NEW BIOMEDICAL TECHNOLOGIES

Effect of Antidigoxin Monoclonal Antibodies on Cardiac Disturbances Caused by High Concentrations of Digoxin

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Effect of high-affinity mouse monoclonal antidigoxin antibodies D22 on digoxin-induced cardiac disturbances is studied in guinea pigs injected with 0.5 mg/kg digoxin. Five minutes after digoxin injection, bradycardia, isolated extrasystoles, conduction disturbances, and changes in the *ST* segment and *QRS* complex appear; digoxin concentration attains 0.2-0.4 μg/ml. Antidigoxin monoclonal antibodies injected 10 min after digoxin slightly reduce heart rate, prevent isolated extrasystoles, improve atrioventricular conduction, and normalize the amplitude of the *ST* segment and the shape of the *QRS* complex. Serum digoxin concentration decreases to 0.015-0.03 μg/ml. These data suggest that monoclonal antidigoxin antibodies can be effectively used in acute digoxin intoxication.

Key Words: *monoclonal antibodies; digoxin; Na,K-ATPase*

Normal function of cardiomyocytes strictly depends on ion contents of extra- and intracellular media, especially on concentrations of Na^+ and K^+ determining electrochemical gradient (membrane potential), which plays a key role in heart electrophysiology. Normal concentrations of Na^+ and K^+ in the extracellular medium are about 440 and 20 mM, respectively, and the corresponding concentrations in the cytoplasm are about 50 and 400 mM, respectively. Moreover, active transport of these ions across the plasma membrane generates action potential, an electrochemical component of myocardial contraction. This transport is executed by transmembrane protein Na,K-ATPase, which can act as receptor, passive ionic channel, and active ATP-dependent membrane ion carrier [6,7,11-13]. Na,K-ATPase heterodimer of a catalytic α-subunit (84-120 kD) and a glycoprotein-bound β-subunit (45 kD) [9]. α-Subunit consist of 8 hydrophobic transmembrane domains connected by extramembrane loops [11]; β-subunit consists of a transmembrane domain, an extracellular and short intracellular fragments. The full α-subunit sequence contains 1018 amino acid residues in the rat and men [9] and 1016 residues in the sheep [14].

It was previously shown by the methods of site-directed mutagenesis and X-ray crystallography that cardiac glycosides, in particular digoxin, bind to the extracellular domain of α_1-subunit (111-122 amino acid residues) [7,8,12,13].

Intoxication caused by overdose or accidental intake of a large dose of digoxin is characterized by abrupt inhibition of Na,K-ATPase, K^+ release from cells, hyperkalemia leading to disturbances of atrioventricular conduction and ventricular tachyarrhythmia [5,17]. Experiments on dogs showed that an increase in digoxin concentration is associated with changes in the depolarization/repolarization ratio.

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These changes include a decrease in ascending slope and plateau length of action potential and enhanced spontaneous diastolic depolarization [17].

MATERIALS AND METHODS

Monoclonal mouse antidigoxin antibodies (ADA) D22, IgG1 isotype were produced and isolated as described previously [1].

Antibodies were purified by affinity chromatography on cyanogen bromide-activated Sepharose CL-6B.

The chromatographic column was equilibrated with a 10-fold excess of physiological buffered saline and 2 ml ADA dialysed against physiological buffered saline was applied to the column. The antibodies were eluted with 0.1 M glicine-HCl buffer, pH 2.7.

Antibody purity was controlled by polyacrylamide gel electrophoresis. Antidigoxin activity was assayed by competitive immunoenzyme assay [1].

Experiments were carried out on male guinea pigs narcotized with Nembutal (40 mg/kg, intraperitoneally). Digoxin (0.025%) was injected into the femoral vein in a dose of 0.5 mg/kg body weight. The signs of digoxin intoxication were recorded in standard lead II 1, 5, 10, 15, 20, 25, and 30 min postinjection at a tape rate of 50 mm/sec.

Blood serum was separated by centrifugation at 6000 rpm. Free digoxin concentration was measured by competitive solid-phase immunoenzyme assay [2] 5 min and 30 min postinjection. To this end, 100 μl serum and 100 μl standard digoxin solutions with concentrations of 0.01, 0.05, 0.1, 0.2, 0.5, and 1 μg/ml were dialyzed for 4 h against 300 μl physiological saline so that the data obtained with serum samples containing only digoxin and digoxin and ADA were comparable. The concentration of digoxin was calculated according to a calibration curve and was considered as free serum digoxin concentration.

TABLE 1. Specificity of Monoclonal ADA D22 [1]

Agent	Cross-reactivity, %
Digoxin	100
Digoxigenin	9
Digitoxin	<0.001
Celanide	100
Digitoxigenin	<0.001

Note. Percentage of cross-reactions is a hapten to analog molar ratio yielding 50% inhibition of specific binding.

Ten minutes after digoxin the animals were injected with 0.5 ml ADA D22 in physiological saline. The effect of monoclonal ADA was evaluated by prevention of the ECG changes typical of digoxin intoxication. Serum concentration of free digoxin was determined after the experiment.

Free serum digoxin concentration in experimental animals (injected with ADA) was compared with that of controls (ADA not injected).

RESULTS

Digoxin (0.025%) was intravenously injected to guinea pigs in a dose of 0.5 mg/kg. Monoclonal ADA (in 0.5 ml physiological saline) were injected 10 min after digoxin in doses 3 (group 1) and 12 mg (group 2). Five minutes after digoxin injection, bradycardia, isolated extrasystoles, conduction disturbances, and changes in the *ST* segment and *QRS* complex appeared (Fig. 1). The concentration of digoxin 5 min postinjection attained 0.2-0.4 μg/ml in control and experimental animals. The ECG abnormalities peaked 10 min postinjection. Control animals died, post-

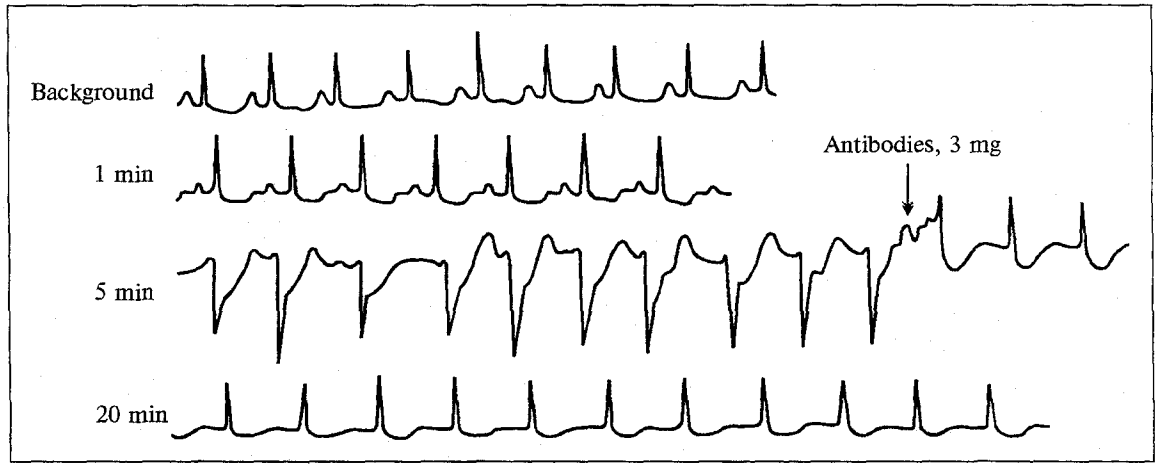

Fig. 1. Standard lead II electrocardiogram in guinea pig. Time after digoxin injection D22 is indicated. Injection of antidigoxin antibodies is indicated by an arrow.

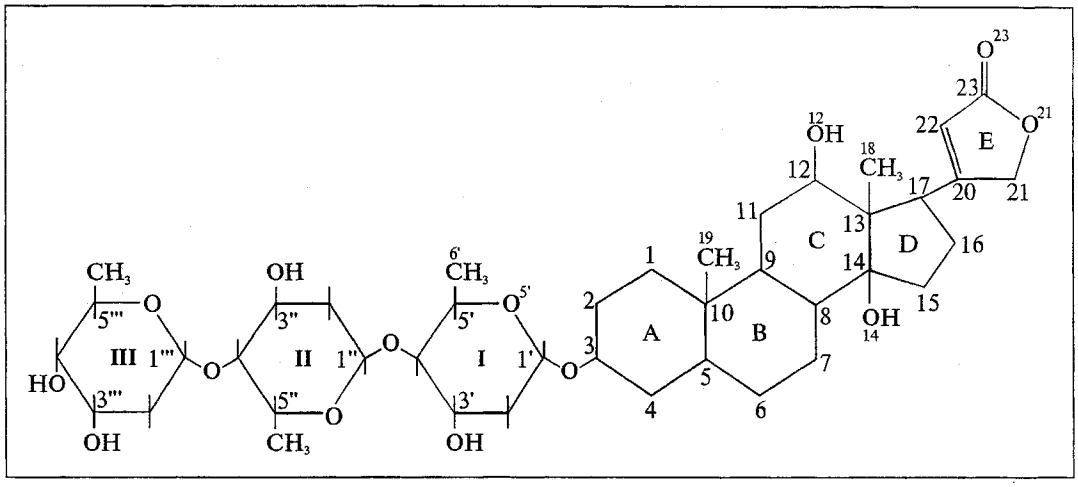

Fig. 2. Chemical structure of digoxin (numbers according to [3]).

mortem digoxin concentration was 0.3 μg/ml. Injection of ADA in a dose of 3 mg induced positive changes in the ECG: heart rate tended to normal, isolated extrasystoles disappeared, atrioventricular conduction, the *ST* segment and *QRS* complex returned to normal (Fig. 1). Serum digoxin concentration decreased to 0.015-0.03 μg/ml. The effect of 12 mg ADA did not differ significantly from that observed in group 1.

Of particular interest is the fact that ADA induced sharp changes in QRS complex (Fig. 1). This can be due to changes in Na,K-ATPase upon digoxin removal from the digoxin—Na,K-ATPase complex and its binding to ADA. It can be also hypothesized that the shape of the *QRS* complex depends on the state of Na,K-ATPase.

Sharp changes in the *QRS* complex suggest that ADA bind Na,K-ATPase-bound rather than free digoxin competing for sterically close binding sites. The binding of free digoxin to ADA should induce slow ECG changes associated with a decrease in free digoxin concentration and a shift in the digoxin—Na,K-ATPase binding reaction toward free digoxin and Na,K-ATPase. The binding of Na,K-ATPase and ADA to sterically distant sites also cannot explain the abrupt shifts in the *QRS* complex; besides, it is at variance with the data on ADA D22 specificity (Table 1). Therefore, the competition between Na,K-ATP and ADD for sterically close binding sites on digoxin molecule seems to be the most credible speculation. There are several binding sites, since ADA D22 are highly specific for digoxin (Table 1), whereas Na,K-ATPase binds all cardiac glycosides.

The data of digoxin X-ray crystallography [3] and amino acid sequences of Na,K-ATPase α_1-subunit from various sources [12] are now available. Published data suggest that cardiac glycosides bind to the following fragment of Na,K-ATPase (numbers according to [13]):

Leu-Cys-Phe-Leu-Ala-Tyr-Gly-Ile-Gln-Ala-Ala-Thr-
105 110
Glu-Glu-Glu-Pro-Gln-Asn-Asp-Asn-Leu-Tyr-Leu-
115 120 125
Gly-Val-Val-Leu-Ser
130

This is confirmed by experiments studying the effect of site-directed amino acid substitution in α_1-subunit (Tyr108Ala, Cys104Ala, Cys104Phe) on ouabain binding to Na,K-ATPase [13].

These data suggest that the contact between lactone ring of digoxin and Cys-Phe fragment plays an important role in the interaction between the glycoside and Na,K-ATPase. Approximate charges on lactone ring atoms are: -0.248 (O^{21}), -0.322 (O^{23}), -0.202 (C^{22}), and +0.389 (C^{23}) (numbers according to [3]). We consider that O^{23} in the lactone ring forms a hydrogen bind with Cys104, inducing stack-interaction between the digoxin lactone ring and the benzyl ring of Phe105. ADA presumably bind to O^{12} in the digoxin molecule, which promotes dissociation of the digoxin—Na,K-ATPase complex.

Our findings demonstrate the possibility of using monoclonal ADA for detoxication in acute digoxin poisoning and illustrate the role of cardiomyocyte membrane Na,K-ATPase in coupling of electrophysiological processes and ionic currents and the effect of digoxin on Na,K-ATPase.

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