# Submolecular Mechanisms Underlying *in Vitro* and *in Vivo* Effect of Cardiac Glycosides on Contractile Activity of Myocardial Myofibrils during Heart Failure

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The development of severe heart failure associated with toxicoallergic myocarditis is accompanied by profound structural and conformational changes in the outer domain of actin (major protein in a thin filament of cardiomyocyte sarcomere). These changes were revealed in subdomains 1 (Cys374 and Cys10) and 2 (Lys61 and Tyr69). Structural and conformational changes in the monomer and protomer of the actin thread during heart failure were energetically forbidden. Variations in the distance between amino acid residues exceeded 0.26 nm. They were partly or completely reversible *in vivo* under the influence of cardiotropic drug refracterin with high antihypoxic activity, as well as *in vitro* after treatment with digitalis preparations optimizing the concentration of ATP.

**Key Words:** *heart failure; thin filament; structural-and-conformational changes; actin; cardiac glycosides*

Under conditions of heart failure (HF) decreased contractile activity of the myocardium is accompanied by impaired force generation and utilization of ATP hydrolysis energy by cardiomyocyte myofibrils [4]. The target of the pathological process and cardiac glycosides in the system of contractile myocardial proteins are actin thin filaments, changes in its diameter, conformation changes in the outer domain, and conversion of the thread into a more rigid, stable, and "frozen" state [2,6,9].

Here we compared the efficiency of structural and conformational recovery of actin *in vitro* and *in vivo* under the influence of cardiac glycoside β-acetyldigoxin (β-AD) and cardiotropic drug refracterin, respectively. Refracterin contains β-AD, nicotinamide dinucleotide, cytochrome C, oxyfedrine, and inosine with direct antihypoxic activity [1,8].

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## MATERIALS AND METHODS

Experiments were performed on isolated myocardial actin from 12 intact Chinchilla rabbits and 46 rabbits with severe HF associated with toxicoallergic myocarditis (TAM). The study was conducted in the autumn-winter period. *In vivo* therapy of TAM rabbits with refracterin and *in vitro* study of the interaction between myocardial actin and β-AD were performed as described elsewhere [4]. Isolation of actin, evaluation of its structural and conformational state and relative position of amino acid residues labeled with fluorescent probes were performed as described previously [6,7]. Fluorescence resonance energy transfer (FRET) in actin was assayed using 1,5-IAEDANS, 5-IAF (attachment to Cys374), DnSCl (attachment to Tyr69), and DDPM (attachment to Cys374 and Cys10) [6,7]. Statistical analysis of small samples with dependent and independent groups was performed by standard methods using SPSS 10 software. The differences were significant at $p<0.05$.

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## RESULTS

In animals with severe HF associated with TAM the 5-day course of refracterin was followed by functional recovery of 3 cardiomyocyte systems responsible for contraction and relaxation. This treatment improved contractile and relaxing properties of the heart (even under conditions of pressure overload) [4,5]. Refracterin restored load-adequate synthesis of macroergic compounds (ATP and creatine phosphate), calcium transport across the cardiomyocyte membranes (sarcolemma, sarcoplasmic reticulum, and mitochondria), and structure and functional activity of myofibrils [4]. The recovery of contractile activity of myocardial myofibrils and economy of energy conversion in refracterin-receiving animals with severe HF was accompanied by derecombination changes in a thin filament.

HF was accompanied by an increase in fluorescence emission of FITC attached to Lys61. It is localized in subdomain 2 near the entrance into the cleft between domains 1 and 2 of actin monomer [12,13]. The Lys61 region becomes open (less screened). In HF polymerization of actin did not decrease FITC-actin fluorescence, while under normal conditions this parameter decreased by 1.4 times. In animals with severe HF the intensity of FITC fluorescence increased not only during fluorescence, but also under conditions of 1,5-IAEDANS excitation at 335 nm. However, 1,5-IAEDANS fluorescence in actin from HF animals was 2-fold below the normal. These changes reflect a sharp increase in entropy and free energy of structural stabilization of Lys61 regions. Moreover, HF is accompanied by a decrease in internal energy of domain 1 in actin. Comparison with healthy and HF animals showed the increase in the distance between Cys374 and Lys61 (by 15%, 0.67 nm) and between Cys374 and Tyr69 in the inner wall of the cleft near the nucleotide-binding site (by 20%, Table 1). The study of myocardial actin from HF animals revealed a 20% increase in the distance between Tyr69 and Cys10 in subdomain 1. It can be hypothesized that the actin monomer is "frozen" in the open state during HF. The cleft between domains is widened. Polymerization of actin (filament formation) does not result in reconstruction of actin molecule, which is observed under normal conditions. This conclusion was derived from measurement of optical density of aromatic amino acid residues [2,3]. Polarization and anisotropy of fluorescent labels attached to Cys374, Lys61, and Tyr69 remain unchanged under these conditions. During HF actin monomer is incorporated into the filament (protomer) and does not undergo conformational changes. Similar results were obtained in circular dichroism studies of actin monomer and actin filament [3]. Our conclusion is supported by the data that in HF animals the distance between Cys374 and Lys61, as well as between Cys374 and Tyr69, is similar to that in the actin monomer of the myocardium. Under normal conditions this distance decreases by 16 and 24.7%, respectively. The distance between Tyr69 and Cys10 decreases by 12%, but does not reach the level typical of the protomer of actin filament in the myocardium of conventionally healthy animals.

The distance between Cys374 and Lys61 in the monomer and protomer of the actin filament reflecting conformational state of domain 1 practically returned no normal under the influence of β-AD and refracterin. The distance between Cys374 and Tyr69 returned no normal only after treatment with refracterin. After *in vitro* treatment with β-AD

**TABLE 1.** Structural-and-Conformational State of the Actin Monomer and Protomer during HF under the Influence of β-AD and Refracterin (*M*±*m*)

| Amino acid residue, state | | Normal | HF | HF+β-AD | HF+refracterin |
|---|---|---|---|---|---|
| Cys374 | monomer | 4.65±0.08 | $5.32\pm0.10^{**}$ | $4.88\pm0.08^{+}$ | $4.73\pm0.10^{++}$ |
| Lys61 | polymer | $3.90\pm0.09^{x}$ | $5.10\pm0.07^{***}$ | $4.15\pm0.09^{++xx}$ | $4.05\pm0.08^{++xx}$ |
| Cys374 | monomer | 2.71±0.11 | 3.58±0.17 | $2.97\pm0.11^{*+}$ | $2.85\pm0.09^{+o}$ |
| Tyr69 | polymer | $2.04\pm0.09^{x}$ | 3.29±0.12 | $1.90\pm0.08^{x}$ | $2.16\pm0.11^{+xo}$ |
| Tyr69 | monomer | 2.95±0.10 | $3.55\pm0.09^{++}$ | $2.85\pm0.08^{+}$ | $3.0\pm0.1^{o}$ |
| Cys10 | polymer | 2.77±0.07 | $2.94\pm0.09^{+x}$ | $2.89\pm0.09^{x}$ | $2.87\pm0.10^{++}$ |
| Cys374 | monomer | 2.65±0.11 | $3.50\pm0.07^{++}$ | $3.15\pm0.11^{*+}$ | $2.85\pm0.09^{+o}$ |
| Cys10 | polymer | $1.72\pm0.10^{x}$ | $3.38\pm0.12^{++}$ | $2.05\pm0.12^{*+}$ | $2.0\pm0.1^{+++x}$ |

**Note.** $^{*}p<0.05$, $^{**}p<0.01$, and $^{***}p<0.001$ compared to normal; $^{+}p<0.05$, $^{++}p<0.01$, and $^{+++}p<0.001$ compared to HF; $^{x}p<0.05$ and $^{xx}p<0.01$ compared to monomer; $^{o}p<0.05$ compared to HF+β-AD.

the distance between Cys374 and Tyr69 in HF animals decreased, but remained above the normal. Refracterin more significantly decreased the distance between Cys374 and Cys10 (Table 1). Probably, conformational changes in actin filament under the influence of cardiac glycosides were cooperatively transduced along the filament. They were not necessarily observed during binding of the test preparation to the protomer. This specific feature is associated with high cooperativeness of the actin filament under normal conditions [12,13] or its recovery in HF animals receiving cardiac glycosides. We conclude that cardiac glycosides reverse or normalize structural and conformational state and conformational mobility of actin during HF.

The *in vitro* effect of β-AD on contractile proteins of the myocardium in HF animals with normal content of ATP and pCa was similar to that observed in *in vivo* study of refracterin. The course of refracterin treatment more significantly improved the structural and conformational state of actin during HF (compared to individual administration of cardiac glycoside *in vitro*, Table 1). These data are consistent with the recovery or improvement of systolic and diastolic function of the heart upon repeated treatment with refracterin during HF associated with TAM. Similar changes were revealed in patients with chronic HF of functional classes III-IV associated with noncoronary myocardial diseases (dilation cardiomyopathy and infectious-and-allergic myocarditis) [10] and chronic forms of coronary heart disease [1]. Our results indicate that significant increase in contractile and relaxing function of the myocardium and improvement of the quality of life in patients with severe HF can be achieved ony via structural and functional recovery of life-supporting systems in the cardiomyocyte and executive apparatus. Cardiac glycosides are safe in patients with severe HF and promote the recovery of functional activity in the energy supply system of cardiomyocytes.

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