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PHOTOCHEMICAL DEGRADATION OF DIGOXIN TESTED BY NA,K-ATPase ACTIVITY

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Abstract

The photochemical degradation of digoxin aqueous solution was obtained by Xe-lamp irradiation. The concentrations of digoxin in irradiated solutions were detected by measurements of Na,K-ATPase activity and by HPLC analysis. The excellent agreement using two independent methods for determination of digoxin concentration in the irradiated samples was achieved.

Introduction

Na,K-ATPase is transmembrane enzyme, which utilize energy liberated from ATP hydrolysis for active transport of monovalent cations across the membranes [1,2]. Digoxin, cardiac glycoside most frequently used to improve cardiac contractility in the treatment of congestive heart failure, acts through the inhibition of Na,K-ATPase [3]. The exposure to light of a great number of organic compounds results in photolytic degradation, leading to their decomposition or to the formation of other toxic compounds. There is lack of data concerning the photochemical degradation of digitalis cardiac glycosides. However, digoxin is known as stable when keeping in the dark and well-closed containers, but it undergoes to the acid catalysed hydrolysis in water solutions [4]. Since digoxin belongs to the group of the specific Na,K-ATPase inhibitors, the aim of this work was to investigate the possibility of the use of Na,K-ATPase assay for the determination of its concentration after the photochemical treatment.

Experimental

Chemicals. All chemicals were of analytical grade. Na,K-ATPase from porcine cerebral cortex was purchased from Sigma Co. Digoxin was obtained from "Zdravlje", Leskovac. Digoxin was irradiated by using Xe lamp of 125 W. The sample was illuminated with light from 220 to 1100 nm, but only the light between 220 and 300 nm was consumed by the molecule.

ATPase assay. Na,K-ATPase activity was determined in 200 μl of the standard incubation medium [4], 2 mg/ml protein and 20 μl of the irradiated digoxin solution. The reaction was started by the addition of ATP at 37 °C. The inorganic orthophosphate (P_i) liberated from the hydrolysis of ATP was measured using modified spectrophotometric procedure [4]. The enzymatic activity in the presence of digoxin was calculated as the percentage of the control value of the standard incubation mixture, without the inhibitor.

Apparatus. HPLC measurements were carried out on a system Hewllet Pacard Series 1100, according to the procedure *Jedlička et al.* [5].

Results and Discussion

Detection of digoxin concentration by Na,K-ATPase assay. Aqueous solution containing $1x10^{-3}$M digoxin and 30% ethanol was irradiated by Xe-lamp for 2.5, 5.0, 7.5, 10.0, 20.0, 40.0 min. The irradiated samples were diluted in the concentration range from $1x10^{-9}$ M to $1x10^{-4}$ M, related to the concentration of the unirradiated sample. The influence of the various irradiation times on digoxin induced inhibition of Na, K-ATPase activity was followed in the above mentioned concentration range as described in *Experimental*. The concentration dependent inhibition curve representing Na,K-ATPase activity vs. digoxin concentration in the unirratiated sample served as the calibration graph for determination of digoxin concentration in irradiated sample. The inhibition curves (activity *vs*. the added digoxin concentration before the photochemical treatment) for various irradiation times are presented in Figure 1. The results show that the increasing of the irradiation time decreased the digoxin-induced inhibition. The concentration of digoxin in irradiated solutions was extrapolated from the concentration dependent inhibition curve of the unirradiated sample and the results are given in Table 1.

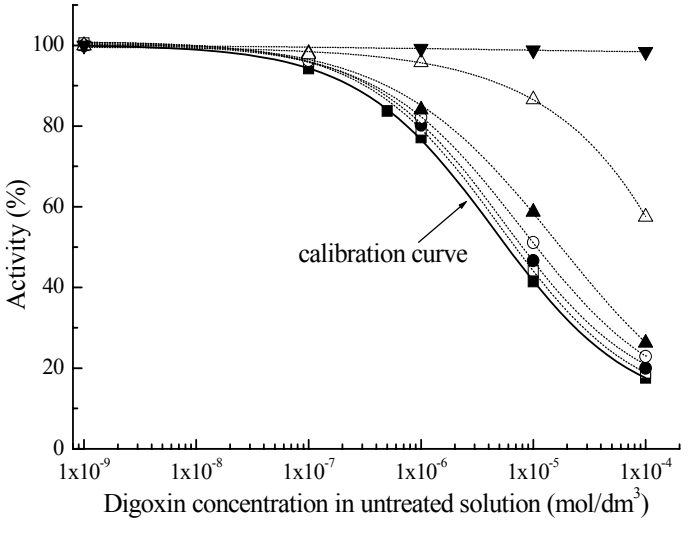

Figure 1. Digoxin-induced inhibition of Na,K-ATPase: unirradiated solution of digoxin (■), solutions of digoxin irradiated for 2.5 min. (□), 5.0 min. (●), 7.5 min. (○), 10.0 min. ▲), 20.0 min. (Δ) and 40.0 min. (▼)

HPLC analysis of irradiated samples. For the comparison, the concentrations of digoxin in irradiated samples were determined by HPLC. It is worthy to notice, that the other degradation products, except the reduced digoxin concentration, were not detected by HPLC analysis. The digoxin concentrations obtained by HPLC analysis are also presented in Table 1, together with the calculated efficiency of degradation. By the applied irradiation energy, over 96% degradation efficiency was achieved already 20 min following the irradiation, since after 40 min irradiation the degradation was complete.

Table 1. Comparison of concentrations of digoxin determined by HPLC and AT-Pase assay in the irradiated samples

Time of irradiation (min.)	digoxin concentration (mol/dm^3)		Efficiency of irradiation (%)
	determined by HPLC	determined by ATPase assay	
0	1.00 x10^{-3}	1.00x10^{-3}	0.0
2.5	8.83 x10^{-4}	8.70x10^{-4}	11.7
5.0	7.23 x10^{-4}	7.32x10^{-4}	27.7
7.5	5.41 x10^{-4}	5.44x10^{-4}	45.9
10.0	3.26 x10^{-4}	3.48x10^{-4}	67.4
20.0	3.76 x10^{-5}	3.87x10^{-5}	96.2
40.0	0	1.24 x10^{-9}	100.0

Conclusion

The determination of digoxin concentration by standard analysis (HPLC) and by the measurement of Na,K-ATPase activity showed the excellent agreement. The measurement of digoxin induced inhibition of Na,K-ATPase activity was considered to be a good test for the determination of the degree of degradation after the photochemical treatment. Moreover, the change of Na,K-ATPase activity can indicate the toxicity of photochemically irradiated digoxin and its degradation products.

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