SPAR as a Tool to Analyse Arterial Blood Pressure Waves, following Fostamatinib and Entospletinib Administration in Radiotelemetry in Rats.

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Introduction

- Typically minimum, maximum and mean values of cardiovascular blood pressure (BP) waveforms (i.e., systolic, diastolic and mean arterial pressure (**MAP**) are examined[1]. More in-depth characterisation of blood pressure waveform data could enhance additional insights in **cardiovascular safety liabilities**[1]. The Symmetric Projection Attractor Reconstruction (**SPAR**) is a novel mathematical method that could enable such detailed waveform analysis[2].
- **Fostamatinib**, a Syk-inhibitor causing hypertension due to off-target VEGFR2-inhibition[3], and **entospletinib**, designed for better selectivity and less adverse effects[4], are presented here as an example.

Aim

Can SPAR metrics provide insights in the cardiovascular safety of fostamatinib and entospletinib, that were not apparent from MAP, HR or PP analysis?

Methods

1. Radiotelemetry

Male Han Wistar rats were instrumented with Stellar telemetry implants. Arterial blood pressure was recorded continuously over two consecutive days, for at least 1h before and up to 23h after daily administration of fostamatinib (20 mg/kg, p.o.) and entospletinib (6 mg/kg, p.o.) or vehicle control (10 mL/kg, p.o.).

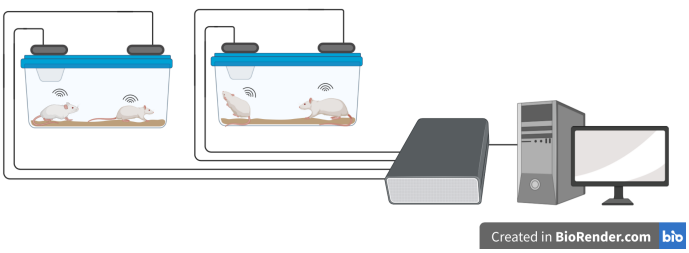
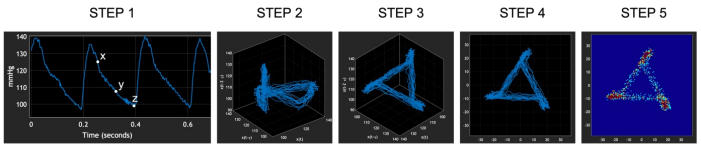


Figure 1: Radiotelemetry. Stellar telemetry implants allow for pair-housing of two instrumented animals and simultaneous, continuous recording of blood pressure, ECG, temperature and activity. (Figure created in Biorender.com)

2. Symmetric Projection Attractor Reconstruction (SPAR)



Step 1	Three points are plotted on the waveform, each separated by a time delay that equals one third of the average duration of the cycle. This set of three delay coordinates (x, y and z) can be placed anywhere on the waveform.
Step 2	Each position of the set of delay-coordinates results in one point in the three-dimensional (3D) space with x-, y- and z-axis. To transform the waveform into a 3D representation in step 2, the three delay coordinates run over the entire recorded time trace. Each pulse of the wave corresponds to one loop in 3D space
Step 3 & 4	Next, the 3D cubicle is rotated and the attractor is viewed down one particular line. The chaotic 3D attractor is transformed into a clearer, symmetrical 2D triangular shape.
Step 5	In the last step, the density (colour) of the attractor reconstruction is added, pointing out where the individual loops overlap and thus providing information on the waveform shape variability. Small morphological changes in the waveform appear amplified in the attractor.

Results

1. Conventional wave analysis

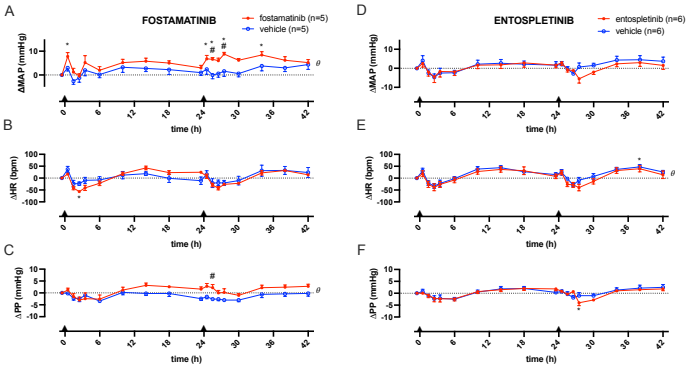


Figure 3: Effects of fostamatinib (A, B and C) and entospletinib (D, E and F) on ΔMAP, ΔHR and ΔPP. Data were normalized to baseline value and presented as mean ± SEM. Black triangles indicate time of drug administration. Statistics: Two-way ANOVA (between groups, θ=p<0.05) and multiple comparisons Sídák (between groups, #=p<0.05) or Dunnett (within group from baseline, *=p<0.05).

2. SPAR analysis

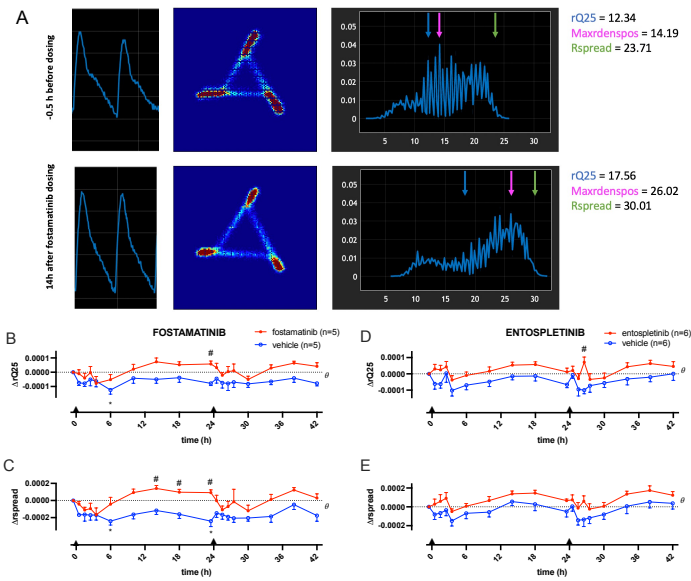


Figure 4: Effects of fostamatinib and entospletinib SPAR metrics. (A) BP wave, associated attractor and radial density plot taken from baseline recording and 14h after fostamatinib administration. Radial density plot measures amount of attractor data contained in different, growing concentric circular sections on the attractor. **(B)** and **(C)** show quantification of fostamatinib-induced changes on attractors: ΔrQ25 and Δrspread. **(D)** and **(E)** show quantification of entospletinib-induced changes on attractors: ΔrQ25 and Δrspread. Data were normalized to attractor size and baseline value and presented as mean ± SEM. Black triangles indicate time of drug administration. Statistics: Two-way ANOVA (between groups, θ=p<0.05) and multiple comparisons Sídák (between groups, #=p<0.05) or Dunnett (within group from baseline, *=p<0.05). Data were normalized to baseline recording and presented as mean ± SEM.

Conclusion and future directions

- SPAR could detect changes in cardiovascular system earlier than MAP, HR or PP could for fostamatinib-treated animals.
- SPAR could detect entospletinib-induced cardiovascular effects that went unnoticed when looking at MAP, HR or PP alone.

→ **Proof of concept: SPAR as a tool for in-depth wave analysis in safety pharmacology**

- Further validation needed with reference compounds and/or *in silico* wave data.

References

[1]Mynard et al. (2020). Frontiers in Physiology [2]Nandi & Aston (2020) Exp. Physiol. [3]Skinner et al. (2014) British Journal of Pharmacology, [4]Currie et al. (2014) Journal of Medicinal Chemistry

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