The effects of nitric oxide donors on vasoactivity and survival in hemorrhagic shock resuscitation with the hemoglobin based oxygen carrier HBOC-201 in swine

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Disclaimer

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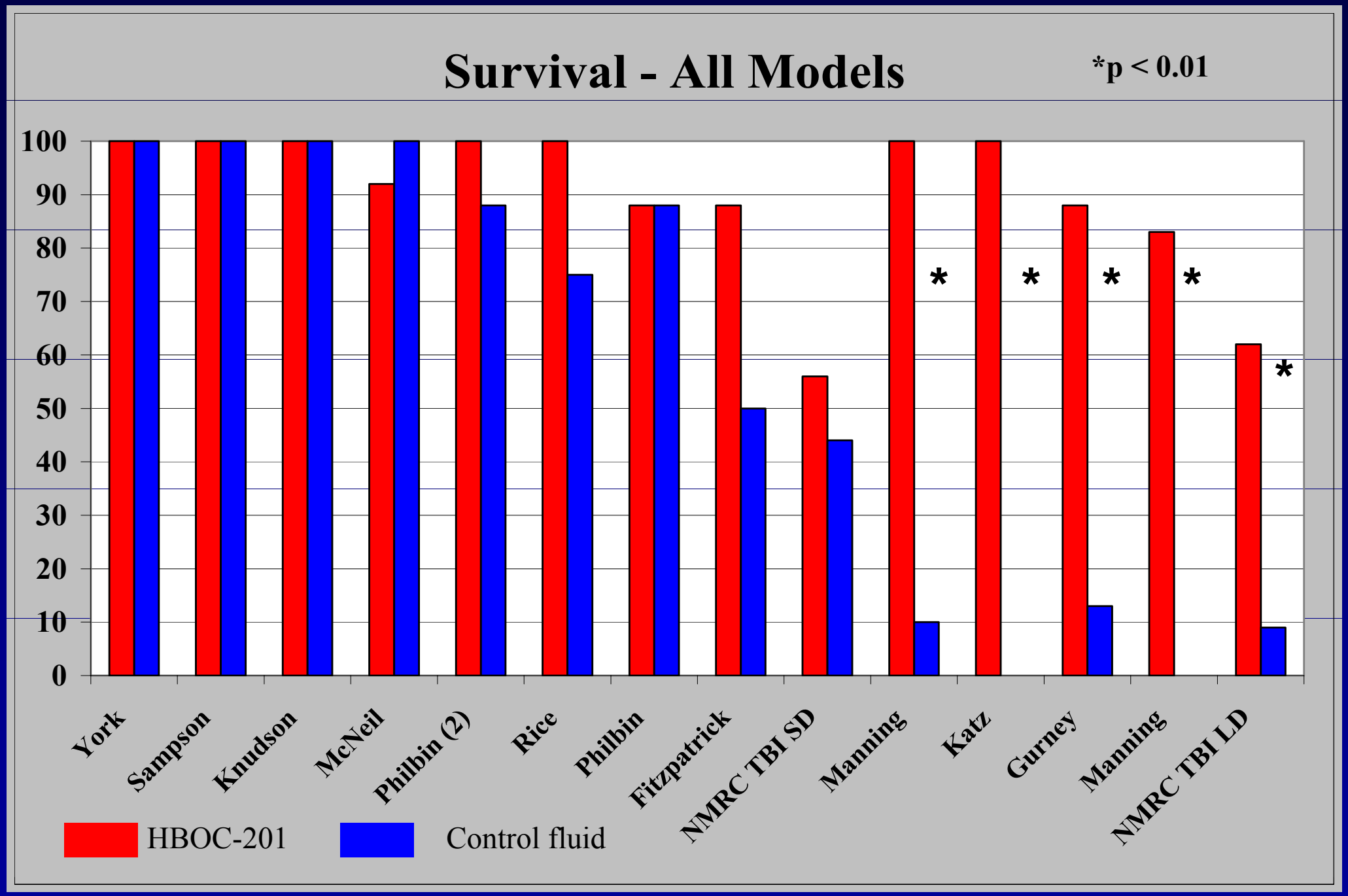
Background

- Hemoglobin based oxygen carriers have been in clinical development for the past 20 years

- Numerous animal studies have shown superiority of HBOC-201 over other resuscitation fluids in pre-hospital trauma care:

  - increased survival
  - increased tissue oxygenation
  - reduced blood transfusion
  - benefits increase logarithmically to injury severity



Less Severe

More Severe

Background

- **HEM-0115 trial:** HBOC-201 vs. RBC in elderly orthopedic patients undergoing elective surgery

  - Blood transfusion avoidance: > 95 % in first 24 hr with HBOC-201
  - Mortality equal
  - BUT: ↑ in hypertensive, cardiac events with HBOC-201

► **FDA puts IND for HBOC-201 on clinical hold**

However,

- in younger patients in this study: no differences

- in severe trauma patients the benefits outweigh the risks
- studies in MI models in dogs have shown ↓ infarct area with HBOC-201 and ↓ troponin elevations

Background

Vasoactivity (i.e. vasoconstriction) associated with HBOC-201 is main reason for clinical hold by FDA.

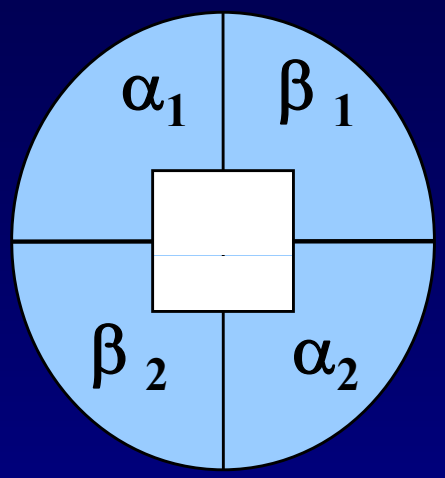
Hypothesis for vasoactivity:

- **Scavenging of vascular endothelial nitric oxide (NO)** by hemoglobin (cell-free, outside of protective red blood cell)

⟶ NO cannot function as mediator in vasodilation

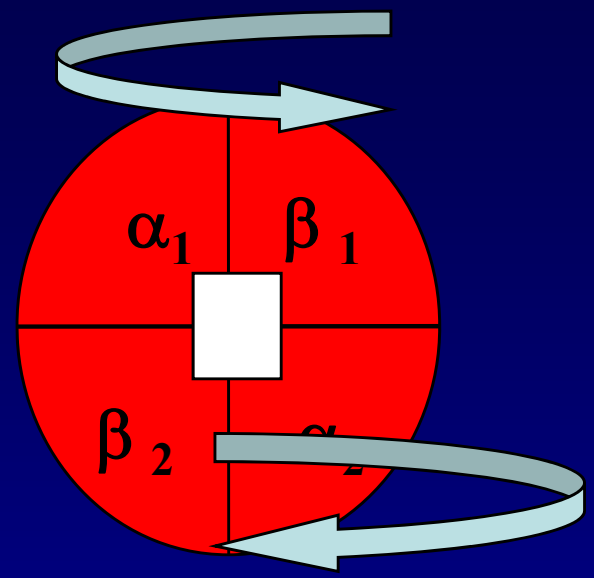
⟶ **Endothelial vasoconstriction**

Hb = allosterically regulated nitrite reductase



O_2 →

50% Saturation = Maximal Nitrite Reductase Activity



DeoxyHb - 'T'ense conformation

Available heme for nitrite reduction

OxyHb- 'R'elaxed conformation

Lower redox potential- (E1/2) better electron donor

oxygenation induces a rotation at the α1β2/ α2β1 interface

Hypothesis

Give an NO donor and vasoactivity will be reduced

- Testing clinically available NO donors in swine models: L-Arginine, sodium nitrite ($NaNO_2$), nitroglycerin (NTG), nitroprusside (NTP)
- L-Arginine: not effective
- Nitroprusside: currently under testing *(complete 10/09)*
- Sodium nitrite: controlled hemorrhage dose escalation *(NR)*
  uncontrolled hemorrhage *(reported here)*
- Nitroglycerin: uncontrolled hemorrhage *(reported here)*

Attenuating Vasoactivity – NMRC Collaborators

- **Vascular rings**
  - C Hai, PhD (Brown University) bovine
  - R Patel, PhD (University of Alabama) rat
- **Intravital microscopy**
  - R Pittman, PhD (Virginia Commonwealth University) anesth. rats, top load & controlled HS
- **Top-load & controlled HS**
  - J Kerby, MD, PhD (University of Alabama) anesth. mice
  - D Duncker, MD, PhD (Erasmus Medical Center, Rotterdam) anesth. mice, awake & anesth. swine
  - A Scultetus, MD (NMRC & USUHS) anesth. swine
  - M Gladwin, MD (NIH, University of Pittsburgh) NO chemistry
  - S Stern, MD (University of Washington) anesth. swine
- **Uncontrolled HS**
  - J Manning, MD and L Katz, MD (University of North Carolina Chapel Hill) anesth. swine, solid organ injury
  - A Scultetus, MD (NMRC & USUHS) anesth. swine, solid organ injury
  - M Gladwin, MD (NIH, University of Pittsburgh) NO chemistry
  - S Stern, MD (University of Washington) anesth. swine, vascular injury
- **Polytrauma**
  - A Scultetus, MD (NMRC & USUHS) anesth. swine, solid organ injury + TBI
  - K Proctor, PhD (University of Miami) anesth. swine, femoral fracture + TBI
- **Artificial vascular chamber**
  - M Kameneva, PhD (University of Pittsburgh)

Materials and Methods

29 Yorkshire Swine (weight 22-33 kg, mean 26.7 kg, ♂ and ♀)

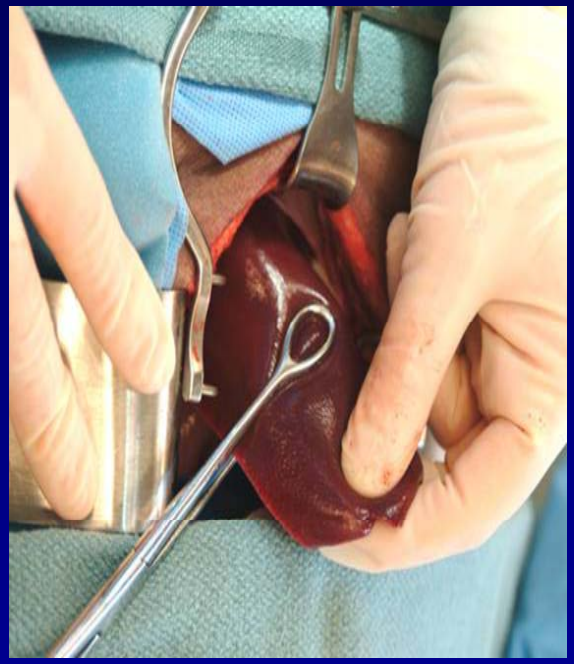
Pre-anesthesia: ketamine + atropine
Intubation, isoflurane anesthesia

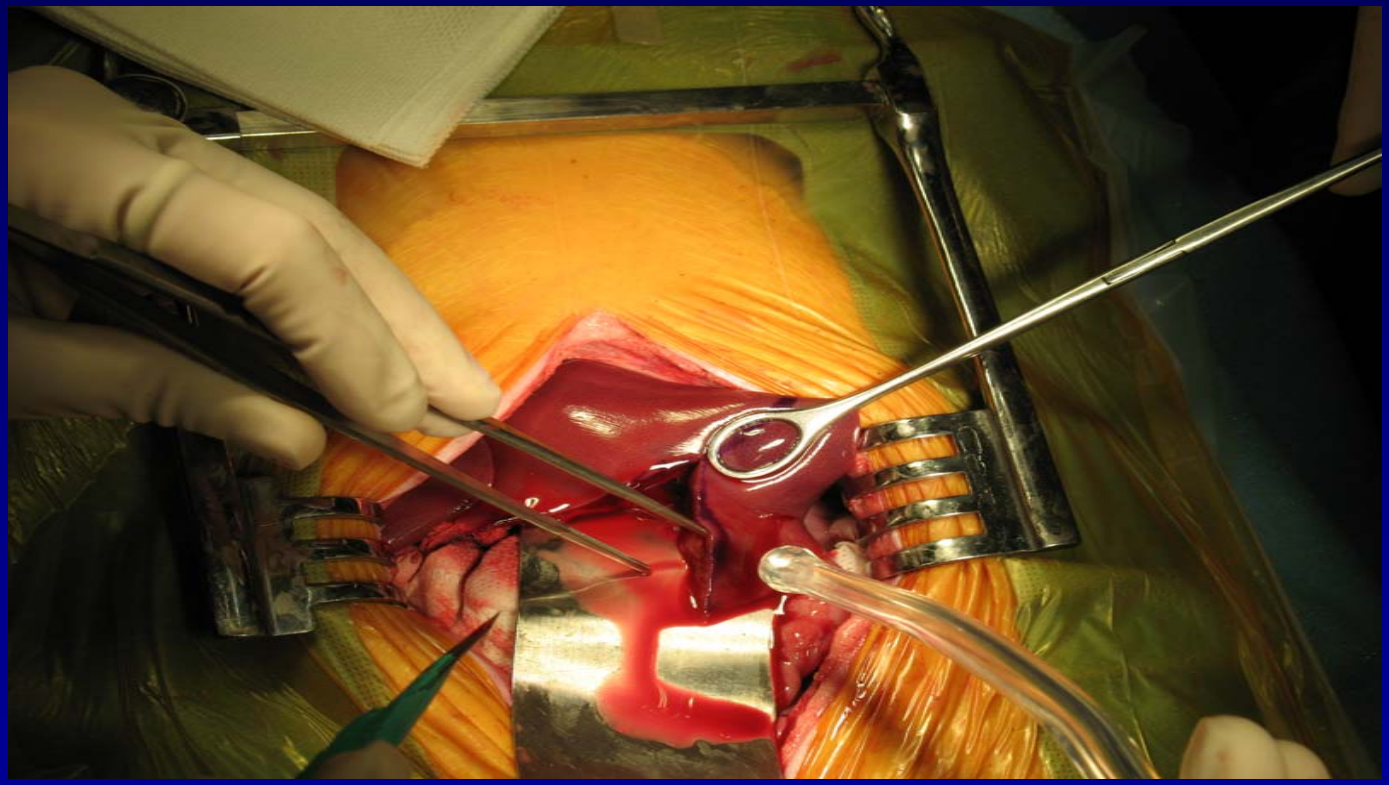
Catheters:

a. external jugular vein (Swan-Ganz, blood sample, infusion)
b. carotid artery (blood pressure, blood sample)

Injury

Uncontrolled hemorrhage from Grade III liver crush/laceration injury



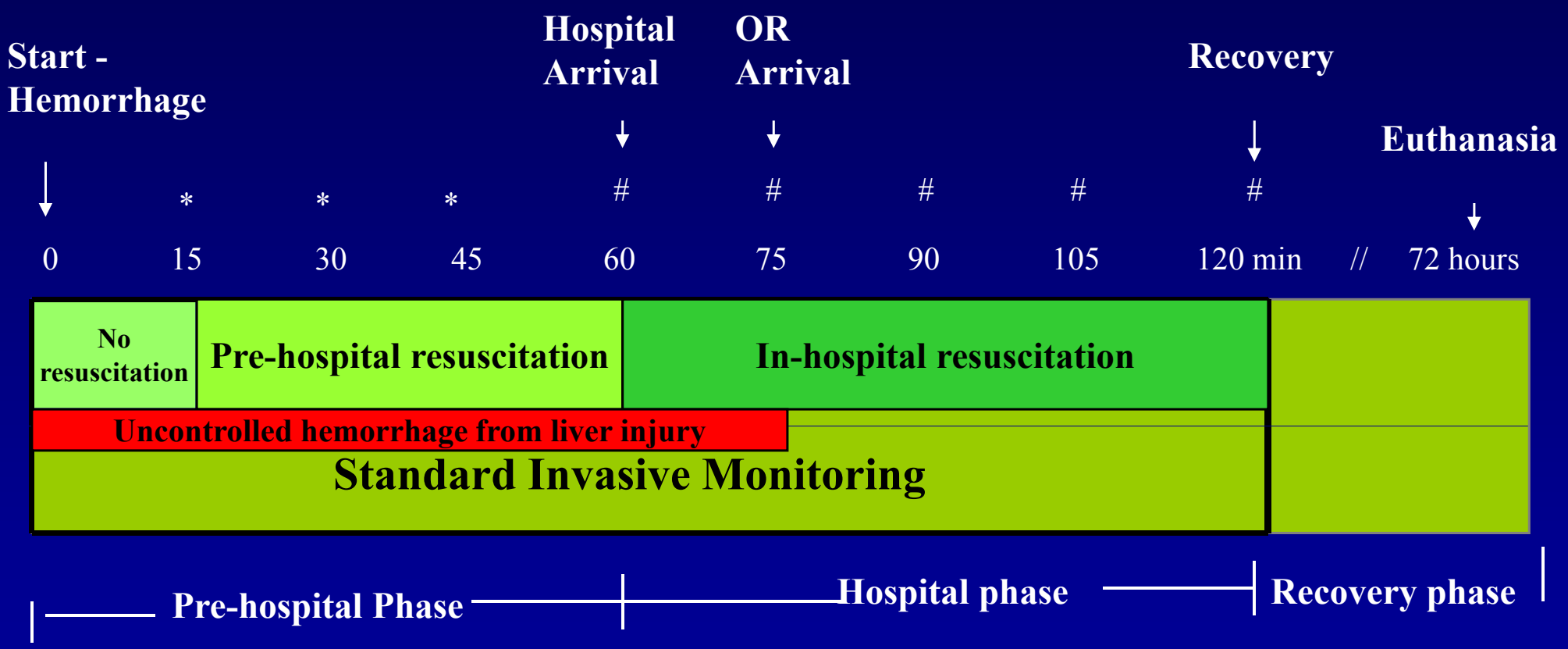


- Left liver lobe
- Crush at 25% length
- And 50% width

- Crush and cut other 50% width
- Hold crush for 1 minute
- Release and cut remaining 50%

Experimental Design

Injury: uncontrolled hemorrhage from Grade III liver crush/laceration, 72-hour survival



* Pre-hospital resuscitation: HBOC-201/HEX alone or with NTG or $NaNO_2$

\# In-hospital resuscitation: Blood or 0.9% saline

Infusions

Infusion sites:

For resuscitation fluid: 9 Fr introducer port in external jugular vein

For NO donor: distal port in Swan-Ganz catheter

► infusion of both substances was separate, but concomitant

Infusion criteria:

	Pre-hospital T0-60	In-hospital – ER arrival T60-75	In-hospital – OR and ICU T75-T120
MAP	≤ 60 mmHg	≤ 60 mmHg	≤ 70 mmHg
HR	≥ T0 + 5%	≥ T0 + 5%	≥ T0 + 5%
Lactate	N/A	≥ 4 mmol/L	≥ 2 mmol/L
Hb	N/A	≤ 7g/dl →blood	≤ 7g/dl →blood

Experimental Groups

Part 1

HBOC-201 + $NaNO_2$ 1.08 μg/kg/min	*n*=4
HEX + $NaNO_2$ 1.08 μg/kg/min	*n*=4

Controls for Part 1 and 2

HBOC-201	*n*=6
HEX	*n*=6

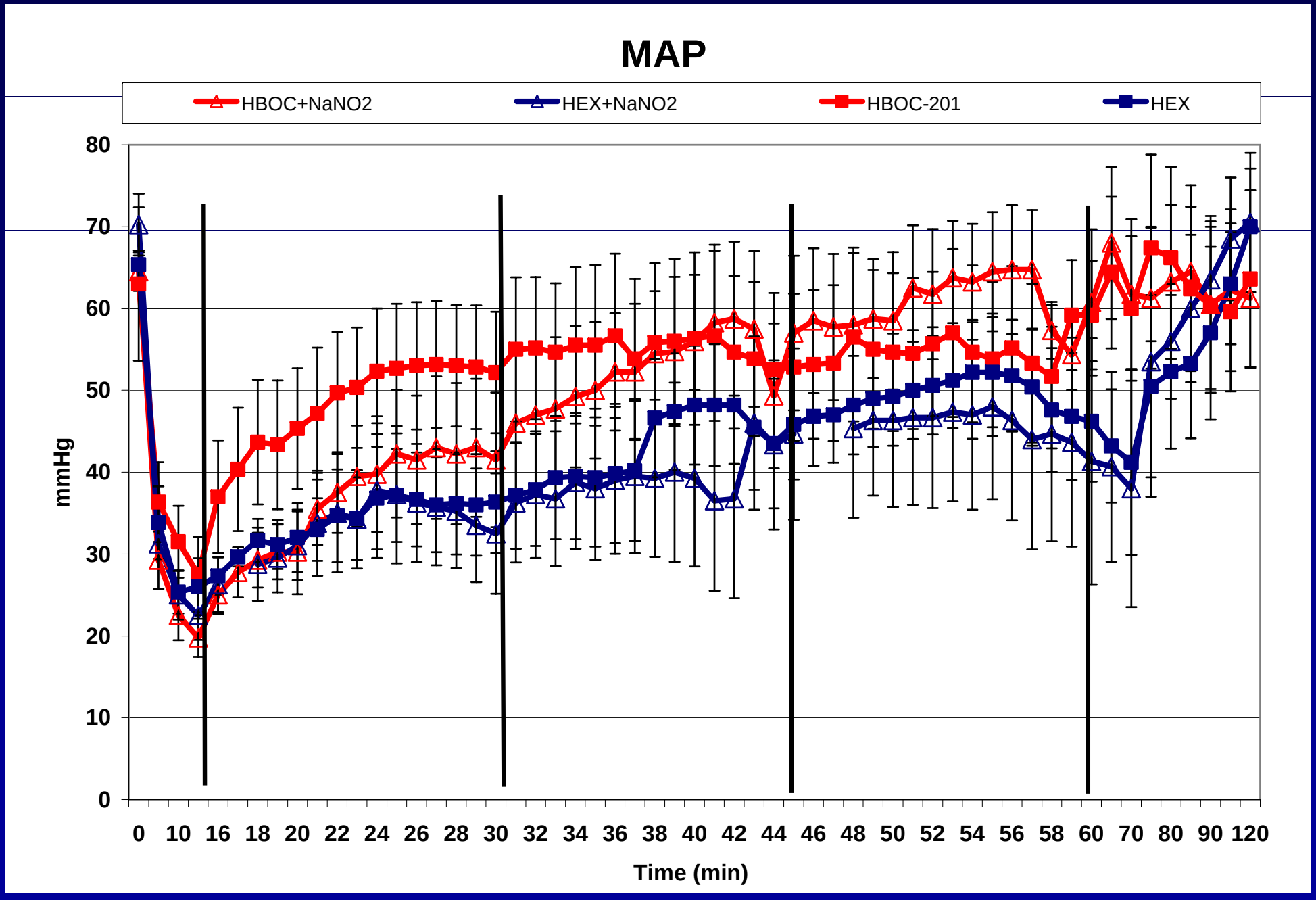
Part 2

HBOC-201 + NTG 5 μg/kg/min	*n*=7

Part 1

HBOC-201 + Sodium Nitrite ($NaNO_2$)

Part 1: Sodium Nitrite study



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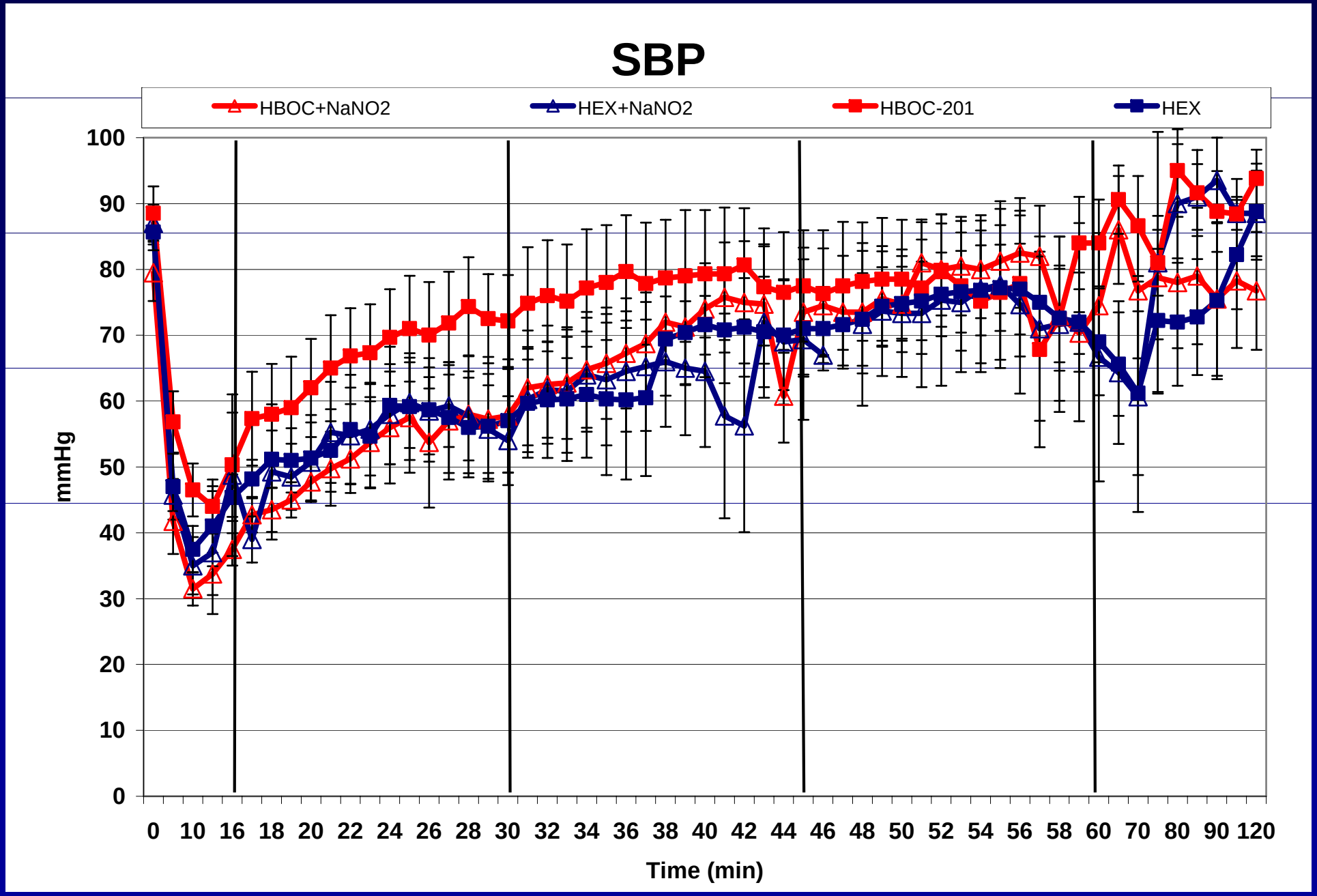

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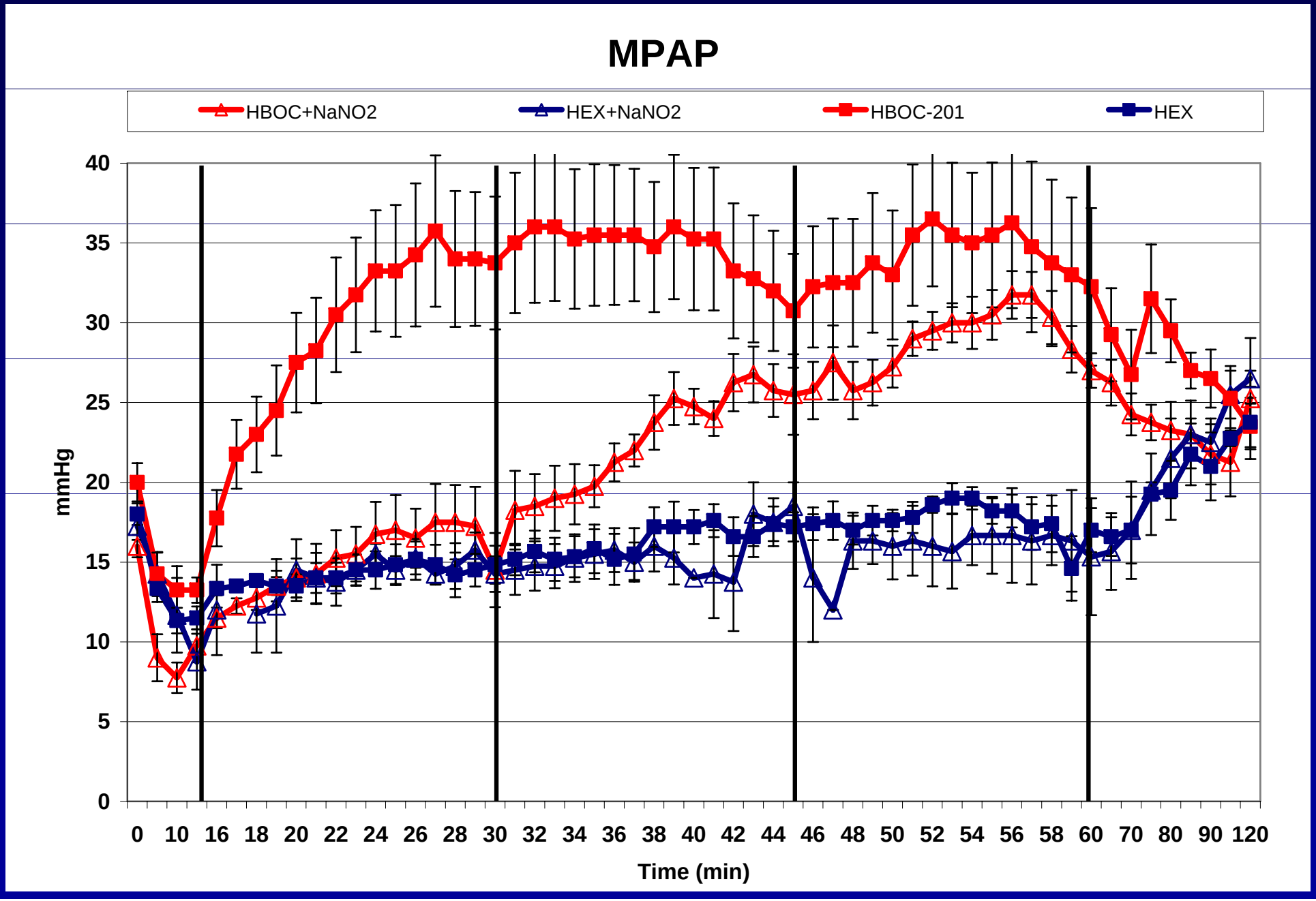

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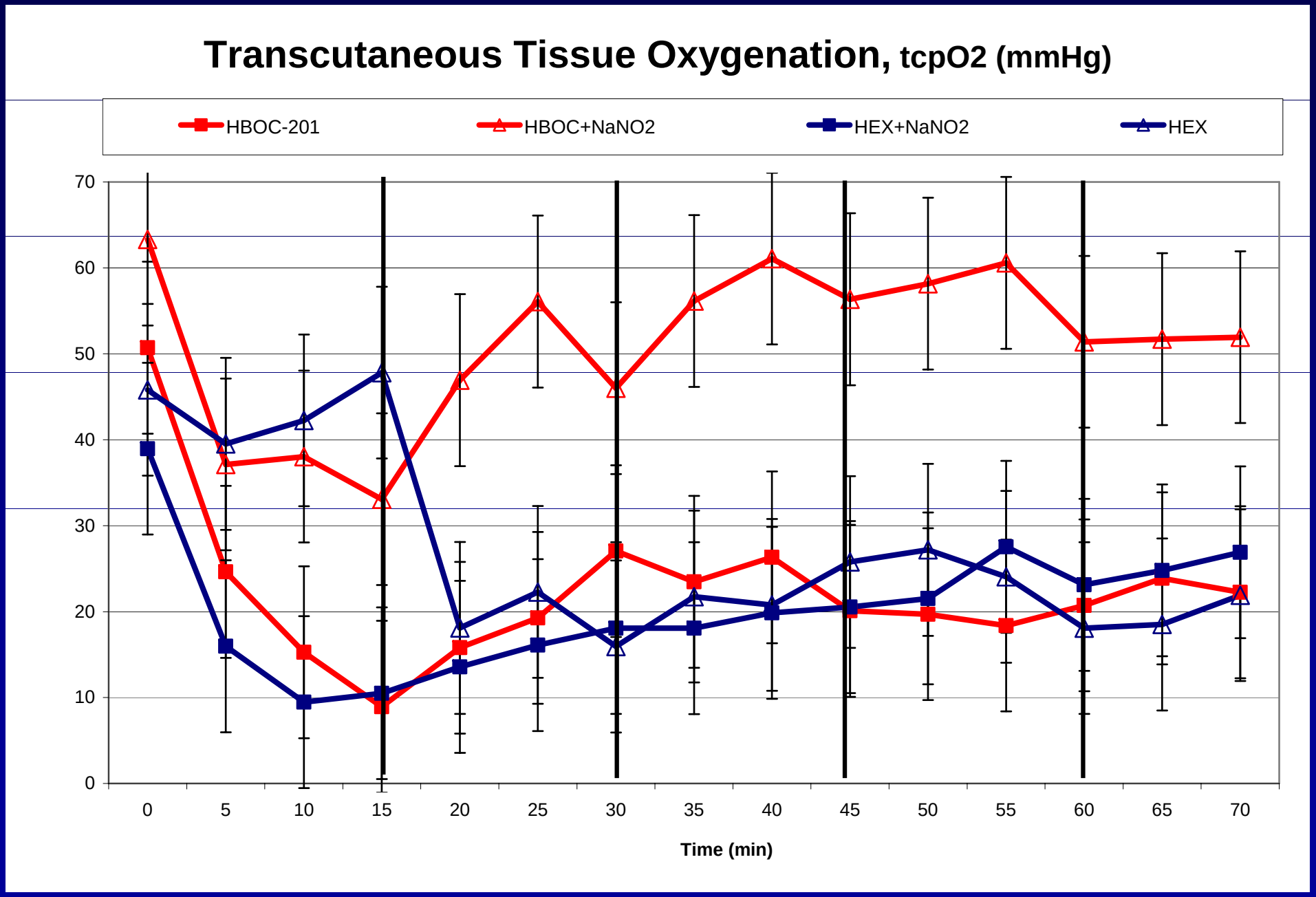
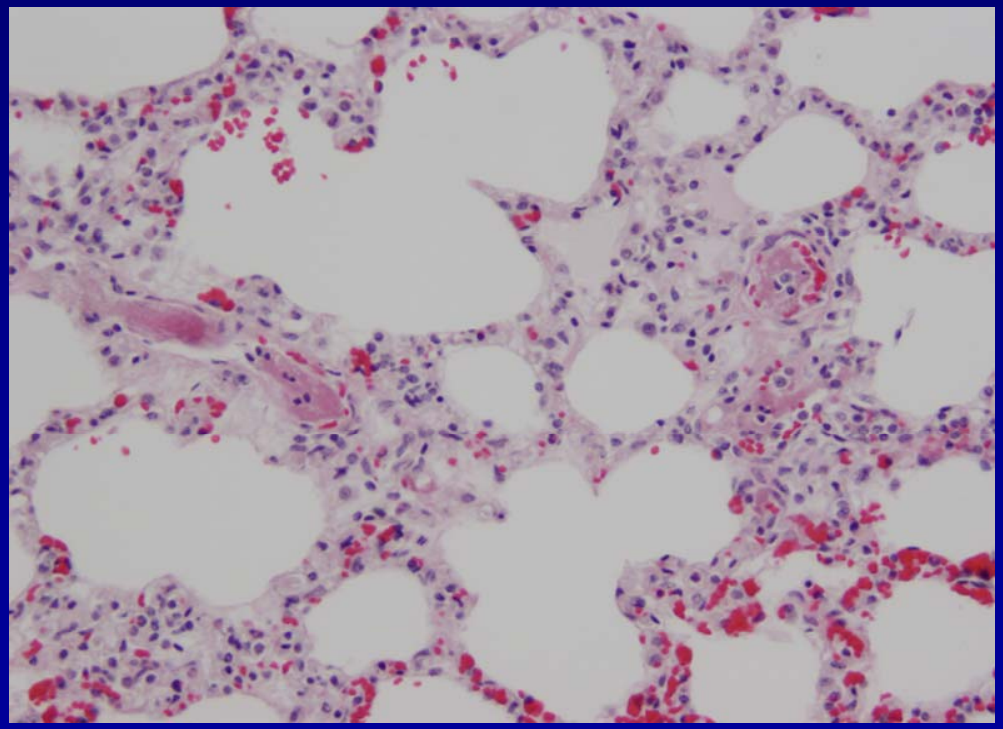
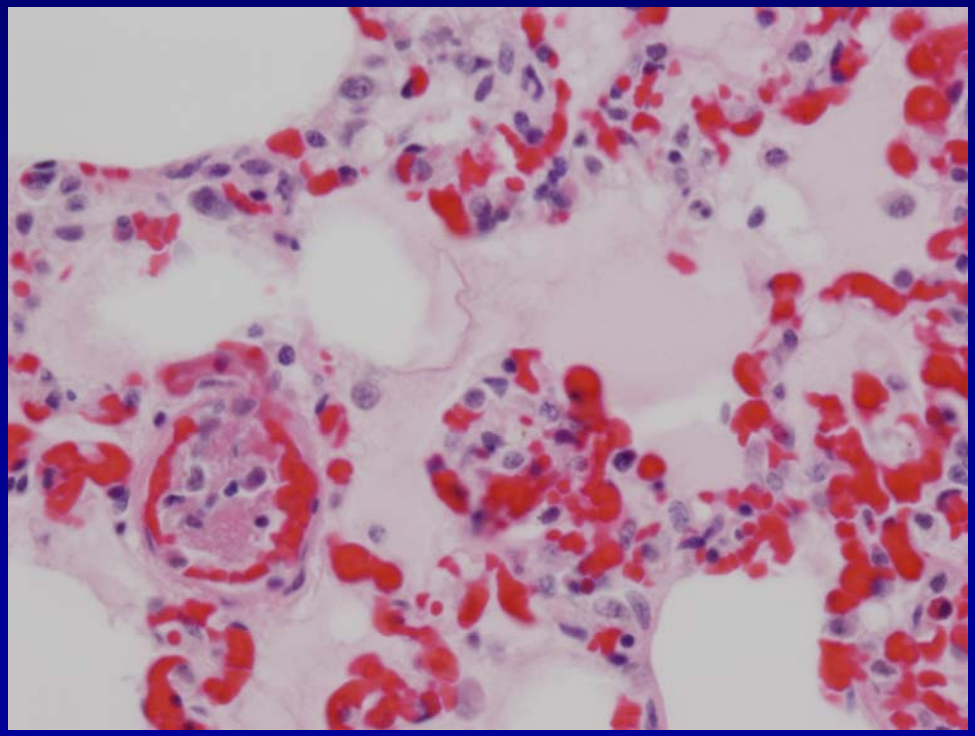


Part 1: Sodium Nitrite study

All HBOC + $NaNO_2$ animals - LUNGS

Macroscopic: edema, congested, heavy
Microscopic: thrombi, alveolar edema





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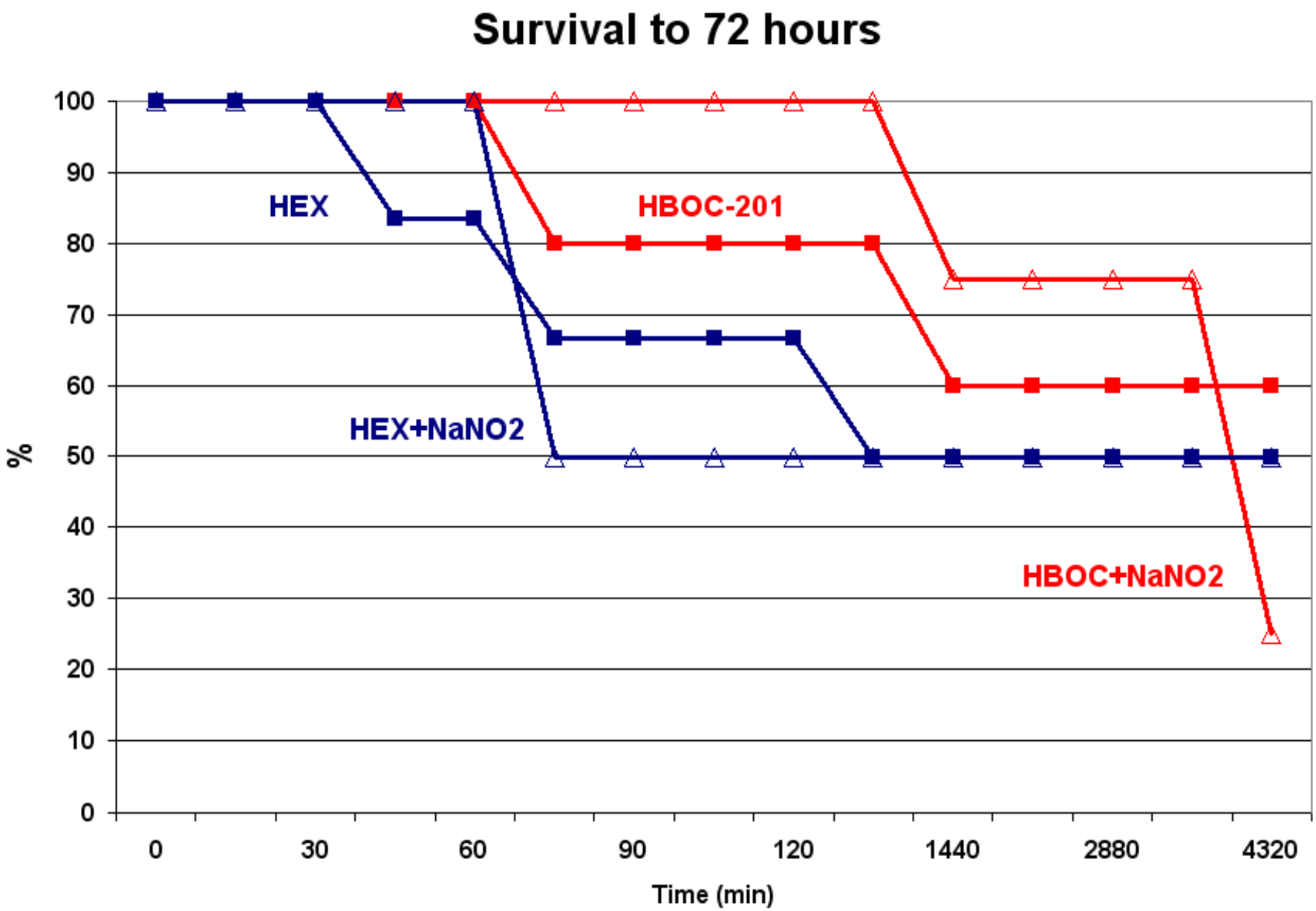


Summary

Addition of sodium nitrite

- Attenuated vasoactivity asssociated with HBOC-201
- **BUT:** compromised survival, due to interactions not yet elucidated

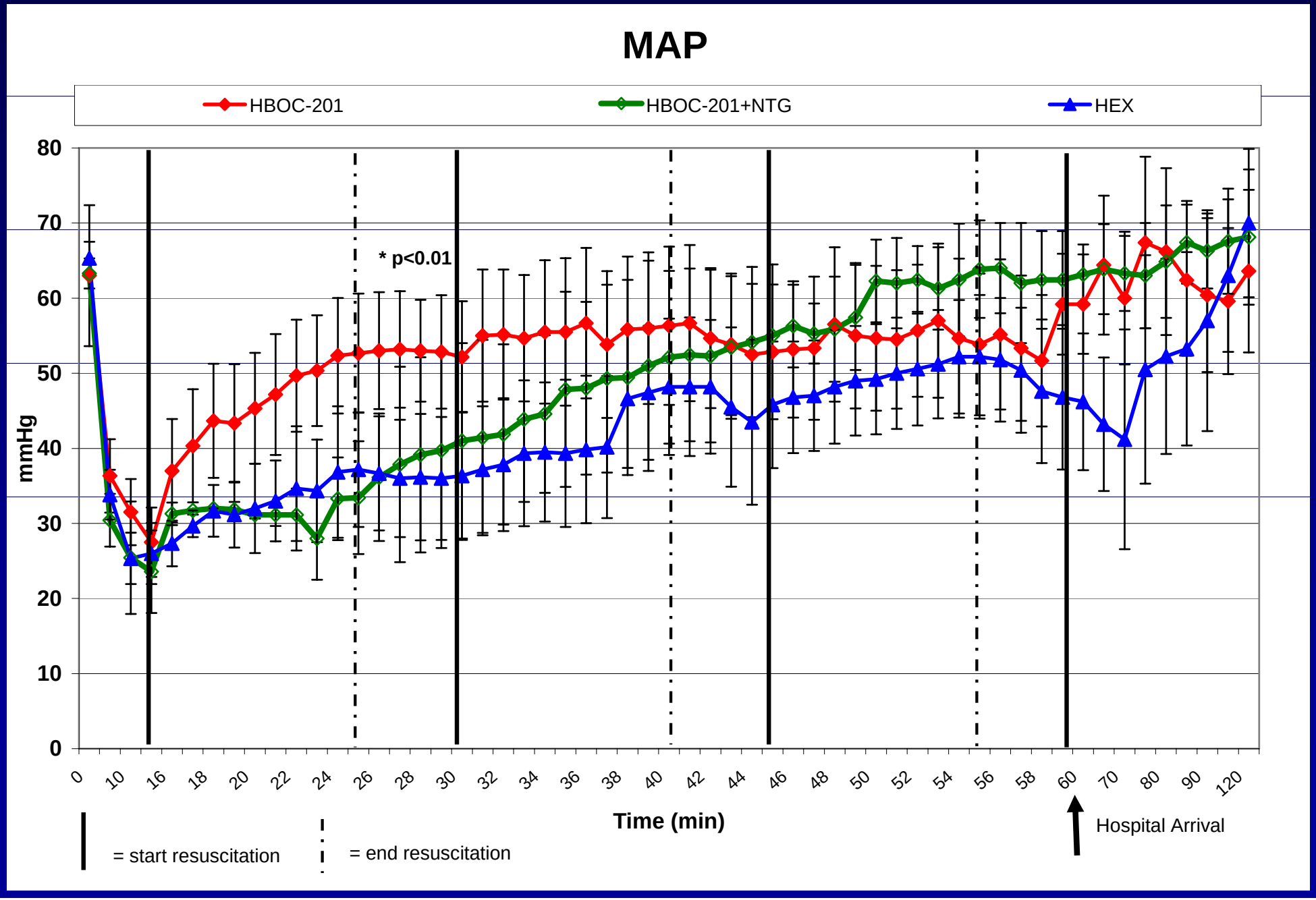
Part 2

HBOC-201 + Nitroglycerin (NTG)

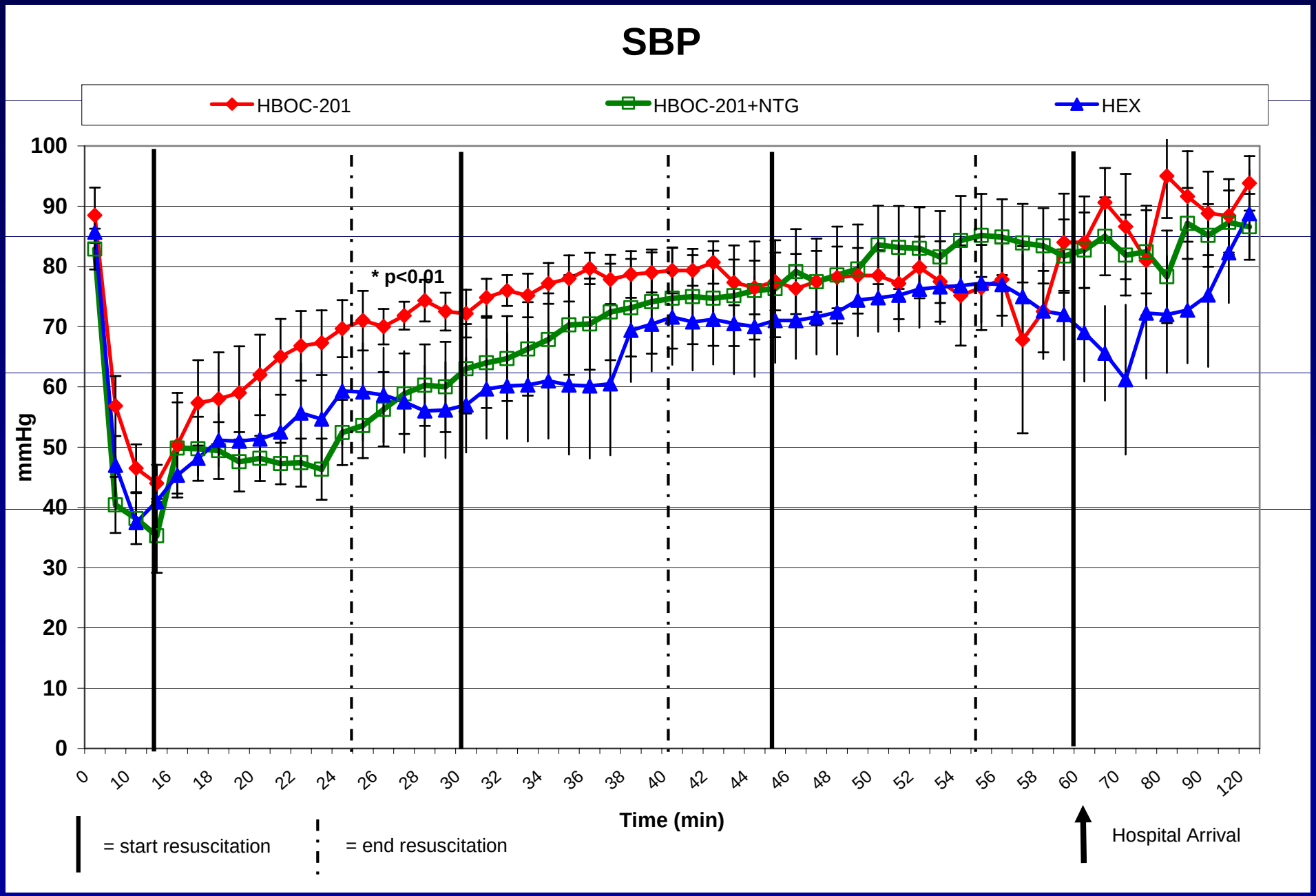
Part 2: NTG study

- Dose: 5 μg/kg/min
- Dose based on clinical use

Part 2: NTG study



Part 2: NTG study



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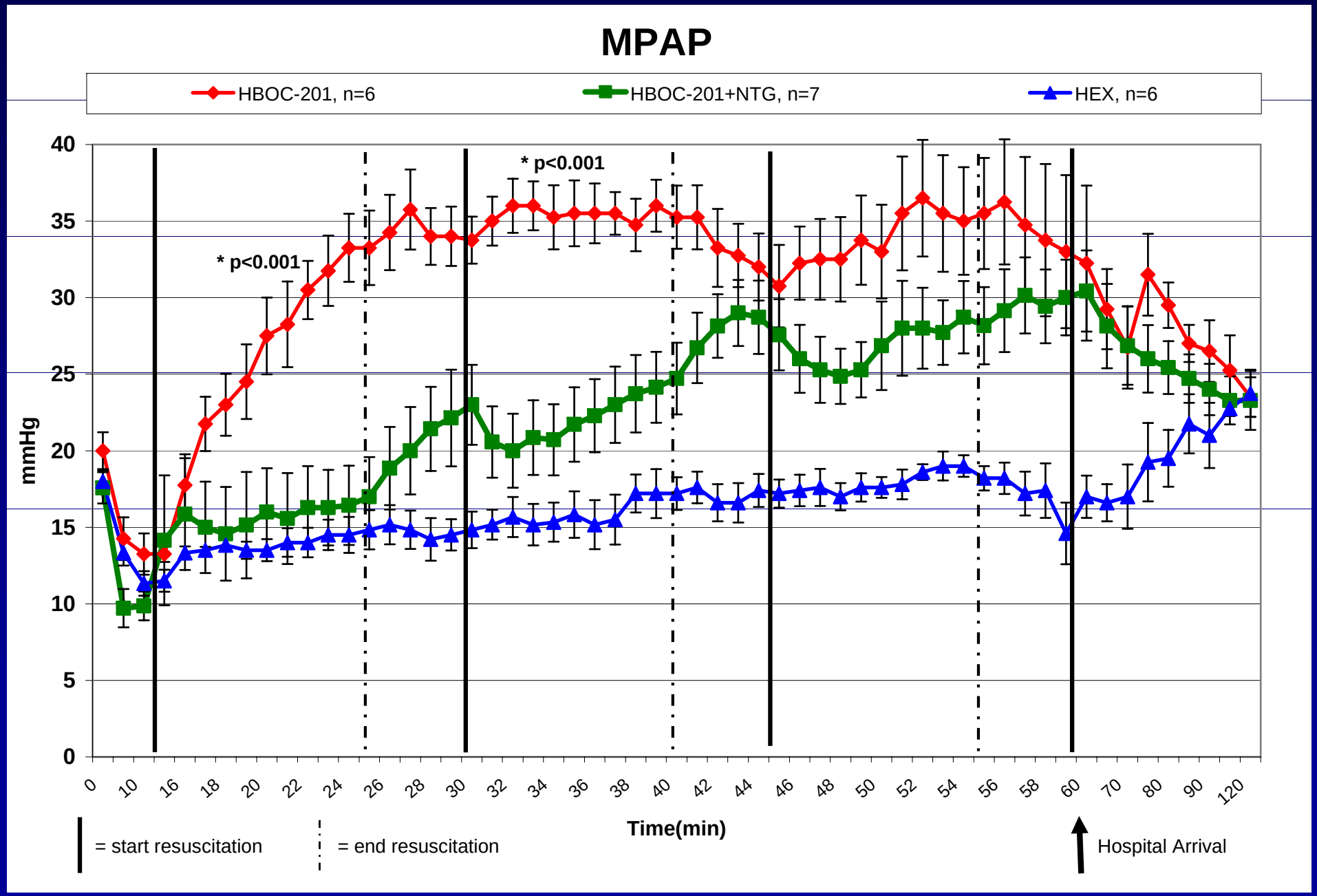

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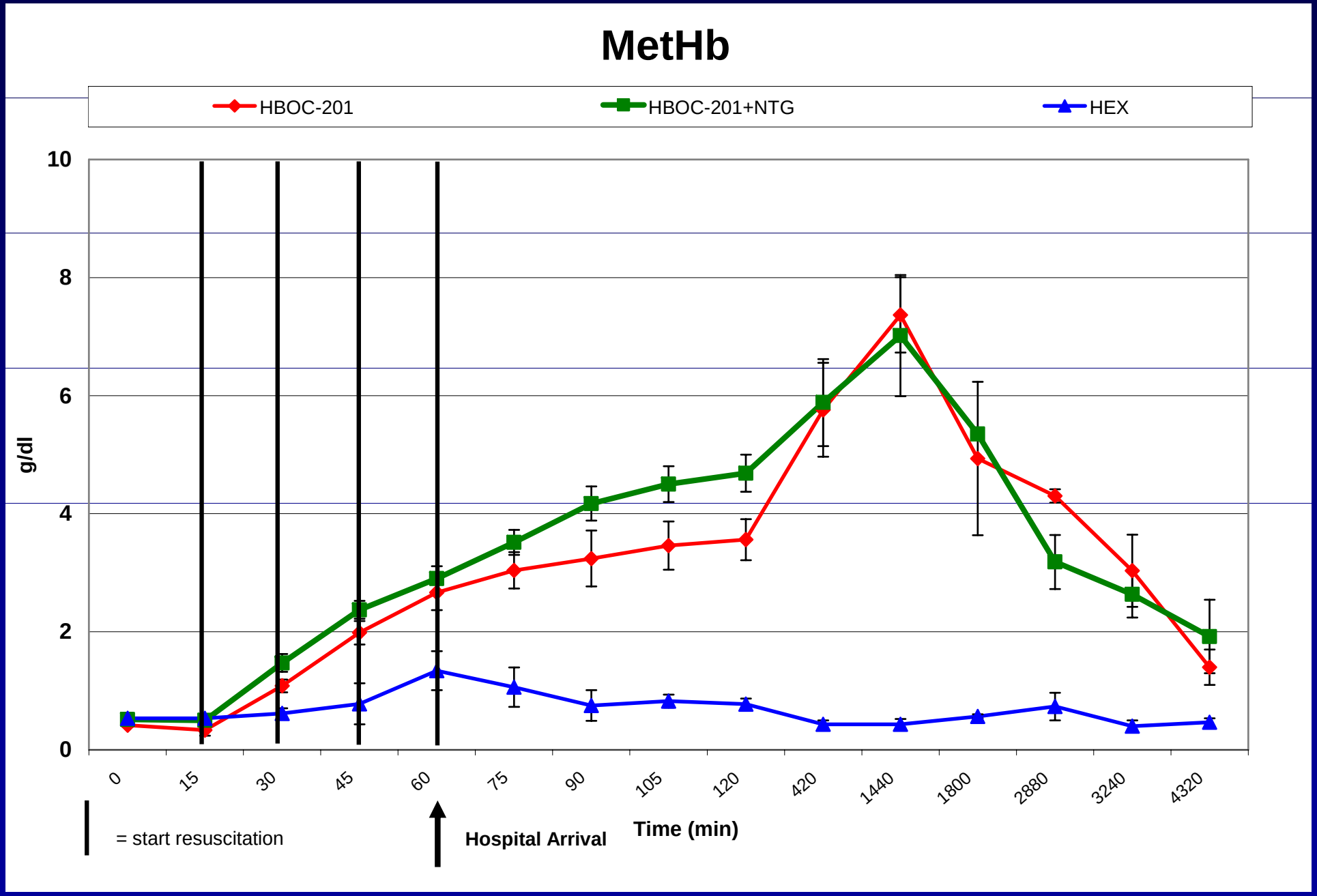

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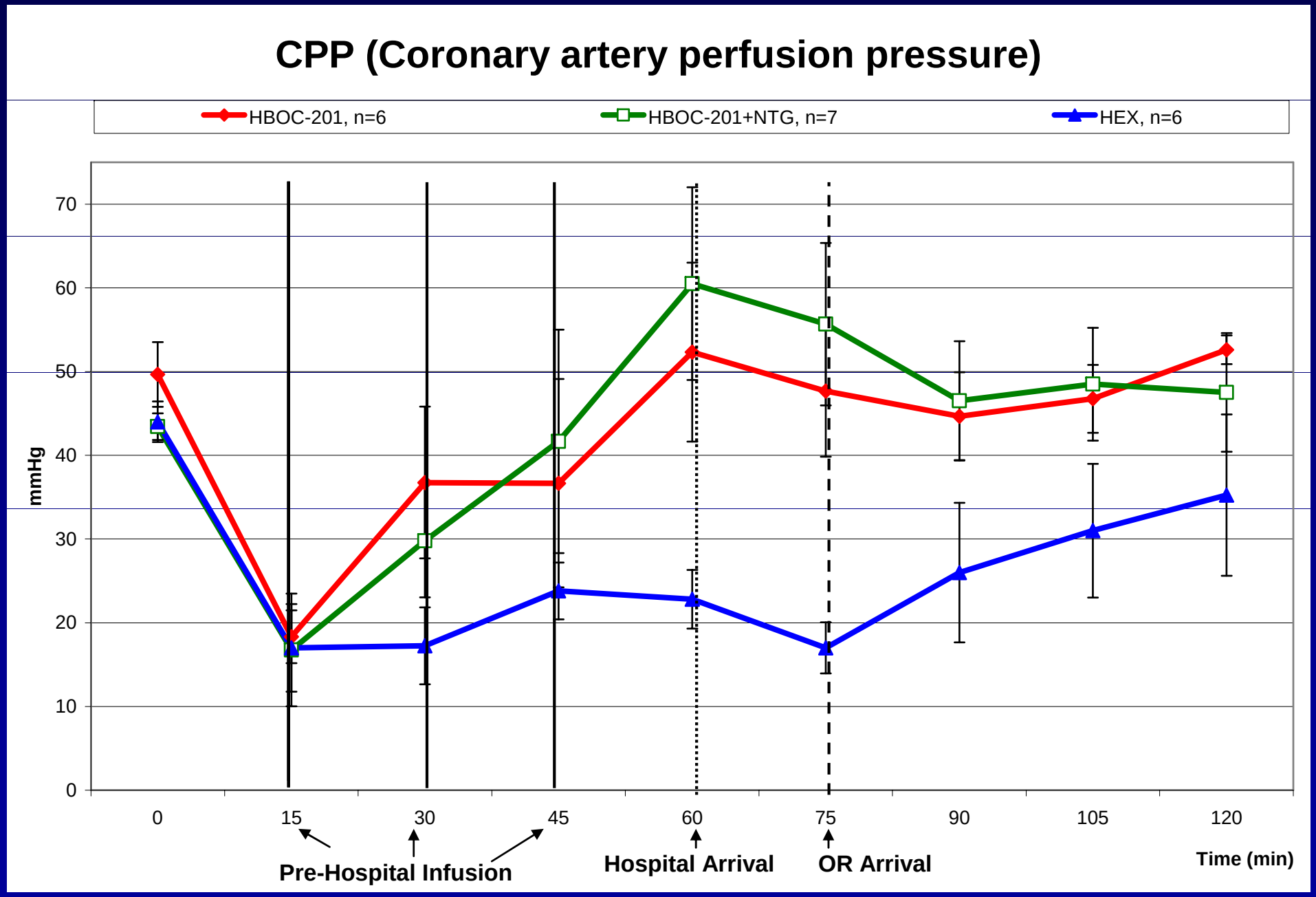

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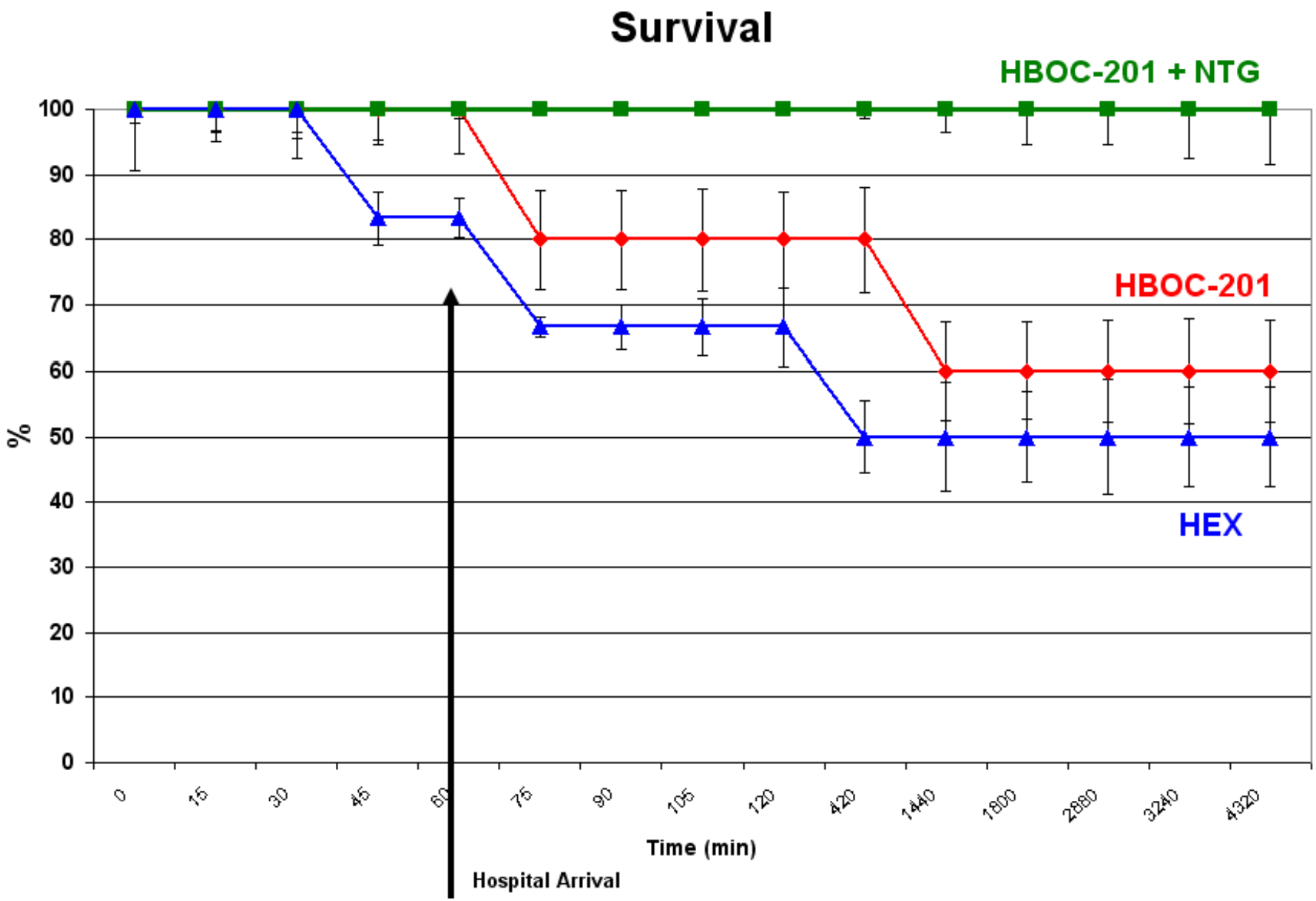

Summary

- NTG reduced pulmonary artery pressure effectively, while allowing systemic arterial pressure to rise within clinically desirable values.
- NTG did not show side effects

Conclusion

- We can control vasoconstriction associated with HBOC-201 in the pre-hospital environment.

Future

Currently:

1. Polytrauma study (TBI + liver injury) with HBOC-201 + NTG in swine
2. NTG stability pilot study (goal: HBOC-201 and NTG in 1 bag)

Next:

1. Toxicology study in rabbits using various doses of NTG with HBOC-201 in three exchange transfusions
2. Cardiotoxicity study in rabbits
3. Swine study mimicking proposed Phase 1b trial
4. Phase 1b trial

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Questions?