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Acute cardiovascular effects of tacrolimus in the isolated guinea pig heart

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Tacrolimus is an immunosuppressive, macrolide antibiotic frequently used to minimize transplant rejections. It has also been used as a topical ointment to treat atopic dermatitis (Kapp *et al.*, 2003), and as an immunosuppressant in kidney transplants in dogs (Griffin *et al.*, 1992). Macrolide antibiotics are known to prolong ventricular repolarization, and to produce the potentially fatal arrhythmia torsade de pointes (TdP).

Reports by Johnson *et al.* (1992) and Hodak *et al.* (1998) describe TdP in patients receiving tacrolimus. TdP is a rapid, polymorphic, ventricular tachycardia produced most often by drugs that lengthen the duration of depolarization and repolarization measured as the QT duration in the electrocardiogram (ECG). Tacrolimus has been shown to increase QTc (QT corrected for heart rate) dispersion in patients with kidney transplants, implying a risk for TdP (Gerhart *et al.*, 2001). A second study by González *et al.* (1999) reported shortening of QTc in patients with liver transplants treated with tacrolimus. The QTc dispersion refers to the difference in durations of QTcs from numerous leads, and is thought to reflect heterogeneity of ventricular repolarization, an electrophysiological substrate for early after depolarizations and re-entrant ventricular arrhythmias. Minematsu *et al.* (1999) demonstrated lengthening of QTc in a use-dependent manner showing counterclockwise hysteresis in anesthetized guinea pigs. Thus, the QTc lengthened more at higher heart rates than at lower heart rates – a pattern opposite to that of most torsadogenic agents, which lengthen QTc in a reverse-use dependent manner.

There are two mechanisms by which tacrolimus may retard ventricular repolarization. Tacrolimus blocks intermediate-conductance K^+ channels (I_K) responsible for repolarizing currents (duBell *et al.*, 1997). It also elevates intracellular Ca^{2+} by its ability to increase conductance in ryanodine release channels and decrease conductance in sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA2) entry channels (Bultynck *et al.*, 2000).

Little is known about the effects of tacrolimus on mechanical properties of the heart. This study was conducted to determine the effects of escalating doses of tacrolimus on electrophysiological and mechanical properties of the heart. All experiments were performed in isolated, perfused guinea pig hearts to prevent obfuscation by metabolites or autonomic innervation.

This study was approved by the ILACUC of QTest Labs, Inc. Ten healthy male guinea pigs (weighing 350–400 g) were used in these experiments. All animals received humane care in accordance with national guidelines and legal regulations. Guinea pigs were anesthetized with sodium pentobarbital (100 mg/kg) and anticoagulated with sodium heparin (100 U) injected i.v., and their hearts were rapidly removed and perfused according to the method of Langendorff (Langendorff, 1903; Hamlin *et al.*, 2004) with the following modifications. The hearts were suspended from a Langendorff apparatus, and perfused at a constant pressure of 80 mmHg with modified Krebs solution (37 ± 1 °C) gassed with 95% oxygen and 5% carbon dioxide. Bipolar transventricular electrodes were used for recording a cardiac electrogram (BIOPAC MP150 Data Acquisition Unit, BIOPAC Systems, Inc., Santa Barbara, CA, USA). A saline-filled balloon was inserted into the left ventricle for recording of isovolumetric left ventricular pressure. This instrumentation permitted recording of RR, PQ, QRS, and QT intervals/durations, and maximum ($dLVP_{max}$) and minimum ($dLVP_{min}$) rate of change of left ventricular pressure.

Baseline electrograms and left ventricular pressure pulses were recorded for 15 min. Following baseline measurements, hearts were perfused for 15 min with 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M tacrolimus (Prograf, Fujisawa, Japan) in 0.1% DMSO ($n = 6$ each) or vehicle ($n = 4$). Recordings were obtained during the final minute of each 15-min interval. The QTc was calculated according to the method of Fridericia (1920). The derivatives of $dLVP_{max}$ (dP/dt_{max}) and $dLVP_{min}$ (dP/dt_{min}) were calculated as measurements of inotropy and lusitropy. Mean values for all hearts exposed to either tacrolimus or vehicle were calculated, and the between-treatment differences were plotted. Significance was determined using a two-way ANOVA with repeated measures design on dose/time. When indicated by a significant F-statistic, differences in specific means were determined using a Tukey's *post hoc* test requiring a $P < 0.05$ for significance.

Bipolar transventricular electrograms and recordings of isovolumetric left ventricular pressure, free from artifacts, were obtained from all preparations. Tacrolimus did not affect RR interval (the reciprocal of heart rate) or QRS duration (data not shown). At low doses tacrolimus did not affect the PQ interval (Fig. 1). Only the highest concentration of tacrolimus (10^{-5} M) affected the PQ interval, causing it to significantly lengthen ($P < 0.001$). Tacrolimus had a similar effect on both QT duration (Fig. 2) and QTc duration (Fig. 3), which increased significantly in the presence of 10^{-5} M tacrolimus ($P = 0.02$ and 0.002, respectively). Likewise, at the highest concentration of tacrolimus (10^{-5} M) $\mathrm{d}P/\mathrm{d}t_{\max}$ decreased significantly ($P < 0.05$; Fig. 4) and $\mathrm{d}P/\mathrm{d}t_{\min}$ ($P < 0.05$; Fig. 4) increased significantly (i.e. became less negative). Lower concentrations of tacrolimus did not affect QT or QTc duration, $\mathrm{d}P/\mathrm{d}t_{\max}$ or $\mathrm{d}P/\mathrm{d}t_{\min}$.

This study evaluated the effects of escalating concentrations of tacrolimus (10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M) on various electrophysiological (e.g. RR, PQ, and QT intervals, QRS duration, QTc) and mechanical (e.g. $\mathrm{d}P/\mathrm{d}t_{\max}$, $\mathrm{d}P/\mathrm{d}t_{\min}$) properties of the heart in an *ex vivo* guinea pig heart model. This model establishes the effects of tacrolimus devoid of metabolites or effects of autonomic innervation, while maintaining a whole-tissue preparation containing all other cardiac properties (e.g. ion channels). A

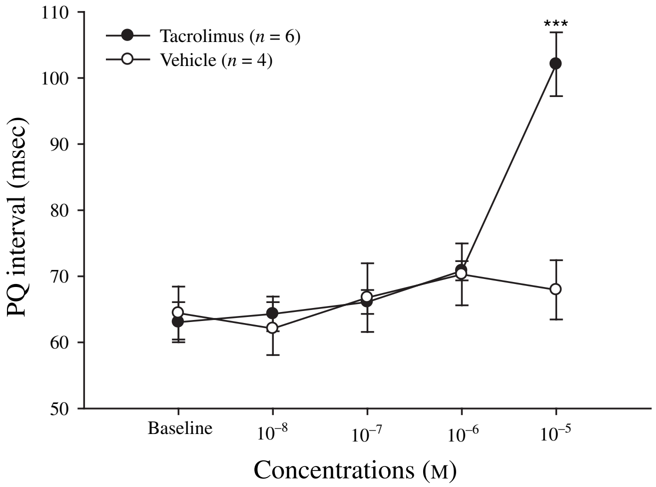

Fig. 1. Concentration–response curve of the effect of escalating doses of tacrolimus ($n = 6$) and vehicle ($n = 4$) on mean PQ interval. Data are given as mean ± standard error of the mean (***$P < 0.001$ relative to vehicle).

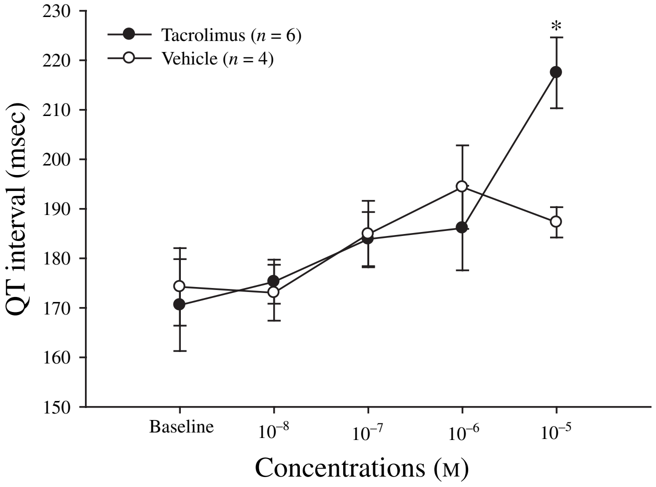

Fig. 2. Concentration–response curve of the effect of escalating doses of tacrolimus ($n = 6$) and vehicle ($n = 4$) on QT interval. Data are given as mean ± standard error of the mean (*$P < 0.05$ relative to vehicle).

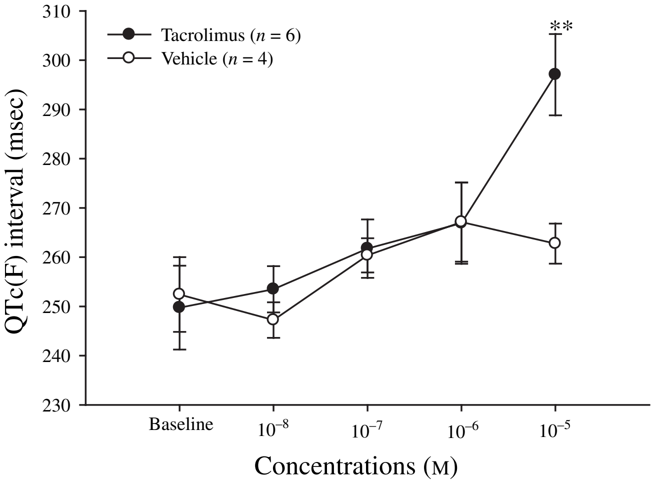

Fig. 3. Concentration–response curve of the effect of escalating doses of tacrolimus ($n = 6$) and vehicle ($n = 4$) on mean QTc interval. Data are given as mean ± standard error of the mean (**$P < 0.01$ relative to vehicle).

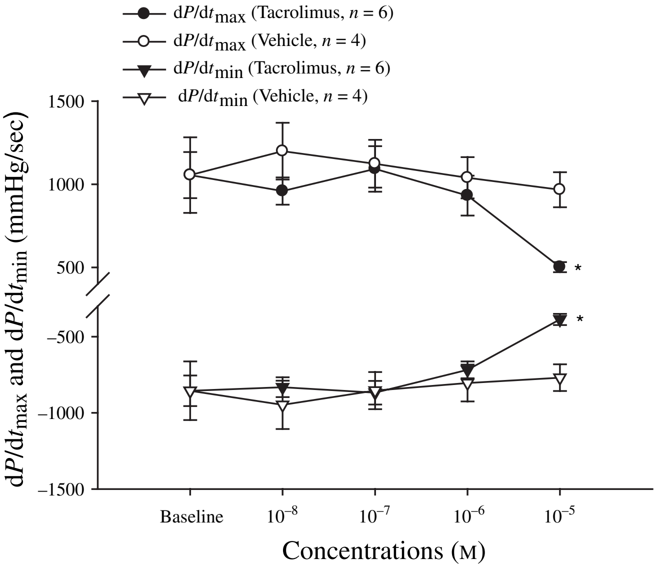

Fig. 4. Effects of escalating doses of tacrolimus ($n = 6$) and vehicle ($n = 4$) on mean $\mathrm{d}P/\mathrm{d}t_{\max}$ and mean $\mathrm{d}P/\mathrm{d}t_{\min}$. Data are given as mean ± standard error of the mean (*$P < 0.05$ relative to vehicle).

guinea pig heart is similar to that of a human or dog, except it lacks the transient outward (I_{TO}) channel (McDermott *et al.*, 2002) that is present in human or canine hearts. Furthermore, it is yet to be determined whether guinea pig ryanodine and SERCA2 channels possess the same FK-binding proteins present in other hearts. This may be of great importance as tacrolimus is thought to exert its physiological effects by binding to the FK-binding protein (FKBP 12.6) (Yin & Ochs, 2003). In the Langendorff preparation, all cardiac tissues were exposed to tacrolimus via the native capillary circulation. An advantage of this preparation over *in vivo* preparations is that a dose range of 10 000 (10^{-8} to 10^{-5} M) could be tested. This optimizes the detection of an effect that might only occur in patients with certain polymorphisms, since it uncovers the potential of tacrolimus to retard ventricular repolarization. This preparation also permits exceptionally accurate measurement intervals. Both inotropy and lusitropy could be measured directly from dP/dt_{max} and dP/dt_{min} (without assumptions implicit in more indirect methods) because the ventricles were contracting isovolumetrically, and at a nearly constant rate, with no effects because of heterometric autoregulation (preload) (Starling, 1918), Anrep effect (afterload) (Anrep, 1912), or chronotropic-inotropy (Bowditch effect) (Bowditch, 1871).

The absence of a change in either RR interval or QRS duration (10^{-8} to 10^{-5} M) suggests that tacrolimus has no direct effect on the rate of diastolic depolarization of the SA node (Wilders *et al.*, 1991), or the velocity of conduction through the ventricular syncytium. This would indicate no effects on the three channels responsible for slow diastolic depolarization of the SA node: the inward rectifying K^+ (I_{K1}) (Wilders *et al.*, 1991; Liu *et al.*, 1998), 'funny' (I_f) (Lipscombe, 2002), and L-type Ca^{2+} (I_{CaL}) (Vinogradova *et al.*, 2000) channels. Of course it is possible that tacrolimus has equal but opposite effects on ion channels, which might produce no net effect. Lengthening of both QT and QTc indicates delayed ventricular repolarization (Zareba & Moss, 2003), and may result from an effect of tacrolimus on any or all of the channels responsible for ventricular repolarization, such as the transient outward K^+ (IK_{TO}), rapidly activating K^+ (I_{Kr}), and delayed rectifier K^+ (I_{Ks}) channels. The absence of an effect of tacrolimus on either dP/dt_{max} or dP/dt_{min} probably indicates little or no direct effect on channels affecting calcium kinetics (I_{CaL}, ryanodine, inositol triphosphate, SERCA2) or binding of calcium to troponin-C.

We have observed in previous studies in dogs anesthetized with morphine–chloralose and exposed to escalating concentrations of tacrolimus, lengthening of both QT and QTc (pers. obs.). In intact dogs we observed an increase in heart rate and dP/dt_{max} and a decrease in dP/dt_{min}. These differences may be related to species differences, to an effect of the Langendorff preparation, to effects of autonomic innervation, or to metabolites of tacrolimus. In a second study in urethane-alpha chloralose-anesthetized guinea pigs, Minematsu *et al.* (1999) reported that i.v. tacrolimus 0.01 or 0.1 mg/kg lengthened QT and QTc intervals, but did not affect heart rate or PQ interval. The authors suggested that the effects of tacrolimus may have been because of altered calcium kinetics produced by an increased opening probability of ryanodine channels. This is consistent with the observation that tacrolimus binds to FKBP 12.6, which then allows calcium to leak from the sarcoplasmic reticulum to the cytosol (Bultynck *et al.*, 2000). Neither dP/dt_{max} nor dP/dt_{min} was measured in this study.

The data presented here are consistent with those reported by Minematsu *et al.* (1999), except for the lengthening of the PQ interval, which was not observed in the earlier study. A possible explanation is that it occurred only at a concentration of 10^{-5} M, which is well above the dose given to the anesthetized guinea pigs. Thus, it appears that the different effects of tacrolimus observed in Langendorff-perfused guinea pig hearts and morphine–chloralose-anesthetized dogs can be explained by species differences rather than by differences in the preparations.

Finally, it appears that tacrolimus, at clinically relevant doses, should have minimal torsadogenic potential; the caveat being that a patient possessing polymorphisms in ion channels important in ventricular repolarization might be susceptible to QTc elongation, potentially leading to TdP.

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