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Effects of Thalidomide on QTc, Inotropy, and Lusitropy in the Isolated Guinea Pig Heart

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Abstract

There has been a resurgence in the use of thalidomide over the past several years; however, little is known about its potential cardiac toxicity. Isolated, perfused guinea pig hearts were exposed to escalating concentrations of thalidomide or vehicle, and changes in RR interval, QT duration and QTc duration, and left ventricular inotropy and lusitropy comparing escalating concentrations of thalidomide with vehicle were sought. RR interval lengthened and QTc prolonged significantly at 10 μ M concentrations. QT did not change. dP/dt_{\max} increased and dP/dt_{\min} decreased in response to thalidomide. Based on results using this preparation, thalidomide has a potential liability for lengthening QTc, but only at concentrations of 10 μ M or greater. It possesses both positive inotropic and positive lusitropic properties.

Key Words: Thalidomide; cardiac toxicity; QTc; torsadogenicity; inotropy; lusitropy.

Introduction

Thalidomide (C₁₃H₁₀N₂O₄, MW 258.2) is a glutamic acid derivative composed of a two-ringed structure with an asymmetric carbon in the glutarimide ring. Before 1962 it was used to prevent nausea in pregnant woman. However, after thalidomide was demonstrated to be teratogenic (1–3), its use was virtually extinguished. Since then, there has been a resurgence (4,5) in its use for the acute management of various conditions, including the cutaneous manifestations of moderate to severe erythema nodosum leprosum through anti-inflammatory/immunomodulatory mechanisms (6,7), graft-vs-host disease (8), multiple myeloma (9), HIV (10), diseases that depend on angiogenesis (11), and advanced congestive heart failure (12). We are aware of no studies describing potential cardiac toxicity, and in particular none to identify a potential for retarding ventricular repolarization indicating a liability for producing torsade de pointes. Because thalidomide counteracts the activity of tumor necrosis factor- α (12), it is under investigation to explore the

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ability to increase the inotropic effect of catecholamines, to increase ejection fraction, and to produce vasoconstriction.

Adverse events related to thalidomide include: (1) drowsiness without depression of ventilation (resulting from activation of diencephalic sleep centers), (2) rash (occurring most commonly in patients with depressed CD4+ T-cell counts and usually controllable with antihistamines), (3) peripheral edema (unknown etiology), (4) paresthesia (present most commonly in elderly females with immunosuppression and caused by distal symmetric axonopathy possibly related to inhibition of tumor necrosis factor- α production or interleukin-1), (5) orthostatic hypotension, and (6) bradycardia (caused putatively by depression of medullary cardioregulatory centers) (13).

Because of the increasing use of thalidomide and the absence of studies exploring possible cardiovascular toxicity, this study was designed to identify potential toxic cardiovascular effects of escalating concentrations of thalidomide on RR, QT, and QTc intervals of the electrocardiogram and on maximal rates of rise and fall (dP/dt_{\max} and dP/dt_{\min}) of left ventricular pressure.

Materials and Methods

This study was approved by the Institutional Laboratory Animal Care and Use Committee of QTest Labs, Inc. Hearts were removed from 18 male guinea pigs weighing 350–450 g. They were anesthetized with sodium pentobarbital (100 mg/kg) and anticoagulated with sodium heparin (100 U) injected intravenously. After anesthesia, an incision was made over the ventral surface of the neck, a tracheotomy was performed, and the animal was ventilated with room air using intermittent positive pressure (small animal respirator, Phipps and Bird, Inc., Richmond, VA). The heart was exposed through a sternotomy. The pericardium was removed and the aorta cleared of any connective tissue. A silk suture (4/0) was placed around the ascending aorta. A blunt, 18-gauge, stainless steel cannula was connected to a three-way stopcock that was connected to a reservoir containing a modified Krebs-Henseleit bicarbonate buffer solution (36–37°C) equilibrated with 95% O₂ and 5% CO₂. The Krebs-Henseleit solution was composed of the following (mM): 118.07 NaCl, 4.69 KCl, 11.05 glucose, 24.99 NaHCO₃, 1.17 MgSO₄, 2.54 CaCl₂,

and 1.18 KH₂PO₄ at pH 7.4 (Model 430 pH/mV/T meter, Corning, New York, NY). The caudal vena cava was clamped just above the diaphragm, and an incision was made in the aorta. The cannula was quickly inserted into the opening and was secured with suture. Then the pulmonary artery was incised and the spontaneously beating heart was perfused through the coronary arteries at 37°C in a noncirculating system with a constant perfusion pressure of 80 mmHg. After the heart was retrogradely perfused, it was carefully excised from the chest, transferred to the Langendorff apparatus, and perfused with the Krebs-Henseleit solution at a constant pressure and temperature (37 ± 0.2°C). All hearts were perfused according to the method of Langendorff (14) and were instrumented to measure left ventricular isovolumetric pressure with a fluid-filled balloon connected to a precision pressure transducer (Model TSD104A, Biopac Systems, Inc., Santa Barbara, CA) inserted through the mitral annulus. A bipolar transventricular electrogram with electrodes placed on the right atrial and the apex of the left ventricle was recorded on Data Acquisition Unit MP150 software (Biopac Systems, Inc., Santa Barbara, CA) with frequency response 0.01–500 Hz (sampling rate of 1 kHz). Temperature and pH were measured just before perfusate entered the aortic cannulae. All hearts were perfused for 15 min with the Krebs-Henseleit solution to permit equilibration. Eight hearts were perfused continuously for 75 additional minutes with vehicle (Krebs-Henseleit solution plus DMSO), and 10 hearts were perfused for 75 additional min with the following escalating concentrations of thalidomide dissolved in 0.1% (v/v) DMSO: 0, 0.01, 0.1, 1.0, 10.0, and 100.0 μ M for 15 min at each concentration. Bipolar, transventricular electrograms, and isovolumetric left ventricular pressures were measured during the last minute of each concentration. The following variables were averaged over three consecutive beats during sinus rhythm: RR interval (ms), QT interval (ms), dP/dt_{\max} (mmHg/s), and dP/dt_{\min} (mmHg/s). QT was measured from the onset of the QRS complex to the place where the T wave returned to isoelectric (baseline). QT was corrected for RR interval by dividing each QT by the cube root of the preceding RR interval (13). The QT corrected for heart rate using the preceding equation, which was proposed by Fridericia, is termed QTc(F). This method was used to correct QT for RR interval because it has

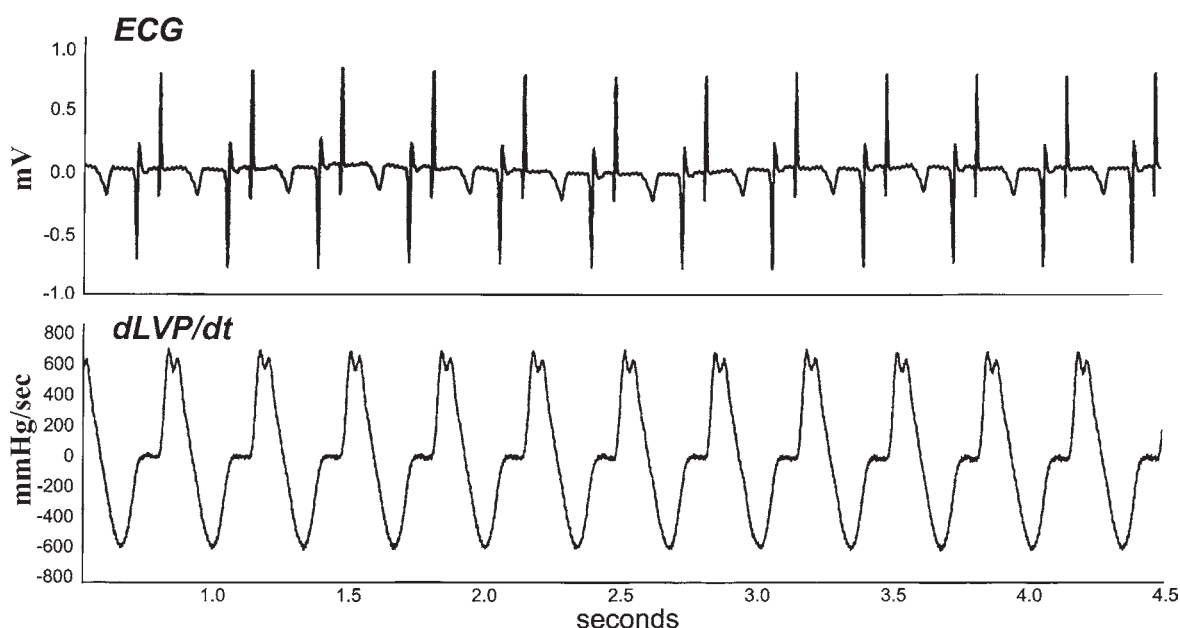


Fig. 1. Recordings (top to bottom) of bipolar transthoracic electrocardiogram and rate of change in left ventricular pressure. Time in seconds appears at the bottom, and either mV or mmHg/s appears at the left. Notice how easily the onset of the QRS complex and the end of the T wave may be identified. The P wave is so large because one of the electrodes is touching the atrium.

been shown in dogs and humans to produce a QTc independent of RR interval over RR intervals varying well beyond those obtained in this study (unpublished data). An average of 3 beats has been shown in dogs to produce mean values of measurements that do not differ from means of 12 consecutive beats (unpublished data); furthermore, the widest difference among the three measurements was <5.4 ms or 20.3 mmHg/s.

Plots were made of physiological variables vs concentrations of thalidomide and for time alone for vehicle (Krebs-Henseleit plus 0.1% DMSO). Because ventricular function deteriorates with time in the Langendorff preparation, differences were calculated between values obtained for hearts exposed to thalidomide and the average values for vehicle at comparable times. The differences that occurred between hearts that were to be exposed to thalidomide and those that were to be exposed to vehicle (i.e., before exposure to thalidomide) are termed baseline differences. An effect of thalidomide was claimed when the difference of the difference between that obtained at any concentration and that obtained during the baseline concentration was significant. Sig-

nificance was sought by a one-way analysis of variance with repeated measures design between that at each concentration (time) and that at the baseline. When indicated by a significant F statistic, specific means were compared by a Tukey post hoc test requiring a $p < 0.05$ for significance. Thus, when significant, the difference between that obtained for thalidomide and vehicle differed significantly from the difference that occurred during baseline.

Results

Electrograms and left ventricular pressures of quality permitting easy measurements were obtained from all hearts (Fig. 1). Plots of each dP/dt vs molar concentration and differences between thalidomide and vehicle (Fig. 2) show that dP/dt_{\max} increased ($p < 0.001$) (indicating positive inotropy) and dP/dt_{\min} decreased (indicating positive lusitropy) in response to thalidomide. The RR interval increased with escalating concentrations of thalidomide of $1 \mu\text{M}$ or greater (Fig. 3), but there were no greater differences between $10\text{-}\mu\text{M}$ and $100\text{-}\mu\text{M}$ concentrations. QT did not change in response to thalidomide. QTc(F) lengthened in an

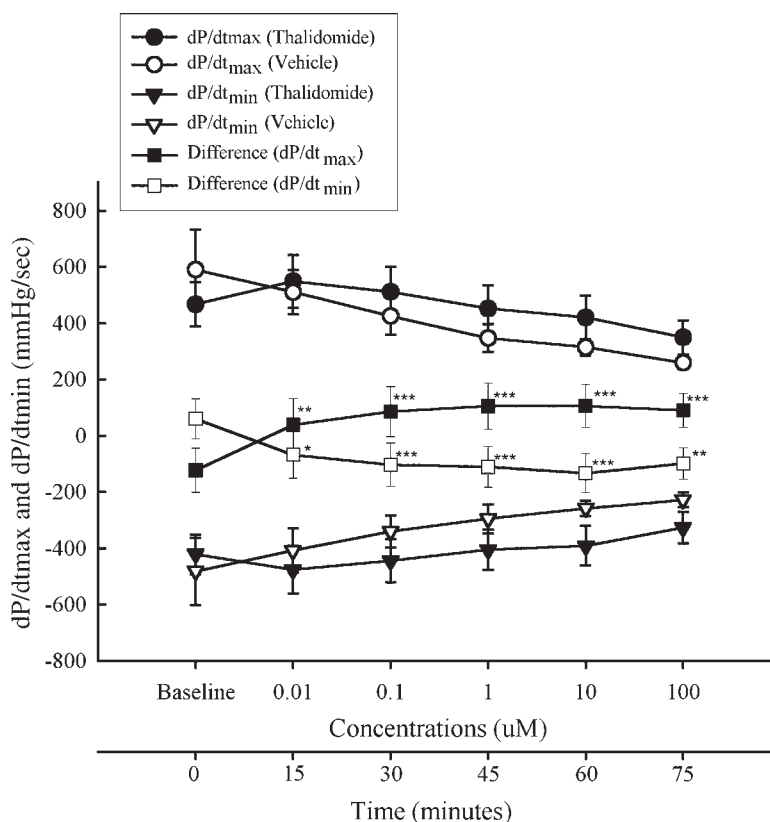


Fig. 2. Plots of mean values and standard error of the mean for dP/dt_{\max} (circles) and dP/dt_{\min} (triangles) for the thalidomide group (closed, $n = 10$) and for the vehicle (open, $n = 8$) vs concentration of test article (for thalidomide) or time (for vehicle). Squares show differences between thalidomide and vehicle for dP/dt_{\max} (closed) and dP/dt_{\min} (open). Asterisks ($*p < 0.05$, $**p < 0.01$, $***p < 0.001$) show where differences differed from baseline. It can be observed that dP/dt_{\max} decreased more slowly for the thalidomide group, manifesting positive inotropy, and that dP/dt_{\min} decreased more slowly for thalidomide, manifesting positive lusitropy.

apparent dose-response relationship at the 10- μM ($p < 0.05$) and 100- μM ($p < 0.01$) concentrations (Fig. 4). The increase in differences in QTc(F) for groups receiving thalidomide and vehicle were 13 ms for the 10- μM concentration and 15.3 ms for the 100- μM concentration. The prolongations of QTc between baseline and the 10- and 100- μM concentrations were 6.1 ms (2.6%) and 13.3 ms (5.6%).

Discussion

This study demonstrates that escalating concentrations of thalidomide, when compared with the vehicle, produce a reduction in heart rate (i.e., lengthening of RR interval), positive inotropy, positive

lusitropy, and prolongation of QTc(F). The method we used for correcting QT for RR interval the method of Fridericia (15) has been shown to be valid in our laboratory. That is, when plots of QTc(F) vs RR are made for varying RR intervals, the relationship is nearly linear and has a slope close to 0.

Inotropy and lusitropy both increased with escalating concentrations of thalidomide or vehicle, but they increased significantly more with thalidomide than with vehicle. Although these changes achieved statistical significance, they were small and probably of little physiological significance. Indeed, the changes in QTc are trivial less than 6%, even at the 100- μM concentration. We did not assay the perfusate for thalidomide, and we do not know how much might

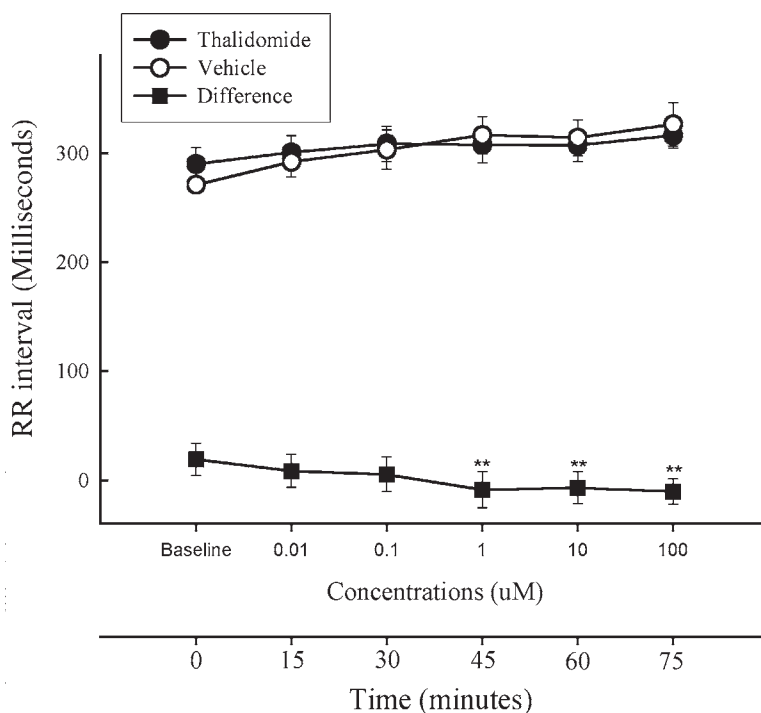


Fig. 3. Plots of RR interval (mean \pm standard error of the mean, $n = 10$ for thalidomide, $n = 8$ for vehicle) vs escalating concentrations for thalidomide (closed circles) and for time with vehicle (open circles). Differences of RRs, thalidomide minus vehicle, are shown (squares). Asterisks (** $p < 0.01$) show where differences differed from baseline; therefore, thalidomide lengthened RR interval (decreased heart rate) more than did vehicle.

have adhered to tubing or glass of the Langendorff apparatus.

Because the ventricles were contracting isovolumetrically, inotropy and lusitropy may be expressed as dP/dt_{\max} and dP/dt_{\min} , respectively (16–18). When RR interval lengthened, we would have expected both inotropy and lusitropy to decrease (chronotropic-inotropism). Therefore, the increases that we observed in both were probably less than the increases that would have occurred with a decrease in heart rate (19). Although differences in dP/dt_{\max} and dP/dt_{\min} increased, inotropic and lusitropic qualities may have been even greater if heart rate had not decreased. There are no data in the literature against which to compare these findings.

The guinea pig heart perfused according to the methods of Langendorff has been used for many studies of electrophysiology (20–24) and ventricular function. In particular, it possesses a complement of ionic channels except I_{T0} (the channel that carries potassium ions from the inside of the cell to the out-

side of the cell during phase 1 of ventricular repolarization) (25) similar to those in the human heart, which affect ventricular repolarization. The Langendorff preparation possesses many advantages and few disadvantages for detecting cardiovascular toxicity and for quantifying therapeutic effects, when compared with other in vivo or in vitro preparations (26–29). It allows identifying effects of a test compound without intervening interactions with the autonomic nervous system or metabolites. The entire heart possesses all tissues (e.g., Purkinje fibers, “M” fiber, endocardium, epicardium) that might be influenced by a test compound (30). Of course, the disadvantage is that if the test compound or metabolites exert toxicity via interactions with the autonomic nervous system, these toxicities would be missed. We have demonstrated that this guinea pig heart–Langendorff preparation has a sensitivity of 1.0 (28 positives of 28 known to lengthen QTc) and a specificity of 1.0 (14 negatives of 14 compounds thought to be negative) for predicting torsadogenicity of a compound in humans (31).

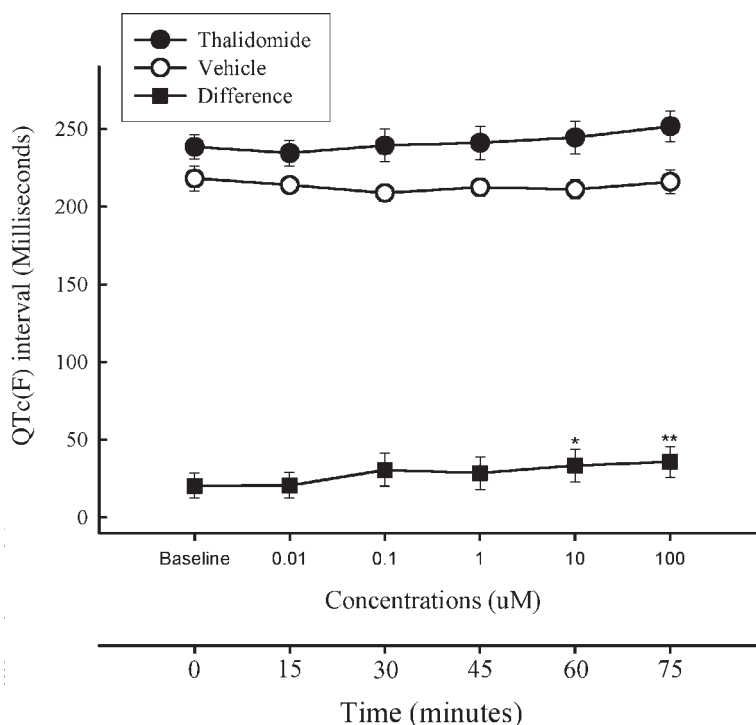


Fig. 4. Plots of QTc(F) (mean \pm standard error of the mean, $n = 10$ for thalidomide, $n = 8$ for vehicle) for thalidomide (closed circles) and vehicle (open circles) vs either concentration (for thalidomide) or time (for vehicle). Differences in QTcs and thalidomide minus vehicle, are shown (squares). Asterisks ($*p < 0.05$, $**p < 0.01$) show where differences between thalidomide and vehicle differed from baseline. The differences of significance indicate QTc(F) lengthened for thalidomide more at 100- μM concentration than at 10- μM concentration.

It is possible that thalidomide *might* possess a torsadogenic property in humans; however, the concentrations at which we observed lengthening of QTc(F) were in excess of 1 μM , probably closer to 10 μM . Thalidomide is from 55 to 66% protein-bound in human plasma (32); therefore, we presume that a concentration of more than 5 μM would be required to prolong QTc(F) in humans. The conventional dose of thalidomide in humans is 250 mg, with a volume of distribution of approx 100 L. Thalidomide has a molecular weight of approx 258 g; therefore, the estimated plasma concentration in humans would be approx 10 μM of free compound. This estimates that, if humans behave in the same way that an isolated perfused guinea pig heart does, then the therapeutic concentration might lengthen QTc(F) by approx 6 ms, or less than 3%. This concentration would place thalidomide in a very low risk category for producing torsade de pointes (33).

Thalidomide is not recommended for women who are pregnant or who are at risk for pregnancy (34) or for men who might become sperm donors (35–37). It is used predominantly in patients with serious, life-threatening diseases (e.g., cancers, heart failure). It is known that patients with heart failure are at an increased risk for developing torsade de pointes from compounds that lengthen QTc (38). Therefore, this study does not exclude a significant torsadogenic potential for thalidomide in patients with heart failure or those receiving other drugs (e.g., erythromycin [39], chloroquine [40]) that may be torsadogenic. Of course, there may be some people with an abnormal hERG (a human homolog of the *Drosophila ether-a-go-go [eag]* gene, which carries the rapid repolarization potassium current) physiology in whom thalidomide might be more torsadogenic. Similarly, this study would not have detected a toxicological effect of metabolites of thalidomide or of interac-

tions between thalidomide and the autonomic nervous system. These, of course, could be explored by *in vivo* studies.

In conclusion, this study demonstrates that thalidomide at concentrations of 0.01 μM to 100 μM produces a slight reduction in heart rate, a slight lengthening of QTc(F), and slight increases in contractility and lusitropy in isolated perfused hearts of healthy guinea pigs. In the absence of information on the cardiovascular effects of thalidomide, these results should provide assurance to prescribers of thalidomide that the compound probably possesses minimal liability to provoke torsade de pointes or other manifestations of cardiac toxicity in humans and that it may actually possess the beneficial effects of positive inotropy and lusitropy.

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