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Structural and Functional Remodeling of Rat Cardiac Tissue in Doxorubicin-Induced Chronic Heart Failure and Its Pharmacological Adjustment by a Newly Synthesized 4-Amino-1,2,4-Triazole Compound

Emily White[1]*, James Green[1], Hannah Black[1]

[1]Department of Pharmaceutical Chemistry, College of Pharmacy, University of Texas at Austin, Austin, USA.

***E-mail** ✉ emily.white@protonmail.com

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ABSTRACT

Bromide 1-(β-phenylethyl)-4-amino-1,2,4-triazolium (Hypertril) exhibits both beta-blocking and nitric oxide–mimetic activities and belongs to toxicity class IV. These pharmacological features make Hypertril a promising therapeutic agent for cardiovascular disorders. The present study aimed to evaluate the cardioprotective properties of Hypertril by assessing its impact on the morpho-functional characteristics of the myocardium in a rat model of chronic heart failure (CHF). Experimental CHF was induced in 80 white outbred rats (190–220 g) through cumulative administration of doxorubicin at a total dose of 15 mg/kg. Following CHF induction, Hypertril and the reference drug metoprolol succinate were administered orally for 30 days at doses of 3.5 mg/kg and 15 mg/kg, respectively. Morphometric evaluation of myocardial cellular architecture was performed using an Axioskop microscope (Zeiss, Germany) in automated mode, employing a macro program developed in the VIDAS-2.5 software environment (Kontron Elektronik, Germany). Treatment with Hypertril in CHF-affected rats resulted in elevated cardiomyocyte nuclear density, enlarged nuclear area, an increased nuclear-to-cytoplasmic ratio, and higher RNA content in both nuclei and cytoplasm compared to untreated controls, suggesting a strong cardioprotective activity of the compound. Moreover, Hypertril demonstrated significantly greater effects ($p < 0.05$) than metoprolol with respect to cardiomyocyte survival density, RNA content, and nuclear-cytoplasmic ratio.

Keywords: Cardioprotection, Hypertril, Chronic heart failure, Metoprolol, Endothelial dysfunction, β-blocker

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Introduction

At the dawn of the 21st century, cardiovascular diseases (CVDs) became increasingly prevalent, ranking among the top three causes of mortality in industrialized nations. Chronic heart failure (CHF) represents one of the most severe complications, accounting for a mortality rate ranging from 10% to 50% among patients with CVDs. Consequently, the search for effective therapeutic agents targeting cardiovascular disorders remains a pressing need in contemporary medicine. According to the European Society of Cardiology, comprehensive management of heart failure should include diuretics, ACE inhibitors, and β-blockers, particularly following myocardial infarction. However, current β-adrenergic blockers often demonstrate suboptimal efficacy, necessitating combination therapy with other agents such as ACE inhibitors, diuretics, or thrombolytics, and may also induce adverse side effects [1].

A characteristic pathological feature of CHF is cardiac remodeling (CR), which contributes to disease progression and encompasses a range of adaptive and structural modifications at the geometric, morpho-functional, and molecular-biochemical levels of the heart [2]. Continuous efforts are directed toward identifying novel agents and therapeutic strategies capable of mitigating CR and halting CHF development. This context prompted the synthesis of a novel compound—bromide 1-(β-phenylethyl)-4-amino-1,2,4-triazolium (provisionally named

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Hypertril)—which exhibits NO-mimetic, β1-adrenergic blocking, antihypertensive, and anti-ischemic effects and is classified as a class IV low-toxicity agent (LD_{50} = 683.4 mg/kg for intragastric administration in rats) [3-5]. The compound was developed collaboratively by SPA "Farmatron" and the Scientific and Technological Complex "Institute of Single Crystals," National Academy of Sciences of Ukraine, which also established laboratory-scale synthesis methods and ampoule solution formulations. Standardization procedures were completed (certificate No. 2, series 020213). Following approval by the State Expert Center of the Ministry of Health of Ukraine, Hypertril successfully passed Phase I clinical trials and is currently in Phase II, being evaluated as an antihypertensive and antianginal agent. The purpose of this study was to assess the cardioprotective efficacy of Hypertril by examining its effects on the morpho-functional parameters of myocardial tissue in rats with experimentally induced CHF.

Materials and Methods

Ethics statement

All experimental procedures adhered to the ARRIVE guidelines, the International Association for the Study of Pain regulations, and the ethical standards for animal research of Zaporozhye State Medical University. The study protocol received approval from the university's Animal Care and Use Committee. Every effort was made to minimize animal discomfort throughout the experiment.

Animal model and grouping

The experiment involved 80 white outbred rats (190–220 g, both sexes) obtained from the breeding facility of the A.A. Bogomolets Institute of Physiology, National Academy of Medical Sciences of Ukraine. Chronic heart failure was induced using the doxorubicin model [6], recognized as an effective method that leads to a severe, progressive form of CHF in most subjects. Doxorubicin ("Ebewe," 50 mg/25 ml; EBEWE Pharma GmbH Nfg. KG, Austria) was diluted with physiological saline and administered intraperitoneally at a cumulative dose of 15 mg/kg (2.5 mg/kg per injection, 0.125 ml/100 g body weight) divided into six doses over 14 days. This regimen resulted in reduced left ventricular contractility, eccentric remodeling, and the development of CHF.

After CHF induction, Hypertril was administered intragastrically at a dose of 3.5 mg/kg once daily for 30 days as a 1% starch mucilage suspension [3]. For comparison, metoprolol succinate (Betaloc ZOK, 47.5 mg tablets, AstraZeneca UK Ltd., Sweden) was given using the same protocol at a dose of 15 mg/kg [6]. The animals were divided into four groups (n = 20 each):

- Intact group – received 1% starch mucilage;
- Control (CHF, untreated) – received 1% starch mucilage;
- CHF + Hypertril – received Hypertril;
- CHF + Metoprolol succinate – received the reference drug.

Morphometric analysis

At the end of the experiment, rats were anesthetized with sodium thiopental (40 mg/kg), and their hearts were excised. The apical portion of each heart was fixed in Carnoy's solution for 24 hours. Following standard dehydration and paraffin embedding procedures (Paraplast, McCormick, USA), 5 μm histological sections were prepared using a Microm-325 rotary microtome (Microm Corp., Germany). The sections were dewaxed in xylene, rehydrated through graded ethanol (100%, 96%, 70%), and rinsed in saline. To visualize RNA, sections were stained for 24 hours with gallocyanine-chromium alum following Einarson's protocol and mounted in EUKITT (O. Kindler GmbH, Germany).

Microscopic examination was performed under transmitted light using an Axioskop microscope (Zeiss, Germany). Images were captured with an 8-bit CCD camera (COHU-4922, COHU Inc., USA) and analyzed using the VIDAS-386 image analysis system (Kontron Elektronik, Germany), digitized on a 256-level gray scale. Approximately 500 myocardial fields were analyzed per series.

Morphometric and densitometric parameters were processed automatically using a macro program in VIDAS-2.5 (Kontron Elektronik, Germany). The following variables were quantified:

- Cardiomyocyte nuclear area (μm²);
- RNA concentration in nuclei (Uod), calculated as the logarithmic ratio of nuclear to intercellular optical density;

- RNA concentration in cytoplasm (Uod), calculated similarly for cytoplasmic regions;
- Nuclear density, representing the number of nuclei per mm² of myocardial tissue;
- Nuclear-cytoplasmic coefficient, indicating the total nuclear area per mm² of myocardial tissue.

All parameters were computed automatically using the VIDAS-2.5 software suite to ensure precision and reproducibility.

Statistical analysis

All data were processed using SPSS 16, Microsoft Excel 2003, and STATISTICA for Windows 7.0 (StatSoft Inc., № AXXR712D833214FAN5). Results were expressed as mean ± standard deviation (SD). Depending on data distribution, comparisons among multiple independent groups were performed using either analysis of variance (ANOVA) for normally distributed data or the Kruskal–Wallis test for non-parametric cases. Correlations between individual parameters were assessed using Pearson's or Spearman's correlation coefficients, as appropriate. Statistical significance was established at $p < 0.05$.

Results and Discussion

Following a 14-day course of doxorubicin and a subsequent 45-day observation period without therapeutic intervention, the myocardium exhibited distinct pathological alterations, including marked circulatory impairment, degenerative changes in cardiomyocytes, and contracture lesions in several cells. Microscopic evaluation revealed disorganization of myofibrillar structure, cytoplasmic rarefaction, and the appearance of perinuclear "clear zones" representing lytic injury. Evidence of necrosis and apoptosis, accompanied by local mononuclear inflammatory infiltration, was also observed. Additionally, pronounced venous congestion and interstitial edema contributed to the disruption of myocardial tissue integrity. These observations were consistent with previously described morphological characteristics of doxorubicin-induced CHF models [6].

Quantitative assessment revealed a significant reduction ($p < 0.05$) in cardiomyocyte nuclear density, reflecting enhanced cell death primarily through apoptosis, a well-known consequence of doxorubicin-induced cardiotoxicity [7]. The loss of cardiomyocytes contributed to small foci of replacement fibrosis, or cardiosclerosis [8, 9]. In agreement with earlier reports [3, 6], surviving mononuclear cardiomyocytes retained some proliferative potential, undergoing karyokinesis and generating binuclear cells. However, a decline in the proportion of mononuclear cardiomyocytes suggested depletion of the myocardium's regenerative capacity.

A noticeable decrease in RNA concentration within both the nuclei and cytoplasm of cardiomyocytes indicated a disruption in biosynthetic processes essential for intracellular repair. Morphological remodeling of cardiac tissue was largely attributed to the suppression of RNA and protein synthesis, leading to compromised regeneration of ultrastructural components. Some cardiomyocytes displayed mild inhibition of biosynthesis, whereas others exhibited prolonged suppression or complete arrest, resulting in atrophy of intracellular elements.

Among the control CHF group, severe interstitial edema and nuclear atrophy were most prominent. The reduction in cardiomyocyte nuclear area compared with intact controls suggested impaired synthesis of intracellular components and protein deficiency, reflecting diminished functional capacity under doxorubicin exposure. These structural alterations, together with increased cardiac mass, implied substitution of contractile myocardium with fibrotic tissue—a hallmark of regenerative-plastic insufficiency leading to heart failure [5]. The predominant cellular damage involved myofibrillar lysis and apoptosis, culminating in diffuse or focal cardiosclerosis. The resulting remodeling process typically followed a dilated pattern, predisposing the heart to congestive failure [1, 6].

Experimental CHF induction caused substantial myocardial histoarchitectural disruption, characterized by a 21.2% reduction in cardiomyocyte nuclear density, a 23% decrease in nuclear area, a 4.5% drop in nuclear RNA concentration, a 14.6% reduction in cytoplasmic RNA concentration, and an 81% decline in the nuclear-cytoplasmic index compared to healthy controls, indicating pronounced myocardial hypertrophy **(Table 1)**. These findings reflected severe ischemic metabolic disturbances, impaired biosynthesis, and increased cell loss through necrosis and apoptosis.

Chronic administration of Hypertril (3.5 mg/kg) to CHF rats demonstrated a marked cardioprotective response, evidenced by a 21% rise in cardiomyocyte nuclear density compared to untreated CHF animals, confirming the compound's potential to mitigate myocardial damage and support cellular recovery.

Table 1. Influence of Hypertril and the reference drug on the morpho-functional characteristics of cardiomyocytes in experimental chronic heart failure (CHF).

Parameter	Intact	CHF (Control)	CHF + Hypertril (3.5 mg/kg)	CHF + Metoprolol (15 mg/kg)
Cardiomyocyte nuclear area (μm²)	12.6 ± 0.21	9.7 ± 0.38	13.0 ± 0.22 *	8.4 ± 0.31
Nuclear density (per 1 mm² of myocardial tissue)	9788 ± 208	7711 ± 215	9326 ± 296 *[1]	7911 ± 166 *
Nuclear-to-cytoplasmic ratio	0.64 ± 0.003	0.12 ± 0.004	0.37 ± 0.003 *[1]	0.11 ± 0.002
RNA concentration in cardiomyocyte nuclei (optical density units, EOP)	0.23 ± 0.001	0.21 ± 0.001	0.25 ± 0.002 *[1]	0.22 ± 0.001
RNA concentration in cardiomyocyte cytoplasm (optical density units, EOP)	0.082 ± 0.001	0.070 ± 0.001	0.103 ± 0.001 *[1]	0.065 ± 0.002 *

*– differences are statistically significant compared with the control CHF group ($p < 0.05$); [1] – differences are statistically significant compared with the metoprolol-treated group ($p < 0.05$).

In rats with CHF receiving Hypertril, the myocardial nuclear area increased by 34 percent relative to the control group ($p < 0.05$). Treatment with Hypertril also resulted in a twofold elevation of the nuclear-cytoplasmic ratio ($p < 0.05$) compared to the untreated CHF group, suggesting attenuation of myocardial hypertrophy. Moreover, Hypertril administration enhanced RNA concentration in the nuclei by 19 percent and in the cytoplasm of cardiomyocytes by 47 percent compared with the control animals, reflecting activation of transcriptional mechanisms and confirming the anti-ischemic and reparative potential of Hypertril [4, 5].

The beneficial influence of Hypertril on myocardial morpho-functional characteristics in rats with chronic heart failure (CHF) is consistent with findings from previous investigations, primarily attributed to its antioxidant and nitric oxide (NO)-mimetic activities. The antioxidant mechanism of Hypertril likely involves the suppression of reactive oxygen species (ROS) production through modulation of adrenergic and nitroxydergic transmitter systems. Based on these observations, it can be inferred that Hypertril not only prevents the generation of superoxide radicals in the adrenaline–adrenochrome system but also limits the formation of cytotoxic NO derivatives, thereby restoring the eNOS/iNOS balance [3-5]. Furthermore, by interacting with NO as a spin-trapping agent, Hypertril contributes to the formation of more stable radical complexes.

It is well established that CHF is associated with profound disruptions in myocardial nitroxydergic regulation. Variations in the levels of NO end products during CHF reveal reduced nitrite concentrations in blood and urine, which is characteristic of endothelial dysfunction in cardiovascular diseases. This decline indicates impaired NO biosynthesis due to suppression of endothelial NO synthase gene expression, depletion of essential cofactors such as L-arginine and tetrahydrobiopterin, oxidation of very low-density lipoproteins, accumulation of peroxynitrite within vascular tissues, weakened antioxidant defense, and elevated endogenous NO inhibitors [7]. Given the crucial vasodilatory role of NO, its insufficiency contributes significantly to the pathogenesis of hypertension, reduced vascular compliance, diminished coronary reserve, left ventricular hypertrophy, and diastolic dysfunction.

In CHF patients, disturbances in endogenous NO metabolism are well documented. Cardiomyocytes express two principal isoforms of nitric oxide synthase (NOS): endothelial (eNOS) and inducible (iNOS). The activity of eNOS depends on myocardial contractile performance, whereas iNOS is upregulated by cytokines. During CHF, oxidative stress reduces eNOS expression and its cofactors, resulting in NO deficiency [10, 11]. Studies demonstrate that cytokines such as IL-1β, TNF-α, and IFN induce NO synthesis via iNOS activation. However, when eNOS expression is suppressed and oxidative stress intensifies, NO degradation increases through its conversion to peroxynitrite by ROS, leading to elevated nitrotyrosine levels [12, 13]. The resulting cytotoxic NO species directly damage myocardial tissue, promote interstitial fibrosis, and enhance the negative inotropic influence of NO, ultimately contributing to cardiac remodeling.

Further upregulation of iNOS augments cytokine-driven NO production, impairing contractility. Fibroblast-to-myofibroblast transformation, driven by transforming growth factor β1 (TGF-β1)—whose expression is modulated by ROS and NO, particularly peroxynitrite—is associated with increased expression of α-smooth muscle actin (α-SMA) and desmin [8, 9, 13, 14]. Links between TGF-β1 expression and the activities of iNOS and NADPH oxidase have also been identified [7, 9, 15]. The proliferation of myofibroblasts plays a major role in fibrotic and sclerotic processes of the myocardium [15-18]. In CHF, ROS and NO participate in apoptosis

induction through both caspase activation and mitochondrial membrane disruption [6, 11, 13, 14, 16-18]. Experimental and clinical CHF models show elevated iNOS activity and increased nitrite and nitrotyrosine levels during decompensated heart failure [6, 8-11].

Therefore, the therapeutic use of agents that enhance NO synthesis in CHF patients is well justified. Recent research indicates that β-blockers not only improve survival and reduce major cardiovascular events in CHF but also exert a favorable influence on NO production [3-6, 10, 19-21]. As a β1-blocker, Hypertril exhibits strong negative chronotropic effects, stabilizing heart rate, ventricular R-wave amplitude, and T-wave repolarization in CHF rats, while reducing the ST-segment amplitude. Its normalization of depolarization duration (QRS complex), repolarization phase (T-wave), and electrical diastole (TP interval) represents an important therapeutic outcome in CHF management [22], indicating its ability to prevent diastolic dysfunction.

Hypertril corrects disturbances in the myocardial L-arginine-NO-NOS axis during ischemia and hypertension. Through its distinct NO-mimetic activity, it enhances eNOS mRNA and protein expression in cardiac and vascular tissues, restoring NO balance and explaining its cardioprotective mechanism. By reinforcing NO's protective effects and reducing its oxidative conversion to peroxynitrite, Hypertril improves cardiomyocyte resilience against injury [4, 5]. Moreover, it protects mitochondrial structures from doxorubicin-induced oxidative damage [3-5], thereby preventing cardiomyocyte apoptosis.

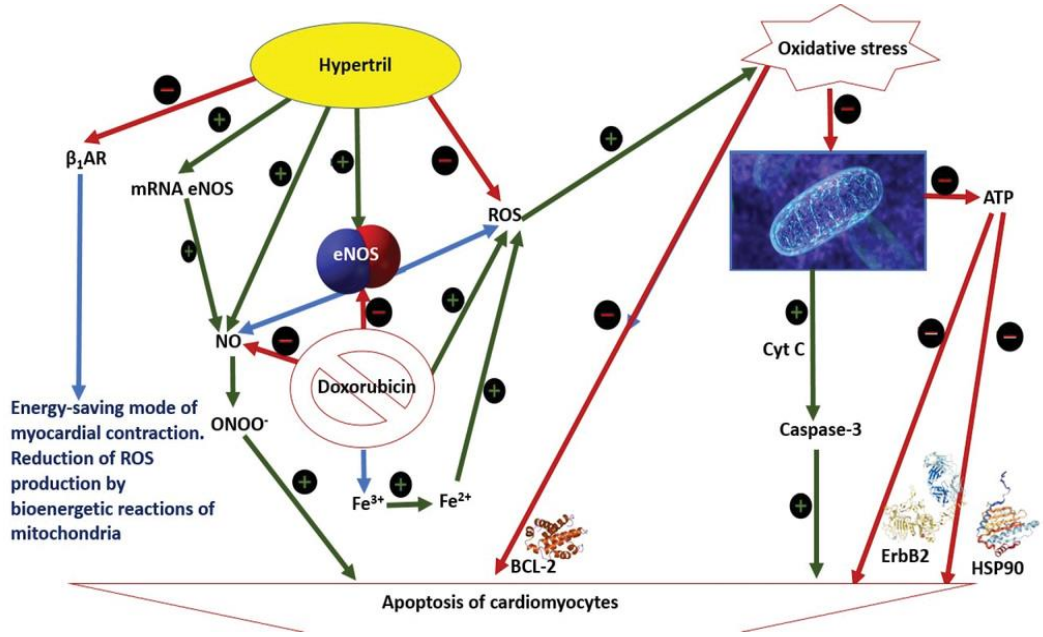

Figure 1. Proposed mechanism illustrating the beneficial influence of the compound "Hypertril" on the morpho-functional characteristics of rat cardiomyocytes in experimental doxorubicin-induced CHF.

Prolonged administration of metoprolol produced a less distinct cardioprotective and anti-ischemic response compared to Hypertril. Parameters such as cardiomyocyte nuclear area and the nuclear-cytoplasmic ratio were lower in CHF rats treated with metoprolol than in untreated controls. Regarding indicators including the density of viable cardiomyocytes, RNA content, and the nuclear-cytoplasmic ratio, Hypertril demonstrated a statistically significant ($p < 0.05$) superiority over metoprolol.

Conclusion

Treatment with Hypertril in CHF animals resulted in marked improvements in myocardial morphology and function, reflected by an increase in cardiomyocyte nuclear density, enlargement of nuclear area, elevation of the nuclear-cytoplasmic index, and higher RNA concentrations in both nuclei and cytoplasm compared with untreated controls. These findings confirm the strong cardioprotective potential of Hypertril, which in terms of therapeutic efficacy, significantly outperforms metoprolol succinate.

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Conflict of Interest: None

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