Introduction

- Vascular endothelium dysfunction is a key component in the pathogenesis of many diseases
  - Ischemia reperfusion (I/R) Injury (i.e. Myocardial Infarct and Stroke)
  - Diabetes
  - Atherosclerosis
- Reduced bioavailability of nitric oxide (NO) coupled with an increase in superoxide (SO) release in blood.
  - SO is converted to hydrogen peroxide ($\text{H}_2\text{O}_2$) by Superoxide Dismutase
- Sources of oxidative stress in I/R injury
  - NADPH Oxidase
    - Source of SO release from leukocytes as well as endothelial cells
    - Activated by PKC (primarily βII and zeta isoforms)
  - eNOS uncoupling
    - Oxidation of tetrahydrobiopterin ($\text{BH}_4$) to dihydrobiopterin ($\text{BH}_2$) leads to increased $\text{BH}_2/\text{BH}_4$ ratio
    - This facilitates a switch from NO production to SO production
NADPH oxidase
COUPLED VS. UNCOUPLED eNOS

**Coupled eNOS**
- O₂
- L-Arg
- L-Cit
- NO
- BH4
- CaM
- FMN
- FAD
- NADP
- NADPH
- e⁻ reductase

**Uncoupled eNOS**
- O₂
- L-Arg
- BH₂
- CaM
- FMN
- FAD
- NADP
- NADPH
- e⁻ reductase
Introduction Cont.

- **Protein Kinase C**
  - Three subfamilies of isozymes
    - Classical (α, βI, βII, and γ)
    - Novel (δ, ε, η, and θ)
    - Atypical (ζ)
  - Mediates PMN activation and endothelial cell response during I/R
  - Activated PKC βII, ζ stimulate NADPH oxidase to release SO from PMNs /endothelial cells
  - Activated PKC βII, δ and ζ inhibit eNOS activity

- **Gö 6983**
  - Broad spectrum PKC inhibitor (MW= 442)
    - Inhibits classical, novel, and atypical PKC isoforms found in PMNs/ endothelial cells
    - Inhibits leukocyte SO release (in vitro)
    - Increases aortic segment NO release (in vitro)
    - Restores post-reperfusion cardiac function (ex vivo)
    - Attenuates leukocyte-endothelial interactions (in vivo)

- Hypothesis: Inhibiting PKC with Gö 6983 at the beginning of reperfusion in femoral I/R will attenuate H₂O₂ release and increase NO release in blood (in vivo).
Methods

- Measure $\text{H}_2\text{O}_2$/NO release from rat femoral veins in vivo

- Male Sprague-Dawley rats (275-325g) one limb undergoes I/R, the other a non-ischemic sham control

- $\text{H}_2\text{O}_2$ or NO microsensors (100 µm, World Precision Instruments (WPI) Inc.) are inserted (via a catheter) inside each femoral vein and are connected to a free radical analyzer (Apollo 4000 from WPI).

- Record NO or $\text{H}_2\text{O}_2$ release every 5 min during:
  - 15 minutes of BASELINE
  - 20 minutes ISCHEMIA (clamped femoral artery and vein)
  - 45 minutes REPERFUSION (clamp released)

- Drug
  - Gö 6983 dissolved in saline (7.4µg/kg I.V. via tail vein, about 200nM in blood)

- The electrical signal (picoamps) was converted to a molar concentration through a calibration curve.

- The changes of NO or $\text{H}_2\text{O}_2$ is expressed by the difference between I/R limb and sham limb
  - Nitric Oxide (NO) – nM
  - Hydrogen Peroxide ($\text{H}_2\text{O}_2$) – µM

- Data analyzed by student’s t-test.
Experimental preparation (in Femoral I/R)

Figure 1. The preparation for measuring $\text{H}_2\text{O}_2$ or NO release from femoral veins.
Results: $\text{H}_2\text{O}_2$ release from femoral veins

Figure 2. A sample trace of $\text{H}_2\text{O}_2$ release from sham and I/R femoral veins
Relative Change: H$_2$O$_2$ Control

Figure 3. Time course of H$_2$O$_2$ (µM) release during reperfusion from rat femoral veins in the control group. A. Anesthetized rats were given saline i.v. at the beginning of reperfusion (n=7). There was a significant increase in H$_2$O$_2$ release from I/R compared to sham femoral veins throughout reperfusion (*p<0.05 from sham, **p<0.01 from sham).

B. Represents relative difference in H$_2$O$_2$ release between I/R and sham femoral veins (*p<0.05 from initial, **p<0.01 from initial).
Results: H$_2$O$_2$ release in Gö 6983, or control groups

![Graph showing H$_2$O$_2$ release during reperfusion](image)

Figure 4. Measurement of H$_2$O$_2$ (µM) release from rat femoral veins during reperfusion. Anesthetized rats were given Gö 6983 (7.4 µg/kg). There was a significant decrease in H$_2$O$_2$ release from I/R veins compared to sham veins during reperfusion in drug treated groups vs. saline control (*p<0.05 from saline).
Figure 5. Time course of NO (nM) release during reperfusion from rat femoral veins in the control group. A. Anesthetized rats were given saline i.v. at the beginning of reperfusion (n=6). There was no significant difference in NO release from I/R veins compared to sham veins during reperfusion. B. Represents relative difference in NO release between I/R and sham femoral veins.
Results: NO release in Gö 6983, or control groups

Figure 6. Measurement of NO (nM) release from rat femoral veins during reperfusion. Anesthetized rats were given Gö 6983 (7.4 μg/kg). There was a significant increase in NO release from I/R veins compared to sham veins during reperfusion with Gö 6983 treated vs. saline control (**p<0.01 from saline).
Physiological Significance

• Reduction in $\text{H}_2\text{O}_2$ release in femoral I/R correlates with improved post-reperfused cardiac function
• Reduction in $\text{H}_2\text{O}_2$ release correlates with attenuation of leukocyte-endothelial interactions in mesenteric circulation
• Data suggests that Gö 6983 may be effective in reducing oxidative stress associated with clinical organ transplantation
Conclusion

1. Gö 6983 significantly decreases H$_2$O$_2$ release during reperfusion in femoral veins subjected to I/R.
2. Gö 6983 significantly increases NO release during reperfusion in femoral veins subjected to I/R.

References


