Detrimental effects of thyroid hormone analog DITPA in the mouse heart: increased mortality with in vivo acute myocardial ischemia-reperfusion

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Submitted 28 May 2010; accepted in final form 23 November 2010

THYROID HORMONE (TH) has profound effects on the heart and cardiovascular system. The biologically active TH, 3,5,3'-triiodothyronine (T3), increases cardiac output through inotropic, chronotropic, and vasodilatory mechanisms (18). A TH analog, 3,5-diiodothyropropionic acid (DITPA), a TH analog, has been proposed to be a safer therapeutic agent than T3. DITPA was identified as a compound that improves postischemic cardiac function. 3,5-Diiodothyropropionic acid (DITPA), a TH analog, has been shown to exert greater positive inotropic than chronotropic effects (32), induces angiogenesis (45), improves vasorelaxation (37), and improves calcium handling (33) in different experimental conditions. These properties of DITPA are thought to contribute to the beneficial effects of the compound in clinical application.

Circulating and cardiac T3 levels are reduced in advanced heart disease, after acute myocardial infarction (AMI), and in patients with cardiopulmonary bypass (14, 15, 18, 24). Clinical and experimental studies have demonstrated that increased circulating levels of T3 improved cardiac contractile function in normal myocardium as well as after acute ischemic injury to the myocardium (8, 18, 26). Recently, DITPA has been in phase II clinical trials for its efficacy as a cardiotonic agent in stable heart failure patients; however, there was no symptomatic benefit in patients despite some improvements in hemodynamic and metabolic parameters (11). DITPA treatment was initially reported to increase baseline cardiac contractility (13, 32); however, administration of DITPA is reported to have diverse effects on postischemic/postinfarct myocardial function (for review, see Refs. 19, 22). Effects of DITPA on fractional shortening and ejection fraction have been mixed, one showing an increase (33, 45) and others no change (19). Thus it is uncertain whether and/or when a clear benefit from DITPA is achievable. It is also unknown whether the inconsistencies are related to experimental protocols or species.

TH can regulate contractile function and heart rhythm via its genomic or nongenomic actions (18, 27). In fact, most of the important regulatory contractile proteins and ion channels are TH responsive (2, 7). Sarco(endo)plasmic reticulum Ca2+-ATPase (SERCA) is positively, while phospholamban (PLB) is negatively, regulated by TH (2). It has been reported that the effects of TH on cardiac contractility as well as rates of contraction and relaxation are mainly mediated by increases in the levels of SERCA2a and decreases in PLB in cardiomyocytes (2). DITPA has been shown to prevent downregulation of SERCA2a proteins following myocardial infarction in rabbits (33). Interestingly, short-term in vitro DITPA pretreatment has been shown to induce upregulation of genes encoding contractile proteins and SERCA2a in isolated rat cardiomyocytes (1). Recent studies have provided evidence that TH can also regulate intracellular survival signaling pathways and enhance the induction of cardioprotective molecules such as heat shock proteins (HSPs) (27). TH-induced HSP70 expression has been
reported to increase the tolerance of the myocardium to ischemia-reperfusion (I/R) and preserve contractile function in rats (28). Mice with increased SERCA expression demonstrate protection against myocardial I/R injury (40), and conversely mice with reduced SERCA expression are susceptible to accelerated myocardial I/R injury (39). Deletion of the inducible 70-kDa HSP genes in mice is reported to impair cardiac contractile function and calcium handling (17).

While murine models are widely used and provide unique opportunity for the use of genetic modification, there are no prior reports evaluating the effects of DITPA in mice with regard to baseline cardiac function and cardioprotection. Pretreatment of a putative cardioprotective agent in animal studies is performed to know the drug effects at baseline and after specific interventions. The cardioprotective effects of T3 or T4 pretreatments have been reported (26, 27, 29–31). The rationale of using DITPA was to avoid the adverse sympathomimetic effects of exogenously administered TH while maintaining the beneficial molecular effects (22). The present study was thus designed to examine the dose-dependent effects of DITPA on the basal physiological and hemodynamic parameters of mice and to explore its potency in cardioprotection with preservation of postischemic myocardial function and viability.

METHODS

This study was reviewed and approved by the Institutional Laboratory Animal Care and Use Committee at The Ohio State University and conforms with the Guidelines for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Pub. No. 85-23, revised 1996).

Mice and DITPA treatment. Young male C57BL/6 mice (16–20 wk) were used in this study. Pretreatment with T3 or T4 is reported to be cardioprotective (26, 27, 29–31); however, there is no such report for DITPA pretreatment. Interestingly, 48-h DITPA or T3 pretreatment is reported to induce upregulation of identical gene encoding contractile proteins and SERCA2a in isolated cultured rat cardiomyocytes (1). DITPA is shown to exert distinct myocardial effects on postischemic rat hearts within 3–7 days of treatment (45). Therefore, considering these early DITPA effects (1, 45) and the well-reported dose-dependence of DITPA (3.75 mg·kg−1·day−1) in rabbits, rats, hamsters, and humans (19, 21, 23, 33, 34, 37), mice were initially assigned to DITPA treatment for 7 days with 3.75 mg·kg−1·day−1 subcutaneously for in vitro experiments. To investigate the detailed in vivo dose-dependent cardiovascular effects of DITPA at baseline and during stress conditions, four incremental doses of DITPA were included for 7-day subcutaneous treatment, 0.937, 1.875, 3.75, and 7.5 mg·kg−1·day−1; these doses are referred to here as DITPA-0.937, DITPA-1.875, DITPA-3.75, and DITPA-7.5, respectively. Stock solutions of DITPA (Sigma; 3.75 mg/ml) were prepared by dissolving the powder in 0.1 N NaOH and diluting with 0.9% saline. The final pH was adjusted to pH 8–9 by titration with 0.1 N HCl before injection. For TH analysis, blood samples from different sets of mice were collected on day 8, and serum TSH, total T3 (T3t), and free T3 (fT3) levels were measured by radioimmunoassay (Endocrine Diagnostic Section, Diagnostic Center for Population and Animal Health, Michigan State University, Lansing, MI).

Langendorff-perfused heart preparation. After 7-day DITPA-3.75 treatment, hearts were isolated from age-matched mice as described previously (40) in parallel with hearts from untreated mice. Briefly, mice were anesthetized with pentobarbital (50 mg/kg ip), and hearts were excised, aorta cannulated, and perfused in a Langendorff mode at a constant pressure of 80 mmHg with a modified Krebs-Henseleit buffer (KHB) equilibrated with 95% O2-5% CO2 at 37°C. The constituents of KHB were (in mmol/l) 120 NaCl, 5.9 KCl, 25 NaHCO3, 1.2 MgCl2, 2.5 CaCl2, 0.5 EDTA, and 16.7 glucose. A fluid-filled balloon was inserted into the left ventricle (LV) across the mitral valve and connected to a pressure transducer, permitting continuous measurement of LV pressure (LVP). Hearts were immersed in a water-jacketed bath maintained at 37°C, and the LV balloon was filled with water to yield a LV diastolic pressure of 3–6 mmHg. Coronary flow was continuously monitored via a Doppler flow probe (T206, Transonic Systems, Ithaca, NY) placed in the aortic perfusion line. Aortic pressure and LVP were recorded on a PowerLab/400 multichannel data acquisition system (ADInstruments; Castle Hill, Australia). The LVP signal was digitally processed (with PowerLab Chart software version 4.2, ADInstruments) to yield diastolic and systolic pressures, LV developed pressure (LVDP), HR, rate-pressure product (RPP = LVDP × HR), and positive and negative change in pressure over time (±dP/dt).

After 30-min equilibration, hearts underwent 30 min of global ischemia followed by 45 min of reperfusion. Blood pressure measurements in conscious animals. Blood pressure (BP) was measured in conscious animals by the tail-cuff method (CODA-2,Kent Scientific, Torrington, CT). Briefly, each animal was acclimatized for at least three practice sessions in three consecutive days before the final BP was recorded. In each session 15 consecutive BP readings were recorded, and the average was used for systolic, diastolic, and mean BP.

Echocardiographic evaluation of LV function. In vivo cardiac dimension and contractile function were evaluated in transthoracic M-mode echocardiography under light isoflurane (1–1.5%) anesthesia as described previously (39). Briefly, with a GE Vivid7 echocardiography system and intraoperative epicardial probe (model i13L; FREQ 14 MHz), the two-dimensional short-axis view was used as a guide and LV M-mode tracings were obtained close to the papillary muscle. LV end-diastolic and end-systolic internal diameter (LVIDd and LVIDs, respectively) were measured with the American Society of Echocardiography leading-edge method (35). LV fractional shortening was calculated as FS (%) = (LVIDd − LVIDs)/LVIDd × 100. Echocardiography was performed in the same animal in the absence and/or presence of DITPA treatment, and also before and after in vivo myocardial I/R.

In vivo myocardial ischemia-reperfusion. In vivo myocardial I/R was performed as described previously (40). Briefly, mice were anesthetized with a mixture of intraperitoneal ketamine (55 mg/kg) and xylazine (15 mg/kg). After adequate anesthesia and aspetic preparations, mice were intubated and ventilated with room air by a MiniVent (type 845, Harvard Apparatus). The respiratory rate was maintained at 125–130 breaths/min with a tidal volume of 0.25 ml for a 25-g mouse. The rectal temperature of the mouse was maintained at 37°C by a thermo heating pad. After the chest was opened and the heart visualized, the left anterior descending coronary artery (LAD) was ligated 2 mm below the tip of the left auricle by a 7-0 silk ligature. Occlusion was confirmed by ST segment elevation in ECG, dramatic changes in myocardial color (red to pallor), and restricted ventricular motion. After 30-min LAD occlusion the knot was released to start reperfusion, and reperfusion was confirmed by return of the pink-red color and motion of the anterior wall of the LV. The chest was closed in layers. When mice resumed a normal breathing pattern and started walking, the ventilator was taken off and mice were transferred to a clean cage with free access to food and water.

Criteria used to determine arrhythmias during in vivo myocardial I/R. Monitoring of ECG during I/R was performed in anesthetized mice with limb lead II with needle electrodes inserted subcutaneously. ECG was recorded 5 min before (for baseline measurements) and throughout 30-min ischemia and for the first 15–20 min of reperfusion at a sampling rate of 2 KHz with PowerLab Chart 5.0 (ADInstruments). The time of 15–20 min of reperfusion was chosen because most of the mice woke up by 10–15 min of reperfusion. The acquired ECG tracings were displayed and analyzed off-line according to the Lambeth Convention guidelines for the analysis of experimental
Table 1. Effects of 7-day DITPA treatment on serum TSH and TH levels in mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>TSH, ng/ml</th>
<th>TT3, nmol/l</th>
<th>FT3, pmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>2.48 ± 0.8</td>
<td>0.70 ± 0.1</td>
<td>14.1 ± 3.0</td>
</tr>
<tr>
<td>DITPA-0.973</td>
<td>2.93 ± 0.4</td>
<td>0.72 ± 0.1</td>
<td>11.8 ± 2.9</td>
</tr>
<tr>
<td>DITPA-1.85</td>
<td>3.16 ± 0.3</td>
<td>0.68 ± 0.1</td>
<td>16.5 ± 3.8</td>
</tr>
<tr>
<td>DITPA-3.75</td>
<td>2.72 ± 0.6</td>
<td>0.66 ± 0.2</td>
<td>16.9 ± 6.0</td>
</tr>
<tr>
<td>DITPA-7.5</td>
<td>2.72 ± 0.4</td>
<td>0.43 ± 0.13</td>
<td>16.4 ± 3.2</td>
</tr>
</tbody>
</table>

Values are means ± SD for n = 5–14 animals/group, TH, thyroid hormone; TT3, 3,5,3’-triiodothyronine; FT3, free TT3; DITPA-0.937, DITPA-1.875, DITPA-3.75, and DITPA-7.5, 3,5-diiodothyropropionic acid (DITPA) at 0.937, 1.875, 3.75, and 7.5 mg·kg⁻¹·day⁻¹, respectively. †P < 0.001 vs. respective untreated value.

RESULTS

Effects of DITPA on TH levels. To determine the effects of DITPA pretreatment on circulating TSH and TH levels in mice, whole serum from DITPA-pretreated mice was analyzed for TSH, TT3, and FT3 levels. As summarized in Table 1, compared with the untreated group there is no significant change in circulating TSH levels with any of the doses of DITPA, but DITPA at the highest dose (DITPA-7.5) significantly decreased TT3 level without any significant effect on FT3 level.

DITPA on cardiac function before and during ex vivo global I/R. To determine the effects of DITPA-3.75 on myocardial function, isolated hearts from both untreated and DITPA-treated mice were investigated for ex vivo baseline and postischemic cardiac parameters. For the ex vivo experiments, we looked for the effects of a widely used DITPA dose, 3.75 mg·kg⁻¹·day⁻¹ subcutaneously for 7 days. Table 2 summarizes the baseline functional parameters for untreated and DITPA-treated isolated hearts determined at the end of the 30-min equilibration period. There was no difference in heart weight-to-body weight ratio between untreated and DITPA-treated mice. Baseline coronary flow (normalized to the wet weight of each heart) was not significantly different between untreated and treated groups; however, DITPA-3.75 significantly impaired baseline cardiac contractile parameters in isolated hearts compared with untreated control hearts.

Figure 1 shows the time course of recovery for coronary flow (Fig. 1A), LV end-diastolic pressure (LVEDP; Fig. 1B), HR (Fig. 1C), and LVEDP (Fig. 1D) in untreated and DITPA-treated hearts subjected to 30-min global ischemia and 45-min reperfusion. Upon reperfusion, coronary flow rate was comparable between untreated and DITPA-3.75 groups (Fig. 1A); however, coronary flow recovery in DITPA-treated hearts at 45 min of reperfusion was significantly higher from their respective preischemic (PI) level (13 ± 2.84 vs. 10 ± 1.63 ml·min⁻¹·g⁻¹ at PI; P < 0.05, n = 8/group). Coronary flow remained unchanged in untreated hearts (11.8 ± 2.25 vs. 11.7 ± 1.48 ml·min⁻¹·g⁻¹ at PI; n = 8/group). While a similar rise in LVEDP was seen in both groups during ischemia, upon reperfusion LVEDP increased and remained elevated above the ischemic level in both untreated and DITPA-treated hearts (Fig. 1B). Upon reperfusion, HR recovered totally in both groups (Fig. 1C); however, LVEDP reduced significantly to a similar extent in both untreated and DITPA-treated hearts (Fig. 1D). Since the absolute baseline HR and LVEDP were different...
between the two groups (Table 2), postischemic data were normalized to their respective PI values (100%). We observed that the time course curves were comparable between the two groups (data not illustrated). There was no difference in LVDP at 45 min of reperfusion between the two groups (35 ± 10.3% of PI in untreated group vs. 34 ± 7.5% of PI in DITPA group; n = 8/group). HR in untreated hearts at 45 min of reperfusion remained unchanged compared with their PI level (96 ± 10% of PI; n = 8/group); however, HR at 45 min of reperfusion in DITPA-treated hearts was significantly higher compared with their PI level (124 ± 22% of PI; P < 0.05, n = 8/group) and also with the untreated group at 45 min of reperfusion (124 ± 22% vs. 96 ± 10% of PI; P < 0.05, n = 8/group).

Effects of DITPA on general physiology and baseline in vivo cardiac parameters. To investigate in vivo dose-dependent effects of DITPA, we randomly assigned the animals for four different doses. Table 3 summarizes the dose-dependent effects of DITPA treatment on general physiology and BP in age-matched animals. There was no effect of DITPA on body temperature and body weight; however, BP was mildly increased with high DITPA doses. Table 4 summarizes the effects of DITPA treatment on echocardiographic parameters of anesthetized mice. DITPA did not affect intrinsic HR; however, it caused marked abnormalities in cardiac parameters with higher doses. LVID of the hearts at both diastole and systole were significantly increased after 7-day DITPA treatment. Importantly, global contractile function of the heart determined as FS (%) was not affected by DITPA-0.937 (Table 4), but it was significantly reduced to 83 ± 5%, 73 ± 13%, and 63 ± 13% of respective baseline value (100%) by DITPA at 1.875, 3.75, and 7.5 mg·kg⁻¹·day⁻¹, respectively.

Effects of DITPA on myocardial infarction and function after in vivo regional I/R. To determine the effects of DITPA on postischemic myocardial salvage, dose-dependent effects of DITPA on myocardial infarction and postischemic cardiac function were evaluated in a clinically relevant model of in vivo myocardial I/R. First, in untreated mice, FS (%) 3 days before LAD ligation was taken as the preischemic (pre-MI) value, and it was compared with the subsequent postischemic (post-MI) value obtained 24 h after reperfusion. Second, in treatment groups, FS (%) after 7-day DITPA treatment was taken as the preischemic (pre-MI or DITPA) value, and it was compared with the subsequent postischemic (post-MI) value obtained 24 h after reperfusion. DITPA pretreatment at any dose had no effect on the myocardial AAR or infarct size compared with the untreated group (Fig. 2A). Table 5 summarizes the post-MI cardiac parameters compared with respective pre-MI levels. In the untreated group, post-MI FS was significantly reduced from the pre-MI level. Post-MI FS was also significantly reduced from the pre-MI level with all doses of DITPA.

Table 5 shows that DITPA treatment
Effects of DITPA on survival and incidences of arrhythmias during in vivo regional I/R. After induction of acute in vivo myocardial I/R, we did not observe any death in the untreated (0/10) or DITPA-1.875 (0/10) groups; however, posts ischemic mortality was significantly higher in DITPA-3.75 (6/20) and DITPA-7.5 (4/10) groups. The Kaplan-Meier survival rate during in vivo I/R was 70% and 60% with DITPA-3.75 and DITPA-7.5, respectively. While most of these mice died during early reperfusion (Fig. 2A), one mouse in the DITPA-0.937 group, DITPA-1.875 mouse that developed heart block during early reperfusion (Fig. 2C) and/or reperfusion. Figure 3 shows ECG tracings for a DITPA-3.75 mouse that developed heart block during early reperfusion and died within 30 min of reperfusion. Figure 3D shows ECG tracings for a DITPA-7.5 mouse that developed conduction defects during early ischemia and died within 15 min of LAD ligation because of heart block. Figure 4 shows ECG tracings during I/R where a DITPA-7.5 mouse developed agonal ventricular rhythm during ischemia and died with severe heart block during early reperfusion. Of note, all of these DITPA-treated mice died with terminal heart blocks.

Effects of DITPA on cardiac proteins. To determine whether the observed cardiac effects with DITPA treatment were associated with alterations in the cardiac proteins, quantitative immunoblotting was performed for Ca²⁺ handling proteins SERCA2a and PLB, and HSP70. Figure 5A shows the representative ECG recordings at baseline, during LAD ligation, and at reperfusion in an untreated mouse under anesthesia. Typically, ST segment elevation develops within minutes of LAD ligation and ST elevation begins to disappear within 15 min of reperfusion. Rhythm disturbances were not observed in any untreated (Fig. 3A) and DITPA-1.875 (Fig. 3B) mice; however, high-DITPA groups displayed abnormal cardiac rhythms during ischemia and/or reperfusion. Figure 3C shows ECG tracings for a DITPA-3.75 mouse that developed heart block during early reperfusion and died within 30 min of reperfusion. Figure 3D shows ECG tracings for a DITPA-7.5 mouse that developed conduction defects during early ischemia and died within 15 min of LAD ligation because of heart block. Figure 4 shows ECG tracings during I/R where a DITPA-7.5 mouse developed agonal ventricular rhythm during ischemia and died with severe heart block during early reperfusion. Of note, all of these DITPA-treated mice died with terminal heart blocks.

DISCUSSION

The major goal of this study was to test the effects of TH analog DITPA pretreatments on cardiac function of mice under physiological and pathological conditions. We observed that DITPA pretreatments in mice resulted in 1) mildly elevated BP and impaired cardiac parameters under normal physiological conditions; 2) no improvements in myocardial infarction and post-MI cardiac function; 3) abnormal cardiac rhythms during in vivo ischemia and/or reperfusion; and 4) higher mortality during in vivo I/R. Importantly, levels of myocardial calcium handling proteins SERCA2a and PLB, and HSP70 remained unchanged after DITPA treatment. To our knowledge, this is the first study that demonstrates the potential cardiac effects of DITPA in a mouse model before and after ex vivo and in vivo I/R.

Animal studies with DITPA have shown that DITPA treatment can cause an increase (13, 21, 32, 33), a decrease (37), or no effect (19) on cardiac contractility and dP/dt. We observed that DITPA at most doses significantly impaired the cardiac function; however, at lower doses, DITPA showed a mild decrease and at high doses a significant decrease in cardiac contractility. This suggests that DITPA has a biphasic effect on cardiac contractility. Further studies are needed to understand this biphasic effect of DITPA on cardiac contractility.
parameters, with increased in vivo LVID at both systole and diastole and decreased FS from the respective baseline value (Table 4). With higher doses, DITPA mildly increased BP (Table 3). Baseline contractile parameters were also impaired in ex vivo isolated hearts from DITPA-treated mice (Table 2). All of these cardiac effects of DITPA were independent of any change in myocardial protein expressions (Fig. 5). DITPA treatment did not affect body weight, temperature, or HR in mice (Tables 3 and 4), and these findings are consistent with the report in rats where DITPA treatment did not alter body weight, heart weight, or HR (37). DITPA also did not increase body temperature in thyroidectomized rats compared with a

### Table 5. Echocardiographic parameters in anesthetized mice before and 24 h after in vivo I/R

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Untreated</th>
<th>DITPA-1.875</th>
<th>DITPA-3.75</th>
<th>DITPA-7.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, bpm</td>
<td>578 ± 53</td>
<td>553 ± 53</td>
<td>577 ± 40</td>
<td>562 ± 29</td>
</tr>
<tr>
<td>LVIDd, cm</td>
<td>0.35 ± 0.04</td>
<td>0.36 ± 0.02</td>
<td>0.38 ± 0.01</td>
<td>0.38 ± 0.04</td>
</tr>
<tr>
<td>LVIDs, cm</td>
<td>0.17 ± 0.03</td>
<td>0.20 ± 0.01</td>
<td>0.23 ± 0.03</td>
<td>0.24 ± 0.04</td>
</tr>
<tr>
<td>LVSPd, cm</td>
<td>0.18 ± 0.02</td>
<td>0.13 ± 0.01</td>
<td>0.14 ± 0.02</td>
<td>0.12 ± 0.02</td>
</tr>
<tr>
<td>FS, %</td>
<td>54 ± 4.3</td>
<td>43 ± 2.2</td>
<td>40 ± 6.6</td>
<td>33 ± 7.1</td>
</tr>
</tbody>
</table>

Values are means ± SD for n = 6 or 7 animals/group before (pre-MI) or 24 h after (post-MI) ischemia-reperfusion (I/R). This data set represents those mice that survived 24-h reperfusion. *P < 0.05, †P < 0.01, ‡P < 0.001 vs. respective pre-MI value; §P < 0.05 vs. untreated post-MI value.

Fig. 3. Representative ECG tracings obtained before (baseline) and during in vivo myocardial ischemia (IS) and reperfusion (RP). A: typical ECG tracings in an untreated mouse during baseline (BL), IS with developing ST elevation (arrow), and RP with disappearing ST elevation. B: ECG tracings of a DITPA-1.875 mouse during BL, IS with developing ST elevation (arrow), and RP with disappearing ST elevation. C: ECG tracings of a DITPA-3.75 mouse showing IS with developing ST elevation (arrow) and low-voltage signals with fatal atrioventricular (A-V) blocks from 5 min of RP. D: ECG tracings of a DITPA-7.5 mouse showing ST elevation (arrow) in early IS followed by low-voltage signals and fatal A-V blocks from 10 min of IS.
Fig. 4. Representative ECG tracings obtained from a DITPA-7.5 mouse during in vivo myocardial ischemia (IS) and reperfusion (RP). Immediately after LAD ligation, ST segment elevation developed within 5 min of IS. At 20 min of IS agonal ventricular rhythm appeared spontaneously, and it progressed to partial A-V block with low-voltage signals at 30 min of IS and finally complete A-V block developed at 10 min of RP.
DITPA-7.5 significantly decreased serum TT3 levels without placebo group (36). Importantly, the effects of DITPA on TH levels have been variable in different species. DITPA treatment has been shown to increase serum fT3 level in cardiomyopathic hamsters without any effect on serum TSH level (19). In thyroidectomized rats, DITPA treatment increased serum TT3 levels compared with placebo; however, TT4 levels remained unchanged (36). Importantly, in clinical trials DITPA decreased serum TSH, TT4, or TT3 levels in heart failure patients (20, 23). In our study, DITPA only at the maximum dose studied (DITPA-7.5) significantly decreased serum TT3 levels without any significant changes in TSH and fT3 levels (Table 1).

Several studies using various experimental models have shown that either acute or chronic pretreatment with T3 before I/R improves postischemic recovery of cardiac function (for review, see Refs. 26, 27). These observations were based on the facts that plasma levels of T3 are low during AMI, during cardiac arrest, and immediately after coronary artery bypass graft (14, 15, 18, 24). Administration of DITPA before or after I/R is reported to have diverse effects on the postischemic/post-MI myocardial function (for review, see Refs. 19, 22). An earlier report in a rat heart failure model showed that DITPA alone did not alter hemodynamics but the combination of DITPA and captopril improved cardiac output and increased \(-dP/dt\) (34). In post-MI rats, long-term DITPA treatment did not improve, but rather significantly decreased, LV \(dP/dt\) (37). In contrast, improved contractile function by DITPA alone was reported in post-MI heart failure of rabbits (33) and in post-MI rats (45). In cardiomyopathic hamsters (19), DITPA treatment did not improve \(\pm dP/dt\) or ejection fraction. Importantly, while an early clinical trial reported that DITPA had no effect on systolic cardiac function but improved diastolic function in heart failure patients (23), recent clinical trials reported that the drug was poorly tolerated and the composite heart failure end point was not improved (11, 20). We observed that DITPA administration significantly and dose-dependently decreased baseline FS under normal physiological conditions (Table 4); and post-MI FS was significantly smaller in the high-DITPA group compared with untreated mice (Table 5). Interestingly, despite comparable ex vivo baseline coronary flow between untreated and DITPA-3.75 mice (Table 2), coronary flow at 45 min of reperfusion was significantly higher from their respective PI level in DITPA-treated hearts but not in untreated hearts (Fig. 1A). Of note, long-term DITPA treatment has been shown to increase in vivo myocardial blood flow in cardiomyopathic hamsters both at baseline and after maximal dilation compared with placebo, without any effect on LV function (19). Increased angiogenesis or reduced loss of arterioles has been implicated in this improved coronary flow (19). Taken together, these findings indicate that the pharmacological effects of DITPA in the heart are diverse in different species at baseline and/or under disease conditions.

TH and its analogs critically regulate cardiac performance by direct and indirect action on myocytes and by genomic and nongenomic mechanisms (3, 5). Nongenomic actions of TH include those on membrane ion channels and pumps (5, 27). The genomic action of TH is mediated through chromatin-associated nuclear TH receptors (TRs) (7, 12). It has been reported that both genomic and nongenomic actions of TH could interface at SERCA2a, where gene expression is genomic and enzyme activity is nongenomic (3, 5). Carr and Kranias (2) suggested that TH directly regulates sarcoplasmic reticulum (SR) Ca2+ handling proteins; thus it controls intracellular Ca2+ homeostasis and cardiac contraction and relaxation. However, decreased \(-dP/dt\) in control rats with long-term T3 treatment has been reported because of augmented SERCA2a enzyme activity (38). Our echocardiographic data demonstrated that DITPA treatment resulted in deleterious effects on the mouse heart under normal physiological conditions by increasing LVIDs and by decreasing global contractile function, and that these effects were evident without any alterations in cardiac SERCA2a and PLB levels. Recently, using proof-of-principle mouse models, we have clearly demonstrated that optimal SERCA function is indispensable for improving postischemic Ca2+ overload, myocardial contracture, and ventricular relaxation in isolated hearts, and also for reducing myocardial infarction following in vivo myocardial I/R (39–41). We observed that DITPA pretreatment did not improve postischemic ventricular relaxation (Fig. 1B) in ex vivo hearts, nor did it reduce
thyroid hormone analog and myocardial function

myocardial infarction or improve postischemic contractile function in vivo (Fig. 2A; Table 5). Thus the potential involvement of SR Ca\(^{2+}\) handling proteins is uncertain in our experiments with short-term DITPA treatment. TH has been reported to upregulate prosurvival signaling pathways by increased expression of cardiac HSP70, and this has been related to the improved postischemic contractile function in isolated rat hearts (28). However, we did not observe any change in the levels of myocardial HSP70 with DITPA or any improvements in the postischemic myocardial function in isolated hearts.

An important and perhaps clinically critical finding of this study is that despite comparable in vivo myocardial infarction, there was a prevalence of fatal heart blocks during I/R in DITPA-treated mice. ECG recordings (Figs. 3 and 4) demonstrated that myocardial conduction defects during in vivo I/R were the terminal cause of mortality. Although the exact mechanisms of action by which high DITPA evokes heart blocks cannot be assessed in our study, it is clearly evident that the high mortality rate during in vivo myocardial I/R is related to DITPA-induced defect in electrical impulse generation and conduction. With ex vivo I/R, HR at 45 min of reperfusion in DITPA-treated mice was significantly higher compared with their respective PI level as well as compared with untreated hearts. In dilated cardiomyopathic hamsters, long-term DITPA treatment is reported to increase HR compared with placebo (19). Importantly, a recent clinical trial with DITPA in heart failure patients revealed mixed primary outcome, with serious extracardiac deleterious effects and increased mortality (11). In that trial, DITPA increased HR (≥10 beats/min) in more patients than with placebo (61% vs. 41%) (11). Of note, DITPA treatment resulted in increased arrhythmias in heart failure patients compared with a placebo group (9% vs. 7%), and there was 4% death in the DITPA group compared with none in the placebo group (11). The composite outcome in that trial was unchanged in 48% patients, while 19% had improved and 33% had worsened outcomes. It is well known that TH exerts marked influences on cardiovascular hemodynamics and function, including effects on cardiac impulse generation and conduction (16) and on arterial BP (18). Elevation of TH concentration is usually accompanied by an increase in cardiac output mediated by the reduction of systemic vascular resistance and chronotropic and inotropic cardiac effects (18). Overt hypothyroidism is associated with diastolic hypertension (4, 9); however, the effects of overt hyperthyroidism on BP are variable (4). We observed that the highest dose of DITPA significantly lowered TT\(_3\) levels (Table 1), and it was concurrent with impaired cardiac function and increased mortality during myocardial I/R. These unexpected and altered in vivo cardiac and hemodynamic parameters with DITPA could be of serious concern for its potential therapeutic safety and efficacy.

The overall concept of myocardial protection from DITPA administration and also for amelioration of subsequent heart failure is that DITPA treatment will induce molecular changes in calcium regulation, such as increases in SERCA expression, and metabolism that can be protective and that these protective molecular changes occur without the sympathetic chro- notropic effects of TH. Unfortunately, at least in the present model, this was not evident, and there was no cardioprotection, with adverse rather than favorable effects seen. This is a major concern in view of the ongoing clinical evaluation of this drug in patients with heart failure (11, 20). The reasons for these diverse effects of DITPA are unknown in different species; various aspects of the signal transduction process including different cardiac contractile proteins, ion channels, TRs, second messengers, and subsequent steps that lead to functional modulation may be involved (2, 3, 5, 7, 27).

DITPA has been shown to bind with almost the same affinity to both TR-\(\alpha\) and TR-\(\beta\) receptors, but the affinity is 100-fold less than the affinity of these receptors for T\(_3\) (32). The action of TH in the heart is mediated predominantly by TR-\(\alpha\)1 and TR-\(\beta\)1 is expressed at low levels (10, 25). TR-\(\alpha\)1 exerts a predominant effect on cardiac impulse generation and mechanical functions, and mice deficient in TR-\(\alpha\)1 are reported to have lower baseline HR and prolonged QRS complex than control mice (44). Importantly, DITPA has been shown to reduce T\(_3\) uptake in rat cardiomyocytes in a time-dependent manner (>50% in 4 h) and to interfere with plasma membrane transport of T\(_3\) (42). However, it is unknown to what extent the inhibitory effect of DITPA on endogenous T\(_3\) uptake and/or plasma transport in the cardiomyocytes results in receptor modulation and thus attenuation or stimulation of overall cardiac function at baseline and/or under disease conditions. A recent review by Davis et al. (6) summarized that the molecular basis for the nongenomic actions of TH analogs is linked to the plasma membrane integrin receptor. While the molecular basis of TH action in the heart continues to be explored, future studies in mouse models with deletion of specific TR isoforms would be required to address the observed multidimensional cardiovascular effects of DITPA.

In the present study, we first demonstrate that DITPA treatment induces intrinsic deleterious cardiovascular effects in mice under normal physiological conditions. We also demonstrate that DITPA treatment has a narrow safety margin, with increased risk of death during acute myocardial I/R. DITPA has also been shown to have a narrow therapeutic window in recent clinical trials (11, 20). Since the cardiac liabilities of a new drug are serious therapeutic concerns in clinical use, caution must be taken in future DITPA therapy in patients with or at risk for myocardial ischemia.

ACKNOWLEDGMENTS

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GRANTS

This work was supported by National Heart, Lung, and Blood Institute Grants HL-63744, HL-65608, and HL-38324 to J. L. Zweier and HL-64140-08 and HL-080551-01 to M. Periasamy.

DISCLOSURES

There are no conflicts of interests or other disclosures.

REFERENCES


AJP-Heart Circ Physiol • VOL 300 • FEBRUARY 2011 • www.ajpheart.org
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