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Original Article

Carbonic anhydrase inhibitors reduce cardiac dysfunction after sustained coronary artery ligation in rats

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ABSTRACT

Background: Two potent carbonic anhydrase (CA) inhibitors with widely differing membrane permeability, poorly diffusible benzolamide (BZ), and highly diffusible ethoxzolamide (ETZ) were assessed to determine whether they can reduce cardiac dysfunction in rats subjected to coronary artery ligation (CAL)-induced myocardial infarction.

Methods and results: Rats with evidence of heart failure (HF) at 32 weeks following a permanent left anterior coronary artery occlusion were treated with placebo, BZ, or ETZ (4 mg kg day^{-1}) for 4 weeks at which time left ventricular function and structure were evaluated. Lung weight/body weight (LW/BW) ratio increased in CAL rats by 17±1% vs. control, suggesting pulmonary edema. There was a trend for BZ and ETZ to ameliorate the increase in LW/BW by almost 50% (9±5% and 9±8%, respectively, versus CAL) (P=.16, NS). Echocardiographic assessment showed decreased left ventricular midwall shortening in HF rats, 21±1% vs. control 32±1%, which was improved by BZ to 29±1% and ETZ to 27±1%, and reduced endocardial shortening in HF rats 38±3% vs. control 62±1%, partially restored by BZ and ETZ to ~50%. Expression of the hypoxia-inducible membrane-associated CAIX isoform increased by ~60% in HF rat hearts, and this effect was blocked by ETZ.

Conclusions: We conclude that CAL-induced myocardial interstitial fibrosis and associated decline in left ventricular function were diminished with BZ or ETZ treatment. The reductions in cardiac remodeling in HF with both ETZ and BZ CA inhibitors suggest that inhibition of a membrane-bound CA appears to be the critical site for this protection.

1. Introduction

Heart failure (HF) remains one of the leading causes of morbidity and mortality worldwide with fibrotic remodeling after myocardial infarction (MI) as the most frequently recognized primary factor [1,2].

Pathologic cardiac hypertrophy diminishes contractile function and commonly progresses to HF [3]. Changes in ion homeostasis, which result from altered expression and/or function of the ion transporters NHE1 Na^+/H^+ exchanger [4,5], AE3 Cl^-/HCO_3^- exchanger [6], and NBC Na^+/HCO_3^- cotransporter [7], and their associated regulatory partners (carbonic anhydrases, CAs) [8,9], contribute to hypertrophic growth. Experimental and clinical studies demonstrate the pathophysiological role of increased NHE1 activity during cardiac ischemia/reperfusion injury and postinjury hypertrophy [10,11]. The proposed cascade of undesirable events, involving NHE1, AE3, and NBC activation, has been attributed to an increased intracellular Na^+ load [6,12–14] and subsequent increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). Augmented $[Ca^{2+}]_i$ then triggers widely recognized Ca^{2+}-dependent intracellular signaling pathways leading to cardiac hypertrophy [15–18].

Interestingly, we have identified a role for CA in the hypertrophic growth of cultured cardiomyocytes exposed to phenylephrine, angiotensin II, or endothelin 1 [8]. Treatment of hypertrophically stimulated cardiomyocytes with the freely diffusible potent CA inhibitor ethoxzolamide (ETZ; Cardrase) diminished the hormonally induced hypertrophy and reversed it after once established. These effects appear to involve enhanced intracellular Na^+ and Ca^{2+} loading [8]. Furthermore, cultured myocytes of CAII-deficient mice did not respond to the same prohypertrophic stimulation, suggesting a role of CAII in promoting cardiac hypertrophy [19].

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We recently reported that failing human hearts (of ischemic and nonischemic origin) in nondiabetic patients are characterized by increased CAII and CAIV expression [9]. Moreover, myocardial CA activation was found to be present in human diabetic ischemic cardiomyopathy (HDIC) [20]. Indeed, CAI and CAII isozymes are overexpressed in HDIC, and the increased myocyte expression of CAII is associated with the NHE1 Na^+/H^+ exchanger hyperphosphorylation in this specific diabetic HF condition [20]. Therefore, on the basis of these findings, it is tempting to speculate that CA inhibition with existing drugs could be rapidly implemented and would be therapeutic for diabetic patients with post-MI HF. Because CA overexpression is identified as a marker of the hypertrophic human heart that progresses toward failure [9], CA inhibition might be an appropriate strategy to moderate the hypertrophic cascade.

The CAIX isozyme is expressed in the mammalian heart [21], localizing to the t-tubules of cardiomyocytes [22]. CAIX, whose gene has a hypoxia-inducible factor (HIF)-responsive element in its promoter region, is overexpressed in hypoxic tumors and is essential in promoting tumor growth and metastasis [23,24]. However, CAIX expression is not limited to cancer but may be also induced in other pathological situations associated with ischemia, fibrosis, vascular remodeling, inflammation, or metabolic disturbances such as HF that leads to activation of the HIF pathway. In the setting of HF, changes in mitochondrial metabolism lead to possibly injurious reactive oxygen species generation and a greater reliance of glycolytic metabolism [25]. CAVB isozyme is a mitochondrial CA involved in cellular metabolism [26] and could be altered in the failing heart.

The functional benefit of CA inhibition has not been evaluated in cardiac dysfunction after experimental MI. In the present study, we examined the effect of CA inhibitors on rat heart function and collagen deposition following MI and subsequent pathological hypertrophic remodeling, induced by left anterior descending (LAD) coronary artery ligation (CAL), and we investigated a potential link of CAIX and CAVB with the development of HF following MI.

2. Methods

Protocols that involved rats were submitted to, reviewed, and approved by the Animal Welfare Committee of La Plata School of Medicine and were performed in accordance with the Guide for the Care and Use of Laboratory Animals (Argentine Republic Law No. 14346), concerning animal protection.

2.1. Animals

A total of 52 three-month-old male Wistar rats, originally derived from Charles River Breeding Farms (Wilmington, MA), were used in these studies. All animals were housed under identical conditions and had free access to standard dry meal and water.

2.2. Experimental protocol and group assignments

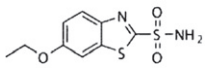
MI was produced in 16 three-month-old Wistar rats by permanent ligation of the LAD coronary artery, according to a method previously described [27]. Thirty six age-matched rats that did not undergo CAL were maintained under identical housing conditions and served as controls. Briefly, the 16 rats subjected to surgery were fully anesthetized by an i.p. injection of Euthanyl (sodium pentobarbital, 35 mg kg^{-1}) and then quickly intubated and ventilated with ambient air using a positive-pressure respirator (Model 680, Harvard). A left thoracotomy was performed via the fourth intercostal space, and the lungs retracted to expose the heart. The LAD coronary artery was ligated with a 7-0 silk suture near its origin. Acute ischemia was deemed successful when the anterior wall of the left ventricle became cyanotic. Atelectatic lung regions near the heart were reinflated by increasing positive end-expiratory pressure, and the thoracotomy site was closed in layers. The animals were then allowed to recover from anesthesia and separated into three experimental groups. All animals survived the surgery and the MI and none died before reaching the time of preplanned terminal measurements either at 3 or 36 weeks. Similar observation of survival and degree of MI achieved by CAL in rats has been reported before [27,28]. Table 1 provides detailed information about group assignments, the number of animals used, the treatments given, and the measurements made in each group. The condition of the animals was monitored on a daily basis. Four rats subjected to CAL-induced MI were used only to determine the infarct size and degree of myocardial damage attained at 3 weeks post-MI and to assess echocardiographic estimates of left ventricular function at 1 and 3 weeks post-MI. These rats were euthanized at 3 weeks later and the heart was removed for histological studies. All of these parameters obtained at 3 weeks post-MI in this group were used for comparison with the functional and histological status of the hearts of the remaining animals subjected to CAL and maintained for 36 weeks.

2.3. CA inhibitors treatment in rats subjected to sustained CAL

Of the 12 rats with CAL and taken out to 36 weeks, 6 had no treatment. Three of these only had pathological examination and the other three additionally had echocardiography and immunoblotting for CAVB and CAIX (described below). The remaining six were either treated with the potent freely diffusible CA inhibitor ETZ (6-ethoxy-1,3-benzothiazole-2-sulfonamide) (n=3) or the poorly membrane-permeable potent CA inhibitor, benzolamide [(5-(benzenesulfonamido)-1,3,4-thiadiazole 2 sulfonamide; BZ)] (n=3) [29,30].

The structures of the two drugs are given below.

Ethoxzolamide **Benzolamide**



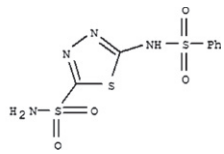


Table 1
Experimental design — 3-month-old Wistar rats

Number of animals	Experimental procedure	Treatment	Length of study	Studies performed/ analysis
24	None	None	36 weeks	Echo — week 1 Echo — week 36
12	None	None	36 weeks	Echo — week 32 Echo — week 36 Histology Pathology Immunoblotting (WB)
4	CAL	None	3 weeks	Echo — week 1 Echo — week 3 Histology Pathology
3	CAL	None	36 weeks	Pathology
3	CAL	None	36 weeks	Echo — week 32 Echo — week 36 Histology Pathology Immunoblotting (WB)
3	CAL	ETZ	36 weeks	Echo — week 32 Echo — week 36 Histology Pathology Immunoblotting (WB)
3	CAL	BZ	36 weeks	Echo — week 32 Echo — week 36 Histology Pathology Immunoblotting (WB)

ETZ, 6-Ethoxy-1, 3 benzothiazole-2-sulfonamide; BZ, 5-(benzenesulfonamido)-1,3,4-thiadiazole 2 sulfonamide; Echo, echocardiographic examination; WB, Western blot.