THE USE OF BOTH ANAESTHETISED AND TELEMETERED CONSCIOUS GUINEA-PIGS IN EARLY SAFETY PHARMACOLOGY: A CASE STUDY WITH SUNITINIB

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INTRODUCTION

The guinea-pig (GP) is recognized as a good surrogate species possessing predictive value in early safety assessment of new chemical entities, in particular to evaluate the potential for delayed ventricular repolarization.
Sunitinib is a recently-approved multi-targeted tyrosine-kinase inhibitor with potential antineoplastic activity, but concerns about cardiac safety have arisen with this agent (e.g. increases in QTc, PR and QRS intervals), likely mediated by multichannel blockade (Cohen *et al.*, *Toxicol. Appl. Pharmacol.* **257(1)**: 74-83 (2011).
In the present study, Sunitinib was administered i.v. in the anaesthetised GP and orally in the conscious telemetered GP. Blood samplings were performed in satellite animals to evaluate Sunitinib exposure in both models.

MATERIALS & METHODS

Anaesthetised Guinea-Pigs:

o Surgery:
- Male Dunkin-Hartley guinea-pigs weighing 320-380g; n=6 in each group.
- Surgery and experiment under sodium pentobarbital anaesthesia (60 mg/kg, ip).
- Animals artificially ventilated with ambient air enriched with oxygen.
- Catheters introduced into a carotid artery and a jugular vein for measurement of arterial blood pressure and administration of sunitinib (or vehicle), respectively.
- Subcutaneous electrodes (lead II) for ECG measurement.
- Body temperature maintained ~37°C using heating pad.
- Arterial blood pressure and ECG signals monitored and analyzed using HEM software (Notocord System, France).

o Administration protocol: 3 successive 15-min iv infusions (2 mL/kg) of increasing doses of Sunitinib (3, 10, and 30 mg/kg) or corresponding vehicle.
o Blood samplings: performed in 3 anaesthetized satellite GP at 15, 30 and 45 min (same design than cardiovascular study). Blood collected from the arterial catheter into lithium heparinate tubes and centrifuged to obtain plasma.
o Statistical Analysis: changes from baseline values compared between the 2 groups using a two-way ANOVA (group, time) with repeated measurements.

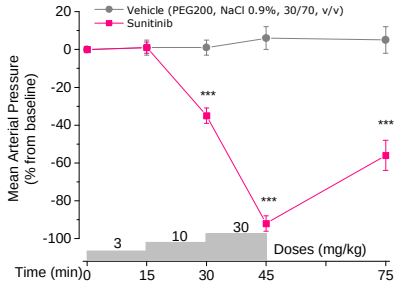
Telemetered Guinea-Pigs:

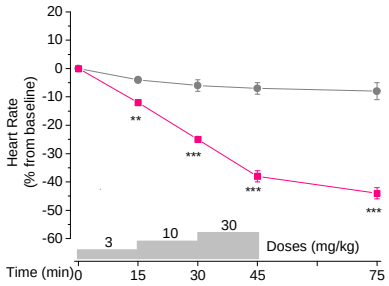
o Surgery:
- Male Dunkin-Hartley guinea-pigs weighing 350-420g; n=7.
- Surgery under isoflurane anaesthesia (2.5 to 5%).
- Telemetric transmitter (TL11M2-C50-PXT, Data Science International, Minnesota, USA) implanted for measurements of arterial blood pressure, heart rate and ECG.
- Body of the transmitter placed on the back of the neck.
- Pressure catheter introduced into the left carotid artery and ECG leads placed subcutaneously in lead II position (right shoulder/lower thorax).
- Arterial blood pressure and ECG signals monitored and analysed using HEM software (Notocord System, France).
- Analgesia: buprenorphine 0.05 mg/kg
- Antibiotic : enrofloxacin 10 mg/kg
- At least 15 days of recovery post-surgery

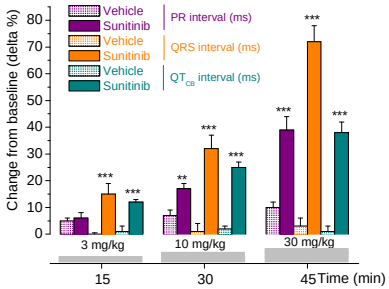
o Administration protocol: oral administration (5 mL/Kg) of vehicle and increasing doses of Sunitinib (30, 90 and 180 mg/kg) in fasted animals.
o Blood samplings: performed in 9 conscious non-instrumented satellite animals (n=3/dose) at 120, 360, 600 and 1440 min post-administration. Blood collected under isoflurane anaesthesia from the orbital sinus into lithium heparinate tubes and centrifuged to obtain plasma.
o Statistical Analysis: two-way ANOVA (treatment, time) with repeated measurements.

RESULTS

Anaesthetised GP







Plasma concentrations of Sunitinib

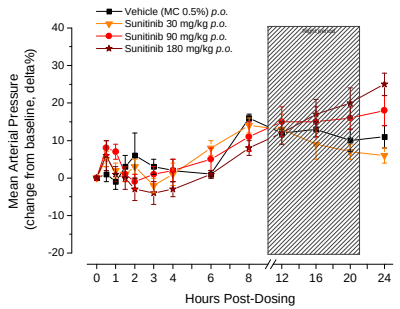
Maximum Drug Plasma Concentration (ng/mL)		
Sunitinib (mg/kg)	***Anaesthetised GP i.v. route***	***Conscious GP p.o. route***
3	**318** (T15)	-
10	**596** (T30)	-
30	**4518** (T45)	**51** (T120)
90	-	**110** (T360)
180	-	**173** (T600)

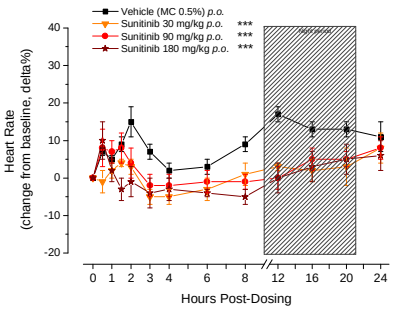
LC-MS/MS method developped at Avogadro (Fontenilles, France)

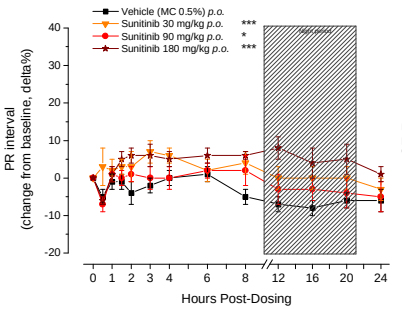
Baseline values

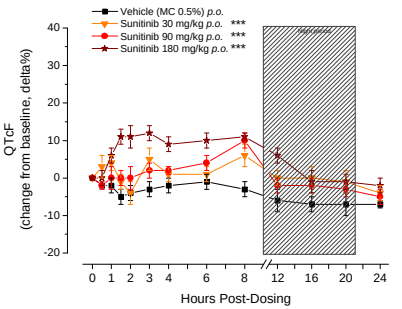
	MAP (mmHg)	HR (bpm)	PR (ms)	QRS (ms)	QT (ms)	QTc Bazett	QTc Fridericia
Anaesthetised GP	**39 ± 2**	**270 ± 5**	**54 ± 1**	**18 ± 1**	**159 ± 3**	**339 ± 4**	**264 ± 4**
Telemetered GP	**61 ± 1**	**207 ± 3**	**66 ± 1**	**18 ± 0**	**146 ± 2**	**272 ± 3**	**221 ± 2**

Telemetered GP









CONCLUSION

In anaesthetised GP, Sunitinib administered i.v. induced marked dose-dependent decreases in arterial pressure and heart rate and increases in PR, QRS and QTc intervals, consistent with its multichannel blockade properties and clinical observations. In conscious telemetered animals, Sunitinib administered p.o. did not induce major effects on haemodynamic parameters and only slight increases in PR and QTc, mainly observed at 180 mg/kg. The comparison of plasma concentrations in both models showed that despite 6-fold higher doses tested orally, the exposure is far lower, illustrating poor oral bioavailability of Sunitinib in this condition. Therefore, although ICH guidelines recommend the use of conscious animals, anaesthetised preparations may allow higher compound exposure and better characterisation of safety profile of new drugs.

ACKNOWLEDGMENTS

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