

## Antiviral potential of distinct heparins against SARS-CoV-2 *in vitro*.

Aline R. Matos<sup>1</sup>; Bráulio C Caetano<sup>1</sup>, Michele G. P. Oliveira<sup>1</sup>; Jéssica S.C.C. Martins<sup>1</sup>, Thiago C. Sousa<sup>1</sup>, Juliana M. Motta<sup>2</sup>, Rodrigo F. Cano<sup>3</sup>, Francisco F. Bezerra<sup>2</sup>, Stephan-Nicolas M.C.G. Oliveira<sup>2</sup>, Ana M.F. Tovar<sup>2</sup>, Mariana S. Pereira<sup>2</sup>, Eduardo Vilanova<sup>2</sup>; Paulo A.S. Mourão<sup>2</sup>, Marilda M Siqueira<sup>1</sup>

1. Laboratório de Vírus Respiratórios e do Sarampo - Instituto Oswaldo Cruz – FIOCRUZ, Rio de Janeiro, Brazil
2. Laboratório de Tecido Conjuntivo, Instituto de Bioquímica Médica Leopoldo de Meis e Hospital Universitário Clementino Fraga Filho, UFRJ, Rio de Janeiro, Brazil
3. Instituto SENAI de Inovação em Biotécnicos e Fibras, Centro de Tecnologia da Indústria Química e Têxtil, Rio de Janeiro, Brazil.

### Background

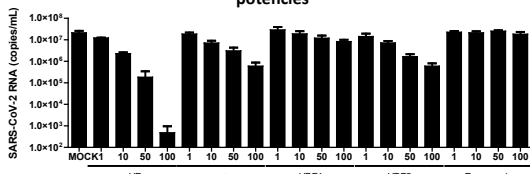
The COVID-19 pandemic is imposing a great burden to public health worldwide. Currently, there are no vaccines or effective therapies against SARS-CoV-2 infection. Thus, investigation of antiviral options is detrimental for development of intervention methods. Heparins, anticoagulant compounds of common clinical use, have previously demonstrated antiviral activity against other coronaviruses and respiratory viruses, through inhibition of virus anchoring in heparan sulfate proteoglycans on host cell membrane. Therefore, the aim of this study was to investigate the potential of traditional porcine and bovine sourced heparins (HP and HB, respectively), the new bovine heparins HBF1 and HBF2 and the low-molecular-weight-heparin enoxaparin antiviral activity against SARS-CoV-2 *in vitro*.

### Methods

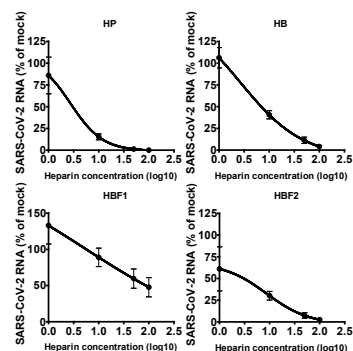
Vero E6 (monkey kidney) and H1299 (human pulmonary) cells were infected with a SARS-CoV-2 isolate in a multiplicity of infection (MOI) of 0.01. Infected cells were pre-incubated with the distinct heparins, in addition to incubation during and post infection, at a range of concentrations, for up to 48 hours post infection. Additionally, cell treatment was performed either pre, during or post infection, as well as virus was incubated with the compounds before infection. Viral RNA was quantified in culture supernatants.

### Results

#### Distinct heparins display dose dependent antiviral activity against SARS-CoV-2 infection in Vero-E6 cell with differential potencies



**Figure 1 – Antiviral activity of distinct heparins on SARS-CoV-2 infected Vero-E6 cells.** Cells were treated (1-100 µg/mL) with distinct heparins (HP, HB, HBF1, HBF2 and enoxaparin) before, during and post-infection. SARS-CoV-2 infections were performed for 1h at a MOI of 0.01. Viral RNA was quantified from the collected supernatants 48 hpi by real time PCR. Bars show mean and SEM of at least three independent experiments.



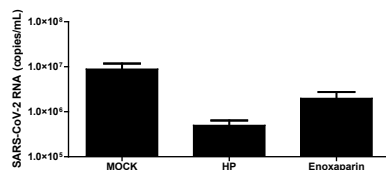
**Figure 2 – Dose response curves for heparins against SARS-CoV-2 in Vero-E6 cells.** Cells were treated (1-100 µg/mL) with distinct heparins (HP, HB, HBF1 and HBF2) before, during and post-infection. SARS-CoV-2 infections were performed for 1h at a MOI of 0.01. Viral RNA was quantified from the collected supernatants 48 hpi by real time PCR. Data point with bars represent mean and SEM of at least three independent experiments.

**Table 1 – IC<sub>50</sub> values of heparins against SARS-CoV-2 *in vitro*.**

Heparin	IC <sub>50</sub> (µg/mL)	Antiviral activity - compared to HP (n-fold)
HP	2,75	1,00
HB	2,63	1,05
HBF1	5,55	0,50
HBF2	8,74	0,31
Enoxaparin	ND	---

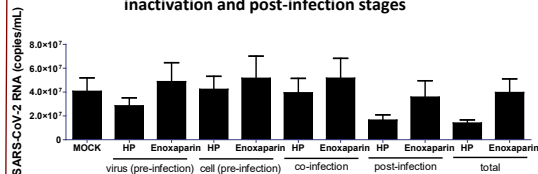
Note: At least 3 independent experiments were performed. Quantified values were analyzed by non-linear regression to determine IC<sub>50</sub> values. The relative potencies were calculated by dividing the HP IC<sub>50</sub> by the IC<sub>50</sub> of the other subtypes.

#### HP and enoxaparin display distinct antiviral activity against SARS-CoV-2 infection in human H1299 cells



**Figure 3 – Antiviral activity of HP and enoxaparin on SARS-CoV-2 infected human H1299 cells.** Cells were treated (10 µg/mL) with HP and enoxaparin before, during and post-infection. SARS-CoV-2 infections were performed for 1h at a MOI of 0.1. Viral RNA was quantified from the collected supernatants 48 hpi by real time PCR. Bars show mean and SEM of three independent experiments.

#### HP anti-SARS-CoV-2 mechanism might involve viral direct inactivation and post-infection stages



**Figure 4 – Time-of-addition experiments with HP and enoxaparin.** Vero-E6 cells were infected with SARS-CoV-2 at MOI of 0.01 and treated with HP and enoxaparin at 10 µg/mL. The following conditions were used: virus (pre-infection), viruses treated for 1 h before infection; cell (pre-infection), cells treated for 1h before infection; co-infection, cells treated during infection, for 1h; post-infection, cells treated for 48 h post-infection. Viral RNA was quantified from the collected supernatants 48 hpi by real time PCR. Bars show mean and SEM of three independent experiments.

### Conclusions

Interestingly, our study reveals that HP, HB, HBF1 and HBF2 possess distinct antiviral potencies activity against SARS-CoV-2 *in vitro*, in contrast to enoxaparin, which in turn has already been used as anticoagulant agent to reverse sepsis induced coagulopathies in COVID-19 patients. Further experiments are being conducted to determine the mechanism of action of these compounds and their potential interaction with the virus.

### Conflict of interest

The presenter states that she has no conflict of interest.