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Thiazolidinedione Drugs Promote Onset, Alter Characteristics, and Increase Mortality of Ischemic Ventricular Fibrillation in Pigs

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Abstract

Purpose—Despite favorable metabolic and vascular effects, thiazolidinedione (TZD) drugs have not convincingly reduced cardiovascular mortality in clinical trials, raising the possibility of countervailing, off-target effects. We previously showed that TZDs block cardiac ATP-sensitive potassium (K_{ATP}) channels in pigs. In this study, we investigated whether TZDs affect onset, spectral characteristics, and mortality of ischemic ventricular fibrillation (VF) and whether such effects are recapitulated by a non-selective K_{ATP} blocker (glyburide) or a mitochondrial K_{ATP} blocker (5-hydroxydecanoate).

Methods—A total of 121 anesthetized pigs were pre-treated with TZD (pioglitazone or rosiglitazone, 1 mg/kg IV, resulting in clinically relevant plasma concentrations), glyburide (1 mg/kg IV), 5-hydroxydecanoate (5 mg/kg IV) or inert vehicle. Ischemia was produced by occlusion of the left anterior descending coronary artery. In a subset of pigs treated with rosiglitazone or vehicle, ischemic preconditioning was performed.

Results—VF developed in all but 6 pigs. In non-preconditioned pigs, onset of VF occurred sooner with pioglitazone (11 ± 3 min, $p < 0.05$) or rosiglitazone (14 ± 3 min, $p = 0.06$) than with vehicle (20 ± 2 min). Defibrillation of VF was successful in 44% of pigs treated with vehicle, compared with 0% with pioglitazone ($p = 0.057$) and 33% with rosiglitazone (NS). After ischemic

preconditioning, defibrillation was successful in 62% of pigs treated with vehicle, compared with 26% treated with rosiglitazone ($p=0.03$). TZDs attenuated slowing of conduction due to ischemia and shifted ECG power spectra during VF toward higher frequencies. All effects of TZDs were recapitulated by glyburide, but not by 5-hydroxydecanoate, supporting an interaction of TZDs with the sarcolemmal K_{ATP} channel.

Conclusion—In a porcine model, TZDs promote onset and increase mortality of ischemic VF, associated with alterations of conduction and VF spectral characteristics. Similar effects in a clinical setting might adversely impact cardiovascular mortality.

Keywords

Thiazolidinedione; K_{ATP} channel; Ischemia; Ventricular fibrillation; Fourier analysis

Introduction

Thiazolidinedione (TZD) drugs have been in clinical use since 1997 for the treatment of type 2 diabetes and are currently prescribed to millions of patients worldwide [1]. These agents exert favorable metabolic and vascular effects, including improvement of insulin sensitivity and glycemic control, increase in HDL cholesterol concentration, reduction in circulating inflammatory markers, improvement in vascular endothelial function, and slowed progression of atherosclerosis as measured by vascular imaging [2]. In light of these salutary effects, it is surprising that TZD treatment has not been demonstrated to reduce cardiovascular mortality [3], and in the case of rosiglitazone may actually increase mortality [4]. These seemingly paradoxical observations might be explained, in part, by TZD-mediated fluid retention and congestive failure. However, the observations raise the question whether other countervailing, off-target effects of TZDs diminish or negate their benefit in clinical practice.

Myocardial ATP-sensitive potassium (K_{ATP}) channels ordinarily open during ischemia. This response is generally believed to be protective: Opening of K_{ATP} channels shortens action potential duration, limiting calcium influx and cellular injury [5]. Increased K_{ATP} conductance also stabilizes the resting membrane potential, thereby reducing injury current between cells and the propensity for triggered arrhythmias. Opening of cardiac K_{ATP} channels is also a necessary condition for the protection conferred by ischemic preconditioning [6]. On the other hand, opening of K_{ATP} channels shortens refractoriness in a transmurally heterogeneous manner, potentially promoting reentrant arrhythmias. A complex interplay among these effects may explain the lack of concordance in the literature as to whether and how pharmacologic K_{ATP} openers and blockers affect the propensity for ischemic arrhythmia [7, 8].

We previously reported that three TZD drugs (troglitazone, pioglitazone, and rosiglitazone) block cardiac sarcolemmal (sarc) K_{ATP} channels in pigs [9]. In the current study, we investigated effects of pioglitazone and rosiglitazone on the onset, spectral characteristics, and mortality of ischemic ventricular fibrillation (VF), and whether such effects are recapitulated by a non-selective K_{ATP} blocker, glyburide, or a mitochondrial K_{ATP} blocker, 5-hydroxydecanoate (5-HD).

Methods

Anesthesia, surgery, and instrumentation (Fig. 1)

Domestic pigs of either sex (mean weight 28 kg) were sedated with ketamine (25 mg/kg IM) and midazolam (0.5 mg/kg IM) and anesthetized with alpha chloralose (100 mg/kg IV induction, 20–30 mg/kg/h IV maintenance) and midazolam (0.35 mg/kg/h IV). After endotracheal intubation, pigs were mechanically ventilated with oxygen-enriched air. Catheters were inserted in the jugular veins and a carotid artery to administer fluids and drugs and measure arterial pressure. Needle electrodes attached to each limb recorded the surface electrocardiogram with band pass filtering (0.5–40 Hz). The chest was opened by median sternotomy and the heart suspended in a pericardial cradle. Umbilical tape was placed around the left anterior descending coronary artery (LAD), just distal to the first diagonal branch, to occlude flow. An ultrasonic flowmeter (Transonic, Ithaca, NY) confirmed cessation of LAD flow during occlusion.

Experimental treatments

In 110 pigs, treatment with pioglitazone, rosiglitazone, or glyburide (each 1 mg/kg, IV) was compared with inert vehicle. Each treatment was administered over 10 min, beginning 90 min prior to sustained coronary occlusion. Pioglitazone (Molcan Chemical, Richmond Hill, ON, Canada) was dissolved in polyethylene glycol 400 and diluted with an equal volume of 0.15 mol/L NaHCO₃ to a final concentration of 1 mg/ml. Rosiglitazone potassium salt (Cayman Chemical, Ann Arbor, MI) was dissolved in DMSO (1:10 w/v) and diluted with distilled water to a final concentration of 2 mg/ml. Glyburide (Sigma-Aldrich, St. Louis, MO) was dissolved in DMSO (1:10 w/v) and then in polyethylene glycol 400 to a final concentration of 2 mg/ml. Experiments with inert vehicle were divided among the vehicles for each active treatment. An additional 11 pigs were treated with 5-hydroxydecanoate (5-HD, Sigma-Aldrich), a putative blocker of mitochondrial K_{ATP} channel. A dose of 5 mg/kg IV was administered 30 min prior to coronary occlusion [10, 11].

Experimental protocols

Figure 2 diagrams the experimental protocols. Protocol A evaluated effects of treatment on time of onset, spectral characteristics, and success of defibrillation of ischemic VF in 51 pigs (vehicle 17, pioglitazone 8, rosiglitazone 12, glyburide 8, 5-HD 6). Ninety minutes after drug or vehicle treatment, the LAD was occluded for up to 60 min. Time from onset of coronary occlusion to onset of VF was noted. Among pigs that developed VF, defibrillation was attempted after 30 s of arrhythmia using epicardial plate electrodes and shocks of 30 J stored energy (up to 2 attempts separated by 10 s) followed by 40 J (up to 8 attempts separated by 10 s). Defibrillation attempts were continued until a stable, perfusing rhythm was restored or until death (i.e., persistent VF despite 10 defibrillation attempts). No drugs or mechanical measures were used as adjuncts to defibrillation.

In a subset of 34 pigs in Protocol A (vehicle, *n*=8; pioglitazone, rosiglitazone, and glyburide, *n*=7 each; 5-HD, *n*=5), the initial 30 s of VF recorded from the six standard limb leads of the surface electrocardiogram data were digitized at 500 Hz. Fast Fourier transformation was performed, and power spectra were computed over the frequency range 2–20 Hz. Median

frequency (bisecting the area of the power spectrum) and edge frequency (below which 95% of the power spectrum area lies) were computed. A composite power spectrum for each pig was computed by averaging spectra from each of the 6 electrocardiographic leads, according equal weight to each lead. A composite power spectrum for each treatment group was computed by averaging spectra from all pigs in that group, according equal weight to each pig.

Protocol B evaluated effects of TZDs on conduction in ischemic myocardium prior to VF in 24 pigs (4 pioglitazone, 5 each rosiglitazone, glyburide, 5-HD, and vehicle). Two pairs of bipolar epicardial electrodes with 3 mm inter-electrode spacing were sutured to the epicardium at the boundaries of the ischemic zone as indicated in Fig. 1. One electrode pair was aligned parallel and one orthogonal to the presumed axis of epicardial fibers [12]. One electrode of each pair was connected to a Grass stimulator (model S8800) through an isolation unit. Stimuli were applied at cycle length 400 ms, duration 5 ms, and amplitude 0.1 mA. The other electrode of each pair served as recording electrode. Conduction time was measured from stimulus to maximal dV/dt of the intrinsicoid deflection of the bipolar electrogram from the recording electrode. Measurements were made at baseline, 90 min after drug or vehicle treatment before onset of ischemia, and then every minute from onset of ischemia to onset of ischemic VF. Time of onset of VF and success of defibrillation were not assessed in Protocol B because of potential effects of rapid pacing during ischemia on these measures.

Protocol C evaluated effects of rosiglitazone ($n=24$) or vehicle ($n=22$) on time of onset and mortality of ischemic VF after ischemic preconditioning. Preconditioning was produced with 2 cycles of 5 min LAD occlusion and 10 min reperfusion, spanning the interval 60–90 min after drug or vehicle treatment. After preconditioning, the LAD was occluded for up to 60 min, time of VF onset was recorded and defibrillation attempted as in Protocol A.

Plasma samples were collected just prior to onset of ischemia to measure concentrations of pioglitazone and its active metabolites M-III and M-IV ($n=6$) or rosiglitazone ($n=19$) using liquid chromatography and mass spectrometry [13, 14]. Plasma glucose was measured before and 90 min after drug treatment in 17 pigs.

Statistics

Data are presented as mean $\pm$ SEM. For continuous variables, active treatments were compared with vehicle using t-tests. Mortality was analyzed using Fisher Exact test. Results were considered significant at the p 0.05 level.

Results

Hemodynamics

Aortic blood pressure ($100/74\pm2/2$ mmHg) and heart rate 92 ± 3 min⁻¹) did not differ between groups and were not significantly affected by any of the treatments.

Drug and glucose concentrations

Prior to ischemia, plasma concentration of pioglitazone was 1.26 ± 0.17 $\mu\text{g/ml}$, pioglitazone M-III 54 ± 13 ng/ml , pioglitazone M-IV 253 ± 20 ng/ml , and rosiglitazone 1.15 ± 0.18 $\mu\text{g/ml}$. These concentrations are similar to those achieved in clinical practice [15–17]. Plasma glucose was unaffected by TZDs (mean 2.9 mmol/L both before and 90 min after treatment) and decreased modestly after glyburide (mean 3.7 mmol/L before and 2.9 mmol/L 90 min after treatment).

Effects of TZD treatment on time to first occurrence of ischemic VF

In the absence of preconditioning (Protocol A), ischemic VF occurred in 42 of 45 pigs. Three pigs did not develop VF (1 each in pioglitazone, glyburide, and vehicle groups). In pigs treated with vehicle, VF occurred at 20 ± 2 min after coronary occlusion (Fig. 3). Compared with vehicle, VF occurred sooner after coronary occlusion in pigs treated with pioglitazone (11 ± 3 min, $p < 0.05$), rosiglitazone (14 ± 3 min, $p = 0.07$), or glyburide (13 ± 3 min, $p < 0.05$).

Effect of TZD treatment on defibrillation success

In the absence of ischemic preconditioning (Protocol A), survival (i.e., successful defibrillation) was reduced among pigs treated with pioglitazone (0%, $p = 0.057$) compared with those treated with vehicle (44%) (Fig. 4). Survival was also lower, although non-significantly, among pigs treated with rosiglitazone (33%) and glyburide (14%). Because defibrillation at maximum energy was unsuccessful in the majority of pigs, defibrillation thresholds could not be computed.

Effect of TZD treatment on spectral characteristics of ischemic VF

To determine whether an adverse effect of TZD treatment on success of defibrillation was related to a change in spectral characteristics of VF, Fourier analysis was performed using data recorded during the first 30 s of VF. Total power (integrated area) of spectra did not differ among treatments. However, both pioglitazone and rosiglitazone shifted power spectra of VF toward higher frequencies, manifest by significantly higher median and 95% edge frequencies, compared with vehicle (Fig. 5). Once again, effects were recapitulated by glyburide.

Effects of TZD treatment on conduction in ischemic myocardium

To determine if the effect of TZD treatment to shift VF power spectra to higher frequencies was accompanied by an effect on conduction in ischemic tissue, conduction time across the ischemic region was measured prior to VF onset (Protocol B). Representative and group data are shown in Figs. 6 and 7. In the absence of ischemia, treatment with rosiglitazone, pioglitazone, or glyburide had no effect on conduction time. As expected, ischemia significantly slowed conduction (prolonged conduction time). However, treatment with pioglitazone or rosiglitazone attenuated the degree of ischemic conduction slowing, in both parallel and orthogonal directions to the presumed fiber axis ($p < 0.05$ compared with vehicle). Glyburide again recapitulated this effect. Considering all pigs in vehicle, TZD, and glyburide groups, there was a significant correlation ($r^2 = 0.24$, $p < 0.05$) between conduction

time during ischemia and time to onset of VF, i.e., suggesting that effects of the drugs on these parameters may be linked.

Effects of rosiglitazone with ischemic preconditioning

After ischemic preconditioning (Protocol C), VF occurred in 44 of 46 pigs. One pig each in vehicle and rosiglitazone groups did not develop VF. Time of onset of VF in preconditioned pigs treated with vehicle (16 ± 3 min) was slightly, but not significantly shorter than in non-preconditioned pigs treated with vehicle (20 ± 2 min). Moreover, time of onset of VF did not differ between preconditioned pigs treated with vehicle or rosiglitazone (18 ± 3 min). Among pigs treated with vehicle, survival (successful defibrillation) did not differ significantly between preconditioned and non-preconditioned pigs in (62% versus 44%). However, among preconditioned pigs survival was poorer among those treated with rosiglitazone than among these treated with vehicle (26% versus 62%, $p=0.03$).

Effects of 5-hydroxydecanoate

Ten of 11 pigs treated with 5-HD developed ischemic VF. Conduction time increased with ischemia by a mean of 86% in pigs treated with 5-HD, similar to vehicle (110% increase, Table 1) and more than in the combined TZD group (39% increase, $p=0.06$ versus 5-HD). On the other hand, mortality (failure of defibrillation) among pigs treated with 5-HD was 80%, similar to the combined TZD group (78%) and higher than vehicle-treated pigs (38%, $p=0.03$). VF onset and VF median frequency with 5-HD were intermediate between and did not differ significantly from combined TZD or vehicle groups.

Discussion

This study demonstrates that TZD drugs at clinically relevant concentrations exert previously unsuspected, adverse electrophysiologic effects during acute myocardial ischemia. Pioglitazone, the predominant TZD in current clinical use, promoted earlier onset of ischemic VF after coronary occlusion and reduced the success of defibrillation once VF occurred. Rosiglitazone produced directionally similar, but less pronounced effects, and significantly reduced success of defibrillation after ischemic preconditioning. The pro-arrhythmic actions of TZDs were associated with attenuation of the slowing of conduction that ordinarily occurs in ischemic myocardium and a shift in the power spectrum of ischemic VF toward higher frequencies.

The effects of pioglitazone and rosiglitazone did not differ significantly from one another with respect to any of the electrophysiologic measures in this study. Although rosiglitazone did not significantly affect success of defibrillation in non-preconditioned pigs, considering preconditioned and non-preconditioned pigs in aggregate, rosiglitazone did reduce success of defibrillation compared with vehicle (29% versus 54%, $p=0.03$), thus mirroring the effect of pioglitazone. Both rosiglitazone and pioglitazone abrogated K_{ATP} effects on action potentials to a similar degree as in a prior study from our laboratory [9]. Thus, it is likely that these agents share common electrophysiologic effects.

Recapitulation of the effects of TZDs by the prototypical K_{ATP} blocker, glyburide, suggests that the pro-arrhythmic actions of TZDs are related to blockade of myocardial K_{ATP}

channels. Three lines of evidence support such a mechanism. First, we have previously observed in vivo that troglitazone, rosiglitazone, and pioglitazone abrogate action potential shortening induced by levcromakalim, a K_{ATP} activator [9]. Second, each electrophysiologic effect of TZDs observed in the present study is concordant with previously characterized effects of pharmacologic K_{ATP} blockade or genetic abrogation of the K_{ATP} channel [8, 18–24]. Third, in vitro studies indicate interaction of TZDs with K_{ATP} . In islet cells, rosiglitazone promotes phosphorylation of the Kir6.2 subunit of K_{ATP} (the same Kir subunit found in cardiac channels) at Ser(385), resulting in inactivation of the channel [25]. Rowe et al. [26] and McKay et al. [27] showed that another TZD, englitazone, also blocks IK_{ATP} through interaction with Kir6.2. Finally, cardiac K_{ATP} channels reconstituted in COS cells were blocked by troglitazone [28]. Although each electrophysiologic effect of pioglitazone and rosiglitazone in the present study was recapitulated by glyburide direct proof that TZDs block K_{ATP} would rest upon demonstration that TZDs block IK_{ATP} in isolated porcine ventricular myocytes.

The mean experimental plasma concentration of pioglitazone (1.26 $\mu\text{g/ml}$) in the present study is similar to C_{max} in patients after an oral dose of 45 mg (1.05–1.33 $\mu\text{g/ml}$), while experimental concentrations of pioglitazone active metabolites M-III and M-IV were somewhat lower than those measured clinically [15, 16]. The mean experimental plasma concentration of rosiglitazone (1.15 $\mu\text{g/ml}$) is within 2-fold of the C_{max} measured in patients after an oral dose of 8 mg (0.60 $\mu\text{g/ml}$) [17]. Thus, it is quite possible that pro-arrhythmic effects observed in the current experiments are relevant to clinical use of TZDs.

The role of K_{ATP} channels in the genesis of ischemic arrhythmias is controversial. On one hand, opening of K_{ATP} channels during ischemia shortens action potential duration and may increase dispersion of refractoriness, thereby promoting reentry. To the extent that a K_{ATP} blocker prevents these effects, its actions may be anti-arrhythmic. On the other hand, opening of K_{ATP} channels during ischemia or with pharmacologic agents may augment outward flux of potassium, stabilize the resting membrane potential, promote repolarization, limit calcium influx, and reduce the propensity for early afterdepolarizations and triggered arrhythmia [19, 29, 30]. Abrogation of these effects by pharmacologic K_{ATP} blockade or in genetic models with defective K_{ATP} channels delays repolarization and promotes calcium overload, early afterdepolarizations, and triggered ventricular arrhythmias [22–24, 31, 32]. Whether the net effect of an agent that blocks K_{ATP} is to promote or suppress ischemic arrhythmias likely depends upon the specific experimental model and conditions. Under the conditions of the current study, the earlier onset of ischemic VF and the refractory nature of VF once established reflect a predominant pro-arrhythmic effect of TZDs.

Opening of K_{ATP} channels ordinarily contributes to slowing of conduction in ischemic myocardium. Conversely, blockade of K_{ATP} channels attenuates the slowing of conduction (maintains faster conduction) in ischemic myocardium [21, 33]. In the present model, treatment with either TZD or glyburide, compared with vehicle, maintained faster conduction in ischemic myocardium prior to the onset of VF. A corresponding facilitation of conduction during ischemic VF may explain the shift of the VF power spectrum toward higher frequencies in pigs treated with TZD or glyburide.

To terminate VF, a shock must fulfill two conditions [34, [35]]: It must halt VF wavefronts by depolarizing a critical volume of myocardial tissue, and it must not activate new wavefronts that reinitiate reentrant VF. In this and previous studies [9], we have shown that TZDs increase ischemic conduction velocity, shorten VF cycle length (reflected by higher spectral frequencies) and prolong refractoriness in ischemic tissue (reflected by longer action potential duration). In combination, these effects may decrease the excitable gap in a reentrant VF circuit [36], thus reducing the likelihood that a defibrillation shock will halt existing VF wavefronts. Moreover, if TZD treatment promotes afterdepolarizations as described above, VF may be re-initiated immediately after a defibrillation shock. Our data do not allow us to distinguish failure to defibrillate from immediate reinitiation of VF, but either mechanism could account for the reduced success of defibrillation and higher mortality of pigs treated with pioglitazone (Protocol A) or rosiglitazone (Protocol C).

While it is plausible that the shift of ischemic VF power spectra to higher frequencies caused more frequent failure of defibrillation among pigs treated with TZDs, this remains uncertain. In fact, with the caveat that determinants of defibrillation may differ in ischemic and non-ischemic VF [37], prior studies under the latter condition have shown an opposite association: higher frequency VF associated with greater success of defibrillation and/or lower defibrillation threshold [38, 39]. An alternative explanation for the present finding that TZDs or glyburide reduce success of defibrillation is that adverse metabolic effects due to K_{ATP} blockade lead to more severe calcium overload and high energy phosphate depletion during ischemia [31, 40], rendering defibrillation futile.

In this study, electrophysiologic effects of TZDs were observed within 90 min of drug administration, making it unlikely that the effects were transcriptional and mediated through nuclear PPAR gamma receptors. More likely, the observed effects of TZDs reflect off-target interaction of the drugs with cell surface K_{ATP} channels. Supporting this supposition, the effects of TZDs were recapitulated by glyburide, which is not a transcriptional regulator. A non-transcriptional mechanism of K_{ATP} blockade is also supported by in vitro studies in non-cardiac cells that demonstrate immediate attenuation of IK_{ATP} upon exposure to TZDs [27, 41].

Because opening of cardiac K_{ATP} channels is a requisite step in the mechanism of ischemic preconditioning, a drug that blocks K_{ATP} during ischemia might be expected to abrogate some of the protective effects of preconditioning. We initially undertook experiments with preconditioning with the goal of assessing effects of TZD treatment on infarct size, the usual readout of preconditioning protection. However, this proved to be impossible because of survivor bias, with significantly lower survival through ischemia and reperfusion in preconditioned pigs treated with rosiglitazone, compared with vehicle. Ischemic preconditioning did not affect the incidence of ischemic VF and tended to shorten the time of onset of VF in vehicle-treated pigs, consistent with prior studies in swine [42, 43]. Moreover, in preconditioned pigs neither VF incidence nor time of onset was influenced by rosiglitazone, consistent with a prior study showing no effect of glyburide on these measures [44].

In a previous study [9] we showed that TZDs abrogate the effects of a K_{ATP} opener, levcromakalim, on the cardiac action potential, providing a readout of effects of TZDs on $sarK_{ATP}$. We also showed that a selective $sarK_{ATP}$ blocker, HMR-1098, promoted onset of VF to the same extent as rosiglitazone. These data suggest that TZDs interact with $sarK_{ATP}$ channels. Glyburide is believed to block both sarcolemmal and mitochondrial (mito) K_{ATP} channels. Therefore, recapitulation of TZD effects by glyburide leaves open the possibility that TZDs also interact with $mitoK_{ATP}$. In fact, a recent study suggests that pioglitazone may activate $mitoK_{ATP}$ [45], based on an observation in rats that reduction in myocardial infarct size by pioglitazone was abrogated by the $mitoK_{ATP}$ blocker 5-HD.

In the present model, we attempted to assess whether TZDs interact with $mitoK_{ATP}$ by examining effects of 5-HD treatment. The data do not exclude such an interaction, but do provide further support for a TZD/ $sarK_{ATP}$ interaction. Slowing of conduction in ischemia has been attributed, in part, to increased extracellular K^+ via efflux through open $sarK_{ATP}$ [21, 33]. The fact that TZDs did, but 5-HD did not attenuate the slowing of conduction with ischemia supports blockade of $sarK_{ATP}$ by TZDs, but does not elucidate a potential effect of TZDs on $mitoK_{ATP}$. Time of onset and median frequency of VF with 5-HD were intermediate between TZD and vehicle groups, and thus neither support nor refute a TZD/ $mitoK_{ATP}$ interaction. Mortality (failure of defibrillation) was increased to a similar extent with 5-HD and TZDs. Although interaction of TZDs with $mitoK_{ATP}$ might account for this similarity, 5-HD might also have promoted metabolic rundown during ischemia and consequent refractory VF through distinct effects on mitochondrial function.

Several other limitations of this study are recognized. We administered drugs acutely, by the intravenous route. It is uncertain whether chronic, oral administration of TZDs would affect ischemic VF in a similar manner. Although plasma concentrations of pioglitazone and rosiglitazone were in clinically relevant ranges, we studied only one dose of each drug; therefore, dose–response relations for outcome variables of interest are undetermined. We cannot determine whether pro-arrhythmia resulted directly from electrophysiologic effects of K_{ATP} blockade, or indirectly from metabolic run-down during ischemia promoted by K_{ATP} blockade. After the onset of VF, we did not employ cardiac massage, adjunctive drug treatment, or any resuscitative measures other than defibrillation, and our examination of effects of TZD treatment on VF characteristics and defibrillation success was limited to the initial 30 s of arrhythmia. However, these experimental conditions mirror the scenario of attempted therapy by an implanted defibrillator, and therefore may have clinical relevance. We studied electrophysiologic effects of TZDs in structurally normal porcine hearts subjected to acute coronary occlusion; it is uncertain whether effects would be similar in the human heart, after healed myocardial infarction, or with other cardiomyopathies that may affect the substrate for arrhythmia. We inferred conduction velocity from pairs of electrodes, rather than arrays. Thus we cannot exclude the possibility that conduction path length, rather than velocity, was affected by interventions. Finally, recapitulation of TZD effects by glyburide does not exclude the possibility of TZD effects on channels other than K_{ATP} . In fact, rosiglitazone has been shown to block conductance of several other cardiac potassium channels in vitro [46], albeit at concentrations much higher than those achieved clinically or in the present study.

The results of the current study raise the following clinical question: Do heretofore unsuspected, off-target, pro-arrhythmic effects of TZDs due to cardiac K_{ATP} blockade account for the dichotomy between the beneficial metabolic and vascular effects of these drugs and the absence of convincing data that they reduce morbidity and mortality? Clinical trials and observational analyses suggest that pioglitazone has neutral to favorable effects [3, 47] while rosiglitazone has neutral to harmful effects on cardiovascular outcomes [3, 4]. However, analyses comparing the two agents provide no consensus that they produce different cardiovascular outcomes [48]. In this and a prior experimental study from our laboratory [9], similar electrophysiologic effects were observed with both pioglitazone and rosiglitazone. It is therefore possible that potential clinical benefits of both agents are offset by shared deleterious, off-target effects related to K_{ATP} blockade.

Sudden cardiac death is a problem of large proportions among diabetics, affecting an estimated 80,000 patients per year in the United States alone [49]. Any effect of diabetic treatments to influence this risk is therefore important. If the present observations that TZDs shorten the time to ischemic VF by approximately 50% and reduce the success of defibrillation also apply to patients treated with the drugs, there could be substantial adverse clinical implications. To date, rigorous clinical electrophysiologic studies with TZD drugs have not been performed, but may be indicated. Moreover, in the development of new insulin-sensitizing drugs it may be important to exclude interaction with cardiac K_{ATP} channels.

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Fig. 1. Instrumentation of the heart. Diagram indicates placement of a coronary occluder on the left anterior descending coronary artery, the resulting ischemic zone of the left ventricle, and positions of bipolar stimulation and recording electrodes oriented parallel and orthogonal to the presumed epicardial fiber axis (for Protocol B)

Fig. 2.

Experimental protocols. Protocol A: 50 pigs (vehicle 17, pioglitazone 8, rosiglitazone 12, glyburide 8, 5-HD 5). Protocol B: 24 pigs (pioglitazone 4; rosiglitazone, glyburide, 5-HD, and vehicle 5 each). Protocol C: 46 pigs (rosiglitazone 24, vehicle 22). FFT = spectral analysis by fast Fourier transformation. Drugs were given 90 min before sustained ischemia, except for 5-HD which was given 30 min before ischemia

Fig. 3.

Effect of TZDs on time from coronary occlusion to first occurrence of ischemic VF: Data are mean $\pm$ SEM for vehicle ($n=16$), pioglitazone ($n=7$), rosiglitazone ($n=12$), and glyburide ($n=7$). VF occurred sooner after coronary occlusion in pigs treated with pioglitazone, rosiglitazone, or glyburide than in pigs treated with vehicle (*, $p < 0.05$ versus vehicle; †, $p=0.06$ versus vehicle)

Fig. 4.

Effect of TZDs on survival from ischemic VF (successful defibrillation). After 30 s of ischemic VF, defibrillation was attempted with epicardial plate electrodes with 2 attempts at energy 30 joules and up to 8 attempts at 40 joules, until a perfusing rhythm was restored or until death. Survival was decreased in pigs treated with pioglitazone, compared with vehicle (*, $p=0.057$)

Fig. 5.

Effect of TZDs on power spectrum of ischemic VF. Power spectra of ischemic VF were obtained by fast Fourier transformation of recordings of the surface electrocardiogram during the initial 30 s of the arrhythmia. Figure shows spectra averaged among pigs in each treatment group (pioglitazone, rosiglitazone, and glyburide, $n=7$ each; vehicle, $n=8$). Compared with vehicle, each drug treatment shifted the power spectrum of ischemic VF toward higher frequencies, with significant increases of median frequency (bisecting the area under the power spectrum) and edge frequency (below which 95% of the area of the power spectrum resides)

Fig. 6.

TZDs attenuate slowing of conduction in ischemic myocardium. Recordings of surface ECG and from paired bipolar stimulating and recording electrodes in ischemic region of left ventricle. Examples from pigs treated with vehicle, rosiglitazone, pioglitazone, and glyburide. *Top:* Recordings after vehicle or drug treatment, but before ischemia. *Bottom:* Recordings during ischemia, before the onset of VF. Conduction time prolonged during ischemia in all groups. However, ischemic prolongation of conduction was less pronounced in pigs treated with TZD or glyburide than with vehicle

Fig. 7.

Effect of TZDs on conduction time across ischemic region. Time for conduction between paired stimulating and recording electrodes oriented parallel (*top*) and orthogonal (*bottom*) to the presumed epicardial fiber axis was measured in pigs treated with vehicle ($n=5$), pioglitazone ($n=4$), rosiglitazone ($n=5$), or glyburide ($n=5$). In each pig, conduction time was normalized to its baseline (pre-treatment) value. Data are mean $\pm$ SE. In the absence of ischemia, treatments had no effect on conduction. Ischemia increased conduction time in all groups, both in parallel and orthogonal directions to presumed fiber axis. However, treatment with pioglitazone, rosiglitazone, or glyburide significantly attenuated the increase in conduction time with ischemia (*, $p < 0.05$ versus vehicle)

Table 1

Effects of 5-hydroxydecanoate on ischemic VF

Group parameter	Vehicle	5-HD	Combined TZD group
Increase in conduction time with ischemia (%)	110±6	86±26 [‡]	40±13
Time of onset of VF (sec)			
mean ± SE	20±2	14±4	13±2
median	20	18	10
Median frequency of VF (Hz)	8.9±0.3	9.7±0.5	9.9±0.2
Mortality (% unsuccessful defibrillation)	38	80 [*]	78

Data are mean ± SE. *N*=5 to 19, depending upon group and measurement (details in Methods). 5-HD, 5-hydroxydecanoate. Combined TZD group, pioglitazone and rosiglitazone.

[‡], *p*=0.06 versus combined TZD group.

^{*}, *p*=0.03 versus vehicle