 ng/ml ($n = 35$) Mv ± s: 1.7 ± 1.9 ng/ml	n.d.–6.3 ng/ml ($n = 27$) Mv ± s: 2.1 ± 1.9 ng/ml

Digitoxin levels in all tissues, particularly those in kidney, varied widely.

Figure 1 depicts the mean values and standard deviations for the subgroup of 27 patients who had received *long-term therapy*. The mean level in the choroid-retina was below that in the myocardium. The highest mean tissue level was found in kidney, the lowest in liver.

No definite correlation was found between postmortem digitoxin levels in serum and levels in choroid-retina, vitreous humor, myocardium, kidney or liver. Furthermore, no discernable correlation was seen between choroid-retinal levels and those in kidney or liver. Figure 2 shows the digitoxin levels in choroid-retina in the therapeutic group compared to those for the "target tissue", the myocardium; here a possible, although rather loose, correlation is evident[2]. Digitoxin levels in the vitreous humor showed no discernable relationship to those in the choroid-retina or to the duration of either the therapy-free or postmortem intervals.

In 4 patients the choroid-retinae of the left and right eyes were examined (Table 2). A close correlation between the values for both eyes was found in each case.

[1] Because of the difficulties involved in post mortem preparation of choroid and retina, these tissues were examined jointly as "choroid-retina"

[2] The correlation coefficient was determined to be $r = 0.65$. However, this may not be very reliable in light of the relatively small – for a statistical analysis – series ($n = 33$)

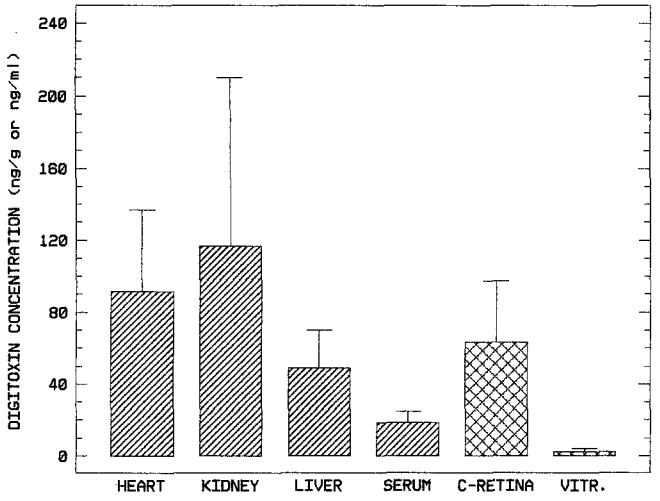

Fig. 1. Mean digitoxin levels (columns) in tissues and body fluids of the group of 27 patients who underwent long-term therapy; the standard deviations are indicated by the lines on the columns ("C-retina" = Choroid-retina, "Vitr." = vitreous humor)

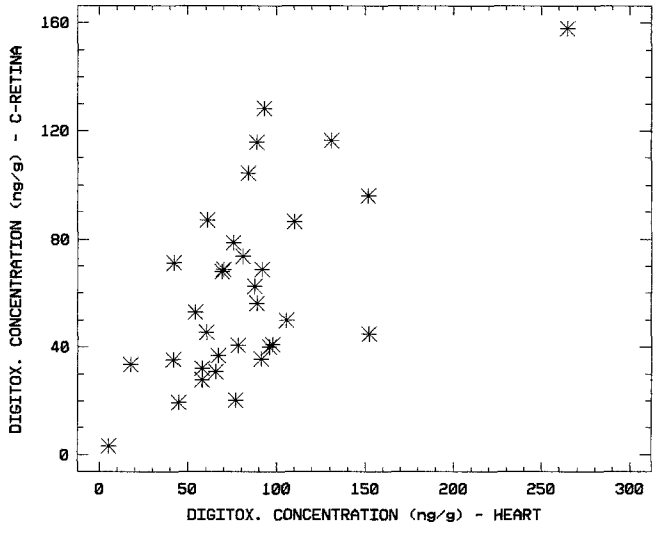

Fig. 2. Digitoxin levels in the choroid-retina ("C-retina") of the therapeutic group in relation to levels in the myocardium ($n = 33$)

Table 2. Comparison of digitoxin levels in the choroid-retina of the right and left eyes in 4 cases (A–D)

Case	Digitoxin levels (ng/g wet weight)	
	Choroid-retina right	Choroid-retina left
A	68.8	68.1
B	40.5	34.5
C	86.6	79.3
D	73.5	67.0

In Figure 3 the mean *digitoxin* levels in the subgroup of 27 patients who had undergone long-term *digitoxin* therapy are compared with *digoxin* levels in a group of 7 patients who had received long-term *digoxin* therapy (digoxin values taken from Ritz et al. [15]). Whereas the

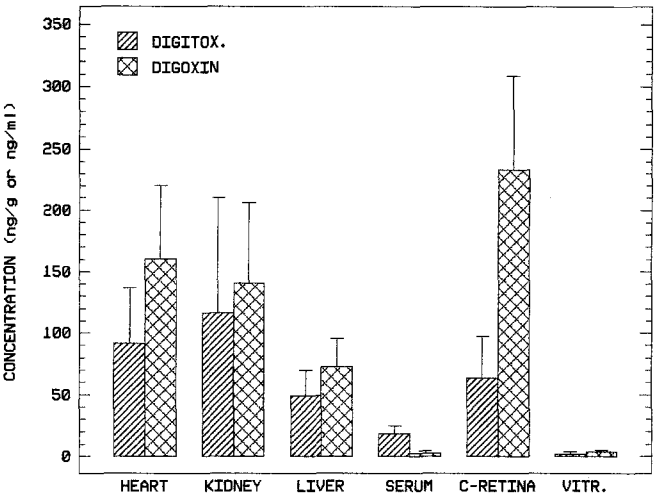

Fig. 3. Comparison of mean *digitoxin* levels in the 27 patients who received long-term *digitoxin* therapy (hatched columns) with the mean *digoxin* levels (cross-hatched columns) in a group of 7 hospital patients that underwent long-term *digoxin* therapy (digoxin values taken from [15]); the lines on the columns indicate the standard deviations ("C-retina" = choroid-retina, "Vitr." = vitreous humor)

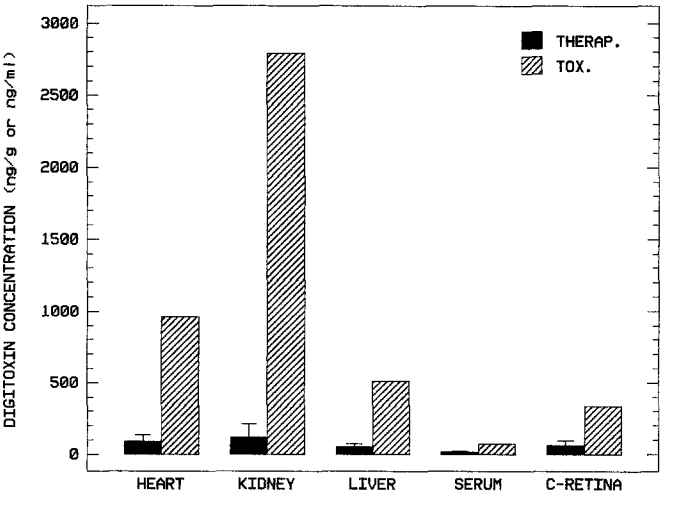

Fig. 4. Comparison of the tissue levels in the case of suicidal digitoxin intoxication (hatched columns) with mean digitoxin levels in the group of 27 patients who underwent long-term digitoxin therapy (dark columns; the lines on the columns indicate standard deviations); "C-retina" = choroid-retina, "Vitr." = vitreous humor

mean *digoxin* level in the choroid-retina clearly exceeded the mean values for all other tissues and body fluids, the mean *digitoxin* level in the choroid-retina was below the levels in myocardium and kidney. For *digoxin*, the ratio of mean digitalis-glycoside tissue levels in myocardium, kidney, liver and choroid-retina was 1:0.88:0.46:1.46, respectively. The corresponding values for *digitoxin* were 1:1.27:0.53:0.69.

2. *Suicidal Intoxication*

In the case of suicidal digitoxin poisoning extremely high digitoxin levels were present in the serum and in all tissues investigated:

- serum: 72.6 ng/ml,
- myocardium: 959.1 ng/g,
- kidney: 2791.1 ng/g,
- liver: 510.6 ng/g,
- choroid-retina: 334.5 ng/g.

In Figure 4 these values are compared with the mean digitoxin levels of the 27 patients who had received long-term digitoxin therapy. Levels in the case of suicidal digitoxin intoxication – including those in the choroid-retina – far exceeded the mean levels in the long-term therapy group.

Discussion

In contrast to the extensive literature on *digoxin* levels in blood and tissues (for review see Aderjan [3]), little information is available on the distribution of *digitoxin* in body fluids and organs following therapeutic doses or intoxication.

Unlike postmortem digoxin blood levels, postmortem digitoxin *blood levels* should be comparable to antemortem blood levels [1–3, 14]. Digitoxin serum levels in our therapeutic group where the upper therapeutic limit was never significantly exceeded (Table 1) confirm this hypothesis. But because of the large overlap between therapeutic and toxic digitoxin levels in blood and the possibility of misleadingly high blood levels before distribution is complete, postmortem digitoxin blood levels should never be evaluated in isolation but always in conjunction with tissues levels.

Mean digitoxin *levels in the myocardium, kidney and liver* in our therapeutic group were well below the corresponding values reported by Aderjan [2] in patients who had received therapeutic doses. This discrepancy is probably due to the disparate methods used for tissue preparation and measurement. Although our absolute values differed, the relative digitoxin distribution we found in heart and liver was similar to that reported by Aderjan [2]. The ratio of the mean digitoxin levels in the left ventricle and liver was 1:0.53 in our long-term therapy group and 1:0.51 in Aderjan [2]. Our digitoxin levels in kidney were not directly comparable to those of Aderjan [2] since he evaluated the renal cortex and pulp separately.

Whereas the ratio between the mean levels in *myocardium, kidney, and liver* in the 27 patients who had received long-term *digitoxin* therapy was 1:1.27:0.53, the corresponding values in a group of patients who had undergone long-term *digoxin* treatment were 1:0.88:0.46 (Digoxin values from Ritz et al. [15]). The significance of the higher mean kidney concentrations for digitoxin is open to question since the kidney levels varied widely and the digoxin group was small ($n = 7$). Despite the discrepancy, the relative levels indicate a similar distribution of digitoxin and digoxin in myocardium, kidney and liver. This accords in essence with Aderjan's findings [2].

Like Aderjan [2], Lukas [12] and Storstein [16], we found a wide variation in tissue concentrations in heart, kidney and liver following therapeutic doses of digitoxin (Table 1).

Digitoxin levels in the vitreous humor of the therapeutic group were relatively low, and were comparable to the digoxin concentrations we measured in this body fluid [15]. The digitoxin levels in the vitreous humor showed no discernable relationship either to levels in the choroid-retina or to the length of the postmortem interval. However, in light of the sizable difference between levels in the retina and the vitreous humor a possible increase in the latter due to postmortem diffusion should be considered in the interpretation of postmortem digitoxin levels in vitreous humor.

The mean digitoxin level in the *choroid-retina* of the long-term therapy patients lay between the mean levels in the myocardium and liver (Fig. 1). A comparison with the levels in patients who had received long-term *digoxin* therapy (Fig. 3) showed that digitoxin apparently accumulates in the choroid-retina to a significantly lesser degree than digoxin, which was present in much higher levels in the choroid-retina than in all other tissues examined. This finding accords with the results of clinical studies showing that diminished ability to differentiate colors due to impaired retinal function occurs at much lower doses of digoxin (already at subtoxic serum levels) than of digitoxin [8].

In contrast to our findings, Duncker and Herzig [7] observed a greater accumulation of digitoxin in the choroid and retina of animals. Apart from the fact that findings in animals are not necessarily valid for humans, the results of these authors are not comparable to our results because digitoxin and digoxin were each administered in a single dose and the animals were killed 1–3 h later. The patients we examined, on the other hand, had undergone long-term therapy.

Why digoxin accumulates more readily in the choroid-retina than the more lipophilic digitoxin, while both show an almost equal distribution pattern in heart, kidney and liver, is difficult to answer. Haustein et al. [8] suspected that binding sites on plasma proteins, which have a much higher affinity for digitoxin than for digoxin, compete with binding sites in the retina, thus reducing the concentration of digitoxin in the retina. However, other organs would also be affected by such a competition between plasma protein and tissue binding. Hence, it is unlikely that this mechanism alone could be responsible for the highly divergent accumulations of digoxin and digitoxin in the choroid-retina.

No reliable correlation was found between digitoxin levels in serum and those in choroid-retina and other tissues. This agrees with the results of Storstein [16], who found no significant correlation between digitoxin levels in serum and myocardium. Furthermore, no discernible correlation was found between levels in the choroid-retina and those in liver and kidney. A possible, but at most slight, correlation was observed between digitoxin levels in the choroid-retina and myocardium (Fig. 2). This lack of, or at best weak, correlation could result from a variably intense, nonspecific binding [4, 6] or from alterations in the tissue levels caused by pathological changes in structure and function of the tissues.

In the *case of suicidal intoxication* digitoxin levels were far higher than the corresponding mean values in

patients who had undergone long-term therapy. The digitoxin tissue concentrations in this case also exceeded the threshold values reported by Aderjan [2] and thus support the hypothesis that the woman died of lethal digitoxin poisoning. The vomitus found near the body and at autopsy can be interpreted as a sign of a central nervous system disorder induced by digitalis intoxication. However, at autopsy electrical burns were found and histological investigation indicated that the survival time would have been short. Thus, electrocution must also be considered as the possible direct cause of death, despite the fact that electrical burns only prove that electrical shock – but not necessarily electrocution – had occurred. Regardless of whether death was caused by electrocution or by digitoxin intoxication, the extremely high digitoxin levels in all organs investigated indicated that intoxication was a primary mechanism leading to death.

The variation in choroid-retinal levels in the therapeutic group was clearly smaller than in the kidney and was comparable to the variation of levels in myocardium and liver (Table 1 and Fig. 1). In the 4 cases in which both eyes were studied, a close correlation was found between the levels in each eye (Table 2). There was no indication of significant changes in choroid-retinal levels due to postmortem diffusion into the vitreous body. In the case of suicidal intoxication, the digitoxin concentration in the choroid-retina far exceeded the choroid-retinal levels of the long-term therapy patients.

These results support the thesis that measurement of digitalis-glycoside levels in the choroid-retina can aid not only in cases of suspected digoxin intoxication [15] but also in cases of suspected digitoxin poisoning. It has already been pointed out [15] that reliable data for comparison must be obtained in studies of large series of therapeutic and toxic cases before ocular tissue levels can be used in the diagnosis of suspected digitalis-glycoside poisoning.

In these first investigations on digitalis-glycoside levels in *human* ocular tissues, concentrations of metabolites were not measured. However, it has been shown that the pattern of digoxin metabolites in the myocardium, especially the relationship between digoxin and digoxigenin, can aid in the evaluation of cases of suspected intoxication [13, 14]. It remains to be determined whether a separation of the metabolites of digoxin and digitoxin in the *choroid-retina* can provide additional information that can facilitate the postmortem diagnosis of digitalis-glycoside poisoning.

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