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RESEARCH ARTICLE

OCT2013, an ischaemia-activated antiarrhythmic prodrug, devoid of the systemic side effects of lidocaine

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Background and Purpose: Sudden cardiac death (SCD) caused by acute myocardial ischaemia and ventricular fibrillation (VF) is an unmet therapeutic need. Lidocaine suppresses ischaemia-induced VF, but its utility is limited by side effects and a narrow therapeutic index. Here, we characterise OCT2013, a putative ischaemia-activated prodrug of lidocaine.

Experimental Approach: The rat Langendorff-perfused isolated heart, anaesthetised rat and rat ventricular myocyte preparations were utilised in a series of blinded and randomised studies to investigate the antiarrhythmic effectiveness, adverse effects and mechanism of action of OCT2013, compared with lidocaine.

Key Results: In isolated hearts, OCT2013 and lidocaine prevented ischaemia-induced VF equi-effectively, but OCT2013 did not share lidocaine's adverse effects (PR widening, bradycardia and negative inotropy). In anaesthetised rats, i.v. OCT2013 and lidocaine suppressed VF and increased survival equi-effectively; OCT2013 had no effect on cardiac output even at 64 $mg{\cdot}kg^{-1}$ i.v., whereas lidocaine reduced it even at 1 $mg{\cdot}kg^{-1}$. In adult rat ventricular myocytes, OCT2013 had no effect on Ca^{2+} handling, whereas lidocaine impaired it. In paced isolated hearts, lidocaine caused rate-dependent conduction slowing and block, whereas OCT2013 was inactive. However, during regional ischaemia, OCT2013 and lidocaine equi-effectively hastened conduction block. Chromatography and MS analysis revealed that OCT2013, detectable in normoxic OCT2013-perfused hearts, became undetectable during global ischaemia, with lidocaine becoming detectable.

Conclusions and Implications: OCT2013 is inactive but is bio-reduced locally in ischaemic myocardium to lidocaine, acting as an ischaemia-activated and ischaemia-selective antiarrhythmic prodrug with a large therapeutic index, mimicking lidocaine's benefit without adversity.

Abbreviations: OCT2013, 2-(2,6-dimethylanilino)-*N*,*N*-diethyl-2-oxoethanamine oxide; VF, ventricular fibrillation.

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KEYWORDS
antiarrhythmic, cardiac, ischaemia, lidocaine, prodrug, ventricular fibrillation

1 | INTRODUCTION

Sudden cardiac death, due to ischaemia-induced lethal ventricular fibrillation (VF), is part of the syndrome of acute myocardial infarction and represents a substantial clinical burden (Huikuri et al., 2002; Myerburg & Junttila, 2012; Wong et al., 2019). There are no antiarrhythmic drugs currently available for out-of-hospital pretreatment, where the greatest impact on mortality would be obtained (Priori et al., 2015). This is partly because no such specific investigation (on out-of-hospital acute myocardial ischaemia-induced VF) has been undertaken. Among the agents previously tested, the patient cohort was acute myocardial infarction survivors but the out-of-hospital cause of sudden cardiac death was not identified in individual cases, with spontaneous VF associated with infarct scarring and/or hypertrophy, and VF cause by a new episode of acute coronary obstruction, both plausible causes. Given that this is the available human data set, one must consider the outcome of drug studies with circumspection. Some drugs lacked efficacy because they increased mortality (CAST Investigators, 1989; Waldo et al., 1996), while the remainder lacked efficacy because doses were kept low to avoid adverse drug reactions (Køber et al., 2000; The Danish Study Group on **Verapamil** in Myocardial Infarction, 1990; The Multicenter Ditiazem Postinfarction Group, 1988). In a smaller cohort of acute myocardial infarction survivors, those at higher risk of VF and sudden cardiac death, **amiodarone** and **mexiletine** are sometimes administered but their risk/benefit ratio is poor (IMPACT Research Group, 1984; Julian et al., 1997; Pandya et al., 2016; Rutledge et al., 1985). It has been proposed that a drug with selectivity of action for the arrhythmogenic ischaemic myocardium may address the unmet therapeutic need to suppress ischaemia-induced VF (Bain et al., 1997; Barrett et al., 1995; Barrett et al., 2000; Farkas et al., 1999; Walker & Guppy, 2003).

We set out to create an ischaemia-activated antiarrhythmic prodrug of **lidocaine**. Lidocaine is a cardiac **$Na_v1.5$** channel blocker (Bean et al., 1983). Its clinical use as an antiarrhythmic is restricted to the in-hospital setting via the i.v. route and it does not increase survival (Herlitz et al., 1997; Martí-Carvajal et al., 2015; Sadowski et al., 1999), in part due to dose-limiting adverse drug reactions in the CNS and on the heart (Aj et al., 2015; Pfeifer et al., 1976). Nevertheless, extensive nonclinical data show that the $Na_v1.5$ channel blocking properties of lidocaine can prevent ischaemia-induced re-entrant conduction and halt lethal arrhythmogenesis during acute myocardial ischaemia (Barrett et al., 1995; Bergey et al., 1982; Canyon & Dobson, 2004; Farkas & Curtis, 2002; Sarraf et al., 2003).

OCT2013 (IUPAC name: 2-(2,6-dimethylanilino)-*N*,*N*-diethyl-2-oxoethanamine oxide) is an aliphatic amine N-oxide that is converted to lidocaine via two-electron reduction (Figure S1) mediated enzymically and non-enzymically by processes inhibited by oxygen (Patterson, 1993). Our hypothesis is that because ischaemia renders myocardial tissue hypoxic (e.g. Marshall et al., 1974), OCT2013 will be converted to lidocaine locally in the reducing environment of the ischaemic myocardium. This may be sufficient to obtain an ischaemia-selective conduction block during ventricular tachyarrhythmias and suppression of VF, mimicking the beneficial effects of lidocaine but without lidocaine's myocardial and extracardiac adverse drug reactions. To test this, we used a multifaceted approach, employing *in vitro*, *ex vivo* and *in vivo* techniques. The findings identified OCT2013 as a first-in-class drug with novel effectiveness and safety.

What is already known

- Sudden cardiac death caused by ischaemia-induced ventricular fibrillation (VF) is an unmet therapeutic need.
- Lidocaine suppresses ischaemia-induced VF, but adverse effects and a narrow therapeutic window limit its utility.

What does this study add

- OCT2013 and lidocaine prevented ischaemia-induced VF equi-effectively, but OCT2013 had no adverse effects.
- OCT2013 was converted to lidocaine in the ischaemic myocardium leading to ischaemia-selective rate-dependent conduction block.

What is the clinical significance

- OCT2013 is a prototype novel ischaemia-activated ischaemia-selective antiarrhythmic prodrug devoid of adverse effects.
- This unique profile has the potential to obtain antiarrhythmic prophylaxis against sudden cardiac death.

2 | METHODS

2.1 | Experimental design

Experiments were performed in line with published guidelines, with randomisation to treatment and blinded investigation and analysis of data (Curtis et al., 2018, 2015). Blinding was achieved in several different ways. To make up drug solutions, stocks of different types were prepared by the person undertaking the experimental lab work (the 'operator'). Solutions were prepared in identical storage containers,

labelled, and a record of the labels written in a blinding book by the operator. A second person then selected a code name (e.g. a single letter) for each solution before they removed the original label of the storage vessel, replaced it with the code name and recorded it in the blinding book. The relabelled stock solutions were used by the operator to prepare solutions for experimentation, guided by a randomisation table (prepared separately) (Curtis et al., 2013). A test solution or other intervention was selected by reference to the randomisation table. Blinding was maintained until after data had been collected, collated and analysed, and any excluded preparations or animals had been replaced according to predetermined exclusion criteria (Hesketh et al., 2020). Investigational endpoints were all determined prior to the start of each study. Other experimental design details varied in line with the model used and variable investigated. Group size selection was based on published principles outlined for pharmacological investigation (Curtis et al., 2018, 2015), which recommend that estimates based on power analysis when effect size is uncertain be increased by 50% to mitigate against type 2 error. Thus, for example, HPLC studies, with their high level of precision, required $n = 6$ hearts per group whereas Langendorff-perfused isolated heart studies investigating Gaussian-distributed physiological variables (heart rate, ECG intervals) required $n = 9$ hearts per group, and studies investigating binomially distributed variables (ischaemia-induced VF) required a sample size of $n = 12$ (or $n = 20$ in order to preclude false negatives when weak effects were anticipated) (Andrag & Curtis, 2013). Details for protocol length, sample size and drug interventions are summarised in Figure S2.

2.2 | ARRIVE, ethical, legal and experimental requirements

All animal surgical procedures and perioperative management were carried out in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication, 8th Edition, 2011), under assurance number A5634-01, and the EU Directive 2010/63/EU or UK Home Office Guide on the Operation of the Animals (Scientific Procedures) Act 1986. Animal housing and husbandry were as previously described (Andrag & Curtis, 2013). Animal studies are reported in compliance with the ARRIVE guidelines (Percie du Sert et al., 2020) and with the recommendations made by the *British Journal of Pharmacology* (Lilley et al., 2020). Imperial College Ethical Review Committee or King's College London Ethical Review Committee, where appropriate, authorised the project licence.

2.3 | Techniques used in the study

2.3.1 | Rat Langendorff-perfused isolated heart technique

Male Wistar rats (290–500 g) were anaesthetised with a lethal dose of sodium pentobarbitone (170 $mg \cdot kg^{-1}$; i.p.) and heparinised with 160 $IU \cdot kg^{-1}$ sodium heparin to prevent blood clotting. Once a surgical level of anaesthesia was confirmed by removal of the pedal reflex, hearts were excised and arrested in ice-cold (4°C) Krebs' perfusion solution modified to contain (mM) NaCl 118.5, $NaHCO_3$ 25.0, $MgSO_4$ 1.2, NaH_2PO_4 1.2, $CaCl_2$ 1.4, KCl 3.0 and glucose 11.1 (perfusate salts purchased from VWR International, UK). Throughout, we refer to this modified Krebs' solution as *start solution*. All perfusion solutions were filtered before use (5 μm pore size) to remove particulate matter. Hearts were perfused via the ascending aorta with start solution warmed to 37°C and gassed with a combination of 95% O_2 and 5% CO_2 to achieve a pH of 7.4. The solution is nominally normoxic, meaning the pO_2 is in the region of 600 mmHg, allowing for normal heart function in the absence of Hb, according to Henry's law and as previously validated (Yamada et al., 1990). Perfusion pressure was approximately 80 mmHg, achieved by delivering solutions via a gravity-fed constant pressure system. Coronary flow ($ml \cdot min^{-1}$) was measured by weighing coronary effluent collected over timed intervals (1 ml = 1 g) and corrected for heart weight ($ml \cdot min^{-1} \cdot g^{-1}$).

In all Langendorff studies, the control groups were perfused with a test solution made from start solution modified to contain drug vehicle (0.1% ethanol).

To detect changes in heart rate and rhythm, a unipolar electrode was inserted into the apex of the heart and was connected to a PowerLab system (Powerlab 4/35 and Animal Bio Amp, ADInstruments, UK; sampling rate 4 kHz) and Labchart software (v.7, ADInstruments, UK). PR interval (ms), QT_{90} interval (QT interval at 90% repolarisation) (ms) and heart rate ($beats \cdot min^{-1}$) were recorded only when hearts were in sinus rhythm.

Induction of regional ischaemia by coronary artery ligation in vitro

In Langendorff experiments that involved regional ischaemia, a 4-0 silk suture (Ethicon) was sewn around the left anterior descending (LAD) coronary artery 1–2 mm below the left atrial appendage (accessed by lifting the left atrium), threaded through a polyethene tube, left loose and later tightened to achieve a region of ischaemia in the range of 35%–60% of the total ventricular weight in order to maximise the likelihood of VF that is dependent on ischaemic zone size (Ridley et al., 1992). Ischaemic zone size was quantitated at the end of the designated period of reperfusion using 1 $mg \cdot ml^{-1}$, Patent blue VF sodium salt in 0.9% NaCl solution, with re-occlusion trapping the dye in the ischaemic zone, and reverting to dye-free test solution to wash out dye from the non-ischaemic zone followed by dissection (Curtis & Hearse, 1989). In experiments where myocardial tissue samples were required for drug extraction and quantitation, contamination from the blue dye was avoided by quantifying ischaemic zone size from per cent reduction in coronary flow at +1 min after left anterior descending coronary artery ligation versus −1 min prior, a method previously validated in this preparation (Curtis & Hearse, 1989). ECG analysis and arrhythmia definitions followed the Lambeth Conventions II guidelines (Curtis et al., 2013).

Induction of global ischaemia via cessation of flow

In Langendorff experiments that involved global myocardial ischaemia, the perfusion inflow line was clamped to cause complete cessation of

coronary flow and hearts were immediately submerged in warmed start solution (37°C) to maintain a constant temperature for the duration of global ischaemia.

Use of an intraventricular balloon for assessment of contractile function

In Langendorff experiments that involved assessment of contractile function, an intraventricular balloon made from compliant and non-elastic material was inserted into the left ventricle by way of the mitral valve, accessed by incising the left atrium (Wilder et al., 2016). The intraventricular balloon was attached to a pressure transducer connected to a PowerLab system (PowerLab 4/35 and Bridge Amp, ADInstruments, UK; sampling rate 4 kHz) and Labchart software (v.7, ADInstruments, UK). The pressure transducer was calibrated each day of use using a sphygmomanometer. The intraventricular balloon was inflated slightly (~0.01 ml) with saline (0.9% NaCl,) until a pressure could just be detected, and the value (arbitrary) on the syringe noted as the 'zero volume', and this value was used as the reference point for later intraventricular balloon inflations (Wilder et al., 2016). The intraventricular balloon was then further inflated with saline until a developed pressure of >100 mmHg was achieved, while diastolic pressure remained <10 mmHg. The volume added to the 'zero volume' to achieve this was recorded and defined as the 'working volume' (Wilder et al., 2016). Diastolic pressure and developed pressure (systolic pressure–diastolic pressure) were recorded from the pressure trace on Labchart at predetermined timepoints according to the experimental protocol (see below). Contractile function was also assessed by construction of a Starling curve, by deflating the balloon to zero volume and then increasing the volume by 0.02 ml increments up to a maximum total volume of 0.14 ml or a diastolic pressure ≤ 20 mmHg (whichever reached first), allowing the resultant changes in developed and diastolic pressures to plateau for at least 5 consecutive beats following cessation of any transient arrhythmia that sometimes occurs with balloon inflation (Wilder et al., 2016). The gradient between pressure (developed and diastolic) and added volume was determined in each heart, and group means calculated.

Use of epicardial pacing to measure ventricular conduction time and conduction block

In Langendorff experiments that involved measurement of ventricular conduction times and conduction block, silver bipolar pacing wires were inserted into the left ventricular apex. The wires were attached to a DS3 Isolated Constant Current Stimulator (DS3, Digitimer) and a Powerlab system (PowerLab 4/35 and Bridge Amp, ADInstruments, UK; sampling rate 4 kHz) connected to Labchart software (v.7, ADInstruments, UK). The current (mA) used to stimulate a heart during an epicardial pacing protocol differed based on the investigational endpoint, but in each case was determined based on the pacing threshold of the given heart. The pacing threshold was the minimum current required to pace the heart at a frequency of 6.7 Hz (400 beats·min^{-1}) and was determined 2 min prior to commencement of an epicardial pacing protocol by incrementally increasing the current supplied to the heart, from 0 mA until the heart began to pace.

Once the pacing threshold value for an individual heart was established, this value was doubled to set the constant current used for the subsequent epicardial pacing protocols. An epicardial pacing protocol was then run to determine myocardial conduction time (ms) and the occurrence of conduction block. The pacing protocol consisted of 6 pacing rates run for sequential 15 s time periods (400, 500, 600, 700, 800 and 900 beats·min^{-1}). Langendorff-perfused isolated hearts were set up for coronary ligation, epicardial pacing wires were placed 2 mm rostral to the apex on the anterior left surface and an ECG electrode was placed 2 mm rostral to apex on the right surface. This ensured that the heart could be paced from within the ischaemic zone and that the ECG electrode was located within the uninvolved zone (UZ) when regional ischaemia was induced. Myocardial conduction time was defined as the delay between the pacing stimulus and the appearance of the subsequent paced ventricular complex (Cascio et al., 1987). Myocardial conduction block was defined as an instance when the pacing stimulus was not followed by a resultant paced ventricular complex (Cascio et al., 1987).

2.3.2 | Antiarrhythmic assessment *in vivo* in anaesthetised rats

When antiarrhythmic effects were evaluated *in vivo*, adult male Sprague Dawley rats, weighing 500–750 g, were anaesthetised using 5% isoflurane to allow for shaving of the chest, under-fore-limb areas, thighs and neck, and intubation via an endotracheal tube (blunted 14G intravenous cannula, Venflon). Intubated animals were attached to a Harvard Ventilator (Harvard Apparatus, Massachusetts, USA) and mechanically ventilated initially with 5% isoflurane at a rate of 90 breaths·min^{-1}, with a tidal volume of 3 ml, before the isoflurane concentration was reduced to a maintenance level of approximately 1.5% for the remainder of the experiment. The rat was repositioned and secured in the left lateral position, and a 3-lead ECG was attached to the animal subcutaneously at the right thigh, right under-fore-limb and left lower abdomen. Rats were positioned and secured in the left lateral position on a warmed surface to maintain body temperature at 37°C. The ECG was connected to a Powerlab system (PowerLab 4/35 and Bridge Amp, ADInstruments, UK; sampling rate 4 kHz) using to Labchart software v.7 (ADInstruments, UK). A 22G intravenous cannula (Venflon) was inserted into the right internal jugular vein for drug administration via a Standard Infusion Only Pump 11 Elite infusion pump (Harvard Apparatus, Massachusetts, USA). Lidocaine and OCT2013 solutions were prepared in Becton Dickinson 10 ml syringes, diluted to the desired concentration in medical grade saline (0.9% NaCl). A thoracotomy via the fourth intercostal space was carried out, ensuring an optimal view of the left atrium, left atrial appendage and left ventricular apex. Ligation of the left anterior descending coronary artery coronary artery was performed using a 6-0 prolene needled suture (Ethicon) 1–2 mm below the corner of the left atrium to achieve a large left ventricular ischaemic region, capable of inducing a high incidence of VF within 5 min of ischaemia onset (Curtis, 1998). Ligation was confirmed through observational changes in left

ventricular contractility and epicardial pallor. The occurrence of VF was determined during 30 min of left anterior descending coronary artery ligation. If VF occurred, up to three attempts at defibrillation were tried using a small custom-built rat defibrillator (Ordodi et al., 2006). If a rhythm incompatible with life, either VF or asystole, was present 2 min after a third shock, the rat was deemed to have not survived. Heart rate and PR and QT_{90} intervals were recorded throughout the protocol at 5 min intervals. Animals alive at the end of the protocol were killed by cervical dislocation.

2.3.3 | Haemodynamic adverse drug reaction assessment *in vivo* in anaesthetised rats

When acute adverse effects of drugs on haemodynamic function were assessed *in vivo*, adult male Sprague Dawley rats, weighing 500–750 g, were anaesthetised, using 5% isoflurane, and the ECG was recorded as in antiarrhythmic *in vivo* studies (above). A left parasternal incision was made, and limited thoracotomy was performed in the fifth intercostal space. A 1.9F Scisense pressure–volume (PV) catheter was then inserted into the heart at the left ventricular apex. Data were collected via the ADVantange acquisition system (Scisense Inc., Ontario, Canada) connected to Labchart software (v.7, ADInstruments, UK). Four ECG cycles were averaged, and 10 pressure–volume loop cycles were averaged and analysed using the pressure–volume loop Analysis Module on Labchart (v.7, ADInstruments, UK). A jugular i.v. line was set up for cumulative dose drug administration (above) for detection of the threshold dose evoking adverse drug reactions. Animals alive at the end of the protocol were killed by cervical dislocation.

2.3.4 | Sarcomere length and intracellular Ca^{2+} in isolated ventricular myocytes

When adverse effects of drugs on contractile function were evaluated in isolated left ventricular myocytes, cells were isolated from male Sprague Dawley rats as previously described (Sato et al., 2005). Myocytes were loaded with the ratiometric Ca^{2+}-sensitive fluorescent dye Fura-2 AM (5 μM) (Invitrogen, Life Technologies Ltd) for 15 min at room temperature. Thereafter, the cell suspension was centrifuged at 400 rpm and myocytes were resuspended in a diluted DMEM containing 1 mM Ca^{2+} and were further incubated at room temperature for at least 30 min to allow for de-esterification of the Ca^{2+} indicator.

Effects of drugs were examined at 37°C. Myocytes were visualised on a TE300 Nikon 40× oil-immersed objective microscope coupled to an IonOptix system (IonOptix Ltd). Cells were initially field-stimulated (20 V) at 1 Hz for a minimum of 2 min to achieve steady-state Ca^{2+} handling in Tyrode's solution containing NaCl 137 mM, KCl 5.4 mM, glucose 10 mM, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulphonic acid 10 mM, $MgCl_2$ 1 mM and $CaCl_2$ 1 mM, pH adjusted to 7.4 with 1 M of NaOH. Only cells with clear striations and non-spontaneous contractions were used. Sarcomere length and intracellular Ca^{2+} were measured simultaneously using the IonOptix MyoCam-S and μStep light source, respectively. A myocyte was positioned parallel within the field of view ensuring that the striation pattern was vertically aligned. The iris of the cell framing adaptor was adjusted around the perimeter of the cell to optimise the selected cell fluorescence. A region of interest was selected to measure a minimum of 10 sarcomeres, maximising measurement accuracy.

Background autofluorescence (using an average from unloaded cells) was subtracted. The ratio was derived conventionally from the emitted fluorescence following excitation at 360 nm divided by that emitted at 380 nm. We used a ratiometric Ca^{2+} indicator to reduce the impact of indicator extrusion and bleaching during experiments.

Transients were analysed using the IonWizard software (v.6.6.10.125 [×64]). For each experimental condition, the amplitudes of 10 fluorescence transients and 10 corresponding contraction profiles were averaged. In addition, time to 90% restitution of systolic Ca^{2+} was used as a measure of diastolic cellular Ca^{2+} uptake (Sankaranarayanan et al., 2017).

2.3.5 | Conduction assessment with neonatal rat ventricular myocyte microelectrode arrays

When microelectrode arrays were used to evaluate the effects of drugs on conduction, neonatal rat ventricular myocytes at age P0–P2 were isolated using a GentleMACs system (Miltenyi Biotec) using the protocol supplied with the enzyme kit. After a pre-plating step to purify the myocytes, 2×10^5 cells were seeded onto microelectrode arrays in a 30 μl drop of medium over the electrodes in the centre of the array. After allowing 30 min for the cells to attach, the medium was topped up. Recordings were taken 3–4 days after plating cells. The microelectrode array array (MultiChannel Systems, GmbH) consisted of an 8 × 8 array of unipolar electrodes (100 μm diameter, 700 μm inter-electrode distance). Any electrode can be used to either record or stimulate. Cells were paced from the top line of electrodes at 1 Hz at 120% of threshold. A custom in-house MatLab code was used to assign local activation times, compute average inter-electrode conduction velocities and draw activation maps (Chowdhury et al., 2018).

2.3.6 | UHPLC–MS/MS measurement of OCT2013 and lidocaine in perfused myocardium

For detection of OCT2013 and lidocaine, the UHPLC–MS/MS used was a Waters Xevo TQS-micro triple quadrupole mass spectrometer with an electrospray ionisation source (operated in positive mode), coupled to an Acquity H Class LC system. Data were acquired using MassLynx V4.1 software. Separations were conducted on a Waters ACQUITY UHPLC™ BEH C_{18} column (2.1 × 50 mm, 1.7 μm), maintained at 40°C. Binary gradient profiles were developed using water (Optima™ LC–MS grade, Fisher) with 0.1% formic acid (LC–MS grade, Fisher) (A) and methanol (Optima™ LC–MS grade, Fisher) with

0.1% formic acid (B) at a flow rate of 200 μl·min^{-1}. Separations were conducted under the following chromatographic: 100% solvent A for 0.5 min, decreased to 10% in 4.5 min and subsequently to 0% in 0.1 min, maintained for 1.9 min before being increased over 0.2 min to 100%. Column equilibration time was 3 min and the total run time of 10 min. The sample injection volume was 20 μl. Mass spectrometer parameters were as follows: - capillary voltage 3.1 kV, desolvation temperature 600°C, cone gas flow 1 L·h^{-1}, desolvation gas flow 1000 L·h^{-1} and dwell time 46 ms per analyte. Analyte quantitation was performed using the multiple reaction monitoring method shown in Table S1.

Stock solutions (1 mg·ml^{-1}) of lidocaine, OCT2013 and internal standard (IS) (lidocaine-d10 or (2-[bis(1,1,2,2,2-pentadeuterioethyl) amino]-*N*-(2,6-dimethylphenyl)acetamide)) used for construction of calibration curves were prepared in methanol and stored at −20°C. These stock solutions were used to generate a calibration graph for OCT2013 and lidocaine over the concentration range 0.1–500 ng·ml^{-1}. A standard calibration curve was constructed for both lidocaine and OCT2013 on the same day that test samples were analysed. The response generated was linear throughout the concentration range used for both analytes (0.1–500 ng·ml^{-1}), and the coefficient of determination (r^2) was 0.999166 for OCT2013 (Figure S3A) and 0.999052 for lidocaine (Figure S3B).

Following completion of each heart perfusion protocol (details below), samples of frozen myocardial ventricular tissue, weighing approximately 300 mg, were dissected and stored within CryoTube Vials (Thermo Scientific, USA) in liquid nitrogen until subsequently prepared for UHPLC–MS/MS analysis. Frozen tissue samples were cut to obtain portions of 20–30 mg in weight. Tissue samples were then transferred into individual round bottom Eppendorf tubes (2 ml) containing 920 μl methanol, 70 μl H_2O_2 (30 g/100 ml, 9.8 M) and 10 μl IS (100 ng·ml^{-1} in methanol) and weighed. These samples were moved into cold plates and homogenised using a TissueLyser II homogeniser for 30 s at 30 Hz before being centrifuged for 5 min at 14,000 rpm at 4°C. Each sample (50 μl) was then transferred into individual UHPLC injection vials containing 250 μl of 0.2% formic acid in water, subsequently vortexed and injected into the UHPLC–MS/MS system (20 μl injection volume).

2.3.7 | Exclusion criteria

In Langendorff experiments, hearts were excluded from analysis if, during perfusion with start solution, coronary flow was <7 or >20 ml·min^{-1}·g^{-1}, or heart rate was <200 beats·min^{-1}, and in the coronary ligation cohort, hearts were excluded if the ischaemic zone size was outside the range of 35%–60% of the total ventricular weight, all as per previous studies (Andrag & Curtis, 2013; Clements-Jewery et al., 2006; Wilder et al., 2016). Excluded hearts were replaced, while maintaining blinding, to maintain equal group sizes, unless their inclusion would not affect the outcome of the study (i.e. in a heart experiencing VF despite a small ischaemic zone of <35%, Curtis & Hearse, 1989), thus mitigating against needless animal use.

2.4 | Protocols for Langendorff experiments

All experimental protocols are summarised in Figure S2.

2.4.1 | Protocol for antiarrhythmic assessment of drugs administered from before the start of ischaemia

Hearts were initially perfused with start solution for 10 min to record baseline values of measured variables and apply exclusion criteria. Variables were then recorded at 5 min intervals for the remainder of the protocol. Test solution, 15 μM lidocaine, 15 μM OCT2013 or control solution ($n = 12$ per group), was introduced from 10 min prior to the onset of 30 min of regional ischaemia, with ischaemic zone size later quantified by the dye method. The occurrence of ventricular arrhythmias was recorded throughout the experiment (Curtis et al., 2013).

2.4.2 | Protocol for antiarrhythmic assessment of drugs administered after the start of ischaemia

Hearts were initially perfused with start solution for 10 min to record baseline values. Regional ischaemia was induced and 10 min later the perfusate was switched to one of five test solutions, 15 μM lidocaine, 15 μM OCT2013, 60 μM lidocaine, 60 μM OCT2013 or control solution ($n = 20$ per group), for a further 20 min before ischaemic zone size was quantified by the flow reduction method.

2.4.3 | Protocol for contractile function assessment (*in vitro*)

With the intraventricular balloon inflated to working volume, hearts were perfused with start solution for 10 min to establish baseline values for diastolic and developed pressures. A Starling curve was constructed. Perfusate was then switched to 15 μM lidocaine, 15 μM OCT2013 or vehicle ($n = 12$ per group) and at 10 min a second Starling curve was constructed. Regional ischaemia was then induced for 30 min followed by reperfusion for 60 min whence a third and final Starling curve was constructed. The ischaemic zone size was then determined by the dye method.

2.4.4 | Protocol for conduction time and conduction block assessment during regional ischaemia

Values of heart rate and coronary flow were recorded 5 min before the start of left ventricular epicardial pacing, and any hearts meeting exclusion criteria were replaced. Conduction time and conduction

block were recorded before and after switch to perfusion with test solution (15 μM lidocaine, 15 μM OCT2013 or vehicle, $n = 9$ per group) and at 5, 10, 20, 30 and 40 min into myocardial ischaemia. Following cessation of the protocol ischaemic zone size was quantified by the blue dye method.

2.5 | Protocols for *in vivo* experiments in anaesthetised rats

All experimental protocols are summarised in Figure S2.

2.5.1 | Protocol for assessment of antiarrhythmic effectiveness *in vivo*

When ischaemia-induced arrhythmias were examined in rats *in vivo*, a bolus of 2 $mg \cdot kg^{-1}$ lidocaine, 2 $mg \cdot kg^{-1}$ OCT2013 or the equivalent volume of saline ($n = 6$ per group) was administered to the animal, immediately followed by the commencement of a 0.5 $mg \cdot kg^{-1} \cdot min^{-1}$ continuous infusion, which remained constant for the remainder of the experiment. Coronary ligation was undertaken 5 min later for a period of 30 min.

2.5.2 | Protocol for assessment of haemodynamic adverse drug reactions *in vivo*

When acute haemodynamic adverse drug reactions were examined *in vivo*, rats were administered sequential i.v. boluses of lidocaine or OCT2013 ($n = 5$ per group), 1 $mg \cdot kg^{-1}$, then a further 1, 2, 4, 8, 16 and 32 $mg \cdot kg^{-1}$, giving total drug doses of 1, 2, 4, 8, 16, 32 and 64 $mg \cdot kg^{-1}$, respectively. The interval between boluses was 2 min. PR, QT_{90} interval and heart rate were recorded just prior to each bolus (i.e. at the end of each 2 min interval).

2.6 | Protocol for isolated myocyte intracellular Ca^{2+} and sarcomere length measurement

The experimental protocol is summarised in Figure S2. Following continuous superfusion with start solution, Tyrode's buffer (2 min at 1 Hz), 10 sarcomere length and fura-2 transients were recorded. Thereafter, remaining on the same myocyte, start solution was switched to test solution, Tyrode's containing 15 μM lidocaine ($n = 35$), 15 μM OCT2013 ($n = 20$) or 60 μM OCT2013 ($n = 20$). The test solution was continuously superfused for 2 min while stimulating the cell at 1 Hz. Following the incubation period, another 10 sarcomere lengths and fura-2 ratio transients were acquired. The experimental solutions were sequentially reversed; thus, on the next selected cell, the experiment started in the solution containing the drug in order to avoid bias relating to possible cell deterioration over time (Sikkel et al., 2013).

2.7 | Protocol for assessment of conduction velocity using microelectrode arrays

The experimental protocol is summarised in Figure S2. Recordings (10 s) were taken in HBSS during perfusion with start solution and after administration of 1, 5, 10, 75 and 100 μM of lidocaine or OCT2013 ($n = 10$ per group).

2.8 | Protocol for measurement of ischaemia-activated conversion of OCT2013 to lidocaine

The experimental protocol is summarised in Figure S2. Male Wistar rat (315–394 g) hearts were Langendorff perfused with start solution (as described above) for 10 min, during which baseline values of coronary flow were recorded. Perfusate was switched to 15 μM OCT2013 ($n = 12$) for 10 min prior to either 30 min of global ischaemia ($n = 6$) or 30 min of time-matched normoxia (normal perfusion) ($n = 6$). Hearts were submerged in warmed start solution for the duration of global ischaemia or time-matched normoxia (37°C). At the end of the protocol, the external surface of the hearts was rapidly washed with warmed start solution and whole hearts were snap frozen with cryogenic tongs previously cooled in liquid nitrogen (approximately −195°C).

2.9 | Data and statistical analysis

The data and statistical analysis comply with the recommendations of the *British Journal of Pharmacology* on experimental design and analysis in pharmacology (Curtis et al., 2018, 2015). Gaussian distributed variables were subjected to *t*-tests (two group comparisons), one-way ANOVA (three or more groups) or two-way ANOVA (changes to variables over time, across three or more groups) followed by either Dunnett's (comparison of groups to one control group) or Sidak's (comparison of two groups with multiple points) post hoc tests (if *F* was significant and data Gaussian). Binomially distributed variables (e.g., arrhythmia incidence) were compared using Fisher's exact test. Survival or 'trend over time' data (e.g., survival or time to VF in anaesthetised rats) were analysed using the log-rank (Mantel–Cox) test. Line gradients (e.g., Starling curve analysis) were compared between groups using linear regression analysis. Statistical significance was set at $P < 0.05$ for all analysis. All statistical analyses were performed on GraphPad Prism software (v.8).

2.10 | Materials

OCT2013 [2-(2,6-dimethylanilino)-*N*,*N*-diethyl-2-oxoethanamine oxide hydrochloride] was synthesised by BioTherics Limited from commercially available lidocaine (Sigma Adrich, UK) according to a method generally used to prepare aliphatic tertiary amine N-oxides (Craig & Purushothaman, 1970) and shown to be authentic and pure by NMR and mass spectroscopy.

Perfusion solutions containing lidocaine hydrochloride (Sigma Aldrich, UK) or OCT2013 were made by diluting concentrated stock solutions composed of drug in a vehicle of 1:4 ethanol (VWR International, UK) and water (PURELAB ELGA Process Water, UK). Patent Blue VF Sodium Salt and Lidocaine-(diethyl-d_{10}) were purchased from Sigma-Aldrich, UK. Optima LC/MS Grade Formic Acid, Optima LC/MS Grade Water and Optima LC/MS Grade Methanol were obtained from Fisher Scientific, UK. $CaCl_2 2H_2O$, Glucose D(+), Methanol, NaCl, $NaHCO_3$, $MgSO_4 7H_20$, NaH_2PO_4 and KCl were purchased from VWR International, UK.

2.11 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in the IUPHAR/BPS Guide to PHARMACOLOGY http://www.guidetopharmacology.org and are permanently archived in the Concise Guide to PHARMACOLOGY 2021/22 (Alexander et al., 2021).

3 | RESULTS

3.1 | OCT2013 prevents ischaemia-induced VF without adverse drug reactions

Pretreatment and continuous perfusion with OCT2013 (15 μM) or lidocaine (15 μM) reduced the incidence of VF (Figure 1a) during 30 min of regional ischaemia in the rat Langendorff preparation. A higher concentration of OCT2013 (60 μM) and a range of concentrations of lidocaine (15 and 60 μM) had similar effects when administered 'post-ligation', from the 10th minute after the onset of ischaemia only (Figure 1e). OCT2013 at 15 and 60 μM caused no adverse drug reactions and was devoid of any pharmacological activity in non-ischaemic tissue (Figure 1b–d + f–h). In contrast, lidocaine evoked concentration-dependent adverse drug reactions including bradycardia (Figure 1b + f), PR interval prolongation (Figure 1c + g) and QT_{90} interval prolongation (Figure 1d). Likewise, in separate studies in the rat Langendorff preparation, 15 μM OCT2013 was devoid of adverse actions on ventricular contractile function, whereas lidocaine had negative inotropic activity (Figure 2b). Lidocaine and OCT2013 provided modest protection against diastolic dysfunction during reperfusion (Figure 2f).

Studies *in vivo* in anaesthetised rats recapitulated the key findings. OCT2013 and lidocaine significantly and similarly reduced the incidence of VF (Figure 3a + c) and increased animal survival (Figure 3b) during 30 min regional ischaemia.

3.2 | OCT2013 has no adversity in cumulative dose acute toxicity assessment

In anaesthetised rats, cumulative doses of lidocaine (2–32 mg·kg^{-1}) increased PR interval (Figure 4a), caused bradycardia (Figure 4b) and reduced cardiac output (Figure 4d) and stroke volume (Figure 4e). At 64 mg·kg^{-1}, lidocaine caused death in all animals from AV block and cardiovascular collapse. In contrast, OCT2013 caused no such adverse drug reactions up to 32 mg·kg^{-1} (Figure 4a–e). At 64 mg·kg^{-1} (>30-fold its effective antiarrhythmic activity in anaesthetised rats), OCT2013 caused a modest PR widening (Figure 4a) without any other adversity (Figure 4b–e).

3.3 | Mechanism of action is ischaemia-selective ventricular conduction block

It was hypothesised that ischaemia would transform OCT2013 from an inert substance to a mimic of lidocaine, with effects typical of those of ischaemia-selective $Na_v1.5$ channel block, and this hypothesis was tested in multiple different mechanistic studies.

First, it was established that properties typical of $Na_v1.5$ channel block exhibited by lidocaine were not shared by OCT2013 during normoxia. In neonatal rat cultured ventricular myocyte monolayers, lidocaine reduced conduction velocity at 50 μM whereas OCT2013 had no effect even at 100 μM (Figure 5a + b).

Next, we tested the hypothesis that in whole hearts (Langendorff perfusion), OCT2013 would mimic the typical rate-dependent effects of lidocaine on conduction (Davis et al., 1986; Matsubara et al., 1987) but, unlike lidocaine, only during regional ischaemia. During normoxia, 15 μM OCT2013 had no effect on conduction time, whereas 15 μM lidocaine caused a conduction slowing at pacing rates of 600–900 beats·min^{-1} (Figure 5c). Conduction time was progressively slowed 5 to 10 min after the onset of regional ischaemia in controls and this effect was enhanced by increasing pacing rates (400–900 beats·min^{-1}) (Figure 5c). OCT2013 and lidocaine exacerbated this slowing at every pacing rate and to a similar degree (Figure 5c). Ischaemia altered the rate dependence such that maximum conduction slowing was obtained by increasing rate from 400 to only 500 beats·min^{-1} (Figure 5c). In the absence of drug or ischaemia, there was no conduction block in any heart at pacing rates up to 900 beats·min^{-1} (Figure 5d). In the absence of ischaemia, there was only one episode of conduction block in one heart perfused with OCT2013, and only when paced at 900 beats·min^{-1}, whereas lidocaine readily caused rate-dependent conduction block, with almost half the group of hearts experiencing some block when paced at 900 beats·min^{-1} (Figure 5d). During ischaemia, rate-dependent conduction block occurred in control hearts. This block was markedly exacerbated by lidocaine within 5 min of ischaemia onset, an effect mimicked precisely by OCT2013 (Figure 5d). By 10 min of ischaemia, when arrhythmia severity peaks in this preparation, lidocaine and OCT2013 caused conduction block in most hearts even at low pacing rates (400–600 beats·min^{-1}), whereas block was less frequent in controls (Figure 5d). However, by 20 min after ischaemia onset, when arrhythmia severity begins to wane, rate-dependent conduction block in controls had become so marked that all three groups resembled one another (Figure 5d). It was necessary to test if OCT2013 altered calcium regulation within ventricular myocytes because of the known

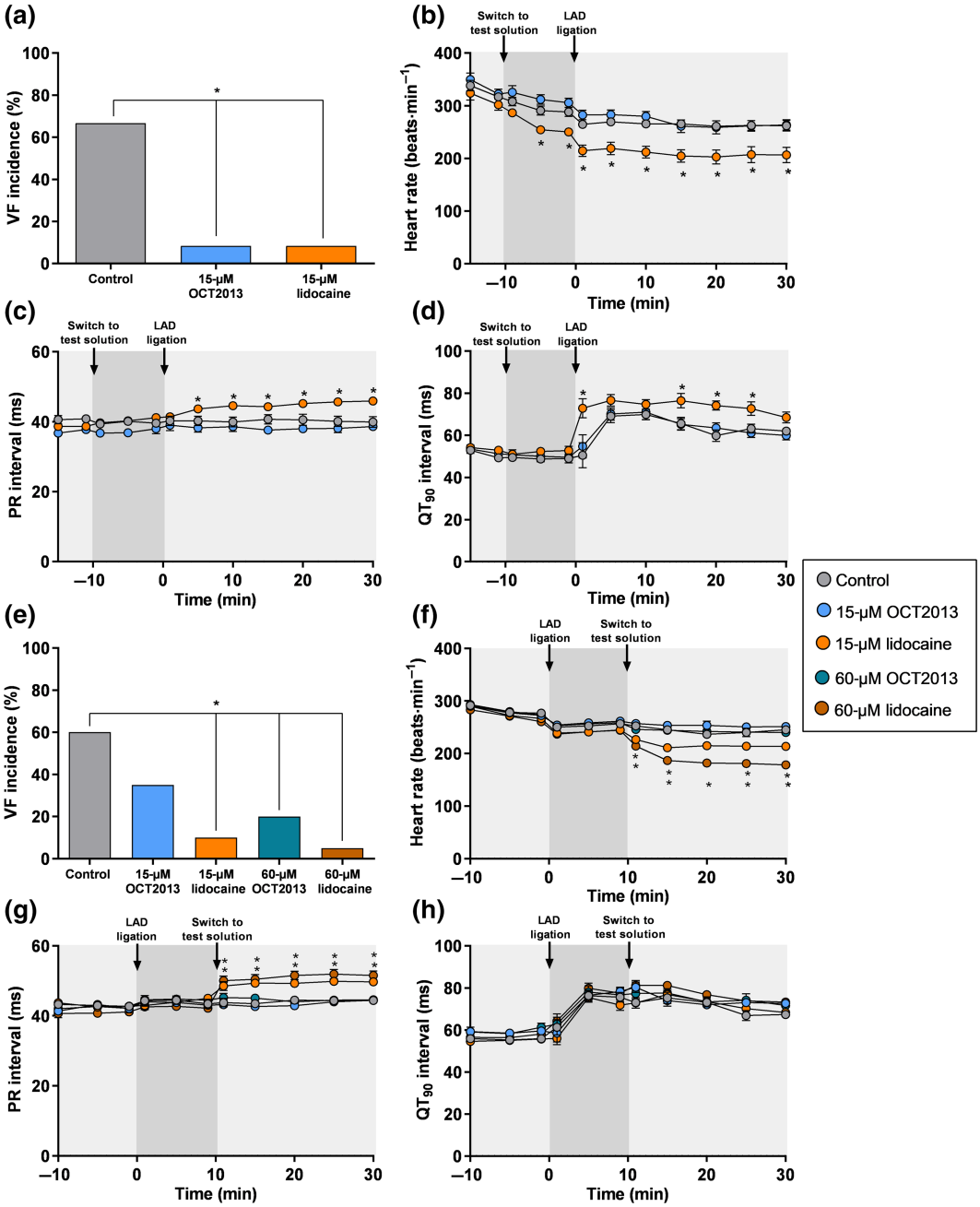


FIGURE 1 Antiarrhythmic and ECG effects of OCT2013 versus lidocaine in rat Langendorff hearts. (a,e) Incidence of ventricular fibrillation (VF), (b,f) heart rate, (c,g) PR and (d,h) QT_{90} intervals during 30 min of regional ischaemia in hearts perfused with Krebs, OCT2013 or lidocaine. (a–d) Perfusion with test solution started 10 min before coronary ligation (onset of ischaemia), $n = 12$ hearts per group were required to compare drugs (15 μM each) versus control group. (e–h) Test solution perfusion commenced at 10 min after ligation. A higher concentration of each drug (60 μM) and increased group sizes to $n = 20$ were implemented in the expectation of weaker drug effects with post-ligation administration. Binomially distributed variables (arrhythmia incidence) were compared using Fisher's exact test. Gaussian distributed variables (mean ± SEM) were subjected to two-way ANOVA followed by Dunnett's post hoc tests (following demonstration that F was significant and data Gaussian). *$P < 0.05$ versus Krebs' control. Abbreviation, LAD, left anterior descending coronary artery

effects of some class 1 antiarrhythmics on cellular sodium and, in turn, calcium homeostasis. Lidocaine at 15 μM caused a reduction in adult myocyte contractility, calcium transient amplitude and diastolic calcium uptake, whereas OCT2013 at 15 or 60 μM did not affect these variables (Figure 6a–i).

3.4 | OCT2013 is an ischaemia-activated prodrug of lidocaine

Because we had shown that ischaemia converted OCT2013 into *a mimic* of lidocaine, we next sought to determine whether ischaemia

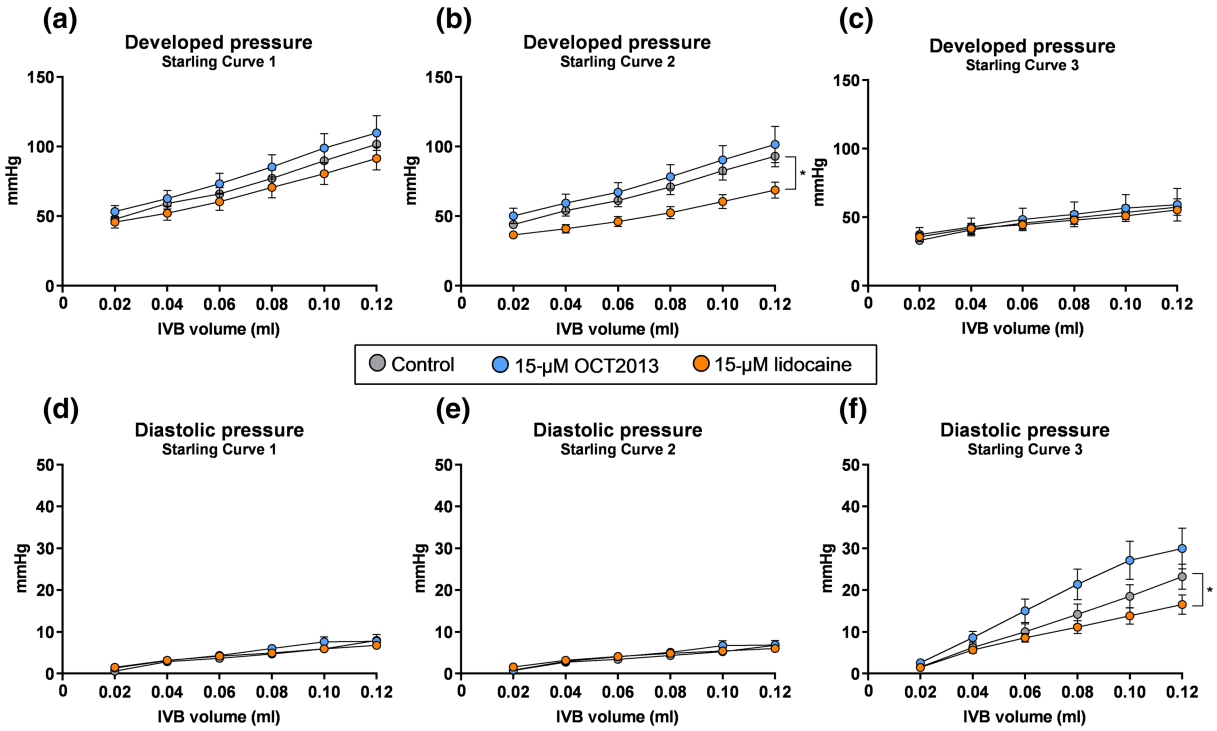


FIGURE 2 Inotropic and lusitropic effects of OCT2013 in the rat Langendorff preparation assessed by intraventricular balloon (IVB) Starling curve analysis. (a–c) Left ventricular developed pressure and (d–f) diastolic pressure at increasing IVB volumes in hearts perfused with Krebs prior to switch to test solution (Starling Curve 1), 10 min after switch to test solution composed of Krebs, 15 μM OCT2013 or 15 μM lidocaine (Starling Curve 2), and at the 60th minute of reperfusion with the same test solution after 30 min regional ischaemia (Starling Curve 3). $n = 12$ per group; mean ± SEM; *$P < 0.05$ versus control (linear regression analysis)

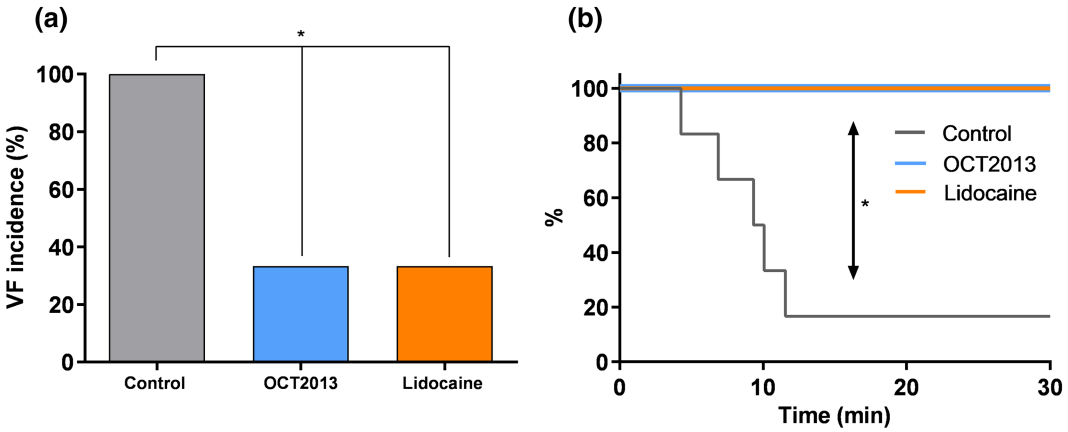


FIGURE 3 Antiarrhythmic effectiveness and survival benefit of OCT2013 *in vivo*. (a) Incidence of ventricular fibrillation (VF) and (b) overall survival in anaesthetised rats administered 2 mg·kg^{-1} (+0.5 mg·kg^{-1}·min^{-1}) OCT2013, lidocaine or saline (control). $n = 6$ per group; binomially distributed variables (arrhythmia incidence) were compared using Fisher's exact test. Survival was analysed using the log-rank (Mantel–Cox) test. *$P < 0.05$ versus control

converted OCT2013 to lidocaine, itself, in Langendorff-perfused rat hearts, using UHPLC–MS/MS analysis. Hearts subjected to global ischaemia showed almost complete loss of OCT2013 trapped in the coronary vasculature during cessation of perfusion compared with the amount of OCT2013 found in the normoxic perfused hearts, and the loss of OCT2013 was accompanied by the appearance of lidocaine (Figure 7) albeit the apparent stoichiometry of the conversion was not 1:1. In contrast, minimal formation of lidocaine (<6% of that in ischaemic hearts) was present in hearts snap frozen during normoxic perfusion with OCT2013, indicating the intrinsic resistance of OCT2013 to bioreduction under normoxic conditions (Figure 7).

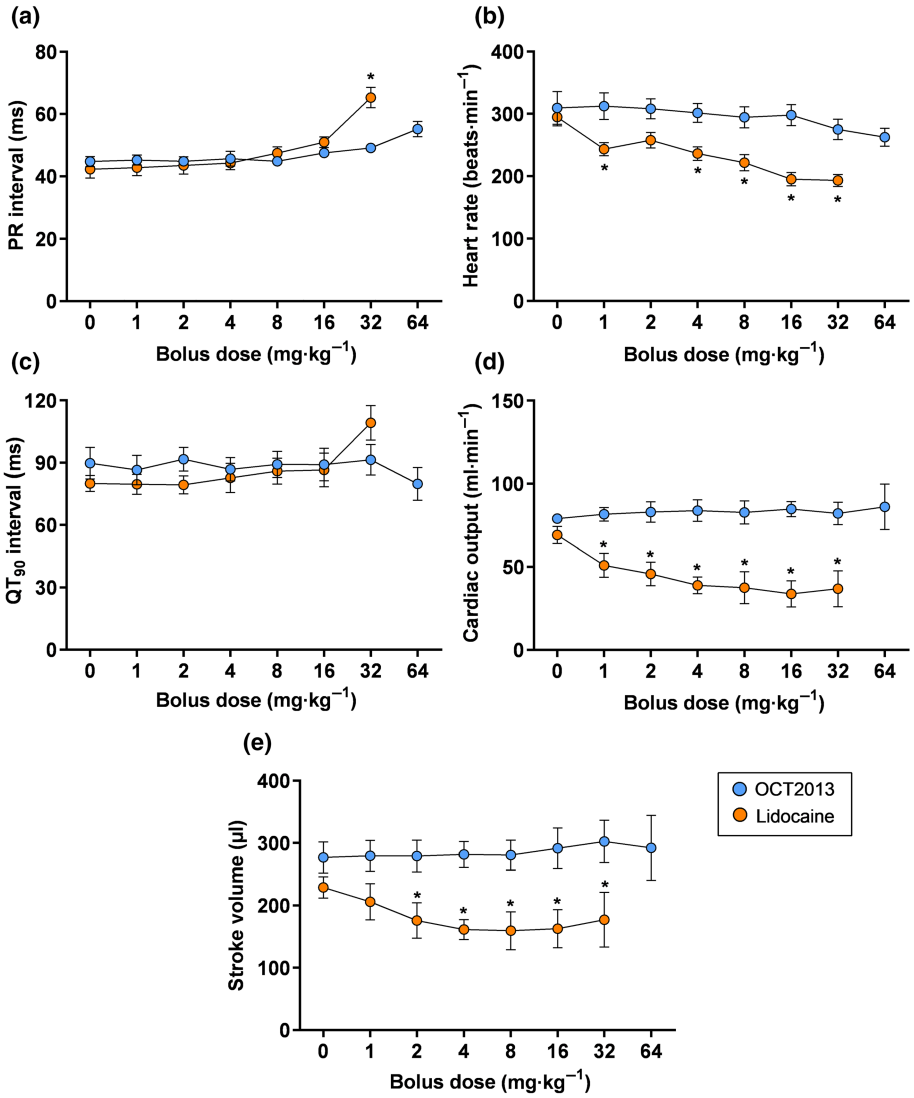


FIGURE 4 Cumulative-dose toxicity of OCT2013 and lidocaine in anaesthetised rats. (a) PR intervals, (b) heart rate, (c) QT_{90} intervals, (d) cardiac output and (e) stroke volume measured approximately 1 min 55 s after sequential escalating drug boluses (1–64 $mg \cdot kg^{-1}$) given at 2-min intervals. No animal survived the 64 $mg \cdot kg^{-1}$ lidocaine dose long enough to permit data recording. $n = 5$ per group; mean ± SEM; variables were subjected to two-way ANOVA followed by Sidak's post hoc tests (if F values were significant and data Gaussian). $^*P < 0.05$

4 | DISCUSSION AND CONCLUSIONS

4.1 | Sudden cardiac death, an unmet therapeutic need

Despite clear therapeutic need (Huikuri et al., 2002; Myerburg & Junttila, 2012; Wong et al., 2019), there have been no novel pharmacological interventions introduced for the treatment of lethal ventricular arrhythmias associated with acute myocardial infarction in decades. Current guidelines support the use of β-adrenoceptor antagonists to reduce the risk of sudden cardiac death in patients diagnosed with ischaemia (Priori et al., 2015), but their impact on sudden cardiac death has been limited (Kezerashvili et al., 2012). Amiodarone (class 3) is the most widely used second-line pharmacological therapy for ventricular arrhythmias, but evidence for mortality reduction is weak (Priori et al., 2015). In addition, amiodarone and mexiletine (class 1b) have narrow, if barely discernible, therapeutic windows owing to serious adverse drug reactions (IMPACT Research Group, 1984; Julian et al., 1997; Pandya et al., 2016; Rutledge et al., 1985). A very safe antiarrhythmic drug would be required to reach lower risk groups (out-of-hospital) that represent a larger numerical cohort of sudden cardiac death victims (Huikuri et al., 2002).

4.2 | The concept of an ischaemia-activated antiarrhythmic prodrug of lidocaine

We sought to address the problem by creating an ischaemia-activated prodrug of an antiarrhythmic. We selected lidocaine for two reasons. Firstly, as a tertiary amine, lidocaine has scope for chemical modification to the corresponding N-oxide, to provide an inert drug that may be converted to active drug in ischaemic tissue (Patterson, 1993), something not possible for some other antiarrhythmics, including mexiletine. Second, although lidocaine itself has a range of adverse drug reactions, it is a highly effective inhibitor of ischaemia-induced VF according to numerous animal model studies (Bergey et al., 1982; Canyon & Dobson, 2004; Farkas & Curtis, 2002; Sarraf et al., 2003), owing to its high affinity for the $Na_v1.5$ Na^+ channel in its activated/inactivated state (Matsubara et al., 1987) and consequent inherent tachycardia and ischaemia selectivity (Davis et al., 1986;

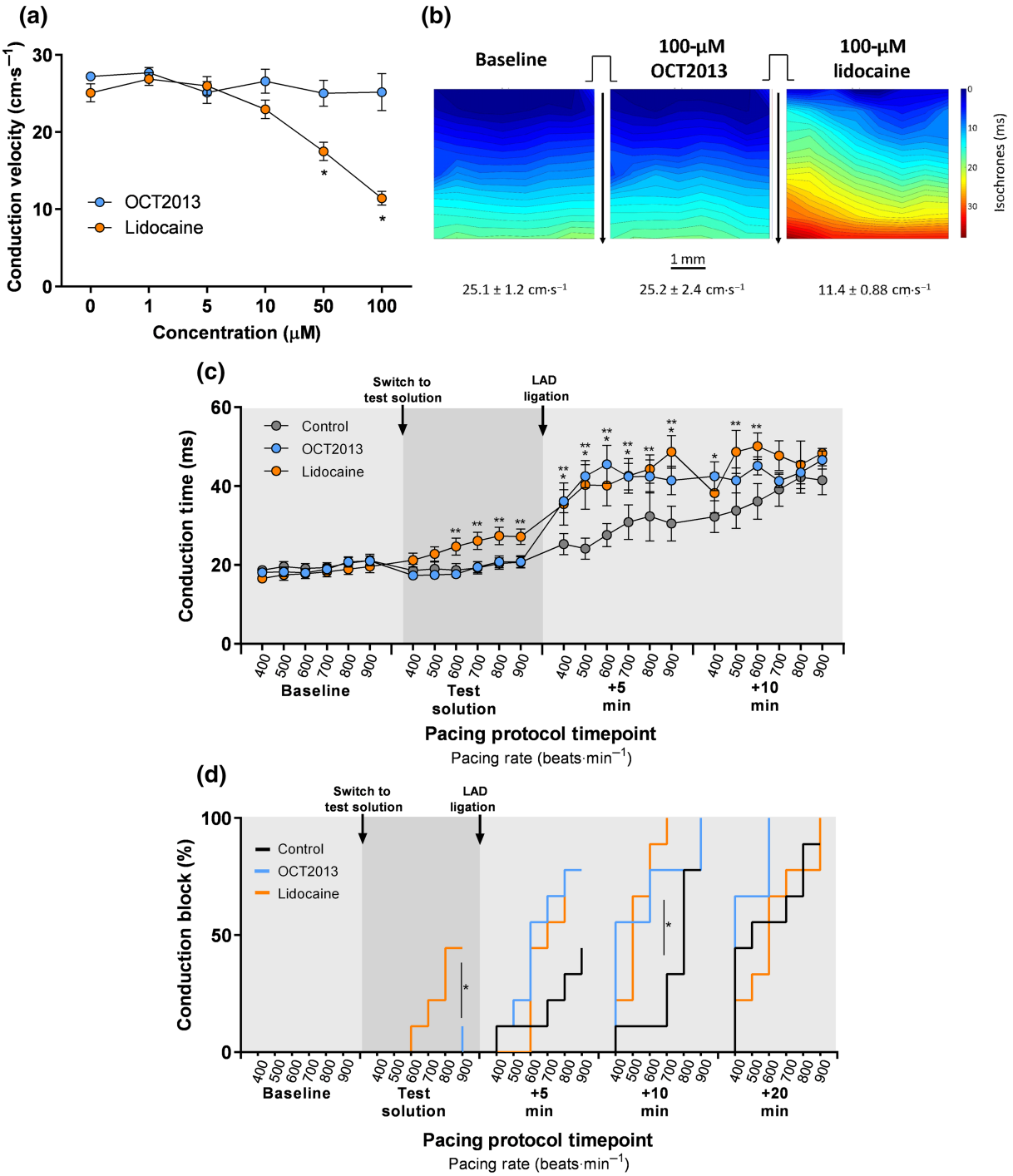


FIGURE 5 Effects of OCT2013 and lidocaine on myocardial conduction in neonatal rat ventricular myocytes seeded onto microelectrode arrays (MEAs) and in whole Langendorff-perfused rat hearts. In microelectrode arrays (MEAs), (a) effects of up to 100-μM OCT2013 or lidocaine ($n = 10$ per group) compared with baseline on conduction velocity (cm·s^{-1}) are shown together with (b) an example of the effect of 100 μM OCT2013 and 100 μM lidocaine. In paced (400–900 beats·min^{-1}) hearts, the effects of 15 μM OCT2013 or 15 μM lidocaine compared with Krebs are shown on (c) conduction time and (d) occurrence of conduction block during different stages of regional ischaemia, $n = 9$ per group. Data are (a–c) mean ± SEM or (d) survival percentage; conduction velocity values were compared by two-way ANOVA followed by Sidak's post hoc test (as F was significant and data Gaussian). Conduction time was evaluated by two-way ANOVA followed by Dunnett's post hoc tests (if F was significant and data Gaussian). Incidence of conduction block was subjected to Kaplan–Meier survival analysis. *$P < 0.05$. Abbreviation, LAD, left anterior descending coronary artery

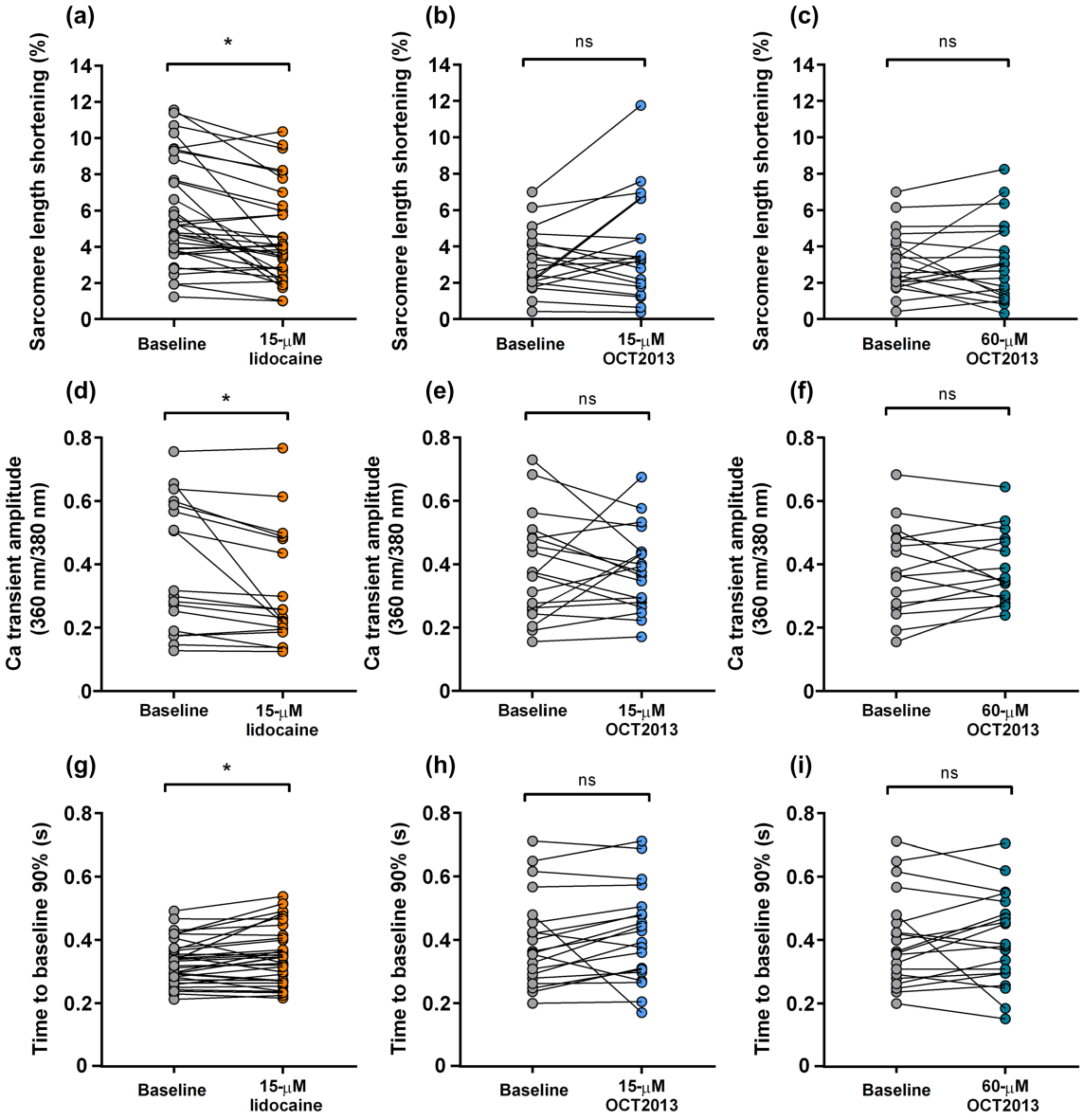


FIGURE 6 Sarcomere shortening and intracellular Ca^{2+} dynamics in adult rat ventricular myocytes in the absence (baseline) and presence of either lidocaine or OCT2013 at the stated concentration. Panels (a–c) sarcomere shortening, (d–f) Ca^{2+} transient amplitude (Fura-2 fluorescence ratio 360 nm/380 nm) and (g–i) time to 90% recovery of Ca^{2+} transient. Measurements were taken 2 min after normal superfusion (baseline) and 2 min after the start of superfusion with test solution (15 μmol·L^{-1} lidocaine, 15 μmol·L^{-1} OCT2013 or 60 μmol·L^{-1} OCT2013). At least 16 single cell recordings were made from 3 hearts, with values averaged from 10 Ca^{2+} transients or 10 sarcomere shortenings; comparison with baseline (no treatment) was undertaken using paired Student's *t*-tests. *$P < 0.05$ versus baseline

Hondeghem, 1987; Hondeghem & Katzung, 1984; Matsubara et al., 1987), which converts ischaemia-induced conduction slowing into antiarrhythmic conduction block (Hondeghem, 1987; Hondeghem & Cotner, 1978). Thus, an ischaemia-activated lidocaine prodrug was conceived as a plausible candidate.

4.3 | Proof of concept

OCT2013 was shown to mimic the ability of lidocaine to suppress ischaemia-induced VF, *in vivo* in the anaesthetised rat and *in vitro*, but without the archetypal adverse drug reactions found to be caused by lidocaine (Wilson et al., 1993) that include bradycardia, PR prolongation, negative inotropy and Ca^{2+} mishandling, effects mediated outside the ischaemic region (Bergey et al., 1982; Canyon & Dobson, 2004; Farkas & Curtis, 2002). During reperfusion, OCT2013 and lidocaine offered some protection against diastolic dysfunction. Chemical analysis of hearts showed that ischaemia resulted in the loss of OCT2013 and the concomitant appearance of lidocaine (hitherto absent during normoxic perfusion). Together, these data confirm that OCT2013 is an ischaemia-activated antiarrhythmic mimic of lidocaine and an ischaemia-activated antiarrhythmic prodrug of lidocaine.

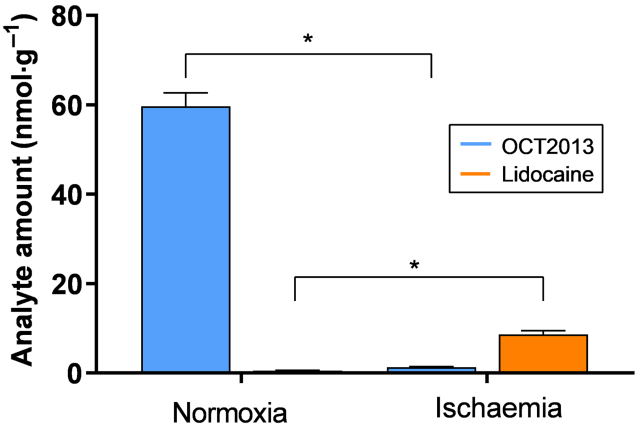


FIGURE 7 The presence of OCT2013 and lidocaine in myocardial tissue. OCT2013 and lidocaine were detected using UHPLC–MS/MS methodology in myocardial tissue samples collected from Langendorff-perfused isolated hearts, perfused with 15 μM OCT2013, after 30 min global ischaemia or time-matched normoxia. Variables ($n = 5$ per group; mean ± SEM) were subjected to one-way ANOVA followed by Dunnett's post hoc tests (as F was significant and data Gaussian). *$P < 0.05$ versus normoxic control

4.4 | Mechanism of antiarrhythmic activity of OCT2013

Further studies identified a plausible mechanism for OCT2013 suppression of ischaemia-induced VF, namely, the ischaemia-selective mimicry of lidocaine's hastening of rate-dependent conduction block during ischaemia. Evidence for this was (i), lack of effect of OCT2013 on conduction velocity during normoxic perfusion compared with rate-dependent conduction slowing by lidocaine and (ii), marked exacerbation of ischaemia-induced rate-dependent conduction slowing versus controls that became rate-dependent conduction block as ischaemia progressed, with OCT2013 and lidocaine possessing almost identical activity. Rate-dependent conduction block progressed more slowly during ischaemia in controls with values matching OCT2013 and lidocaine values 20 min after ischaemia onset, a time when the severity of ischaemia-induced arrhythmias diminishes in this model (Curtis, 1998). Thus, OCT2013 mimicked the actions of lidocaine that are attributable Na^+ channel block and which account for its antiarrhythmic effects (Kléber et al., 1986), hastening conduction block in the ischaemic region to narrow the temporal window of vulnerability to re-entry, the well-established basis for the antiarrhythmic action of lidocaine during ischaemia (Hondeghem, 1987; Hondeghem & Cotner, 1978), while lacking any measurable pharmacological activity during normoxia.

4.5 | Effectiveness and safety of OCT2013 *in vivo*

Key findings were confirmed in the anaesthetised rat, with OCT2013 devoid of effect on important haemodynamic variables including cardiac output, stroke volume, heart rate and PR interval, at doses up to 16 times that which suppressed ischaemia-induced VF (2 mg·kg^{-1}). In contrast, lidocaine produced adverse effects at 1 mg·kg^{-1}. The fact that all rats treated with 64 mg·kg^{-1} of lidocaine died (of conduction block with slow atrial activity continuing but ventricular standstill) while all rats treated with OCT2013 at the same dose survived, highlights the substantial differences in the translational therapeutic window of these drugs.

4.6 | Effectiveness of post-ligation administration

Previous studies have shown that i.v. verapamil given post-ligation can access the ischaemic region of the rat heart via collateral vessels and accumulate there, with this accounting for its post-ligation antiarrhythmic activity (Curtis et al., 1984). This is despite residual collateral flow in rat hearts being the *lowest* among animal species, amounting to a maximum of only ≈6% of the non-ischaemic flow (Maxwell et al., 1987). In the present study, OCT2013 was likewise effective when administered post-ligation, albeit less potently than by pretreatment (as was the case with verapamil *in vivo*, Curtis et al., 1984), consistent with a requirement for time to access and accumulate in the ischaemic region via collaterals, and subsequently be converted to lidocaine to exert its effect (Curtis, 1998). Benefit was, nevertheless, obtainable. This is encouraging because thrombolysis candidates with AMI are an obvious cohort for initial human efficacy testing, meaning that OCT2013 would need to be able to obtain benefit when administration is begun after the onset of regional ischaemia.

4.7 | Study limitations

The rat Langendorff preparation has several limitations. These include the small heart size (1 g), fast sinus rate (>300 beats·min^{-1}) and absence of blood and functional innervation. The rat ventricle does not respond to the actions of delayed rectifying potassium channel blockers, meaning that if a drug has actions on this current, the characteristic prolongation of ventricular action potential duration (APD) and QT interval are not manifested (Curtis, 1998). This means that if a drug has relevant pharmacological activity on this molecular target, the effects will not be identified if the research is not recapitulated in a species that expresses the functional channel. The rat heart also has a propensity for spontaneous defibrillation owing to the relationship between the size of the heart, the APD and the conduction velocity (Curtis, 1998). Although this may be disconcerting, it does not affect assessment of drug effects on the initiation of VF and the incidence of VF. These limitations, together with the model's advantages, are well documented (Curtis, 1998). However, the conclusions of the present study do not rely on Langendorff data and key findings were recapitulated in a whole animal model. Although we detected no proarrhythmia with OCT2013 or lidocaine, and although lidocaine is not associated with proarrhythmia during acute myocardial ischaemia in humans (Martí-Carvajal et al., 2015), the potential for proarrhythmia with OCT2013 would need to be ruled out, as it would for any novel antiarrhythmic drug. Addressing this would

best be undertaken using a standard safety pharmacology approach, that is, dog telemetry, closer to first-in-human studies. Finally, all contractile activity in the ischaemic zone is lost within a minute of the onset of regional ischaemia in controls in the rat heart (Wilder et al., 2016). This means that if a drug were to adversely affect contractile function in the ischaemic zone, during ischaemia, it would not be possible to detect this in the rat heart. Future studies will seek to confirm key findings in a second species with different translational relevance and undertake chronic toxicity assessment and pharmacokinetic characterisation after single and repeat dosage *in vivo*.

5 | CONCLUSIONS

OCT2013 is a first-in-class antiarrhythmic prodrug, with ischaemia-selective actions that mimic those of lidocaine, resulting in VF prevention, but without adversity at ≥16 times the effective dose *in vivo* and ≥7 the effective concentration *in vitro* (these numbers representing the translational therapeutic index in each setting). This is far better than the profile of lidocaine, whose equivalent value was <1 according to the data in the present study, and another clinically used class 1b drug, mexiletine (value < 2 according to recent data derived from the *in vitro* rat heart model used in the present study; Hesketh et al., 2020). There are no drugs tested in animal models of sudden cardiac death that have shown a profile as promising as that of OCT2013 since assessment began in the 1960s, to our knowledge.

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AUTHOR CONTRIBUTIONS

Conceptualisation: SO, LHP, MJC, MBS. Methodology: LMH, MBS, JW, FM, LP, MJC. Investigation: LMH, MBS, LMS, PT, FM, RAC, KNT, JW. Data analysis: LMH, MBS, FM, JW, CDEW, LP, MJC. Funding acquisition: LMH, MBS, MJC. Supervision: MJC, MBS, KTM, NSP. Writing—original draft: LMH, MBS, LHP, MJC. Writing—review and editing: LMH, MBS, LHP, MJC, RAC, KNT, JF, FM, KTM, SO, NSP.

CONFLICT OF INTEREST

SO, LHP, MJC and MBS are shareholders in BioTherics Limited, a private company that holds a patent on the use of OCT2013 as an antiarrhythmic. SO is a director of BioTherics Limited. All other authors have no conflict of interest to declare.

DECLARATION OF TRANSPARENCY AND SCIENTIFIC RIGOUR

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research as stated in the *BJP* guidelines for Design and Analysis and Animal Experimentation, and as recommended by funding agencies, publishers and other organisations engaged with supporting research.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

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